ISSN 0284-186X

Volume 57/ No 1/2018

ACTA ONCOLOGICA

Special Issue: Papers at the occasion of the 40-years anniversary of DBCG – the Danish Breast Cancer Cooperative Group and the 16th Acta Oncologica Symposium, Aarhus, Denmark – January 18–19, 2018

www.tandfonline.com/ionc

Taylor & Francis
Taylor & Francis Group

Papers at the occasion of the 40-years anniversary of DBCG – the Danish Breast Cancer Cooperative Group and the 16th Acta Oncologica Symposium, Aarhus, Denmark - January 18-19, 2018

Editorial
Is DBCG abreast of new developments?
Birgitte V. Offersen, Bent Ejlertsen, Eva Balslev, Henrik Flyger, Anne-Marie Gerdes, Mads K. Hansen, Lisbet R. Hølmich, Maj-Britt Jensen, Bent Kristensen, Anne-Vibeke Laenkholm, Henning T. Mouridsen, Mette H. Nielsen, Jens Overgaard, Malgorzata Tuxen, Ilse Vejborg & Peer Christiansen
Pages: 1-2

Article
Forty years of landmark trials undertaken by the Danish Breast Cancer Cooperative Group (DBCG) nationwide or in international collaboration
Bent Ejlertsen, Birgitte Vrou Offersen, Jens Overgaard, Peer Christiansen, Maj-Britt Jensen, Niels Kroman, Ann Søgaard Knoop & Henning Mouridsen
Pages: 3-12

Article
The clinical database and implementation of treatment guidelines by the Danish Breast Cancer Cooperative Group in 2007–2016
Maj-Britt Jensen, Anne-Vibeke Laenkholm, Birgitte V. Offersen, Peer Christiansen, Niels Kroman, Henning T. Mouridsen & Bent Ejlertsen
Pages: 13-18

Article
Breast conserving surgery versus mastectomy: overall and relative survival—a population based study by the Danish Breast Cancer Cooperative Group (DBCG)
Peer Christiansen, Stina Lyck Carstensen, Bent Ejlertsen, Niels Kroman, Birgitte Offersen, Anne Bodilsen & Maj-Britt Jensen
Pages: 19-25

Article
Two years of tamoxifen or no adjuvant systemic therapy for patients with high-risk breast cancer: long-term follow-up of the Copenhagen breast cancer trial
Maj-Britt Jensen, Jens Fabricius Krarup, Torben Palshof, Henning T. Mouridsen & Bent Ejlertsen
Pages: 26-30

Article
Neoadjuvant letrozole for postmenopausal estrogen receptor-positive, HER2-negative breast cancer patients, a study from the Danish Breast Cancer Cooperative Group (DBCG)
Signe Korsgaard Skriver, Anne-Vibeke Laenkholm, Birgitte Bruun Rasmussen, Jürgen Handler, Bo Grundtmann, Tove Filtenborg Tvedskov, Peer Christiansen, Ann S. Knoop, Maj-Britt Jensen & Bent Ejlertsen
Pages: 31-37

Article
Intrinsic subtypes and benefit from postmastectomy radiotherapy in node-positive premenopausal breast cancer patients who received adjuvant chemotherapy – results from two independent randomized trials
Tinne Laurberg, Trine Tramm, Torsten Nielsen, Jan Alsner, Silje Nord, Simen Myhre, Therese Sørlie, Samuel Leung, Cheng Fan, Charles Perou, Karen Gelmon, Jens Overgaard, David Voduc, Aleix Prat & Maggie Chon U. Cheang
Pages: 38-43

Article
The ability of PAM50 risk of recurrence score to predict 10-year distant recurrence in hormone receptor-positive postmenopausal women with special histological subtypes
Anne-Vibeke Laenkholm, Maj-Britt Jensen, Jens Ole Eriksen, Wesley Buckingham, Sean Ferree, Torsten O. Nielsen & Bent Ejlertsen
Pages: 44-50

Article
Characterization of basal-like subtype in a Danish consecutive primary breast cancer cohort
Savvas Kinalis, Finn Cilius Nielsen, Maj-Lis Talman, Bent Ejlertsen & Maria Rossing
Pages: 51-57

Article
Molecular subtyping of breast cancer improves identification of both high and low risk patients
Maria Rossing, Olga Østrup, Wiktor W. Majewski, Savvas Kinalis, Maj-Britt Jensen, Ann Knoop, Niels Kroman, Maj-Lis Talman, Thomas V. O. Hansen, Bent Ejlertsen & Finn C. Nielsen
Pages: 58-66

Article
Aurora kinase A as a possible marker for endocrine resistance in early estrogen receptor positive breast cancer
Anne E. Lykkesfeldt, Benedikte R. Iversen, Maj-Britt Jensen, Bent Ejlertsen, Anita Giobbie-Hurder, Birgit E. Reiter, Tove Kirkegaard & Birgitte B. Rasmussen
Pages: 67-73

Review
Triple negative breast cancer – prognostic role of immune-related factors: a systematic review
Elisabeth Specht Stovgaard, Dorte Nielsen, Estrid Hogdall & Eva Balslev
Pages: 74-82

Article
An inter-observer Ki67 reproducibility study applying two different assessment methods: on behalf of the Danish Scientific Committee of Pathology, Danish breast cancer cooperative group (DBCG)
Anne-Vibeke Laenkholm, Dorthe Grabau, Maj-Lis Møller Talman, Eva Balslev, Anne Marie Bak Jylling, Tomasz Piotr Tabor, Morten Johansen, Anja Brügmann, Giedrius Lelkaitis, Tina Di Caterino, Henrik Mygind, Thomas Poulsen, Henrik Mertz, Gorm Søndergaard & Birgitte Bruun Rasmussen
Pages: 83-89

Article
Standardized assessment of tumor-infiltrating lymphocytes in breast cancer: an evaluation of inter-observer agreement between pathologists
Trine Tramm, Tina Di Caterino, Anne-Marie B. Jylling, Giedrius Lelkaitis, Anne-Vibeke Lænkholm, Péter Ragó, Tomasz P. Tabor, Maj-Lis M. Talman, Emmanouela Vouza & On behalf of the Scientific Committee of Pathology, Danish Breast Cancer Group (DBCG)
Pages: 90-94

Article
Clinical and molecular characterization of BRCA-associated breast cancer: results from the DBCG
I. M. H. Soenderstrup, A. V. Laenkholm, M. B. Jensen, J. O. Eriksen, A. M. Gerdes, T. V. O. Hansen, T. A. Kruse, M. J. Larsen, I. S. Pedersen, M. Rossing, M. Thomassen & B. Ejlertsen
Pages: 95-101

Article
Influence of intra-tumoral heterogeneity on the evaluation of BCL2, E-cadherin, EGFR, EMMPRIN, and Ki-67 expression in tissue microarrays from breast cancer
Trine Tramm, Marianne Kyndi, Flemming B. Sørensen, Jens Overgaard & Jan Alsner
Pages: 102-106

Article
DBCG hypo trial validation of radiotherapy parameters from a national data bank versus manual reporting
Carsten Brink, Ebbe L. Lorenzen, Simon Long Krogh, Jonas Westberg, Martin Berg, Ingelise Jensen, Mette Skovhus Thomsen, Esben Svitzer Yates & Birgitte Vrou Offersen
Pages: 107-112

Article
The potential benefits from respiratory gating for breast cancer patients regarding target coverage and dose to organs at risk when applying strict dose limits to the heart: results from the DBCG HYPO trial
Martin Berg, Ebbe L. Lorenzen, Ingelise Jensen, Mette S. Thomsen, Christina Maria Lutz, Lasse Refsgaard, Henrik D. Nissen & Birgitte V. Offersen
Pages: 113-119

Article
Concurrent new drug prescriptions and prognosis of early breast cancer: studies using the Danish Breast Cancer Group clinical database
Deirdre Cronin-Fenton, Timothy L. Lash, Thomas P. Ahern, Per Damkier, Peer Christiansen, Bent Ejlertsen & Henrik T. Sørensen
Pages: 120-128

Article
Influence of specific comorbidities on survival after early-stage breast cancer
Marianne Ewertz, Lotte Holm Land, Susanne Oksbjerg Dalton, Deirdre Cronin-Fenton & Maj-Britt Jensen
Pages: 129-134

Article
Mortality and recurrence rates among systemically untreated high risk breast cancer patients included in the DBCG 77 trials
Maj-Britt Jensen, Torsten O. Nielsen, Ann S. Knoop, Anne-Vibeke Laenkholm, Eva Balslev & Bent Ejlertsen
Pages: 135-140

Article
The occurrence of fractures after adjuvant treatment of breast cancer: a DBCG register study
Bent Kristensen, Bent Ejlertsen, Maj-Britt Jensen, Henning T. Mouridsen & for the Danish Breast Cancer Cooperative Group
Pages: 141-145

Article
Quality of life and care needs in women with estrogen positive metastatic breast cancer: a qualitative study
Gitte Lee Mortensen, Ivan Bredbjerg Madsen, Randi Krogsgaard & Bent Ejlertsen
Pages: 146-151

Letter
Breast cancer patient advocacy in Denmark
Eva Bundesen
Pages: 152-153

Letter
Provision of data from the clinical database and of biological material from the tumor bank of the Danish Breast Cancer Cooperative Group 2008–2017
Henning Mouridsen, Peer Christiansen, Maj-Britt Jensen, Anne-Vibeke Laenkholm, Henrik Flyger, Birgitte Offersen, Ilse Vejborg & Bent Ejlertsen
Pages: 154-156

Letter
Palliative treatment with carboplatin as late line therapy to patients with metastatic breast cancer
Lena H. Rosvig, Sven T. Langkjer, Ann Knoop & Anders B. Jensen
Pages: 156-159

Letter
A Danish national effort of BRCA1/2 variant classification
Inge Søkilde Pedersen, Ane Y. Schmidt, Birgitte Bertelsen, Anja Ernst, Christian Liebst Toft Andersen, Torben Kruse, Maria Rossing & Mads Thomassen
Pages: 159-162

Letter
The accuracy of preoperative staging of the axilla in primary breast cancer: a national register based study on behalf of Danish Breast Cancer Group (DBCG)
Kristine Hamran, Linnea Langhans, Ilse Vejborg, Tove Filtenborg Tvedskov & Niels Kroman
Pages: 162-166

Letter
Axillary lymph node dissection in breast cancer patients after sentinel node biopsy
Christina Jessing, Linnea Langhans, Maj-Britt Jensen, Maj-Lis Talman, Tove Filtenborg Tvedskov & Niels Kroman
Pages: 166-169

Letter
Occult breast cancer in breast reduction specimens
Lotte Meyer, Camilla Bille, Thomas Foged & Anne Marie Bak Jylling
Pages: 170-173

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 1–2
https://doi.org/10.1080/0284186X.2017.1408960

Taylor & Francis
Taylor & Francis Group

EDITORIAL

Is DBCG abreast of new developments?

Birgitte V. Offersen[a,b], Bent Ejlertsen[c,d], Eva Balslev[e], Henrik Flyger[f], Anne-Marie Gerdes[g], Mads K. Hansen[h], Lisbet R. Hølmich[i], Maj-Britt Jensen[c], Bent Kristensen[j], Anne-Vibeke Laenkholm[k], Henning T. Mouridsen[c], Mette H. Nielsen[l], Jens Overgaard[b], Malgorzata Tuxen[m], Ilse Vejborg[n] and Peer Christiansen[o]

[a]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark; [c]Danish Breast Cancer Group (DBCG) Secretariat and Statistical Office, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [d]Department of Oncology, Copenhagen University Rigshospitalet, Copenhagen, Denmark; [e]Department of Pathology, Herlev University Hospital, Herlev, Denmark; [f]Department of Breast Surgery, Herlev University Hospital, Herlev, Denmark; [g]Department of Clinical Genetics, Copenhagen University Rigshospitalet, Copenhagen, Denmark; [h]Lillebaelt Hospital, Vejle, Denmark; [i]Department of Plastic Surgery, Herlev University Hospital, Herlev, Denmark; [j]Department of Clinical Physiology & Nuclear Medicine, Herlev University Hospital, Herlev, Denmark; [k]Department of Surgical Pathology, Zeeland University Hospital, Slagelse, Denmark; [l]Department of Oncology, Odense University Hospital, Odense, Denmark; [m]Department of Oncology, Herlev University Hospital, Herlev, Denmark; [n]Department of Radiology, Copenhagen University Rigshospitalet, Copenhagen, Denmark; [o]Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark

The Danish Breast Cancer Group (DBCG) was established in 1977 by innovative and visionary clinicians and researchers diagnosing, treating and investigating breast cancer. The vision was to gather all existing expertise in breast cancer in a national multidisciplinary network to focus on optimal diagnosis, treatment and follow-up to these patients, preferably as part of relevant evidence-generating trials. The beginning to a comprehensive and large database was established, and since then nearly all Danish breast cancer patients have been registered with data regarding diagnosis, treatment and follow-up. As part of the multidisciplinary strategy, a DBCG Board was established to oversee DBCG-related strategies including approving national therapy guidelines proposed by DBCG committees for radiology, surgery, pathology, radiation therapy, systemic therapy, nuclear medicine, genetics and translational research. These committees have representatives from all relevant Danish departments treating breast cancer patients with the aim to constantly ensure consistency in diagnosis, therapy, follow-up and research of patients all over the country. In a worldwide perspective, DBCG is highly unique, since it is truly exceptional that experts from a whole country meet on a regular basis to discuss and agree on guidelines and develop and participate in trials. The structure of DBCG has subsequently served as the template for other Danish Multidisciplinary Cancer Groups.

Through this unique national multidisciplinary collaboration, DBCG has been leading in ensuring Danish patients diagnosis and therapy at the highest international level. So it is relevant to ask, if we can relax, lean back and simply congratulate ourselves on mission accomplished, or is it time to seize the torch and move forward? The DBCG Board does not think so. On the contrary, it is a clear goal to maintain the high standards and in addition constantly develop new treatment strategies to contribute to further enhancement of the guidelines both nationwide, Nordic and internationally. Therefore, at this 40-year anniversary the DBCG Board has identified challenges that need special focus in the next decade to maintain the high standard and quality of DBCG. Importantly, the strength of DBCG is highly based on the nationwide set-up, and since Denmark is a small country it is more important than ever that the nationwide aspect of DBCG is ensured in the future. Through the nationwide platform, the DBCG and its committees must support a multidisciplinary approach towards all breast cancer patients so they are treated according to DBCG guidelines and offered relevant trials. It is a DBCG goal that within the next decade all Danish patients operated for invasive breast cancer or ductal carcinoma *in situ* (DCIS) will be offered at least one evidence-generating trial, and such trials should be investigator initiated through the relevant DBCG committees and the DBCG Board. It is pivotal that these trials are offered to all patients, thus they must be open in all departments treating Danish patients. After sustained intensification for three decades, focus in the recent decade has shifted towards de-escalation of treatments, and as the prognosis fortunately has improved over the years due to early diagnosis in which implementation of nationwide mammography played a major role and due to improvements in treatment, it has become increasingly important to establish criteria for selection of the individual patient for certain therapies. Therefore, it is also a goal for DBCG to ensure that the DBCG initiated trials will explore criteria for selection of patients to more or less therapy, maybe even omission of therapy, which is otherwise provided today. These criteria may be focused on risk of recurrence, but they may also focus on the patient's individual risk of late morbidity related to a certain therapy. In this way, studies and trials will be initiated examining the individual

CONTACT Birgitte V. Offersen birgoffe@rm.dk Department of Oncology, Aarhus University Hospital, Aarhus, Denmark

gain and harm from that therapy. Such data are highly needed to provide optimal information to the individual patient during the process of shared decision making.

During the recent decade more and more unnecessary obstacles have hampered fast access to individual patient and treatment data from the DBCG database. In the 1970s virtually no rules existed to protect patients when performing research, and fortunately international declarations and Ethical Committees have now provided guidelines for research, which also are incorporated in the DBCG rules. However, during the last decade more and more paper work has been requested from authorities to control what is being investigated. It has reached a point, where it may take more than 6 months from a researcher is ready to start a project before access to data is granted, and that is not acceptable. Therefore, together with the Danish Comprehensive Cancer Center (DCCC) DBCG will fight the increasing bureaucracy to the benefit of all cancer databases in Denmark. Any unnecessary obstacle encountered performing research must be identified and through DCCC Danish politicians must be made aware of this and action will be demanded.

Fortunately, improvements of therapy occur, as also new focus areas treating patients with invasive breast cancer or DCIS emerge. As part of the Danish National Cancer Plan IV much interest is now paid to research in late morbidity, but data on late morbidity have until now not been part of routine reporting to the DBCG database. Therefore, expanding the database with such data has very high priority in the years to come, and new technologies should be implemented as part of this, for example, it should be possible to report patient reported outcome directly to the database. DBCG will adopt artificial intelligence and support machine learning and other new technologies to obtain data from the patients in an efficient way. Furthermore, a constant focus will be maintained on collecting missing data from the Danish departments, because high quality research is impossible if the data quality is poor.

In conclusion, the DBCG has a high focus on maintaining and further develop the current level of all aspects of patients treated for breast cancer or DCIS. Every effort will be done to develop and support investigator initiated trials as well as also nationwide prospective cohort studies. The overall goal is to offer every Danish patient diagnosis, treatment and follow-up at the highest international level in all parts of Denmark. Detailed data related to the patient's course in the hospital and during follow-up will be collected to document the current status at any time. The DBCG committees and Board will always strive to be one step ahead and ensure a systematic and thorough implementation of new standards in Denmark to the benefit of our patients.

ORCID

Henrik Flyger http://orcid.org/0000-0001-8441-0805

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 3–12
https://doi.org/10.1080/0284186X.2017.1408962

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Forty years of landmark trials undertaken by the Danish Breast Cancer Cooperative Group (DBCG) nationwide or in international collaboration

Bent Ejlertsen[a,b], Birgitte Vrou Offersen[c,d], Jens Overgaard[d], Peer Christiansen[e], Maj-Britt Jensen[a], Niels Kroman[f], Ann Søgaard Knoop[b] and Henning Mouridsen[a]

[a]Danish Breast Cancer Cooperative Group (DBCG) Secretariat and Statistical Office, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [b]Department of Oncology, Copenhagen University Rigshospitalet, Copenhagen, Denmark; [c]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark; [d]Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark; [e]Department of Breast Surgery, Aarhus University Hospital, Aarhus, Denmark; [f]Department of Breast Surgery, Copenhagen University Rigshospitalet, Copenhagen, Denmark

ABSTRACT

Background: Over the past 40 years the Danish Breast Cancer Cooperative Group (DBCG) has made significant contributions to improve outcome and to make treatment of patients with early breast cancer more tolerable through nationwide and international trials evaluating loco-regional and systemic treatments. These trials have been instrumental to establish standards for the treatment of early breast cancer.

Methods: The DBCG 82 trials had a global impact by documenting that the significant gain in loco-regional recurrence from postmastectomy radiation added to systemic therapy was associated with a reduction in distant recurrence and mortality in high-risk pre- and postmenopausal patients. The DBCG trials comparing breast conserving surgery and radiotherapy with mastectomy and more recently the trial of internal mammary node irradiation also had a major impact of practice. The trials initiated by the DBCG 40 years ago on tamoxifen and cyclophosphamide based chemotherapy became instrumental for the development of adjuvant systemic therapy not only due to their positive results but by sharing these important data with other members of the Early Breast Cancer Trialist' Collaborative Group (EBCTCG). Trials from the DBCG have also been important for highlighting the relative importance of anthracyclines and taxanes in the adjuvant setting. Furthermore, DBCG has made a major contribution to the development of aromatase inhibitors and targeted adjuvant treatment for human epidermal growth factor receptor 2 positive breast cancers.

Results: The substantial impact of these treatment improvements is illustrated by a 46.7% 10-year overall survival of early breast cancer patients treated in 1978–1987 compared to 71.5% for patients treated 2008–2012.

Conclusions: The trials conducted and implemented by the DBCG appear to have a major impact on the substantial survival improvements in breast cancer.

ARTICLE HISTORY
Received 8 September 2017
Accepted 1 November 2017

Introduction

This paper describes how mortality has improved among Danish breast cancer patients in conjunction with improved quality of loco-regional and systemic therapeutic interventions obtained by the major scientific contributions by Danish Breast Cancer Cooperative Group (DBCG) to clinical research.

Loco-regional treatment studies

Mastectomy and radiotherapy

In the early 1970s the Danish standard treatment of early breast cancer patients was simple mastectomy followed by loco-regional radiotherapy as demonstrated in the Copenhagen Breast Cancer study, where patients were randomly allocated to simple mastectomy followed by radiotherapy or extended radical mastectomy [1–4]. In the DBCG 77 trials the standard treatment was total mastectomy and axillary sampling with the addition of loco-regional radiotherapy in high-risk patients (node-positive and/or invasion to the pectoral fascia and/or T3 or T4 tumors). No systemic treatment was recommended. Orthovoltage was applied ad modum McWhirter, and in 4 out of 5 Danish departments hypofractionation was used due to shortage of radiation capacity [5]. The hypofractionation schedule was a minimum dose of 36.6 Gy in 12 fractions, 2 fractions weekly, or 40.92 Gy/22 fractions, 5 fractions weekly, based on the Ellis NSD formula [6]. A considerable morbidity from hypofractionated orthovoltage radiotherapy was apparent at the time when the early results emerging from adjuvant systemic treatments promulgated the theory proposed by Fisher, e.g., that breast cancer predominantly is systemic disease [7,8]. Thus, there was a shift away from the Halsted theory of

CONTACT Bent Ejlertsen Bent.Ejlertsen@regionh.dk Department of Oncology, Building 2501 Rigshospitalet, Blegdamsvej 9, Copenhagen Ø, Denmark

breast cancer being a loco-regional disease, and aggressive surgery/radiation as the only road to cure [9]. The DBCG 82B trial included high-risk premenopausal patients assigned to CMF and DBCG 82C included high-risk postmenopausal patients assigned to one year of tamoxifen, and patients in both trials were randomized to loco-regional radiotherapy versus no radiotherapy. An ethical prerequisite was that in the presence of systemic treatment the patients would not benefit from radiotherapy. As part of the DBCG 82 trial a consensus was reached and implemented nationwide by oncologists specialized in radiotherapy regarding target definition and radiation treatment techniques. The target comprised regional lymph nodes, including the internal mammary nodes (IMN), and chestwall. Electrons was applied against the chestwall plus IMN and the energy was selected according to the distance from skin to pleura by ultrasound, thus the resulting radiation doses to the heart were relatively low. The DBCG 82 trials demonstrated a significant reduction in the risk of loco-regional recurrence and mortality (Table 1) by postmastectomy radiotherapy, irrespective of menopausal status and number of positive lymph nodes [10–13]. These results were confirmed by the EBCTCG meta-analyzes and have widely been implemented in international guidelines [14,15].

Breast conservation

The DBCG 82TM (tumorectomy versus mastectomy) trial (Table 1) compared modified mastectomy, or breast conserving surgery (BCS) with residual breast radiotherapy, or BCS with residual breast and regional node radiotherapy [16,17]. No significant difference was seen between the two treatment groups regarding 10-year recurrence-free and 20-year overall survival, $p = .94$ and $p = .24$, respectively. The use of BCS has increased to encompass more than 70% of patients with early breast cancer, as a result of the DBCG 82TM, the associated EBCTCG meta-analysis, earlier diagnosis accomplished by mammography screening, neo-adjuvant systemic treatment, and the use of oncoplastic techniques [18,19]. A population-based study performed by DBCG has documented a high risk of reoperation after BCS for non-palpable breast lesions [20]. Radioactive seed has now been introduced as an alternative to the hook wire localization of non-palpable lesions based on results from a large randomized trial [21].

Fractionation of radiotherapy

The poor results obtained by hypofractionation in the DBCG 77 trials and lack of data supporting superiority of hypofractionation compared to normofractionation, caused a reluctance of the DBCG towards moderate hypofraction in the adjuvant breast cancer setting. In 2002 and 2008, large trials from the UK and Canada testing moderate hypofraction versus normofractionation for breast only radiotherapy showed no difference in local control and a trend towards less late radiation morbidity using hypofractionation [22,23]. However, the patients in these trials were not treated with modern chemotherapy nor boost, and in the Canadian trial patients with large breasts had been excluded. The DBCG HYPO trial was therefore initiated in 2009 to clarify the possible implications from large breast size, use of tumor bed boost following adjuvant anthracyclines and taxanes. In March 2014, the DBCG standard was modified to 40 Gy in 15 fractions for breast only radiotherapy following a safety analysis including 1883 patients randomized in the DBCG HYPO trial to 40 Gy in15 fractions compared to 50 Gy in 25 fractions.

In the UK and NL data from the above mentioned trials were extrapolated to patients treated with loco-regional radiotherapy, however, in DBCG this was not accepted. Thus, in 2015 the DBCG Skagen Trial 1 was initiated to introduce moderately hypofractionated loco-regional radiotherapy by randomizing high-risk patients between 40 Gy/15 fractions versus 50 Gy/25 fractions [24,25]. The primary endpoint was arm lymphedema, and secondary endpoints were other morbidities and pattern of recurrence. As of November 2017 around 1100 patients are included, and the trial is expected to accrue around 3000 patients from Europe and Australia.

Partial breast irradiation

In breast recurrences are most often located close to the original tumor bed and the risk of local recurrence has even in high risk patients decreased over decades [26]. Partial breast irradiation (PBI) consequently emerged as an attractive option to lower the radiation burden in selected patients as recently demonstrated in the UK IMPORT LOW trial [27,28]. From 2009 to 2016 the DBCG PBI trial randomized 882 patients to partial versus whole breast radiotherapy and both groups received 40 Gy/15 fractions using the same technique as in the IMPORT Low trial. Early, yet unpublished, results from the DBCG PBI trial regarding morbidity, the primary endpoint, and risk of recurrence are in line with the IMPORT Low trial, and therefore external beam PBI using 40 Gy/15 fractions has since April 2016 been adopted as a standard for selected patients by DBCG.

Treatment of the axilla

During the past 40 years axillary surgery has changed from axillary sampling to axillary lymph node dissection (ALND) and to sentinel node (SN) procedure. Radiotherapy was recommended to lymph node positive patients in the 77 program and this was reinforced by the results obtained in the DBCG 82 postmastectomy trials [10,11]. In a 20-year period ALND partly replaced axillary node level 1 irradiation in node positive patients but the approach of extending radiotherapy to supraclavicular and IMN has been confirmed by others [29,30]. Concern about the possible harmful effect on the heart by IMN radiotherapy and of anthracyclines led in 2003 through 2014 to avoidance of IMN radiotherapy after left-sided breast cancer while IMN radiotherapy was continued following node positive right-sided breast cancer (Table 1). With median 8.9 years follow up an overall survival benefit was shown corresponding to the results in two major trials [29–31]. A meta-analysis of these three studies is in process,

Table 1. Loco-regional treatment, 10-year loco-regional recurrence, disease-free and overall survival.

Study	Regimens	*N*	LRR (%)	DFS; 95% CI	OS; 95% CI
DBCG 82B	Mastectomy + RT	852	9	48% (45; 52)	54% (51; 58)
Overgaard [10]	Mastectomy	856	32	34% (30; 37)	45% (42; 48)
DBCG 82C	Mastectomy + RT	686	8	36% (32; 40)	45% (41; 49)
Overgaard [11]	Mastectomy	689	35	24% (21; 28)	36% (33; 40)
DBCG 82TM	Lumpectomy + RT	381	7	60% (55; 64)	73% (68; 77)
Blichert-Toft [17]	Mastectomy	350	11	61% (56; 66)	71% (66; 76)
DBCG IMN	IMN + RT	1492	[#]2.2	#27% (25;30)	#76% (74; 78)
Thorsen [31]	IMN (no RT)	1597	[#]2.6	#30% (27;32)	#72% (70; 75)

LRR: loco-regional recurrence; DFS: disease-free survival; OS: overall survival; CI: confidence interval; RT: radiotherapy.
[#]DFS and OS at eight years follow-up.

but international guidelines already have included IMN radiotherapy in their recommendations to high-risk breast cancer patients.

When the SN procedure was introduced just after the turn of the millennium it primarily saved patients with negative nodes from unnecessary morbidity of ALND, but the AMAROS trial suggested that axillary radiotherapy may be substituted for ALND in patients with a positive SN [32–35]. The need of ALND in node positive patients was recently re-challenged and DBCG joined the Swedish led phase 3 SENOMAC trial to evaluate the safety of omitting ALDN in patients with one or two positive SN [36].

Long-term outcome and morbidity after loco-regional therapy

The results obtained by BCS and radiotherapy in the DBCG 82TM trial were confirmed in a successive DBCG 89 cohort with similar recurrence and mortality at 20 years [37]. In a small long-term study with a median of 12 years follow-up 88% of the patients were satisfied with their cosmetic outcome, and poor cosmesis was correlated with use of chemotherapy, large breast size and smoking [38]. Local recurrences continued to occur up to 20 years after diagnosis, and a local recurrence in patients <45 years significantly increased the risk of breast cancer mortality, whilst local recurrence among older patients did not [39]. Patients who following a local recurrence developed distant failure could not be identified by classical histopathological parameters or approximated intrinsic subtypes [40]. Efforts to predicting the risk of late morbidity are being continued as part of the DBCG HYPO and PBI trials.

A seven gene profile predicting gain from radiotherapy has been developed using tumor tissue from patients in the DBCG 82 trials and validated in an independent data set [41,42]. Further studies are in progress with the goal to select high risk patients for omission of radiotherapy. Late morbidity was evaluated in a subgroup of patients treated in the DBCG 82 trials [43]. A highly significant gain from loco-regional radiotherapy was seen in patients with 1–3 and ≥4 metastatic nodes and translated into a survival benefit [11–13]. Further analyzes showed that the gain from radiotherapy was highly heterogeneous depending on immunohistochemical approximated intrinsic subtypes [44].

Very severe late treatment related morbidities are heart disease and second cancer. With 12 years median follow up the DBCG 82 trial did not indicating an increased risk of radiation induced heart disease, but extensive research on heart disease in Danish and Swedish breast cancer patients documented a dose–response relationship between mean heart radiation dose and risk of major coronary event [45–47]. Recently a hypothesis was proposed correlating smoking and anthracyclines with a very high risk of radiation induced heart disease [48,49]. Second cancer has also been intensely investigated in patients treated according to DBCG guidelines, and second lung cancer is by far the largest risk, the magnitude is around 1:200 in every irradiated breast cancer patient [50]. The results showed that >90% of those patients developing second lung cancer were smokers.

Systemic treatment

Adjuvant endocrine treatment

Along with its metabolites, tamoxifen competes with estrogens for binding to the estrogen receptor (ER) and the early results of a CBCT study suggested adjuvant tamoxifen could reduce breast cancer recurrence and prolong survival following surgery of early breast cancer [51]. The benefits from adjuvant tamoxifen observed in postmenopausal high-risk (node positive and/or T3) patients were validated in the DBCG 77C trial (Table 2). One year of tamoxifen 30 mg daily reduced the risk of recurrence and mortality in patients with ER positive cancers and no benefit was observed in patients with ER negative and PR positive breast cancer [52,53]. One year of tamoxifen 30 mg daily, the standard in the DBCG 89C trial (Table 2), was not inferior to two years of tamoxifen, and a sequence of tamoxifen for 6 months followed by megestrol acetate for six months in postmenopausal high-risk patients [54]. When the Swedish Breast Group trial in 1996 published a beneficial effect from extending tamoxifen from two to five years participants in DBCG 89C still on treatment were offered to extend treatment which may have biased the overall results [55].

The DBCG 82B trial (Table 2) was in pre- and peri-menopausal high-risk patients unable to demonstrate a reduction in the risk of recurrence or mortality from adding one year of tamoxifen to nine cycles of four-weekly intravenous CMF [56]. Corresponding to our results, the first overview published by the EBCTCG in 1988 was unable to demonstrate a benefit from tamoxifen in patients younger than 50 years [57]. With longer treatment duration and follow-up, the benefit of tamoxifen was however by the EBCTCG shown to be largely independent of age, nodal status, and prior

Table 2. Adjuvant endocrine treatment.

Study	Regimens	*N*	DFS HR; 95% CI	OS HR; 95% CI
CBC 02	Tamoxifen two years	164	0.74; 0.53–1.05[a]	0.79; 0.63–0.99
Jensen [51]	Placebo	153		
DBCG 77C	Tamoxifen one year	868	0.87; 0.77–0.98	0.83; 0.73–0.94[b]
Knoop [52]	Control	848		
DBCG 82B	CMF + Tamoxifen	320	0.93; 0.76–1.15	1.05; 0.85–1.30
Andersson [56]	CMF	314		
DBCG 89C	Tamoxifen one year	554		
Andersen [54]	Tamoxifen two years	535	1.04; 0.89–1.22	0.99; 0.85–1.15
	TAM→Megace	526	1.11; 0.94–1.30	1.05; 0.90–1.23
IES	Tamoxifen	615		
Bliss [73]	Exemestane	584	0.84; 0.71–0.99	0.79; 0.66–0.94
BIG 1–98	Tamoxifen	2459		
Regan [71]	Letrozole	2463	0.86; 0.78–0.96	0.87; 0.77–0.999
	TAM→Letrozole[c]	1548	1.07; 0.92–1.25	1.10; 0.90–1.33
	Letrozole→TAM[c]	1540	1.06; 0.91–1.23	0.97; 0.80–1.19
FACE	Anastrozole	2075	0.93; 0.80–1.07	0.98; 0.82–1.17
Smith [76]	Letrozole	2061		
SOLE	Cont. letrozole	2441		
Colleoni [78]	With breaks	2443	1.08; 0.93–1.26	0.85; 0.68–1.07

DFS: disease-free survival; OS: overall survival; CI: confidence interval; HR: hazard ratio.
[a]Breast cancer recurrence.
[b]Breast cancer mortality.
[c]Versus five years of letrozole; TAM: tamoxifen.

administration of chemotherapy and five years of tamoxifen was introduced as a DBCG standard in 1998 [58,59]. The results from aTTOM and ATLAS demonstrated a further incremental benefit from extending tamoxifen to 10 years [60,61]. While tamoxifen in the eighties was considered less effective in premenopausal patients, chemotherapy was thought to be particular effective and the prevailing view at that time was that this was due to ovarian function suppression (OFS) by chemotherapy [62]. This hypothesis was to some degree confirmed in DBCG 89B that showed a similar DFS and mortality from OFS and CMF [63]. In an exploratory subset analysis the treatment effect largely seemed independent of age, nodal status, tumor size, histological type, malignancy grade, and PR status. However, in the subset with discordant hormone receptor status (either ER or PR negative tumors), CMF resulted in a significant reduction of DFS events and mortality. Several trials support that OFS either alone or in combination with tamoxifen improve outcome similarly to what is achieved with CMF and anthracycline based chemotherapy [64]. Combined the SOFT and TEXT trials demonstrated that OFS with either tamoxifen or an aromatase inhibitor (AI) may lower the risk of recurrence in high-risk premenopausal hormone receptor positive breast cancer patients [65,66]. The cohort study linked to the DBCG 89B trial implied a long-term detrimental effect following OFS corresponding to the data of a long term follow-up of 30,000 participants in the Nurses' Health Study showing an increased mortality despite a reduced risk of breast- and ovarian cancer from ovariectomy in conjunction with hysterectomy due to benign disease [67,68].

In postmenopausal patients aromatase inhibitors have been evaluated in several large trials, and five years of an aromatase inhibitor further reduces mortality by 15% compared to five years of tamoxifen [69]. The breast international group (BIG) 1–98 study compared five years of tamoxifen with five years of letrozole or the two drugs in sequence (each for two to three years) in postmenopausal patients (Table 2). Initial treatment with letrozole reduced the risk of recurrence and mortality [70,71]. The intergroup exemestane study (IES) recruited postmenopausal women who after receiving adequate local and adjuvant systemic therapy remained free of disease after two to three years of tamoxifen (Table 2). Switch to exemestane for the remainder of five years endocrine treatment reduced the risk of recurrence and mortality [72,73]. A pronounced benefit of upfront aromatase inhibition has been shown in patients with a lobular histology and in patients at a high risk of relapse [74,75]. A comparable result from letrozole and anastrozole was obtained in the Femara versus Anastrozole Clinical Evaluation (FACE) trial (Table 2) and from exemestane and anastrozole in NCIC MA.27 [76,77]. Ongoing clinical trials are evaluating the role of extending endocrine treatment after five years of an aromatase inhibitor and in the meanwhile the study of letrozole extension (SOLE) has shown (Table 2) that continuous extended treatment may be substituted by intermittent aromatase inhibition [78]. Only a small group of postmenopausal patients, i.e., node negative patients older than 60 with grade 1 tumors $\leq$10 mm, will without systemic treatment achieve an age-appropriate survival [79].

Adjuvant HER2 targeted treatment

The addition of a one-year course of trastuzumab to chemotherapy in human epidermal growth factor 2 (HER2) positive disease significantly reduced disease recurrences and mortality in several trials including HERceptin Adjuvant (HERA) trial [80–82]. HERA enrolled 5081 patients; hereof 133 by DBCG, from 2001 to 2005 were adjuvant trastuzumab was introduced as a standard (Table 3). DBCG contributed to international but unfruitful efforts to further improve outcome by extending duration of trastuzumab to two years in HERA and concurrent or sequential addition of lapatinib in the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (ALTTO) trial [83,84]. DBCG

Table 3. Adjuvant HER2 targeted treatment.

Study	Regimens	*N*	DFS HR; 95% CI	OS HR; 95% CI
HERA	Trastuzumab two years	1700	0.77; 0.69-0.87	NA
Cameron [83]	Trastuzumab one year	1702	0.76; 0.68–0.86	0.74; 0.64–0.86
	Control	1697		
ALTTO	Lapatinib + T	2093	0.84; 0.70–1.02	0.80; 0.62–1.03
Piccart [84]	T→lapatinib	2091	0.96; 0.80–1.15	0.91; 0.71–1.16
	Lapatinib	2100	1.34; 1.13–1.60	1.36; 1.09–1.72
	Trastuzumab	2097		
APHINITY	Pertuzumab	2400	0.81; 0.66–1.00	0.89; 0.66–1.21
Minckwitz [85]	Control	2404		
ExteNet	Neratinib one year	1420	0.67; 0.50–0.91	NA
Chan [86]	Control	1420		

DFS: disease-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval; T: trastuzumab; NA: non-available.

Table 4. Adjuvant chemotherapy.

Study	Regimens	*N*	DFSHR; 95% CI	OSHR; 95% CI
DBCG 77B	Ctx	181	0.62; 0.46–0.83	0.70; 0.52–0.95
Ejlertsen [89]	Oral CMF	193	0.70; 0.53–0.93	0.70; 0.52–0.94
	Control	187		
DBCG 77B	Ctx	424	0.95; 0.77–1.16	1.09; 0.92–1.29
Ejlertsen [89]	Oral CMF	423		
DBCG 82C	TAM + CMF	709	0.82; 0.71–0.93	0.95; 0.85–1.08
Ejlertsen [78]	TAM	736		
DBCG 89D	CEF	615	0.84; 0.71–0.99	0.79; 0.66–0.94
Ejlertsen [95]	CMF	584		
DBCG READ	EC→D	1001	1.00; 0.78–1.28	1.15; 0.83–1.59
Ejlertsen [101]	DC	1011		
BIG 2–98	4A→3CMF	481	0.81; 0.67–0.99	0.85; 0.67–1.11
Oakman [103]	3A→3D→4CMF	960	1.02; 0.84–1.23	0.96; 0.76–1.21
	4AC→4CMF	487		
	4AD→4CMF	959		

DFS: disease-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval; Ctx: oral cyclophosphamide; C: cyclophosphamide; F: fluorouracil; M: methotrexate; A: doxorubicin; E: epirubicin; D: docetaxel TAM: tamoxifen.

investigators enrolled 87 patients in the Adjuvant Pertuzumab and Herceptin in Initial Therapy (APHINITY) trial, which showed a statistically significant reduction in DFS events from the addition of pertuzumab to trastuzumab-based adjuvant therapy [85] but the clinical benefit has yet to be established. Finally, the EXTEnded NEratinib Trial (ExteNET) had participation of 112 patients though DBCG and showed that one year of neratinib, an irreversible tyrosine kinase inhibitor of HER1, HER2 and HER4, compared to placebo further significantly improved DFS when given after one year of trastuzumab-based (neo)adjuvant therapy in HER2-positive breast cancer [86]. One year of trastuzumab added to adjuvant chemotherapy remains standard of care in patients with early-stage HER2-positive breast cancer but neratinib may become an option to some patients [87].

Adjuvant chemotherapy

The achievements of the DBCG with adjuvant chemotherapy has recently been reviewed [88]. In brief, the DBCG 77B trial (Table 4) showed significant and clinical important reduction in the risk of recurrence and mortality from single agent oral cyclophosphamide and from CMF in premenopausal patients with high-risk (node-positive or T3) breast cancer [89]. Adjuvant CMF was in 1982 selected as a standard in high-risk premenopausal breast cancer by DBCG and subsequently in 1985 by the first NIH Consensus development Conference [90]. In a retrospective analysis patients with core-basal and luminal B breast cancers appeared to derive the largest benefit from cyclophosphamide-based chemotherapy [91]. No apparent benefit was observed from the addition of CMF to tamoxifen among postmenopausal high-risk breast cancer patients in an early analysis of DBCG trial 82C [92] and no clear benefit was shown from adjuvant chemotherapy in the first Oxford overview published in 1988 [57]. A differential benefit however was shown in patients with ER negative breast cancer and adjuvant chemotherapy was in 1989 extended to high-risk postmenopausal patients younger than 70 with ER negative breast cancer. Furthermore, invasive ductal carcinomas with malignancy grade 2 and 3 were irrespective of tumor size and lymph node status included in the premenopausal high-risk group by 1989.

With longer follow-up CMF was associated with a significant improvement in DFS [92,93]. Apart from menopausal status, the DBCG 77B and 82C trials had identical selection criteria, but while 77B trial used classic CMF with oral cyclophosphamide a four-weekly intravenous CMF regimen was used in DBCG 82B and C trials, and a three-weekly CMF regimen was used in the succeeding DBCG 89B and D trials. A population-based DBCG study demonstrated that shifting from classical CMF in DBCG 77B to four-weekly or three-weekly i.v. CMF was associated with a 30% increased risk of a DFS event [94]. Furthermore, the four-weekly regimen as used in DBCG 82B was associated with a 40% increase in mortality.

Danish breast cancer cooperative group 89D showed an incremental reduction in recurrence and mortality from

substituting methotrexate with epirubicin [95]. The advantage of anthracycline-containing three-drug combinations over CMF was confirmed by others and by meta-analysis conducted by EBCTCG, while standard AC (doxorubicin and cyclophophamide) or EC (epirubicin and cyclophosphamide) for four cycles was not superior to classic CMF [96]. HER2 and *TOP2A* were assessed retrospectively in 767 of the 980 Danish patients included in DBCG 89D. Topoisomerase IIα, the enzyme encoded by *TOP2A*, is a direct target for anthracyclines and essential for resolving topological DNA constraints [97,98]. Alteration of *TOP2A* copy number was in 89D associated with an incremental benefit from epirubicin [99]. This was confirmed in a prospectively planned joint analysis of 89D and four other phase three trials [100] but only a trend toward greater benefit was show for patients with *HER2*-amplified tumors. The DBCG 07-READ compared six cycles of docetaxel and cyclophosphamide with three cycles of epirubicin and cyclophosphamide followed by three cycles of docetaxel and confirmed no overall benefit from adjuvant epirubicin in patients with early and *TOP2A*-normal breast cancer [101]. Other mechanisms of action have been proposed including protection from apoptosis by tissue inhibitor of matrix metalloproreinases-1 (TIMP-1). A highly significant interaction was shown in 89D between epirubicin and a classifier constructed by combining lack of TIMP-1 expression and/or *TOP2A* alteration [102].

In BIG 2-98 (Table 4) a further reduction in the risk of recurrence was obtained from adding a taxane to sequential anthracycline and CMF but not from substitution of cycles of cyclophosphamide and doxorubicin with docetaxel and doxorubicin [103]. Similarly a further reduction in breast cancer mortality appeared in the EBCTCG meta-analysis from the addition of a taxane to a standard AC, while the substitution of cycles or drugs with a taxane was not associated with a reduction in mortality [96]. ECOG 1199 in a factorial 2 by two design showed a superior benefit from weekly paclitaxel compared to three-weekly and from three-weekly docetaxel compared to weekly, and overall no difference between docetaxel and paclitaxel [104].

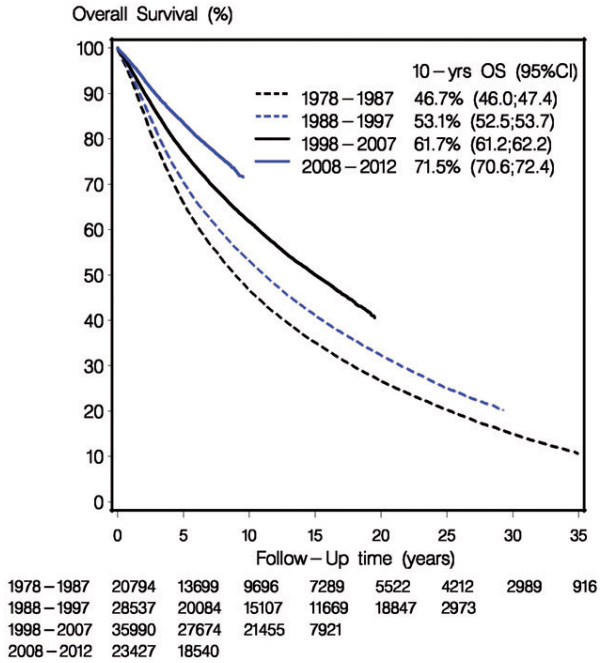


Figure 1. Overall survival (OS) according to period of diagnosis. All patients, irrespective of adjuvant treatment.

Results

Overall survival is in Figure 1 presented according to period of diagnosis for all patients with operable invasive breast cancer, irrespective of the received loco-regional and systemic treatment. A major and clinical important improvement in prognosis according to period of diagnosis is apparent with a decrease in 10 year mortality from 53.3% in 1978–1987 to 28.5% for those diagnosed in 2007–2015 (Figure 1).

A temporary setback in the otherwise continuous improvement in prognosis occurred in the second half of the 1978–1987 period as a consequence of a less effective adjuvant CMF regimen and omission of postmastectomy radiotherapy in patients randomized to the control group in the DBCG 82B&C trials. The improvements obtained in the succeeding periods can hardly be attributed to a single treatment or trial (Figure 2). The proportion of patients receiving adjuvant treatment increased from none to more than 80%, the proportion of patients who received chemotherapy in combination with endocrine therapy and/or trastuzumab increased and the distinct treatment regimens evolved as described in Figure 2 and the preceding sections.

Period	1978 – 1987	1988 – 1997	1998 – 2007	2008 – 2015
Endocrine therapy				
Premenopausal	None		Tamoxifen 5-yr	Tamoxifen 10-yr
Postmenopausal	Trial only	Tamoxifen 1-yr	Tamoxifen 5-yr	AI or AI->TAM 5-yr
Anti-HER2 therapy	None	None	Trial only	Trastuzumab 1-yr
Chemotherapy				
Premenopausal	Trial only	CMF or CEF	CEF	EC->taxane
Postmenopausal	None	CMF in ER negative	CEF in ER negative	EC->taxane

AI: aromatase inhibitor; C: cyclophosphamide; M: methotrexate; E: epirubicin; F: fluorouracil

Figure 2. Patients and treatments in successive decades.

Discussion

In this nationwide and population-based study we have demonstrated a significant improvement of the prognosis following early breast cancer in four successive decades. Furthermore, the present study indicates that the substantial reduction in mortality from to 53.3% to 28.5% in first 10 years after breast cancer is closely connected to results obtained in clinical trials and in particular to those obtained by the Danish Breast Cancer Cooperative Group nationwide or in internal collaboration. In a study accompanying our study in this issue a quite fast implementation of guideline modification is shown over the last decade and equivalent results have previously been shown for the preceding three decades [105,106].

Several issues should be considered when interpreting this study. First, the population-based design of our clinical database and the prospective and comprehensive registration of patients and treatments minimized the risk of bias. Second, patients above 70 at diagnosis of breast cancer and patients with multimorbidity were not included in phase 3 trials and results from clinical trials may not have been implemented fully in non-eligible patients. Third, life-expectancy has gradually been increasing during the last four decades and may in part explain our results. Finally, earlier diagnosis and alterations in the biology of the disease may have contributed to reductions in mortality.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Niels Kroman http://orcid.org/0000-0002-6631-2993

References

[1] Kaae S, Johansen H. Does simple mastectomy followed by irradiation offer survival comparable to radical procedures? Int J Radiat Oncol Biol Phys. 1977;2:1163–1166.
[2] McWhirter R. Simple mastectomy and radiotherapy in the treatment of breast cancer. Br J Radiol. 1955;28:128–139.
[3] Dahl-Iversen E, Toiassen T. Radical mastectomy with parasternal and supraclavicular dissection for mammary carcinoma. Ann Surg. 1963;157:170–173.
[4] Johansen H, Kaae S, Johansen H, et al. Extended radical mastectomy versus simple mastectomy followed by radiotherapy in primary breast cancer. A fifty-year follow-up to the Copenhagen breast cancer randomised study. Acta Oncol. 2008;47:633–638.
[5] McWhirter R. The value of simple mastectomy and radiotherapy in the treatment of cancer of the breast. BJR. 1948;21:99–610.
[6] Ellis F. Dose, time and fractionation: A clinical hypothesis. Clin Radiol. 1969;20:1–7.
[7] Bentzen S, Overgaard M. Early and late normal tissue injury after postmastectomy radiotherapy. Recent Results Cancer Res. 1993;130:59–78.
[8] Fisher B. Laboratory and clinical research in breast cancer – a personal adventure: the David A. Karnofsky memorial lecture. Cancer Res. 1980;40:3863–3874.
[9] Halsted WS. I. The results of radical operations for the cure of carcinoma of the breast. Ann Surg. 1907;46:1.
[10] Overgaard M, Hansen PS, Overgaard J, Danish Breast Cancer Cooperative Group 82b Trial, et al. Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. N Engl J Med. 1997;337:949–955.
[11] Overgaard M, Jensen MB, Overgaard J, et al. Postoperative radiotherapy in high-risk postmenopausal breast-cancer patients given adjuvant tamoxifen: Danish breast cancer cooperative group DBCG 82c randomised trial. Lancet. 1999;353:1641–1648.
[12] Overgaard M, Nielsen HM, Overgaard J. Is the benefit of postmastectomy irradiation limited to patients with four or more positive nodes, as recommended in international consensus reports? A subgroup analysis of the DBCG 82 b&c randomized trials. Radiother Oncol. 2007;82:247–253.
[13] Nielsen HM, Overgaard M, Grau C, et al. Study of failure pattern among high-risk breast cancer patients with or without postmastectomy radiotherapy in addition to adjuvant systemic therapy: long-term results from the Danish breast cancer cooperative group DBCG 82 b and c randomized studies. J Clin Oncol. 2006;24:2268–2275.
[14] McGale P, Taylor C, Correa C, et al. Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta-analysis of individual patient data for 8135 women in 22 randomised trials. Lancet. 2014;383:2127–2135.
[15] Curigliano G, Burstein HJ, P Winer E, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol. 2017;28:1700–1712.
[16] Blichert-Toft M, Brincker H, Andersen JA, et al. A Danish randomized trial comparing breast-preserving therapy with mastectomy in mammary carcinoma. Preliminary results. Acta Oncol. 1988;27:671–677.
[17] Blichert-Toft M, Nielsen M, Düring M, et al. Long-term results of breast conserving surgery vs. mastectomy for early stage invasive breast cancer: 20-year follow-up of the Danish randomized DBCG-82TM protocol. Acta Oncol. 2008;47:672–681.
[18] Clarke M, Collins R, Darby S, et al. Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;366:2087–2106.
[19] Christiansen PM, Carstensen SL, Ejlertsen B, et al. Breast conserving surgery versus mastectomy: overall and relative survival – a population based study by the Danish breast cancer cooperative group. Acta Oncol. 2017 [Nov 23]; [7 p.]. DOI:10.1080/0284186X.2017.1403042
[20] Langhans L, Jensen MB, Talman MM, et al. Reoperation rates in ductal carcinoma in situ vs invasive breast cancer after wire-guided breast-conserving surgery. JAMA Surg. 2017;152:378–384.
[21] Langhans L, Tvedskov TF, Klausen TL, et al. Radioactive seed localization or wire-guided localization of nonpalpable invasive and in situ breast cancer: a randomized, multicenter, open-label trial. Ann Surg. 2017;266:29–35.
[22] Whelan T, MacKenzie R, Julian J, et al. Randomized trial of breast irradiation schedules after lumpectomy for women with lymph node-negative breast cancer. J Natl Cancer Inst. 2002;94:1143–1150.
[23] Bentzen SM, Agrawal RK, Aird EG, START Trialists' Group, et al. The UK standardisation of breast radiotherapy (START) Trial B of radiotherapy hypofractionation for treatment of early breast cancer: a randomised trial. Lancet. 2008;371:1098–1107.
[24] Nielsen HM, Friis RB, Linnet S, et al. Loco-regional morbidity after breast conservation and axillary lymph node dissection for early breast cancer with or without regional nodes radiotherapy, perspectives in modern breast cancer treatment: the Skagen Trial 1 is active. Acta Oncol. 2017;56:713–718.
[25] Francolini G, Thomsen MS, Yates ES, et al. Quality assessment of delineation and dose planning of early breast cancer patients included in the randomized Skagen Trial 1. Radiother Oncol. 2017;123:282–287.

[26] Poortmans PM, Arenas M, Livi L. Over-irradiation. Breast. 2017;31:295–302.
[27] Offersen BV, Overgaard M, Kroman N, et al. Accelerated partial breast irradiation as part of breast conserving therapy of early breast carcinoma: a systematic review. Radiother Oncol. 2009;90:1–13.
[28] Coles CE, Griffin CL, Kirby AM, IMPORT Trialists, et al. Partial-breast radiotherapy after breast conservation surgery for patients with early breast cancer (UKIMPORT LOW trial): 5-year results from a multicentre, randomised, controlled, phase 3, non-inferiority trial. Lancet. 2017;390:1048–1060.
[29] Poortmans PM, Collette S, Kirkove C, et al. Internal mammary and medial supraclavicular irradiation in breast cancer. N Engl J Med. 2015;373:317–327.
[30] Whelan TJ, Olivotto IA, Parulekar WR, et al. Regional nodal irradiation in early-stage breast cancer. N Engl J Med. 2015;373:1878–1879.
[31] Thorsen LB, Offersen BV, Danø H, et al. DBCG-IMN: a population-based cohort study on the effect of internal mammary node irradiation in early node-positive breast cancer. J Clin Oncol. 2016;34:314–320.
[32] Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol. 2014;15:1303–1310.
[33] Friis E, Galatius H, Garne JP. Organized nation-wide implementation of sentinel lymph node biopsy in Denmark. Acta Oncol. 2008;47:556–560.
[34] Christiansen P, Friis E, Balslev E, et al. Sentinel node biopsy in breast cancer: five years experience from Denmark. Acta Oncol. 2008;47:561–568.
[35] Husted Madsen A, Haugaard K, Soerensen J, et al. Arm morbidity following sentinel lymph node biopsy or axillary lymph node dissection: a study from the Danish breast cancer cooperative group. Breast. 2008;17:138–147.
[36] de Boniface J, Frisell J, Andersson Y, et al. Survival and axillary recurrence following sentinel node-positive breast cancer without completion axillary lymph node dissection: the randomized controlled SENOMAC trial. BMC Cancer. 2017;17:379.
[37] Lyngholm CD, Laurberg T, Alsner J, et al. Failure pattern and survival after breast conserving therapy. Long-term results of the Danish Breast Cancer Group (DBCG) 89TM cohort. Acta Oncol. 2016;55:983–992.
[38] Lyngholm CD, Christiansen PM, Damsgaard TE, et al. Long-term follow-up of late morbidity, cosmetic outcome and body image after breast conserving therapy. A study from the Danish Breast Cancer Cooperative Group (DBCG). Acta Oncol. 2013;52:259–269.
[39] Laurberg T, Lyngholm CD, Christiansen PM, et al. Long-term age-dependent failure pattern after breast-conserving therapy or mastectomy among Danish lymph-node-negative breast cancer patients. Radiother Oncol. 2016;120:98–106.
[40] Laurberg T, Alsner J, Tramm T, et al. Impact of age, intrinsic subtype and local treatment on long-term locol-regional recurrence and breast cancer mortality among low-risk breast cancer patients. Acta Oncol. 2017;56:59–67.
[41] Tramm T, Kyndi M, Myhre S, et al. Relationship between the prognostic and predictive value of the intrinsic subtypes and a validated gene profile predictive of loco-regional control and benefit from post-mastectomy radiotherapy in patients with high-risk breast cancer. Acta Oncol. 2014;53:1337–1346.
[42] Tramm T, Mohammed H, Myhre S, et al. Development and validation of a gene profile predicting benefit from postmastectomy radiotherapy in patients with high-risk breast cancer: a study of gene expression in the DBCG 82 b&c cohort. Clin Can Res. 2014;20:5272–5280.
[43] Højris I, Andersen J, Overgaard M, et al. Late treatment-related morbidity in breast cancer patients randomized to postmastectomy radiotherapy and systemic therapy versus systemic therapy alone. Acta Oncol. 2000;39:355–372.
[44] Kyndi M, Overgaard M, Nielsen HM, et al. High local recurrence risk is not associated with large survival reduction after postmastectomy radiotherapy in high-risk breast cancer: a subgroup analysis of DBCG 82 b&c. Radiother Oncol. 2009;90:74–79.
[45] Højris I, Overgaard M, Christensen JJ, et al. Morbidity and mortality of ischaemic heart disease in high-risk breast-cancer patients after adjuvant postmastectomy systemic treatment with or without radiotherapy: analysis of DBCG 82b and 82c randomized trials. Lancet. 1999;354:1425–1430.
[46] McGale P, Darby S, Hall P, et al. Incidence of heart disease in 35,000 women treated with radiotherapy for breast cancer in Denmark and Sweden. Radiother Oncol. 2011;100:167–175.
[47] Darby S, Ewertz M, McGale P, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med. 2013;368:987–998.
[48] Taylor C, Correa C, Duane F, et al. Estimating the risks of breast cancer radiotherapy: evidence from modern radiation doses to the lungs and heart and from previous randomized trials. JCO. 2017;35:1641–1649.
[49] Rehammar JC, Jensen MB, McGale P, et al. Risk of heart disease in relation to radiotherapy and chemotherapy with anthracyclines among 19,464 breast cancer patients in Denmark, 1977–2005. Radiother Oncol. 2017;123:299–305.
[50] Grantzau T, Thomsen MS, Vaeth M, et al. Risk of second primary lung cancer in women after radiotherapy for breast cancer. Radiother Oncol. 2014;111:366–373.
[51] Jensen MB, Krarup JF, Palshof T, et al. Two years of tamoxifen or no adjuvant systemic therapy for patients with high-risk breast cancer: long-term follow-up of the Copenhagen breast cancer trial. Acta Oncol. 2017 [Nov 22]; [5 p.]. DOI:10.1080/0284186X.2017.1400179
[52] Knoop AS, Bentzen SM, Nielsen MM, et al. Value of epidermal growth factor receptor, HER2, p53, and steroid receptors in predicting the efficacy of tamoxifen in high-risk postmenopausal breast cancer patients. JCO. 2001;19:3376–3384.
[53] Knoop AS, Laenkholm AV, Jensen MB, Danish Breast Cancer Cooperative Group, et al. Estrogen receptor, Progesterone receptor, HER2 status and Ki67 index and responsiveness to adjuvant tamoxifen in postmenopausal high-risk breast cancer patients enrolled in the DBCG 77C trial. Eur J Cancer. 2014;50:1412–1421.
[54] Andersen J, Kamby C, Ejlertsen B, et al. Tamoxifen for one year versus two years versus 6 months of Tamoxifen and 6 months of megestrol acetate: a randomized comparison in postmenopausal patients with high-risk breast cancer (DBCG 89C). Acta Oncol. 2008;47:718–724.
[55] Swedish Breast Cancer Cooperative Group. Randomized trial of two versus five years of adjuvant tamoxifen for postmenopausal early stage breast cancer. J Natl Cancer Inst. 1996;88:1543–1549.
[56] Andersson M, Kamby C, Jensen MB, et al. Tamoxifen in high-risk premenopausal women with primary breast cancer receiving adjuvant chemotherapy. Report from the Danish breast cancer co-operative group DBCG 82B trial. Eur J Cancer. 1999;35:1659–1666.
[57] Early Breast Cancer Trialists' Collaborative Group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. N Engl J Med. 1988;319:1681–1692.
[58] Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet. 1998;351:1451–1467.
[59] Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, Gray R, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:771–784.
[60] Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet. 2013;381:805–816.

[61] Gray RG, Rea D, Handley K, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. J Clin Oncol. 2013;31(15_Suppl):5. DOI:10.1200/jco.2013.31.15_suppl.5

[62] Brincker H, Rose C, Rank F, et al. Evidence of a castration-mediated effect of adjuvant cytotoxic chemotherapy in premenopausal breast cancer. JCO. 1987;5: 1771–1778.

[63] Ejlertsen B, Mouridsen HT, Jensen MB, et al. Similar efficacy for ovarian ablation compared with cyclophosphamide, methotrexate, and fluorouracil: from a randomized comparison of premenopausal patients with node-positive, hormone receptor-positive breast cancer. JCO. 2006;24:4956–4962.

[64] Cuzick J, Ambroisine L, Davidson N, et al. Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials. Lancet. 2007;369:1711–1723.

[65] Pagani O, Regan MM, Walley BA, et al. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med. 2014;371:107–118.

[66] Francis PA, Regan MM, Fleming GF, et al. Adjuvant ovarian suppression in premenopausal breast cancer. N Engl J Med. 2015;372:436–446.

[67] Ejlertsen B, Jensen MB, Mouridsen HT, et al. DBCG trial 89B comparing adjuvant CMF and ovarian ablation: similar outcome for eligible but non-enrolled and randomized breast cancer patients. Acta Oncol. 2008;47:709–717.

[68] Parker WH, Feskanich D, Broder MS, et al. Long-term mortality associated with oophorectomy compared with ovarian conservation in the nurses' health study. Obstet Gynecol. 2013;121: 709–716.

[69] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet. 2015;386:1341–1352.

[70] Mouridsen H, Giobbie-Hurder A, Goldhirsch A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med. 2009;361:766–776.

[71] Regan MM, Neven P, Giobbie-Hurder A, BIG 1-98 Collaborative Group; International Breast Cancer Study Group (IBCSG), et al. Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8·1 years median follow-up. Lancet Oncol. 2011;12:1101–1108.

[72] Coombes RC, Hall E, Gibson LJ, Intergroup Exemestane Study, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med. 2004;350:1081–1092.

[73] Bliss JM, Kilburn LS, Coleman RE, et al. Disease-related outcomes with long-term follow-up: an updated analysis of the intergroup exemestane study. J Clin Oncol. 2012;30:709–717.

[74] Metzger Filho O, Giobbie-Hurder A, Mallon E, et al. Relative effectiveness of letrozole compared with tamoxifen for patients with lobular carcinoma in the big 1-98 trial. JCO. 2015;33: 2772–2779.

[75] Rasmussen BB, Regan MM, Lykkesfeldt AE, et al. Adjuvant letrozole versus tamoxifen according to centrally-assessed ERBB2 status for postmenopausal women with endocrine-responsive early breast cancer: supplementary results from the BIG 1-98 randomised trial. Lancet Oncol. 2008;9:23–28.

[76] Smith I, Yardley D, Burris H, et al. Comparative efficacy and safety of adjuvant letrozole versus anastrozole in postmenopausal patients with hormone receptor-positive, node-positive early breast cancer: final results of the randomized phase III femara versus anastrozole clinical evaluation (FACE) trial. JCO. 2017;35:1041–1048.

[77] Goss PE, Ingle JN, Pritchard KI, et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27–a randomized controlled phase III trial. JCO. 2013;31:1398–1404.

[78] Colleoni M, Luo W, Karlsson P, et al. SOLE (Study of Letrozole Extension): a phase III randomized clinical trial of continuous vs intermittent letrozole in postmenopausal women who have received 4–6 years of adjuvant endocrine therapy for lymph node-positive, early breast cancer (BC). J Clin Oncol. 2017;35(15_Suppl):503. DOI:10.1200/JCO.2017.35.15_suppl.503

[79] Christiansen P, Bjerre K, Ejlertsen B, et al. Mortality rates among early-stage hormone receptor-positive breast cancer patients: a population-based cohort study in Denmark. J Natl Cancer Inst. 2011;103:1363–1372.

[80] Gianni L, Dafni U, Gelber RD, Herceptin Adjuvant (HERA) Trial Study Team, et al. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. Lancet Oncol. 2011;12:236–244.

[81] Perez EA, Romond EH, Suman VJ, et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. JCO. 2014;32:3744–3752.

[82] Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011;365:1273–1283.

[83] Cameron D, Piccart-Gebhart MJ, Gelber RD, et al. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. Lancet. 2017;389:1195–1205.

[84] Piccart-Gebhart M, Holmes E, Baselga J, et al. Adjuvant lapatinib and trastuzumab for early human epidermal growth factor receptor 2-positive breast cancer: results from the randomized phase III adjuvant lapatinib and/or trastuzumab treatment optimization trial. J Clin Oncol. 2016;34:1034–1042.

[85] von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N Engl J Med. 2017;377:122–131.

[86] Chan A, Delaloge S, Holmes FA, et al. Neratinib after trastuzumab-based adjuvant therapy in patients with HER2-positive breast cancer (ExteNET): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2016;17: 367–377.

[87] Martin M, Holmes FA, Ejlertsen B, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a multicentre, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2017 [Nov 13]. DOI:10.1016/S1470-2045(17)30717-9

[88] Ejlertsen B. Adjuvant chemotherapy in early breast cancer. Dan Med J. 2016;63:pii:B5222.

[89] Ejlertsen B, Mouridsen HT, Jensen MB, et al. Cyclophosphamide, methotrexate, and fluorouracil; oral cyclophosphamide; levamisole; or no adjuvant therapy for patients with high-risk, premenopausal breast cancer. Cancer. 2010;116:2081–2089.

[90] National Institutes of Health Consensus Development Conference Statement: Adjuvant Chemotherapy for Breast Cancer. September 9–11, 1985. CA Cancer J Clin. 1986;36:42–47.

[91] Nielsen TO, Jensen MB, Burugu S, et al. High-risk, premenopausal luminal A breast cancer patients derive no benefit from adjuvant cyclophosphamide-based chemotherapy: Results from the DBCG77B clinical trial. Clin Cancer Res. 2017;23:946–953.

[92] Mouridsen HT, Rose C, Overgaard M, et al. Adjuvant treatment of postmenopausal patients with high risk primary breast cancer. Results from the Danish adjuvant trials DBCG 77 C and DBCG 82 C. Acta Oncol. 1988;27:699–705.

[93] Ejlertsen B, Jensen MB, Elversang J, et al. One year of adjuvant tamoxifen compared with chemotherapy and tamoxifen in postmenopausal patients with stage II breast cancer. Eur J Cancer. 2013;49:2986–2994.

[94] Ejlertsen B, Mouridsen HT, Jensen MB. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in premonopausal patients with node-positive breast cancer: indirect comparison of dose

and schedule in DBCG trials 77, 82, and 89. Acta Oncol. 2008;47:662–671.
[95] Ejlertsen B, Mouridsen HT, Jensen MB, et al. Improved outcome from substituting methotrexate with epirubicin: results from a randomised comparison of CMF versus CEF in patients with primary breast cancer. Eur J Cancer. 2007;43:877–884.
[96] Peto R, Davies C, Godwin J, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012;379:432–444.
[97] Baxter J, Sen N, Martínez VL, et al. Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes. Science. 2011;331:1328–1332.
[98] Kellner U, Sehested M, Jensen PB, et al. Culprit and victim – DNA topoisomerase II. Lancet Oncol. 2002;3:235–243.
[99] Knoop AS, Knudsen H, Balslev E, et al. Retrospective analysis of topoisomerase IIa amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil: Danish Breast Cancer Cooperative Group. J Clin Oncol. 2005;23:7483–7490.
[100] Di Leo A, Desmedt C, Bartlett JM, et al. HER2 and TOP2A as predictive markers for anthracycline-containing chemotherapy regimens as adjuvant treatment of breast cancer: a meta-analysis of individual patient data. Lancet Oncol. 2011;12:1134–1142.
[101] Ejlertsen B, Tuxen MK, Jakobsen EH, et al. Adjuvant cyclophosphamide and docetaxel with or without epirubicin for early TOP2A-normal breast cancer: DBCG 07-READ, an open-label, phase III, randomized trial. JCO. 2017;35:2639–2646.
[102] Ejlertsen B, Jensen MB, Nielsen KV, et al. HER2, TOP2A, and TIMP-1 and responsiveness to adjuvant anthracycline-containing chemotherapy in high-risk breast cancer patients. JCO. 2010;28:984–990.
[103] Oakman C, Francis PA, Crown J, et al. Overall survival benefit for sequential doxorubicin-docetaxel compared with concurrent doxorubicin and docetaxel in node-positive breast cancer–8-year results of the breast international group 02-98 phase III trial. Ann Oncol. 2013;24:1203–1211.
[104] Sparano JA, Wang M, Martino S, et al. Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med. 2008;358:1663–1671.
[105] Jensen MB, Ejlertsen B, Mouridsen HT, et al. Improvements in breast cancer survival between 1995 and 2012 in Denmark: the importance of earlier diagnosis and adjuvant treatment. Acta Oncol. 2016;55:24–35.
[106] Møller S, Jensen MB, Ejlertsen B, et al. The clinical database and the treatment guidelines of the Danish breast cancer cooperative group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 13–18
https://doi.org/10.1080/0284186X.2017.1404638

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The clinical database and implementation of treatment guidelines by the Danish Breast Cancer Cooperative Group in 2007–2016

Maj-Britt Jensen[a], Anne-Vibeke Laenkholm[b], Birgitte V. Offersen[c], Peer Christiansen[d], Niels Kroman[e], Henning T. Mouridsen[a] and Bent Ejlertsen[a]

[a]Danish Breast Cancer Cooperative Group (DBCG) Secretariat and Statistical Office, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [b]Department of Surgical Pathology, Zealand University Hospital, Slagelse, Denmark; [c]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark; [d]Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark; [e]Department of Breast Surgery, Copenhagen University Hospital Herlev, Copenhagen, Denmark

ABSTRACT

Background: Since 40 years, Danish Breast Cancer Cooperative Group (DBCG) has provided comprehensive guidelines for diagnosis and treatment of breast cancer. This population-based analysis aimed to describe the plurality of modifications introduced over the past 10 years in the national Danish guidelines for the management of early breast cancer. By use of the clinical DBCG database we analyze the effectiveness of the implementation of guideline revisions in Denmark.

Methods: From the DBCG guidelines we extracted modifications introduced in 2007–2016 and selected examples regarding surgery, radiotherapy (RT) and systemic treatment. We assessed introduction of modifications from release on the DBCG webpage to change in clinical practice using the DBCG clinical database.

Results: Over a 10-year period data from 48,772 patients newly diagnosed with malignant breast tumors were entered into DBCG's clinical database and 42,197 of these patients were diagnosed with an invasive carcinoma following breast conserving surgery (BCS) or mastectomy. More than twenty modifications were introduced in the guidelines. Implementations, based on prospectively collected data, varied widely; exemplified with around one quarter of the patients not treated according to a specific guideline within one year from the introduction, to an almost immediate full implantation.

Conclusions: Modifications of the DBCG guidelines were generally well implemented, but the time to full implementation varied from less than one year up to around five years. Our data is registry based and does not allow a closer analysis of the causes for delay in implementation of guideline modifications.

ARTICLE HISTORY
Received 15 September 2017
Accepted 7 November 2017

Introduction

In 1977 the Danish Breast Cancer Cooperative Group (DBCG) launched a nationwide breast cancer database accompanying multidisciplinary guidelines for diagnostic, treatment and follow-up of breast cancer [1,2]. These initiatives have improved the quality of diagnostic procedures and all aspects of breast cancer treatment in Denmark. Furthermore, continued development and standardization of procedure and treatment strategies have significantly contributed to an improvement of the prognosis in breast cancer and has become a model for the construction of multidisciplinary cancer groups [3,4]. Improvement in the quality of care by clinical guidelines has been shown repeatedly but it is less clear how effectively guidelines are maintained and how long time it takes to implement modifications [5].

Several potential barriers may delay implementation of evidence-based guidelines but the awareness of guidelines from the DBCG is promoted by the involvement of relevant professionals, a joint conception of the guidelines and a clinical database and a quality ensurance system [6]. Partnerships between those who produce guidelines and those who use them are likely to enhance their relevance and implementation, and the guidelines of the DBCG, therefore, have been authored by scientific committees encountering all breast centers in Denmark [7].

Methods

Study population

Since 2006, all patients with a record of a first invasive breast tumor in the Danish National Pathology Registry have been registered in the clinical database of DBCG.

Guidelines

National guidelines are continuously modified and available from the website of DBCG (www.dbcg.dk). The guidelines are

CONTACT Maj-Britt Jensen mj@dbcg.dk Danish Breast Cancer Cooperative Group Secretariat, Rigshospitalet, Section 2501, Blegdamsvej 9, Copenhagen DK2100, Denmark

Supplemental data for this article can be accessed here.

prepared and agreed upon in the Scientific Committee of each of the specialties. All Danish centers are represented in the committees. The guidelines are finally approved by the DBCG board.

Organization of the database

The clinical DBCG database comprises a web-based open source data entry and display module with pages adapted for each of the specialties involved. Remote data entry is accessible from all Danish hospital units involved in diagnosis and treatment of breast cancer patients. The modules encompass data validation on data entry and cross validations connected queries directed to specific departments. The database and query system is updated on a daily basis. Treatment guideline algorithms based on reported data on patient characteristics and prognostic factors are built into the system. The data entry and query system has been extended to non-Danish centers participating in randomized DBCG clinical trials.

Histopathology

The reported histopathological data included histological type according to WHO [8], tumor size, examination of tumor margins, invasion into skin or deep resection line, malignancy grade, number of nodes examined, hereof tumor positive, vascular invasion, estrogen (ER) and/or progesterone status, HER2 status, TOP2A status (2007–2013) and Ki67 (from 2009). Additional analysis and definitions are described in details elsewhere [3,9].

Treatment

The data of therapeutic interventions included type of breast (mastectomy or breast conserving surgery (BCS)) and axillary (sentinel node (SN) or axillary lymph node dissection (ALND)) surgery, oncoplastic procedures, radiotherapy (RT) (target, dose, number of fractions), systemic therapy (type, doses, duration), hematological toxicities and other adverse events and the results of the follow-up studies.

Supplementary data

Each patient is registered with a unique civic registration number assigned to each citizen in Denmark [10]. Through linkage to nationwide Danish health registries, complete and continuously updated data on vital status and emigration (Danish Civil Registration System), cause of death (Danish Causes of Death Registry), pathology reports (National Pathology Registry), other malignancy (Danish Cancer Registry) and hospitalizations (National Patient Registry) are retrieved. From the National Patient Registry data, an algorithm has been set up to assign a Charlson comorbidity index to each registered patient [11].

Results

From 2007 to 2016 records from 48,772 patients with malignant breast tumors was entered into DBCG's clinical database, and 42,197 of these patients were diagnosed with an invasive carcinoma following BCS or mastectomy.

Risk assessment

From 1977 to 1989 patients were classified as high-risk if node-positive, tumor size >5 cm or tumor invasion in skin or deep fascia or otherwise as low-risk [7]. The high-risk group was gradually extended and in 2013 a prognostic model was introduced to allow de-escalation of chemotherapy among postmenopausal patients with ER positive breast cancers. This model was constructed using prospectively recorded data on recurrence and survival from 6529 patients who in 1996–2004 as the sole adjuvant systemic treatment received tamoxifen, an aromatase inhibitor or the two in sequence. Using multivariable fractional polynomials a highly performing prognostic index was constructed [12]. In 2017, risk assessment was further refined by inclusion of molecular subtypes (PAM50) [13].

Surgery

Since 2002 the preferred surgical procedure has been BCS combined with SN assessment, and more than 70% of the patients with invasive breast cancer received a BCS in 2016. Guidelines for surgical margin was changed in 2013, see Supplementary Table 1 [14]. ALND is limited to node positive cases and cases not eligible for the SN technique. Primarily based on the results from the ACOSOG Z0011 trial DBCG guidelines (Table 1) for ALND following SN was changed in December 2011 [15]. Figure 1(A) shows the distribution of axillary surgery in patients with micrometastases only in SN. Omission of ALND increased slowly from 16% in 2007 to 21% in 2010, before the guideline was changed in December 2011, rose to 74% after adoption of the revised guideline and ALND was omitted in around 95% from 2013 to 2016.

Pathology

HER2 assessment was in Denmark introduced for identification of eligible participants to the HERceptin Adjuvant (HERA) trial [16,17]. As a results from HERA and other adjuvant trastuzumab trials HER2 assessment was in 2005 introduced to a restricted population and the guidelines were revised in 2008 (Table 1) and has since April 2010 been a standard prognostic and predictive factor comprising all breast cancer patients [18,19]. The change introduced April 2010 in guidelines for adjuvant therapy included patients 60 years or older with ER and HER2 positive breast cancer, and shifted from endocrine therapy alone to combined treatment with chemotherapy, trastuzumab and an aromatase inhibitor. Figure 1(B) shows the distribution of HER2 status according to year of inclusion for this subgroup of patients. The proportion of patients registered with HER2 status increased from 74% in 2007 to 94% in 2011 and 99% in 2016.

ER is registered as a continuous variable allowing for use of different cut-points [20,21]. Very few patients are registered with ER 1–9% (approximately 65 patients per year).

Table 1. Changes 2007–2016 in guidelines selected for data presentation.

Treatments	Specification of guideline changes	Introduction
ALND following SN	Omitted in case of micrometastases only	Dec 2011
HER2	Implementation of national guidelines for standardization and interpretation of HER2 staining procedures according to ASCO CAP with modifications in 2013	Sep 2008 Feb 2014
	Trastuzumab if HER2 positive and HR− or HR+ and <60 years	Jan 2007
	Trastuzumab if HER2 positive irrespective of other risk factors	Apr 2010
Fractionation	48 Gy/24 Fr changed to 50 Gy/25 Fr	Jan 2009
	BCS, breast only RT, no boost (two centers): 40 Gy/15 Fr	Feb 2010
	BCS, breast only RT, >40 years: 40 Gy/15 Fr	Apr 2014
	BCS, breast only RT: 40 Gy/15 Fr	2014–2016
Target volume	Loco-regional RT: IMN included left sided also	Jun 2014
Endocrine treatment	Upfront letrozole to postmenopausal patients	Jan 2009
Chemotherapy	Inclusion of taxanes (sequential EC followed by a taxane)	Jan 2007

ALND: axillary lymph node dissection; SN: sentinel node; IHC: Immunohistochemistry; ISH: *In situ* hybridization; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; Fr: fractions; BCS: breast conserving surgery; RT: radiation therapy; IMN: internal mammary nodes.

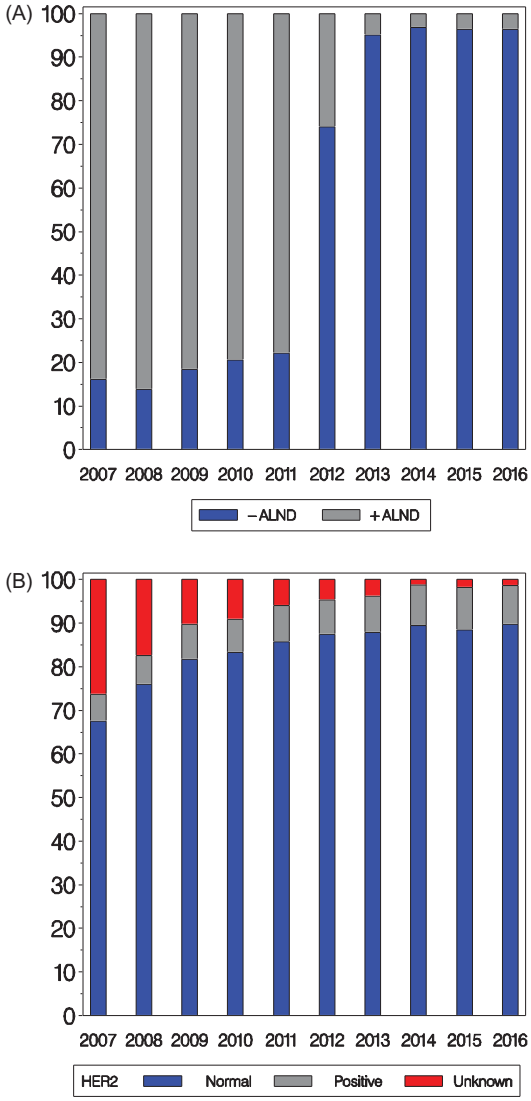


Figure 1. Panel A: Distribution in percent of use of ALND according to year of surgery for patients with invasive breast cancer with micrometastases only in SN ($N = 4869$ for the period 2007–2016). Panel B: Distribution in percent of HER2 status according to year of surgery for patients with ER positive disease 60 years or older at diagnosis ($N = 22{,}209$).

Ki67 has been assessed for most patients since 2009, but was not introduced in treatment guidelines (Supplementary Table 2) due to lack of standardization (methodology, reproducibility) [22].

Radiotherapy

Since 1999 postoperative RT has been recommended following BCS and following mastectomy if node positive (macrometastasis) to women less than 70 years. The age criterion was modified in 2008, Supplementary Table 3. In patients ≥75 years the treatment has been based on an individual evaluation, with a high focus on patient shared decision making.

The fractionation schedules have been changed several times during the last decade, Table 1. Figure 2(A) shows the distribution of different schemes according to time of RT for patients with invasive breast cancer, who had breast only RT and not included in a randomized trial. A marked change from 98 to 81% treated with 48 Gy/24 fractions (Fr) in 2007 and 2008 to 2% in 2009 was seen following the change in guideline January 2009, where the DBCG recommendation was modified to 50 Gy/25 Fr to ensure comparability with other countries. Hypofractionation based on 40 Gy/15 Fr was partly introduced for selected patients treated with breast only RT in the guidelines in 2010, but implemented earlier (1% in 2007, 13% in 2008). In 2014 and 2016 the corresponding figures were 79 and 97%.The division in the years 2009–2015 is a result of successively implementation for different patient groups, but also a different approach in different centers.

From 2003 all high risk breast cancer patients treated with loco-regional RT had the internal mammary nodes (IMN) included in the RT fields in right sided breast cancer, but not in left sided. The IMN target generally included the nodes in caudal direction to the intercostal space IV. In 2014 all patients, irrespective of laterality, receiving loco-regional RT according to DBCG guideline had the IMN included [23–25]. Figure 2(B) shows the proportion of node-positive patients with IMN included according to year of RT for left sided patients, with a marked change in 2014 (76% IMN included) and very few patients not having IMN in the target in 2016 (6%).

Systemic treatment

Systemic treatment is not recommended to postmenopausal women with T1 tumors in the absence of other risk factors [26]. The group of patients receiving systemic treatment has

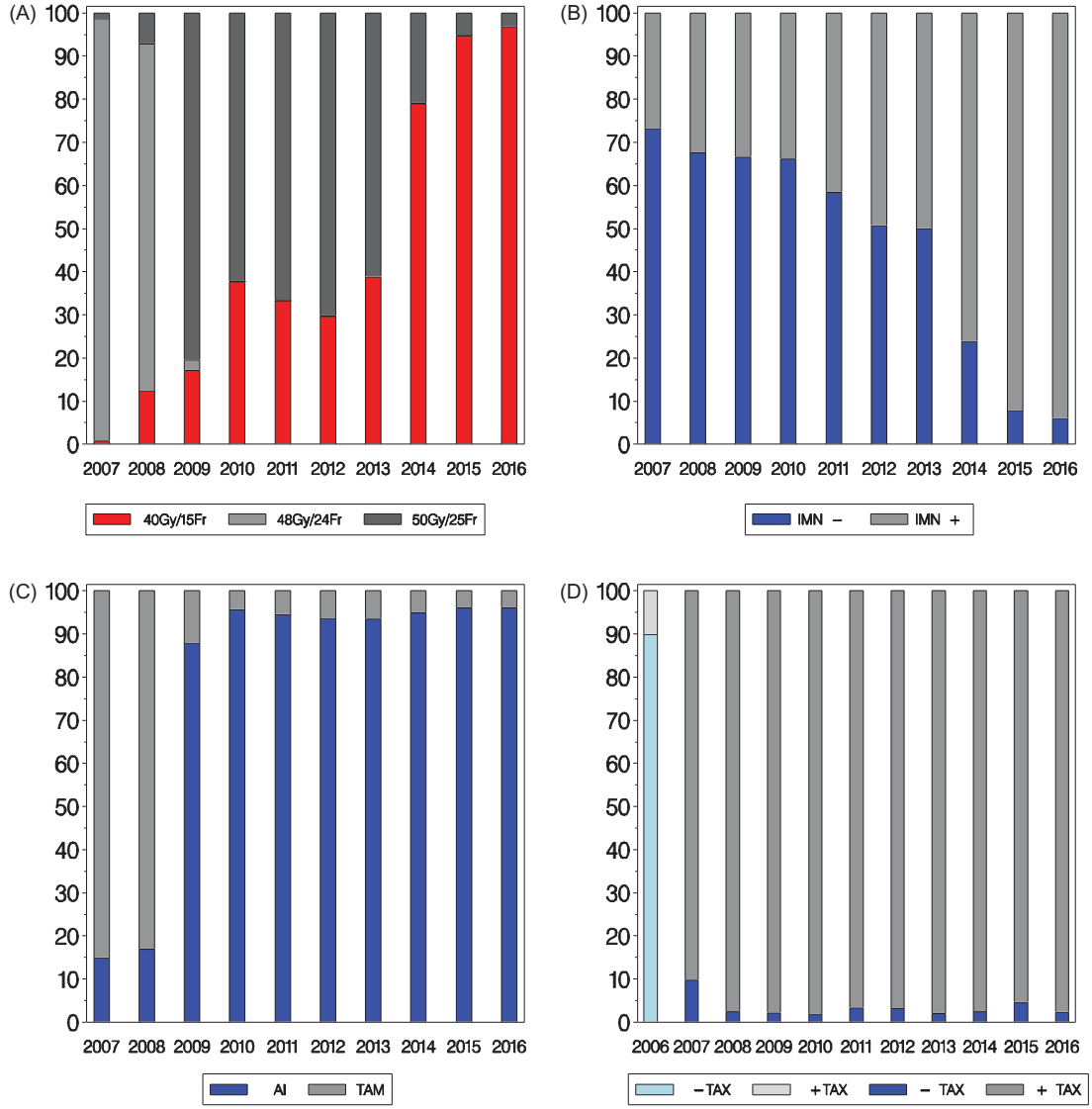


Figure 2. Panel A: Distribution in percent of fractionation used for invasive breast cancer patients with breast only radiation therapy according to year of radiation ($N = 15{,}005$; patients in RT trials not included). Panel B: Distribution in percent of inclusion of IMN (internal mammary nodes) according to year of radiation for patients with loco-regional radiotherapy and left-sided breast cancer ($N = 5805$). Panel C: Distribution in percent of up-front treatment with either tamoxifen (TAM) or an aromatase inhibitor (AI) according to year of inclusion for postmenopausal patients allocated to endocrine treatment ($N = 17{,}314$; patients in trials for systemic treatment not included). Panel D: Distribution in percent of high-risk patients allocated to chemotherapy according to year of inclusion and whether chemotherapy was taxane based ($N = 14{,}457$).

gradually increased, Supplementary Table 4. In 2007 the recommended endocrine therapy was five years of tamoxifen in premenopausal and sequential tamoxifen-aromatase inhibitor in postmenopausal patients [27]. Up-front letrozole to postmenopausal patients was recommended from January 2009 (Table 1) [28]. Figure 2(C) shows postmenopausal patients not included in randomized trial allocated to endocrine therapy according to up-front treatment and year of inclusion. From 16% with up-front AI before the guideline was applicable, the vast majority received an aromatase inhibitor up-front thereafter with 88% in 2009 and 96% in 2010 and 2016. Other changes concerning endocrine treatment are listed in Supplementary Table 4.

Adjuvant trastuzumab has since 2010 been recommended to all patients with HER2 overexpressing or amplified breast cancer. In the period 2006–2010 trastuzumab was only recommended to patients who also were recommended chemotherapy [16]. The administration of trastuzumab has changed from weekly to three-weekly and from intravenous to subcutaneous administration. Also, pertuzumab in combination with trastuzumab has been introduced in the neoadjuvant setting [29].

Throughout 2007–2016 the recommended adjuvant chemotherapy has been three-weekly cycles of EC (600, 90 mg/m^2) followed by either three-weekly cycles of docetaxel (100 mg/m^2) or nine weekly cycles of paclitaxel (80 mg/m^2) [30,31]. Figure 2(D) shows the chemotherapy regimen with or without inclusion of a taxane for this time period. Also, 2006 has been listed to highlight the change by January 2007. In 2006 10% had taxane based CT, changing to 90% in 2007 and stabilizing thereafter with 98% in 2008 and 97% in 2016. The use of paclitaxel has gradually

increased from none to the vast majority of patients. Neoadjuvant chemotherapy has throughout the 10 year period been an option and has since 2016 been encouraged for patients with HER2 positive and to patients with ER and HER2 negative breast cancers. Zoledronic acid (eight times with six months intervals) has been recommended since 2014.

Discussion

In its fourth decade, the DBCG continued to refine multidisciplinary guidelines and further develop the comprehensive clinical database to allow an evaluation of the implementation of the guidelines. More than twenty revisions were introduced in 2007 through 2016 in the DBCG guidelines which reflect the high level of activity within this multidisciplinary group. The revisions monitored in this study were all successfully implemented within a reasonable short timeframe probably facilitated by easily assessable guidelines placed jointly with an online decision support system on the webpage of the responsible cooperative group. Accessibility and applicability has repeatedly been highlighted as the most important factors for implementation of guidelines [32,33].

This study in addition indicates that time from announcement of a breast cancer guideline revision until it is fully implemented may vary from less than one year to more than two years according to the type and setting of the treatment. Guideline revisions dealing with change of systemic treatment were implemented within less than a year while more than two years passed before revisions concerning locoregional treatment were implemented. Also, treatment strategies were to some extent introduced before established in guidelines. There seem to have been the same degree of evidence behind all revision. Upfront treatment with an aromatase inhibitor instead of tamoxifen was based on results from the BIG 1–98 [28], the decision to recommend the addition of a taxane to adjuvant chemotherapy was based on a systematic review [31], omission of ALDN in patients with micrometastases only in SN was based on the ACOSOG Z0011 trial [15] and the revision on irradiation of left-sided IMN was based on a large cohort study [23]. In contrast, only the guideline revisions on systemic treatment were accompanied by a health technology assessment (HTA) to facilitate reimbursement [34].

We are in this study able exactly to indicate the sequence of events. First, the introduction of revisions in the guidelines of the DBCG is announced on the DBCG website setting an official date for their introduction. Second, data prospectively documenting the degree of implementation could for each revision be extracted from the clinical DBCG database. In contrast, implementation of guidelines is frequently evaluated retrospectively using questionnaires [35].

Some limitations should be considered when interpreting our study. We only monitored a small sample of revisions introduced in the last decade, and those included were better defined than those remaining. We did not assess comorbidity in this study and multimorbidity involves a variety of challenges and may to some degree have limited implementation [36]. Further, regional differences have not been investigated.

In conclusion, the guidelines of the DBCG to a large extent ensures homogenous diagnostic and treatment strategy across all centers and implementation of guideline modifications is generally successful although time from introduction to implementation varies across the different disciplines.

Disclosure statement

The authors report no conflict of interest.

ORCID

Niels Kroman http://orcid.org/0000-0002-6631-2993

References

[1] Andersen KW, Mouridsen HT. Danish Breast Cancer Cooperative Group (DBCG). A description of the register of the nation-wide programme for primary breast cancer. Acta Oncol. 1988;27:627–647.
[2] Blichert-Toft M, Christiansen P, Mouridsen HT. Danish Breast Cancer Cooperative Group–DBCG: history, organization, and status of scientific achievements at 30-year anniversary. Acta Oncol. 2008;47:497–505.
[3] Mouridsen HT, Bjerre KD, Christiansen P, et al. Improvement of prognosis in breast cancer in Denmark 1977–2006, based on the nationwide reporting to the DBCG registry. Acta Oncol. 2008;47:525–536.
[4] Jensen MB, Ejlertsen B, Mouridsen HT, et al. Improvements in breast cancer survival between 1995 and 2012 in Denmark: the importance of earlier diagnosis and adjuvant treatment. Acta Oncol. 2016;55(2):24–35.
[5] Grimshaw JM, Russell IT. Effect of clinical guidelines on medical practice: a systematic review of rigorous evaluations. Lancet. 1993;342:1317–1322.
[6] Francke AL, Smit MC, de Veer AJ, et al. Factors influencing the implementation of clinical guidelines for health care professionals: a systematic meta-review. BMC Med Inform Decis Mak. 2008;8:38.
[7] Møller S, Jensen MB, Ejlertsen B, et al. Danish Breast Cancer Cooperative Group. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.
[8] Lakhani SR, Ellis IO, Schnitt SJ, et al. WHO classification of tumours of the breast. 4th ed. Lyon: International Agency for Research on Cancer; 2012.
[9] Kiaer HW, Laenkholm AV, Nielsen BB, et al. Classical pathological variables recorded in the Danish Breast Cancer Cooperative Group's register 1978–2006. Acta Oncol. 2008;47:778–783.
[10] Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol. 2014;29:541–549.
[11] Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–383.
[12] Ejlertsen B, Jensen MB, Mouridsen HT. Excess mortality in postmenopausal high-risk women who only receive adjuvant endocrine therapy for estrogen receptor positive breast cancer. Acta Oncol. 2014;53:174–185.
[13] Laenkholm AV, Jensen MB, Eriksen JO, et al. The PAM50 risk of recurrence score predicts 10 year distant recurrence in a comprehensive Danish cohort of 2558 postmenopausal women allocated

to 5 year of endocrine therapy for hormone receptor positive early breast cancer. J Clin Oncol. 2018;57: in press.
[14] Houssami N, Macaskill P, Marinovich ML, et al. Meta-analysis of the impact of surgical margins on local recurrence in women with early-stage invasive breast cancer treated with breast-conserving therapy. Eur J Cancer. 2010;46:3219–3232.
[15] Giuliano AE, Hunt KK, Ballman KV, et al. Axillary dissection vs. no axillary dissection in women with invasive breast cancer and sentinel node metastasis a randomized clinical trial. JAMA. 2011;305:569–575.
[16] Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Herceptin Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med. 2005;353:1659–1672.
[17] Cameron D, Piccart-Gebhart MJ, Gelber RD, et al. Herceptin Adjuvant (HERA) Trial Study Team. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. Lancet. 2017;389:1195–1205.
[18] Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med. 2007;131:18–43.
[19] Wolff AC, Hammond ME, Hicks DG, et al. American Society of Clinical Oncology; College of American Pathologists. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.
[20] Viale G, Regan MM, Maiorano E, et al. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1–98. J Clin Oncol. 2007; 2:3846–3852.
[21] Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28:2784–2795.
[22] Laenkholm AV, Grabau D, Talman ML, et al. An inter observer Ki67 reproducibility study applying two different assessment methods. On behalf of the Danish Scientific Committee of Pathology, Danish Breast Cancer Cooperative Group (DBCG). Acta Oncol. 2018; in press.
[23] Thorsen LB, Thomsen MS, Overgaard M, et al. Quality assurance of conventional non-CT-based internal mammary lymph node irradiation in a prospective Danish Breast Cancer Cooperative Group trial: the DBCG-IMN study. Acta Oncol. 2013;52:1526–1534.
[24] Thorsen LB, Thomsen MS, Berg M, et al. CT-planned internal mammary node radiotherapy in the DBCG-IMN study: benefit versus potentially harmful effects. Acta Oncol. 2014;53:1027–1034.
[25] Thorsen LB, Offersen BV, Danoe H, et al. DBCG-IMN: a population-based cohort study on the effect of internal mammary node irradiation in early node-positive breast cancer. J Clin Oncol. 2016;34:314–321.
[26] Christiansen P, Bjerre K, Ejlertsen B, et al. Mortality rates among early-stage hormone receptor–positive breast cancer patients: a population-based cohort study in Denmark. J Natl Cancer Inst. 2011;103:1363–1372.
[27] Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med. 2004;350:1081–1092.
[28] BIG 1–98 Collaborative Group, Mouridsen H, Giobbie-Hurder A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med. 2009;361:766–776.
[29] von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N Engl J Med. 2017;377:122–131.
[30] Francis P, Crown J, Di Leo A, et al. Adjuvant chemotherapy with sequential or concurrent anthracycline and docetaxel: Breast International Group 02–98 randomized trial. J Natl Cancer Inst. 2008;100:121–133.
[31] Bria E, Nistico C, Cuppone F, et al. Benefit of taxanes as adjuvant chemotherapy for early breast cancer: pooled analysis of 15,500 patients. Cancer. 2006;106:2337–2344.
[32] Davis DA, Taylor-Vaisey A. Translating guidelines into practice. A systematic review of theoretic concepts, practical experience and research evidence in the adoption of clinical practice guidelines. CMAJ. 1997;157:408–416.
[33] Fischer F, Lange K, Klose K, et al. Barriers and strategies in guideline implementation-a scoping review. Healthcare (Basel). 2016;4:36.
[34] Banta D, Kristensen FB, Jonsson E. A history of health technology assessment at the European level. Int J Technol Assess Health Care. 2009;25(1):68–73.
[35] Nicholson BD, Mant D, Neal RD, et al. International variation in adherence to referral guidelines for suspected cancer: a secondary analysis of survey data. Br J Gen Pract. 2016;66:e106–e113.
[36] Austad B, Hetlevik I, Mjølstad BP, et al. Applying clinical guidelines in general practice: a qualitative study of potential complications. BMC Fam Pract. 2016;17:92.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 19–25
https://doi.org/10.1080/0284186X.2017.1403042

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Breast conserving surgery versus mastectomy: overall and relative survival—a population based study by the Danish Breast Cancer Cooperative Group (DBCG)

Peer Christiansen[a], Stina Lyck Carstensen[b], Bent Ejlertsen[c], Niels Kroman[d], Birgitte Offersen[e], Anne Bodilsen[f] and Maj-Britt Jensen[b]

[a]Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark; [b]Danish Breast Cancer Cooperative Group Secretariat, Copenhagen University, Rigshospitalet, Copenhagen, Denmark; [c]Danish Breast Cancer Cooperative Group Secretariat, Department of Oncology, Copenhagen University, Rigshospitalet, Copenhagen, Denmark; [d]Department of Breast Surgery, Copenhagen University Hospital, Herlev, Denmark; [e]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark; [f]Department of Surgery, Horsens Regional Hospital, Horsens, Denmark

ABSTRACT

Background: Observational studies have pointed at a better survival after breast conserving surgery (BCS) compared with mastectomy. The aim of the present study was to evaluate whether this remains true when more extensive tumor characteristics and treatment data were included.

Methods: The cohort included patients registered after primary surgery for early invasive breast cancer in the database of the Danish Breast Cancer Cooperative Group, in the period 1995–2012. The cohort was divided into three groups: (i) patients who primarily had a mastectomy, (ii) patients treated by BCS, and (iii) patients who primarily had BCS and then mastectomy [intention to treat (ITT) by BCS]. The association between overall mortality and standard mortality ratio (SMR) and risk factors was analyzed in univariate and multivariate Poisson regression models.

Results: A total of 58,331 patients were included: 27,143 in the mastectomy group, 26,958 in the BCS group, and 4230 in the BCS-ITT group. After adjusting for patient and treatment characteristics, the relative risk (RR) was 1.20 (95% CI: 1.15–1.25) after mastectomy and 1.08 (95% CI: 1.01–1.15) after BCS first and then mastectomy, as compared to BCS. Statistically significant interactions were not observed for age, period of treatment, and nodal status, but patients with Charlson's Comorbidity Index (CCI) score 2+ had no increased mortality after mastectomy, as opposed to patients with CCI 0–1. Loco-regional radiation therapy (RT) in node positive patients did not reduce the increased risk associated with mastectomy [RR = 1.28 (95% CI 1.19–1.38)].

Conclusion: Patients assigned to BCS have a better survival than patients assigned to mastectomy. Residual confounding after adjustment for registered characteristics presumably explained the different outcomes, thus consistent with selection bias. Diversities in RT did not appear to explain the observed difference in survival after BCS and mastectomy.

ARTICLE HISTORY
Received 27 September 2017
Accepted 3 November 2017

Introduction

The randomized trials conducted in the 1980-ies showed an equal outcome after breast conserving surgery (BCS) combined with radiation therapy (RT) and mastectomy [1–3]. Since then BCS has become the preferred method.

Recent results from observational studies [4–7] has pointed at a better survival after BCS compared with mastectomy. Accordingly, it has been argued that it may no longer be appropriate to offer women suitable for BCS the choice of mastectomy [8]. All of these studies have limitations, as they all more or less lacked significant prognostic variables characterizing tumor biology and treatment. The present material from the Danish Breast Cancer Cooperative Group (DBCG) was from a population-based cohort including variables comprehensively describing patient and tumor characteristics and the given treatment.

The aim of the present study is on a large population-based material to compare the outcome for Danish breast cancer patients treated by mastectomy and BCS. The comparison takes the available prognostic variables into a multivariate analysis and includes an intention to treat analysis.

Material and methods

Since the establishment in 1977, DBCG has provided standard diagnostic treatment algorithms for early breast cancer. Data on diagnosis, therapy, and follow-up on newly diagnosed breast cancer patients have been collected prospectively in the DBCG registry by the use of standardized forms [9].

The cohort used in this study, included patients who had surgery for invasive breast cancer in the period 1995–2012. Patients preoperatively diagnosed with distant metastatic,

CONTACT Peer Christiansen peerchri@rm.dk Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark
Supplemental data for this article can be accessed here.

locally advanced breast cancer, or synchronous bilateral breast cancers were not included in the cohort. Furthermore, patients who had received neo-adjuvant therapy or patients with unknown nodal status were not included in the cohort.

The cohort was divided into three groups: (i) patients with assigned mastectomy as the first surgical procedure, (ii) patients with breast conservation as the definitive surgical procedure, and (iii) patients assigned with an initial BCS followed by mastectomy as a second or later procedure. The definitive surgical procedure is detailed registered in DBCG and we retrieved information on prior surgery from the Danish National Patients Registry and on prior examination of tissue from the Danish National Pathology Registry. Vital status and emigration was retrieved from the Danish Civil Registration System and mortality in the general Danish female population by age and calendar period was obtained from Statistics Denmark.

Comorbidity was described by Charlson's Comorbidity Index (CCI) [10], based on data from hospital contacts using International Classification of Disease (ICD-8 and ICD-10) data up to 10 years prior to the breast cancer diagnosis date.

Statistics

Primary and secondary end-points were standardized mortality ratios (SMR) and overall survival. Overall survival was defined as time from surgery until death from any cause.

Estimated median follow-up time was quantified in term of Kaplan–Meier estimates [11]. SMR, was calculated as the ratio of the observed number of death among patients to the expected number of deaths. The expected number of deaths was estimated by multiplying the survival time accrued from the study cohort by the mortality rates of the general population of women matched by age (1 year groups) and calendar period (1 year groups). Estimates of SMR greater than unity indicates that breast cancer patients have a higher mortality rate than women in the general population. Kaplan–Meier estimates were used to estimate overall survival.

The association between mortality and risk factors was analyzed in univariate and multivariate Poisson regression models. In the multivariate analysis, we adjusted for: age at operation categorized in year intervals (18–44, 45–54, 55–64, 65–74, 75+), year of operation divided into the three periods (1995–2001, 2002–2007, 2008–2012), comorbidity described by CCI categories (0, 1, 2, 3+), tumor size categorized in intervals (0–10 mm, 11–20 mm, 21–30 mm, 31–50 mm, 51 + mm), number of positive lymph nodes in four nodal categories (0, 1–3, 4–9, $\geq$10), histological type (ductal, lobular, other), malignancy grade (I, II, III), percent of ER-positive tumor cells [negative (0–9%), positive (10–100%)], lymphovascular invasion (LVI) with two levels (yes versus no), multifocality with three levels (multifocal, unifocal, and unknown), chemotherapy, RT and endocrine therapy each with three levels (yes, no and unknown). Unknowns were included in most appropriate categories, unless otherwise specified. Furthermore, the results of the full multivariate model were compared with a corresponding model, where age, tumor size and number of positive lymph nodes were included as continuous variables. The results of these analyses did not alter the overall results noticeably. Likewise was the results not altered by including HER2 in the multivariate models. Hence, HER2 was not included and all variables were treated as categorical in the final multivariate model. Interactions between operation and age, year of operation, CCI, nodal status, and systemic therapy were investigated in separate models. An intention to treat analysis as well as an analysis based on the outcome operation was included.

p values $<.05$ were considered significant, and p values were two-tailed. The statistical analyses were performed in R version 3.2.3 [12].

Results

The population of surgical treated female breast cancer patients included in all 68,842 patients (Supplementary Figure S1). After exclusion of patients with locally advanced, distant metastatic, and bilateral breast cancer, and furthermore patients who were not surgical treated and patients who had neoadjuvant chemotherapy, the material consisted of 61,199 surgical treated patients. Lymph node status was not available in 2868 cases (4.7% of the total), and these patients were not included in the study. Thus, the material included 58,331 breast cancer patients, of which 31,373 were treated by mastectomy and 26,958 by BCS. In the mastectomy group, there were 4230 who initially had breast conservation, but within 3 months had a mastectomy as a second or third procedure.

Patient characteristics are given in Table 1. BCS increased from 24.4% in the period 1995–2001 to 68.0% in the period 2008–2012. In the 75+ age group, 23.6% had BCS. Compared to the mastectomy groups, patients in the BCS group (all $p < .0001$) had smaller tumors (median 23 versus 15 mm, respectively), had fewer lymph node metastases (57.1 versus 34.5%), and were more often hormone receptor positive (73.7 versus 83.1%).

Survival is depicted in Figure 1. The estimated median potential follow-up time was 11.5 years. The 10-year survival rates were 57% after primary mastectomy and 82% in patients having final BCS. Patients who had initially BCS but eventually were mastectomized had a 10-year overall survival of 74%, thus closer to the result in the BCS group.

The differences in survival between the various surgical groups are further elucidated in Table 2 showing both univariate and multivariate risk estimates for overall mortality and SMR. As shown, the mortality was increased in patients treated by mastectomy, although adjusting for the various prognostic risk factors reduced the difference between the groups. Of further notice was an only modest difference between patients, by whom the intention was to do BCS, but later were converted to mastectomy, and those patients, in whom breast conservation succeeded: relative risk (RR) 1.08 (95% CI 1.01–1.15) in the adjusted model.

The impact on mortality of the patient, tumor, and treatment variables included are given in Table 3, presenting the results from the multivariate Poisson model on the intention

Table 1. Characteristics of 58,331 Danish breast cancer patients surgically treated in the period 1995–2002.

Characteristic	Mastectomy (final)		BCS (final)		BCS and mastectomy		
	N	%	*N*	%	*N*	%	Total
	27,143	44.4	26,958	44.1	4230	6.9	58,331
Year of inclusion							
1995–2001	12,735	47	4846	18	2249	53	19,830
2002–2007	9030	33	8907	33	1148	27	19,085
2007–2012	5378	20	13,205	49	833	20	19,416
Age at operation							
18–44	2598	10	2631	10	672	16	5901
45–54	5227	20	6446	24	1239	29	12,912
55–64	6869	25	9497	35	1167	28	17,533
65–74	6656	25	6496	24	823	20	13,975
75+	5793	21	1888	7	329	8	8010
CCI							
0	21,172	78	22,210	82	3572	84	46,954
1	3443	13	2907	11	375	9	6725
2	1565	6	1275	5	185	4	3025
3+	963	4	566	2	98	2	1627
Tumor size (mm)							
0–10	2304	9	7063	26	1133	27	10,500
11–20	8842	33	13,893	52	1592	38	24,327
21–30	8371	31	4879	18	808	19	14,058
31–50	5471	20	949	4	443	11	6863
51+	1807	7	47	0	129	3	1983
Unknown	342	1	117	0	125	3	584
Positive lymph nodes							
0	11,640	43	17,656	66	2409	57	31,705
1–3	8825	33	7333	27	1194	28	17,352
4–9	3936	15	1427	5	402	10	5765
10+	2742	10	542	2	225	5	3509
Histological type							
Ductal	21,744	80	22,143	82	3204	76	47,091
Lobular	3487	13	2277	8	632	15	6396
Other	1699	6	2357	9	359	9	4415
Unknown	213	1	181	1	35	1	429
Grade (ductal and lobular)							
I	6159	24	8776	36	990	256	15,925
II	10,624	42	9969	41	1532	40	22,125
III	6177	25	4861	20	761	20	11,799
Unknown	2271	9	814	3	553	14	3638
Estrogen receptor							
Negative (0–9%)	6068	22	4023	15	905	21	10,996
Positive (10–100%)	19,999	74	22,412	83	3104	73	45,515
Unknown	1076	4	523	2	221	5	1820
HER2 receptor							
Normal	7046	76	15,003	88	1063	78	23,112
Positive	2228	24	1999	12	302	22	4529
Unknown	17,869		9956		2865		30,690
Lymphovascular invasion							
No	19,919	73	23,893	89	3162	75	46,974
Yes	5108	19	1952	7	488	12	7548
Unknown	2116	8	1113	4	580	14	3809
Multifocality							
Multifocal	4302	16	741	3	832	20	5875
Unifocal	20,897	77	25,170	93	2878	68	48,945
Unknown	1944	7	1047	4	520	12	3511

BCS and mastectomy = patients initially assigned to BCS, but finally having mastectomy. Differences between groups were statistically significant for all variables with p values $<.0001$.

to treat populations. Patients primarily assigned to mastectomy had 21% increased risk in overall mortality and 19% increase in SMR compared to BCS.

Interactions between surgical treatment and selected predictive variables are depicted in Figure 2. Significant interactions were not observed for age, period of treatment, and nodal status. HER2 was primarily registered for patients included in the latter half of the study inclusion period, with the majority of patients with HER2-positive tumors receiving HER2-targeted treatment. Inclusion of HER2 in the multivariate models did not alter the overall results. However, there was a highly significant interaction between type of operation and CCI. Those with CCI-score 2+ had no increased mortality after mastectomy, as opposed to patients with CCI 0–1. Similar results were observed for overall survival and for the ITT groups (data not shown). Careful review of CCI groups reveled that chemotherapy and RT were less often given to patients with comorbidity (Supplementary Table S1).

Finally, the complex interaction between surgical type and both nodal status and RT was evaluated (Supplementary Table S2). No statistical significant interactions were observed ($p = .93$). Indication for loco-regional RT has varied over the years for the present study cohort. Comparing patients with macrometastases, loco-regional RT did not reduce the increased risk [RR = 1.28 (95% CI 1.19–1.38)], with an almost identical estimate for patients with macrometastases and no indication for loco-regional RT, i.e., mastectomy no RT versus BCS breast only RT, [RR = 1.27 (95% CI 1.14–1.42)]. Similar results were observed for overall survival and for the ITT groups (data not shown).

Discussion

The present study has shown a higher risk of mortality in breast cancer patients treated by mastectomy compared with patients having breast conserving therapy. Patients going through an attempt of breast conservation prior to mastectomy had a considerable better outcome than those primarily assigned to mastectomy. Adjusting for various prognostic risk factors decreased the excessive mortality after mastectomy to ~20%. The increased risk seems independent of age and period of treatment. Differences in RT could not explain the different outcome between mastectomy and BCS. The observed differences seem to some degree to rely on residual confounding.

The present results confirm previous findings from register based studies of an up to 20–30% better survival in patients treated by BCS compared to mastectomy. The largest study based on data from the Surveillance, Epidemiology, and End Results (SEER) database [4], included more than 130,000 patients treated in the period 1998–2008 with tumor size up to 4 cm and ≤3 positive lymph nodes. In the multivariate analysis in that study, the hazard ratio (HR) for survival was 1.31 after BCS compared with mastectomy alone. The HR was even higher when comparing BCS with mastectomy combined with RT (1.47). The study had some major limitations, as it did not include information on systemic therapy, and the tumor characteristics were limited to hormone receptor status, grade, and lymph node status, with no information on LVI or HER2 status.

Similar results were reported from one Canadian [5], further two American [6,7], and two Norwegian studies [13,14]. These studies also all lacked some essential information on tumor biology and on treatment, especially on systemic treatment. Contrary to this, a study from the Netherlands Cancer Registry including around 37,000 patients treated in the period 2000–2004 had more prognostic factors included

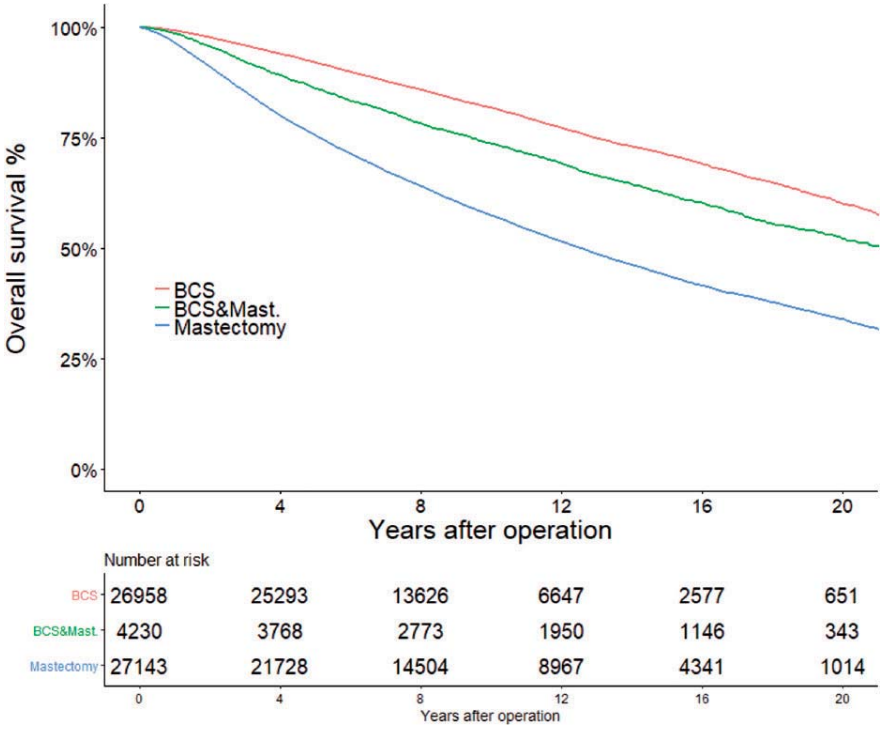


Figure 1. Overall survival in the three groups: patients primarily assigned to mastectomy, patients primarily assigned to BCS, and patients primary assigned to BCS but who finally had mastectomy (BCS & Mast).

Table 2. Univariate and multivariate risk estimates for overall mortality and SMR.

	Unadjusted values				Adjusted values			
	Overall mortality		SMR		Overall mortality		SMR	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
M (ITT) versus BCS (ITT)	2.43	2.36–2.51	1.38	1.34–1.42	1.21	1.17–1.26	1.19	1.15–1.23
M (final) versus BCS (final)	2.46	2.38–2.54	1.44	1.39–1.48	1.23	1.18–1.28	1.20	1.15–1.25
BCS&M versus BCS (final)	1.51	1.43–1.60	1.28	1.21–1.36	1.10	1.03–1.17	1.08	1.01–1.15
M (ITT) versus BCS (final)	2.64	2.55–2.72	1.46	1.41–1.50	1.25	1.20–1.31	1.22	1.17–1.27

M = mastectomy; M (ITT) = patients primary allocated to mastectomy; M (final) = patents finally having mastectomy; BCS (ITT) = patients primary allocated to BCS; BCS (final) = patients finally having BCS; BCS&M = patients initially allocated to BCS but finally having mastectomy.

in their analysis. Thus, information on tumor size, nodal status, and grade were available for most patients, but data regarding hormone receptor status was incomplete and lacking for a large proportion of the patients. Also, the study did not report on HER2 status. The study population was restricted to subgroups T0–2, N0–1. Adjuvant systemic treatment was to only given to ~50% of the patients included, and among patients undergoing mastectomy, all who had RT were excluded. Overall, the adjusted HR for 10-year survival was 0.81 in the mastectomy group, but looking at subgroups a significant benefit was only observed in the T1N0 group. Later, the Dutch group reported in a second study on patients with T1–2, N2 breast cancer [15]. The study included 3700 patients and found an overall adjusted HR at 0.88. Again significance was not found in all subgroups, and in this case it was restricted to the T2N2 group. The same group very recently published a third paper also based on data from the Netherlands Cancer Registry [16]. In that study, the population included 129,692 patients treated in the period 1999–2012, thus including the populations in the previous two studies. Interestingly, the last study report slightly diverging results compared to the previous results: no survival benefit after BCS in the T1–2N2 group while BCS was superior to mastectomy in all T1–2N0–1 subgroups. After stratification there was no difference in patients younger than 50 years and in patients with comorbidity.

The two first studies from the Netherlands, which were known to us when the present study was planned, included more prognostic risk factors in the multivariate analysis than in the aforementioned studies and gave less convincing indications for a better survival after BCS. Based on these observations, and as we were able to include more prognostic factors than presented in previous studies, we expected to find a less pronounced difference in outcome between BCS and mastectomy. But as shown, this was not the case. Even after adjustment for a wide and more complete range of prognostic factors, than in all other population-based studies so far, patients treated by BCS seemed to have a better outcome. It has been argued that it is the RT given to all BCS patients, as opposed to patients treated by mastectomy, which explains the observed differences [16,17], but the present results clearly reject that hypothesis.

Table 3. The impact on mortality of the patient, tumor, and treatment variables evaluated in a multivariate Poisson model on the intention to treat populations.

	Overall mortality			SMR		
Variable	RR	95% CI	*p* value	RR	95% CI	*p* value
Operation			<.0001			<.0001
Mastectomy versus BCS	1.21	1.17–1.26		1.19	1.15–1.23	
Age at operation			<.0001			<.0001
Age at operation 18–44 versus 55–64	0.71	0.67–0.76		4.13	3.88–4.40	
Age at operation 45–54 versus 55–64	0.70	0.67–0.74		1.56	1.49–1.64	
Age at operation 65–74 versus 55–64	1.56	1.50–1.62		0.62	0.60–0.65	
Age at operation 75+ versus 55–64	2.45	2.34–2.57		0.34	0.33–0.36	
Treatment program			<.0001			<.0001
Year of inclusion 2002–2007 versus 1995–2001	0.80	0.77–0.83		1.02	0.99–1.06	
Year of inclusion 2008–2012 versus 1995–2001	0.56	0.53–0.59		0.90	0.86–0.95	
CCI			<.0001			<.0001
CCI 1 versus 0	1.49	1.44–1.55		1.51	1.45–1.57	
CCI 2 versus 0	1.66	1.58–1.75		1.72	1.63–1.81	
CCI 3+ versus 0	2.33	2.18–2.48		2.53	2.37–2.69	
Tumor size			<.0001			<.0001
Tumor size 0–10 versus 11–20[a] mm	0.79	0.75–0.83		0.82	0.78–0.86	
Tumor size 20–30 versus 11–20[a] mm	1.19	1.15–1.23		1.19	1.15–1.23	
Tumor size 30–50 versus 11–20[a] mm	1.33	1.27–1.38		1.32	1.27–1.38	
Tumor size 50+ versus 11–20[a] mm	1.54	1.44–1.64		1.56	1.46–1.66	
Positive lymph nodes			<.0001			<.0001
Positive lymph nodes 1–3 versus 0	1.38	1.33–1.43		1.35	1.30–1.40	
Positive lymph nodes 4–9 versus 0	2.12	2.02–2.22		2.20	2.10–2.30	
Positive lymph nodes 10+ versus 0	3.12	2.96–3.29		3.47	3.29–3.65	
Estrogen receptor (ER) status			<.0001			<.0001
ER negative versus positive	1.20	1.14–1.25		1.19	1.14–1.25	
ER unknown versus positive	1.06	0.99–1.13		1.07	1.00–1.15	
Lymphovascular invasion			<.0001			<.0001
Lymphovascular invasion yes versus no[a]	1.22	1.18–1.27		1.26	1.21–1.31	
Histological type			.0001			<.0001
Lobular versus ductal	0.99	0.95–1.04		0.99	0.95–1.03	
Other type[a] versus ductal	0.89	0.84–0.94		0.87	0.83–0.92	
Grade			<.0001			<.0001
Grade 2[a] versus 1	1.14	1.10–1.19		1.14	1.10–1.18	
Grade 3 versus 1	1.38	1.32–1.45		1.40	1.33–1.46	
Focality			<.0001			.04
Multifocal versus unifocal	1.01	0.97–1.06		1.04	1.00–1.09	
Unknown versus unifocal	1.17	1.11–1.22		1.05	1.00–1.11	
RT			<.0001			<.0001
Unknown versus no	1.11	1.04–1.19		1.14	1.07–1.22	
Yes versus no	0.83	0.80–0.87		0.93	0.89–0.97	
Chemotherapy			<.0001			<.0001
Unknown versus no	1.65	1.53–1.78		1.94	1.79–2.09	
Yes versus no	0.81	0.77–0.85		0.95	0.90–1.00	
Endocrine therapy			<.0001			<.0001
Unknown versus no	1.70	1.58–1.83		1.78	1.65–1.91	
Yes versus no	0.78	0.75–0.82		0.84	0.81–0.88	

[a]Unknown included in group.

Previous Danish studies have pointed at a higher recurrence risk and higher mortality in younger patients [18,19] and among these a worse outcome after BCS compared to mastectomy [18,20]. These studies were done on patients treated in the 1990-ties. The present results on a somewhat later treated cohort, where the proportion of patients treated by BCS increased over time, do not confirm that. The increased use of and improvement in systemic treatment and RT are probably the main explanation for this change in outcome results. The previous studies had longer observation periods and they showed a significant increased mortality even after 10 years observation and we cannot rule out that a longer observation period in the present study would have changed the picture somewhat, although we do not believe the general picture would have been markedly changed, as we consider our results robust.

It is obvious that confounding by indication is in play. Comorbidity has a strong impact with poorer survival [21], and we found that patients with more comorbidity were preferably treated by mastectomy, reducing the survival in this group. Also, the proportion of patients with higher risk tumors was increased. Adjusting for prognostic risk factors reduced the RR after mastectomy, but not completely. The observation that patients, who were initially assigned to BCS, but had a final mastectomy, had a significantly better outcome than patients primarily assigned to mastectomy and only had a slight increase in mortality relative to what was seen in the BCS group, strongly infers that there is some residual confounding, which is not accounted for in our study, and it seems more pronounced in patients with tumors belonging to the more favorable end of the spectrum and to patients without comorbidity. The minor differences observed between the two groups assigned to BCS might be related to recent findings showing that patients undergoing mastectomy secondary to BCS because of insufficient margins, had an increased risk of distant metastasis [22].

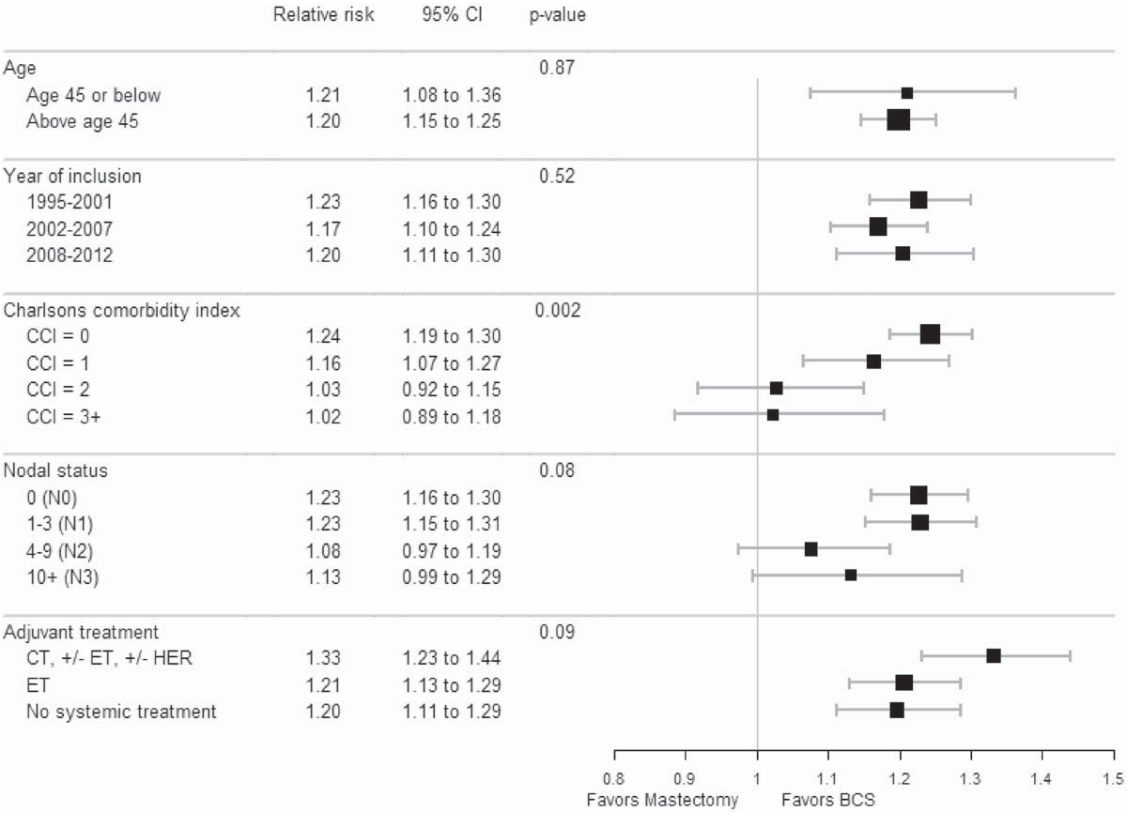


Figure 2. Forrest plot showing interactions between selected risk factors and the risk of mortality (SMR) and the final surgical procedure.

In theory, there are possible explanations for a worse prognosis among the group of patients treated by mastectomy, not accounted for in this study. Some patients selected for mastectomy have characteristics which give them a higher risk of recurrence, including dense breasts [23] and widespread DCIS in the surroundings [24]. Some studies [25–27] have also indicated that multifocal tumors have a more aggressive biology, although others have given diverging results [25,27–29]. It has been speculated that tumors detected by mammography screening preferably treated by BCS have a much more favorable prognosis and therefore should be considered a major confounding factor. Unfortunately, we did not have information on mammography screening that allowed us to include that in our study, but one of the previously cited studies from Norway looked specifically on the impact of detection mode on the difference in outcome between the surgical treatment groups and found a survival benefit after BCS independent of whether the tumors were screen detected or had presented clinically [14]. Multifocality/multicentricity was included in the present study, but the distribution among the groups was very skew, as multifocality has been considered a contraindication for BCS. Therefore, the adjustment did probably not fully adjust for this difference.

Finally, one cannot rule out that there are some adverse effects of mastectomy contrary to BCS. Mastectomy is a more extensive procedure and leads to more tissue damage and a more pronounced inflammatory response [30]. This could have a negative effect by suppressing the immune system and promoting growth of residual tumor cells and in the circulation for instance by angiogenesis of dormant avascular micrometastases and surgery-induced activity of single malignant cells [31,32].

Several issues should be considered when interpreting this study. First, we were able to identify patients in whom BCS was attempted but who went on to secondary mastectomy. Patients with repeat surgery had a significantly lower mortality compared to patients with a primary mastectomy, but still not as favorable as patients with BCS only. Second, detailed diagnostic and treatment characteristics were recorded prospectively, and adjusting for these factors partly explained the difference in outcome observed after BCS and mastectomy. Third, CCI could be estimated using administrative data and in patients with a CCI score of 0 or 1 mastectomy as first choice of surgery was associated with higher mortality and SMR while a CCI score of $\geq$2 was not. Fourth, our study comprises a large and population-based cohort allowing calculation of mortality relative to the Danish female population. Our study was however confounded and we are unable to determine whether the difference in outcome observed after controlling for repeat surgery and adjusting for other patient and treatment characteristics is a real effect or constitutes residual confounding. Our study had other limitations including lack of information on breast density, presence of DCIS, and HER2 status for a major part of the population.

In conclusion, patients assigned to BCS have a better survival than patients assigned to mastectomy. Residual confounding after adjustment for registered characteristics presumably explained the different outcomes, thus consistent

with selection bias. Diversities in RT do not seem to explain the observed difference in survival after BCS and mastectomy.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. N Engl J Med. 2002;347:1233–1241.

[2] Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med. 2002;347:1227–1232.

[3] Blichert-Toft M, Nielsen M, Düring M, et al. Long-term results of breast conserving surgery vs. mastectomy for early stage invasive breast cancer: 20-year follow-up of the Danish randomized DBCG-82TM protocol. Acta Oncol. 2008;47:672–681.

[4] Agarwal S, Pappas L, Neumayer L, et al. Effect of breast conservation therapy vs mastectomy on disease-specific survival for early-stage breast cancer. JAMA Surg. 2014;84132:1–8.

[5] Fisher S, Gao H, Yasui Y, et al. Survival in stage I-III breast cancer patients by surgical treatment in a publicly funded health care system. Ann Oncol. 2015;26:1161–1169.

[6] Hwang ES, Lichtensztajn DY, Gomez SL, et al. Survival after lumpectomy and mastectomy for early stage invasive breast cancer: the effect of age and hormone receptor status. Cancer. 2013;119:1402–1411.

[7] Chen K, Liu J, Zhu L, et al. Comparative effectiveness study of breast-conserving surgery and mastectomy in the general population: a NCDB analysis. Oncotarget. 2015;6:40127–40140.

[8] Johns N, Dixon JM. Should patients with early breast cancer still be offered the choice of breast conserving surgery or mastectomy? Eur J Surg Oncol. 2016;42:1636–1641.

[9] Møller S, Jensen M-B, Ejlertsen B, et al. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.

[10] Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–383.

[11] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17:343–346.

[12] R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015. Available from: https://www.R-project.org/

[13] Hartmann-Johnsen OJ, Kåresen R, Schlichting E, et al. Survival is better after breast conserving therapy than mastectomy for early stage breast cancer: a registry-based follow-up study of Norwegian women primary operated between 1998 and 2008. Ann Surg Oncol. 2015;22:3836–3845.

[14] Hofvind S, Holen Å, Aas T, et al. Women treated with breast conserving surgery do better than those with mastectomy independent of detection mode, prognostic and predictive tumor characteristics. Eur J Surg Oncol. 2015;41:1417–1422.

[15] van Maaren MC, de Munck L, Jobsen JJ, et al. Breast-conserving therapy versus mastectomy in T1-2N2 stage breast cancer: a population-based study on 10-year overall, relative, and distant metastasis-free survival in 3071 patients. Breast Cancer Res Treat. 2016;160:511–521.

[16] Lagendijk M, van Maaren MC, Saadatmand S, etet al. Breast conserving therapy and mastectomy revisited: Breast cancer-specific survival and the influence of prognostic factors in 129,692 patients. Int J Cancer. 2017;142:165–175.

[17] Onitilo AA, Engel JM, Stankowski RV, et al. Survival comparisons for breast conserving surgery and mastectomy revisited: community experience and the role of radiation therapy. Clin Med Res. 2015;13:65–73.

[18] Laurberg T, Lyngholm CD, Christiansen P, et al. Long-term age-dependent failure pattern after breast-conserving therapy or mastectomy among Danish lymph-node-negative breast cancer patients. Radiother Oncol. 2016;120:98–106.

[19] Lyngholm CD, Laurberg T, Alsner J, et al. Failure pattern and survival after breast conserving therapy. Long-term results of the Danish Breast Cancer Group (DBCG) 89 TM cohort. Acta Oncol. 2016;55:983–992.

[20] Laurberg T, Alsner J, Tramm T, et al. Impact of age, intrinsic subtype and local treatment on long-term local-regional recurrence and breast cancer mortality among low-risk breast cancer patients. Acta Oncol. 2017;56:59–67.

[21] Land LH, Dalton SO, Jensen M-B, et al. Impact of comorbidity on mortality: a cohort study of 62,591 Danish women diagnosed with early breast cancer, 1990-2008. Breast Cancer Res Treat. 2012;131:1013–1020.

[22] Bodilsen A, Bjerre K, Offersen BV, et al. The influence of repeat surgery and residual disease on recurrence after breast-conserving surgery: a Danish Breast Cancer Cooperative Group study. Ann Surg Oncol. 2015;22:476–485.

[23] Olsson Å, Sartor H, Borgquist S, et al. Breast density and mode of detection in relation to breast cancer specific survival: a cohort study. BMC Cancer. 2014;14:229.

[24] Bodilsen A, Bjerre K, Offersen BV, et al. Importance of margin width in breast-conserving treatment of early breast cancer. J Surg Oncol. 2016;113:609–615.

[25] Tiong LU, Parkyn R, Walters D, et al. Dilemma in multifocal breast cancer - largest versus aggregate diameter. ANZ J Surg. 2011;81:614–618.

[26] Wolters R, Wöckel A, Janni W, et al. Comparing the outcome between multicentric and multifocal breast cancer: what is the impact on survival, and is there a role for guideline-adherent adjuvant therapy? A retrospective multicenter cohort study of 8,935 patients. Breast Cancer Res Treat. 2013;142:579–590.

[27] Joergensen LE, Gunnarsdottir KA, Lanng C, et al. Multifocality as a prognostic factor in breast cancer patients registered in Danish Breast Cancer Cooperative Group (DBCG) 1996-2001. Breast. 2008;17:587–591.

[28] Bendifallah S, Werkoff G, Borie-Moutafoff C, et al. Multiple synchronous (multifocal and multicentric) breast cancer: clinical implications. Surg Oncol. 2010;19:e115–e123.

[29] Ataseven B, Lederer B, Blohmer JU, et al. Impact of multifocal or multicentric disease on surgery and locoregional, distant and overall survival of 6,134 breast cancer patients treated with neoadjuvant chemotherapy. Ann Surg Oncol. 2015;22:1118–1127.

[30] Jawa RS, Anillo S, Huntoon K, et al. Interleukin-6 in surgery, trauma, and critical care part II: clinical implications. J Intensive Care Med. 2011;26:73–87.

[31] Retsky M, Demicheli R, Hrushesky WJM, et al. Reduction of breast cancer relapses with perioperative non-steroidal anti-inflammatory drugs: new findings and a review. Curr Med Chem. 2013;20:4163–4176.

[32] Demicheli R, Osaro E, Retsky M, et al. Protocol for a randomised, multicentre, double blinded phase III study of perioperative ketorolac in women of African descent with operable breast cancer. Jacobs J Intern Med. 2016;2:17.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 26–30
https://doi.org/10.1080/0284186X.2017.1400179

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Two years of tamoxifen or no adjuvant systemic therapy for patients with high-risk breast cancer: long-term follow-up of the Copenhagen breast cancer trial

Maj-Britt Jensen[a], Jens Fabricius Krarup[a], Torben Palshof†, Henning T. Mouridsen[a] and Bent Ejlertsen[a,b]

[a]Danish Breast Cancer Cooperative Group (DBCG), Secretariat and Statistical Office, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [b]Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: The Copenhagen Breast Cancer Trial (CBCT) randomly assigned patients with early breast cancer to two years of tamoxifen or placebo and we evaluated the effect over the following four decades.

Patient and methods: Between 1975 and 1978, 327 patients with primary breast cancer were randomly assigned to two years of daily placebo or tamoxifen. Survival statistics was collected from the Danish Civil Registration System.

Results: The five-year invasive breast cancer recurrence (BCR) rate was 43.2% in the placebo arm and 31.9% in the tamoxifen arm. Compared with the placebo arm the hazard ratio for a BCR event was 0.73 in the tamoxifen arm ($p = .07$). With an estimated median follow-up on overall survival of 40.9 years, 154 and 145 patients had died in the placebo and tamoxifen arm, respectively. After adjustment for baseline characteristics a significant reduction in mortality was obtained from tamoxifen (HR 0.79; $p = .04$).

Conclusion: Two years of adjuvant tamoxifen resulted in a sustained reduction in mortality in pre- and postmenopausal high-risk breast cancer patients with long-term follow-up data.

ARTICLE HISTORY
Received 14 September 2017
Accepted 24 October 2017

Introduction

In 1975, when the Copenhagen Breast Cancer Trial (CBCT) was initiated, the clinical and experimental experience of tamoxifen was rather limited. An association between estrogen receptor content and endocrine treatment effect was suggested in 1971 and the first report on the clinical effect of tamoxifen in advanced breast cancer was published the same year [1,2]. No published data from the adjuvant setting was accessible and just one other adjuvant tamoxifen trial was being initiated by McGuire and colleagues [3]. Several additional trials evaluating tamoxifen as an adjunct to local treatment of early breast cancer were launched in the late seventies including the DBCG 77C, NSABP B-09, and NATO trials [4–6]. The benefits of tamoxifen in the adjuvant setting was first reviewed by Mouridsen and Palshof in 1983 and later confirmed at the Consensus Development Conference in 1985 as well as the first EBCTCG meta-analysis published in 1988 [7–9].

Tamoxifen has been shown to reduce the risk of recurrence beyond the duration of treatment and there is evidence supporting that this 'carry-over' benefit persists for at least 10 years after completion of 5 years of tamoxifen [10–12]. Moreover, a carry-over benefit persisting beyond 25 years has been suggested following only one or two years of tamoxifen [13,14]. The aim of the this study was to follow the effect of two years of tamoxifen over 40 years which was expected to be the life span for most patients who were postmenopausal at diagnosis.

Patient and methods

The Copenhagen Breast Cancer Trial (CBCT), was a double-blind placebo-controlled phase-3 randomized trial. Identical looking tamoxifen and placebo tablets were provided by Imperial Chemical Industries (ICI), the sponsor of the trial. Patients were randomized to tamoxifen (10 mg) or identical looking placebo and both were administered three times daily. Randomization was stratified according to menopausal status. The organization of CBCT has previously been described in detail [15].

Patients

CBCT included women who achieved complete resection of an invasive unilateral adenocarcinoma of the breast by simple mastectomy (without axillary dissection) and subsequently received adjuvant radiotherapy to the chest wall and regional lymph nodes (40.92 Gy in 22 fractions, 5 fractions

CONTACT Maj-Britt Jensen mj@dbcg.dk Danish Breast Cancer Cooperative Group Secretariat, Building 2501 Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark
†Deceased.

per week) as specified in the preceding CBCT trial [16]. Patients were required to be without evidence of advanced disease by physical examination, radiography of the chest and bone scintigraphy and without previous or concomitant thromboembolic disease, chronic hepatitis, clinically significant or untreated hypertension or heart disease and malignant disease.

Estrogen receptor status

The ER content of the primary tumor was measured in histologically verified tumor tissue by a dextran-coated charcoal assay in a single laboratory (Johan Daehnfeldt). Tumors with at least 10 fmol/mg cytosol protein were classified as ER positive. A later re-evaluation revealed a suboptimal sensitivity and tumors originally classified as ER negative might have been weakly ER positive [17].

Follow-up

Treatment-related adverse events and findings on clinical and laboratory examination were recorded every 3 months the first two years and then every 6 months for the next three years. Bone scintigraphy was done yearly and radiography of the chest and contralateral breast was done every 18 months. The Danish Civil Registration System (CRS) contains continuously updated information on vital status and emigrations and through linkage to CRS by each participant's unique civic registration number a complete follow-up until 1 August 2017 was retrieved on survival [18].

Statistical analysis

The statistical office of the DBCG undertook a central review and analysis of data. The primary endpoint of the original study was time to breast cancer recurrence (BCR) defined as time from randomization to any first event of invasive ipsi- or contralateral breast recurrence, local or regional invasive recurrence or distant recurrence. As inclusion in this study was completed 39 years ago, we as the primary endpoint chose overall survival (OS) defined as time from randomization to death irrespective of cause of death. Follow-up time was quantified in terms of a Kaplan–Meier estimate of potential follow-up [19].

OS was analyzed unadjusted by the Kaplan–Meier method and groups were compared using the log-rank test. Cumulative incidence of BCR was analyzed with death without recurrence as competing event. Expected survival was calculated by applying age and calendar year specific female mortality figures of the general Danish population to the corresponding person-years of the patient cohort. Hazard ratios [HR] were estimated from the Cox proportional hazards regression model (OS) and the Fine-Gray proportional hazards subdistribution model (BCR) to quantify the effect of treatment regimen and to explore interactions. Interactions between treatment and the covariates were investigated in separate models. The assumptions of proportional hazards were assessed by Schoenfeld residuals, and by including a time-dependent component in the model. Associations between treatment regimen and other characteristics were analyzed by chi-square. p values are two-tailed. Statistical analyses were done with the statistical software SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Table 1. Patient and tumor characteristics.

	Tamoxifen		Placebo		
	N	%	*N*	%	*p* value
Total	164		153		
Age					.19
≤40	26	16	13	9	
41–50	48	29	50	33	
51–60	56	34	50	33	
61–70	34	21	40	26	
Menopausal status					.72
Premenopausal	77	47	65	42	
Perimenopausal	35	21	36	24	
Postmenopausal	52	32	52	34	
Estrogen receptor					.59
Positive	62	38	65	42	
Negative	39	24	30	20	
Unknown	63	38	58	38	
Stage					.33
Stage 1	54	33	39	25	
Stage 2	67	41	67	44	
Stage 3	43	26	47	31	

Ethics and role of sponsor

The funders designed the study and were responsible for data collection. The DBCG statistical office was responsible for linking to the CRS, the final decision regarding manuscript content and submission. Placebo or tamoxifen (Nolvadex®) 10 mg (Imperial Chemical Industries (ICI), UK) was taken orally, three times daily continuously for two years.

Results

CBCT was open from March 1975 through March 1978, and this analysis was conducted 39 years after closure of recruitment. Median estimated potential follow-up was 6.3 years for BCR and 40.9 years for OS. Among 317 participants, 164 were randomized to tamoxifen and 153 to placebo, and patient characteristics are shown in Table 1. Information on ER content was available from 196 patients and 127 (65%) were classified as ER positive.

A total of 133 events of local- or distant BCR were observed during the clinical follow-up and Figure 1(A) shows the cumulative incidence curves for BCR. In the intent to treat (ITT) analysis ($N = 317$), the overall unadjusted HR for BCR in the tamoxifen group compared with the placebo group was 0.73; 95% confidence interval [CI] 0.52 to 1.02; $p = .07$. When adjusting for baseline characteristics, including ER, stage and menopausal status, the benefit of tamoxifen remained unchanged (adjusted HR 0.74; CI 0.53–1.05; $p = .09$). BCR remained largely unchanged following exclusion of ER-negative patients (adjusted HR 0.72; CI 0.49–1.08), and Figure 1(B) shows the CI-curves for patients with ER positive or unknown tumors.

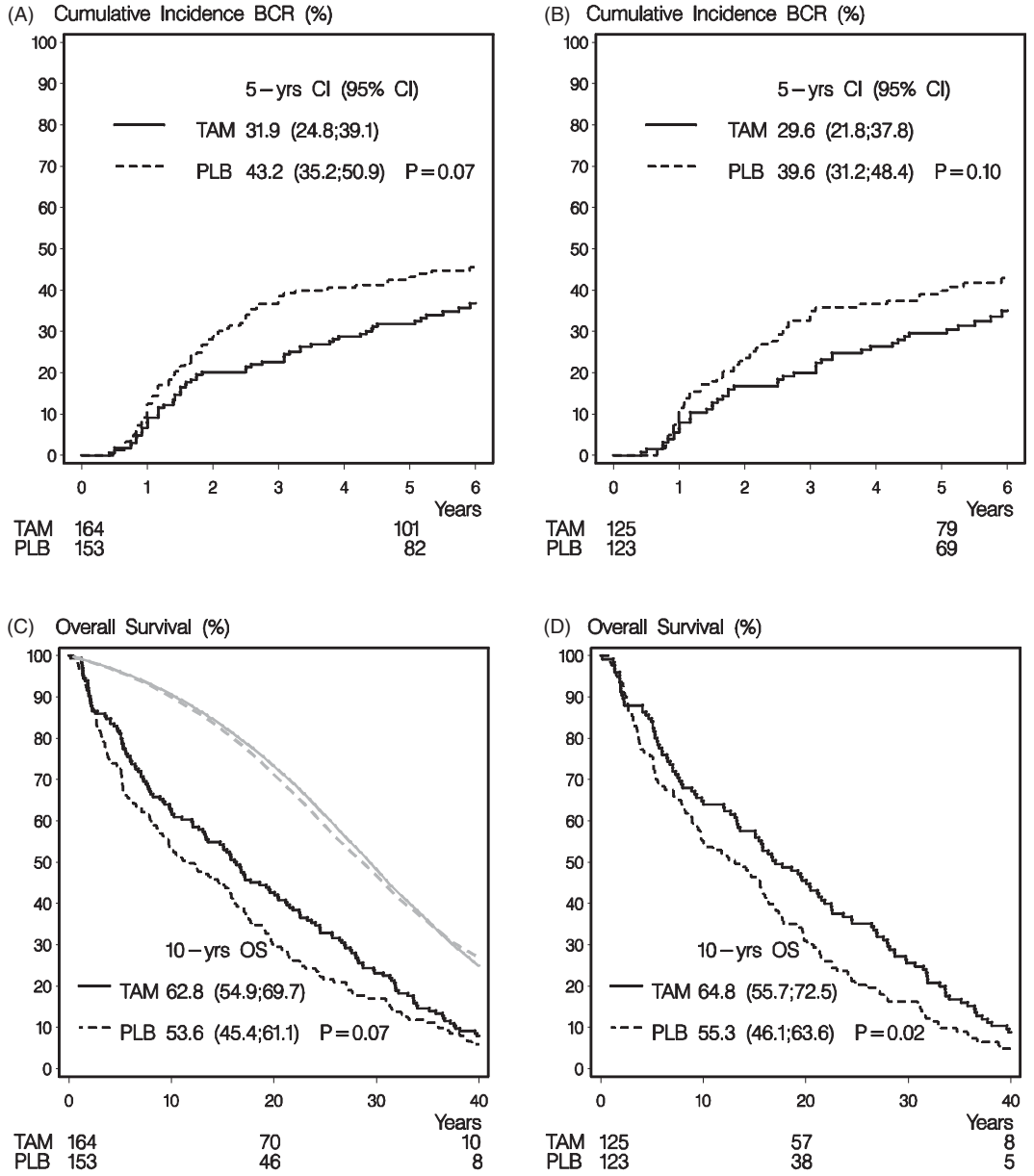


Figure 1. Panel A shows cumulative incidence for Breast Cancer Recurrence (BCR) of the 317 patients included in the intent to treat analysis who were randomly allocated to tamoxifen or placebo. Panel B shows the estimates in patients with confirmed ER positive or ER unknown tumors. Panel C shows the Kaplan–Meier estimates of overall survival of the 317 patients included in the intent to treat analysis who were randomly allocated to tamoxifen or placebo. The gray curves show the expected survival for the 317 patients, applying mortality figures of the general Danish population. Panel D shows the Kaplan–Meier estimates of overall survival in patients with confirmed ER positive or ER unknown tumors. Number of patients at risk are given below x-axes.

By data cutoff, 299 (94%) of the 317 patients had died and 154 and 145 deaths occurred in the placebo and tamoxifen group, respectively. The bottom lines in Figure 1(C) shows a trend towards a reduction in survival from tamoxifen (HR 0.81; CI 0.65–1.07; $p = .07$). After adjustment for baseline characteristics, a significant survival benefit was obtained from tamoxifen in the ITT population (adjusted HR 0.79, CI 0.63–0.99; $p = .04$). For comparison, the survival is in the two upper curves shown for age-matched women in the entire Danish population (Figure 1(C)). The effect of tamoxifen remained largely unchanged by the exclusion of ER negative patients (adjusted HR 0.72; CI 0.56–0.94) as shown in Figure 1(D). As shown in Figure 2, we found no statistical evidence of heterogeneity on the effect of tamoxifen from menopausal status, ER status or stage.

Discussion

This is the longest follow-up ever of an adjuvant tamoxifen trial, and the sustained reduction in mortality from two years of tamoxifen shown in this 40-year analysis of the Copenhagen Breast Cancer Trial has important implications. In particular the long-lasting survival benefit from two years of tamoxifen confirms that patients with pronounced side effects from endocrine treatment can be reassured that even

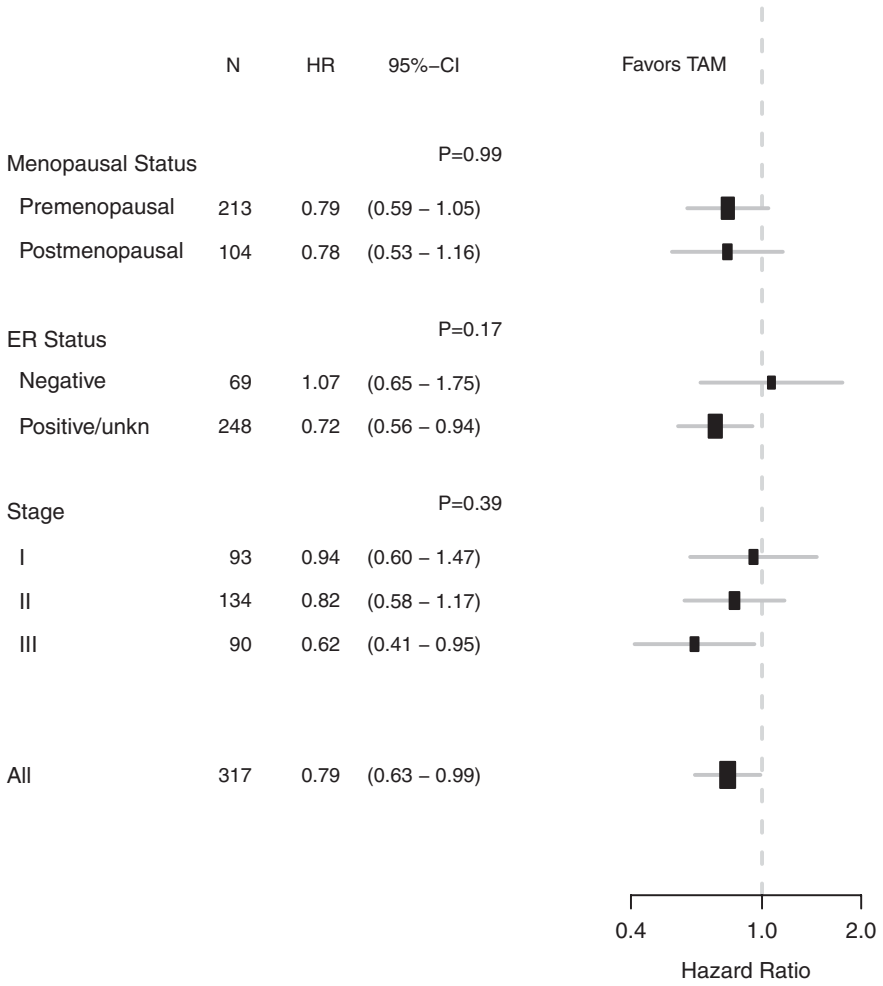


Figure 2. The forest plot illustrates exploratory subgroup analysis of overall survival according to menopausal status, ER status and Stage. Hazard ratios (HRs) refer to adjusted estimates obtained in the multivariate analysis of the intent-to-treat population. CI indicates 95% confidence interval. *p*-values are for test of heterogeneity of treatment effect.

a couple of years of treatment are worthwhile. The reduction in mortality appeared in pre- and postmenopausal patients but may not include patients with ER negative tumors.

This study has several strengths, including its placebo-controlled design with provision of study drugs free of charge. Moreover the importance of achieving local control of breast cancer was already well recognized in the seventies, and as a result of the first Copenhagen breast cancer trial, patients in this study received a mastectomy followed by radiotherapy [16,20]. At the time of enrollment into CBCT patients already had been assigned a unique 10-digit civic registration number, and we obtained a complete follow-up on survival from the CRS who ever since on a daily basis has updated information on migration and vital status [18].

CBCT only included 317 patients and the small sample size limited our ability to evaluate the benefit from tamoxifen in subgroups. Several other limitations should be taken into account when interpretation the results of this study. First, the clinical work-up only continued for eight years from inclusion of the first patient leaving a somewhat short follow-up for breast recurrences. Second, tamoxifen was in CBCT only given for two years and an incremental benefit would be achievable by continuation for five or even 10 years. Third, a further benefit can be achieved in postmenopausal women from substituting tamoxifen with an aromatase inhibitor or giving tamoxifen and an aromatase inhibitor in a sequence [21,22]. Fourth, tissue specimens for hormone receptor assays were not available from about 40% of the patients randomly assigned to treatment in CBCT. By todays standard a low availability, but it must be taken into account that the possibilities to predict anti-estrogen treatment only was hypothesized when the CBCT began [23]. Fifth, the standards for the biochemical hormone receptor test used in this study was revised along the way and finalized after recruitment was completed [24,25]. Finally, long term and close to lifelong follow-up is inevitably associated with non-proportional hazards. A closer investigation of the carryover effect could potential be possible by dividing the follow-up period in intervals, but this is in the current study prohibited by the small sample size of our study [26].

In conclusion, two years of tamoxifen improves outcome in high-risk breast cancer patients. The impact on mortality is long-lasting implying that tamoxifen possess cytotoxic ability. This study confirms that the same degree of benefit seems to be achieved in pre- as well as in postmenopausal breast

cancer patients but the benefit may be restricted to patients with estrogen receptor positive breast cancer.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Jensen EV, Block GE, Smith S, et al. Estrogen receptors and breast cancer response to adrenalectomy. Natl Cancer Inst Monogr. 1971;34:55–70.

[2] Cole MP, Jones CT, Todd ID. A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. Br J Cancer. 1971;25:270–275.

[3] Hubay CA, Pearson OH, Marshall JS, et al. Adjuvant chemotherapy, antiestrogen therapy and immunotherapy for stage II breast cancer: 45-month follow-up of a prospective, randomized clinical trial. Cancer. 1980;46(12 Suppl):2805–2808.

[4] Fisher B, Redmond C, Brown A, et al. Treatment of primary breast cancer with chemotherapy and tamoxifen. N Engl J Med. 1981;305:1–6.

[5] Rose C, Thorpe SM, Mouridsen HT, et al. Antiestrogen treatment of postmenopausal women with primary high risk breast cancer. Breast Cancer Res Tr. 1983;3:77–84.

[6] Baum M, Brinkley DM, Dossett JA, et al. Improved survival among patients treated with adjuvant tamoxifen after mastectomy for early breast cancer. Lancet. 1983;2:450.

[7] Mouridsen HT, Palshof T. Adjuvant systemic therapy in breast cancer; a review. Eur J Cancer Clin Oncol. 1983;19:1753–1770.

[8] Consensus conference. Adjuvant chemotherapy for breast cancer. JAMA. 1985;254:3461–3463.

[9] Early Breast Cancer Trialists' Collaborative Group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. N Engl J Med. 1988;319:1681–1692.

[10] Stewart HJ, Prescott RJ, Forrest AP. Scottish adjuvant tamoxifen trial: a randomized study updated to 15 years. J Natl Cancer Inst. 2001;93:456–462.

[11] Rutqvist LE, Johansson H. Stockholm Breast Cancer Study Group. Long-term follow-up of the randomized Stockholm trial on adjuvant tamoxifen among postmenopausal patients with early stage breast cancer. Acta Oncol. 2007;46:133–145.

[12] Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, Gray R, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:771–784.

[13] Knoop AS, Laenkholm AV, Jensen MB, Danish Breast Cancer Cooperative Group, et al. Estrogen receptor, Progesterone receptor, HER2 status and Ki67 index and responsiveness to adjuvant tamoxifen in postmenopausal high-risk breast cancer patients enrolled in the DBCG 77C trial. Eur J Cancer. 2014;50:1412–1421.

[14] Ekholm M, Bendahl PO, Fernö M, et al. Two years of adjuvant tamoxifen provides a survival benefit compared with no systemic treatment in premenopausal patients with primary breast cancer: long-term follow-up (>25 years) of the Phase III SBII:2pre trial. JCO. 2016;34:2232–2238.

[15] Palshof T, Mouridsen HT, Daehnfeldt JL. Adjuvant endocrine therapy of primary operable breast cancer. Report on the Copenhagen breast cancer trial. In: Mouridsen H T and Palshof T, editors. Breast cancer–experimental and clinical aspects. Oxford (UK): Pergamon Press; 1979. p. 183–8.

[16] Kaae S, Johansen H. Simple mastectomy plus postoperative irradiation by the method of McWhirter for mammary carcinoma. Ann Surg. 1969;170:895–899.

[17] Thorpe SM, Poulsen HS, Pedersen KO, et al. Quality control of receptor analyses of breast cancer tissue in Denmark. Acta Oncol. 1988;27:621–625.

[18] Schmidt M, Pedersen L, Sørensen HT. The Danish civil registration system as a tool in epidemiology. Eur J Epidemiol. 2014;29:541–549.

[19] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17:343–346.

[20] EBCTCG (Early Breast Cancer Trialists' Collaborative Group), McGale P, Taylor C, Correa C, et al. Effect of radiotherapy after mastectomy and axillary surgery on 10-year recurrence and 20-year breast cancer mortality: meta-analysis of individual patient data for 8135 women in 22 randomised trials. Lancet. 2014;383:2127–2135.

[21] BIG 1-98 Collaborative Group, Mouridsen H, Giobbie-Hurder A, Goldhirsch A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med. 2009;361:766–776.

[22] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet. 2015;386:1341–1352.

[23] Horwitz KB, McGuire WL, Pearson OH, et al. Predicting response to endocrine therapy in human breast cancer: a hypothesis. Science. 1975;189:726–727.

[24] EORTC Breast Cancer Co-operative Group. Revision of the standards for the assessment of hormone receptors in human breast cancer; report of the second E.O.R.T.C. Workshop, held on 16-17 March, 1979, in the Netherlands cancer institute. Eur J Cancer. 1980;16:1513–1515.

[25] Koenders A, Thorpe SM. Standardization of steroid receptor assays in human breast cancer–I. Reproducibility of estradiol and progesterone receptor assays. Eur J Cancer Clin Oncol. 1983;19:1221–1229.

[26] Dignam JJ, Dukic V, Anderson SJ, et al. Hazard of recurrence and adjuvant treatment effects over time in lymph node-negative breast cancer. Breast Cancer Res Treat. 2009;116:595–602.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 31–37
https://doi.org/10.1080/0284186X.2017.1401228

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Neoadjuvant letrozole for postmenopausal estrogen receptor-positive, HER2-negative breast cancer patients, a study from the Danish Breast Cancer Cooperative Group (DBCG)

Signe Korsgaard Skriver[a], Anne-Vibeke Laenkholm[b], Birgitte Bruun Rasmussen[c], Jürgen Handler[d], Bo Grundtmann[e], Tove Filtenborg Tvedskov[f], Peer Christiansen[g], Ann S. Knoop[a], Maj-Britt Jensen[h] and Bent Ejlertsen[a,h]

[a]Department of Oncology, Rigshospitalet, Copenhagen, Denmark; [b]Department of Surgical Pathology, Zealand University Hospital, Slagelse, Denmark; [c]Department of Pathology, Herlev Hospital, Herlev, Denmark; [d]Department of Breast Surgery, Sygehus Soenderjylland, Aabenraa, Denmark; [e]Department of Breast Surgery, Ringsted Sygehus, Ringsted, Denmark; [f]Department of Breast Surgery, Rigshospitalet, Copenhagen, Denmark; [g]Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark; [h]Danish Breast Cancer Cooperative Group Secretariat, Rigshospitalet, Copenhagen, Denmark

ABSTRACT

Introduction: Neoadjuvant endocrine treatment (NET) is a low-toxicity approach to achieve operability in locally advanced breast cancer, and to facilitate breast conservation in early breast cancer, particular in patients with highly estrogen receptor (ER) positive and HER2-negative disease. Here, we report the results obtained by neoadjuvant letrozole in patients with early breast cancer in a phase-II design.

Material and methods: A total of 119 postmenopausal women with ER-positive, HER2-negative operable breast cancer were assigned to four months of neoadjuvant letrozole before definitive surgery. Sentinel node or diagnostic fine needle aspiration cytology procedure was performed prior to treatment and the women were assessed prior, at two months, and before surgery with clinical examination, mammography and ultrasonography. Surgical specimens were examined for pathological response. Primary outcome was pathological and clinical response.

Results: The per protocol population consisted of 112 patients. Clinical response was evaluated in 109 patients and pathological response in 108. Overall a mean decrease in tumor size was 15% ($p \leq .0001$). One patient had complete pathological response and 55% of patients had partial pathological response. ER at 100%, ductal subtype, tumor size below 2 cm and lymph node–negative status was significantly associated with a better response to NET and malignancy grade 3 with a poorer response to NET. One patient progressed during treatment and received neoadjuvant chemotherapy. Eight patients received adjuvant chemotherapy due to lack of response.

Conclusion: Neoadjuvant aromatase inhibitor therapy is an acceptable strategy in selected postmenopausal patients with ER-rich and HER2-negative early breast cancer with ductal histology and should be considered when chemotherapy either isn't indicated or feasible.

ARTICLE HISTORY
Received 15 September 2017
Accepted 30 October 2017

Introduction

Neoadjuvant endocrine treatment (NET) is an low-toxicity approach to achieve operability in locally advanced breast cancer, particular in patients with highly estrogen receptor (ER) positive and HER2-negative disease [1]. NET is furthermore increasingly used in patients with earlier-stage operable breast cancer for down staging to allow a less mutilating surgery; and as an research tool to obtain prognostic and predictive information using tumor response [2]. Third generations aromatase inhibitors, such as letrozole, are in postmenopausal women preferred over receptor modulators such as tamoxifen due to higher response rates [3–5]. Pathological complete response (pCR) has been the most commonly used endpoint in neoadjuvant trials, but a low pCR rate in ER-positive breast cancer has together with variable defined pCR criteria made the use of pCR challenging in patients with ER-positive breast cancer [2]. A pCR following neoadjuvant chemotherapy (NCT) is associated with decreased mortality, but has not been validated as a surrogate endpoint for event-free or overall survival [6]. Primary surgery continues to be the standard in patients with ER-positive and HER2-negative breast cancer and change in standard practice towards increasing use of NET will demand definitive survival data from phase-III trials comparing NET to adjuvant treatment or to NCT.

The Danish Breast Cancer Cooperative Group (DBCG) [7] set up a phase-III trial comparing adjuvant letrozole for five years with neoadjuvant letrozole for four months combined with adjuvant letrozole to a total of five years following stable disease or response and combined with adjuvant

CONTACT Signe Korsgaard Skriver signe.korsgaard.skriver@regionh.dk Department of Oncology, section 5073, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Supplemental data for this article can be accessed here.

chemotherapy following progressive disease. The original phase-III design was abandoned due to slow accrual and the trial was converted to a single arm phase-II trial. Here, we report the pathological and clinical results obtained by four months of neoadjuvant letrozole.

Material and methods

Study design

Initiated in 2009 the study was designed as a randomized phase-III study, at nine institutions in Denmark. In brief, at time of study initiation the primary study objective was to assess if letrozole was superior to surgery as primary therapy for early-stage ER-positive breast cancer in postmenopausal women. Eligible patients were randomized to definitive surgery followed by adjuvant letrozole for five years against letrozole for four months before definitive surgery followed by adjuvant letrozole to a total of five years in patients with responsive or stationary tumors. Patients with tumor progression should be considered for adjuvant chemotherapy.

On 10th October 2010, key changes were made due to poor accrual and the modified design allowed recruitment to neoadjuvant letrozole for four months in a phase-II study without randomization.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients gave written informed consent. The study and later amendment was approved by the National Committee on Health Research Ethics. The trial is registered on the ClinicalTrials.gov website NCT00908531.

Patients

Postmenopausal women with histological confirmed, invasive ER-positive, HER2-negative, operable breast cancer were eligible for the study. Eligible patients meet the following criteria: tumor size $\geq$1 cm, age $\geq$60 years, Eastern Cooperative Oncology Group score 0–2 and Charlson comorbidity index 0–2. Patients with prior cytotoxic treatment including aromatase inhibitors and patients with prior malignant disease were excluded. Patients was registered in the DBCG database and updated prospectively.

Treatment

Patients were treated with neoadjuvant letrozole 2.5 mg daily for four months. Treatment was discontinued if disease progression was suspected on ultrasonography, in case of severe toxicity, or if the patient withdrew consent.

Assessment

Patients underwent tumor evaluation upon study entry, after two months, and prior to surgery consisting of breast palpation, mammography and ultrasonography. Blood samples and core biopsies from the tumor along with the sentinel node (SN) procedure or diagnostic fine needle aspiration cytology (FNAC) from axillary lymph nodes were obtained before initiation of trial medication.

Surgery

After the neoadjuvant period, patients underwent mastectomy or breast conserving surgery. In case of lymph node involvement diagnosed prior to NET by SN or FNAC or in cases with progressive disease, patients were reassessed with SN prior to surgery. Patients who were initial lymph node positive underwent axillary lymph node dissection (ALND) irrespective of SN status prior to surgery and likewise in all cases with positive SN prior to surgery regardless of initial axillary status.

Endpoints

Following the amendment clinical response and pathological response, the original secondary endpoints, became the primary endpoints. Clinical response was assessed by ultrasonography and according to RECIST 1.1 defined as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [8] and furthermore evaluated on a continues scale of relative tumor reduction from baseline until surgery. Pathological response was defined as loss of tumor cells $\geq$30% according to a modified Miller–Payne scale used by DBCG [9]. On the modified scale response grade 1 equals no invasive tumor cells present; pathological complete response (pCR). Grade 2 more than 90% loss of tumor cells and grade 3 between 30% and 90% reduction in tumor cells are considered partial response. Grade 4 is defined as less than 30% loss of tumor cells and is considered no response.

Biomarkers was assessed centrally using international standards [10–12]. The percentage of ER-positive cells by nuclear staining was registered and ER-positive status was defined as nuclear staining $\geq$10%. HER2-positive status was defined as HER2 3 + staining or HER2 gene amplification (ratio gen/cen $\geq$2) by FISH. In cases with multiple testing, the assessment with the highest count of ER, progesterone receptor (PgR) and Ki67 was used. In cases lacking central review or central review failed due to lack of tumor tissue, local assessment was used in the analysis.

Statistical analyses

Each factor was analyzed by univariate logistic regression to evaluate the association between the variable and response to NET. Factors were included in univariate models both categorical and continuously to investigate the functional form. Unknowns were included in separate categories. Odds ratio (OR) was estimated with a 95% confidence interval, using the category with highest number of patients as reference group, except for PgR to align it with ER. Multivariate analyzes including all characteristics were applied to assess the adjusted odd ratios. Association with outcome were tested with Pearson's Chi-squared test, unknowns were excluded. Difference in relative tumor change was tested with the Wilcoxon Rank Sum test. Tumor change during NET was

tested with a paired *t*-test. The distribution of ER, PgR and Ki67 did not meet the assumption of normality, and due to their heavy-tailed distribution the sign test was chosen to test for changes during NET. Level of significance was set to 5%. No new power calculations were made for the altered primary endpoints when the study was converted to a single arm study. All analyses performed with SAS Enterprise Guide version 7.11 (Cary, NC, USA).

Results

Between July 2009 and November 2012, a total of 119 patients were registered to receive letrozole, hereof 64 patients in the phase-III and 55 in the phase-II part. Two patients withdrew consent and two were tested HER2-positive after randomization, but before study initiation, thus 115 patients constituted the intention-to-treat population. An additional two patients were after initiation of letrozole diagnosed with a HER2-positive tumor and one was diagnosed with primary lung cancer and discontinued letrozole early and were excluded from the per-protocol population ($n = 112$), supplementary figure A. Patient's basic characteristics are summarized in Table 1.

In total, 111 (99%) patients completed four months of neoadjuvant letrozole as planned, one patient discontinued letrozole following progressive disease at the two months checkup.

Clinical response

Clinical response to letrozole was available for 109 patients. Two had CR (2%), 36 had PR (33%), 57 had SD (52%) and 14 had PD (13%). Concerning changes in tumor size 63 (58%) had a 1 to 75 mm reduction in tumor size (mean 8.1 mm), 20 (18%) had an increase of 1–20 mm (mean 6.7 mm), and 26 (24%) had no change. Correspondingly, the relative changes for the 63 patients were a mean decrease in tumor size of 35% (2–100%). Twenty had a mean increase of 31% (3–75%). Overall, neoadjuvant letrozole lead to a mean decrease in tumor size of 15% (CI: 8–21%; $p \leq .0001$) (Figure 1).

Pathological response

Pathological tumor response was available for 108 patients. One patient (0.9%) had pathological complete response (grade 1), seven (6%) had minimal residual disease (grade 2), 52 (48%) had moderate residual disease (grade 3), and 48 patients (44%) had no response (grade 4).

Fourteen patients were clinical node positive ($n = 14$) verified by FNAC, and 37 of the 97 who had a SN biopsy prior to NET were classified as SN positive, while 60 were classified as SN negative. One patient was not staged prior to treatment (Table 1). Of the 51 node-positive patients, 22 underwent a second SN prior to surgery and hereof 20 continued to ALND, 27 underwent ALND only and two were neither restaged nor underwent ALND. Of the 51 patients with initial lymph node involvement, 35 were verified as lymph node positive at time of surgery.

Table 1. Patients- and tumor characteristics of 112 Danish early Breast Cancer patients treated with neoadjuvant letrozole between 2009 and 2012.

	Patients ($n = 112$)	
Variable	*n*	%
Age (years)		
60–69	66	59
70–89	46	41
Tumor size (mm)[a]		
<20	50	45
>20	62	55
Histological subtype		
Ductal invasive	82	73
Other invasive[b]	30	27
Malignancy grade[c]		
1	42	45
2	47	50
3	5	5
Axillary node status prior to treatment[d]		
Negative	60	54
Positive	51	46
Unknown	1	1
Estrogen receptor status (%)		
10–99	19	17
100	93	83
Progesterone receptor status (%)		
10–99	70	63
100	29	26
Unknown	13	12
Ki67 index (%)		
<14	62	55
≥14	27	24
Unknown	23	21

Data reported as *n* (col %).
[a]Range of tumor size: 11–100 mm.
[b]lobular tumors $n = 12$, Mucinous carcinomas $n = 8$, Tubular carcinomas $n = 2$, Medullary carcinomas $n = 1$, not specified $n = 7$.
[c]$n = 94$ (only lobular and ductal tumors graded).
[d]Axillary status assessed by either diagnostic fine needle aspiration cytology (FNAC) or sentinel node (SN), one patient had earlier breast surgery rendering both SN and FNAC impossible.

Of the 20 patients with progressive disease, 12 were initial lymph node positive and are included above. The remaining eight were initially node negative, five were restaged prior to surgery with SN, and were all still node negative and three were not restaged nor underwent ALDN. SN was removed prior to NET and none of 14 clinical node positive patients obtained a pCR in the axillary lymph nodes.

Biomarker response

ER status ($n = 110$) changed from mean 96 (10–100) to mean 93 (1–100), mean difference 3%, $p = .01$. PgR status ($n = 51$) changed from mean 56 (0–100) to 17 (0–100), mean difference 39%, $p \leq .0001$. Ki67 index ($n = 87$) changed from mean 14% (0–90%) to 8% (1–95%), mean difference 6%, $p \leq .01$. Two patients (1.8%) were reclassified as HER2-positive after the neoadjuvant period.

Factors associated with clinical and pathological response

The ORs for clinical response and pathological response and their association to tumor size, histological tumor type, grade, axillary status, Ki67, ER and PgR are summarized in Table 2. ER positivity at 100% was significantly associated with tumor reduction, however, not to pathological response. Tumors smaller than 20 mm had a better response both clinically and pathological than tumors above 20 mm. Ductal tumors had a

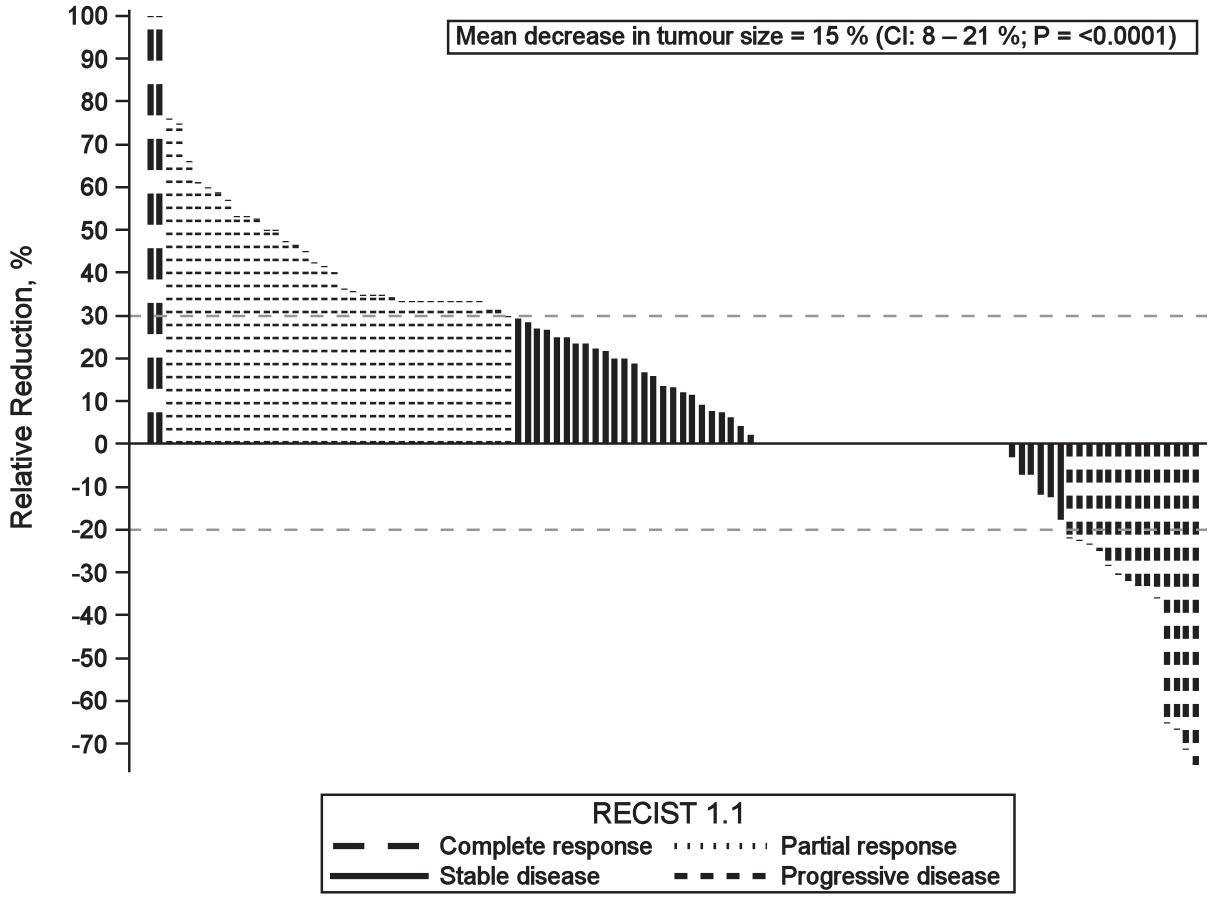


Figure 1. Relative reduction (%) in tumor size for each of the 109 patients with records of clinical outcome (one bar per patient). No change ($n = 26$) visualized by a straight line, negative values symbolizes growth ($n = 20$) and positive values tumor reduction ($n = 63$). Groups represent clinical response according to RECIST 1.1; Complete response ($n = 2$), Partial response ($n = 36$), Stable disease ($n = 57$) and Progressive disease ($n = 14$).

significantly better pathological response to treatment than the other invasive subtypes, and node negative patients had a better clinical response. Malignancy grade 3 was associated with poorer response to NET than malignancy grade 1 and 2. None of the other tested variables were statistically significantly associated with neither clinical nor pathological outcome. When explanatory factors were tested as continuously they did not provide the model with a significant better fit and when tested in multivariate analyzes odds ratios were not significantly altered (data not shown). Neither changes in biomarkers nor clinical response did significantly associate with pathological response (Table 3).

Association between clinical and pathological response

Assessment of both clinical and pathological outcome was done in 106 patients. Thirty-nine patients (37%) had both clinical and pathological response. Nineteen (18%) had pathological response, but no reduction in the tumor size evaluated by ultrasonography. Twenty-four (23%) had ultrasonic regression, but no pathological response, and 24 patients (23%) had neither clinical nor pathological response.

Twenty of the 106 (19%) patient had an increase in tumor size on ultrasonography, 10 of them had pathological response. OR for pathological response if growth seen on ultrasonography is 0.62 (CI: 0.22–1.70), and for no change the OR is 0.4 (CI: 0.15–1.05) $p = .16$. Corresponding OR for clinical response according to RECIST 1.1; OR for pathological response if PD is 0.29 (0.08–1.04) and for SD 0.56 (0.24–1.32), $p = .14$ (Table 3).

Adjuvant treatment

In the adjuvant setting, eight (7%) patients received adjuvant chemotherapy and four of these resumed endocrine treatment after completion of chemotherapy. Adjuvant chemotherapy was justified by absence of a pathological response combined with clinically stabile or progressive disease (seven patients) or a mixed response with clinical but no pathological response (one patient). Endocrine treatment was continued without chemotherapy in 103 (92%) patients and one patient did not receive any adjuvant systemic treatment. In one patient, adjuvant tamoxifen was initiated following progression during NET and neoadjuvant chemotherapy. This patient developed bone metastasis within one year of the initial diagnosis. Adjuvant treatment regimens are shown in supplementary figure A and B according to clinical and pathological response.

Table 2. Univariate analyses for factors associated with clinical and pathological outcome after neoadjuvant treatment with letrozole in early breast cancer patients.

	Clinical response[a]				Relative tumorreduction[c]		Pathological Response			
Variable	n[e]	%	Odds ratio (95% CI)	p[b]	Median	p[d]	n[f]	%	Odds ratio (95% CI)	p[b]
Age (years)				.10		.31				.51
60–69	27	41	1.00		19		35	53	1.00	
70–89	11	26	0.50 (0.21–1.15)		7		25	60	1.30 (0.60–2.85)	
Tumor size (mm)				.04		.04				.06
<20	22	46	2.38 (1.06–5. 32)		27		32	65	2.08 (0.96–4.54)	
≥20	16	26	1.00		2		28	47	1.00	
Subtype				.72		.09				.03
Ductal	28	36	1.00		19		48	62	1.00	
Other invasive	10	32	0.85 (0.35–2.06)		0		12	39	0.38 (0.16–0.89)	
Malignancy grade				.98		.006				.36
1	14	39	1.09 (0.43–2.79)		23		21	64	0.94 (0.36–2.47)	
2	14	37	1.00		16		26	65	1.00	
3	0	0	<0.001		−27		1	25	0.18 (0.02–1.90)	
Axillary node status prior to treatment				.002		.01				.99
Negative	28	47	1.00		25		32	55	1.00	
Positive	9	18	0.25 (0.10–0.60)		0		27	55	1.00 (0.46–2.14)	
Unknown	1	100	–		33		1	100	–	
Estrogen receptor status (%)				.17		.02				.19
10–99	4	21	0.44 (0.14–1.43)		0		8	42	0.52 (0.19–1.41)	
100	34	38	1.00		18		52	58	1.00	
Progesterone receptor status (%)				.49		.21				.75
0–99	22	33	0.65 (0.26–1.61)		9		35	52	0.75 (0.30–1.86)	
100	12	43	1.00		23		16	59	1.00	
Unknown	4	33	0.67 (0.16–2.74)		0		9	75	2.06 (0.48–9.39)	
Ki67 index (%)				.36		.17				.09
0–13	24	40	1.00		19		34	57	1.00	
≥14	8	30	0.63 (0.24–1.67)		0		10	37	0.45 (0.18–1.14)	
Unknown	6	27	0.56 (0.19–1.64)		8		16	76	2.45 (0.79–7.55)	

Data reported as n (row %).
[a]Clinical response = Partial response and Complete response according to RECIST 1.1.
[b]Pearson's Chi square.
[c]Relative reduction (%) from baseline till surgery.
[d]Wilcoxon Rank Sum.
[e]total n evaluated = 109.
[f]total n evaluated = 108.

Table 3. Association between biomarker change and clinical tumor change to pathological outcome after neoadjuvant treatment with letrozole in early breast cancer patients.

	Total		Pathological response			
Variable	n	% col	n	% row	Odds ratio (95% CI)	p value[a]
Estrogen Receptor						.55
Increase	10	9	4	40	0.62 (0.14–2.72)	
Decrease	25	23	13	52	1.00	
No change	71	65	41	58	1.26 (0.51–3.15)	
Missing	2	2	2	100	–	
Progesterone Receptor						.30
Increase	5	5	2	40	0.32 (0.05–2.19)	
Decrease	34	32	23	68	1.00	
No change	7	7	3	43	0.36 (0.07–1.89)	
Missing	59	56	31	53	0.53 (0.22–1.28)	
KI67 index						.23
Increase	24	22	9	38	0.53 (0.20–1.44)	
Decrease	49	45	26	53	1.00	
No change	12	11	8	67	1.77 (0.47–6.66)	
Missing	23	21	17	74	2.51 (0.85–7.43)	
Relative tumor change						.16
Increase	20	19	10	50	0.62 (0.22–1.70)	
Decrease	63	59	39	62	1.00	
No change	23	22	9	39	0.40 (0.15–1.05)	
Response according to RECIST 1.1						.14
Progressive disease	14	13	5	36	0.29 (0.08–1.04)	
Complete or partial response	38	36	25	66	1.00	
Stable disease	54	51	28	52	0.56 (0.24–1.32)	

[a]Excluding unknowns.

Discussion

Our study shows that neoadjuvant aromatase inhibitor therapy in selected postmenopausal patients with ER-rich and HER2-negative early breast cancer leads to a modest clinical and pathological result in around half of the patients. Overall, only a 15% decrease in mean tumor size was achieved. One single patient (<1%) achieved a complete pathological response and overall 55% had partial pathological response. On the other hand, only one patient experienced progressive disease at the two months check up and went on to receive neoadjuvant chemotherapy; however it is important to note that the progression did not lead to the cancer being inoperable, due to locally advanced disease or dissemination. We confirm the heterogeneity in response to NET described by others, and in particular that preoperative finding of a 100% ER-positive tumor was associated with clinical response to NET [6,13]. Patients with ductal tumors achieved a better pathological response as compared to patients with other histological types. A lobular histology have previously been shown to predict not only a poorer response to NET but also to neoadjuvant chemotherapy [14,15]. Node-negative patients achieved a better response than node-positive patients and patients with a tumor with malignancy grade 3 achieved a reduced pathological response compared to malignancy grade 1 and 2. Overall, reduced tumor size detected by ultrasonography correlated well with pathological response, whereas tumor growth poorly predicted pathological response. A possible explanation is that an inflammatory response to NET can be mistaken as tumor growth.

The strengths of our study include prospectively planned diagnostic procedures, treatment, and follow-up according to national guidelines of a nationwide cooperative group. All endpoints were pre-planned.

This study has several potential limitations. As the study changed from a randomized phase-III study to a single arm phase-II study with no control group confounding issues may have been introduced. Tumor response and pCR were secondary endpoints in the original phase-III trial and became the primary endpoints when the trial was converted to a single arm phase-II study. Eight patients received chemotherapy following a less favorable outcome from NET, but we are unable to evaluate the possible benefits. Although, most patients with ER-positive and HER2-negative breast cancer do not benefit from chemotherapy, patients with endocrine non-responsive disease may potentially behave differently. Another limitation is the small sample size of this study resulting in limited power, especially when dividing patients into subgroups.

Aromatase inhibitors are the treatment option of choice; however, the optimal duration of treatment is yet to be determined. Letrozole for four months was used in this study and while the most substantial response is obtained during the first four months continuation beyond four months may result in further tumor shrinkage [5,16–18]. Arguably, NET for a pragmatic individualized timeframe will to a large extend maximize treatment benefit in responders but also increase the risk of clinical important treatment failure.

In conclusion, in postmenopausal patients with early breast cancer letrozole given in four months prior to surgery seems to lead to a limited clinical and pathological response but could be considered as a neoadjuvant treatment modality in selected cases where chemotherapy either is not indicated or feasible.

Disclosure statement

One author has reported potential conflict of interest via manuscript central – Ann S Knoop: Lilly Eli (Fee), Pfizer (advisory board + teaching fee), Roche – advisory board and research grant).

None of the other authors have conflict of interests.

Funding

This work was funded by Novartis (Protocol No. CFEM EDK0110) and the Danish Council for Strategic Research (Reference No. 2101-07-0022), Programme Commission on Health, Food and Welfare.

ORCID

Signe Korsgaard Skriver http://orcid.org/0000-0003-1895-0963
Tove Filtenborg Tvedskov http://orcid.org/0000-0002-7568-1790

References

[1] Horobin JM, Preece PE, Dewar JA, et al. Long-term follow-up of elderly patients with locoregional breast cancer treated with tamoxifen only. Br J Surg. 1991;78:213–217.
[2] Kaufmann M, von Minckwitz G, Mamounas EP, et al. Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol. 2012;19:1508–1516.
[3] Gnant M, Thomssen C, Harbeck N. St. Gallen/Vienna 2015: a brief summary of the consensus discussion. Breast Care. 2015;10:124–130.
[4] Dixon JM, Jackson J, Renshaw L, et al. Neoadjuvant tamoxifen and aromatase inhibitors: comparisons and clinical outcomes. J Steroid Biochem Mol Biol. 2003;86:295–299.
[5] Charehbili A, Fontein DBY, Kroep JR, et al. Neoadjuvant hormonal therapy for endocrine sensitive breast cancer: a systematic review. Cancer Treat. Rev. 2014;40:86–92.
[6] Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014;384:164–172.
[7] Christiansen P, Ejlertsen B, Jensen M-B, et al. Danish Breast Cancer Cooperative Group. Clin Epidemiol. 2016;8:445.
[8] Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer Oxf Engl 1990. 2009;45:228–247.
[9] Ogston KN, Miller ID, Payne S, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. Breast Edinb Scotl. 2003;12:320–327.
[10] Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst. 2011;103:1656–1664.
[11] Hammond MEH, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. J Clin Oncol 2010;28:2784–2795.
[12] Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast

cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31:3997–4013.

[13] Ellis MJ, Suman VJ, Hoog J, et al. Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype–ACOSOG Z1031. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29:2342–2349.

[14] Delpech Y, Coutant C, Hsu L, et al. Clinical benefit from neoadjuvant chemotherapy in oestrogen receptor-positive invasive ductal and lobular carcinomas. Br J Cancer. 2013; 108:285–291.

[15] Loibl S, Volz C, Mau C, et al. Response and prognosis after neoadjuvant chemotherapy in 1,051 patients with infiltrating lobular breast carcinoma. Breast Cancer Res Treat. 2014; 144:153–162.

[16] Krainick-Strobel UE, Lichtenegger W, Wallwiener D, et al. Neoadjuvant letrozole in postmenopausal estrogen and/or progesterone receptor positive breast cancer: a phase IIb/III trial to investigate optimal duration of preoperative endocrine therapy. BMC Cancer. 2008;8:62.

[17] Dixon JM, Renshaw L, Macaskill EJ, et al. Increase in response rate by prolonged treatment with neoadjuvant letrozole. Breast Cancer Res Treat. 2009;113:145–151.

[18] Carpenter R, Doughty JC, Cordiner C, et al. Optimum duration of neoadjuvant letrozole to permit breast conserving surgery. Breast Cancer Res Treat. 2014;144:569–576.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 38–43
https://doi.org/10.1080/0284186X.2017.1401735

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Intrinsic subtypes and benefit from postmastectomy radiotherapy in node-positive premenopausal breast cancer patients who received adjuvant chemotherapy – results from two independent randomized trials

Tinne Laurberg[a*], Trine Tramm[b*], Torsten Nielsen[c], Jan Alsner[a], Silje Nord[d], Simen Myhre[d], Therese Sørlie[d], Samuel Leung[c], Cheng Fan[e], Charles Perou[e], Karen Gelmon[c], Jens Overgaard[a], David Voduc[c], Aleix Prat[f,g] and Maggie Chon U. Cheang[h]

[a]Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark; [c]British Columbia Cancer Agency, Vancouver, British Columbia, Canada; [d]Department of Genetics, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [e]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, USA; [f]Department of Medical Oncology, Hospital Clinic, Barcelona, Spain; [g]Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [h]Clinical Trials and Statistics Unit, Institute of Cancer Research, London, UK

ABSTRACT

Background: The study of the intrinsic molecular subtypes of breast cancer has revealed differences among them in terms of prognosis and response to chemotherapy and endocrine therapy. However, the ability of intrinsic subtypes to predict benefit from adjuvant radiotherapy has only been examined in few studies.

Methods: Gene expression-based intrinsic subtyping was performed in 228 breast tumors collected from two independent post-mastectomy clinical trials (British Columbia and the Danish Breast Cancer Cooperative Group 82b trials), where pre-menopausal patients with node-positive disease were randomized to adjuvant radiotherapy or not. All patients received adjuvant chemotherapy and a subgroup of patients underwent ovarian ablation. Tumors were classified into intrinsic subtypes: Luminal A, Luminal B, HER2-enriched, Basal-like and Normal-like using the research-based PAM50 classifier.

Results: In the British Columbia study, patients treated with radiation had an overall significant lower incidence of locoregional recurrence compared to the controls. For Luminal A tumors the risk of loco-regional recurrence was low and was further lowered by adjuvant radiation. These findings were validated in the DBCG 82b study. The individual data from the two cohorts were merged, the hazard ratio (HR) for loco-regional recurrence associated with giving radiation was 0.34 (0.19 to 0.61) overall and 0.12 (0.03 to 0.52) for Luminal A tumors.

Conclusions: In both postmastectomy trials, patients with Luminal A tumors turned out to have a significant lower incidence of loco-regional recurrence when randomized to adjuvant radiotherapy, leaving no indication to omit postmastectomy adjuvant radiation in pre-menopausal high-risk patients with Luminal A tumors. It was not possible to evaluate the effect of radiotherapy among the other subtypes because of limited sample sizes.

ARTICLE HISTORY
Received 3 October 2017
Accepted 1 November 2017

Introduction

In the past two decades, there has been a growing focus on breast tumor heterogeneity, and genomic studies have defined five major intrinsic subtypes of importance: Luminal A, Luminal B, Basal-like, HER2-enriched, and Normal-like [1,2]. Intrinsic subtype was initially discovered by global-gene expression profiling, later a 50-gene profile (PAM50) was developed to be applied on formalin-fixed paraffin-embedded tumor tissue [3] and developed as a qualitative assay that utilizes gene expression data, weighted together with clinical variables to generate a risk category and numerical score, to assess a patient's risk of distant recurrence of disease [4].

The benefit of administering adjuvant radiation therapy (RT) in combination with adjuvant systemic chemotherapy was first demonstrated by two independent randomized trials: The British Columbia (BC) Randomized Radiation trial [5] and the Danish Breast Cancer Group (DBCG) protocol 82b [6]. After 10 years of follow up, women in the DBCG 82b trial assigned to chemotherapy plus RT had a 23% reduction in the rate of loco-regional recurrence (LRR) and a 9% reduction in mortality. A similar effect was demonstrated in the BC trial after 15 years of follow-up: patients treated with RT had a 33% reduction of LRR and a 29% reduction in mortality from breast cancer. These findings have had a profound impact on the indication of RT, and all high-risk patients, particularly

CONTACT Maggie Chon U. Cheang Maggie.cheang@icr.ac.uk Clinical Trials and Statistics Unit, The Institute of Cancer Research, London, UK
*These authors contributed equally to the work.

Supplemental data for this article can be accessed here.

those with node involvement more than four positive nodes, often receive adjuvant RT regardless of tumor characteristics and adjuvant systemic treatment. However, there is still a substantial portion of patients who will develop loco-regional relapse.

The study of the intrinsic molecular subtypes of breast cancer has revealed differences among them in terms of prognosis and response to chemotherapy and endocrine therapy [7–13]. To a lesser extent, studies have tried to clarify if intrinsic subtypes may affect the effect of RT [14–16].

Here, we aim to test if intrinsic subtypes have predictive impact on the effect of postmastectomy RT among young lymph-node–positive patients treated with systemic therapy. We first tested the intrinsic subtypes in the BC-trial and then validated our findings in a subset of patients from the DBCG 82b trial.

Patients and methods

Patient populations

A detailed description of the trials is found in Supplementary Table 1. The British Columbia (BC) trial enrolled 318 high-risk pre-menopausal patients from 1979 to 1986 [5]. The inclusion criterion was pathological examined lymph-node-positive disease. All patients were treated with mastectomy and axillary dissection; adjuvant systemic treatment was cyclophosphamide-methotrexate and 5-flurouracil (CMF). The patients were randomized to postmastectomy RT or no RT. The dose of RT was 37.5 Gy (given in 16 fractions) through two tangential fields of the chest wall and 35 Gy through an anterior supraclavicular–axillary field with a posterior axillary boost. Finally, the internal mammary field received a dose of 35 Gy. All the fields were treated with cobalt-60. In addition patients with estrogen receptor positive tumor were sub-randomized to receive ovarian ablation induced by radiation and prednisolone. Twenty years clinical follow-up was obtained for all patients.

The DBCG 82b trial enrolled 1708 high-risk premenopausal patients from 1982 to 1989 [6]. The inclusion criterion was lymph-node-positive disease and/or tumor size larger than 5 cm and/or invasion of tumor to surrounding skin or pectoral fascia. Like the BC trial, all patients had mastectomy, axillary dissection, received adjuvant CMF and were randomized to postmastectomy RT or no RT. The intended dose of RT was 55 Gy (given in 25 fractions) or 53 Gy (given in 22 fractions) delivered through an anterior electron field to the chest wall and internal mammary nodes and an anterior photon field against the supraclavicular, infraclavicular and axillary regions. The use of posterior axillary fields was advised in patients in whom the ratio of the anterior to posterior diameter was too large to limit the maximal absorbed dose. The closing date for the assessment of recurrence and vital status was 1 January, 2012. The potential median observation time was 25.1 years.

Gene expression profiling

A flowchart for the patients included is shown in Supplementary Figure 1. From the 318 enrolled patients in BC-trial, 159 (50%) had formalin-fixed paraffin-embedded (FFPE) tissues available for RNA extraction. The gene expression profiles of the PAM50 genes essential for intrinsic subtype classification were collected using Nanostring nCounter® system [13,17]. Expression of each gene was normalized relative to the expression of the five housekeeper genes including *ACTB, MRPL19, PSMC4, RPLP0* and *SF3A*. In 145/159 cases, intrinsic subtyping by PAM50 was technically successful.

To enhance the comparability between the studies only material from DBCG 82b-patients with lymph-node positive disease were included. Fresh frozen tumor (FFT) samples were available from 83 patients. Extraction of mRNA from FFT and microarray analysis was performed as described previously [18]. Whole gene expression profiles were obtained using the Applied Biosystem Human Genome Survey Microarray v2.0 (Applied Biosystem, Foster City CA). Microarray data was $\log_2$-transformed and quantile normalized. The 83 patients are part of a previously published data set (GEO: GSE24117).

In our 148 samples, we have 49 ER-positive patients, 74 ER-negative patients, and 25 patients without ER status. To match the clinicopathological heterogeneity of the training cohort, we use all the 49 ER positive patients, and randomly select 49 ER-negative from the 74 (subsetting), calculate the average expression of these 49 pairs of samples, and use this average expression as the normalization vector. Instead of row (gene) median centering, we subtract this normalization vector from the whole data matrix (148 samples) and use the residue as our normalized data matrix to perform PAM50 analysis [3,19].

Statistical analysis

Primary endpoint was local–regional relapse (LRR) for both trials, defined as relapse in the ipsilateral chest wall or an axillary, internal mammary or supraclavicular lymph-node. Cumulative incidence curves for LRR were plotted using a competing risk model, considering distant metastasis and death as competing events. Crude hazard ratios (HR) were computed for all end-points using Cox proportional hazards regression. Patient and clinicopathological parameters were compared by chi-squared test. All tests were two-tailed and p value $<.05$ were considered significant. All statistical tests were performed using STATA version 12.1 (Stata Corp, College Station, TX) and R 3.0.1.

Results

The patient and clinicopathological parameters had a similar distribution within the randomization arms in both studies (Table 1). Similar distributions were also found between patients from the study cohorts and the original BC and DBCG 82b trials, except for lymph node status and tumor size (DBCG 82b) and malignancy grades (BC) (Supplementary Table 2).

In the BC cohort, 39% patients were assigned as Luminal A (56/145), 16% Luminal B (23/145), 17% HER2-E (25/145),

Table 1. Distribution of patient and clinicopathological parameters among patients from the BC and DBCG 82b study cohorts.

	BC trial							DBCG 82b trial						
	All	(%)	RT	(%)	NoRT	(%)	*p* value	All	(%)	RT	(%)	NoRT	(%)	*p* value
Patients (N)	145	(100%)	69	(48%)	76	(52%)		83	(100%)	45	(54%)	38	(46%)	
Ovarian ablation	35	(24%)	19	(28%)	16	(21%)	.36	0	(0%)	0	(0%)	0	(0%)	
Age							.58							.94
<41	41	(28%)	18	(26%)	23	(30%)		15	(18%)	8	(18%)	7	(18%)	
41-	104	(72%)	51	(74%)	53	(70%)		68	(82%)	37	(82%)	31	(82%)	
Tumor size (mm)							.65							.64
<21	40	(28%)	16	(23%)	24	(32%)		24	(29%)	12	(27%)	12	(32%)	
21–50	82	(57%)	41	(59%)	41	(54%)		46	(55%)	27	(60%)	19	(50%)	
>50	9	(6%)	4	(6%)	5	(7%)		13	(16%)	6	(13%)	7	(18%)	
Unknown	14	(10%)	8	(12%)	6	(8%)		0	(0%)	0	(0%)	0	(0%)	
Lymph node status							.80							.44
1–3 positive	84	(58%)	38	(55%)	46	(61%)		42	(51%)	21	(47%)	21	(55%)	
>3 positive	49	(34%)	25	(36%)	24	(32%)		41	(49%)	24	(53%)	17	(45%)	
Unknown	12	(8%)	6	(9%)	6	(8%)		0	(0%)	0	(0%)	0	(0%)	
Malignancy grade							.53							.78
Grade 1	16	(11%)	7	(10%)	9	(12%)		10	(12%)	4	(9%)	6	(16%)	
Grade 2	51	(35%)	25	(36%)	26	(34%)		47	(57%)	26	(58%)	21	(55%)	
Grade 3	55	(38%)	29	(42%)	26	(34%)		23	(28%)	13	(29%)	10	(26%)	
Unknown	23	(16%)	8	(12%)	15	(20%)		3	(4%)	2	(4%)	1	(3%)	
Histopathology														
Ductal carcinoma	131	(90%)	63	(91%)	68	(89%)	.71	70	(84%)	39	(87%)	31	(82%)	.52
ER-pos	79	(54%)	38	(55%)	41	(54%)	.89	55	(66%)	31	(69%)	24	(63%)	.58
HER2-pos	22	(15%)	14	(20%)	8	(11%)	.10	26	(31%)	13	(29%)	13	(34%)	.60
Intrinsic subtype							.17							.77
LumA	56	(39%)	26	(38%)	30	(39%)		30	(36%)	18	(40%)	12	(32%)	
LumB	23	(16%)	10	(14%)	13	(17%)		15	(18%)	6	(13%)	9	(24%)	
Her2-E	25	(17%)	17	(25%)	8	(11%)		12	(14%)	7	(16%)	5	(13%)	
Basal-like	27	(19%)	12	(17%)	15	(20%)		16	(19%)	9	(20%)	7	(18%)	
Normal-like	14	(10%)	4	(6%)	10	(13%)		10	(12%)	5	(11%)	5	(13%)	

19% Basal-like (27/145) and 10% as Normal-like (14/145). In the DBCG 82b validation-cohort of 83 patients, the distribution of intrinsic subtypes was similar to the BC-study ($p = .94$); 36% patients were assigned to Luminal A (30/83), 18% to Luminal B (15/83), 14% to HER2-E (12/83), 19% to Basal-like (16/83) and 12% to Normal-like (10/83) (Table 1).

Association of locoregional recurrence with radiation therapy, stratified by intrinsic subtypes

Overall, adjuvant RT decreased the locoregional recurrence significantly in both BC study and DBCG 82b study (Figure 1). After 20 years of follow-up, the cumulative incidence proportion of LRR was 15% in the BC trial among women assigned to RT compared to 36% in the control group, giving a 22% (95CI: 8-36%) absolute risk reduction of LRR associated with RT (HR = 0.35 (0.17–0.72). A similar effect was demonstrated in the DBCG 82b trial, wherein the 20-years risk of LRR was 11% in the RT arm versus 37% in the control, giving a 26% (8-44%) absolute LRR risk reduction, HR = 0.30 (0.11–0.83). In the BC study, patients with Luminal A tumors had a significant reduced risk of LRR (4% in the RT arm vs. 31% in the control arm) when treated with RT, giving a 20-years absolute LRR risk difference of 27% (9–46%), HR = 0.17 (0.01–0.92) (Figure 2). A reduction of LRR was also found among the Basal-like cases (Figure 3). No statistically significant difference of LRR was observed between the radiation- and control arm at 20 years for patients with Luminal B and the few HER2-E tumors, respectively (Figure 3).

In the DBCG 82b validation-cohort, among patients with Luminal A tumors, those who received RT had a significantly reduced risk of LRR (6% in the RT arm vs. 42% in the control arm). The 20-years absolute LRR risk difference was 36% (6-66%), HR = 0.12 (0.01–1.02) (Figure 2). LRR risk differences did not reach statistical significance, with hazard ratio confidence intervals crossing 1.0 observed between the radiation and control arm at 20 years for patients with Luminal B, Basal-like and HER2-E tumors respectively (Figure 3)

An overall estimate was calculated by merging the individual data from the two trials. Adjuvant RT reduced the incidence of LRR significant in the merged cohort (HR = 0.33 (0.18–0.60)) (Figure 3). The overall estimate within each intrinsic subtype generally favorable outcome was observed in the RT arm. This benefit was greatest for Luminal A (HR = 0.12 (0.02–0.60)) and to a lesser extent for the Basal-like tumors. In the smaller Luminal B and HER2-E tumor subsets, no significant differences were observed in the risk of LRR between the RT – and control arm.

Discussion

We studied intrinsic subtyping of patients from the original post-mastectomy randomized radiation studies, BC- and DBCG 82b-trial, and confirmed that our translational study had demonstrated a reduced risk of LRR associated with RT among young high-risk patients treated with adjuvant systemic therapy as the original trials.

Our data supports that premenopausal lymph-node-positive patients with Luminal A tumors do benefit from post-mastectomy RT. The other intrinsic subtypes generally have favorable outcomes in the RT arm, but because of the low

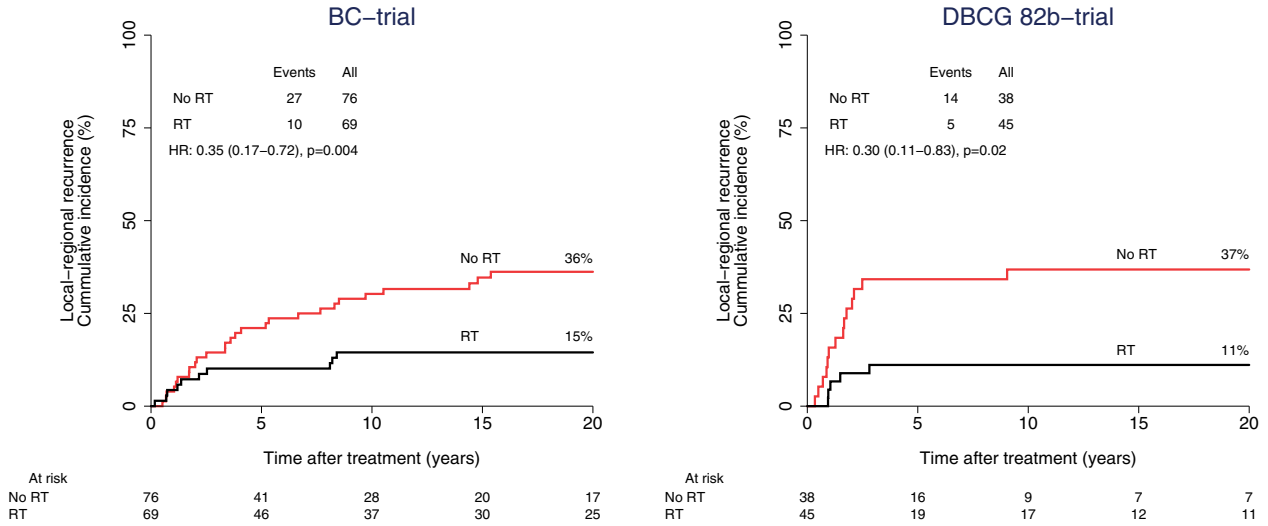


Figure 1. Loco-regional recurrence as a function of randomization assignment to adjuvant postmastectomy radiotherapy (RT) within the study cohorts of the BC-trial (left) and the DBCG 82b-trial (right).

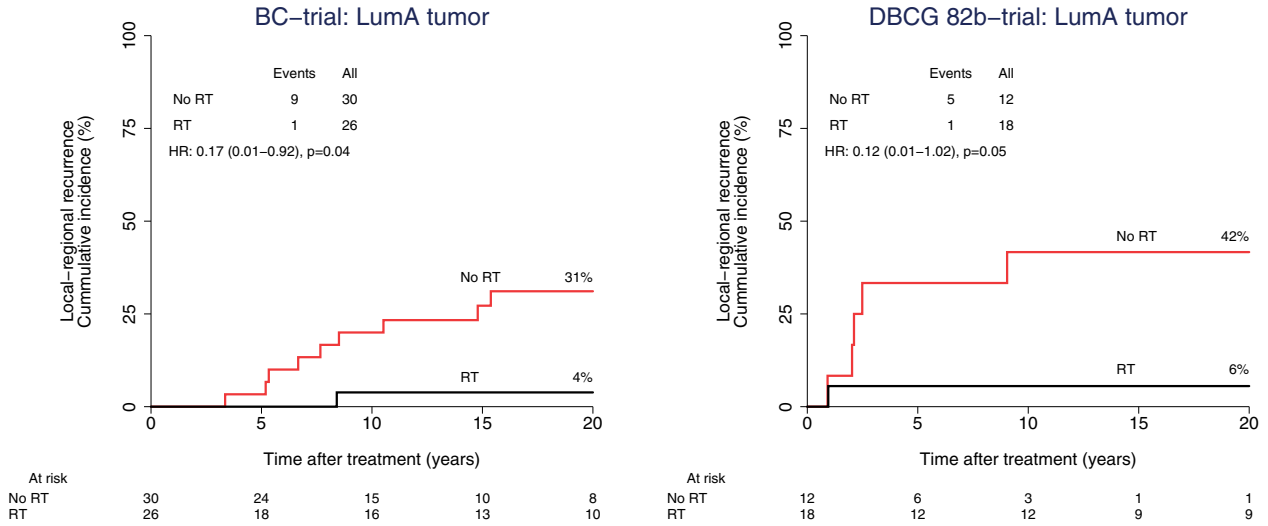


Figure 2. Loco-regional recurrence among patients with a Luminal A tumor as a function of randomization to adjuvant postmastectomy radiotherapy (RT) within the BC-trial (left) and the DBCG 82b-trial (right).

numbers within each of these subgroups, it was not possible to prove the benefit of RT.

On a larger material from the DBCG 82b and 82-c trials, molecular subtypes were approximated by an immuno-histochemical panel of estrogen, progesterone and HER2 [20]. Luminal A-like tumors had beneficial effect of RT (3% in RT arm vs. 32% in the control arm), and the 15-year overall survival was improved from 33% vs. 44%, HR = 0.78 (0.64–0.93). They did also find an equivalent (3% vs. 48%) association between RT and LRR among Luminal B-like tumors (defined as estrogen and/or progesterone receptor positive and HER2 positive).

However, Liu et al. found among post-menopausal lymph-node-negative patients receiving tamoxifen and randomized to ± RT, which intrinsic subtype classification had prognostic impact on the risk of developing local failure, but was not predictive of benefit from RT [14]. Interestingly, the author observed no effect of RT among low-risk patients older than 60 patients with Luminal A tumors. These opposite findings of RT effects on Luminal tumors in our study may reflect the a priori prognosis of the different study populations. In the current study and the Kyndi paper, the cohort consisted of high-risk lymph-node-positive patients, whereas in the Liu study, all the patients had lymph-node negative disease. One could speculate that in the first case the less aggressive tumor type, Luminal A, had beneficial effect of RT, whereas it is more doubtful with the more aggressive tumor subtypes, because those patients suffer from distant metastases. Luminal A tumors are local slow growing and have such a good prognosis after adjuvant systemic treatment, which the patients do not develop LRR and as a consequence do not obtain any beneficial effect from RT. It is also likely that the Luminal A tumors harbor further heterogeneity in regard to cellular radiosensitivity. In a previous study, a differential

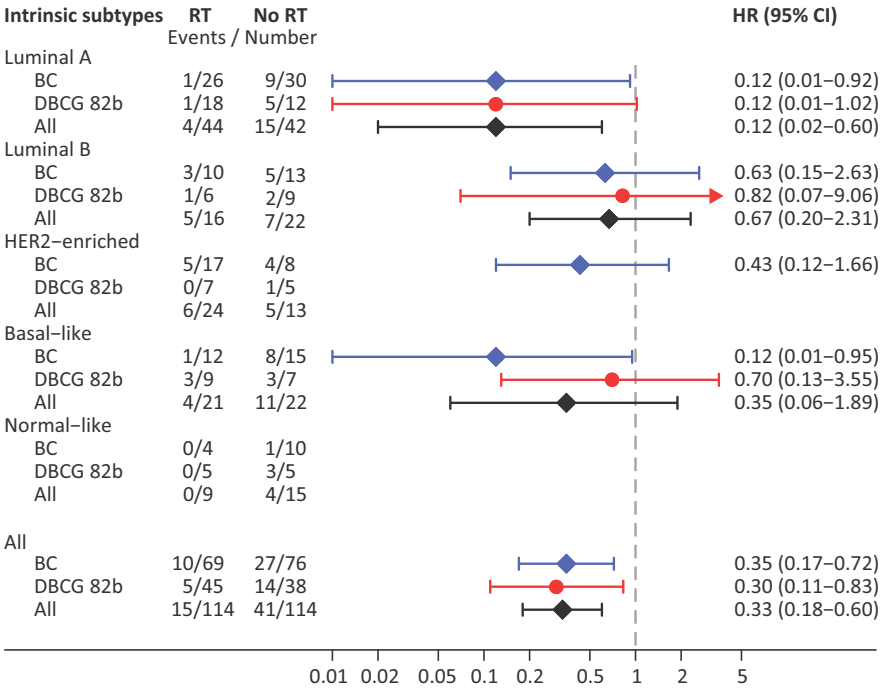


Figure 3. Forest plot showing the association between radiotherapy (RT) and the incidence of local-regional recurrence within different intrinsic subtype subgroups. BC-trial (Blue bar), DBCG 82b-trial (Red bar) and Merged data (Black bar). In subgroups with no events, HR cannot be estimated, nor can the overall HR.

effect of postmastectomy RT in Luminal A tumors has been observed, when examining a 7-gene profile predictive of response to postmastectomy RT (DBCG-RT profile) [16].

Recently, Sjøgren et al also reported no predictive value of intrinsic subtype related to RT among lymph-node negative patients randomized to ± RT after breast conservative surgery, but low-risk patients with Luminal A tumors had beneficial effect of RT [15]. The inconsistent results of the subanalysis restricted to older low-risk Luminal A patients could be due to all patients receiving tamoxifen in the paper from Lui et al, whereas only 8% received adjuvant systemic treatment in the Sjøgren study.

A limitation of our present study was the low number of patients with available material in comparison with the total number of patients accrued in the original trial. Another limitation is that at the time of enrollment in the trials the standard treatment for all premenopausal high-risk patients was adjuvant CMF; if the patients were treated today they would have received anthracycline and/or taxane-based systemic treatment. We also acknowledge that the expression profiles were obtained from two different technology platforms: the expression profiles from the BC-study were based on FFPE-derived RNA analyzed on the Nanostring nCounter whereas that from the DBCG 82b trial was based on frozen-tissue derived RNA applied to whole genome microarrays. However, despite different gene technologies applied, the PAM50 intrinsic calls and their association of outcome to radiation therapy were similar in the two trials. Hence our results suggest that PAM50 assignments are robust across technology platforms and patient populations, as Luminal A tumors did benefit from RT in both studies.

In summary, we demonstrate using material from two independent randomized trials that postmastectomy RT significantly decreases local-regional recurrences among pre-menopausal high-risk patients treated with chemotherapy. Because of limited material, when breaking into the major intrinsic molecular subtypes, it was only possible to evaluate the effect of RT among patients with Luminal A tumors. In both trials, RT lowered the risk of LRR, and thus there is no molecular subtype indication to omit adjuvant chest wall radiation in pre-menopausal high-risk patients with Luminal A tumors treated by mastectomy.

Acknowledgments

We thank Sherman Lau at the Genetic Pathology Evaluation Centre (Vancouver) for his assistance in RNA extraction of samples from the BC trial.

Disclosure statement

C.M.P. is equity stock holders of, and T.O. consults for BioClassifier LLC. C.M.P., T.O. and M.C.U.C. have filed a patent on the PAM50 assay. T.T, J.A., S.M, T.S. and J.O holds a patent for a gene signature associated with efficacy of radiotherapy in breast cancer (international patent publication no. WO 2013/132354A2). The patent is not related to the present work.

Funding

This work was supported by funds from ReThink Breast Cancer Career Development Award for MCU Cheang, The Danish Council for Strategic Research (DBCG-TIBCAT) and CIRRO (Danish Center for Interventional Research in Radiation Oncology).

ORCID

Jan Alsner http://orcid.org/0000-0002-5395-3193

References

[1] Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature. 2000;406:747–752.

[2] Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98:10869–10874.

[3] Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol. 2009;27:1160–1167.

[4] Gnant M, Filipits M, Greil R, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 risk of recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. Ann Oncol. 2014;25:339–345.

[5] Ragaz J, Jackson SM, Le N, et al. Adjuvant radiotherapy and chemotherapy in node-positive premenopausal women with breast cancer. N Engl J Med. 1997;337:956–962.

[6] Overgaard M, Hansen PS, Overgaard J, et al. Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. Danish breast cancer cooperative group 82b Trial. N Engl J Med. 1997;337:949–955.

[7] Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res. 2010;16:5222–5232.

[8] Sestak I, Dowsett M, Zabaglo L, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. J Natl Cancer Inst. 2013;105:1504–1511.

[9] Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. Clin Cancer Res. 2014;20:511–521.

[10] Cheang MC, Voduc KD, Tu D, et al. Responsiveness of intrinsic subtypes to adjuvant anthracycline substitution in the NCIC.CTG MA.5 randomized trial. Clin Cancer Res. 2012;18:2402–2412.

[11] Chia SK, Bramwell VH, Tu D, et al. A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. Clin Cancer Res. 2012;18:4465–4472.

[12] Nielsen TO, Jensen M-B, Burugu S, et al. High-Risk Premenopausal luminal A breast cancer patients derive no benefit from adjuvant cyclophosphamide-based chemotherapy: results from the DBCG77B clinical trial. Clin Cancer Res. 2017; 23:946–953.

[13] Jørgensen CLT, Nielsen TO, Bjerre KD, et al. PAM50 breast cancer intrinsic subtypes and effect of gemcitabine in advanced breast cancer patients. Acta Oncol. 2014;53:776–787.

[14] Liu F-F, Shi W, Done SJ, et al. Identification of a low-risk luminal A breast cancer cohort that may not benefit from breast radiotherapy. J Clin Oncol. 2015;33:2035–2040.

[15] Sjöström M, Lundstedt D, Hartman L, et al. Response to radiotherapy after breast-conserving surgery in different breast cancer subtypes in the Swedish breast cancer group 91 radiotherapy randomized clinical trial. J Clin Oncol. 2017;35: 3222–3229.

[16] Tramm T, Kyndi M, Myhre S, et al. Relationship between the prognostic and predictive value of the intrinsic subtypes and a validated gene profile predictive of loco-regional control and benefit from post-mastectomy radiotherapy in patients with high-risk breast cancer. Acta Oncol. 2014;53:1337–1346.

[17] Fortina P, Surrey S. Digital mRNA profiling. Nat Biotechnol. 2008;26:293–294.

[18] Myhre S, Mohammed H, Tramm T, et al. In silico ascription of gene expression differences to tumor and stromal cells in a model to study impact on breast cancer outcome. PLoS One. 2010;5:e14002.

[19] Zhao X, Rødland EA, Tibshirani R, et al. Molecular subtyping for clinically defined breast cancer subgroups. Breast Cancer Res. 2015;17:29

[20] Kyndi M, Sorensen FB, Knudsen H, et al. Estrogen receptor, progesterone receptor, HER-2, and response to postmastectomy radiotherapy in high-risk breast cancer: the Danish breast cancer cooperative group. J Clin Oncol. 2008;26:1419–1426.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 44–50
https://doi.org/10.1080/0284186X.2017.1403044

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The ability of PAM50 risk of recurrence score to predict 10-year distant recurrence in hormone receptor-positive postmenopausal women with special histological subtypes

Anne-Vibeke Laenkholm[a], Maj-Britt Jensen[b], Jens Ole Eriksen[a], Wesley Buckingham[c], Sean Ferree[c], Torsten O. Nielsen[d] and Bent Ejlertsen[b,e]

[a]Department of Surgical Pathology, Zealand University Hospital, Slagelse, Denmark; [b]Danish Breast Cancer Cooperative Group, Rigshospitalet, Copenhagen, Denmark; [c]NanoString Technologies Inc, Seattle, WA, USA; [d]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; [e]Danish Breast Cancer Cooperative Group, Department of Oncology, Rigshospitalet, Copenhagen, Denmark

ABSTRACT

Introduction: The Prosigna-PAM50 risk of recurrence (ROR) score has been validated in randomized clinical trials to predict 10-year distant recurrence (DR) in hormone receptor-positive breast cancer. Here, we examine the ability of Prosigna for predicting DR at 10 years in a subgroup of postmenopausal breast cancer patients with special histological subtypes.

Methods: Using the population based Danish Breast Cancer Group database, follow-up data were collected on all patients diagnosed from 2000 to 2003 with estrogen receptor (ER)-positive and human epidermal growth factor receptor 2 (HER2) normal breast cancer who by nationwide guidelines were treated with 5 year of endocrine therapy ($N = 2558$). Among patients with 1 to 3 positive lymph nodes or a tumor size >20 mm, we identified 1570 with invasive ductal carcinoma (IDC) and 89 with special histological subtypes (apocrine, medullary, mucinous, papillary, secretory, tubular, neuroendocrine) who were tested with Prosigna. Fine and Gray models were applied to determine the prognostic value of the Prosigna-PAM50 ROR score for DR special subtypes as compared to IDC.

Results: Median follow-up for DR was 9.2 year and for OS 15.2 year. The 10-year DR rate for the special subtypes was 9.2% (95% CI: 4.0% to 17.2%) as compared to 13.7% (95% CI: 11.9% to 15.7%) for IDC. The 10-year OS was 74.2% (95% CI: 63.7% to 82.0%) for the special subtypes and 75.4% (95% CI: 73.2% to 77.4%) for IDC. Prosigna showed a statistical significant association of the continuous ROR score with risk of DR for both IDC and the special subtypes (IDC: $p < .0001$; special subtypes: $p = .01$).

Conclusion: In the present study, we demonstrated that Prosigna-PAM50 continuous ROR score added significant prognostic information for 10-year DR in postmenopausal patients with special subtypes (tumor size >20 mm or 1 to 3 positive lymph nodes) and ER-positive, HER2-normal early breast cancer.

ARTICLE HISTORY
Received 8 October 2017
Accepted 1 November 2017

Introduction

Invasive breast cancer encompasses a heterogeneous group of tumors with specific morphologic and phenotypic features [1]. At least 90% of breast cancers present histologic characteristics allowing their assignment to one of 21 histologic subtypes endorsed by the WHO [1]. The vast majority of breast cancers are classified as invasive ductal (IDC) or invasive lobular carcinomas (ILC) and less than 8% are invasive tumors with other specific or special histologic features, for example, invasive apocrine, medullary, mucinous, neuroendocrine, papillary, secretory and tubular carcinomas.

Some of these special subtypes are characterized by good long-term outcome [2,3] but published follow-up data are based on small series of patients due to the rarity of these tumors [1]. A better understanding at the molecular level by the application of genomic assays to a sufficiently large cohort might provide improved treatment guidance for these patients.

Previous reports have linked the ER-negative basal-like and claudin-low genomic profiles with metaplastic carcinoma, carcinomas with medullary features, and the molecular apocrine subtype with increased androgen receptor signaling [4–7]. However, the majority of the histological special subtypes, that is, tubular, mucinous, neuroendocrine or papillary carcinomas are ER-positive and are thought to belong to luminal intrinsic molecular subtypes [4,8]. Several multigene assays have been evaluated for the prognostication of early-stage node-negative disease and have confirmed the clinical utility of these tests [9–17]. The Prosigna-PAM50 ROR score adds significant prognostic information above standard clinico-pathological factors in postmenopausal ER-positive HER2 normal, node-negative as well as node-positive (1 to 3 positive lymph nodes) patients [11,15,18]. However, little is

CONTACT Anne-Vibeke Laenkholm anlae@regionsjaelland.dk Department of Surgical Pathology, Ingemannsvej 48, Zealand University Hospital, 4200 Slagelse, Denmark

Supplemental data for this article can be accessed here

known about the performance of the test when applied on tumors of special subtypes.

In this study, we evaluated the ability of the Prosigna-PAM50 ROR score to predict distant recurrence at 10 years in a subgroup of clinically ER-positive postmenopausal breast cancer patients with special subtypes as compared to IDC following allocation without adjuvant chemotherapy to 5 years of endocrine therapy.

Material and methods

The organization of the DBCG and current study cohort has previously been described in detail [18–20]. The study was approved by the National Danish Ethics Committee (H-D-2007-0034).

Patient population

The patients included in this study were all postmenopausal women who by national Danish guidelines from 2000 through 2003 were allocated to 5 years of endocrine treatment as the only adjuvant systemic treatment following a first diagnosis of ER-positive invasive breast cancer. Eligible patients for this DBCG cohort were 50 years or older and met at least one of the following risk criteria: a tumor size >20 mm (any subtype), ductal histology with malignancy grade 2 or 3; or one to three positive nodes (any subtype). Surgery, radiotherapy and systemic treatment were predetermined and have previously been described as well as the external quality assurance program for ER immunohistochemical staining procedure that was attended by all the Danish pathology departments [19,21]. The prespecified ER cutoff value at the time was $\geq$10% positive nuclear staining regardless of staining intensity. The histopathological tumor classification was performed by dedicated breast pathologists at the Danish departments of pathology representing real world diagnostics.

For the present subgroup analysis, only patients with carcinomas $>$20 mm and/or 1–3 positive lymph nodes, and with either rare subtypes (other than ILC) or IDC were included (Supplementary Figure 1).

Central assessment of HER2

Formalin-fixed, paraffin-embedded (FFPE) tumor samples from primary excisional surgery specimens were collected at the Department of Surgical Pathology, Zealand University Hospital, and tissue microarrays (TMA) were constructed with 4×1.5 mm tumor tissue cores from, whenever possible, the tumor periphery and then split onto two separate recipient blocks. HER2 status was established centrally on TMAs by standard recommendations [22].

Prosigna analysis

The Prosigna algorithm calculates the tumor-specific molecular subtype (luminal A, luminal B, HER2-enriched or basal like) and calculates a ROR score (1-100 scale) based on a 46 gene subset of the 50 target genes (PAM50) with inclusion of a proliferation score (mean expression of an 18-gene subset of the 50 genes) and tumor size [23]. The Prosigna ROR score can be used as a continuous score or it can categorize ER-positive, HER2-negative postmenopausal breast cancer patients with 0–3 positive lymph nodes into one of three risk groups, or even one of two (low/intermediate versus high) if data are sparse, to determine 10-year risk of recurrence [10,11,24].

RNA extraction and Prosigna testing were performed blinded to any clinical data, following standard operating procedures [11,15,25,26] at the Department of Surgical Pathology, Zealand University Hospital. Once testing of the samples was complete, Prosigna results were transferred to the data manager for preparation of the analysis data set, also blinded to patient data. The analysis data set was then transferred to the DBCG for merging with clinical data and survival analysis according to a prespecified statistical plan.

Endpoints

The primary endpoint was time to distant recurrence (DR) defined as the interval from breast cancer surgery until distant recurrence or death from breast cancer as a first event. Overall survival (OS) was a secondary endpoint. For OS, complete follow-up was achieved until 31 May 2017 by linkage to the Danish Central Population Registry.

Statistical analyses

The statistical analysis was executed by the DBCG statistical office. Categorical characteristics were compared by χ^2 or Fisher's exact test. Follow-up time was quantified in terms of a Kaplan-Meier estimate of potential follow-up. Kaplan–Meier estimates were calculated for OS, and cumulative incidences for DR, handling secondary carcinomas, contralateral breast cancer and death as a first event from causes other than breast cancer as competing risk events. Univariate and multivariate regression analyses were performed for DR and OS. For competing risk analyses, the Fine-Gray subdistribution hazard model was used, while for overall survival the Cox proportional hazard model was employed. Factors included in the multivariable analyses were age (continuous), tumor size (transformed to log(cm)), number of positive lymph nodes (0, 1, 2, 3), histological type and grade (1, 2, 3 and not graded) and for DR also lymphovascular invasion and ER expression (continuous, % positive tumor cells). The assumption of proportional hazards was assessed by Schoenfeld residuals and by including a time-dependent component in the model. The hazard rates of ER and grade were not proportional and were each modeled for early and late periods ($<$5 years, $\geq$5 years). Further, the ROR score as a continuous measure (20 point change) and molecular subtype were included in separate models. All p values are two-sided. Statistical analyses were done using SAS v9.4 (SAS Institute, Inc., Cary, NC) and R v3.2.2.

Results

We identified 2558 HER2 normal cases hereof 89 cases of special subtypes and 1570 cases of IDC with tumor size >20 mm or 1 to 3 positive lymph nodes (Supplementary Figure 1). With an estimated median potential follow-up for DR of 9.2 years and for OS of 15.2 years, 191 DR events and 666 deaths were recorded. The median age was 62 years (range, 50 to 89) for IDC and 65 (range, 50 to 87) for the special subtypes ($p = .03$). Patients in the study cohort were postmenopausal and were without adjuvant chemotherapy assigned to five years of tamoxifen, an aromatase inhibitor (AI) or a sequence of these drugs for early ER-positive breast cancer as described elsewhere [18].

Histological subtype

The histopathological classification of the special subtypes is presented in Table 1 and included mucinous: $N = 51$ (2.0%); tubular: $N = 16$ (0.6%); papillary: $N = 12$ (0.5%); medullary: $N = 5$ (0.2%); apocrine: $N = 3$ (0.1%) and neuroendocrine: $N = 1$ (0.01%). It should be noted that pure papillary carcinomas are rare and that some cases of the invasive variant of solid papillary carcinoma might be included in this subgroup [1]. In addition, both the medullary and apocrine subtype predominantly but not exclusively are ER negative [1,27]. Overall, the proportion of the individual histopathological subtypes in this study are in accordance with WHO classification of tumors of the breast [1]. Patient characteristics for both special subtypes and IDC are shown in Table 2; In general patients with a special subtype had larger tumor size, but fewer positive lymph nodes. The overall 10-year DR rate for the special subtypes was 9.2% (95% CI: 4.0% to 17.2%) as compared to 13.7% (95% CI: 11.9% to 15.7%) for IDC. The 10-year OS was 74.2% (95% CI: 63.7% to 82.0%) for the special subtypes and 75.4% (95% CI: 73.2% to 77.4%) for IDC.

Table 1. Histological special subtypes and distribution of molecular intrinsic subtype including DR events and Deaths (any cause) ($N = 89$).

Histological subtypes	$N = 89$	Luminal A	Luminal B	HER2Enriched	Basallike	DR events	Deaths any cause
Apocrine	3	0	1	2	0	1	1
Medullary	5	0	1	1	3	1	2
Mucinous	51	23	26	2	0	4	28
Papillary	12	2	8	2	0	1	5
Secretory	1	1	0	0	0	0	0
Tubular	16	16	0	0	0	0	5
Neuroendocrine	1	0	1	0	0	0	0

Table 2. Patient characteristics. Special subtypes (except for invasive lobular carcinoma) are not graded according to national guidelines [www.dbcg.dk].

	IDC ($N = 1570$)	(%)	Special subtypes ($N = 89$)	(%)	p
Number of positive lymph nodes					.0005
0	425	(27%)	42	(47%)	
1	636	(40%)	30	(34%)	
2	324	(21%)	11	(12%)	
3	185	(12%)	6	(7%)	
Tumor size (mm)					.0002
≤10	100	(6%)	6	(7%)	
11–20	560	(36%)	16	(18%)	
21–30	680	(43%)	41	(46%)	
>30	230	(15%)	26	(29%)	
Grade					
1	562	(36%)	6	(7%)	
2	790	(50%)	4	(4%)	
3	218	(14%)	0	(0%)	
Not done	0	(0%)	79	(89%)	
Lymphovascular invasion					.10
Present	221	(14%)	7	(8%)	
Absent	1349	(86%)	82	(92%)	
ER expression level					.54
10–59%	161	(10%)	6	(7%)	
60–89%	335	(21%)	17	(19%)	
90–99%	415	(27%)	27	(30%)	
100%	638	(41%)	39	(44%)	
Positive[a]	21	(1%)	0	(0%)	
Molecular subtype					[b].052
Luminal A	863	(55%)	42	(47%)	
Luminal B	620	(39%)	37	(42%)	
HER2Enriched	73	(5%)	7	(8%)	
Basallike	14	(1%)	3	(3%)	
ROR group					
Low/intermediate (≤40)	532	(34%)	29	(33%)	
High (>40)	1038	(66%)	60	(67%)	

[a]ER ≥10% the exact percentage unknown;
[b]Fisher's Exact Test.

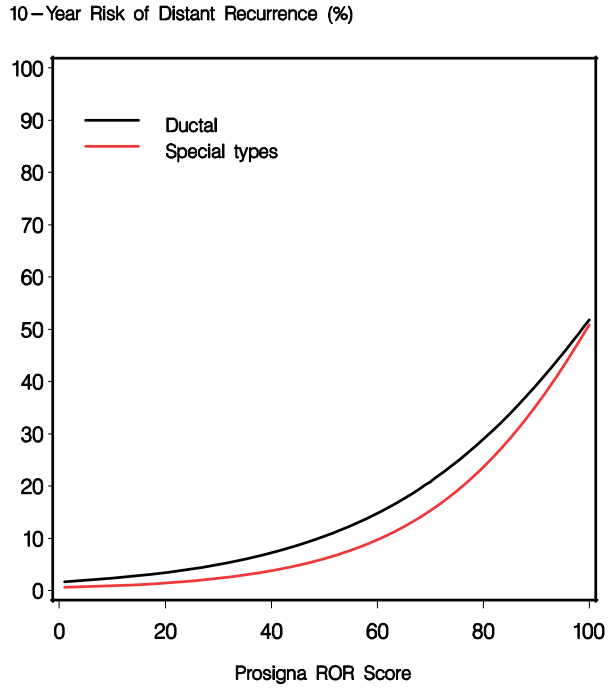


Figure 1. Continuous relationship between 10-year risk of distant recurrence and the continuous PAM50-Prosigna ROR score by IDC and special subtypes. The figure shows for continuous ROR score (stated on *x*-axis) the 10-year risk of distant recurrence in percent (stated on *y*-axis) for each of the two subgroups, modeled by the Fine-Gray subdistribution hazards model.

Prosigna ROR score

The distribution of ROR score (low/intermediate vs. high) based on previously defined cutoff levels [11] is presented in Table 2. Due to the small sample size for special type analyzes, cases within the range of the low/intermediate ROR score were merged. Prosigna showed a statistical significant association of the continuous ROR score with risk of DR not only, as expected, for IDC, but also for the special subtypes (IDC: $p < .0001$; special subtypes: $p = .01$) (Figure 1). Associations were maintained in adjusted estimates (Table 3). For the categorical ROR score no events were registered for the special subtypes in the low/intermediate risk group as compared to IDC with 10-year DR of 3.8% (95% CI: 2.3 to 6.0). In the high-risk group, the 10-year DR rate for the special subtypes was 13.1% (95% CI: 5.7% to 23.8%) and 19.9% (95% CI: 16.3% to 21.7% for IDC (Figure 2(A)). For OS, the prognostic information for both the special histological subtypes and IDC were somewhat lower, and while highly statistical significant for IDC, neither in univariate nor multivariate analysis was a significant association for ROR score found in the small subgroup of patients with special subtypes (Figure 2(C)). For both univariate and multivariate analyzes, and considering both DR and OS, no statistically significant heterogeneity in estimates of the ROR score could be found.

PAM50 intrinsic subtypes

Almost half of the patients with rare subtypes ($N = 42$, 47%) were assigned to Luminal A, while 37 (42%) were assigned to Luminal B. Only a minor proportion of the patients ($N = 7$, 8%) had a HER2-enriched or a basal like ($N = 3$, 3%) subtype as shown in Table 1. The subtype assignment for IDC was comparable with the special subtypes ($p = .052$). Table 1 illustrates the distribution of the special subtypes with relation to the molecular subtypes, number of events and number of deaths (any cause). The tubular carcinomas are all luminal A, whereas both the mucinous and papillary carcinomas are distributed as luminal A and B with a few tumors allocated to the HER2-enriched subtype. As expected, the tumors with medullary features are mainly basal-like and the apocrine subtype mainly HER2 enriched.

Table 3. Hazard Ratio (HR) estimates from multivariate analyzes for distant recurrence and overall survival.

	IDC		Special subtypes	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Distant recurrence				
ROR Cont	1.78 (1.49–2.13)	<.0001	2.83 (1.28–6.26)	.01
Overall survival				
ROR Cont	1.32 (1.21–1.45)	<.0001	1.11(0.79–1.55)	.56
Subtype				
Luminal B vs Luminal A	1.76 (1.41–2.21)	<.0001	1.23 (0.58–2.63)	.64

The DR rate was significantly lower ($p < .0001$) in luminal A, IDC as compared to luminal B, with an absolute 10-year DR rate of 7.4% (95% CI: 5.5% to 9.5%) for the luminal A as compared to 20.7% (95% CI: 17.2% to 24.5%) for the luminal B subtype (Figure 2(B)). For the special subtypes, the number of events was limited ($N = 7$), with no events registered for the Luminal A molecular subtype; for Luminal B the absolute 10-year DR rate was 13.1% (4.0% to 27.9%). For OS, there was a statistical significant ($p < .0001$) better outcome in patients with Luminal A as compared to Luminal B (Figure 2(D)) for IDC, and a similar trend is seen for patients with special types, although not significant ($p = .21$). Similarly, results are shown from the adjusted estimates for Luminal B as compared to Luminal A in Table 3. No statistical heterogeneity in effect of Luminal B compared to Luminal A according to type (IDC vs. special) was identified, with $P_{interaction} = .55$ in multivariate analysis.

Discussion

The present study showed that the Prosigna-PAM50 continuous ROR score added significant prognostic information in postmenopausal breast cancers patients with special ER-positive and HER2-normal histological subtypes. Furthermore, we found no evidence to support a differential impact on prognosis by ROR-score or molecular subtype as determined by Prosigna among patients with a special histological subtype as compared to IDC.

Some caution should be exercised when interpreting our results. First, the number of cases with special subtypes, even in this large study set, was limited. Given that half of the special subtypes were mucinous one could expect that this subtype would drive the results for the entire group, but a formal analysis was prohibited by the small sample size. However, no statistical heterogeneity for neither luminal subtype (B vs. A) nor ROR score according to type (mucinous vs other special subtypes) was observed (data not shown).

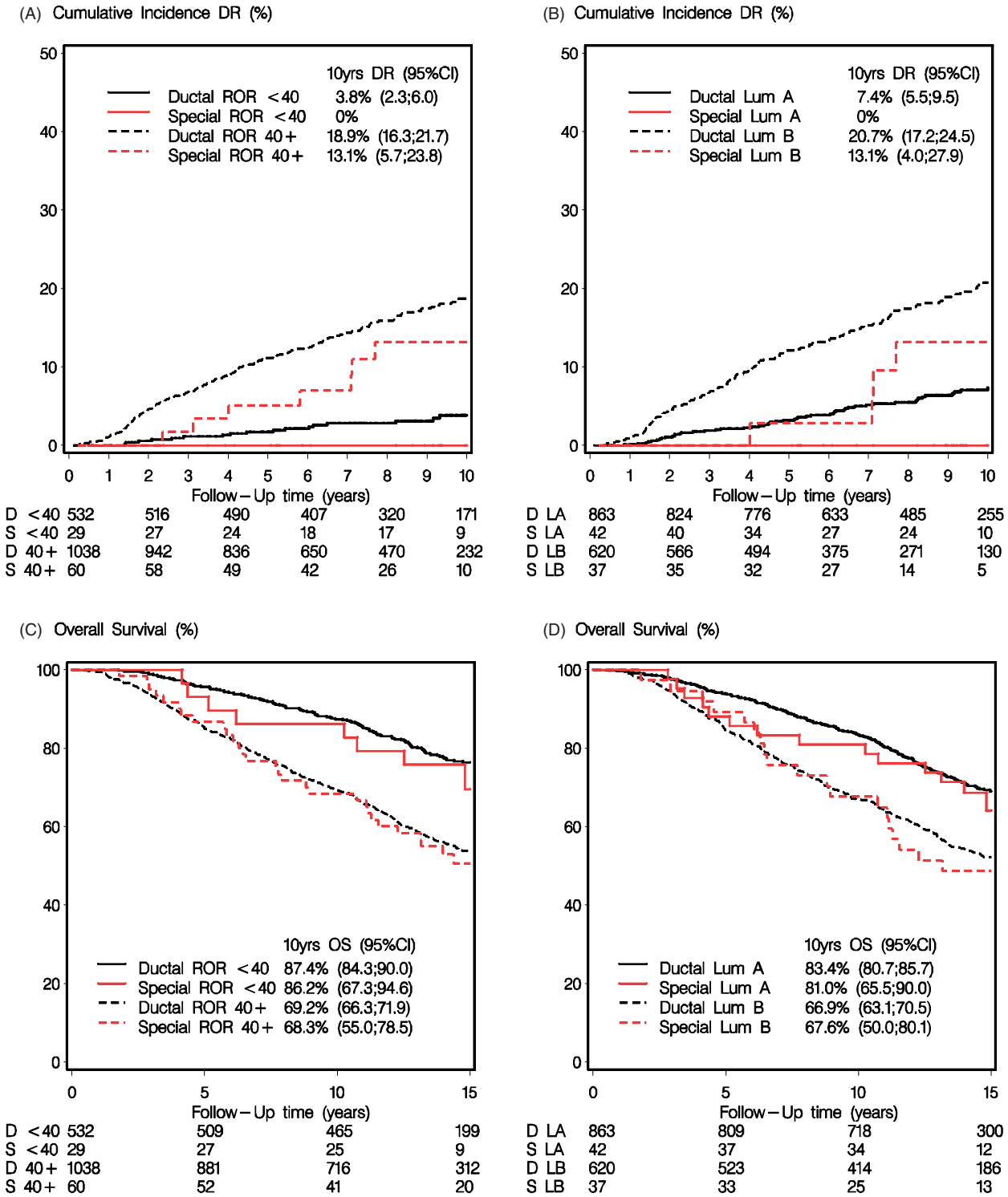


Figure 2. Cumulative incidence and overall survival according to ROR score low/intermediate versus high (A + C) and molecular subtype (B + D) for IDC and special types (black = ductal, red = special types).

The small sample size probably in addition explain why the continuous ROR provided a significant result for DR but not for ROR groups or intrinsic subtypes and neither for OS, although a similar pattern was observed. Strengths of our study include a formal prospective–retrospective design with long and detailed clinical follow up. In addition, the histopathological subclassification of breast tumors was performed by experienced breast pathologists at the Danish departments of pathology dedicated to diagnostic breast pathology.

Several multigene assays can robustly identify early stage node negative patients at sufficiently low risk of 10-year DR that they can be safely spared chemotherapy [9–17,28]. The patients recommended for molecular testing are primarily patients with ductal histology and little is known about the benefit of applying these tests in patients with breast

carcinomas of special subtypes. Due to their relative rarity, these subtypes are difficult to study outside of very large patient cohorts, as are available through the DBCG.

In daily practice, the histological special subtypes are mainly classified by light microscopy on standard haematoxylin–eosin (H&E) stained sections according to specific morphological growth patterns [1]. Correct histological diagnosis is dependent on the experience of the pathologist as well as standardized sampling of tumor sections for conclusive diagnosis since some of these tumors have mixed growth pattern. Previous studies have shown only moderate agreement (Cohen's K) between observers with respect to the histopathological classification of breast tumors [29]. Not many immunohistochemical biomarkers are available to support the morphological classification apart from loss of E-cadherin as indication of ILC, the presence of androgen receptor and Gross Cystic Disease Fluid Protein in apocrine carcinomas, the myoepithelial cytokeratins in metaplastic carcinomas and synaptophysin and chromogranin A in neuroendocrine tumors. Correct histological classification of malignant breast tumors is mandatory to guide both optimal surgery and postoperative systemic treatment [1]. Also, the increased application of neoadjuvant treatment for tumor down-staging emphasizes the need for accurate tumor subclassification even further, since low proliferative, highly ER-positive, HER2-negative tumors (luminal A-like tumors) respond poorly to neoadjuvant treatment [30]. Heng et al. [31] demonstrated that integration of gene signatures associated with morphological features might add important information with respect to drug resistance and potential targeted treatment options. In addition, the intrinsic molecular subtypes seem to show different patterns of metastasis with luminal A tumors metastasizing primarily to bone [32,33].

On the other hand, both Bomeisl et al. [34] and Turashvili et al. [35] questioned whether the OncotypeDX recurrence score (RS) was a necessary supplement for breast cancers with favorable histology. In the latter, Turasvili et al. did not identify any tumors with high RS among their cohort of 57 patients with special histological subtypes consisting of mucinous, papillary (encapsulated or solid) and tubular carcinomas [35]. This might be explained by the fact that OncotypeDX RS is based on a supervised signature that was not trained on special types.

In our study, both the mucinous and papillary group of tumors were classified into three of the four intrinsic molecular subtypes, excluding only the basal-like type. Given that the cohort was selected based on hormone receptor positivity, it is perhaps not surprising that few cases, apart from three of the five medullary carcinomas, were basal-like by Prosigna. The DBCG used a 10% cut point for estrogen receptor positivity; other cohorts employing the less stringent 1% or Allred three cut points have been found to include larger numbers of basal cases [36].

In conclusion, when applied to histological special subtypes, the continuous PAM50/Prosigna ROR score in particular remain prognostic for distant recurrence in a large population-based series of hormone receptor positive women treated with adjuvant endocrine therapy.

Disclosure statement

Anne-Vibeke Laenkholm, Maj-Britt Jensen and Bent Ejlertsen have received research funding from Nanostring Technologies Inc. Sean Ferree and Wesley Buckingham: Employment and stockholders, Nanostring Technologies Inc. Torsten O. Nielsen: Advisory role for Nanostring Technologies Inc. Torsten O. Nielsen also declare stock or other ownership for Bioclassifier. Jens Ole Eriksen declare stock or Other ownership for Novo Nordisk.

Funding

The authors thank Nanostring Technologies Inc. for financial support of the study.

References

[1] WHO Classification of Tumours of the Breast, 4th edition. Washington: WHO; 2012.
[2] Di Saverio S, Gutierrez J, Avisar E. A retrospective review with long term follow up of 11,400 cases of pure mucinous breast carcinoma. Breast Cancer Res Treat. 2008;11:541–547.
[3] Oakley GJ III Tubbs RR, Crowe J, et al. HER-2 amplification in tubular carcinoma of the breast. Am J Clin Pathol. 2006;126:55–58.
[4] Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature. 2012;486:346–352.
[5] Prat A, Parker JS, Karginova O, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 2010;12:R68.
[6] Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;21:2750–2767.
[7] Reis-Filho JS, Pusztai L. Gene expression profiling in breast cancer: classification, prognostication, and prediction. Lancet. 2011;378:1812–1823.
[8] Weigelt B, Horlings HM, Kreike B, et al. Refinement of breast cancer classification by molecular characterization of histological special types. J Pathol. 2008;16:141–150.
[9] Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351:2817–2826.
[10] Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. Jco. 2013;1:2783–2790.
[11] Gnant M, Filipits M, Greil R, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. Ann Oncol. 2014;25:339–345.
[12] Sestak I, Cuzick J, Dowsett M, et al. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal cancer study group 8 and arimidex, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. J Clin Oncol. 2015;33:916–922.
[13] Sgroi DC, Sestak I, Cuzick J, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol. 2013;4:1067–1076.
[14] Buus R, Sestak I, Kronenwett R, et al. Comparison of EndoPredict and EPclin with Oncotype DX Recurrence Score for prediction of risk of distant recurrence after endocrine therapy. J Natl Cancer Inst. 2016;108:1–7.
[15] Gnant M, Sestak I, Filipits M, et al. Identifying clinically relevant prognostic subgroups of postmenopausal women with node-

positive hormone receptor-positive early-stage breast cancer treated with endocrine therapy: a combined analysis of ABCSG-8 and ATAC using the PAM50 risk of recurrence score and intrinsic subtype. Ann Oncol. 2015;26:1685–1691.

[16] Dubsky P, Brase JC, Jakesz R, et al. The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2-breast cancer patients. Br J Cancer. 2013;109:2959–2964.

[17] Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. N Engl J Med. 2016;375:717–729.

[18] Laenkholm AV, Jensen MB, Eriksen JO, et al. The PAM50 Risk of Recurrence Score Predicts 10year distant recurrence in a comprehensive Danish cohort of 2558 postmenopausal women allocated to 5yr of endocrine therapy for hormone receptor positive early breast cancer. J Clin Oncol. 2018;57, In press.

[19] Ejlertsen B, Jensen MB, Mouridsen HT, et al. Excess mortality in postmenopausal high-risk women who only receive adjuvant endocrine therapy for estrogen receptor positive breast cancer. Acta Oncol. 2014;53:174–185.

[20] Christiansen P, Ejlertsen B, Jensen MB, et al. Danish Breast Cancer Cooperative Group. Clep. 2016;8:445–449.

[21] Talman ML, Rasmussen BB, Andersen J, et al. Estrogen Receptor analyses in the Danish Breast Cancer Cooperative Group. History, methods, prognosis and clinical implications. Acta Oncologica. 2008;47:789–794.

[22] Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.

[23] Nielsen T, Wallden B, Schaper C, et al. Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. BMC Cancer. 2014;14:177.

[24] Wallden B, Storhoff J, Nielsen T, et al. Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. BMC Med Genomics. 2015;8:54.

[25] Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res. 2010;16:5222–5232.

[26] NanoString Technologies S. Prosigna Package Insert. Seattle (WA): NanoString Technologies; 2013.

[27] Martinez SR, Beal SH, Canter RJ, et al. Medullary carcinoma of the breast: a population-based perspective. Med Oncol. 2011;28:738–744.

[28] Sestak I. Identifying biomarkers to select patients with early breast cancer suitable for extended adjuvant endocrine therapy. Breast Care (Basel). 2017;12:146–151.

[29] Stenkvist B, Bengtsson E, Eriksson O, et al. Histopathological systems of breast cancer classification: reproducibility and clinical significance. J Clin Pathol. 1983;36:392–398.

[30] Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014;384:164–172.

[31] Heng YJ, Lester SC, Tse GM, et al. The molecular basis of breast cancer pathological phenotypes. J Pathol. 2017;241:375–391.

[32] Kennecke H, Yerushalmi R, Woods R, et al. Metastatic behavior of breast cancer subtypes. JCO. 2010;28:3271–3277.

[33] Sihto H, Lundin J, Lundin M, et al. Breast cancer biological subtypes and protein expression predict for the preferential distant metastasis sites: a nationwide cohort study. Breast Cancer Res. 2011;13:R87.

[34] Bomeisl PE, Thompson CL, Harris LN, et al. Comparison of Oncotype DX Recurrence Score by histologic types of breast carcinoma. Arch Pathol Lab Med. 2015;139:1546–1549.

[35] Turashvili G, Brogi E, Morrow M, et al. The 21-gene recurrence score in special histologic subtypes of breast cancer with favorable prognosis. Breast Cancer Res Treat. 2017;165:65–76.

[36] Sheffield BS, Kos Z, Asleh-Aburaya K, et al. Molecular subtype profiling of invasive breast cancers weakly positive for estrogen receptor. Breast Cancer Res Treat. 2016;155:483–490.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 51–57
https://doi.org/10.1080/0284186X.2017.1398837

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Characterization of basal-like subtype in a Danish consecutive primary breast cancer cohort

Savvas Kinalis[a], Finn Cilius Nielsen[a], Maj-Lis Talman[b], Bent Ejlertsen[c,d] and Maria Rossing[a]

[a]Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [b]Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [c]Denmark Danish Breast Cancer Cooperative Group, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [d]Department of Clinical Oncology, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: Transcriptome analysis enables classification of breast tumors into molecular subtypes. *BRCA1/2* predisposed patients are more likely to suffer from a basal-like subtype and this group of patients displays a more distinct phenotype and genotype. Hence, in-depth characterization of this separate entity is needed.

Material and methods: Molecular subtyping was performed on a consecutive and unselected series of 1560 tumors from patients with primary breast cancer. Tumors were classified by the 256 gene expression signature (CIT) and associated with basic clinical characteristics and aggregated expression levels in the hallmark gene sets.

Results: Of the 1560 samples, 168 were classified basal-like and 120 patients were screened for *BRCA1/2* mutations, resulting in 19 *BRCA1/2* carriers, 95 non-carriers and six patients carried variants of unknown significance. The *BRCA1/2* carriers were significantly younger and there were no carriers above 60 years of age. The tumors showed a loss in DNA-repair profile, as well as an upregulation in proliferative cancer signaling pathways. A robust molecular signature for identification of the *BRCA1/2* - carriers was infeasible in the current cohort. Patients with a basal like breast cancer had the lowest median age and the largest median tumor size. They were almost exclusively diagnosed in disease stage III.

Conclusions: Basal-like subtype is indeed a separate entity compared with other molecular breast cancer subtypes and the clinical course for this patient group should reflect the aggressiveness of this cancer. Taken together, patients being diagnosed with a basal-like breast cancer are in the youngest segment of breast cancer patients and are mainly diagnosed in stage III disease.

ARTICLE HISTORY
Received 15 September 2017
Accepted 25 October 2017

Introduction

Breast cancer is the most common malignancy in women and despite considerable advances in early detection, diagnosis, and treatment, breast cancer remains one of the leading causes of cancer death [1]. Breast tumors are heterogeneous and are the first solid malignancy where specific molecular treatment factors were introduced [2–4]. Breast tumors can be classified into at least four intrinsic subtypes. One of the key factors distinguishing the different subtypes is receptor status; Luminal A and Luminal B are estrogen receptor (ER) and progesterone (PR)-positive and receptor tyrosine-protein kinase erbB-2 (ERBB2)-negative. The ERBB2 positive subtype is characterized by the ERBB2 positive samples and finally the Basal-like subtype is predominated by receptor negative samples [5]. Based on signaling pathways, copynumber alterations, histopathological and clinical features, including metastatic sites and relapse free survival, Guedj et al. refined the taxonomy by introducing six stable molecular subtypes assigned with normal-like, luminal A, luminal B, luminal C, molecular apocrine and basal-like [6]. The classifier provided by Guedj at al. (CIT classifier) captures the characteristics of the four major subgroups of breast cancer patients [7] and at the same time enables finer clustering associated with distinct clinical and molecular characteristics, e.g., the molecular apocrine subtype is an ER/PR/HER2-negative but Androgen receptor positive subcluster with a poor prognosis. Moreover the CIT classifier identifies the normal-like samples which share many molecular features with the luminal A subtype, including low proliferation, however the normal-like subgroup exhibits improved prognostic behavior [6]. Furthermore, the CIT classifier is readily available as open-source software that uses microarray expression data as input. Another advantage of running the classifier based on microarray data is their reusability, i.e., proliferation index and receptor status can be computed based on the same microarray data.

The basal-like subtype is the most distinct type of the intrinsic subgroups and has more common molecular features with squamous cell lung cancer than with the luminal A or B subtypes [7,8]. Hence, this supports the hypothesis

CONTACT Maria Rossing caroline.maria.rossing@regionh.dk Center for Genomic Medicine, Rigshospitalet, Blegdamsvej 9, Copenhagen DK-2100, Denmark

that basal-like subtype should be considered a separate entity when breast cancer is assessed [9]. The neoplastic cells of basal-like breast cancers generally express genes in common with normal basal or myoepithelial cell profile of the breast [3,10]. Furthermore, basal-like cancers are predominated by the lack of expression of hormone receptors; ER, PR and of ERBB2 – receptors [11]. If diagnosed by immunohistochemistry (IHC), the assigned diagnose is triple-negative breast cancer (TNBC), still the terminologies tend to be used interchangeably, whether it is IHC or molecular signature derived tumor classification. This is unfortunate because the therapeutic responses differ between basal-like tumors and other TNBC that are non-basal-like [12]. All though the basal-like subgroup has shown to be a more homogenous group on the molecular level than the TNBC diagnosed by immunohistochemistry, patients assigned with this subtype have demonstrated some diversity in their outcome [13]. In fact, a recent study proposed a molecular signature for identification of two separate groups within the basal-like subtype, with significant differences in patient survival outcomes [14]. Basal-like breast cancers are known for their high proliferation rate and their aggressive behavior and patients suffering from this molecular subtype have a poor prognosis and a short-term disease free survival (DFS) as well as overall survival (OS) [12,15].

Studies have shown a higher prevalence of *BRCA1/2* predisposing mutations among patients with TNBC and basal-like subtype [15,16] and patients with either type of these two breast cancer types are frequently *BRCA1* carriers [17,18]. Normal functioning BRCA1/2 proteins suppress genome instability by promoting the homologous recombination repair (HRR)-system [19,20]. Homologous recombination repair is essential to avoid DNA double-strand breaks, often caused by UV light and metabolic processes, and is a major error-free DNA-repair pathway [21,22]. Therefore, basal-like subtype, enriched in patients with germline pathogenic variants in *BRCA1/2*, may reflect deficiency in HRR and loss of DNA-repair mechanism in their molecular profiles in comparison to the remaining subtypes [23].

In a Danish cohort of more than 1500 prospective and consecutive primary breast cancer patients, we aimed to characterize the basal-like cancers according to basic clinical parameters and molecular hallmarks of cancer [24]. Furthermore, we examined if a distinct molecular profile can identify the *BRCA1/2* carriers among the basal-like tumors.

Material and methods

Patients and tumor samples

The sample cohort consisted of 1560 female patients diagnosed with breast cancer of stage I–III. The patients were clinically and diagnostically assessed at Rigshospitalet between 2014 and 2017. Fresh tumor specimens, extracted during surgery, were inspected by pathologists and tumor biopsies of around 100 mg were stored in RNALater (Thermo Fisher Scientific, Waltham, MA, USA). The Danish data Protection Agency (jr. no.: 2012-58-0004) and Danish Breast Cancer Group (jr. no.: DBCG-2015-14) approved the study.

Gene expression and subclass analyses

RNA was isolated using the AllPrep DNA/RNA purification kit (Qiagen, Hilden, Germany). The integrity of the RNA was measured using the Agilent RNA 6000 Nano Kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). RNA was reverse transcribed and used for cRNA synthesis, labeling and hybridization with GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. In short, arrays were washed and stained with phycoerytrin conjugated streptavidin using the Affymetrix Fluidics Station 450, and scanned in the Affymetrix GeneArray 3000 7G scanner to generate fluorescent images. Cell intensity files (.CEL files) were generated in the GeneChip Command Console Software (AGCC; Affymetrix, Santa Clara, CA, USA). The probe level data (.CEL files) were transformed into expression measures using R version 3.4.1 (https://www.R-project.org/). Raw intensity .CEL files were preprocessed by quantile normalization, and probe summaries were extracted via robust multi-array average (RMA). The CIT classifier [6] was applied to the probe expression data, by assigning each sample, a molecular subtype; normal-like, luminal A, luminal B, luminal C, molecular apocrine and basal-like. Gene expression values were deduced from the probe expression values, by taking the median probe expression value, in case more than one probe corresponded to the same gene. Each of the CIT subtypes was given a score on each of the hallmark gene sets from the Molecular Signatures Database (MsigDB). The score corresponded to the mean value of the expressions of the genes contained in each hallmark gene set and the expressions of all samples from each subtype. The average expression of normal samples (from Rigshospitalet) was deducted from the expression values of the rest of the samples before the aggregation; hence the normal samples were used for centering.

It should be noted that the subtypes derived by the CIT classifier correlate to the ones from the PAM50 classifier, with few differences; CIT normal-like subgroup does not correspond to normal breast tissue but exhibits similar expression profiles to the luminal A subgroup. The CIT normal-like samples would classify as PAM50-luminal A. The CIT luminal C and molecular apocrine subtypes include samples with overexpressed HER2; however, the two subtypes represent patients with distinct ER status (positive and negative, respectively). CIT luminal A and B largely overlap with the PAM50 luminal A and B subtypes.

Blood sample and germline mutation screening

Genomic DNA was isolated using the ReliaPrep Large Volume HT gDNA Isolation Kit (Promega, Madison, WI, USA) and a Tecan Freedom EVO HSM2.0 Workstation according to the manufacturer's instructions. Mutation screening was done by the breast cancer-predisposing gene-panel as previously described [25]. Sequencing was performed on a MiSeq (Illumina, San Diego, CA, USA) to an average depth of at least 100×. Sequencing data were analyzed using Sequence Pilot (JSI Medical Systems, Ettenheim, Germany), where variants

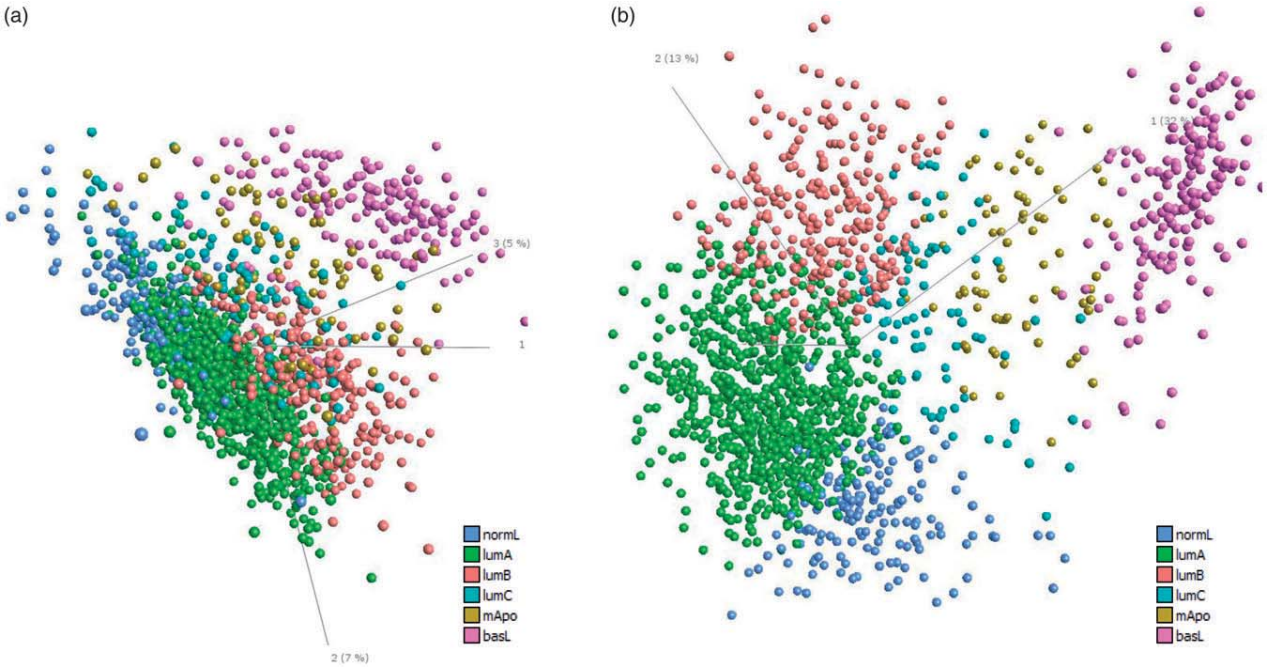


Figure 1. The three most variable components of the principal component analysis (PCA) of the gene expression profiles. (a) All genes of the Affymetrix platform are considered and (b) only genes from the 256 gene signature (CIT) are considered. The distinction between the basal-like and the more heterogeneous luminal subtypes is clearly illustrated by the PCA plot, even before selecting for the CIT gene signature.

are called if the non-reference base frequency was above 25%. Variants are numbered according to the following GenBank accession numbers: NM_007294 (BRCA1) and NM_000059 (BRCA2) using the guidelines from the Human Genome Variation Society (www.hgvs.org/mutnomen). All class 3–5 variants were verified by Sanger sequencing on an ABI 3730 DNA Analyzer using DNA purified from a second blood sample.

Statistical analysis

The data processing was performed using the R software (https://www.R-project.org/) and packages from the Bioconductor project [26]. Cell intensity files were processed with the R package *simpleaffy* (http://bioinformatics.picr.man.ac.uk/simpleaffy/). Molecular subtypes were predicted using package *citbcmst* (https://CRAN.R-project.org/package=citbcmst). Linear models were fit with R package *limma* [27]. The principal component analysis (PCA) visualizations and the heatmaps were performed in the Qlucore Omics Explorer™ software (Qlucore AB, Lund, Sweden). The rest of the graphics were done using the R package *ggplot2* [28].

Results

Subtype distribution

The patient cohort consisted of 1560 consecutive primary breast cancers in stage I–III. The distribution of subclasses were derived from the 256 gene signature (referred to as the CIT classifier further on), and resulted in 161 normal-like (normL), 777 luminal A (lumA), 284 luminal B (lumB), 93 luminal C (lumC), 77 molecular apocrine subtype (mApo) and 168 basal-like (basL) samples. The intrinsic subtypes were depicted in the PCA plots of gene expression values, where the differentiation of the basal-like group compared to the rest of the groups was evident (Figure 1(a)). The deviation appeared even before reducing the gene set, from all the genes contained in the Affymetrix platform, to the 256 gene signature, which is illustrated in Figure 1(b). The heterogeneous luminal types were harder to distinct, which is also clearly shown in both Figure 1(a,b).

Clinical characteristics

The 168 samples classified as basal-like subtype included some ER and HER2 positive samples based on IHC, substantiating the fact that basal-like subtype are not identical to TNBC. In particular, among the 168 patients; 10 had HER2 positive status and 158 had negative status, 56 were ER positive with a cutoff of 1% and 112 were negative, 31 were ER positive with a cutoff of 9% and 137 were negative. Patients with basal-like breast cancer were significantly younger at the time of diagnosis compared to other patient groups (see Figure 2(a)). Tumor size did not vary significantly across the subtypes (see Figure 2(b)). Apart from normal-like, age at diagnosis was inversely correlated to tumor size e.g., the younger the age at diagnosis, the larger the tumor size. We correlated clinical stage at the time of diagnosis to the six individual molecular subclasses, and the relative comparison showed common trends. Hence, normal-like and luminal A breast cancers were predominantly diagnosed at stage II, luminal B and C as well as molecular apocrine subtype were more often diagnosed at stage III rather than stage II. However, patients with basal-like cancers were almost exclusively diagnosed with stage III disease (see Figure 2(c)).

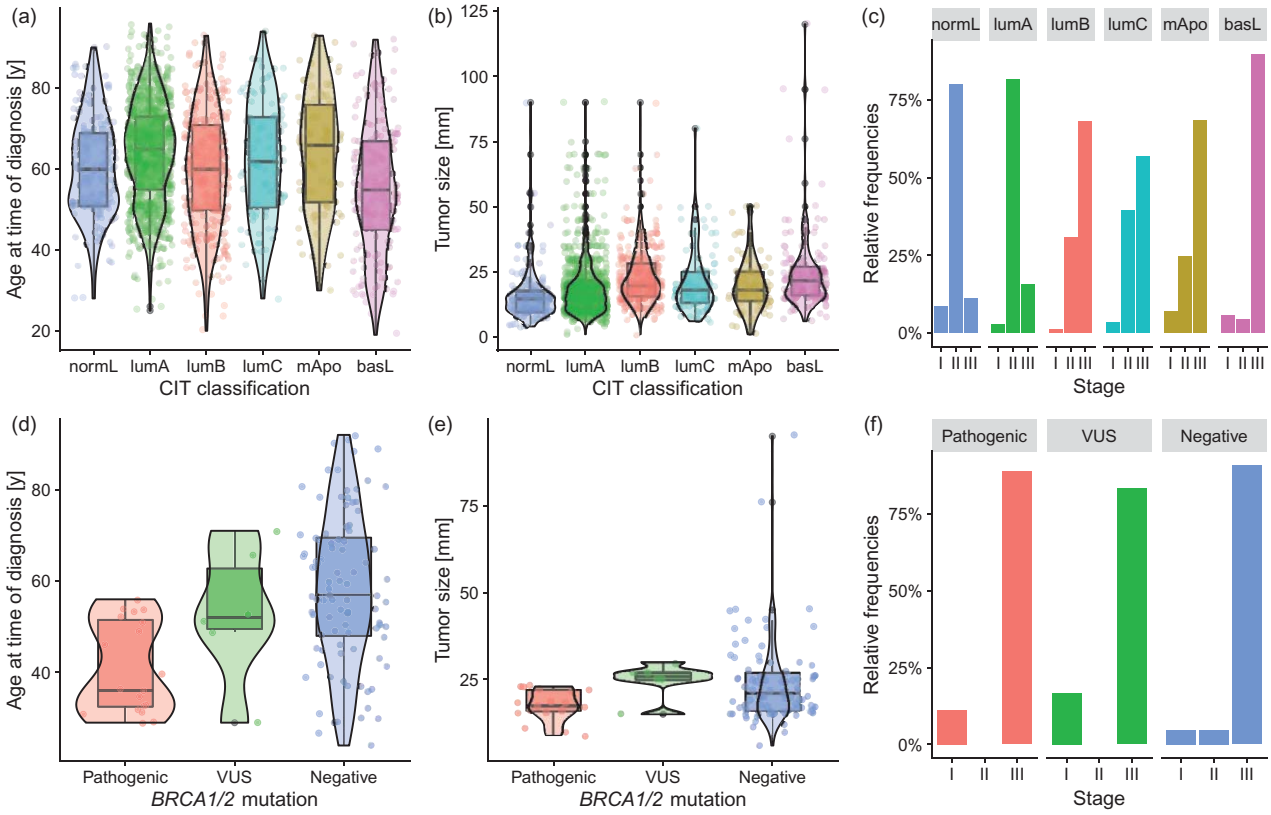


Figure 2. (a) Violin and box plots of the age distribution at the time of diagnosis (y-axis) of molecular subtypes (x-axis). (b) Violin and box plots of the tumor size (y-axis) of molecular subtypes (x-axis). (c) Relative frequency distribution chart of disease stage (x-axis) and relative frequency of occurrence (y-axis). The chart is shown separately for each subtype. (d) Violin and box plots of age distribution at the time of diagnosis (y-axis) of *BRCA1/2* carriers and non-carriers (x-axis). VUS: variant of unknown significance. (e) Violin and box plots of tumor size (y-axis) of *BRCA1/2* carriers (x-axis). (f) Relative frequency distribution chart of disease stage (x-axis) and the relative frequency of occurrence (y-axis). The chart is shown separately for group of patients (carriers and non-carriers).

Hallmark characterization

To unravel the basal-like portrait we examined the signaling pathways from the well-established hallmarks of cancer by assigning a subtype score to each pathway (Figure 3). The heatmap clearly depicts that the basal-like cancers were receptor-negative (see estrogen and androgen response pathways) and shows a clear upregulation of molecular profile related to the proliferative cancer signaling pathways (see E2F targets, mitotic spindle, KRAS and WNT). In addition, the TP53 tumor suppressor pathway was downregulated. Basal-like and molecular apocrine subtypes were clearly distinct by their metabolic and protein secreting pathways. Immune response pathways were upregulated in basal-like similar to molecular apocrine and luminal C. One of the DNA repair pathways was downregulated; however two other DNA repair pathways were upregulated. Thus, this indicates that neoplastic cells from basal-like samples have lost their cellular modeling and developmental features. In conclusion, the molecular hallmarks paint a portrait that the basal-like samples in our cohort were neoplastic, with highly proliferative features and clear immuno-response profiles.

BRCA1/2 predisposed patients

As part of our diagnostic pipeline our basal-like patients were offered genomic screening to detect if they were carriers of a *BRCA1* or *BRCA2* pathogenic variants. One of the hypotheses in the present study was to identify distinct features between the *BRCA1/2* carriers opposed to non-carriers, also diagnosed with a basal-like breast cancer. At the time of data collection for the present study, 120 patients were screened for genetic variants in *BRCA1/2* out of a total of 168 breast cancer patients with a basal-like subtype. Forty-eight patients did not enter the screening procedure since a blood samples were not obtained at the day of surgical procedure, which was the rule for inclusion in this clinical prospective study. The results of the screening showed that 19 patients were pre-disposed, 6 had a variant of unknown significance (VUS) and 95 were *BRCA1/2* negative. We explored the clinical characteristics of the *BRCA1/2* carriers in comparison to non-carriers with a basal-like subtype.

The clinical characteristics of the screened patients show that the *BRCA1/2* carriers were significantly younger than the *BRCA1/2* non-carriers (see Figure 2(d)). Neither tumor size nor stages were significantly different in *BRCA1/2* carriers as compared to non-carriers (see Figure 2(e,f)).

To extend upon the observation that *BRCA1/2* carriers were significantly younger than the *BRCA1/2* non-carriers, we sought to identify differentially expressed genes between the two patient groups. An additive linear model with age and pathogenic mutations as the response variables was fit to the gene expression data. The age was divided into groups of over 60 years of age and younger patients with a

Figure 3. Heatmap of scaled mean expression values of molecular subtypes for each hallmark gene set. Expressions level of genes contained in each hallmark and the expressions of all samples from each subtype are aggregated, by the mean value. The color ranges from bright green for the lowest expression values to bright red for the highest. Heatmap of the scaled mean expression values of patients above 60 years of age (*BRCA1/2* non-carriers, assigned α) and *BRCA1/2* carriers, assigned β, for each hallmark gene set. Expressions of genes contained in each hallmark and expressions of samples from each group are aggregated, by the mean value. The color ranges from bright green for the lowest expression values to bright red for the highest.

pathogenic mutation in *BRCA1/2*. The contrasts of gene expression profiles between these two patient groups were examined and differentially expressed genes for that contrast and the corresponding p and q-values were computed. The histogram of the p-values resembled a uniform distribution. The analysis showed that *IMPDH2* gene was the most differentiated (q-value $=0.063$) and upregulated in the patients >60 years of age and without a pathogenic mutation. To pursue possible differences in molecular signaling pathways between the two clinically distinct subclusters we generated a heatmap based on the hallmark signatures (see Figure 3). The main differences in the BRCA1/2-carriers compared to the non-carriers were a clear loss in DNA-repair mechanism and relative upregulation in KRAS, Hedgehog and WNT_BETA_CATENIN – signaling pathways. Furthermore, the proliferation rate was altered among the two sub-clusters and the heatmaps demonstrate a higher proliferation in the non-carrier-cluster. The ER-response signals were relatively higher in the BRCA1/2-carrier cluster.

Discussion

The prognostic and predictive features associated with a basal-like breast cancer, as well as distinct molecular profiles, indicate that this subtype should be considered a separate entity. In particular, as it has been shown, that patients with basal-like breast cancer have a profound increase in the risk of recurrence and mortality [12]. In this study, we examined the clinical features of consecutive breast cancer patients and established that basal-like subtype is diagnosed in significantly younger patients who are almost exclusively in stage III disease. We further exploited the molecular hallmarks of basal-like samples and confirmed that this subtype is distinguished by its high proliferative signaling pathways. However, we received a complex signal in the DNA repair pathways, although we expected them to be lost since this subtype is known to have a higher proportion of *BRCA1/2* carriers.

We exploited the basal-like subtype by exploring the hallmark gene sets of two clinically separate phenotypes and found that the downregulation of DNA – repair mechanisms is more substantiated among the *BRCA1/2* carriers. The differences in the hallmark gene sets were not adequate to establish a robust molecular signature for future identification of *BRCA1/2* based on their molecular signature alone. Moreover, no significant correlation was established between age at diagnosis, pathogeny of mutations and gene expression profile. Since the comparison of the two basal-like subgroups did not generate a clear signature at the molecular level it may possibly imply that there are other genes, not yet annotated, that predispose to same type of distinct breast cancer type [29]. However, a previous Danish study including >180 samples and close to a third ($n = 55$) of *BRCA1/2* carriers were somewhat closer in building a molecular signature for identification of mutation carriers, although it was concluded that further validation is needed [30]. Additionally, the sample size of our study is not scaled appropriately as the number of patients carrying a pathogenic mutation ($n = 19$) is largely lower, compared to the number of genes under consideration ($n = 20545$). Intriguingly though, none of the hereditary basal-like cancers was identified in the patient group above 60 years of age. However, we cannot conclude that patients above a certain age with a basal-like subtype are not at risk of being predisposed. Repeating this comparison in a larger study would be of great interest. Comparing our cohort and number of patients with a pathogenic *BRCA1/2* carrier, we found that previous studies included a much higher frequency (up to 60% of *BRCA1/2* carriers; data not shown) [30,31]. With a larger patient cohort and more *BRCA1/2* carriers in particular, we would expect to predict when there is high probability that the patient is a non-carrier of pathogenic mutation. However, the results are somewhat unique since they reflect a true clinical setting as all tissue samples were obtained and analyzed within the 10 d of the surgical procedure from primary breast cancer patients. Overall, patients with basal-like breast cancer are at great risk of recurrent disease and death. Thus, the clinical course for this patient group should be tailored and reflect the aggressiveness of this cancer. In line with this, upfront identification of *BRCA1/2* carriers could lead to stratified treatment with poly(ADP-ribose) polymerase inhibitors (PARP-inhibitors) [32] and result in a greater implementation of precision medicine for this challenging group of patients.

Taken together, we established that basal-like subtype is diagnosed in significantly younger patients who are almost exclusively in stage III disease. Patients with a basal like breast cancer had the lowest median age and the largest median tumor size. The BRCA1/2 carriers were significantly younger and we did not identify any carriers above 60 years of age.

Acknowledgments

We would like to thank Line Offenbach Jacobsen, Søren Petersen and Bettina Marsot Andersen for excellent laboratory assistance.

Disclosure statement

The authors have no conflicts of interest to declare.

Funding

The authors received no funding for this study.

Reference

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66:7–30.
[2] Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2000 breast tumours reveals novel subgroups. Nature. 2012;486:346–352.
[3] Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature. 2000;406:747–752.
[4] Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98:10869–10874.
[5] Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA. 2003;100:8418–8423.
[6] Guedj M, Marisa L, de RA, et al. A refined molecular taxonomy of breast cancer. Oncogene. 2012;31:1196–1206.
[7] Cancer Genome Atlas Network.Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61–70.
[8] Prat A, Adamo B, Fan C, et al. Genomic analyses across six cancer types identify basal-like breast cancer as a unique molecular entity. Sci Rep. 2013;3:3544.
[9] Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast. 2015;24: S26–S35.
[10] Page MJ, Amess B, Townsend RR, et al. Proteomic definition of normal human luminal and myoepithelial breast cells purified from reduction mammoplasties. Proc Natl Acad Sci USA. 1999;96:12589–12594.
[11] Prat A, Adamo B, Cheang MC, et al. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist. 2013;18:123–133.
[12] Banerjee S, Reis-Filho JS, Ashley S, et al. Basal-like breast carcinomas: clinical outcome and response to chemotherapy. J Clin Pathol. 2006;59:729–735.
[13] Bertucci F, Finetti P, Birnbaum D. Basal breast cancer: a complex and deadly molecular subtype. Curr Mol Med. 2012;12:96–110.
[14] Milioli HH, Tishchenko I, Riveros C, et al. Basal-like breast cancer: molecular profiles, clinical features and survival outcomes. BMC Med Genomics. 2017;10:19.

[15] Schneider BP, Winer EP, Foulkes WD, et al. Triple-negative breast cancer: risk factors to potential targets. Clin Cancer Res. 2008;14:8010–8018.
[16] Foulkes WD, Stefansson IM, Chappuis PO, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst. 2003;95:1482–1485.
[17] Jung J, Kang E, Gwak JM, et al. Association between basal-like phenotype and BRCA1/2 germline mutations in Korean breast cancer patients. Curr Oncol. 2016;23:298–303.
[18] Peshkin BN, Alabek ML, Isaacs C. BRCA1/2 mutations and triple negative breast cancers. Breast Dis. 2010;32:25–33.
[19] Moynahan ME, Chiu JW, Koller BH, et al. Brca1 controls homology-directed DNA repair. Mol Cell. 1999;4:511–518.
[20] Prakash R, Zhang Y, Feng W, et al. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb Perspect Biol. 2015;7:a016600.
[21] Bouwman P, Jonkers J. The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nat Rev Cancer. 2012;12:587–598.
[22] Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends Cell Biol. 2016;26:52–64.
[23] Weigman VJ, Chao HH, Shabalin AA, et al. Basal-like breast cancer DNA copy number losses identify genes involved in genomic instability, response to therapy, and patient survival. Breast Cancer Res Treat. 2012;133:865–880.
[24] Liberzon A, Birger C, Thorvaldsdottir H, et al. The molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1:417–425.
[25] Jonson L, Ahlborn LB, Steffensen AY, et al. Identification of six pathogenic RAD51C mutations via mutational screening of 1228 Danish individuals with increased risk of hereditary breast and/or ovarian cancer. Breast Cancer Res Treat. 2016;155:215–222.
[26] Huber W, Carey VJ, Gentleman R, et al. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Meth. 2015;12:115–121.
[27] Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47.
[28] Wickham H. ggplot2: Elegant graphics for data analysis. New York (NY): Springer-Verlag; 2009.
[29] Nielsen FC, van Overeem HT, Sorensen CS. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer. 2016;16:599–612.
[30] Larsen MJ, Kruse TA, Tan Q, et al. Classifications within molecular subtypes enables identification of BRCA1/BRCA2 mutation carriers by RNA tumor profiling. PLoS One. 2013;8:e64268.
[31] Jonsson G, Staaf J, Vallon-Christersson J, et al. The retinoblastoma gene undergoes rearrangements in BRCA1-deficient basal-like breast cancer. Cancer Res. 2012;72:4028–4036.
[32] Hiller DJ, Chu QD. Current status of poly(ADP-ribose) polymerase inhibitors as novel therapeutic agents for triple-negative breast cancer. Int J Breast Cancer. 2012;2012:829315.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 58–66
https://doi.org/10.1080/0284186X.2017.1398416

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Molecular subtyping of breast cancer improves identification of both high and low risk patients

Maria Rossing[a], Olga Østrup[a], Wiktor W. Majewski[a], Savvas Kinalis[a], Maj-Britt Jensen[b], Ann Knoop[c], Niels Kroman[d], Maj-Lis Talman[e], Thomas V. O. Hansen[a], Bent Ejlertsen[b,c]* and Finn C. Nielsen[a]*

[a]Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [b]Danish Breast Cancer Cooperative Group, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [c]Department of Clinical Oncology, Copenhagen University Hospital, Copenhagen, Denmark; [d]Department of Breast Surgery, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [e]Department of Pathology, Copenhagen University Hospital, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: Transcriptome analysis enables classification of breast tumors into molecular subtypes that correlate with prognosis and effect of therapy. We evaluated the clinical benefits of molecular subtyping compared to our current diagnostic practice.

Materials and methods: Molecular subtyping was performed on a consecutive and unselected series of 524 tumors from women with primary breast cancer ($n = 508$). Tumors were classified by the 256 gene expression signature (CIT) and compared to conventional immunohistochemistry (IHC) procedures.

Results: More than 99% of tumors were eligible for molecular classification and final reports were available prior to the multidisciplinary conference. Using a prognostic standard mortality rate index (PSMRi) developed by the Danish Breast Cancer Group (DBCG) 39 patients were assigned with an intermediate risk and among these 16 (41%) were furthermore diagnosed by the multi-gene signature assigned with a luminal A tumor and consequently spared adjuvant chemotherapy. There was overall agreement between mRNA derived and IHC hormone receptor status, whereas IHC Ki67 protein proliferative index proved inaccurate, compared to the mRNA derived index. Forty-one patients with basal-like (basL) subtypes were screened for predisposing mutations regardless of clinical predisposition. Of those 17% carried pathogenic mutations.

Conclusion: Transcriptome based subtyping of breast tumors evidently reduces the need for adjuvant chemotherapy and improves identification of women with predisposing mutations. The results imply that transcriptome profiling should become an integrated part of current breast cancer management.

ARTICLE HISTORY
Received 15 September 2017
Accepted 24 October 2017

Introduction

Despite the early detection and improvement of treatment over the last decades, breast cancer is still the second leading cause of cancer-related death in women [1]. Transcriptomic and genomic profiling has enabled classification of breast cancer into intrinsic molecular subtypes and breast cancer is no longer considered a single disease [2–6]. The subtypes are biologically distinct entities with specific prognostic and therapeutic features. The pivotal study proposed five subclasses: (i) the ER-receptor positive and human epidermal growth factor receptor 2 (HER2)-receptor negative tumors i.e., luminal A (lumA), luminal B (lumB) and normal breast-like subclass, (ii) the HER2-receptor positive tumors: HER2-like subclass and (iii) the ER- and HER2-receptor negative tumors called the basal-like (basL)subclass [2–5]. Four of the subclasses can be distinguished by a 50-gene molecular classifier (PAM50) which has been developed as a commercial FDA approved platform (Prosigna®) [7]. Recent taxonomies optimized the subclasses by applying integrative genomic analysis [8,9] and Guedj et al. [9] refined the subclasses by introducing six stable molecular subtypes based on genomic rearrangement and the expression of 256 transcripts. This taxonomy is remarkably robust and has been validated in nearly 3000 breast cancer samples, showing a high correlation between clinical characteristic and patient outcome [9]. The CIT-classifier is however not validated in randomized trials like, e.g., the PAM50/Nanostring and the MammaPrint signatures [10,11]. The PAM50 and the CIT signatures are based on RNA isolated from tissue stored under very different conditions and include an uneven number of subgroups, e.g., the PAM50 do not comprise the normal-like (normL) or the molecular apocrine (mApo) subgroups; thus a direct comparison of the PAM50 signature derived from microarray data is irrelevant. Based on survival data, other commercial molecular algorithms have emerged, that score the samples into low, medium or high clinical risk of recurrent disease [7,12–14]. However, a comprehensive genomic study

CONTACT Maria Rossing caroline.maria.rossing@region.dk Center for Genomic Medicine, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark
*Equal contribution

Supplemental data for this article can be accessed here.

integrating both genetic and epigenetic alterations concluded that breast cancers are biologically defined by five intrinsic subtypes and that clinical heterogeneity can be explained by subsets within the subtypes [15]. We chose to implement the CIT-classifier as a supplement to the existing diagnostic set-up, as it was built on an open-source and high-throughput platform (Affymetrix U133A gene expression microarrays) and included all of the intrinsic subtypes [16,17]. Moreover the transcriptomic data could provide any additional signatures, e.g., receptor status, without excess costs.

Still, in daily clinical practice the definition of molecular subtypes are only important if this knowledge improves the standard of care. It is well-established that the biological hallmark of luminal A subtype is low proliferation, high expression of the ESR1 gene and a favorable clinical outcome [4,5,18]. Since 2011, the St Gallen international expert consensus panel has recommended merely endocrine therapy in patients with luminal A disease [19,20]. Attempts have been made indirectly to approximate luminal A - like subtype by the use of IHC biomarkers - ER and/or PGR positive, HER2 negative and low Ki67 protein staining [20–22,]. Classification with only four biomarkers may however not entirely recapitulate the intrinsic subtype of breast cancer [18,23], so from 2014, we optimized our diagnostic work-up and implemented the six-class taxonomy on all patients undergoing breast cancer surgery at Rigshospitalet as a supplement to the existing procedures (Figure 1). Subsequently (in the current year), the Danish Breast Cancer Group (DBCG) introduced their revised guidelines recommending a PAM50 classifier for patients at intermediate risk.

Here, we present the data from the first year of systematic molecular diagnostics of unselected breast cancer patients. The aim was to determine the feasibility of microarray-based transcriptomic profiling and to evaluate the reliability of molecular subtyping compared to our traditional stratification of the patients. Moreover, we analyzed the presumptive benefits of molecular subtyping for selection of patients eligible for genetic screening of six breast/ovarian cancer predisposing genes. Taken together we report that transcriptome based subtyping of breast tumors reduces the need for adjuvant chemotherapy and improves identification of women with predisposing mutations compared to the conventional work-up.

Material and methods

Patients and tumor samples

Over 11 months (2014–2015) consecutive female breast cancer patients (Stage I–III) were included in the study cohort provided they received breast and histopathological assessment at Rigshospitalet. The study was approved by

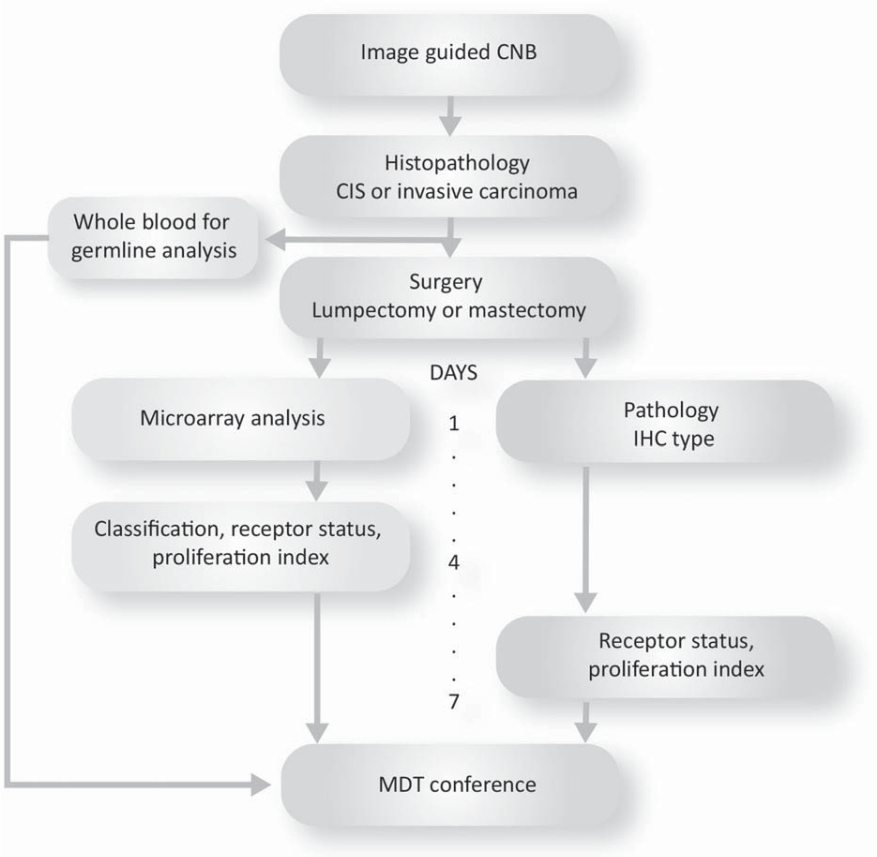


Figure 1. Complete diagnostic work-up. A workflow illustrating the routine assessment of consecutive breast cancer patients enrolled in the complete diagnostic work-up including both standard histopathological evaluation and microarray analysis. In addition, blood samples were obtained for screening of germline predisposition, in case of a basal-like subtype, receptor negative profile or if patients were under the age of 40. A final report on molecular subtyping was available for clinical decision at the following multidisciplinary conference.

The Danish data Protection Agency (jr. no.: 2012-58-0004) and DBCG, (jr. no.: DBCG-2015-14). Following surgical resection, fresh tumor specimens were evaluated by designated pathologists and tumor biopsies (≈100 mg) were stored in RNALater (Thermo Fisher Scientific Co., Waltham, MA,USA). A neighboring tumor section was sampled for verification of invasive tumor tissue in each case. Screening for genetic predisposing mutations followed the guidelines of DBCG (In short: breast cancer before the age of forty, both breast and ovary cancer, two first-degree relatives diagnosed before the age of fifty or three first-degree relatives with at least one before the age of fifty). Following implementation of molecular subgroups, patients with either a TNBC or a basL subtype were likewise tested for predisposing mutations.

Clinical risk assessment

According to the treatment algorithm of DBCG, patients 60 years or older with a node negative T1, ER positive and HER2 negative breast cancer are assigned to the low risk group and is not recommended adjuvant systemic treatment. Patients in the intermediate and high risk groups with ER positive breast cancer are recommended adjuvant endocrine therapy with tamoxifen or an aromatase inhibitor according to their menopausal status [24]. One year of trastuzumab combined with three weekly cycles of eprirubicin and cyclophosphamide followed by three weekly cycles of docetaxel were recommended to patients with HER2 overexpressed or amplified tumors [25]. In postmenopausal patients with intermediate or high risk ER positive breast cancer a prognostic standard mortality rate index (PSMRi) is used to allocate patients to adjuvant chemotherapy. The PSMRi was built by the DBCG, from the data of 6529 postmenopausal patients, post-surgery for ER positive breast cancer were allocated to five years of an aromatase inhibitor or tamoxifen [26]. Patients without excess mortality by PSMRi are not recommended for adjuvant chemotherapy.

Gene expression analysis

RNA was isolated using the AllPrep DNA/RNA purification kit (Qiagen, Hilden, Germany) and the QIACube workstation according to the manufacturer's instructions. The integrity of the RNA was measured using the Agilent RNA 6000 Nano Kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The purified RNA was immediately analyzed on arrays. RNA was reverse transcribed and used for cRNA synthesis, labeling and hybridization with GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. The arrays were washed and stained with phycoerytrin conjugated streptavidin using the Affymetrix Fluidics Station 450 and the arrays were scanned in the Affymetrix GeneArray 3000 7G scanner to generate fluorescent images. Cell intensity files (.CEL files) were generated in the GeneChip Command Console Software (AGCC; Affymetrix). Raw intensity .CEL files were preprocessed by quantile normalization and gene summaries were extracted via robust multi-array average (RMA). The probe level data (.CEL files) were transformed into expression measures using R version 3.2.2 (https://www.R-project.org/). Expression values of each sample were combined in a batch with expression measures of 12 selected samples from the study. Subsequently, the values were batch corrected against CIT 'core set' using 'ComBat' implemented in 'sva' R package [27,28]. After such processing, breast cancer subtyping was performed using CIT predictor [9] incorporated in 'citbcmst' R package (http://CRAN.R-project.org/package=citbcmst). The subtypes are assigned to the closest centroids computed on 375 probe sets. To test the in-house settings, we used the CIT 'core set', and found compliance of the assigned subclasses. Microarray data are available as .CEL files in the online repository Array Express (accession number: E-MTAB-5724). It was noted, that the subtypes derived by the CIT classifier correlate to the ones from the PAM50 classifier, with few differences; CIT normal-like subgroup does not correspond to normal breast tissue but exhibits similar expression profiles to the luminal A subgroup. The CIT normal-like samples would classify as PAM50-luminal A. The CIT luminal C (lumC) and molecular apocrine subtypes include samples with overexpressed HER2; however the two subtypes represent patients with distinct ER status (positive and negative, respectively). CIT luminal A and B largely overlap with the PAM50 luminal A and B subtypes.

Proliferative index and receptor profile

All samples were assigned with a relative proliferative index (PI), comprising expression values of 79 genes encoding proliferative and cell cycle markers (100 probe sets). Previous in-house analysis revealed difference in PI between normal tissue and malignant tumor samples (data not shown) and normal tissue samples expressed a PI below five, thus a cutoff value was set at 5.5 and a PI $\geq$5.5 ensured that the tissue was malignant. Samples with a PI $<$5.5 were subjected to a cancer-type classifier consisting of 641 probe sets and more than 2400 samples were derived [29]. Samples classified as normal tissue by mRNA profile were excluded from further analysis. Confirmed tumor samples with PI $<$6.5 were assigned as low proliferative in the subsequent analysis. The expression value thresholds for receptor status were set by default as (intermediate-positive): HER2 (11.001–11.5001), estrogen receptor (ER) (9–9.5), and progesterone receptor (PGR) (6–7) For MKI67 expression status, the average expression value of two probe sets were used (212023_s_at and 212021_s_at).

Statistical analysis

Principal component analysis (PCA) analysis and visualization of the data were performed in the Qlucore Omics Explorer™ software (Qlucore AB, Lund, Sweden). Correlation calculations, plots and boxplots were generated in R. We applied the Anderson-Darling for normality-test of the data derived from the proliferative index (PI). Testing for overrepresentation of germline mutations among basL-subtypes was done by using Fisher's exact test.

Histopathological diagnosis and ER, HER2 and Ki67 protein immunohistopathological analysis

Standard histopathological diagnosis of breast cancer samples was performed by a designated pathologist by light microscopy on glass slides from formalin fixed, paraffin embedded tissue blocks, according to the WHO-classification recommendations [30]. Analyses for ER, Ki67 protein and HER2 were performed by immunohistochemistry (IHC) using tissue micro array technique (TMA), with two cores of 2 mm from the invasive front of each tumor, as previously reported [31]. Staining for ER (SP1, diluted 1:25), Ki67 protein (MIB1; murine monoclonal antibody 1) and HER2 (4B5), all from Ventana Medical Systems, were carried out according to the manufacturer's instructions. Scoring of ER and Ki67 protein were semi quantitative with a positive cutoff point of >1% for ER positive tumor. Scoring of HER2 was performed as described by Hansen et al. [32] following the national guidelines (www.dbcg.dk). Online available datasets for comparison of percentage of positive Ki67 protein-cells were downloaded from www.ebi.ac.uk/arrayexpress/ (accession numbers; E-GEOD- 43358, -76040 and -76250).

Germline mutation screening

Genomic DNA was isolated using the ReliaPrep Large Volume HT gDNA Isolation Kit (Promega, Madison, WI, USA) and a Tecan Freedom EVO HSM2.0 Workstation according to the manufacturer's instructions. Germline mutation screening was performed using a gene panel consisting of six breast cancer-predisposing genes, including *BRCA1, BRCA2, CDH1, PTEN, RAD51C* and *TP53* as described by Jonson et al. [33]. Sequencing was performed on a MiSeq (Illumina, San Diego, CA, USA) to an average depth of at least 100×. Sequencing data were analyzed using Sequence Pilot (JSI medical systems, Ettenheim, Germany), where variants were called if the non-reference base frequency was above 25%. Variants are numbered according to the following GenBank accession numbers: NM_007294 (BRCA1), NM_000059 (BRCA2), NM_004360 (CDH1), NM_000314 (PTEN), NM_058216 (RAD51C) and NM_000546 (TP53) using the guidelines from the Human Genome Variation Society (www.hgvs.org/mutnomen). All variants, except well-known polymorphisms and neutral variants were verified by Sanger sequencing on an ABI 3730 DNA analyzer using DNA purified from a second blood sample.

Results

Baseline characteristics

Clinical characteristics of the five hundred and eight patients and basic histopathological features of their tumor samples are summarized in Table 1. More than 80% of the patients were aged 50 or older and the majority were diagnosed with stage I and II breast cancer. In total 524 tumor samples were included in the study. Sixteen of the patients had two tumors at the time of diagnosis and one sample was incorrectly preserved for RNA-extraction and could not undergo molecular classification, resulting in a total of 523 tumor samples which were finally eligible for molecular analysis.

Table 1. Clinical characteristics of patients and basic histopathological features of primary tumor samples.

Patients[a]	Number	%
Age at diagnosis ($n = 508$)		
<50 years	93	18.3
≥50 years	415	81.7
Size of primary tumor (508)		
<2 cm	303	59.6
2.1–5 cm	189	37.2
>5 cm	16	3.1
Positive Lymph Nodes ($n = 508$)		
0	300	59.1
1–3	143	28.1
≥4	63	12.4
N/A	2	0.4
Malignancy grade[b] ($n = 524$)		
1	39	7.4
2	329	62.8
3	137	26.1
N/A	19	3.6
Treatment ($n = 508$)		
No adjuvant therapy	29	5.7
CT[c]+ET(+T, if HER2+)	224	44.1
ET	193	38.0
CT (+T, if HER2+)	55	10.8
N/A	7	1.4

[a]Patients with two tumors: $n = 16$. Tumor samples: $n = 524$.
[b]Malignancy grade is assigned for lobular and ductal carcinomas.
[c]EC (Epirubicin + Cyklofosfamid) ×3 → Docetal ×3
CT: chemotherapy; ET: tamoxifen; T: trastuzumab; N/A: not applicable; HER2: human epidermal growth factor receptor 2

Molecular subtyping of breast cancer

Molecular subtyping was initiated on the day of the primary breast surgery and the diagnostic report was completed within six days and available prior to the clinical multidisciplinary conference (Figure 1). Only samples with proliferative index (PI) >5.5 were considered for subtype classification. Samples with PI <5.5 ($n = 54$) were classified by our cancer-type classifier [29] and samples classified as normal tissue were excluded from the analysis ($n = 3$), resulting in 520 tumor samples for molecular subtyping. The distribution of molecular subtypes in the study cohort is outlined in Table 2, together with treatment regimen for patients with ER-positive samples. Close to 15% of the samples could not be classified as a core-class and were consequently assigned as 'mixed', meaning that they consisted of two or three subtypes. Among the 76 mixed samples, 70 fell between the luminal subtypes where the lumA/normL were the most frequent ($n = 29$) combination. The remaining six mixed samples fell between either Basl/mApo or mApo/lumC. The spatial distribution of molecular subtypes and the WHO morphological classes is depicted in the principal component analysis (Figure 2(A)). Molecular subtypes were not associated with neither the WHO stage (Figure 2(B)) nor the histological subtype (invasive ductal (78%), invasive lobular (11%), mucinous, tubular and others) and molecular subtype (Figure 2(C)).

Table 2. Distribution of the molecular subtypes of 520 samples.[a]

Subtype	Basal-like	Molecular apocrine	Luminal C	LuminalB	LuminalA	Normal-like	Mixed
All ($n = 520$)	50 (9.6%)	28 (5.4%)	40 (7.7%)	76 (14.6%)	182 (35.0%)	68 (13.1%)	76 (14.6%)
ER positive[b] ($n = 461$)	14 (3.0%)	10 (2.2%)	39 (8.5%)	75 (16.3%)	182 (39.5%)	67 (14.5%)	74 (16.1%)
HER2 positive[c] ($n = 69$)	0 –	17 (24.6%)	18 (26.1%)	17 (24.6%)	5 (7.2%)	2 (2.9%)	10 (14.5%)
No adjuvant therapy	1 (7.1%)	–	1 (2.6%)	2 (2.7%)	17 (9.3%)	3 (4.5%)	4 (5.4%)
ET	–	1 (10.0%)	9 (23.1%)	11 (14.7%)	105 (57.7%)	37 (55.2%)	32 (43.2%)
CT[d] (+T, if HER2+)	1 (7.1%)	1 (10.0%)	–	–	1 (0.5%)	–	–
CT[d]+ET (+T, if HER2+)	11 (78.6%)	8 (80.0%)	29 (74.4%)	60 (80.0%)	59 (32.4%)	26 (38.8%)	36 (48.6%)
N/A	1 (7.1%)	–	–	2 (2.7%)	–	1 (1.5%)	2 (2.7%)

[a]In total 524 tumor samples were included in the cohort. One sample was incorrectly preserved for RNA-extraction and three samples were not classified as they consisted of normal tissue (0.8%).
[b]ER + determined by IHC ($n = 461$).
[c]HER2 status determined by immune histochemistry and fluorescent *in situ* hybridization.
[d]EC (Epirubicin + Cyklofosfamid) × 3 → Docetal × 3.
CT: chemotherapy; N/A: not applicable; ET: tamoxifen; T: trastuzumab; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2.

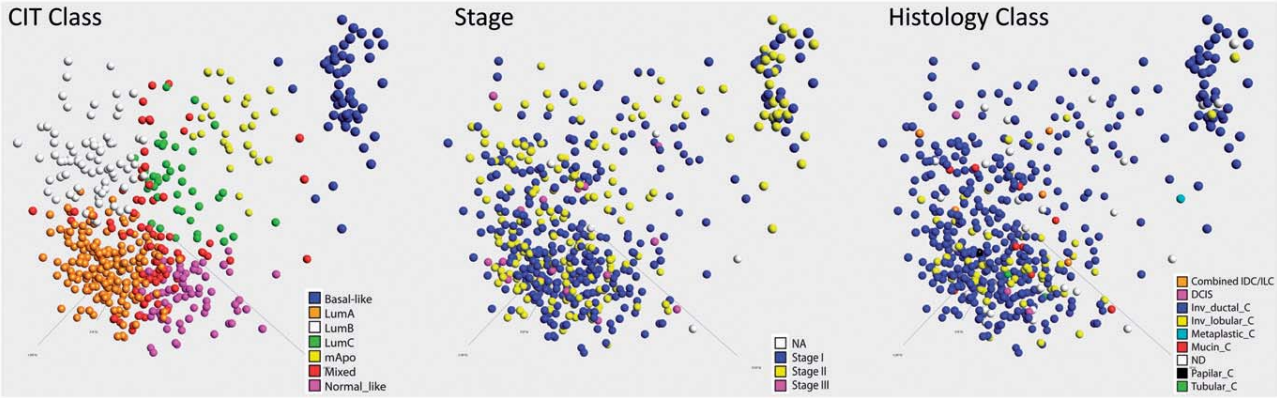


Figure 2. Principal component analysis of subtypes, stages and histopathological diagnosis. (A) A principal component analysis (PCA) showing the distribution of 520 breast cancer samples according to six molecular subtypes based on the expression profiles of the 375 probe sets. The subclasses cluster was assigned as separate entities with the samples classified as mixed located in the areas between the core clusters. The basal-like subtype, representing the double negative breast cancer samples, forms its own distinct cluster. (B) The distribution of patient stage from I–III in the spectra of subclasses clearly illustrates that assigned subclass is not dependent on patient stage. (C) The scattering of the assigned WHO histopathological diagnosis in the allocated molecular subclasses demonstrates that there is no association between molecular subgroup and histopathological diagnosis.

Receptor status defined by IHC and expression data

We compared the status of hormone receptors assigned by IHC and/or fluorescent *in situ* hybridization (FISH) with their microarray derived status (Supplementary Table 1). ER-positive breast cancer samples by IHC and those assigned by expression array overlapped to a large extent independently of the threshold levels (Supplementary Figure 1(A)). The algorithm identified 91% (420/461) of the IHC-positive samples with the 1% cutoff, whereas 33 (IHC 9%) and 41 (IHC 1%) samples, respectively, were not assigned as positive by the array. HER2 receptor status by IHC was scored according to international guidelines, where score 0 and 1 are considered negative, 2 is equivocal (considered positive by a HER2/centromere 17 ratio $\geq$2 by FISH) and 3 is considered as positive [34]. In total, 71 samples were HER2-positive by IHC and FISH and 42 samples were HER2-positive by array (Supplementary Table 1). There was a strong correlation between samples assigned as negative by array and scored 0–2 by IHC, whereas the correlation between HER2 positive samples was less profound (Supplementary Figure 1(B,C)). Twenty-seven out of 71 IHC/FISH positive samples (38%) did not exhibit elevated mRNA expression and were subsequently reevaluated by a designated pathologist. This provided an explanation in 70% of cases ($n = 19$) and were related to multiple tumors and intratumor heterogeneity or border-line assessments.

Proliferation index and Ki67 protein expression

Ki67 protein expression (IHC) is of some prognostic significance in breast cancer [35,36] and we compared the analytical validity of the analysis to a PI based on gene expression profiles [29]. In addition to our own data set, we examined three online available datasets ($n = 368$ samples

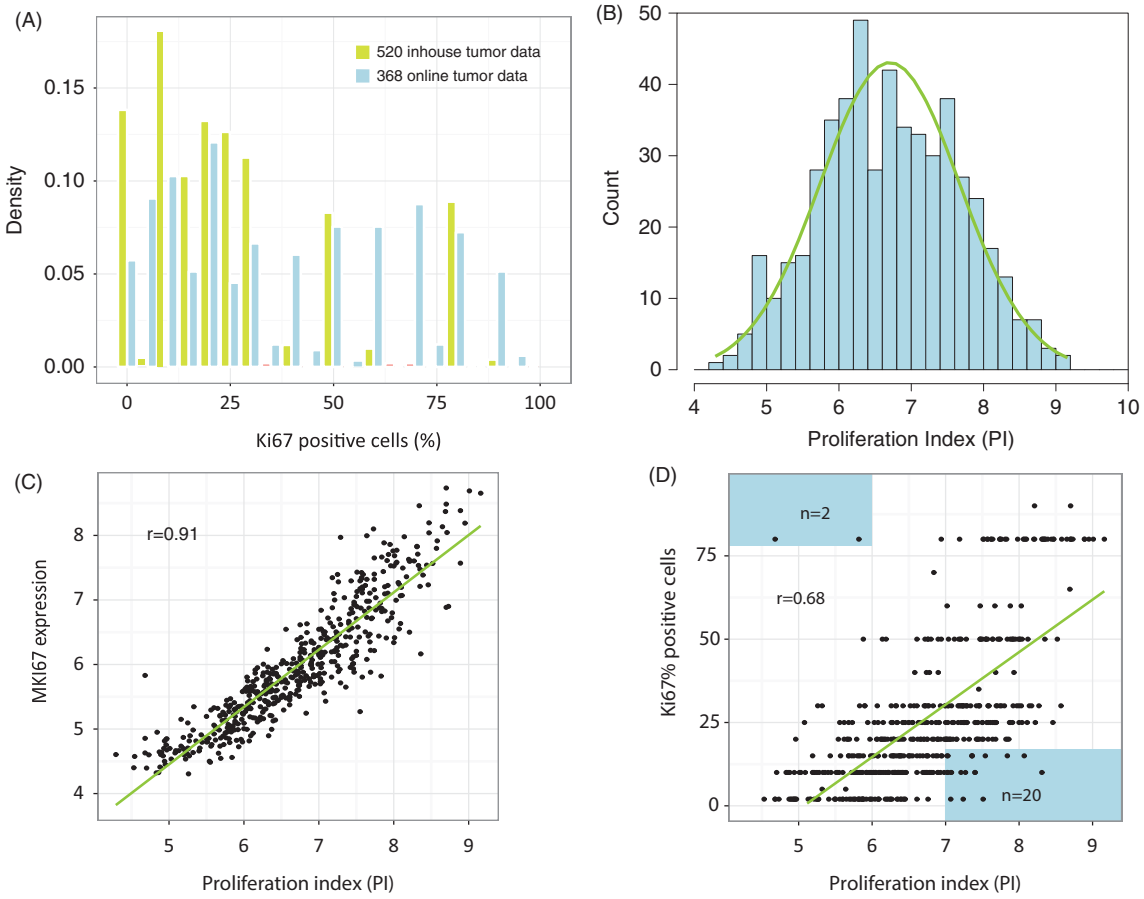


Figure 3. Correlation of Ki67 protein by immunohistochemistry (IHC) and expression profile. (A) The distribution of Ki67 protein positive cells from each representative tumor slide showing peaks from 5–30, 50 and 80% (in-house data to the left and online data to the right). (B) The distribution of the proliferative index (PI) extracted from the microarray analysis from each tumor specimen resembles that of a normal distribution. (C) The correlation of the PI and MKI67 protein encoding Ki67 shows a high correlation of $r = 91$. (D) The correlation of Ki67 protein positive cells by IHC and PI from array analysis shows a reduced correlation of just $r = 0.68$. Twenty-two samples (marked in square boxes) were found to have negative correlation and rendered further investigation.

in total) hereby confirming the distribution of the percentages of Ki67 protein positive cells, although identification of 60 and 70% nearly did not occur as the in-house opposed the on-line data (Figure 3(A)). In contrast to scoring of Ki67 by IHC, signature based assessment of proliferation is based on the expression levels of 79 transcripts and thus provides an objective, quantitative and reproducible proliferation value. The distribution of PI in our cohort was seen to approach that of a normal Gaussian distribution, as $p = .04$ by the applied test for normality (Figure 3(B)). Furthermore, there was a strong correlation, $r = 0.91$, between the PI levels (calculations) and the transcript levels of Ki67 protein (Figure 3(C)). On the contrary, the percentage of Ki67 protein positive cells showed a weak correlation with PI ($r = 0.68$;Figure 3(D)). There was discrepancy in 20 tumors that had 15% or less of Ki67 protein positive cells, but were scored with high PI (>7). A review of the individual histopathological reports clarified four of the cases; one sample was due to fibroadenomatosis and three were due to multiple tumors. Two tumors had 80% Ki67 protein positive cells but low PI and the review of the discrepancy could only be clarified for the one, since it was due to multiple tumors, whereas a plausible explanation for the second sample remains unknown.

Luminal A subtyping

To explore possible benefits of implementing the 256-gene expression signature for subtyping in a routine diagnostic work-up of breast cancer patients, we focused on the patient group who might benefit from omitting chemotherapy. Based on the prognostic standard mortality rate index (PSMRi), 39 women were annotated as being in the intermediate-low risk group. Of the 39 patients, 16 (41%) had a luminal A subtype and were treated solely with endocrine therapy. In our consecutive cohort, a total of 195 patients were treated with endocrine therapy alone (Table 2). Hence, as a direct consequence of implementing molecular subtyping, we showed an increase of 9% (16/179) in comparison to the original diagnostic set-up, in the total number of patients treated with endocrine therapy alone.

Identifying genetic predisposition by molecular subtype

As a part of the diagnostic work-up, screening for pathogenic germline mutations in breast/ovarian cancer predisposing genes (*BRCA1, BRCA2, CDH1, PTEN, RAD51C* and *TP53*) was performed. In total, 70 patients were screened, resulting in identification of eight pathogenic *BRCA1/BRCA2* mutations

(Supplementary Table 2). Noteworthy, seven of the eight pathogenic mutations were assigned to the basL-subtype and 17% (7/41) of the patients with a basL-subtype were carriers of a pathogenic *BRCA1/BRCA2* mutation. Nine out of the 50 patients with a basL-like subtype did not deliver a blood-sample for genetic screening. In addition, 12 out of 23 receptor-negative tumors, predominated by mApo subtype, were screened resulting in the identification of a single pathogenic *BRCA1* mutation. Finding of a pathogen germline mutation is significantly enriched in a basL-like subtype in comparison to a receptor-negative, non-basL-like subtype ($p < .05$). Moreover, 17 patients were screened due to young age (<40 years) among patients with a non- basL-like subtype (lumA ($n = 4$), lumB ($n = 6$), lumC ($n = 3$), mApo ($n = 2$), normL ($n = 1$) and mixed ($n = 1$)), did not carry any germline mutations in the six genes analyzed. Thus, the results indicate that basL-subtype is a predictor of patients at a greater risk of carrying a *BRCA1/BRCA2* germline predisposing mutation.

Discussion

For more than a decade, several studies have shown that primary breast cancers can be classified according to specific molecular based signatures into intrinsic subtypes. Accordingly, breast cancer classification is moving towards the molecular classification based on whole-transcriptome profiling. Molecular taxonomies comprise important information regarding diagnostics, treatment and clinical outcome [12,37]. Accommodating this evolution, the microarray based molecular subtyping (CIT-classification) was implemented as a supplement to our routine diagnostic and clinical setting for all primary breast cancer patients undergoing surgery at Rigshospitalet, Copenhagen University Hospital, Denmark.

Strengths of our study include that RNA-yield and downstream gene expression analysis from fresh or frozen biospecimens are superior to formalin-fixed paraffin-embedded (FFPE) tissues and the reduced gene expression from FFPE stored samples may tend to underestimate expression of important biomarkers [38,39]. However, classifications by multi-gene signatures tend to perform reasonablywell on both FFPE and fresh frozen tissue [40]. A corner-stone in subtyping of breast cancer is a precise measurement of proliferation rate as well as ER and HER2-status. We found some concordance (91%) between ER positive samples comparing IHC and mRNA results; however expression array failed to detect a number of IHC assigned ER-positive samples. The advantage of IHC staining is the ability to capture a single ER-positive cell among 100 tumor cells, excluding any surrounding or normal breast cancer tissue in the examination. We tried to minimize the normal tumor contamination by letting the resection of tumor-biopsies for transcriptome analysis solely handled by our specialized breast cancer pathologist. However, the risk of normal tissue contamination, especially among small size tumors, are a possible limitation of our study and a small number of samples were not included in the study at all, when the pathologist had evaluated the tumor to be too minute to spare material or ensured high tumor cell content in the sampling.

Our result, as well as others, clearly depicts the limitations of Ki67 protein staining used for quantitative measurements, with a specific threshold, probably due to the heterogeneous expression on IHC [41–43]. In regard to the proliferation rate, the St. Gallen consensus rapport is complex, since clinicpathological luminal A-like subtype definition is dependent on Ki67 protein staining with a cutoff that varies between laboratories [20]. We have shown that Ki67 protein expression is nearly distributed as normal Gaussian and well-correlated with the PI index, whereas Ki67 protein assessment by histopathological methods is biased. However, the poor correlation between expression levels and IHC in our study may be more prominent since Ki67 protein merely was evaluated based on two TMA cores as opposed to a whole slide section. Stressing the fact that these results are obtained from a routine diagnostic and clinical setting, expression array derived measurements for proliferation is superior to standard IHC.

The choice of method for luminal A subclass identification may depend on the available molecular platforms at-hand. As discussed in the above section, IHC for luminal Alike classification is suboptimal since it is dependent on the Ki67 protein index. Indeed, it is by now established that multi-gene signatures are superior to single-biomarker subtype classification and a subset of 50 genes was found to be the minimum number of genes in order to robustly identify the four basic intrinsic subtypes (luminal A, luminal B, HER2-enriched and basL) without compromising precision [7,18,23]. To ensure that the majority of the biological heterogeneity was contained in each consecutive sample, we chose a comprehensive taxonomy model for optimal distinction between the intrinsic subclasses. Moreover, the multi-gene platform enables an illustrative presentation of each sample according to the 'reference cluster'. This may be in contrast to the smaller gene-panels, where edgy samples remain unrecognized. Indeed, this is relevant when considering the heterogeneity of the large ER+/PGR + groups.

An interesting group is a group of mixed samples. Close to 15% of our samples could not be assigned with a core class and might therefore represent a challenge when conveying research data into clinical practice. Similarly in the comparative French cohort, one third of samples were not assigned with the same subclass in all three classification algorithms and therefore represented a mixed group. Moreover, the fraction of non-tumor cells may dilute the intrinsic subclass-signal and questions have been raised, whether the norm-like subgroup is in fact an artifact due to high-contest of non-tumor cells [44]. Still, we cannot exclude that the fraction of normal tissue content in our samples results in the relatively large number of both mixed and normal-like samples, which remains a weaker point of the methodology of our study. To address this important issue, Guedj et al. [9] estimated the rate of non-diploid cells and their distribution within the subgroups by SNP array data and showed that normL ranked third and LumA and LumB consisted of the highest fraction of non-diploid cells. They validated the SNP-array data by histological estimates of non-tumor cell fraction and found that the SNP-array based assessments of non-diploid cells were in fact lower than

pathological tumor cell content hereby substantiating that normL is a recognized subgroup [9]. It is reasonable to assume that low-risk patients suffering from a normL tumor also can be spared chemotherapy, all though long-term follow-up and additional samples are needed to test this hypothesis.

It is well-founded that the 'triple-negative' phenotype and/or the basL subtype are associated with the risk of a germline *BRCA1* mutation [2,5,45]. As depicted in Figure 1, all patients under 40 years of age with a basL subtype or receptor negative tumor were eligible for screening of pathogenic germline mutations. We found an overrepresentation of *BRCA1,* and to some extent, *BRCA2* mutations in the basL subtype, since 17% of the patients were genetically predisposed to breast cancer. The original retrospective study identified 11% *BRCA1* germline mutation carriers among 268 breast cancer patients with a basL phenotype [2]. This is in agreement with our prospective cohort where 12% (5/41) of the basL subtype subsequently was found to harbor a *BRCA1* germline mutation. While only half as many patients with a non-basL substype were screened ($n = 23$ vs. $n = 41$) it is remarkable, that we merely identified a single predisposing *BRCA1* mutation in these patients. It is obvious to suggest, that the young patients with a non-basL subtype most likely harbor other predisposing genomic alterations other than the genes included in our NGS-panel (*BRCA1, BRCA2, CDH1, PTEN, RAD51C* and *TP53*). An ongoing large-scale screening and validation of candidate breast cancer predisposition genes aim to identify exactly which genes it will be [46].

In this prospective study, close to 100% of the samples were eligible for analysis demonstrating that microarray based classification is suitable for clinical practice. Moreover, time is an important parameter in cancer diagnostics and here we showed that an array-based signature is only a few-day procedure from surgery to clinical report.

To the best of our knowledge, this study is one of the first reporting the results of implementing a six subclass array-based classification into a diagnostic setting as a supplement to the existing diagnostic work-up. The cohort is increasing prospectively and ongoing retrospective evaluation of the results will be carried out with rational intermissions. This study has focused on the two extremes of the benefits of molecular subtyping; the identification of intermediate risk patients with not only a luminal A , but also with a basL tumor and a greater risk of being genetically predisposed. Future studies should unravel the clinical relevant characteristics of the remaining subgroups by subsequent mutational testing, prognostic outcome and treatment-regime, since this may possibly pinpoint the breast cancer patients that could benefit from an optimized personalized treatment and follow-up regimen.

Acknowledgments

We thank Susanne Smed, Maria Gushchina and Julie Fisker Nielsen for excellent laboratory assistance.

Disclosure statement

None of the authors have any potential conflicts of interest.

Funding

The study was supported by the Capital Region of Denmark.

ORCID

Niels Kroman http://orcid.org/0000-0002-6631-2993

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66:7–30.
[2] Foulkes WD, Stefansson IM, Chappuis PO, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst. 2003;95:1482–1485.
[3] Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature. 2000;406:747–752.
[4] Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98:10869–10874.
[5] Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA. 2003;100:8418–8423.
[6] Sotiriou C, Neo SY, McShane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci USA. 2003;100:10393–10398.
[7] Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol. 2009;27:1160–1167.
[8] Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2000 breast tumours reveals novel subgroups. Nature. 2012;486:346–352.
[9] Guedj M, Marisa L, de RA, et al. A refined molecular taxonomy of breast cancer. Oncogene. 2012;31:1196–1206.
[10] Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. N Engl J Med. 2016;375:717–729.
[11] Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. J Clin Oncol. 2013;31:2783–2790.
[12] van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002;347:1999–2009.
[13] Sparano JA, Paik S. Development of the 21-gene assay and its application in clinical practice and clinical trials. J Clin Oncol. 2008;26:721–728.
[14] Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351:2817–2826.
[15] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61–70.
[16] Farmer P, Bonnefoi H, Becette V, et al. Identification of molecular apocrine breast tumours by microarray analysis. Oncogene. 2005;24:4660–4671.
[17] Smid M, Wang Y, Zhang Y, et al. Subtypes of breast cancer show preferential site of relapse. Cancer Res. 2008;68:3108–3114.
[18] Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast. 2015;24(Suppl 2):S26–S35.
[19] Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies–improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol. 2015;26:1533–1546.
[20] Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St

Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013;24:2206–2223.
[21] Prat A, Cheang MC, Martin M, et al. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol. 2013;31:203–209.
[22] Viale G, Regan MM, Maiorano E, et al. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol. 2007;25:3846–3852.
[23] Prat A, Parker JS, Fan C, et al. PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. Breast Cancer Res Treat. 2012;135:301–306.
[24] Christiansen P, Bjerre K, Ejlertsen B, et al. Mortality rates among early-stage hormone receptor-positive breast cancer patients: a population-based cohort study in Denmark. J Natl Cancer Inst. 2011;103:1363–1372.
[25] Ejlertsen B, Tuxen MK, Jakobsen EH, et al. Adjuvant cyclophosphamide and docetaxel with or without epirubicin for early TOP2A-normal breast cancer: DBCG 07-rEAD, an open-label, phase III, randomized trial. J Clin Oncol. 2017;35:2639–2646.
[26] Ejlertsen B, Jensen MB, Mouridsen HT. Excess mortality in postmenopausal high-risk women who only receive adjuvant endocrine therapy for estrogen receptor positive breast cancer. Acta Oncol. 2014;53:174–185.
[27] Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics. 2007;8:118–127.
[28] Leek JT, Johnson WE, Parker HS, et al. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics. 2012;28:882–883.
[29] Vikesa J, Moller AK, Kaczkowski B, et al. Cancers of unknown primary origin (CUP) are characterized by chromosomal instability (CIN) compared to metastasis of know origin. BMC Cancer. 2015;15:151.
[30] Lakhani SR, Ellis IO, Schnitt SJ, et al. WHO Classification of Tumors of the Breast. IARC: Lyon; 2012.
[31] Rossing HH, Talman ML, Laenkholm AV, et al. Implementation of TMA and digitalization in routine diagnostics of breast pathology. APMIS. 2012;120:341–347.
[32] Hansen TV, Vikesaa J, Buhl SS, et al. High-density SNP arrays improve detection of HER2 amplification and polyploidy in breast tumors. BMC Cancer. 2015;15:35.
[33] Jonson L, Ahlborn LB, Steffensen AY, et al. Identification of six pathogenic RAD51C mutations via mutational screening of 1228 Danish individuals with increased risk of hereditary breast and/or ovarian cancer. Breast Cancer Res Treat. 2016;155:215–222.
[34] Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med. 2007;131:18–43.
[35] Thor AD, Liu S, Moore DH, et al. Comparison of mitotic index, in vitro bromodeoxyuridine labeling, and MIB-1 assays to quantitate proliferation in breast cancer. J Clin Oncol. 1999;17:470–477.
[36] Viale G. Pathological work up of the primary tumor: getting the proper information out of it. Breast. 2011;20(Suppl 3):S82–S86.
[37] van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002;415:530–536.
[38] Greytak SR, Engel KB, Bass BP, et al. Accuracy of molecular data generated with FFPE biospecimens: lessons from the literature. Cancer Res. 2015;75:1541–1547.
[39] Williams PM, Li R, Johnson NA, et al. A novel method of amplification of FFPET-derived RNA enables accurate disease classification with microarrays. J Mol Diagn. 2010;12:680–686.
[40] Mittempergher L, de Ronde JJ, Nieuwland M, et al. Gene expression profiles from formalin fixed paraffin embedded breast cancer tissue are largely comparable to fresh frozen matched tissue. PLoS One. 2011;6:e17163.
[41] Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. 2007;25:5287–5312.
[42] Yerushalmi R, Woods R, Ravdin PM, et al. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11:174–183.
[43] Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101:736–750.
[44] Prat A, Parker JS, Karginova O, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 2010;12:R68.
[45] Lakhani SR, Reis-Filho JS, Fulford L, et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res. 2005;11:5175–5180.
[46] Southey MC, Park DJ, Nguyen-Dumont T, et al. COMPLEXO: identifying the missing heritability of breast cancer via next generation collaboration. Breast Cancer Res. 2013;15:402.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 67–73
https://doi.org/10.1080/0284186X.2017.1404126

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Aurora kinase A as a possible marker for endocrine resistance in early estrogen receptor positive breast cancer

Anne E. Lykkesfeldt[a], Benedikte R. Iversen[b], Maj-Britt Jensen[c], Bent Ejlertsen[c], Anita Giobbie-Hurder[d], Birgit E. Reiter[a], Tove Kirkegaard[e] and Birgitte B. Rasmussen[b]

[a]Unit of Cell Death and Metabolism, Danish Cancer Society Research Center, Copenhagen, Denmark; [b]Department of Pathology, Herlev University Hospital, Herlev, Denmark; [c]Danish Breast Cancer Cooperative Group (DBCG), Rigshospitalet, Copenhagen, Denmark; [d]International Breast Cancer Study Group (IBCSG), Department of Biostatistics & Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA; [e]Department of Surgery, Zealand University Hospital, Koege, Denmark

ABSTRACT

Background: Cell culture studies have disclosed that the mitotic Aurora kinase A is causally involved in both tamoxifen and aromatase inhibitor resistant cell growth and thus may be a potential new marker for endocrine resistance in the clinical setting.

Material and methods: Archival tumor tissue was available from 1323 Danish patients with estrogen receptor (ER) positive primary breast cancer, who participated in the Breast International Group (BIG) 1-98 trial, comparing treatment with tamoxifen and letrozole and both in a sequence. The expression of Aurora A was determined by immunohistochemistry in 980 tumors and semi quantitively scored into three groups; negative/weak, moderate and high. The Aurora A expression levels were compared to other clinico-pathological parameters and outcome, defined as disease-free survival (DFS) and overall survival (OS).

Results: High expression of Aurora A was found in 26.9% of patients and moderate in 57.0%. High expression was significantly associated with high malignancy grade and HER2 amplification. High Aurora A expression was significantly more frequent in ductal compared to lobular carcinomas. We found no significant association between Aurora A expression and DFS or OS and no evidence of interaction between Aurora A expression and benefits from tamoxifen versus letrozole.

Conclusions: Aurora A expression in breast tumors was associated with high malignancy grade III and with HER2 amplification. A trend as a prognostic factor for OS was found in patients with high Aurora A expression. No predictive property was observed in this study with early breast cancer.

ARTICLE HISTORY
Received 6 September 2017
Accepted 8 November 2017

Background

Adjuvant endocrine therapy has improved survival of estrogen receptor α (ER) positive breast cancer patients, but recurrent disease is found in 19.1 and 22.7% of the patients at 10 years follow-up after five years of treatment with aromatase inhibitor or tamoxifen, respectively [1]. Thus, new markers are required to identify the ER positive breast cancer patients who may need supplementary treatment, e.g., tailored biological treatment.

Studies of cell culture models mimicking ER positive breast cancer resistant to the antiestrogens tamoxifen and fulvestrant and also to aromatase inhibitors have disclosed that Aurora kinases are important for growth of both antiestrogen and aromatase inhibitor resistant breast cancer cells [2–4]. Aurora kinases (A, B and C) are key regulators of mitosis and multiple signaling pathways [5,6]. Gene amplification and protein overexpression of Aurora kinases have been found in both hematologic malignancies and solid tumors and deregulation of Aurora kinases has been linked to tumorigenesis [7]. Aurora A is consistently associated with cancers and Aurora B may also contribute to tumorigenesis, whereas the role of Aurora C is not yet clarified [8].

Aurora A has been found to be a prognostic marker indicating poor prognosis in ER positive node-negative breast cancer [9] and Aurora A outperforms other proliferation markers including the surrogate tissue-based proliferation marker Ki-67 [10,11].

Screening analyses with a library of 195 kinase inhibitors identified the dual Aurora A and B inhibitor JNJ-7706621 as a preferential and efficient inhibitor of a panel of tamoxifen and aromatase inhibitor resistant breast cancer cell lines derived from the ER positive breast cancer cell lines MCF-7 and T47D by long term treatment with the endocrine agent [2,3]. siRNA (small interfering RNA) mediated specific knock-down of Aurora A and B revealed that Aurora A, but not B, was important for growth of the tamoxifen resistant breast cancer cells. Furthermore, sensitivity to tamoxifen treatment was restored in tamoxifen resistant cell lines with siRNA mediated knock-down of Aurora A or by treatment with the Aurora A/B inhibitor JNJ-7706621 [2], indicating a causal role for Aurora A in tamoxifen resistance. The finding that

CONTACT Birgitte B. Rasmussen Birgittte.Bruun.Rasmussen@regionh.dk Department of Pathology, Herlev University Hospital, Herlev Ringvej 75, 2730 Herlev, Denmark

tamoxifen resistant breast cancer cell lines express increased level of Aurora A compared to their parental cell lines [2,12] and that ectopic overexpression of Aurora A renders ERα positive breast cancer cell lines less sensitive to tamoxifen treatment via phosphorylation of ERα, suggest that high expression level of Aurora A may be a marker for tamoxifen resistance [12]. In agreement, our pilot study with 244 patients, who received adjuvant tamoxifen therapy, disclosed that high tumor expression level of Aurora A was associated with reduced disease-free survival (DFS) [2]. Today, treatment with aromatase inhibitor is the recommended adjuvant endocrine therapy for postmenopausal breast cancer patients and high expression level of Aurora A may also be a marker for reduced benefit from adjuvant aromatase inhibitor treatment, as our cell culture model studies have shown that Aurora A and also Aurora B are important for growth of aromatase inhibitor resistant breast cancer cells [3]. Therefore, in this study, we have evaluated whether high expression level of Aurora A is associated with shorter DFS and overall survival (OS) in 980 Danish postmenopausal breast cancer patients who have participated in BIG 1–98, a randomized phase III clinical trial comparing adjuvant endocrine therapy with tamoxifen, the aromatase inhibitor letrozole or sequential tamoxifen and letrozole. We have also investigated whether Aurora A is a predictive marker for response to treatment with tamoxifen and with letrozole.

Material and methods

Patients

Archival formalin-fixed and paraffin-embedded primary tumor tissues from Danish breast cancer patients participating in the international randomized double-blinded clinical phase III trial, Breast International Group trial 1-98 (BIG 1-98), were included in the study. All patients gave written, informed consent before inclusion in the study.

In the BIG 1-98, ER-positive, early breast cancer patients were randomized to five years of tamoxifen or letrozole monotherapy, or sequential treatment, two years tamoxifen or letrozole followed by three years of letrozole or tamoxifen [13]. In total, 1396 Danish patients were randomized in BIG 1-98 between 1998 and 2003 and primary tumors from 1323 patients were available for tissue microarray (TMA) preparation as previously described [14]. The study was conducted according to the Helsinki declaration and approved by The Danish National Committee on Biomedical Ethics in Denmark in 1997 (KF02 1 178/97) and an addendum approved in 2004 (KF 12 – 142/04). The Danish Data Protection Agency also provided an approval (RH-2015-166).

Immunohistochemical (IHC) analysis and evaluation

TMAs were constructed from formalin-fixed paraffin-embedded tumor blocks, with two cores of 2 mm tissue from each tumor. Immunohistochemical staining of Aurora A was performed with the procedure described in our pilot study [2]. The Aurora A primary antibody (Cell Signaling Technologies, Danvers, MA,USA, 4718) was selected for its usefulness in both western blot analysis and immunohistochemistry. The specificity of the antibody was documented by knocking-down of the expression of the 48 kDa Aurora A protein in human breast cancer MCF-7 cells treated with Aurora A specific silencing RNA constructs, as shown in Thrane et al. [2]. In the present validation study, the enhancement system was improved. Antigen retrieval was performed in a microwave oven for 15 min in Envision FLEX Target Retrieval Solution High pH (50x) (DAKO Denmark A/S, Glostrup, Denmark Cat. no K8004), Aurora A primary antibody was diluted 1:100 and applied over night at 4 °C. High Definition Detection HRP Polymer System (AH Diagnostics A/S, Tilst, Denmark, Cat. no 954D-30) was used for enhancement and staining was performed with DAB substrate (DAKO, Cat. no K3468). Nuclei were counterstained with hematoxylin before mounting in pertex.

Aurora A expression in tumor cells was evaluated in both cores from each tumor with a modified Allred procedure [15], including scoring of the fraction of Aurora A positive tumor cells and of Aurora A expression intensity. Regarding the fraction of Aurora A positive tumor cells, three levels were defined (1 point: 0–10%; 2 points: 11–50% and 3 points: 51–100% of tumor cells), as well as three levels of Aurora A expression intensity (1 point: low; 2 points: medium and 3 points: high). These two parameters combined resulted in a three-tiered score (0–2 points: negative or weak; 3–4 points: moderate and 5–6 points: high; Figure 1). Cores with less than 100 tumor cells were excluded from the analysis.

Statistical analysis

All clinical data were collected and monitored by the International Breast Cancer Study Group (IBSCG) Data Management Center (Buffalo, NY, USA) and the Danish Breast Cancer Cooperative Group (DBCG). Baseline data including data on follow-up until October 2010 [16] were provided by the IBCSG datacenter. The statistical analyses were conducted at DBCG by the author MJ.

Follow-up time was quantified in terms of a Kaplan-Meier estimate of potential follow-up. Kaplan-Meier plots were used to illustrate the primary end-point DFS, defined as the time from randomization to the earliest of any of the following events: recurrence of the disease at a local, regional or distant site; a new invasive cancer in the contralateral breast; a new secondary non-breast cancer or death without a previous cancer event. Secondary end-point was OS, defined as the time from randomization to death, irrespective of cause of death. Time to event outcomes DFS and OS were analyzed according to the intention to treat principle (ITT). Follow-up in the sequential treatment arms was censored at two years, i.e., at the time of scheduled treatment change.

Baseline characteristics were compared using the two-sided Fisher's exact test (excluding unknown) or Wilcoxon rank sum test. The associations of Aurora A expression and time to event endpoints, DFS and OS, were analyzed by Kaplan-Meier estimates, and statistical significance was estimated by log-rank test stratified by two- or four-arm random assignment option and treatment arm (tamoxifen vs.

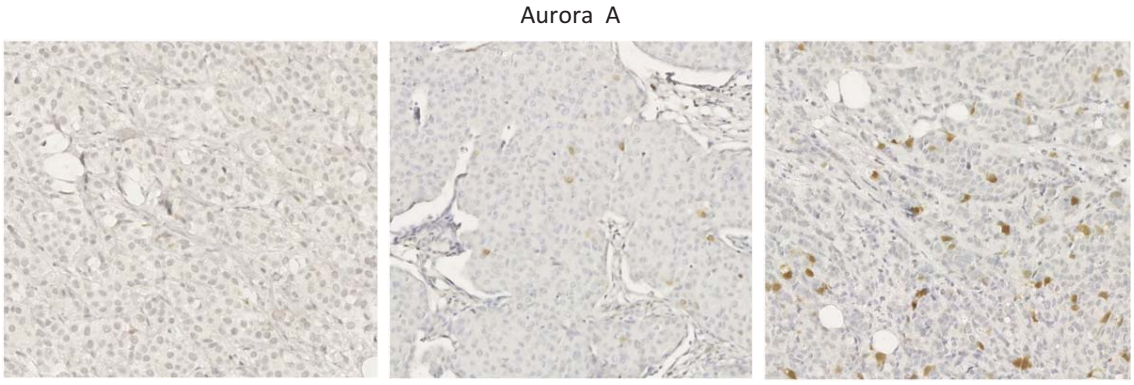


Figure 1. Representative pictures showing immunohistochemical staining for Aurora A: negative/weak, moderate and high staining.

letrozole). Hazard ratio (HR) estimates were obtained from analysis by the Cox proportional hazards ratio model. Multivariate analysis included age at randomization, tumor size, histological type and grade, nodal status, ER and HER2 status and random assignment. The HER2 normal population was used to estimate treatment effect. Level of statistical significance was set to 5%.

Results

Aurora A expression and correlation to other tumor markers

In this study, archival primary tumor tissues from 1323 of the 1396 Danish breast cancer patients enrolled in the BIG 1-98 study were investigated. By central assessment, 1244 patients were ER-positive (>1%). Aurora A data were available from 980 of the ER-positive patients. The exclusion of 416 patients was mainly because of lack of tumor samples or too few tumor cells in the TMA-cores.

Clinico-pathological parameters for included and excluded patients are presented in Table 1. The excluded patients had a higher frequency of small tumors (0–20 mm) than the included patients (54.3 vs. 46.7%). The estimated median potential follow-up time was nine years. 353 patients experienced a recurrence and 252 had died. In the letrozole arm, slightly but significantly more patients were included ($p = .04$) as compared to the other treatment arms (tamoxifen alone, tamoxifen followed by letrozole and letrozole followed by tamoxifen, data not shown).

High Aurora A expression was found in 26.9% of the study population, moderate expression in 57.0% (Table 2). High expression of Aurora A was more frequent in invasive ductal carcinomas than in lobular carcinomas ($p < .0001$). The majority of tumors with high malignancy grade III and with HER2 amplification expressed high Aurora A level ($p < .0001$). Tumors with intermediate ER scores (Allred 3–6) were less frequently expressing high Aurora A compared to tumors with high ER expression ($p < .0001$). With regard to expression of Aurora A and treatment arms, the patients were well balanced (data not shown).

Aurora A as a prognostic factor

The association of Aurora A expression and DFS and OS is shown by Kaplan-Meier estimates (Figure 2). DFS at five year follow-up was 77.6% (95% CI 70.3–83.4) for negative or weak expression, 81.0% (95% CI 77.5–84.0) for moderate expression and 76.3% (95% CI 70.7–81.0) for high expression; the corresponding numbers for OS were 85.9% (95% CI 79.4–90.5) versus 89.8% (95% CI 87.0–92.0) versus 85.8% (95% CI 81.0–89.5). No significant association was found between Aurora A expression and DFS (HR 1.22 (CI 0.96–1.55), $p = .28$) whereas OS was borderline significant (HR 1.41 (1.07–1.87), $p = .06$; Table 3) for patients with high Aurora A expression compared to the moderate group. Restricting the patient population by excluding patients with amplified HER2 revealed no statistical significant association between Aurora A expression and DFS and OS ($p = .51$ and .13; Table 3). Adjusted analysis (multivariate analysis) included ER status (Allred scores 3–6 vs. 7–8), tumor grade, lymph node status, histological type, age and tumor diameter as covariates and neither the whole group of patients nor the HER2 normal subpopulation disclosed significant difference (Table 3).

Aurora A as a predictive marker for treatment with letrozole versus tamoxifen

In the group of patients with HER2 normal expression, no statistical significant difference in DFS (HR 0.95 (CI 0.72–1.25), $p = .70$) or OS (HR 1.03 (CI 0.73–1.44), $p = .89$) was found in relation to treatment with letrozole versus tamoxifen (Table 4). Treatment effect heterogeneity (letrozole versus tamoxifen) according to Aurora A expression levels negative/weak, moderate or high, did not show significant effects, $p = .20$ and .89 for DFS and OS, respectively.

Discussion

In this large subset of BIG 1-98, we were unable to demonstrate a prognostic or predictive effect of Aurora A expression. Our results are in contrast to previous results including a prior pilot study from our group [2,9–12,17]. Our pilot study included both HER2 positive and negative patients [2] and in

Table 1. Patient characteristics in study population and patients excluded from the total cohort of Danish patients in BIG 1-98.

	Study population		Excluded		Total		
Characteristic	*N*	(%)	*N*	(%)	*N*	(%)	*p* value
All	980	(100.0)	416	(100.0)	1396	(100.0%)	
Tumor size, mm[a]							
0–20	458	(46.7)	226	(54.3)	684	(49.0)	.01
21–50	495	(50.6)	173	(41.6)	668	(47.9)	
51+	26	(2.7)	17	(4.1)	43	(3.1)	
Histological type[a]							
Invasive ductal	827	(84.5)	341	(82.0)	1168	(83.7)	.11
Invasive lobular	122	(12.4)	67	(16.1)	189	(13.5)	
Other	30	(3.1)	8	(1.9)	38	(2.7)	
Tumor grade							
I	213	(21.7)	97	(23.3)	310	(22.2)	.44
II	504	(51.4)	213	(51.2)	717	(51.4)	
III	135	(13.8)	47	(11.3)	182	(12.0)	
Unknown	128	(13.1)	59	(14.2)	187	(13.4)	
Positive nodes[a]							
0	347	(35.4)	158	(38.0)	505	(36.2)	.07
1–3	427	(43.7)	152	(36.5)	579	(41.5)	
4–9	129	(13.2)	69	(16.6)	198	(14.2)	
10+	76	(7.8)	37	(8.9)	113	(8.1)	
ER							
Allred scores							
0–2	0	(0.0)	32	(7.7)	32	(2.3)	.04
3–6	258	(26.3)	87	(20.9)	345	(24.7)	
7–8	722	(73.7)	177	(42.5)	899	(64.4)	
Unknown	0	(0.0)	120	(28.8)	120	(8.6)	
Aurora A							
Negative or weak	157	(16.0)	5	(1.2)	162	(11.6)	.39
Moderate	559	(57.0)	15	(3.6)	574	(41.1)	
High	264	(26.9)	12	(2.9)	276	(19.8)	
Missing	0	(0.0)	384	(92.3)	384	(27.5)	
HER2							
Normal	880	(89.8)	280	(67.3)	1160	(83.1)	.07
Amplified	97	(9.9)	19	(4.6)	116	(8.3)	
Unknown	3	(0.3)	117	(28.1)	120	(8.6)	

[a]One patient was omitted because of lack of data.

Table 2. Aurora A's association with other prognostic variables.

	Aurora A								
	Negative/weak		Moderate		High		Total		
Characteristic	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*p* value
All	157	(100.0)	559	(100.0)	264	(100.0)	980	(100)	
Tumor size, mm[a]									
0–20	61	(38.9)	282	(50.4)	115	(43.6)	458	(46.7)	.003
21–50	87	(55.4)	264	(47.2)	144	(54.9)	495	(50.6)	
51+	9	(5.7)	13	(2.3)	4	(1.5)	26	(2.7)	
Histological type[a]									
Invasive ductal	118	(75.2)	469	(83.9)	240	(91.3)	827	(84.5)	<.0001
Invasive lobular	35	(22.3)	70	(12.5)	17	(6.4)	122	(12.4)	
other	4	(2.5)	20	(3.6)	6	(2.3)	130	(12.4)	
Tumor grade									
I	53	(33.8)	139	(24.9)	21	(8.0)	213	(21.7)	<.0001
II	65	(41.5)	307	(54.9)	132	(50.0)	504	(51.4)	
III	6	(3.8)	36	(6.4)	93	(13.8)	135	(13.8)	
Unknown	33	(21.0)	77	(13.8)	18	(6.8)	128	(13.1)	
Positive nodes[a]									
0	44	(28.0)	198	(35.4)	105	(39.8)	347	(35.4)	.12
1–3	77	(49.0)	248	(44.5)	102	(38.6)	427	(43.7)	
4–9	25	(15.9)	73	(13.1)	31	(11.7)	129	(13.2)	
10+	11	(7.0)	39	(7.0)	26	(9.8)	76	(7.8)	
ER									
Allred scores 3–6	66	(42.0)	124	(22.2)	68	(24.7)	258	(25.8)	<.0001
Allred scores 7–8	91	(58.0)	435	(77.8)	196	(64.4)	722	(74.2)	
HER2									
Normal	149	(94.9)	525	(93.9)	206	(78.0)	880	(89.9)	<.0001
Amplified	7	(4.5)	32	(5.7)	58	(22.0)	97	(9.9)	
Unknown	1	(0.6)	2	(0.4)	0	(0.0)	3	(3.0)	

[a]One patient was omitted because of lack of data.

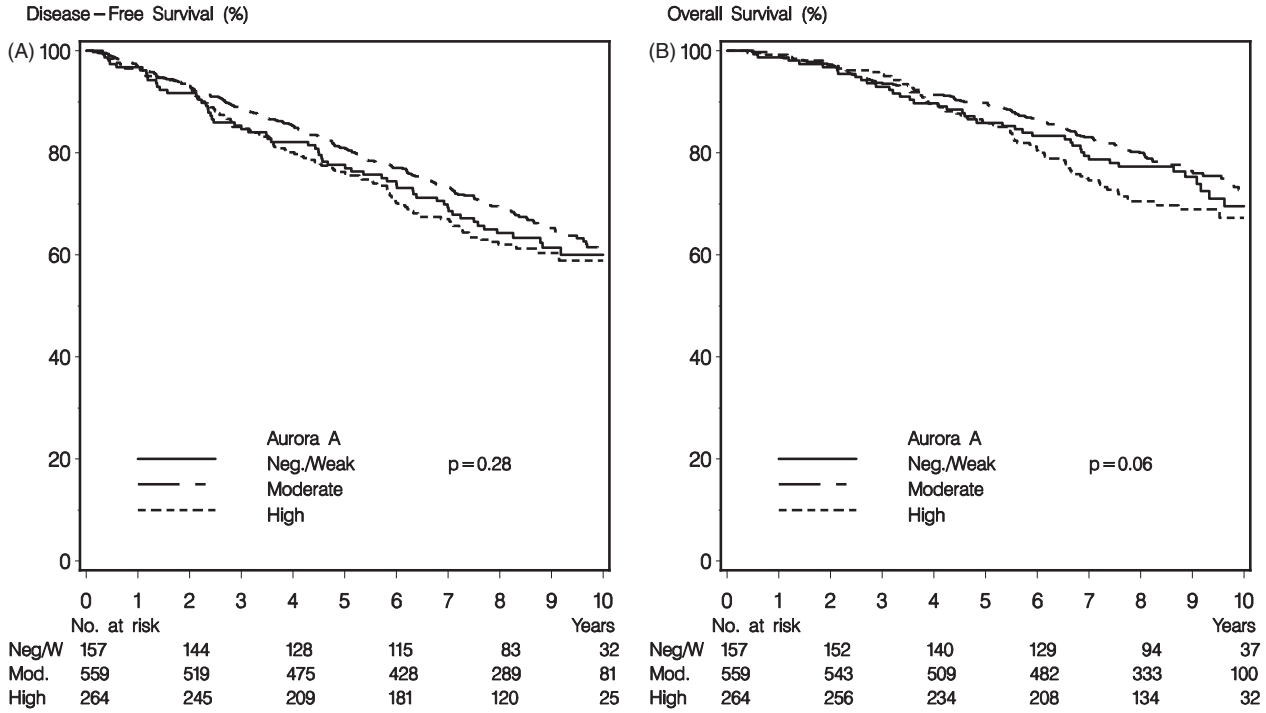


Figure 2. Kaplan-Meier survival curves demonstrating DFS (A) and OS (B) for patients with high, moderate and negative/weak Aurora A expression. *p* values are procured from log-rank testing.

Table 3. Association between Aurora A expression and disease-free survival (DFS) and overall survival (OS).

			Un-adjusted[a]			Adjusted[b]		
Population	Response	Aurora A	HR	(95%CI)	*p* value	HR	(95% CI)	*p* value
All (*N*= 980)	DFS	Negative/weak	1.09	(0.82–1.46)	.28	1.02	(0.75–1.39)	.54
		Moderate	1.00	(reference)		1.00	(reference)	
		High	1.22	(0.96–1.55)		0.86	(0.66–1.14)	
	OS	Negative/weak	1.11	(0.78–1.57)	.06	0.98	(0.68–1.42)	.98
		Moderate	1.00	(reference)		1.00	(reference)	
		High	1.41	(1.07–1.87)		1.03	(0.75–1.42)	
HER2 negative or unknown (*N* = 883)	DFS	Negative/weak	1.13	(0.83–1.53)	.51	1.04	(0.75–1.43)	.94
		Moderate	1.00	(reference)		1.00	(reference)	
		High	1.16	(0.88–1.52)		0.97	(0.71–1.32)	
	OS	Negative/weak	1.10	(0.76–1.59)	.13	0.97	(0.65–1.43)	.68
		Moderate	1.00	(reference)		1.00	(reference)	
		High	1.39	(1.01–1.90)		1.16	(0.81–1.64)	

[a]Cox proportional hazards model stratified for random assignment option and treatment arm.
[b]Cox proportional hazards model stratified for random assignment option and treatment arm. Model was adjusted for ER status, tumor grade, lymph node status, histological type and tumor size.

Table 4. Treatment effect of letrozole versus tamoxifen [a].

			Adjusted[b]			
Population	Response	Aurora A Subgroup	HR	(95% CI)	*p*	*p* het[c]
HER2 negative or unknown (*N* = 883)	DFS		0.95	(0.72–1.25)	.70	
	DFS	Negative/weak	0.64	(0.33–1.22)		.20
		Moderate	1.17	(0.81–1.69)		
		High	0.78	(0.44–1.37)		
	OS		1.03	(0.73–1.44)	.89	
	OS	Negative/weak	0.78	(0.35–1.71)		.47
		Moderate	1.24	(0.78–1.95)		
		High	0.83	(0.42–1.63)		

[a]Analysis in mono-therapy arms only (follow-up in sequential treatment arms truncated two years after randomization).
[b]Cox proportional hazards model adjusted for ER status, tumor grade, lymph node status, histological type, tumor size, age and HER2 status.
[c]Test for treatment effect heterogeneity.

the BIG 1-98 patients, we found a trend for significance of Aurora A expression as a prognostic marker for OS in the univariate analysis including all patients, $p = .06$. However, when we omitted patients with amplified HER2, no significant association between Aurora A expression and DFS or OS was observed. High Aurora A expression was associated with high tumor grade (grade III compared to I) and high Aurora A level was more frequent in ductal compared to lobular tumors (29.0 vs. 13.9%). The higher frequency of high Aurora A expression in ductal carcinomas is in agreement with a study with 1359 ER positive breast tumors, in which high Aurora A expression was of found in 27% of the ductal and in 12.8% of the lobular tumors [10]. Our finding of more frequently high Aurora A expression in HER2 positive tumors compared to HER2 negative tumors was seen also in other studies [10,18]. The association between Aurora A expression and the independent variables; histological type, tumor grade and HER2 status may explain the lack of significance in the multivariate analysis.

The BIG 1-98 study included 8010 patients with early breast cancer, and reduction in recurrence and mortality was obtained by letrozole monotherapy compared to tamoxifen monotherapy [16]. We have analyzed tumors from a subgroup of only the Danish patients who had participated in the BIG 1-98 study. Overall there was no significant treatment effect comparing tamoxifen and letrozole. This may be due to more extensive cross-over from tamoxifen to letrozole in Denmark when it became evident that letrozole was superior to tamoxifen. Our analysis for potential treatment effect heterogeneity according to Aurora A expression level did not reveal significant difference. Of note, centrally reviewed Ki-67 labeling index of 2685 primary tumors from the BIG 1-98 study receiving monotherapy disclosed that treatment benefit from letrozole versus tamoxifen was greater among patients with high Ki-67 than among patients with low Ki-67 [19].

Several studies have shown that the mitotic Aurora kinase A is a poor prognostic marker in breast cancer [9–11,17,18,20], and we found borderline significance for reduced OS in this group of breast cancer patients with early breast cancer treated with adjuvant endocrine therapy. Aurora A is not only a cell cycle regulator but is also involved in many different signaling cross-talks within the cells [5–7], including activation of the ER via phosphorylation [12]. Our finding in this study which included 980 early breast cancer patients showed that Aurora A did not show significance as prognostic or predictive marker for response to tamoxifen or to letrozole. This indicates that Aurora A overexpression alone may not be sufficient to render breast cancer cells resistant to adjuvant endocrine therapy. It should be mentioned that our cell culture models have been established from the human breast cancer cell lines MCF-7 and T47D, both of which are established from breast cancer metastases. MCF-7 and T47D cells respond to treatment with tamoxifen and with aromatase inhibitor, but few cells survive treatment. Resistant cell lines have been established from colonies of surviving cell, which have acquired the ability to grow in presence of either tamoxifen or an aromatase inhibitor [2,21,22]. Thus, the cell culture models in which Aurora A has a causal role for resistant cell growth mimic patients with advanced disease who after an initial response to the endocrine therapy regress during treatment. This study indicates that the mechanisms, which render early breast cancer resistant to treatment may differ from the mechanisms involved in acquired resistance. Determination of Aurora A expression in primary breast tumors from patients with advanced disease and in the corresponding metastases developed after an initial response to treatment with either tamoxifen or an aromatase inhibitor may clarify whether high Aurora A expression is associated with acquired resistance.

In summary, we have found high Aurora A expression in 26.9% of the 980 breast tumors included in this study. Aurora A expression was associated with ductal carcinomas, high malignancy grade and HER2 amplification. A trend was found for decreased OS in patients with high Aurora A expression. In this study with early breast cancer, Aurora A was not a predictive marker neither for response to tamoxifen nor to letrozole. We suggest that high expression of Aurora A may be specific for acquired resistance to treatment with tamoxifen and with aromatase inhibitor.

Acknowledgments

We kindly acknowledge the participating patients, investigators, study nurses and thank the Danish Pathology Departments for supplying paraffin embedded tumor material: Aalborg Sygehus, Aarhus Hospital, Bispebjerg Hospital, Esbjerg Sygehus, Gentofte Hospital, Herlev Hospital, Hillerød Hospital, Hjørring Sygehus, Holstebro Sygehus, Hvidovre Hospital, Nykøbing Falster sygehus, Naestved Sygehus, Odense Hospital, Randers Sygehus, Rigshospitalet, Roskilde Sygehus, Skive Sygehus, Slagelse Sygehus, Svendborg Sygehus, Sønderborg Sygehus, Vejle Sygehus and Viborg Sygehus. Thanks to Annette Bartels and Nils Brünner from Section for Molecular Disease Biology, University of Copenhagen for the help rendered regarding immunohistochemical analysis.

Disclosure statement

No potential conflicts of interest were disclosed.

Funding

This study was supported by Danish Cancer Society, grant no. R72-A4510-13-S2.

References

[1] Early Breast Cancer Trialists' Collaborative Group: Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of randomized trials. Lancet. 2015;386:1341–1352.

[2] Thrane S, Pedersen AM, Thomsen MBH, et al. A kinase inhibitor screen identifies Mcl-1 and Aurora kinase A as novel treatment targets in antiestrogen-resistant breast cancer cells. Oncogene. 2015;34:4199–4210.

[3] Hole S, Pedersen AM, Lykkesfeldt AE, et al. Aurora kinase A and B as new treatment targets in aromatase inhibitor-resistant breast cancer cells. Breast Cancer Res Treat. 2015;149:715–726.

[4] Larsen SL, Yde CW, Laenkholm AV, et al. Aurora B is important for antiestrogen resistant cell growth and a potential biomarker for tamoxifen resistant breast cancer. BMC Cancer. 2015;15:239.

[5] Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nat Rev Cancer. 2017;17:93–115.
[6] Sasai K, Treekitkammonkol T, Kai K, et al. Functional significance of Aurora kinase-p53 protein interactions. Front Oncol. 2016;6:247.
[7] Saeki T, Ouchi M, Ouchi T. Physiological and oncogenic Aurora-A pathway. Int J Biol Sci. 2009;5:758–762.
[8] Dar AA, Goff LW, Majid S, et al. Aurora kinase inhibitors – rising stars in cancer therapeutics? Mol Cancer Therap. 2010;9:267–278.
[9] Siggelkow W, Boehm D, Gebhard S, et al. Expression of Aurora kinase A is associated with metastasis-free survival in node-negative breast cancer patients. BMC Cancer. 2012;12:562.
[10] Ali HR, Dawson SJ, Blows FM, et al. Aurora kinase A outperforms Ki67 as a prognostic marker in ER-positive breast cancer. Br J Cancer. 2012;106:1798–1806.
[11] Yamamoto S, Yamamoto-Ibusuki M, Yamamoto Y, et al. A comprehensive analysis of Aurora A; transcript levels are the most reliable in association with proliferation and prognosis in breast cancer. BMC Cancer. 2013;13:217.
[12] Zheng XQ, Guo JP, Yang H, et al. Aurora-A is a determinant of tamoxifen sensitivity through phosphorylation of ERα in breast cancer. Oncogene. 2014;33:4985–4996.
[13] Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med. 2005;353:2747–2757.
[14] Henriksen KL, Rasmussen BB, Lykkesfeldt AE, et al. Semi-quantitative scoring of potentially predictive markers for endocrine treatment of breast cancer: a comparison between whole sections and tissue microarrays. J Clin Pathol. 2007;60:397–404.
[15] Allred DC, Harvey JM, Berardo M, et al. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1998;11:155–168.
[16] Regan MM, Neven P, Giobbie-Hurder A, et al. Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8.1 years median follow-up. Lancet Oncol. 2011;12:1101–1108.
[17] Nadler Y, Camp RL, Schwartz C, et al. Expression of Aurora A (but not Aurora B) is predictive of survival in breast cancer. Clin Cancer Res. 2008;14:4455–4462.
[18] Cirak Y, Furuncuoglu Y, Yapicier O, et al. Aurora A overexpression in breast cancer patients induces taxane resistance and results in worse prognosis. J BUON. 2015;20:1414–1419.
[19] Viale G, Giobbie-Hurder A, Regan MM, et al. Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrine-responsive breast cancer: results from Breast International Group trial 1-98 comparing adjuvant tamoxifen with letrozole. J Clin Oncol. 2008;26:5569–5575.
[20] Xu J, Xing W, Zhou W, et al. Aurora-A identifies early recurrence and poor prognosis and promises a potential therapeutic target in triple negative breast cancer. PLoS One. 2013;8:e56919.
[21] Thrane S, Lykkesfeldt AE, Larsen M, et al. Estrogen receptor α is the major driving factor for growth in tamoxifen-resistant breast cancer and supported by HER/ERK signaling. Breast Cancer Res Treat. 2013;139:71–80.
[22] Hole S, Pedersen AM, Hansen S, et al. New cell culture model for aromatase inhibitor-resistant breast cancer shows sensitivity to fulvestrant treatment and cross-resistance between letrozole and exemestane. Int J Oncol. 2015;46:1481–1490.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 74–82
https://doi.org/10.1080/0284186X.2017.1400180

Taylor & Francis
Taylor & Francis Group

REVIEW

Triple negative breast cancer – prognostic role of immune-related factors: a systematic review

Elisabeth Specht Stovgaard[a], Dorte Nielsen[b], Estrid Hogdall[c] and Eva Balslev[a]

[a]Deparment of Pathology, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark; [b]Department of Oncology, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark; [c]Department of Pathology, Molecular Unit, Herlev and Gentofte Hospital, University of Copenhagen, Herlev, Denmark

ABSTRACT

Purpose: Treatment of breast cancer has been increasingly successful in recent years with the advent of HER2-receptor targeted treatment and endocrine treatment. However, the triple negative subgroup of breast cancer (TNBC) (estrogen-, progesterone- and HER2-receptor negative) still lacks targeted treatment options. TNBC is a type of breast cancer that often affects younger women, and generally has a worse prognosis than other types of breast cancer. Recently, the complex role of the immune system in cancer growth, elimination and metastasis has been the object of increased attention. There is hope that a more detailed understanding of the intricate roles of the constituents of the immune system, will hold potential both as prognostic or predictive markers of cancer progression, but also as treatment targets for a wide range of tumors, including TNBC. The aim of this review is to provide an overview of the cellular immune microenvironment in TNBC, and to highlight areas in which TNBC may differ from other types of breast cancer.

Material and methods: A search of PubMed was made using the terms 'triple negative breast cancer' and 'tumor infiltrating lymphocytes', 'CD8', 'CD4', 'B cells', 'natural killer cells', 'macrophages', myeloid derived suppressor cells', 'dendritic cells', 'immune check point inhibitor', 'CTLA-4' and 'PD-L1'.

Results: We find that whilst factors such as TILs and certain subgroups of TILs (e.g., CD8 + and regulator T-cells) have been extensively researched, none of these markers are currently applicable to routine clinical practice. Also, TNBC differs from other types of breast cancer with regards to cellular composition of the immune infiltrate and PD-L1 expression, and the prognostic significance of these.

Conclusions: Immune-related factors have the potential as both prognostic and predictive biomarkers for new treatments targeting the immune system in breast cancer. However, multivariate analyses, taking other well-known factors into account, are required to determine the true value of these biomarkers. Also, differences between TNBC and other types of breast cancer may have implications for treatment and use of immune-related factors as biomarkers.

ARTICLE HISTORY
Received 15 September 2017
Accepted 28 October 2017

Introduction

Triple negative breast cancer

Triple negative breast cancer (TNBC) is defined by ≤1% estrogen receptor (ER) positive tumor cells, progesterone receptor (PR) negativity, and normal HER2-receptor expression (HER2 normal by immunohistochemistry (IHC) or *in situ* hybridization (ISH) analysis separately or combined). In the literature, TNBC and basal-like breast cancer is often used interchangeably, however, the two terms are not totally overlapping, as basal-like breast cancer can be receptor-positive in rare cases. TNBC constitutes 15–20% of all breast carcinomas [1]. TNBC affects younger women more often and has a worse prognosis than breast cancer in general, due to a combination of more aggressive clinical behavior and lack of molecular targets for therapy [2].

Due to lack of targeted treatment, there is a need for new treatment options, and amongst these there are hopes that the emerging field of immunotherapy will provide efficient treatment strategies for this aggressive cancer.

Prognostic factors in TNBC

Apart from the extensively documented clinico-pathological risk factors such as node-status, tumor size, grade and proliferation rate (ki-67), there are no prognostic and predictive biomarkers suitable for clinical use for TNBC [3]. There is hope that a more detailed knowledge of the interaction between tumor cells and the immune system might lead to clinically useful biomarkers. Among these are tumor infiltrating lymphocytes (TILs) and the prevalence of other cells from the immune system as well as biomarkers related to the immune/tumor interaction, such as PD-L1.

However, in breast cancer, there are differences in the prognostic significance of immune cells according to breast cancer subtype, likewise, the expression and significance of

CONTACT Elisabeth Specht Stovgaard elisabethidaspecht@gmail.com Department of Pathology, Herlev and Gentofte Hospital, University of Copenhagen, Herlev Ringvej, DK-2730 Herlev, Denmark

immune checkpoint markers such as PD-L1 may not have the same significance in TNBC as in other, less aggressive breast cancers [4–8].

Aim of this review

The purpose of this review is to give an overview of the composition of cells of the immune system and of biomarkers of immune checkpoint inhibitors in the tumor microenvironment and their significance in TNBC. Hereby elucidating which cells and which biomarkers related to the immune system play the most important role in the interaction between tumor and immune system, it is necessary to investigate the tumor microenvironment to discover which immune cells are present, and what their prognostic and predictive values are. This will clarify if known cancer immune treatments will be effective, but will also potentially inform us of other possible targets for therapy.

Material and methods

A search of PubMed was made using the terms 'triple negative breast cancer' and 'tumor infiltrating lymphocytes', 'CD8', 'CD4', 'B cells', 'natural killer cells', 'macrophages', 'myeloid derived suppressor cells', 'dendritic cells', 'immune check point inhibitor', 'CTLA-4' and 'PD-L1'. Only articles written in English including at least 50 patients were included, unless there were no studies on the subject except with less than 50 patients.

Significance of TILs in TNBC

When considering the inflammatory infiltrate in cancers, TILs have been the main focus of much of recent research. TILs have been shown both in breast cancer in general and in TNBC in particular to be a strong prognostic indicator. Loi et al. were the first to show that each 10% increment in intratumoral and stromal TILs was associated respectively with a 27% and 17% reduced risk of death in the TNBC group of a study including 2009 breast cancer patients. Loi et al. later confirmed these results in a study of 134 TNBCs, where they also showed that a high number of TILs was a significant predictor of distant recurrence, and that each 10% increase in TILs was associated with a 13% reduction in relative risk of distant recurrence [9].

In a meta-analysis of the prognostic value of TILs in TNBC including 8 studies with a total of 2987 patients, it was found that cancers rich in TILs were associated with a 30% reduced risk of recurrence, a 22% reduction in distant recurrence and a 34% reduced risk of death [10].

TILs have also been shown to be predictive of response to neoadjuvant chemotherapy (NAC). Denkert et al. were the first to show a positive association between TILs and response to NAC in a study of over 1000 breast cancer patients [11]. Denkert et al. later confirmed that TILs are also associated with pathological complete remission (pCR) to NAC when looking at the TNBC group alone [12], and these results have been confirmed by others [13].

To facilitate the use of TILs as a prognostic marker in the clinical setting, an International TILs Working Group published guidelines to allow for a more standardized evaluation of this parameter [14], with minor modifications added in 2017 [15]. However, despite these efforts, interobserver variance is still deemed too great to allow for TIL evaluation to be introduced in routine clinical practice [16].

Subpopulations of TILs

With regards to subtypes of TILs the strongest evidence for effect on outcome has been found for T-lymphocytes. T-lymphocytes are the most predominant type of lymphocytes in the tumor microenvironment, constituting up to 75% of TILs [17]. In the following, we discuss subtypes of TILs with different impacts on prognosis. A recurring paradox in this area is that despite the functional heterogeneity of TIL subtypes, the very general parameter of TIL evaluation on H and E (Hematoxylin Eosin) stains is still a strong prognostic factor [14]. Also, some TIL subtypes are known to downregulate the immune system. However, their presence in some cancers seems to infer a better prognosis [18,19]. This somewhat arbitrary effect is taken as a sign that the presence of these cells is an expression of a robust immune response, including natural feedback mechanisms [14].

CD8+-T lymphocytes

CD8 + T-lymphocytes differentiate into cytotoxic T-lymphocytes (CTLs) upon recognition of antigen and play a key role in the adaptive immunological defense against foreign agents and tumor cells. In TNBC, as in many other cancer types, tumors rich in CD8 + T-lymphocytes are associated with a better prognosis (Table 1) [10,20–22]. CD8 + infiltrates are seen in 60% of TNBCs [23]. Some evidence suggests that the effect of CD8 + T-cells is more powerful in hormone receptor negative breast cancers. In a study of 1854 breast cancer samples, Baker et al. [4] only found independent prognostic significance of CD8 + T-cells in ER-negative breast cancers ($p = .03$), whereas the same could not be shown for ER positive tumors.

CD4 + T helper cells

CD4 + T helper cells can differentiate into a variety of subtypes upon activation, and their function is to modulate the activity and differentiation of the immune system through modulation of, e.g., B-cells, CD8 + -T cells and macrophages [24]. The main subgroups that have been investigated are T-helper cells (TH1), follicular T helper cells and regulator T-lymphocytes.

TH1 are the principal source of interferon-γ, and follicular T-helper cells (Tfh) are a relatively newly described subgroup of CD4 + T-cells. Both subgroups have shown improved survival in some hormone receptor positive breast cancers, but, as yet, not in TNBC [17].

Regulator T-lymphocytes (Tregs) are a subpopulation of CD4 + T-lymphocytes with the immune phenotype CD4+, CD25+, Fox3P+. In breast cancer, TNBCs have the highest

Table 1. Prognostic value of T-lymphocyte subtypes.

	Number of patients	Phenotype	Evaluation method	CD8+	CD4 + TH1	CD4 + Tfh	CD4 + Treg (FOXP3+)
Mahmoud et al. [20]	1902 of which 261 basal-like	Basal-like and ER/PR/HER2 +/−	IHC for CD8	CD8-high associated with better breast cancer specific survival			
Miyashita et al. [21]	110	TNBC	IHC for CD8 and FOXP3	CD8-high better pCR rates (41 vs 18%, $p = .03$)			Low CD8+/FOXP3+ ratio associated with lower pCR (44 vs 14%, $p = .002$)
Matsumoto et al. [22]	232	TNBC	IHC for CD8 and CD4	Better DFS with high intratumoral CD8+ TILs: 0.48 (CI: 0.27–0.83, $p = .01$)			
Baker et al. [23]	1953, of which 268 ER negative	ER/PR/HER2 +/−, histological grade high/low	IHC for CD8	Recurrence rate for CD8+ high, ER-negative: 0.70 (CI: 0.5–1.0, $p = .03$)			
Gu-Trantien et al. [17]	70	ER/PR/HER2 +/−	Flow cytometry and gene expression analysis		Prognostic significance in TNBC not yet shown	Prognostic significance in TNBC not yet shown	
Yeung et al. [18]	164	TNBC	IHC, immunofluorescence, NanoString gene expression				High Treg associated with improved survival (HR: 0.33, 95% CI 0.17–0.66, $p = .002$)
Liu et al. [28]	3992, of which 630 TNBC	ER/PR/HER2 +/−, basal-like or TNBC, non basal-like	IHC				Basal-like tumors high in FOXP3+ had significantly better survival. No effect on TNBC, non-basal-like

amounts of FOXP3 + cells (70%) compared to other types of breast cancer [23]. The function of Tregs in the normal immune environment is to regulate and suppress immune responses to prevent autoimmune reactions. Traditionally, it has been believed that, in the tumoral environment, regulatory T-cells can suppress the effect of other effector cells, thus preventing an effective immune response to tumor [25]. Previously, it was thought that high levels of Tregs were associated with a worse prognosis. However, several recent studies have shown the opposite in a variety of cancers, including TNBC (Table 1) [18,26–28]. It is, as yet, unknown what the exact mechanism behind this positive effect of Tregs is, but in colorectal cancer, certain subsets of Tregs were associated with a better prognosis than others [29]. A similar study has not, to our knowledge, been performed in breast cancer, but Syed et al. showed the accumulation of a subset of Treg cells with immunosuppressive characteristics in breast cancer tumor microenvironment, compared to normal tissue [30].

Natural killer cells

Natural killer cells (NK) recognize and eliminate foreign cells lacking the MHC class 1 molecule, necessary for activation of CD8 + lymphocytes [31]. Studies of breast cancers in general and TNBC cell lines in particular, have shown that tumor cells are capable of downregulating their 'visibility' to NK cells through modulation of their receptors and inhibitory factors in the microenvironment [32,33]. Studies of breast cancer have shown NK cells to be associated with a better prognosis, but there has been little research regarding differences between breast cancer subtypes (Table 2) [34,35].

B-cells

B-lymphocytes have not been shown to have the same degree of significance as T-lymphocytes, with studies showing both worse, better, or unaffected prognoses [36,37]. However, some evidence suggests, that the B-lymphocyte population in the basal-like subtype might have more significance than in other types of breast cancer, where Iglesia et al. showed that metastasis- and DFS correlated better with B-cell gene expression signatures for basal-like and HER-2 enriched cancers, than for other subtypes (Table 2) [38].

Other cells from the immune system

Tumor-associated macrophages

Tumor-associated macrophages (TAMs) play a key role in regulating the interaction between the immune system and cancer [39]. Two subtypes are relevant, M1 and M2, where M1 is an efficient antigen presenter and produces inflammatory cytokines, whereas M2 macrophages participate in dampening of inflammation, angiogenesis and tumor progression [40].

In breast cancer in general and in TNBC, TAMs are mostly associated with a worse prognosis (Table 2) [37,41,42]. Campbell et al. showed that proliferating macrophages are associated with hormone-receptor negativity ($p = .00001$ for ER and $p = .002$ for PR), and with basal-like cancer, but not with HER2 status [42].

Also, the composition of macrophage subtypes seems to be different in TNBC. Stewart et al. showed that basal-like breast cancer cells had a greater ability than the less aggressive luminal breast cancer type, to drive macrophage differentiation in cell cultures, and to induce polarization towards both M1 and M2 phenotype, creating a population of macrophages distinct from the population found in cell cultures with breast cancer cells of a less aggressive type [43].

Evidence also suggests that the prevalence of the M2-phenotype is more prevalent in TNBC/basal-like breast cancers than in hormone receptor positive breast cancers, as shown by Medrek et al. in a study of 144 breast cancer samples, where high densities of CD163 + macrophages in tumor stroma (CD163 is a biomarker for M2 macrophages) were associated with TNBC/basal-like cancers, higher grade and larger size. However, the study only included 15 patients with TNBC/basal-like tumors [5].

Dendritic cells

Dendritic cells (DCs) are professional antigen presenting cells (APCs) that participate in the activation of adaptive immune cells, e.g., T-cells. However, tumor infiltrating DCs often show an aberrant phenotype with lower expression of costimulatory molecules, blunted antigen cross representation and upregulation of regulatory molecules, pointing towards factors in the tumor environment blunting the stimulatory effect of DCs, turning them towards a protumorigenic effect. A subgroup of DCs often seen in tumors are the plasmacytoid dendritic cells (pDCs), which are often associated with a worse prognosis, tumor tolerance and upregulation of Treg [6,44–47]. In breast cancers, higher numbers of pDC were found in TNBC than in less aggressive tumor types in 151 patients with non-metastatic cancer (Table 2) [6]. Clinical trials in breast cancer patients with dendritic cell vaccines are ongoing, some in combination with chemotherapy, but so far results have been negligible [48].

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells. Their main function is to inhibit the immune system through secretion of inhibitory cytokines and other substances [49]. Circulating MDSCs in peripheral blood have been shown to be elevated in breast cancer patients in all stages of the disease, and to be positively correlated with stage and metastasis [50,51]. Most of the research of MDSCs in the tumor microenvironment has been performed in murine models, and very little research exits on MDSCs in human breast cancer tissue, but one study showed MDSCs to be expanded in breast cancer tumor tissue as opposed to normal tissue and was not particularly associated with hormone receptor negativity. However, this study only included 23 breast cancer patients [52].

Table 2. Other cells from the immune system.

	Number of patients	Phenotype	Evaluation method	NK-cells	B-cells	Macrophages	Dendritic cells (DC)
Ascierto et al. [35]	14, of which 9 TNBC	ER/PR/HER2 +/−	IHC and gene expression profiling	Increased expression of NK-activating genes in patients without recurrence			
Mahmoud et al. [37]	1902, of which 288 basal-like	ER/PR/HER2 +/−	IHC		Total CD20+ B-cell count independently associated with outcome (HR = 0.75, CI = 0.58–0.96)		
Iglesia et al. [39]	855, of which 140 basal-like	Classified according to gene expression profiles	Gene expression analysis		B-cells associated with improved metastasis free survival in basal-like		
Murri et al. [43]	168, of which 35 ER/PR negative	ER/PR +/−, HER2 not evaluated	IHC			No significant effect of macrophages in multivariate analysis	
Campbell et al. [44]	216, of which 29 basal-like	ER/PR/HER2 +/−	IHC			Proliferating macrophages associated with 75% increased risk of death ($p = .048$)	
Medrek et al. [5]	144, of which 15 TNBC/basal-like	ER/PR/HER2 +/−	IHC and gene expression profiling			Macrophages an independent risk factor for BCSS (HR = 0.12, CI, 0.02–0.72, $p = 0.02$)	
Treilleux et al. [49]	255	ER/PR/HER2 +/−	IHC				OS at 58 months: 93% vs 73% ($p = .05$) for plasmacytoid DC high tumors
Sisirak et al. [6]	60, of which 12 TNBC	ER/PR/HER2 +/−	IHC and flow cytometry				Dendritic cells associated with TNBC

Table 3. PD-L1.

Author	Number of patients	Phenotype	Evaluation method	CTLA4	PD-L1
Yu et al. [61]	130	ER/PR/HER2 +/−	IHC	CTLA4 expression independent predictor of shorter DFS (HR 2.17, $p = .03$) and OS (HR 2.82, $p = .01$)	
Botti et al. [72]	238	TNBC	IHC		PD-L1 expression associated with better DFS ($p = .04$)
Zhang et al. [8]	2546 (metaanalysis)	ER/PR/HER2 +/−	–		PD-L1 expression associated with worse OS (HR = 1.76, CI:1.09–2.82, $p = .02$).
Wang et al. [64]	443, of which 34 TNBC, non-basal-like, 69 basal-like	ER/PR/HER2 +/−	IHC		PD-L1 expression associated with better RFS (HR: 0.39, CI = 0.22–0.86, $p = .02$)
Li et al. [73]	136	TNBC	IHC		Stromal PD-L1 expression associated with better DFS ($p = .05$)
Mori et al. [74]	248	TNBC	IHC		High PD-L1 expression and low TILs independent prognostic factor of RFS and OS (for RFS: HR = 4.7, CI 1.6–12.7, $p = .01$; for OS: HR = 8.4, CI 2.3–30.3, $p = .02$)

Immune checkpoints as a prognostic factor and treatment target

The function of immune checkpoints in the non-cancerous environment is to regulate the proliferation and activities of cytotoxic cells to prevent autoimmune reactions. In the cancerous environment, these mechanisms are adopted by cancer cells to render immune cells anergic, and unable to eliminate tumor cells.

Immunotherapy, which mainly focuses on blocking immune-regulating proteins that suppress the anticancer tumor response, has proven effective in many different cancer types, including melanoma and lung cancer [53–55].

There are currently numerous ongoing clinical studies involving breast cancer patients and various immunotherapeutic treatment strategies [56]. The most established forms of immunotherapy are centered around the Programmed Death 1 receptor (PD-1) and its ligand PD-L1, and also CTLA-4.

CTLA-4

CTLA-4 blockade is effective in the treatment of melanoma, but is not yet well researched in breast cancer [56,57]. CTLA-4 can be expressed by cancer cells and plays a key role in 'switching off' the immune response of T-cells by progressively blocking the co-stimulatory signals from APCs, needed by T-cells to react to the antigens they are presented to [56,58]. In breast cancer, CTLA-4 has been shown to be an independent predictor of shorter DFS (hazard ratio (HR) 2.176, 95% CI 1.084–4.437, $p = .029$) and OS (HR 2.820, CI 1.337–5.950, $p = .007$) in 130 patients (see Table 3) [59], but differences in CTLA-4 expression in subtypes of breast cancer have not been described.

PD-1 and PD-L1

PD-1 is expressed in activated T-lymphocytes, but also in B-lymphocytes, mononuclear cells, NK cells and some DCs [60]. When PD-1 binds to its ligand PD-L1, it serves to down-regulate T-cell activity, thus playing an important role in harnessing autoimmune reactions in the normal body [61]. PD-L1 is expressed in a variety of solid tumors, including breast cancer, colorectal cancer, melanoma and lung cancer [62–65].

PD-L1 is commonly expressed in TNBC. In a study including 35 triple-negative, non-basal-like tumors and 69 basal-like tumors, high expression of PD-L1 was found in 31% and 33%, respectively [62]. Mittendorf et al. found higher expression of PD-L1 in TNBC, than in other cancer types, using RNA sequencing ($p = .001$) [66], but there have been conflicting results as to whether there is a positive correlation between basal-like/hormone-receptor negative cancers and PD-L1 expression compared to other breast cancer subtypes [7,8,67,68].

Another area with conflicting results is the impact of high expression of PD-L1 on prognosis, with recent evidence pointing towards PD-L1 expression being associated with improved survival (Table 3) [69]. This somewhat arbitrary effect is thought to be explained by high expression of PD-L1 being an indicator of a more robust immune response to tumor. However, other studies have reported worse outcomes with high PD-L1 expression, as described below. A meta-analysis including 5 studies composing 2546 patients with breast cancer of all types found association between shorter OS and PD-L1 overexpression (HR = 1.76, 95% CI 1.09–2.82; $p = .02$), but also found association between TNBC and higher levels of PD-L1 expression [8]. However, one of the studies included in this meta-analysis also studied outcome in subtypes, concluding that PD-L1 was a significant

predictor of OS in the basal-like subtype (HR: 2.60, CI: 1.016–6.652, $p = .046$) [7].

Interestingly, Wang et al. reported no effect on outcome for high expression of PD-L1 in general in breast tumors, except for the basal-like subtype, where it was associated with better recurrence free survival (RFS) (HR = 0.39, CI = 0.22–0.86, $p = .018$) [62]. Other studies, exclusively including TNBC have also found diverging results, with Botti et al. showing better DFS for tumors expressing PD-L1 in 238 TNBCs ($p = .04$), while there was no effect on OS [69] and Li et al. showing only association between stromal PD-L1 expression and DFS ($p = .04$), while there was no effect on OS or with regards to tumoral expression of PD-L1 [70]. Mori et al. found no effect on OS with PD-L1 alone in TNBC, but high expression of PD-L1 in combination with low numbers of TILs was an independent prognostic factor of both RFS and OS (for RFS: HR = 4.7, CI 1.6–12.7, $p = .0067$; for OS: HR = 8.4, CI 2.3–30.3, $p = .019$) [71]. Clinical trials targeting the PD-1-PD-L1 pathway to treat TNBC patients are ongoing, and preliminary results have been promising [72,73].

A high expression of PD-L1 is associated with higher chance of response to PD-L1 blockade in several tumor types. However, even tumors with very low expression do respond, but less frequently. Selecting patients for treatment with PL-L1 blockade based on PD-L1 expression, and if so, which cut-point to use, is therefore debated [54].

Discussion

Biomarkers related to the immune system have been demonstrated to be of prognostic significance in many tumor types, including breast cancer. However, even TILs, the most thoroughly evaluated parameter, is not yet ready for clinical use, due to interobserver variability and lack of standardization.

The composition of immune cells in the tumor microenvironment of TNBC in some ways resemble that of breast cancer in general, however, there are differences in the prevalence and prognostic significance of several of the cells, and the exact impact on prognosis is not yet known. The composition of the cellular tumor microenvironment is therefore currently mostly of scientific interest, and is not yet ready to be utilized as prognostic indicators in the clinical setting.

The immune checkpoint markers are also correlated with other known prognostic factors, and multivariate analyses taking other known prognostic factors into account has, in some cases, not been performed in past research, but are needed to assess the true value of immune biomarkers in the prognostic evaluation of patients.

More research in this area may be important for our understanding of the role of the immune system in different tumor types. Moreover, treatments targeting the immune system are being developed, and the immune biomarkers may become essential as predictive factors for selecting patients that are likely to benefit from treatment. It remains to be determined which markers, and which cut-points, are optimal. Research in PD-L1 and other biomarkers has, so far given diverse and sometimes contradictory results. This might be due to different cut-of points and methods of evaluation, but also because of different qualities in the antibodies utilized in histological evaluation. Perhaps the future direction of research in this area should focus on expression of biomarkers on a molecular level, as this could lead to more uniform results, which will potentially lead to a better understanding of prognosis and of which patients may benefit from immunotherapy.

Immunotherapy would seem to be a promising treatment modality in TNBC. First, TILs are generally more predominant in this subgroup [74,75]. Secondly, the hormone receptor negative/basal-like subtypes have been considered the most likely candidates to benefit from immunotherapy, due to their high levels of mutations, resulting in a larger number of neo-antigens, which have been shown to be immunogenic [76–79]. TNBC has few targeted treatment options, and further research into immunotherapy for this disease may lead to significant improvements in the treatment and prognosis for these patients.

Disclosure statement

The authors report no conflicts of interest.

ORCID

Elisabeth Specht Stovgaard http://orcid.org/0000-0002-5784-3610

References

[1] Reis-Filho JS, Tutt ANJ. Triple negative tumours: a critical review. Histopathology. 2007;52:108–118.
[2] Bianchini G, Balko JM, Mayer IA, et al. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13:674–690.
[3] Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies – improving the management of early breast cancer: St Gallen international expert consensus on the primary therapy of early breast cancer 2015. Ann Oncol. 2015;26:1533–1546.
[4] Baker K, Lachapelle J, Zlobec I, et al. Prognostic significance of CD8+ T lymphocytes in breast cancer depends upon both oestrogen receptor status and histological grade. Histopathology. 2011;58:1107–1116.
[5] Medrek C, Pontén F, Jirström K, et al. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer. 2012;12:306.
[6] Sisirak V, Faget J, Gobert M, et al. Impaired IFN-α production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression. Cancer Res. 2012;72:5188–5197.
[7] Li Z, Dong P, Ren M, et al. PD-L1 expression is associated with tumor FOXP3(+) regulatory T-Cell infiltration of breast cancer and poor prognosis of patient. J Cancer. 2016;7:784–793.
[8] Zhang M, Sun H, Zhao S, et al. Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. Oncotarget. 2017;8:31347–31354.
[9] Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25:1544–1550.
[10] Ibrahim EM, Al-Foheidi ME, Al-Mansour M. The prognostic value of tumor-infiltrating lymphocytesin triple-negative breast cancer: a meta-analysis. Breast Cancer Res Treat. 2014;148:467–476.

[11] Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28:105–113.

[12] Denkert C, von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2–positive and triple-negative primary breast cancers. J Clin Oncol. 2015;33:983–991.

[13] Ono M, Tsuda H, Shimizu C, et al. Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer. Breast Cancer Res Treat. 2012;132:793–805.

[14] Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an international TILs working group 2014. Ann Oncol. 2015;26:259–271.

[15] Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors. Adv Anat Pathol. 2017;24:235–251.

[16] Denkert C, Wienert S, Poterie A, et al. Standardized evaluation of tumor-infiltrating lymphocytes in breast cancer: results of the ring studies of the international immuno-oncology biomarker working group. Mod Pathol. 2016;29:1155–1164.

[17] Gu-Trantien C, Loi S, Garaud S, et al. $CD4^{+}$ follicular helper T cell infiltration predicts breast cancer survival. J Clin Investig. 2013;123:2873–2892.

[18] Yeong J, Thike AA, Lim JCT, et al. Higher densities of Foxp3+ regulatory T cells are associated with better prognosis in triple-negative breast cancer. Breast Cancer Res Treat. 2017;163:21–35.

[19] West NR, Kost SE, Martin SD, et al. Tumour-infiltrating FOXP3(+) lymphocytes are associated with cytotoxic immune responses and good clinical outcome in oestrogen receptor-negative breast cancer. Br J Cancer. 2013;108:155–162.

[20] Mahmoud SMA, Paish EC, Powe DG, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29:1949–1955.

[21] Miyashita M, Sasano H, Tamaki k. Tumor-infiltrating CD8+ and FOXP3+ lymphocytes in triple-negative breast cancer: its correlation with pathological completeresponse to neoadjuvant chemotherapy. Breast Cancer Res Treat. 2014;148:525–534.

[22] Matsumoto H, Thike AA, Li H, et al. Increased CD4 and CD8-positive T cell infiltrate signifies good prognosis in a subset of triple-negative breast cancer. Breast Cancer Res Treat. 2016;156:237–247.

[23] Stanton SE, Disis ML. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. J Immunother Cancer. 2016;4:59.

[24] Ahn SG, Jeong J, Hong S, et al. Current issues and clinical evidence in tumor-infiltrating lymphocytes in breast cancer. J Pathol Transl Med. 2015;49:355–363.

[25] Nishikawa H, Sakaguchi S. Regulatory T cells in tumor immunity. Int J Cancer. 2010;127:759–767.

[26] Sun X, Feng Z, Wang Y, et al. Expression of Foxp3 and its prognostic significance in colorectal cancer. Int J Immunopathol Pharmacol. 2017;30:201–206.

[27] Liu S, Foulkes WD, Leung S, et al. Prognostic significance of FOXP3+ tumor-infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. Breast Cancer Res. 2014;16:432.

[28] Lee S, Cho EY, Park YH, et al. Prognostic impact of FOXP3 expression in triple-negative breast cancer. Acta Oncol. 2013;52:73–81.

[29] Saito T, Nishikawa H, Wada H, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med. 2016;22:679–684.

[30] Syed Khaja AS, Toor SM, El Salhat H, et al. Preferential accumulation of regulatory T cells with highly immunosuppressive characteristics in breast tumor microenvironment. Oncotarget. 2017;8:33159–33171.

[31] Mamessier E, Bertucci F, Sabatier R, et al. Breast cancer cells shun NK-cells anti-tumor immunity. Oncoimmunology. 2012;1:366–368.

[32] Mamessier E, Sylvain A, Thibult M-L, et al. Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. J Clin Investig. 2011;121:3609–3622.

[33] Park IH, Yang HN, Lee KJ, et al. Tumor-derived IL-18 induces PD-1 expression on immunosuppressive NK cells in triple-negative breast cancer. Oncotarget. 2017;8:32722–32730.

[34] Ascierto ML, Idowu MO, Zhao Y, et al. Molecular signatures mostly associated with NK cells are predictive of relapse free survival in breast cancer patients. J Transl Med. 2013;11:145.

[35] Tian W, Wang L, Yuan L, et al. A prognostic risk model for patients with triple negative breast cancer based on stromal natural killer cells, tumor-associated macrophages and growth-arrest specific protein 6. Cancer Sci. 2016;107:882–889.

[36] Mahmoud SMA, Lee AHS, Paish EC, et al. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. Breast Cancer Res Treat. 2012;132:545–553.

[37] Mohammed ZMA, Going JJ, Edwards J, et al. The role of the tumour inflammatory cell infiltrate in predicting recurrence and survival in patients with primary operable breast cancer. Cancer Treat Rev. 2012;38:943–955.

[38] Iglesia MD, Vincent BG, Parker JS, et al. Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific breast and ovarian cancer. Clin Cancer Res. 2014;20:3818–3829.

[39] Ostuni R, Kratochvill F, Murray PJ, et al. Macrophages and cancer: from mechanisms to therapeutic implications. Trends Immunol. 2015;36:229–239.

[40] Mantovani A, Biswas SK, Galdiero MR, et al. Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol. 2013;229:176–185.

[41] Murri AMA, Hilmy M, Bell J, et al. The relationship between the systemic inflammatory response, tumour proliferative activity, T-lymphocytic and macrophage infiltration, microvessel density and survival in patients with primary operable breast cancer. Br J Cancer. 2008;99:1013–1019.

[42] Campbell MJ, Tonlaar NY, Garwood ER, et al. Proliferating macrophages associated with high grade, hormone receptor negative breast cancer and poor clinical outcome. Breast Cancer Res Treat. 2011;128:703–711.

[43] Stewart DA, Yang Y, Makowski L, et al. Basal-like breast cancer cells induce phenotypic and genomic changes in macrophages. Mol Cancer Res. 2012;10:727–738.

[44] Tran Janco JM, Lamichhane P, Karyampudi L, et al. Tumor-infiltrating dendritic cells in cancer pathogenesis. J Immunol. 2015;194:2985–2991.

[45] Ito T, Yang M, Wang Y-H, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J Exp Med. 2007;204:105–115.

[46] Palucka K, Coussens LM, O'Shaughnessy J. Dendritic cells, inflammation, and breast cancer. Cancer J. 2013;19:511–516.

[47] Treilleux I, Blay J-Y, Bendriss-Vermare N, et al. Dendritic cell infiltration and prognosis of early stage breast cancer. Clin Cancer Res. 2004;10:7466–7474.

[48] Gelao L, Criscitiello C, Esposito A, et al. Dendritic cell-based vaccines: clinical applications in breast cancer. Immunotherapy. 2014;6:349–360.

[49] Markowitz J, Wesolowski R, Papenfuss T, et al. Myeloid-derived suppressor cells in breast cancer. Breast Cancer Res Treat. 2013;140:13–21.

[50] Diaz-Montero CM, Salem ML, Nishimura MI, et al. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009;58:49–59.

[51] Bergenfelz C, Larsson A-M, von Stedingk K, et al. Systemic monocytic-MDSCs are generated from monocytes and correlate with disease progression in breast cancer patients. PLoS One. 2015;10:e0127028.

[52] Toor SM, Syed Khaja AS, El Salhat H, et al. Myeloid cells in circulation and tumor microenvironment of breast cancer patients. Cancer Immunol Immunother. 2017;66:753–764.

[53] Hu X, Huang W, Fan M. Emerging therapies for breast cancer. J Hematol Oncol. 2017;10:98.
[54] Nishino M, Ramaiya NH, Hatabu H, et al. Monitoring immune-checkpoint blockade: response evaluation and biomarker development. Nat Rev Clin Oncol. 2017;14:655–668.
[55] Pianko MJ, Liu Y, Bagchi S, et al. Immune checkpoint blockade for hematologic malignancies: a review. Stem Cell Investig 2017;4:32.
[56] Voutsadakis IA. Immune blockade inhibition in breast cancer. Anticancer Res. 2016;36:5607–5622.
[57] Daud A. Current and emerging perspectives on immunotherapy for melanoma. Semin Oncol. 2015;42(Suppl 3):S3–S11.
[58] Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161:205–214.
[59] Yu H, Yang J, Jiao S, et al. Cytotoxic T lymphocyte antigen 4 expression in human breast cancer: implications for prognosis. Cancer Immunol Immunother. 2015;64:853–860.
[60] Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219–242.
[61] Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25:9543–9553.
[62] Wang Z-Q, Milne K, Derocher H, et al. PD-L1 and intratumoral immune response in breast cancer. Oncotarget. 2017;8:51641–51651.
[63] Koganemaru S, Inoshita N, Miura Y, et al. Prognostic value of programmed death-ligand 1 expression in patients with stage III colorectal cancer. Cancer Sci. 2017;108:853–858.
[64] Minghui Z, Dalong W, Qi S, et al. Prognostic significance of PD-L1 expression and 18F-FDG PET/CT in surgical pulmonary squamous cell carcinoma. Oncotarget. 2017;8:51630–51640.
[65] Vilain RE, Menzies AM, Wilmott JS, et al. Dynamic changes in PD-L1 expression and immune infiltrates early during treatment predict response to PD-1 blockade in melanoma. Clin Cancer Res. 2017;23:5024–5033.
[66] Mittendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res. 2014;2:361–370.
[67] Muenst S, Schaerli AR, Gao F, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat. 2014;146:15–24.
[68] Zawlik I, Gablon N, Szymanska B, et al. Immune checkpoints in aggressive breast cancer subtypes. Neoplasma. 2016;63:768–773.
[69] Botti G, Collina F, Scognamiglio G, et al. Programmed death ligand 1 (PD-L1) tumor expression is associated with a better prognosis and diabetic disease in triple negative breast cancer patients. Int J Mol Sci. 2017;18:E459.
[70] Li X, Wetherilt CS, Krishnamurti U, et al. Stromal PD-L1 expression is associated with better disease-free survival in triple-negative breast cancer. Am J Clin Pathol. 2016;146:496–502.
[71] Mori H, Kubo M, Yamaguchi R, et al. The combination of PD-L1 expression and decreased tumor-infiltrating lymphocytes is associated with a poor prognosis in triple-negative breast cancer. Oncotarget. 2017;8:15584–15592.
[72] Nanda R, Chow LQM, Dees EC, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase Ib KEYNOTE-012 study. J Clin Oncol. 2016;34:2460–2467.
[73] Gibson J. Anti-PD-L1 for metastatic triple-negative breast cancer. Lancet Oncol. 2015;16:e264.
[74] Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol. 2013;31:860–867.
[75] Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes. JAMA Oncol. 2016;2:1354–1360.
[76] Haricharan S, Bainbridge MN, Scheet P, et al. Somatic mutation load of estrogen receptor-positive breast tumors predicts overall survival: an analysis of genome sequence data. Breast Cancer Res Treat. 2014;146:211–220.
[77] Brown SD, Warren RL, Gibb EA, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome Res. 2014;24:743–750.
[78] Cancer Genome Atlas Network TCGA. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61–70.
[79] Luen S, Virassamy B, Savas P, et al. The genomic landscape of breast cancer and its interaction with host immunity. Breast. 2016;29:241–250.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 83–89
https://doi.org/10.1080/0284186X.2017.1404127

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

An inter-observer Ki67 reproducibility study applying two different assessment methods: on behalf of the Danish Scientific Committee of Pathology, Danish breast cancer cooperative group (DBCG)

Anne-Vibeke Laenkholm[a], Dorthe Grabau[b]†, Maj-Lis Møller Talman[c], Eva Balslev[d], Anne Marie Bak Jylling[e], Tomasz Piotr Tabor[f], Morten Johansen[g], Anja Brügmann[h], Giedrius Lelkaitis[c], Tina Di Caterino[i], Henrik Mygind[a], Thomas Poulsen[j], Henrik Mertz[k], Gorm Søndergaard[l] and Birgitte Bruun Rasmussen[d]

[a]Department of Surgical Pathology, Zealand University Hospital, Slagelse, Denmark; [b]Department of Pathology, Skåne University Hospital, Lund, Sweden; [c]Department of Pathology, Rigshospitalet, Copenhagen, Denmark; [d]Department of Pathology, Herlev Hospital, Herlev, Denmark; [e]Department of Pathology, Odense University Hospital, Odense, Denmark; [f]Department of Pathology, Lillebaelt Hospital, Vejle, Denmark; [g]Department of Pathology, North Denmark Regional Hospital, Hjørring, Denmark; [h]Department of Pathology, Aalborg University Hospital, Aalborg, Denmark; [i]Department of Pathology, Hospital of South West Jutland, Esbjerg, Denmark; [j]Department of Pathology, Sygehus Soenderjylland, Sønderborg, Denmark; [k]Department of Pathology, Regional Hospital of Randers, Randers, Denmark; [l]Department of Pathology, Region Hospital of Viborg, Viborg, Denmark

ABSTRACT

Introduction: In 2011, the St. Gallen Consensus Conference introduced the use of pathology to define the intrinsic breast cancer subtypes by application of immunohistochemical (IHC) surrogate markers ER, PR, HER2 and Ki67 with a specified Ki67 cutoff (>14%) for luminal B-like definition. Reports concerning impaired reproducibility of Ki67 estimation and threshold inconsistency led to the initiation of this quality assurance study (2013–2015). The aim of the study was to investigate inter-observer variation for Ki67 estimation in malignant breast tumors by two different quantification methods (assessment method and count method) including measure of agreement between methods.

Material and methods: Fourteen experienced breast pathologists from 12 pathology departments evaluated 118 slides from a consecutive series of malignant breast tumors. The staining interpretation was performed according to both the Danish and Swedish guidelines. Reproducibility was quantified by intra-class correlation coefficient (ICC) and Lights Kappa with dichotomization of observations at the larger than (>) 20% threshold. The agreement between observations by the two quantification methods was evaluated by Bland–Altman plot.

Results: For the fourteen raters the median ranged from 20% to 40% by the assessment method and from 22.5% to 36.5% by the count method. Light's Kappa was 0.664 for observation by the assessment method and 0.649 by the count method. The ICC was 0.82 (95% CI: 0.77–0.86) by the assessment method vs. 0.84 (95% CI: 0.80–0.87) by the count method.

Conclusion: Although the study in general showed a moderate to good inter-observer agreement according to both ICC and Lights Kappa, still major discrepancies were identified in especially the mid-range of observations. Consequently, for now Ki67 estimation is not implemented in the DBCG treatment algorithm.

ARTICLE HISTORY
Received 14 September 2017
Accepted 1 November 2017

Introduction

The classification of malignant breast tumors into molecular intrinsic subtypes by DNA microarrays (luminal A, luminal B, HER2-enriched and basallike) were introduced by Perou et al. [1]. The study contributed with new insights into the molecular landscape of breast cancer providing important prognostic information concerning both disease free survival and overall survival for each of the molecular intrinsic subtypes with luminal B tumors having poorer outcome than luminal A tumors. The main difference between luminal A and luminal B is related to the higher expression of proliferation related genes in luminal B tumors [2] and it has been confirmed that genes indicative of high tumor cell proliferation are the major contributors of poor prognosis in various prognostic gene assays [3]. Since multigene testing is an expensive procedure not applicable in every pathology laboratory immunohistochemical staining (IHC) for the proliferation marker Ki67 has been an obvious choice. Ki67 is expressed in the cell nucleus during cell cycle in the G1, S, G2 and M phase but not in the G0 phase. Several studies have documented the prognostic and predictive value of Ki67 expression as a continuous variable in both the adjuvant and neoadjuvant setting [4–6]. Cheang et al. [7] showed that a clinical relevant ki67 IHC cut point could be determined

CONTACT Anne-Vibeke Laenkholm anlae@regionsjaelland.dk Department of Surgical Pathology, Zealand University Hospital, Slagelse, Ingemannsvej 48, 4200 Slagelse, Denmark
†Dorthe Grabau has died from severe illness.

from ROC curves to separate luminal A and luminal B tumors as compared to the PAM50 gene expression signature as gold standard. The initial training set consisting of 144 tumors identified a Ki67 threshold of 13.25% separating the luminal A-like and luminal B-like tumors in two distinct prognostic groups. Validation of this finding by IHC in a larger population of ER positive HER2 normal cases ($N = 2276$) confirmed the initial results with a cutoff value of 14%. The study was performed on tissue microarrays (TMA) and a population with excellent prognosis was identified indicating that postmenopausal patients with luminal A-like tumors might be spared chemotherapy. Encouraged by the results from this study the 2011 St. Gallen Consensus Conference [8] introduced the use of pathology to define the intrinsic breast cancer subtypes by application of IHC surrogate markers (ER, PR, HER2 and Ki67) with a specified Ki67 cutoff (>14%) for luminal B-like definition. The Ki67 cutoff value for luminal B-like classification was further changed to ≥20% at the 2013 St. Gallen Consensus Conference [9] based on the work of Prat et al [10]. In addition, the International Ki67 in Breast Cancer working group published recommendations for Ki67 assessment for both the pre-analytical, analytical and post-analytical phase emphasizing the need for standardization of procedures [11].

Since 2010, Ki67 IHC has been performed on all malignant breast tumors in Denmark. Due to concerns about the reproducibility of Ki67 interpretation and quantification the Danish Scientific Committee of Pathology in the DBCG initiated and finalized a Ki67 quality assurance study during a two-year period (2013–2015). Eleven out of twelve Danish departments of pathology involved in breast cancer diagnostics participated in the study in collaboration with Department of Pathology, Skåne University Hospital, Lund, Sweden.

The aim of the study was to investigate inter-observer variation for Ki67 estimation in malignant breast tumors by two different methods according to the national guidelines for Ki67 staining interpretation in Denmark and Sweden including measure of agreement between methods.

Material and methods

Material

Fourteen experienced breast pathologists evaluated 118 slides from a consecutive series of malignant breast tumors as part of this quality assurance study.

The Ki67 staining was performed centrally with CONFIRM anti-Ki67 Rabbit Monoclonal Primary Antibody 30-9 (Roche/Ventana a/s) according to standard procedure (http://www.nordiqc.org/). The slides were circulated among the observers and evaluated locally by standard light microscopy on full tumor sections. Five samples had missing observations by the assessment method and six samples by the count method resulting in a total number of 1647/1646 observations for the assessment method and count method, respectively.

Staining interpretation

Positive ki67 staining was defined as any brown stain in the nucleus above background.

The staining interpretation was performed according to national guidelines:

The Danish interpretation guideline recommends a semi-quantitative evaluation of Ki67 nuclear staining in hotspot areas with notation of the percentage of Ki67 positive invasive tumor cells in 5–10% intervals (assessment method).

The Swedish interpretation guideline recommends calculation of 200 invasive tumor cells in hotspot areas with notation of the number of Ki67 positive tumor cells in percentage (count method). Figure 1 represents an example of nuclear Ki67 staining with variable nuclear staining intensity.

Statistics

The statistical analysis was performed by the DBCG statisticians. The distribution of observations by the assessment and count methods is presented in histograms and inter-observer variability is visualized in box-plots. Intra-class correlation

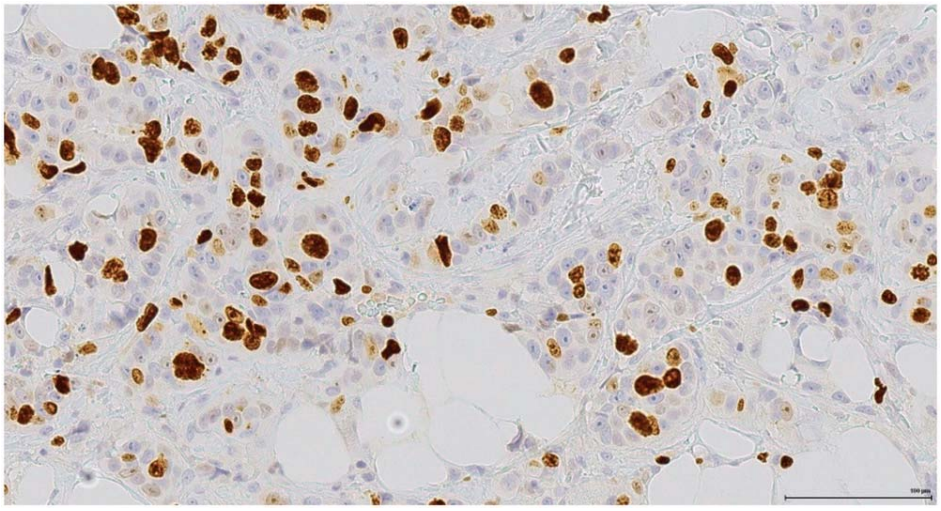


Figure 1. Nuclear Ki67 immunohistochemical staining demonstrating variation in staining intensity.

coefficient (ICC) was used as a summary measure of inter-observer reproducibility and for this purpose the two-way random effect model was applied [12]. The ICC has a range of 0–1, with one denoting the highest agreement. Since there are no standard values for acceptable reliability of ICC, it was decided that a prespecified value of ICC > 0.80 was to be considered indicative of good agreement in this study as compared to kappa statistics with 0.8–1.0 regarded as almost perfect [13–15].

Following dichotomization of observations at the larger than (>) 20% threshold the agreement between individual observers was calculated as the proportion of overall positive and negative agreement and as Light's Kappa [16]. The Kappa statistics is not defined in case of missing observations; hence, the dataset was reduced to 113 samples evaluated by all observers and by both methods. The agreement between observations done by the assessment and count methods was evaluated in a Bland–Altman plot. The plot depicts the difference between observations of the same sample by the same observer against the mean of observations [17]. If the differences have constant mean and variance the limits of agreement (LoA) can readily be added to the plot. For the Ki67 observations the mean and the variance of differences were clearly not constant over the range of observations and for this reason variance stabilizing logit and arcsine transformations were evaluated. By arcsine transformation variance homogeneity was obtained, whereas a linear trend of differences remained. Therefore arcsine transformation was considered as a valid model to be used for predictions of the outcome of one method, by knowing the observation of the other method.

In case of transformed observations the Bland–Altman plot and associated LoA do not offer an easy visual interpretation. To make a clinically meaningful presentation of the prediction interval this was determined according to Carstensen et al. [18] and back-transformed to the original scale.

Results

Observations by the assessment method were in general skewed towards lower values compared to observations by the count method (Figure 2). The assessment method had a lower mean value of 35.3% (95% CI: 33.97–36.65) vs. 37.4% (95% CI: 36.12–38.67) for the count method but a larger dispersion: The standard deviation was 27.8 vs. 26.4 and distance between the 10%-percentile and the 90%-percentile was 75-percentage-points vs. 74-percentage-points. As can be seen from the confidence intervals the means of the two methods are not statistically significantly different.

For the fourteen raters the median ranged from 20% to 40% by the assessment method and from 22.5% to 36.5% by the count method (Figures 3(A,B)).

The ICC was 0.82 (95% CI: 0.77–0.86) by the assessment method vs. 0.84 (95% CI: 0.80–0.87) by the count method.

For Ki67 observations dichotomized at the 20% threshold 57.4% were positive by the assessment method whereas 67.6% were positive by the count method. It is shown that

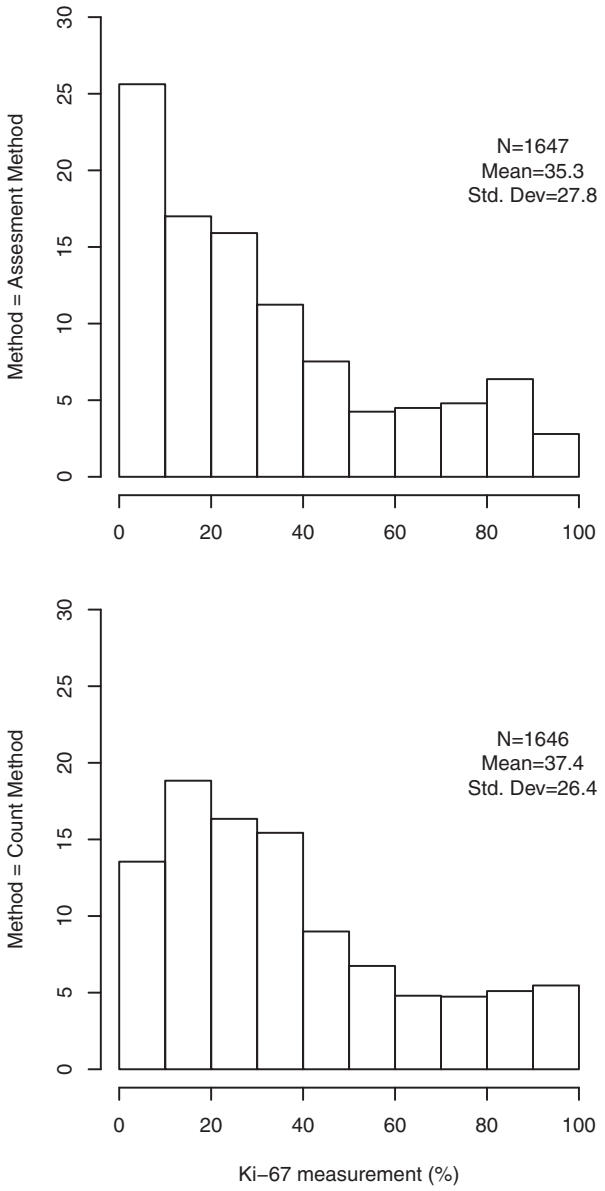


Figure 2. Distribution of registrations by the assessment and count methods on the original scale as percent positively stained tumor cells.

195 (28%) of the observations ≤20% by the assessment method was estimated as above 20% by the count method and 26 (3%) of the observations above 20% by the assessment method was estimated as ≤20% by the count method (Table 1). The proportion of between-method agreement of individual observers for observations above 20% ranged from 0.73 to 0.96 (median 0.87), and, as more observations were classified as positive by both methods, the proportion of positive between-method agreement (range 0.78–0.97, median 0.90) was larger than the proportion of between method agreement for observations ≤20% (0.65–0.91, median 0.83). Light's Kappa was 0.66 for observation by the assessment method and 0.65 by the count method ($n = 1582$).

A Bland–Altman plot (Figure 4(A)) visualized the agreement between the count and assessment methods. It is seen that the difference between observations is small at the

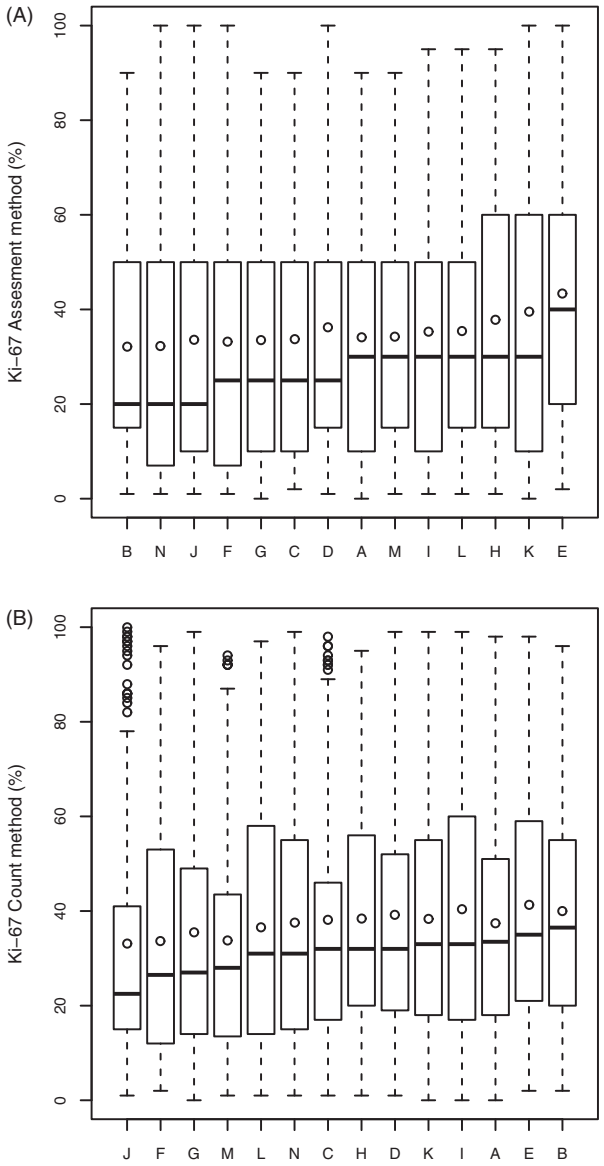


Figure 3. Distribution of Ki67 observations by the (A) assessment and (B) count methods according to rater. The bottom and top edges of the box are located at the sample 25th and 75th percentiles. The center horizontal line is drawn at the 50th percentile (median) and the circle illustrates the mean value.

Table 1. Observations of Ki-67 by the assessment and count methods dichotomized at the >20% threshold*.

	Count method		
Assessment method	≤20%	>20%	Total
≤20%	507	195	702
>20%	26	918	944
Total	533	1113	1646

*Proportion of overall agreement: (507 + 918)/(507 + 918 + 195 + 26) = 0.866.
Proportion of positive agreement: (2*918)/(2*918 + 195 + 26) = 0.893.
Proportion of negative agreement: (2*507)/(2*507 + 195 + 26) = 0.821.

lower and upper end of the scale but large in central part of the scale. At the low end of the scale observations by the count method tend to be larger than observations by the assessment method (as seen by the regression line), whereas the opposite tends to be the case in the high end of the scale. Due to this trend in differences and the absence of constant variance, the fitted LoA are not reliable. In Figure 4(B), the regression line and prediction limits are back-transformed from the arcsine-scale. The regression line in Figure 4(B) shows that at the 20% threshold for the assessment method the prediction of the count method is 23.4% (95% PI: 8.2–43.5%), vice versa at the 20% threshold for the count method the prediction of the assessment method is 16.7% (95% PI: 2.6–36.8%)

An observation of 5% by the assessment method has 95% chance of being Ki67 negative (≤20%) on the count scale, whereas an observation of 10% by the assessment method has 95% chance of being Ki67 negative on the assessment scale.

An observation of 33% by the assessment method has 95% chance of being Ki67 positive (>20%) on the count scale, whereas an observation of 40% by the assessment method has 95% chance of being Ki67 positive on the assessment scale.

Discussion

Despite of controversies concerning Ki67 standardization of interpretation and cutoff levels the Ki67 IHC labeling index is generally accepted as an important prognostic factor in ER positive breast cancer being the main discriminator in classification of luminal A-like and luminal B-like breast cancer. In this study the count method resulted in a higher proportion of cases with Ki67 > 20% as compared to the assessment method (Figure 2). Also, the study showed almost similar kappa values (0.66 for the assessment method and 0.65 for the count method) consistent with similar level of disagreement for both methods although the prespecified ICC level was achieved especially for the count method by 0.84 (95% CI: 0.80–0.87).

The magnitude of estimators of agreement (kappa or ICC) is conventionally interpreted as follows: 0 (absent), 0–0.19 (poor), 0.20–0.39 (weak), 0.30–0.59 (moderate), 0.60–0.79 (good), and ≥0.80 (almost complete agreement). This interpretation is however arbitrary without objective argumentation for the specified intervals. Consequently, despite of kappa values of 0.664 for the assessment method and 0.649 for the count method in the present study and as such indicating good inter-observer agreement according to conventional kappa interpretation, it must be reconsidered whether this level of agreement is acceptable when it relates to clinical treatment decision. An alternative approach for kappa interpretation has been suggested with kappa values above 0.8 as recommended prior to clinical implementation [19].

With respect to the measure of agreement between methods the Bland–Altman plot confirmed that the highest agreement in Ki67 observations in this study was in the very low and very high end of the scale with impaired agreement in the mid-range of observations (Figure 4(A,B)). This resulted in regrouping of a large number of observations depending on quantification method (Table 1) which is critical since a major part of Ki67 estimations in daily routine diagnostics are in the range of 10–30%. These findings in combination with the

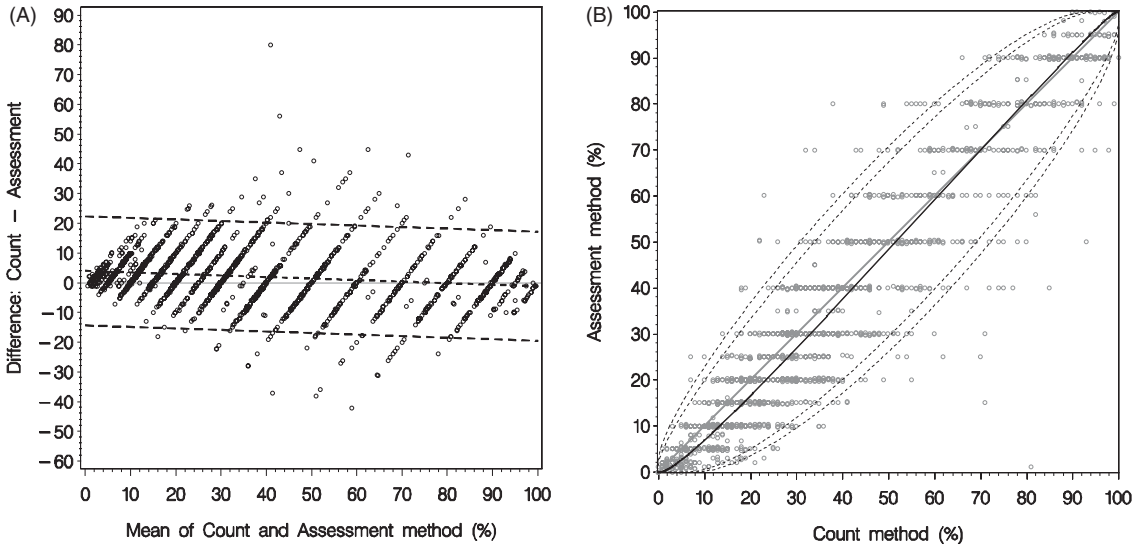


Figure 4. Ki67 observations done by the assessment and count methods ($n = 1646$). (A) Bland–Altman plot. The variance of the difference between observations is clearly not constant; hence the suggested limits of agreement (dashed lines) are not meaningful. For ease of interpretation overlapping observations are made visible by adding random noise. (B) Two-way prediction limits for observations. The regression line (solid line) and the 90% and 95% prediction interval (dashed lines) are back-transformed from the arcsine-scale. For ease of interpretation overlapping observations are made visible by adding random noise to values observed by the assessment method.

fact that the inter-observer agreement (kappa value and ICC) for both methods were almost identical lead to the conclusion that implementation of a prespecified Ki67 cutoff level was not advisable, thus in line with the 2017 St. Gallen Consensus Conference publication [20]. The presented results in this study are in accordance with those of others documenting impaired Ki67 inter-observer agreement [13,21–24]. Leung et al. [15] and Polley et al. [23] showed that calibration and standardization of scoring methods on TMA and core needle biopsies improved ICC to above 0.90 but still major discrepancies persisted around clinical important cutoff values. In addition, other studies have documented high level of inter-observer variability in the gray zone area of Ki67 index of 10–30% [21,24–26]. The concerns regarding interpretation of Ki67 are related to several contributing factors other than inter-observer reproducibility and lack of standardization of staining interpretation. Also inter-laboratory discrepancies with relation to IHC platform, choice of Ki67 antibody (clone) and IHC detection system are to be considered [22,27]. The Swedish survey by Ekholm et al. [27] reported good inter-laboratory reproducibility for Ki67 with central review. However, the Swedish laboratories had lab-specific thresholds for Ki67 and a lower agreement was reported between observers and central review when the lab-specific cutoff levels were used ($\kappa = 0.57$). In the recent paper by Focke et al. [22] on behalf of the German Breast Screening Pathology Initiative, thirty European pathology laboratories stained serial sectioned TMA slides according to local routine protocols. Central Ki67 assessment was performed reporting the proportion of tumors classified as luminal A-like after dichotomizing observations at the $\geq$14% threshold. The study showed a huge inter-laboratory variation in luminal A-like classification ranging from 17% to 57% ($p < .0001$).

The strength of this study is related to the fact that experienced breast pathologists performed the staining interpretation in accordance to the national guidelines in Denmark and Sweden.

Further, the staining procedure was done centrally and the staining interpretation was performed on full sections. There are however some potential limitations to be considered. Standard light microscopy was applied in the present study since automated image analysis is not standard procedure for Ki67 estimation in the Danish departments of pathology. Recent promising results regarding improvement of Ki67 reproducibility by computer assisted image analysis warrants further investigation of this method [28,29]. Also, this study did not include neither complete subtype classification by IHC surrogate markers nor validation by PAM50 gene expression as gold standard. Recent studies have demonstrated that classification of the intrinsic subtypes by molecular gene expression profiling is superior to classification by IHC surrogate markers [30,31].

Consequently, when implemented as part of the surrogate IHC panel for the intrinsic subtypes the documented inconsistency in Ki67 estimation in combination with the lack of agreement concerning Ki67 thresholds [8,9,20,32] might course either under- or over treatment on the individual patient level.

Based on the present study the Scientific Committee of Pathology in the DBCG concluded that for now Ki67 IHC index should not be introduced in the DBCG treatment algorithm. Due to the documented level of evidence (1B) the PAM50 multigene test was included in the national DBCG guidelines in 2017 and is presently offered to a subset of the Danish postmenopausal ER+, HER2 negative breast cancer patients (www.dbcg.dk) [33,34].

In conclusion, although the study in general showed good inter-observer agreement according to both ICC and Lights Kappa, still major discrepancies were identified in especially the mid-range of observations. The study confirmed the importance of standardization and validation of procedures

prior to implementation of (bio) markers for treatment guidance in national guidelines (http://www.nordiqc.org/) [35–37].

Acknowledgments

The authors thank Stina Lyck Carstensen* and Karsten Bjerre for assistance with the statistical analyses.

*Danish Breast Cancer Group Secretariat, Rigshospitalet, Copenhagen, Denmark.

Disclosure statement

Maj-Lis Møller Talman and Tomasz Piotr Tabor have received honoraria from Roche education session. Other authors declare that the have no potential conflict of interest.

References

[1] Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747–752.
[2] Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351:2817–2826.
[3] Wirapati P, Sotiriou C, Kunkel S, et al. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast Cancer Res. 2008;10:R65.
[4] de Azambuja E, Cardoso F, de Castro G, Jr., et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer. 2007;96:1504–1513.
[5] Luporsi E, Andre F, Spyratos F, et al. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: analytical and critical review. Breast Cancer Res Treat. 2012;132:895–915.
[6] Yerushalmi R, Woods R, Ravdin PM, et al. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11:174–183.
[7] Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101:736–750.
[8] Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22:1736–1747.
[9] Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013;24:2206–2223.
[10] Prat A, Cheang MC, Martin M, et al. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol. 2013;31:203–209.
[11] Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103:1656–1664.
[12] Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull. 1979;86:420–428.
[13] Polley MY, Leung SC, McShane LM, et al. An international Ki67 reproducibility study. J Natl Cancer Inst. 2013;105:1897–1906.
[14] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–174.
[15] Leung SCY, Nielsen TO, Zabaglo L, et al. Analytical validation of a standardized scoring protocol for Ki67: phase 3 of an international multicenter collaboration. NPJ Breast Cancer. 2016;2:16014.
[16] Light RJ. Measures of response agreement for qualitative data: some generalizations and alternatives. Psychol Bull. 1971;76:365–377.
[17] Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8:135–160.
[18] Carstensen B. Comparing methods of measurement: extending the LoA by regression. Stat Med. 2010;29:401–410.
[19] McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb). 2012;22:276–282.
[20] Curigliano G, Burstein HJ, E PW, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol. 2017;28:1700–1712.
[21] Varga Z, Diebold J, Dommann-Scherrer C, et al. How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists. PLoS One. 2012;7:e37379.
[22] Focke CM, Burger H, van Diest PJ, et al. Interlaboratory variability of Ki67 staining in breast cancer. Eur J Cancer. 2017;84:219–227.
[23] Polley MY, Leung SC, Gao D, et al. An international study to increase concordance in Ki67 scoring. Mod Pathol. 2015;28:778–786.
[24] Shui R, Yu B, Bi R, et al. An interobserver reproducibility analysis of Ki67 visual assessment in breast cancer. PLoS One. 2015;10:e0125131.
[25] Cserni G, Voros A, Liepniece-Karele I, et al. Distribution pattern of the Ki67 labelling index in breast cancer and its implications for choosing cut-off values. Breast. 2014;23:259–263.
[26] Hida AI, Oshiro Y, Inoue H, et al. Visual assessment of Ki67 at a glance is an easy method to exclude many luminal-type breast cancers from counting 1000 cells. Breast Cancer. 2015;22:129–134.
[27] Ekholm M, Grabau D, Bendahl PO, et al. Highly reproducible results of breast cancer biomarkers when analysed in accordance with national guidelines – a Swedish survey with central re-assessment. Acta Oncol. 2015;54:1040–1048.
[28] Roge R, Riber-Hansen R, Nielsen S, et al. Proliferation assessment in breast carcinomas using digital image analysis based on virtual Ki67/cytokeratin double staining. Breast Cancer Res Treat. 2016;158:11–19.
[29] Stalhammar G, Fuentes Martinez N, Lippert M, et al. Digital image analysis outperforms manual biomarker assessment in breast cancer. Mod Pathol. 2016;29:318–329.
[30] Hequet D, Callens C, Gentien D, et al. Prospective, multicenter French study evaluating the clinical impact of the Breast Cancer Intrinsic Subtype-Prosigna(R) Test in the management of early-stage breast cancers. PLoS One. 2017;12:e0185753.
[31] Viale G, de Snoo FA, Slaets L, et al. Immunohistochemical versus molecular (BluePrint and MammaPrint) subtyping of breast carcinoma. Outcome results from the EORTC 10041/BIG 3-04 MINDACT trial. Breast Cancer Res Treat. 2017. doi: 10.1007/s10549-017-4509-9.
[32] Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies – improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol. 2015;26:1533–1546.
[33] Laenkholm AV, Jensen MB, Eriksen JO, et al. The PAM50 risk of recurrence score predicts 10 year distant recurrence in a comprehensive Danish cohort of 2558 postmenopausal women allocated to 5 yr of endocrine therapy for hormone receptor positive early breast cancer. J Clin Oncol. 2018;57:in press.
[34] Gnant M, Sestak I, Filipits M, et al. Identifying clinically relevant prognostic subgroups of postmenopausal women with node-positive hormone receptor-positive early-stage breast cancer treated with endocrine therapy: a combined analysis of ABCSG-8 and ATAC using the PAM50 risk of

recurrence score and intrinsic subtype. Ann Oncol. 2015;26: 1685–1691.

[35] Krop I, Ismaila N, Stearns V. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American society of clinical oncology clinical practice focused update guideline summary. J Oncol Pract. 2017;13:763–766.

[36] Harris LN, Ismaila N, McShane LM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American society of clinical oncology clinical practice guideline. J Clin Oncol. 2016;34:1134–1150.

[37] Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst. 2009;101:1446–1452.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 90–94
https://doi.org/10.1080/0284186X.2017.1403040

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Standardized assessment of tumor-infiltrating lymphocytes in breast cancer: an evaluation of inter-observer agreement between pathologists

Trine Tramm[a], Tina Di Caterino[b], Anne-Marie B. Jylling[b], Giedrius Lelkaitis[c], Anne-Vibeke Lænkholm[d], Péter Ragó[e], Tomasz P. Tabor[f], Maj-Lis M. Talman[c] and Emmanouela Vouza[g]; On behalf of the Scientific Committee of Pathology, Danish Breast Cancer Group (DBCG)

[a]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Pathology, Odense University Hospital, Odense, Denmark; [c]Department of Pathology, Rigshopitalet, Copenhagen University Hospital, Copenhagen, Denmark; [d]Department of Surgical Pathology, Zealand University Hospital, Slagelse, Denmark; [e]Department of Pathology, Regional Hospital Central Jutland, Viborg, Denmark; [f]Department of Clinical Pathology, Lillebaelt Hospital, Vejle, Denmark; [g]Department of Pathology, Hospital of Southern Jutland, Sønderborg, Denmark

ABSTRACT

Introduction: In breast cancer, there is a growing body of evidence that tumor-infiltrating lymphocytes (TILs) may have clinical utility and may be able to direct clinical decisions for subgroups of patients. Clinical utility is, however, not sufficient for warranting the implementation of a new biomarker in the routine practice, and evaluation of the analytical validity is needed, including testing the reproducibility of decentralized assessment of TILs. The aim of this study was to evaluate the inter-observer agreement of TILs assessment using a standardized method, as proposed by the International TILs Working Group 2014, applied to a cohort of breast cancers reflecting an average breast cancer population.

Material and methods: Stromal TILs were assessed using full slide sections from 124 breast cancers with varying histology, malignancy grade and ER- and HER2 status. TILs were estimated by nine dedicated breast pathologists using scanned hematoxylin–eosin stainings. TILs results were categorized using various cutoffs, and the inter-observer agreement was evaluated using the intraclass coefficient (ICC), Kappa statistics as well as individual overall agreements with the median value of TILs.

Results: Evaluation of TILs led to an ICC of 0.71 (95% CI: 0.65–0.77) corresponding to an acceptable agreement. Kappa values were in the range of 0.38–0.46 corresponding to a fair to moderate agreement. The individual agreements increased, when using only two categories ('high' vs. 'low' TILs) and a cutoff of 50–60%.

Discussion: The results of the present study are in accordance with previous studies, and shows that the proposed methodology for standardized evaluation of TILs renders an acceptable inter-observer agreement. The findings, however, indicate that assessment of TILs needs further refinement, and is in support of the latest St. Gallen Consensus, that routine reporting of TILs for early breast cancer is not ready for implementation in a clinical setting.

ARTICLE HISTORY
Received 15 September 2017
Accepted 1 November 2017

Introduction

Varying presence of an inflammatory response is observed in virtually all neoplasms. Since the 1940s, it has been speculated that the inflammatory response in breast cancer could be associated with prognostic significance, based on the observation of how medullary carcinomas, often accompanied by a well-pronounced lymphocytic infiltrate, seemed to be associated with an extremely good prognosis despite an otherwise low differentiated appearance [1]. Later, lymphocytic infiltration has been described as a prognostic variable in more frequent histological types of breast cancer [2].

In comparison to e.g., malignant melanoma and lung cancer, breast cancer is considered non-immunogenic, but especially triple negative (TNBC) and HER-positive cancers have been shown to have higher levels of tumor-infiltrating lymphocytes (TILs) [3], and a clinical impact of TILs in breast cancers has been shown to be particularly evident in these subtypes. The prognostic impact of TILs has been proven in thousands of TNBC [3–5] and HER2 positive cancers [6], and a linear relationship has been described for TNBC and HER2 positive cancers with a 15–20% reduction in distant metastasis rate and mortality for each 10% increase in TILs [5]. A predictive impact have also been described, e.g., in the FinHer study, where patients with HER2 positive tumors with high TILs levels were found to derive greater benefit from Trastuzumab than patients with low TILs levels; thus indicating a positive predictive impact of a preexisting host antitumor immune response [7]. Finally, high level of TILs has been associated with higher rates of pathological complete response (pCR) after neoadjuvant chemotherapy [8,9].

CONTACT Trine Tramm tramm@oncology.au.dk Department of Pathology, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, Plan 1, C112, DK-8200 Aarhus N, Denmark

Supplemental data for this article can be accessed here.

Table 1. Histopathological tumor characteristics.

	N	%
Histology type		
Invasive ductal carcinoma	104	84
Invasive lobular carcinoma	16	13
Other types	4	3
Estrogen receptor status[a]		
Positive	110	89
Negative	13	10
Missing	1	1
HER2 status		
Positive	35	28
Negative	83	67
Missing	6	5
Malignancy grade		
I	30	24
II	66	53
II	24	19
Missing	4	3

[a]1% cut off.

There is as such a growing body of evidence that TILs have clinical validity and utility in breast cancer and may be able to direct clinical decisions for a group of patients. Clinical utility is, however, not sufficient for warranting the implementation of a new biomarker in the clinical practice, and a thorough evaluation of the analytical validity is needed in order to describe the robustness of the test including accuracy and inter-observer reproducibility [10].

The International TILs Working Group 2014 has formulated recommendations for evaluation of TILs in breast cancer [11], and the inter-observer agreement between pathologists in TILs have been examined for TNBC and HER2 positive cancers [12,13].

The purpose of this study was to evaluate the inter-observer agreement of TILs assessment using the standardized method as proposed by the International TILs Working Group 2014 [11] applied to a cohort of breast cancers reflecting an average breast cancer population, including estrogen receptor (ER) positive and HER2 negative cancers.

Material and methods

Stromal tumor infiltrating lymphocytes were assessed using full slide sections from a total number of 124 breast cancers. The distribution of ER-status, HER2-status, malignancy grade and histological type of the 124 carcinomas was comparable to an average Caucasian population (Table 1) [14].

From each formalin fixed, paraffin embedded (FFPE) block, one full slide, HE-stained section (3 μm thick) were scanned at 20× magnification using a Nanozoomer 2.0HT (Hamamatsu Photonics K.K., Hamamatsu City, Japan), and the scanned sections were uploaded and made available for analysis using the free downloadable program NDP.view2 by Hamamatsu. Digitalized HE sections were chosen in order to facilitate distribution of the material, and evaluation of the scanned HE-sections was considered comparable to evaluation of the actual glass slides by light-microscopy. The evaluation was based on the recommendations from the TILs working group 2014 [11]. In short, TILs encompass both lymphocytes and plasma cells. According to the international recommendations, only stromal TILs within the borders of the invasive tumor were evaluated, and areas of crush artifacts, necrosis, and previous core biopsy sites were excluded. The estimation was semiquantitative, assessing an average TILs score in the tumor area for the full section, with no evaluation of hotspots. TILs were recorded as a continuous variable, and hereafter, the amount of TILs was categorized into various 2- or 3 grade categorizations using different cut offs (A: 0–10%, 11–39%, ≥40%; B: 0–20%, 21–49%, ≥50%; C: <50% vs. ≥50% or D: <60% vs. ≥60%). Each TILs value describes the 'area of stromal tissue occupied by TILs/total area of intra-tumoral stromal area'.

Nine dedicated breast pathologists evaluated the sections independently, and the results from each pathologist were kept confident with no individual feedback provided to the participants.

Statistical analysis

Inter-observer agreement for the evaluation of TILs reported as a continuous score was assessed via the intraclass coefficient (ICC) calculated using a mixed model. Fleiss kappa values (adaptation of Cohen's kappa for 3 or more raters) were used for assessing the inter-observer agreement, when evaluating TILs as categorical data [15]. Missing values were replaced by the mean of the measurements for the sample for calculation of Kappa values and ICC. Finally, a concordance analysis was performed by calculating for each pathologist the overall agreement with the median rating (number of samples in agreement/total number of samples), and this was repeated for the four different groupings of TILs (A: 0–10%, 11–39%, ≥40%; B: 0–20%, 21–49%, ≥50%; C: <50% vs. ≥50% or D: <60% vs. ≥60%). The kappa values is a commonly used method for assessing interrater agreement, and were designed to take account of the possibility of guessing [16], but the values are difficult to interpret. Similarly, no standard values for an acceptable agreement for ICC exist, but a statistically significant ICC of 0.70 has previously been used as an endpoint for a successful evaluation of TILs [12,17]. Percent agreement is a basic measurement of inter-observer agreement, where the effect of chance in achieving agreement between raters is not accounted for. It was decided to report the different measures of inter-observer agreement in order to clarify the inter-observer variability from different angles. Statistical analysis was performed using STATA-version 11.2 (StataCorp, College Station, TX).

Results

The 124 cases evaluated by nine pathologists led to 1107 observations with nine missing values. The mean TILs values for each observer ranged from 10 to 23%, and some observers seemed to have a higher individual threshold or scaling (Supplementary Figure 1 shows the mean and standard deviation of the TILs values for each observer). However, single outliers also contributed to the inter-observer variability. Figure 1(A) shows how the observers occasionally reported a high or a low value in opposition to the rest of the observers, with a TILs value differing substantially from the mean

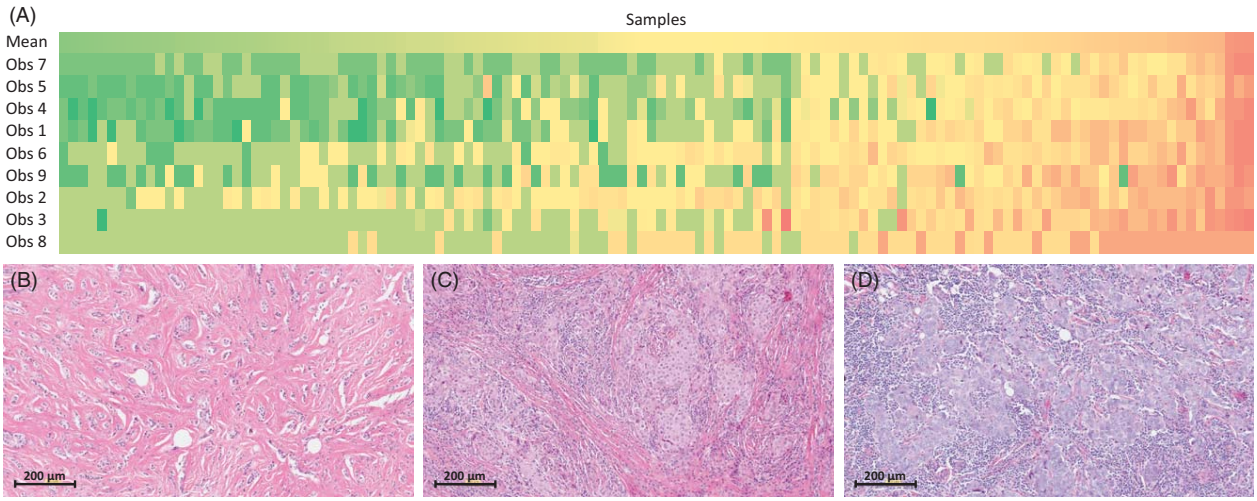


Figure 1. (A) Heat map showing graphically the individual recordings of TILs sorted in columns from left to right according to ascending mean stromal TILs values shown in the top row. The rows underneath represents the nine observers recordings arranged from top to bottom according to increasing individual mean values. Single outliers are represented by, e.g., red pixels among otherwise green or yellow pixels or vice versa. (B) A carcinoma with very low mean TILs level (0.03), (C) a carcinoma with a mean TILs level around the 50–60% cut off (0.56) and (D) a carcinoma with a high mean TILs level (0.83). The black bars in (B–D) measures 200 µm (HE, original magnification 100×).

Table 2. Interobserver agreement in assessment of tumor-infiltrating lymphocytes (TILs).

Intraclass coefficient (ICC)	0.71 (95% CI: 0.65–0.77)
Fleiss' kappa values	
TILs 0–10%, 11–39%, ≥40%	0.41
TILs 0–20%, 21–49%, ≥50%	0.36
TILs <50% vs. ≥50%	0.48
TILs <60% vs. ≥60%	0.44
Concordance rates	
TILs 0–10%, 11–39%, ≥40%	0.79 (range: 0.60–0.94)
TILs 0–20%, 21–49%, ≥50%	0.82 (range: 0.54–0.92)
TILs <50% vs. ≥50%	0.93 (range: 0.81–0.99)
TILs <60% vs. ≥60%	0.95 (range: 0.77–0.99)

value for that particular sample. Figure 1(B–D) illustrates three different tumors with varying mean values of TILs.

The ICC was 0.71 (95% CI: 0.65–0.77) (Table 2). The ICC describes to which degree the variance in the measurements can be attributed to the actual biological differences in comparison to the variance delivered by the fact that different pathologist rates differently. Interpretation of the obtained ICC thus means that 71% of the variance in the present results can be attributed to variance caused by inter-tumoral differences, but the remaining 29% will be due to artifacts attributable to inter-observer variability. The pre-specified endpoint of a statistically significant ICC was not completely met, since the 95% confidence interval included 0.70.

Kappa values for the different categories of TILs (A: 0–10%, 11–39%, ≥40%; B: 0–20%, 21–49%, ≥50%; C: <50% vs. ≥50% or D: <60% vs. ≥60%) were 0.41, 0.36, 0.48 and 0.44, respectively (Table 2). This corresponds to a fair to moderate agreement according to the criteria by Landis and Koch [18].

Furthermore, the overall agreement between each observer's recordings compared to the median value for the samples was calculated for each of the different categorizations. The mean value of these overall agreements is listed in Table 2. The agreements were poorest, when dividing the TILs into 3-grade categories (0.79 and 0.82, respectively), whereas the overall agreement for each observer increased substantially when using only two categories (0.93 and 0.95, respectively).

For 118/124, both ER and HER2 status were available (Table 1). For all combinations of ER and HER2, samples with high as well as low TILs values were found, though ER+/HER2– were found to have a lower mean TILs value than the other subtypes with 95% confidence intervals only overlapping with ER+/HER2+ samples (Supplementary Table 1).

Discussion

The results of our reproducibility study are in accordance with two previous studies [12,13], and shows that the internationally proposed methodology for standardized evaluation of TILs [11] renders an acceptable agreement among observers.

Recently, the International Immuno-oncology Biomarker Working Group conducted two ring studies with the purpose of evaluating the inter-observer agreement for decentralized assessment of TILs in a total number of 120 HER2-positive and TN breast cancers [12]. The pre-specified endpoint (ICC > 0.7) was not reached with statistical significance (ICC: 0.70; 95% CI: 0.62–0.78), since the 95% confidence interval did indeed include 0.70, but the agreement was found to be relatively good. A similar acceptable ICC of 0.62 was found in a smaller series of 75 TNBC [13]. The presented study is the first to evaluate the standardized assessment of TILs in a broader range of breast cancer also encompassing ER+/HER2-cancers, and shows an ICC and Kappa values similar to the previous findings.

The inter-observer variability can be attributed to different issues as, e.g., different individual thresholds/scaling differences as could be seen in Supplementary Figure 1. Intratumoral heterogeneity may also contribute to variations in the recordings, and may especially contribute to presence of

single outliers. According to the international standardized guidelines, it is attempted to evaluate the average TILs level on a full slide section and not concentrate on hot spots. It is, however, highly likely that the eye is drawn towards hot spots, which may affect the registration. Lymphocytic infiltration within a normal lobule or around areas of ductal carcinoma *in situ* within or outside the tumor-area should not be included in the assessment of TILs, but may erroneously lead to overestimation of TILs levels. Incorrect registrations may lead to single outliers, and finally, various tissue components (apoptosis, individual cell necrosis and stromal fibroblasts, etc.) may be misinterpreted as TILs. Though the use of immunohistochemical (IHC) stainings may assist in discriminating between intra-tumoral lymphocytes and other tissue components, it is at present not recommended to include IHC in the assessment.

A more thorough discrimination of the specific subpopulations of TILs and ratio between the various inflammatory cell types using IHC or gene expression profiling may prove to have clinical implications in terms of prognosis and predictive value regarding immune-modulating therapy [19], but evaluation of this aspect was not within the scope of this study.

The use of digital analysis to optimize the evaluation of TILs is at present not recommended. It is, however, highly likely that practice as well as machine learning algorithms may improve the inter-observer agreement. This was shown in the 2nd ring-study by Denkert et al. [12], where the reproducibility improved after the introduction of a specifically designed software program guiding the pathologist to evaluation in predefined screening areas and returning immediate feedback for each TILs value entered in the system.

The use of digitalized HE-sections could, on the other hand, be regarded as a weakness in this study, and may have introduced variations in the evaluation due to factors related to each observer's availability of suitable IT-solutions (resolution of the computer-screen, speed of the internet, etc.). Furthermore, the quality of each scanned slide may also have affected the evaluation in a negative way.

In this study, the concordance for each observer was found to improve substantially, when using a single cut off (either 50% or 60%), indicating that separation between tumors with 'low' vs. 'high' level of TILs may be more reproducible and safer to use in a daily setting. A 50–60% cut off has been used in several studies, and tumors with high levels of TILs have been designated 'lymphocytic predominant' [3,4,7]. The use of a single cut off could perhaps facilitate the implementation of TILs assessment in a routine setting. A weakness of this study is that the vast majority of the tumors had very low levels of TILs. It would have been preferable to have a higher number of tumors with TILs levels around the 50–60% cut off level.

The results of this study finally showed that ER+/HER2 negative tumors had lower mean TILs levels than HER2 + (and ER−/HER) tumors, and this is in accordance with other studies [3,20]. However, high levels of TILS (>60%) were found even among ER+/HER2– tumors, indicating that some ER+/HER2– tumors may also be considered immunogenic.

A prerequisite for introducing a new biomarker into the daily clinical practice is that the test – besides being sensitive, specific and reproducible – is robust and preferably as non-laborious as possible. Considering this, the evaluation of TILs on HE-section is pragmatic, cost-effective and easy to implement. One of the strengths of this study is that it is performed on full slide sections, and that the participating observers represents pathologists situated nationwide and with various years of experience. The results as such reflect the variability that can be expected, when performing a decentralized assessment of TILs in a representative cohort of breast cancers.

In conclusion, the results of the present study are in accordance with previous studies, and shows that the proposed methodology for standardized evaluation of TILs renders an acceptable inter-observer agreement. The agreement increased when dichotomizing the tumors into samples with 'high' or 'low' levels of TILs. The findings, however, indicate that the assessment needs further refinement and support the latest St. Gallen Consensus [21] that routine reporting of TILs for early breast cancer is not ready for implementation in the daily clinical setting.

Disclosure statement

TT has filed a patent for a gene signature associated with efficacy of radiotherapy in breast cancer (International Patent Publication No. WO 2013/132354A2). The patent is not related to the present work.

AVL, TPT and TT have received royalties from Roche A/S for lectures given.

No potential conflicts of interest were disclosed by the other authors.

References

[1] Moore OS, Jr, Foote FW, Jr. The relatively favorable prognosis of medullary carcinoma of the breast. Cancer. 1949;2:635–642.

[2] Aaltomaa S, Lipponen P, Eskelinen M, et al. Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer. 1992;28A:859–864.

[3] Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol. 2013;31:860–867.

[4] Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol. 2014;32:2959–2966.

[5] Ibrahim EM, Al-Foheidi ME, Al-Mansour MM, et al. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: a meta-analysis. Breast Cancer Res Treat. 2014;148: 467–476.

[6] Ali HR, Provenzano E, Dawson SJ, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol. 2014;25:1536–1543.

[7] Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25:1544–1550.

[8] Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28:105–113.

[9] Salgado R, Denkert C, Campbell C, et al. Tumor-infiltrating lymphocytes and associations with pathological complete response

and event-free survival in HER2-positive early-stage breast cancer treated with lapatinib and trastuzumab: a secondary analysis of the NeoALTTO trial. JAMA Oncol. 2015;1:448–454.

[10] Wein L, Savas P, Luen SJ, et al. Clinical validity and utility of tumor-infiltrating lymphocytes in routine clinical practice for breast cancer patients: current and future directions. Front Oncol. 2017;7:156.

[11] Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26:259–271.

[12] Denkert C, Wienert S, Poterie A, et al. Standardized evaluation of tumor-infiltrating lymphocytes in breast cancer: results of the ring studies of the international immuno-oncology biomarker working group. Mod Pathol. 2016;29:1155–1164.

[13] Swisher SK, Wu Y, Castaneda CA, et al. Interobserver agreement between pathologists assessing tumor-infiltrating lymphocytes (TILs) in breast cancer using methodology proposed by the International TILs Working Group. Ann Surg Oncol. 2016;23:2242–2248.

[14] O'Brien KM, Cole SR, Tse CK, et al. Intrinsic breast tumor subtypes, race, and long-term survival in the Carolina Breast Cancer Study. Clin Cancer Res. 2010;16:6100–6110.

[15] Gisev N, Bell JS, Chen TF. Interrater agreement and interrater reliability: key concepts, approaches, and applications. Res Social Adm Pharm. 2013;9:330–338.

[16] Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychol Bull. 1968;70:213–220.

[17] Denkert C, von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol. 2015;33:983–991.

[18] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–174.

[19] Savas P, Salgado R, Denkert C, et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat Rev Clin Oncol. 2016;13:228–241.

[20] Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes. JAMA Oncol. 2016;2:1354–1360.

[21] Curigliano G, Burstein HJ, P Winer E, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol. 2017;28:1700–1712.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 95–101
https://doi.org/10.1080/0284186X.2017.1398415

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Clinical and molecular characterization of *BRCA*-associated breast cancer: results from the DBCG

I. M. H. Soenderstrup[a], A. V. Laenkholm[a], M. B. Jensen[b], J. O. Eriksen[a], A. M. Gerdes[c], T. V. O. Hansen[d], T. A. Kruse[e], M. J. Larsen[e], I. S. Pedersen[f], M. Rossing[d], M. Thomassen[e] and B. Ejlertsen[a,g]

[a]Department of Surgical Pathology Region Zealand, Zealand University Hospital, Slagelse, Denmark; [b]Danish Breast Cancer Group, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [c]Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [d]Center for Genomic Medicine, Rigshospitalet Copenhagen University Hospital, Copenhagen, Denmark; [e]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark; [f]Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark; [g]Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: In breast cancer (BC) patients a cancer predisposing *BRCA1/2* mutation is associated with adverse tumor characteristics, risk assessment and treatment allocation. We aimed to estimate overall- (OS) and disease-free survival (DFS) according to tumor characteristics and treatment among women who within two years of definitive surgery for primary BC were shown to carry a mutation in *BRCA1/2* .

Material and methods: From the clinical database of the Danish Breast Cancer Group we included 141 *BRCA1* and 96 *BRCA2* BC patients. Estrogen receptor and HER2 status were centrally reviewed on paraffin-embedded tumor tissue. Information on risk reducing surgery was obtained from the Danish Pathology and Patient Registries and included as time-dependent variables in Cox proportional hazard models.

Results: Ten-year OS and DFS for *BRCA1* BC patients were 78% (95% CI 69–85) and 74% (95% CI 64–81). Ten-year OS and DFS for *BRCA2* BC were 88% (95% CI 78–94) and 84% (95% CI 74–91). *BRCA1* BC patients as compared to *BRCA2* BC patients had a higher risk of BC relapse or non-breast cancer within ten years of follow-up, independent of ER status (adjusted HR 2.78 95% CI 1.28–6.05, $p = .01$), but *BRCA* mutation was not associated with OS (adjusted HR 1.98, 95% CI 0.87–4.52, $p = .10$). In multivariate analysis, including both *BRCA1* and *BRCA2* carriers, no chemotherapy was associated with a higher risk of death (adjusted OS HR 3.58, 95% CI 1.29–9.97, $p = .01$) and risk reducing contralateral mastectomy (RRCM) was associated with a significantly reduced risk of death (adjusted OS HR 0.42, 95% CI =0.21–0.84, $p = .01$).

Conclusion: Difference in OS between *BRCA1* and *BRCA2* BC patients could be ascribed to tumor-biology. *BRCA1* BC patients may have a shorter ten-year DFS than *BRCA2* BC patients. Chemotherapy and risk reducing contralateral mastectomy reduce mortality for both *BRCA1* and *BRCA2* BC patients.

ARTICLE HISTORY
Received 15 September 2017
Accepted 21 October 2017

Background

In around 80% of *BRCA* carriers, breast cancers (BC) will be high grade invasive ductal carcinomas of no special type (NST) [1–3]. Medullary features and abundance of lymphocytes in the tumor-microenvironment is more often present in *BRCA1* carriers and less than 25% express estrogen (ER) or progesterone receptors (PR) and only around 10% express human epidermal growth factor receptor 2 (HER2) [4,5]. The majority of *BRCA2* BCs are ER/PR positive. Compared with sporadic BC a higher proportion are high grade but without other histopathological characteristics different from sporadic BC [4,5]. Identification of a cancer predisposing *BRCA* mutation at the diagnosis of BC may influence decisions about treatment and cancer prophylaxis. Recent moderate-sized population-based studies have shown a higher mortality in *BRCA* BC patients if chemotherapy is omitted, indicating that allocation of chemotherapy by standard guidelines have not been optimal for this subgroup of BC patients [6,7]. However, risk reducing salpingo-oophorectomy (RRSO) and risk reducing contralateral mastectomy (RRCM) are recommended in *BRCA1/2* mutation carriers since they are associated with reduced risk of BC and ovarian cancer and a better overall survival (OS) [8].

Here we report ten-year survival estimates and associates of treatment-, patient- and tumor characteristics with survival in a large cohort of *BRCA1/2* BC patients with detailed prospectively collected clinical data from the national database of The Danish Breast Cancer Group (DBCG).

Method and materials

Patients

The Danish Data Protection Agency (2009-41-3611) and the Danish Ethical Committee (registration number 33483) approved the study.

CONTACT Ida Marie Heeholm Soenderstrup idms@regionsjaelland.dk Department of Surgical Pathology, Zealand University Hospital, Ingemannsvej 48, 4200 Slagelse, Denmark

Danish patients are registered for health care and administrative purposes by a unique personal identification number (CPR) in national clinical medical registries [9]. The CPR was used to cross-link information from registries. Hospital admissions, outpatients, surgery and diagnosis have been registered in The Danish National Patients Registry (DNPR) [10] since 1977, pathological procedures in the Danish Pathology Register (DPR) since 1997 [11] and cancer incidences in The Danish Cancer Registry (CR) since 1943 [12]. Since 1977, Danish women with primary BC have been registered in the DBCG database including prospectively registered clinical data. Family history of breast- and ovarian cancer was added in the Hereditary Breast and Ovarian Cancer (HBOC) register in 1999 [13]. Patients suspected to carry a high-risk mutation and referred for genetic counseling were registered with a unique family-number. National guidelines from 2001 proposed referral for genetic counseling in families with two first-degree relatives with BC under the age of fifty or ovarian cancer, three first degree relatives with BC, breast and ovarian cancer in the same individual and male BC. For high-risk patients undergoing mutational screening, the finding of a deleterious *BRCA1/2* mutation was subsequently registered [14,15].

By standard clinical guidelines [16] early stage I–III BC patients undergo mastectomy or breast-conserving surgery and SN procedure if clinical node-negative, followed by radiation therapy and adjuvant systemic treatment. Patients older than 60 at diagnosis with small tumor size ($\leq$10 mm), node negative, ER positive ($\geq$10%), HER2 negative and grade I ductal or grade I–II lobular tumors are classified as low risk and not eligible for systemic treatment. This group amounts to approximately 8% and consequently approximately 90% of early BC patients receives systemic treatment. Trastuzumab was introduced in 2005 [17] and taxanes in 2007 [18]. Over time, surgery has become less extensive with breast-conserving surgery, smaller margins and introduction of the SN procedure in 2001 [17,19]. However, primary mastectomy was recommended since 2001 in BC patients with *BRCA1/2* mutation [14].

Patients had clinical follow-up every three months, the first year after BC, every sixth months, two to five years after BC and once a year, 6–10 years after BC or until a first event. Death before clinical follow-up, distant- or local relapse, contralateral BC or non-breast cancer were registered as an event.

BRCA1/2 mutational status was updated until July 2011 and BC surgery until February 2012. Data on clinical follow-up, vital status, treatment, surgery and pathology were updated until September 2016. Information about vital status by linkage to the Danish Civil Registration System [9] is complete until September 2016.

Patients were excluded if they had *BRCA1/2* mutation screening performed more than two years after date of definitive surgery for primary BC, if they had stage four disease at diagnosis, previous malignant disease (other than non-melanoma skin cancer) or no clinical follow-up.

RRSO was defined as removal of both ovaries. RRCM was defined as bilateral mastectomy before BC diagnosis, bilateral mastectomy after primary breast-conserving surgery or contralateral mastectomy after primary ipsilateral mastectomy. Dates of operation for RRSO and RRCM were retrieved from DNPR by searching ICD8/ICD10 codes for prophylactic procedures combined with codes for surgical removal of ovaries or mastectomy. Search in DPR was performed during April and May 2017 to review pathology reports from all patients not found to have risk reducing surgery in DNPR, if there was uncertainty of indication for mastectomy/salpingo-oophorectomy or uncertainty about 'site' of RRSO/RRCM. Mastectomy or salpingo-oophorectomy associated with a malignant diagnosis were not considered. If no information was found in the registers, we assumed the patient did not have risk reducing surgery. Information on other malignancy during follow-up was registered in the DBCG database, however date of primary ovarian-, fallopian tube- or peritoneal cancer during and after end of clinical follow-up was retrieved from CR. Data from CR were updated until 31 December 2014.

Among 1507 *BRCA1/2* mutation carriers, 597 were diagnosed with primary invasive BC and 583 had definitive surgery. Median time from BC to *BRCA* testing was 0.7 years (240 days and 251 days for *BRCA1* and *BRCA2,* respectively). Forty-three patients were tested before BC diagnosis (twenty-seven *BRCA1* and sixteen *BRCA2*). In 285 patients the *BRCA1/2* mutational screening was performed less than two years after surgery. Three patients with stage IV disease, eighteen with previous malignancy and twenty-seven patients with no clinical follow-up were excluded, resulting in 237 BC patients with a *BRCA1/2* mutation (141 *BRCA1* and 96 *BRCA2*) included for survival analysis (Figure 1).

BRCA1/2 screening

BRCA1/2 mutational screening was offered as a diagnostic test from 1997 in East Denmark and from 1999 in Western Denmark. *BRCA1/2* mutation screening and mutation variant classification was performed in three centers as previously described [20]. Methods used were denaturing high performance liquid chromatography or temperature gradient electrophoresis and protein truncation test. Validation was performed with Sanger sequencing, or Sanger sequencing and multiplex ligation-dependent probe amplification.

IHC/ISH analysis and tumor morphology

Immunohistochemical (IHC) and *in situ* hybridization analysis was performed on formalin-fixed paraffin-embedded (FFPE) tumor tissue to obtain accurate ER and HER2 status. FFPE blocks with primary BC tissue were collected from Danish pathology departments. IHC and ISH analysis were performed on tissue-micro-arrays (TMA) with up to four 1.5 mm cores from each tumor. Four micrometer sections from TMA blocks were stained with validated antibodies for ER (clone SP1 RTU with visualization system Ultraview or Optiview, Ventana) and HER2 (clone 4B5 RTU, visualization system Ultraview, Ventana). The tumor was reported as ER positive if $\geq$1% of positive nuclear staining. HER2 IHC was scored according to standardized guidelines [21]. In HER2 equivocal cases, HER2 gene amplification was determined with a gene-protein assay

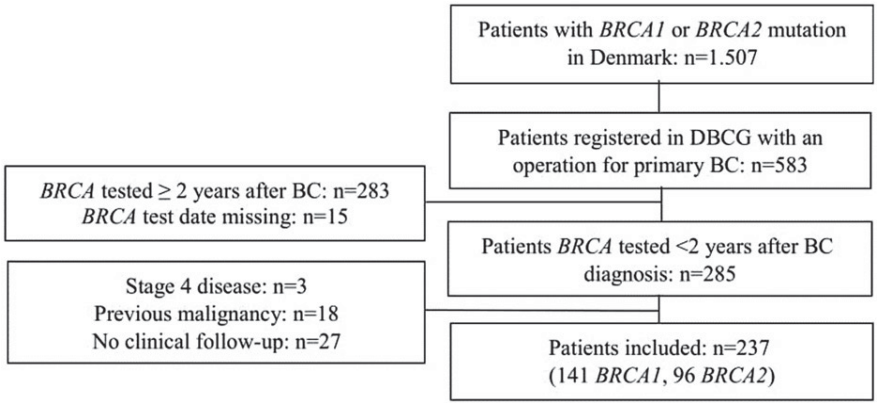


Figure 1: Patients flowchart.

(INFORM Dual ISH DNA Probe cocktail, Ventana) and scored according to guidelines [21]. Malignancy grade and histological subtype were determined with standard light microscopy on full-size four micrometer sections from HE stained FFPE tumor sections. Grading was performed according to the modified Bloom-Richardson-Elston system [22] and histological subtype according to WHO classification of tumors [23].

Statistical analysis

Disease-free survival (DFS) was defined as the time from definitive BC surgery to invasive loco-regional recurrence, distant metastasis, contralateral invasive breast cancer, second primary non-breast invasive cancer or death irrespective of cause. Overall survival was calculated as elapsed time from BC surgery to death. DFS and OS were analyzed unadjusted by the Kaplan-Meier method and the log-rank test to compare groups. Considering contralateral BC, local- and distant relapse and death as first event competing events, ten-year cumulative incidence of non-breast cancer was calculated in competing risk analysis.

Hazard ratios (HR) were estimated from the Cox proportional hazard regression model to quantify effects and to explore interactions. The assumption of proportional hazards (PH) was tested by plotting Schoenfeld residuals against time and by testing correlation between ranked failure times and Schoenfeld residuals. A time-dependent component was included for ER status to comply with proportional hazards. *BRCA* status (*BRCA1* vs. *BRCA2*), ER status (positive vs. negative), positive lymph node metastases (no vs. yes), adjuvant chemotherapy (yes vs. no) and RRCM were included in multivariate analysis. RRSO and RRCM were included as time-dependent covariates. Missing values were imputed with the most frequent value in the remaining patients. Sensitivity analysis was performed with a model where patients with missing values were excluded and the resulting hazard ratios were not significantly different. We used two-sided p value with 5% significance level.

Differences of distribution of patient and tumor characteristics between subgroups were assessed with Chi-square test or Fishers exact test where appropriate.

Results

Treatment, patient- and tumor-characteristics are presented in Table 1. ER, PR and HER2 status were obtained from the central pathology review or retrieved from DBCG clinical database (ER in ten and HER2 in eight patients). Mean age at diagnosis was 42 and 46 years for *BRCA1* and *BRCA2* carriers, respectively. The majority were premenopausal at diagnosis with invasive ductal and HER2 negative tumors. *BRCA1* tumors were ER negative/TN in contrary to *BRCA2* tumors which were mainly ER positive. The majority of *BRCA1* tumors were high grade and lymph node negative while almost two thirds of *BRCA2* patients were lymph node positive. A mastectomy was the primary surgery in just over half irrespective of the involved gene but the uptake on RRCM was somewhat higher in *BRCA1* carriers (Table 1). The vast majority received adjuvant chemotherapy and RRSO. Median time to RRSO and RRCM after BC surgery was 1.3 and 1.6 years (minimum 12 and zero days respectively to maximum 10.6 years) for both *BRCA1* and *BRCA2* patients, respectively.

Median potential follow-up time for OS and DFS was 9.01 (*BRCA1* 9.01, *BRCA2* 8.52) and 7.15 years (*BRCA1* 7.02, *BRCA2* 7.15), respectively. Of *BRCA1* patients, eighteen (13%) had local- or distant recurrence, five (4%) had contralateral breast cancer, three died before follow-up (2%) and seven had non-breast cancer (5%). Nine (9%) *BRCA2* patients had local- or distant relapse, one died before clinical follow-up (1%) and two had non-breast cancer (2%). Ten-year cumulative incidence of local- or distant relapse, contralateral BC and death as first event or non-breast cancer was 19.0 (95%CI 13–27) and 6.9% (95% CI 2.9–13.4) for *BRCA1* and 13.4 (95% CI 6.6–22.6) and 2.2% (95% CI 0.4–7.0) for *BRCA2* carriers, respectively. A higher proportion of events in *BRCA1* carriers were non-breast cancer (36%) compared to *BRCA2* carriers (16%). Two patients had ovarian cancer, both *BRCA1* carriers. Cancers other than BC and ovarian cancer were lung cancer (one *BRCA2* carrier), gastrointestinal cancer (two *BRCA1* carriers, one *BRCA2* carrier), malignant melanoma (two *BRCA1* carriers) and unknown type (one *BRCA1* carrier). DFS was significantly worse in *BRCA1* compared to *BRCA2* carriers (Figure 2, Table 3) and adjusted for lymph node status, adjuvant chemotherapy and RRCM, *BRCA1* mutation was

Table 1. Patient and tumor characteristics.

	Patients			
	BRCA1		*BRCA2*	
Variable	*n* 141	% 59	*n* 96	% 41
Age				
18–39	66	47	31	32
40–60	62	44	52	54
>60	13	9	13	14
Year of diagnosis				
1998–1999	1	1	1	1
2000–2004	29	21	20	21
2005–2009	86	61	58	60
2010–2011	25	18	17	18
Menopausal status at diagnosis				
Premenopausal	111	79	64	67
Postmenopausal	29	21	32	33
Unknown	1	1	0	0
Histology				
Ductal	129	91	85	89
Lobular	0	0	5	5
Other	12	9	6	6
Tumor size				
≤10 mm	19	13	17	18
11–20 mm	64	45	40	42
21–50 mm	56	40	36	38
>50 mm	2	1	3	3
Grade				
1	3	2	8	8
2	29	21	40	42
3	97	69	41	43
Non-ductal/non-lobular histology	12	9	6	6
Missing	0	0	1	1
Positive lymph nodes				
0	82	58	34	35
1–3 positive	41	29	47	49
≥ 4 positive	18	13	14	15
Unknown	0	0	1	1
Bilateral breast cancer				
Yes	2	1	3	3
No	136	97	92	96
Unknown	3	2	1	1
ER status				
Positive	44	31	76	79
Negative	97	69	20	21
PR status				
Positive	35	25	67	70
Negative	106	75	29	30
HER2 status				
Positive	6	4	6	6
Negative	134	95	89	93
Unknown	1	1	1	1
TN				
Yes	91	65	19	20
No	50	35	77	80
Surgery				
Mastectomy	76	54	54	56
Breast conserving surgery	65	46	42	44
Systemic therapy				
None	2	1	1	1
Chemotherapy alone	106	75	23	24
Chemotherapy and endocrine therapy	28	20	59	61
Endocrine therapy alone	5	4	13	14
Radiation therapy				
Yes	84	60	72	75
No	42	30	16	17
Unknown	15	11	8	8
RRCM				
Yes	95	67	52	54
No	46	33	44	46
RRSO				
Yes	132	94	90	94
No	9	6	6	6
RRCM only				
Before BC surgery	0	0	0	0
≤2 years after BC surgery	3	2	1	1
>2 years after BC surgery	2	1	0	0
RRSO only				
Before BC surgery	3	2	6	6
≤2 years after BC surgery	26	18	28	29
>2 years after BC surgery	13	9	5	5
RRSO and RRCM				
Before BC surgery	1	1	0	0
≤2 years after BC surgery	66	47	33	34
>2 years after BC surgery	23	16	18	19

ER: estrogen receptor; HER2: human epidermal growth factor Receptor 2; PR: progesterone receptor; TN: triple negative, RRCM: risk reducing contralateral mastectomy; RRSO: risk reducing salpingo-oophorectomy.

associated with a significantly higher hazard (HR 2.90 95% CI 1.4–5.9, $p = .003$). When ER was included in the model the association was still significant (HR 2.78 95% CI 1.28–6.05, $p = .01$). *BRCA1/2* status was not statistical significantly associated with higher risk of death, however a trend towards worse OS was observed.

RRCM was not statistical significantly associated with DFS, however, significantly lower risk of death was observed in both unadjusted and adjusted analysis. Eighteen percent of patients that did not have RRCM versus three percent of patients that did, were older than 60 years at BC diagnosis.

Of patients who had previously undergone RRSO, one patient was diagnosed with primary peritoneal cancer 14.6 years after. One patient with intact ovaries had ovarian cancer. There were no incidences of ovarian cancer after an event of relapse or contralateral BC. No association of RRSO with DFS or OS was observed in univariate analysis.

Patients not treated with chemotherapy showed a trend towards worse outcome for both DFS and OS (Table 2 and Figure 2) and in multivariable analysis a statistical significant effect was seen for OS, with a similar trend for DFS although not significant ($p = .06$; Table 3). Patients not treated with chemotherapy were >10% ER positive postmenopausal and over 50 years, except one 48-year old premenopausal patient.

Discussion

In this nationwide study of *BRCA*-associated BCs in Denmark, *BRCA1* carriers were shown to have a significantly shorter DFS compared to *BRCA2* carriers after primary BC surgery. The majority underwent RRSO and around half RRCM after BC diagnosis. Risk reducing contralateral mastectomy significantly improved survival, but not RRSO. RRCM was associated with a more than 50 percent reduction in risk of death. The uptake of RRCM has increased through the years and our results confirm that *BRCA1/2* positive BC patients benefits from RRCM after BC. However, patients not undergoing RRCM were older, which of course influences OS and age

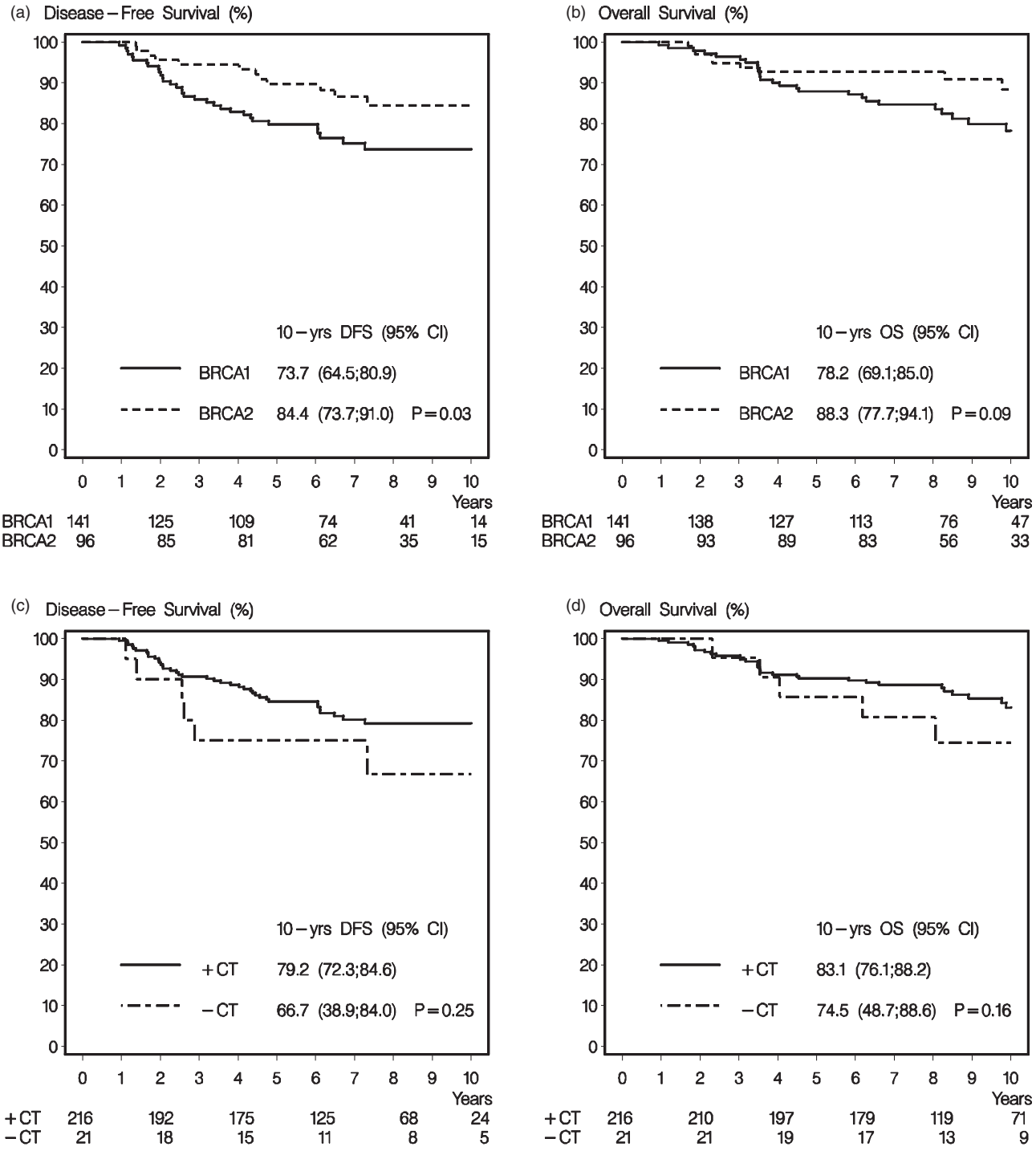


Figure 2: (a,b) DFS and OS according to *BRCA1/2* status; (c,d) Disease free- and overall survival according to adjuvant treatment with or without chemotherapy.

could have confounded the results to some extent. There was no association with DFS which is surprising since RRCM is associated with and mediated by lower risk of contralateral BC [8]. Finally, omission of chemotherapy was in this cohort of *BRCA* carriers with BC patients associated with a higher mortality.

A strength of our study is that clinical data were registered prospectively with detailed information on treatment and follow-up. Secondly we did central review of ER and HER2 status on tissue from 99% of all patients included, resulting in ER/HER2 status with standardized methods. Thirdly we retrieved information on the individual level from the DNPR and DPR registries containing complete information on risk reducing surgery.

There are limitations in the sense that even a nationwide study is restricted by sample size. The study-design contains a potential risk of bias however, we restricted the risk of longevity bias by excluding patients identified as a *BRCA* carrier more than two years after BC diagnosis. Our study was at risk of selection bias as *BRCA* mutation screening was limited

Table 2. Ten-year OS and DFS according to tumor- and patient characteristics.

	BRCA1/2	
	OS 10 year, % (95% CI)	DFS 10 year, % (95% CI)
All	82 (76–87)	78 (71–83)
BRCA mutation		
2	88 (78–94)	84 (74–91)
1	78 (69–85)	74 (64–81)
ER		
Positive	85 (75–91)	79 (68–86)
Negative	80 (70–87)	77 (67–84)
HER2		
Negative	82 (76–88)	79 (72–84)
Positive	83 (46–95)	64 (30–85)
TN		
No	85 (75–91)	78 (69–85)
Yes	80 (70–87)	77 (67–85)
Positive lymph nodes		
No	86 (76–92)	82 (72–89)
Yes	79 (69–86)	73 (64–81)
Tumor size		
0–20 mm	87 (79–92)	80 (70–86)
>20mm	75 (63–84)	75 (65–83)
Grade		
1	83 (27–97)	86 (33–98)
2	82 (66–91)	80 (68–88)
3	82 (73–88)	75 (66–82)
Not ductal/lobular	89 (64–97)	88 (61–97)
Chemotherapy		
No	74 (49–89)	67(39–84)
Yes	83 (76–88)	79 (72–85)

ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; TN: triple negative; OS: overall survival; DFS: disease free survival.

Table 3. Prognostic impact of tumor- and patient characteristics.

	Overall survival			
	Univariate		Multivariate	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
BRCA mutation				
2	1	.09	1	.10
1	1.87 (0.91–3.87)		1.98 (0.87–4.52)	
ER < seven years follow-up				
Positive	1	.03	1	.03
Negative	2.3 (1.05–5.15)		2.81 (1.09–7.27)	
ER > seven years follow-up				
Positive	1	.33		–
Negative	0.5 (0.13–2.06)		–	
HER2				
Negative	1	.90		–
Positive	0.91 (0.22–3.82)		–	
Positive lymph nodes				
No	1	.20	1	.08
Yes	1.54 (0.79–3.00)		1.86 (0.94–3.68)	
Tumor size				
0–20 mm	1	.07		–
>20 mm	1.84 (0.96–3.52)		–	
Chemotherapy				
Yes	1	.20	1	.01
No	1.86 (0.77–4.47)		3.58 (1.29–9.97)	
RRCM				
No	1	.01	1	.01
Yes	0.43 (0.22-0.85)		0.42 (0.21–0.84)	
RRSO				
No	1	.54		
Yes	0.74 (0.29–1.89)			
	Disease free survival			
BRCA mutation				
2	1	.04	1	.01
1	2.02 (1.04–3.90)		2.78 (1.28–6.05)	
ER < seven years follow-up				
Positive	1	.32	1	.79
Negative	1.36 (0.74–2.50)		1.11 (0.53–2.30)	
ER > seven years follow-up				
Positive	1	.69		–
Negative	0.61(0.05–6.91)		–	
HER2				
Negative	1	.36		–
Positive	1.67 (0.60–4.66)		–	
Positive lymph nodes				
No	1	.10	1	.02
Yes	1.66 (0.91–3.03)		2.14 (1.14–4.00)	
Tumor size				
0–20 mm	1	.25		–
>20 mm	1.41 (0.79–2.54)		–	
Chemotherapy				
Yes	1	.29	1	.06
No	1.64 (0.69–3.88)		2.48 (0.95–6.48)	
RRCM				
No	1	.28	1	.25
Yes	0.72 (0.39–1.32)		0.70 (0.38–1.29)	
RRSO				
No	1	.82	–	–
Yes	0.91 (0.42–2.00)			

ER: estrogen receptor; HER2: human epidermal growth factor Receptor 2; PR: progesterone receptor; TN: triple negative, RRCM: risk reducing contralateral mastectomy; RRSO: risk reducing salpingo-oophorectomy.

by patient and family characteristics and to some extent by patients own choice of genetic counseling. Although our study urge caution when omission of adjuvant chemotherapy is considered, we are unable to estimate to what extent this could reduce mortality. We have previously, however, shown a corresponding excess mortality if adjuvant chemotherapy is omitted in patients with sporadic BC where individual trials and meta-analysis have demonstrated a benefit from chemotherapy [24,25].

Because of the rarity of germline *BRCA1/2* mutations in BC [26,27], several survival studies have been conducted retrospectively and in smaller cohorts influenced by biases [28,29]. Higher quality studies in larger *BRCA1/2* and population-based cohorts, with limited selection and longevity bias, have been reported more recently; Huzarski et al. [30] reported an independent and worse prognostic association of *BRCA1* status with survival and a trend towards better survival after chemotherapy (HR 0.42 95% CI 0.12–1.5, $p = .18$) in *BRCA1* carriers compared to sporadic BCs. Goodwin et al. [6] found a significantly higher risk associated with no chemotherapy treatment in *BRCA2* versus sporadic BCs (HR 3.62 95% CI 1.46–8.99, $p = .01$) and similarly Jonasson et al. [7] reported that among patients not treated with chemotherapy, *BRCA2* mutation was associated with an increased risk (HR 2.38, 95% CI 1.31–4.34, $p = .005$). This was not shown in the chemotherapy treated group indicating that *BRCA2* BC patients derived larger benefit from chemotherapy. In summary these results are a strong indication of higher benefit from chemotherapy in *BRCA* BCs and for inclusion of *BRCA* mutation as a criteria for chemotherapy treatment. However, if these results are specific for the afore mentioned, founder mutations and age-groups, should be considered.

In conclusion, *BRCA* mutation type may influence risk of relapse, contralateral BC and incidence of other malignancy,

but not mortality. Risk reducing contralateral mastectomy was associated with a clear survival benefit. Investigation of interaction between *BRCA* carrier status and effect of chemotherapy is out of the scope of this study and would require a comparable control group. However, results are in line with previous reports of the highly beneficial effect of chemotherapy in *BRCA* BC patients.

Acknowledgments

We would like to thank Lejla Majdanac for her excellent assistance in collecting historical data from Rigshospitalet, Copenhagen University Hospital.

Disclosure statement

AMG participated in an advisory board meeting about *BRCA* testing in ovarian cancers in February 2016, funded by AstraZeneca.

Funding

The study was supported financially by Region Zealand, Region of Southern Denmark, The Danish Cancer Research Foundation, Møbelarkitekt Aksel Clausens Foundation, Landsforeningen mod Brystkraeft, Karen A. Tolstrups Foundation and Axel Muusfeldts Foundation.

References

[1] Larsen MJ, Thomassen M, Gerdes AM, et al. Hereditary breast cancer: clinical, pathological and molecular characteristics. Breast Cancer (Auckl). 2014;8:145–155.
[2] Johannsson OT, Idvall I, Anderson C, et al. Tumour biological features of BRCA1-induced breast and ovarian cancer. Eur J Cancer. 1997;33:362–371.
[3] Armes JE, Egan AJ, Southey MC, et al. The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. Cancer. 1998;83:2335–2345.
[4] Mavaddat N, Barrowdale D, Andrulis IL, et al. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol Biomarkers Prev. 2012;21:134–147.
[5] Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. J Natl Cancer Inst. 1998;90:1138–1145.
[6] Goodwin PJ, Phillips KA, West DW, et al. Breast cancer prognosis in BRCA1 and BRCA2 mutation carriers: an International Prospective Breast Cancer Family Registry population-based cohort study. J Clin Oncol. 2012;30:19–26.
[7] Jonasson JG, Stefansson OA, Johannsson OT, et al. Oestrogen receptor status, treatment and breast cancer prognosis in Icelandic BRCA2 mutation carriers. Br J Cancer. 2016;115:776–783.
[8] Li X, You R, Wang X, et al. Effectiveness of prophylactic surgeries in BRCA1 or BRCA2 mutation carriers: a meta-analysis and systematic review. Clin Cancer Res. 2016;22:3971–3981.
[9] Pedersen CB. The Danish Civil Registration System. Scand J Public Health. 2011;39:22–25.
[10] Schmidt M, Schmidt SA, Sandegaard JL, et al. The Danish National Patient Registry: a review of content, data quality, and research potential. J Clin Epidemiol. 2015;7:449–490.
[11] Bjerregaard B, Larsen OB. The Danish Pathology Register. Scand J Public Health. 2011;39:72–74.
[12] Gjerstorff ML. The Danish Cancer Registry. Scand J Public Health. 2011;39:42–45.
[13] Christiansen P, Ejlertsen B, Jensen MB, et al. Danish Breast Cancer Cooperative Group. J Clin Epidemiol. 2016;8:445–449.
[14] Danish Breast Cancer Group Guideline. hereditary Breast- and Ovarian Cancer. 2016. [Internet]. Available from: http://dbcg.dk/PDF%20Filer/Kapitel_19_HBOC_23.09.2016.pdf
[15] Thomassen M, Hansen TV, Borg A, et al. BRCA1 and BRCA2 mutations in Danish families with hereditary breast and/or ovarian cancer. Acta Oncol. 2008;47:772–777.
[16] Danish Breast Cancer Group Guideline. Clinical guidelines, DBCG. Copenhagen, Denmark: DBCG; 2017.
[17] Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med. 2005;353:1659–1672.
[18] Eckhoff L, Nielsen M, Moeller S, et al. TAXTOX - a retrospective study regarding the side effects of docetaxel given as part of the adjuvant treatment to patients with primary breast cancer in Denmark from 2007 to 2009. Acta Oncol. 2011;50:1075–1082.
[19] Jensen MB, Ejlertsen B, Mouridsen HT, et al. Improvements in breast cancer survival between 1995 and 2012 in Denmark: the importance of earlier diagnosis and adjuvant treatment. Acta Oncol. 2016;55(Suppl 2):24–35.
[20] Pedersen IS, Schmidt AY, Bertelsen B, et al. A Danish national effort of BRCA1/2 variant classification. Acta Oncol. 2018.
[21] DBCG retningslinjer/vejledninger: Patologi. Copenhagen, Denmark: DBCG. Available from: http://dbcg.dk/PDF%20Filer/Kap_3_Patologi_22_juni_2017.pdf
[22] Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer. 1957;11:359–377.
[23] Lakhani SR, Ellis IO, Schnitt SJ, et al, editors. WHO classification of tumours of the breast. 4th ed. Vol. 4. WHO/IARC; 2012.
[24] Ejlertsen B, Jensen MB, Mouridsen HT. Excess mortality in postmenopausal high-risk women who only receive adjuvant endocrine therapy for estrogen receptor positive breast cancer. Acta Oncol. 2014;53:174–185.
[25] Peto R, Davies C, Godwin J, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012;379:432–444.
[26] Gerdes AM, Cruger DG, Thomassen M, et al. Evaluation of two different models to predict BRCA1 and BRCA2 mutations in a cohort of Danish hereditary breast and/or ovarian cancer families. Clin Genet. 2006;69:171–178.
[27] Winter C, Nilsson MP, Olsson E, et al. Targeted sequencing of BRCA1 and BRCA2 across a large unselected breast cancer cohort suggests that one-third of mutations are somatic. Ann Oncol. 2016;27:1532–1538.
[28] van den Broek AJ, Schmidt MK, van 't Veer LJ, et al. Worse breast cancer prognosis of BRCA1/BRCA2 mutation carriers: what's the evidence? A systematic review with meta-analysis. PLoS One. 2015;10:e0120189.
[29] Bordeleau L, Panchal S, Goodwin P. Prognosis of BRCA-associated breast cancer: a summary of evidence. Breast Cancer Res Treat. 2010;119:13–24.
[30] Huzarski T, Byrski T, Gronwald J, et al. Ten-year survival in patients with BRCA1-negative and BRCA1-positive breast cancer. J Clin Oncol. 2013;31:3191–3196

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 102–106
https://doi.org/10.1080/0284186X.2017.1404128

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Influence of intra-tumoral heterogeneity on the evaluation of BCL2, E-cadherin, EGFR, EMMPRIN, and Ki-67 expression in tissue microarrays from breast cancer

Trine Tramm[a,b], Marianne Kyndi[b], Flemming B. Sørensen[a], Jens Overgaard[b] and Jan Alsner[b]

[a]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark

ABSTRACT

Introduction: The influence of intra-tumoral heterogeneity on the evaluation of immunohistochemical (IHC) biomarker expression may affect the analytical validity of new biomarkers substantially and hence compromise the clinical utility. The aim of this study was to examine the influence of intra-tumoral heterogeneity as well as inter-observer variability on the evaluation of various IHC markers with potential prognostic impact in breast cancer (BCL2, E-cadherin, EGFR, EMMPRIN and Ki-67).

Material and methods: From each of 27 breast cancer patients, two tumor-containing paraffin blocks were chosen. Intra-tumoral heterogeneity was evaluated (1) within a single tumor-containing paraffin block ('intra-block agreement') by comparing information from a central, a peripheral tissue microarray (TMA) core and a whole slide section (WS), (2) between two different tumor-containing blocks from the same primary tumor ('inter-block agreement') by comparing information from TMA cores (central/peripheral) and WS. IHC markers on WS and TMA cores were evaluated by two observers independently, and agreements were estimated by Kappa statistics.

Results: For BCL2, E-cadherin and EGFR, an almost perfect intra- and inter-block agreement was found. EMMPRIN and Ki-67 showed a more heterogeneous expression with moderate to substantial intra-block agreements. For both stainings, there was a moderate inter-block agreement that improved slightly for EMMPRIN, when using WS instead of TMA cores. Inter-observer agreements were found to be almost perfect for BCL2, E-cadherin and EGFR (WS: $\kappa > 0.82$, TMAs: $\kappa > 0.90$), substantial for EMMPRIN ($\kappa > 0.63$), but only fair to moderate for Ki-67 (WS: $\kappa = 0.54$, TMAs: $\kappa = 0.33$).

Conclusions: BCL2, E-cadherin and EGFR were found to be homogeneously expressed, whereas EMMPRIN and Ki-67 showed a more pronounced degree of intra-tumoral heterogeneity. The results emphasize the importance of securing the analytical validity of new biomarkers by examining the intra-tumoral heterogeneity of immunohistochemical stainings applied to TMA cores individually in each type of cancer.

ARTICLE HISTORY
Received 15 September 2017
Accepted 6 November 2017

Introduction

Individualizing cancer therapy is the pivot of current cancer research, and large scale gene-expression analyses are carried out in the search for potential prognostic and predictive biomarkers. Immunohistochemical (IHC) stainings, highlighting protein expression, represent an economical and readily available alternative to gene expression analysis, and may potentially aid the integration of new prognostic/predictive markers into the daily clinical practice in an easy and standardized way. Studies encompassing large number of patients are, however, necessary to corroborate the actual relevance of the different markers in order to secure the clinical validity. The tissue microarray (TMA) procedure, examining multiple tiny paraffin-embedded tumor biopsies, facilitates analysis of such large-scaled IHC studies [1] and reduces the expenses. One of the most frequently presented concerns, when using TMAs instead of whole slide sections (WS), is that the small-sized TMA cores may not be representative for the tumor bulk [2–4]. The influence of intra-tumoral heterogeneity on the evaluation of biomarker expression may thus affect the analytical validity of the biomarker and hence the clinical utility. Publications on biomarkers differ, nevertheless, substantially in the use of either WS or TMAs, and when TMAs are used, it is often not well-described, which area of the tumor the TMA core has been sampled from (e.g., invasive front or center of tumor). We have previously shown a fine agreement between central TMA cores and WS for the estrogen receptor and the HER-2 receptor, as well as an acceptable agreement for the progesterone receptor and for carbonic anhydrase 9 after staining just a single central or peripheral TMA core biopsy [5,6].

In this study, we have examined a larger panel of biomarkers with potential prognostic value in breast cancer (BCL2, E-cadherin, EGFR, EMMPRIN, and Ki-67), using both TMAs and WS in order to examine the influence of intra-tumoral heterogenity on the interpretation of these markers.

CONTACT T Tramm tramm@oncology.au.dk Department of Pathology, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, Plan 1, C112, DK-8200 Aarhus N, Denmark

Supplemental data for this article can be accessed here.

B-cell lymphoma 2 (BCL2) is an anti-apoptosis protein shown to be of independent prognostic value in breast cancer [7]. BCL2 is an estrogen responsive gene and the prognostic role may be subtype specific [8]. Previous studies showing the prognostic value of BCL2 have used either WS [8] or TMAs [9]. E-cadherin is an adhesion molecule, which is absent in the majority of invasive lobular carcinomas of the breast. In invasive ductal carcinomas, loss of E-cadherin may be an independent, negative prognostic indicator [10]. EGFR expression is linked to especially triple negative breast cancer (TNBC)/basal-like tumors, and has been found to be associated with an inferior prognosis [11]. The presence of EGFR overexpression may represent a potential target in TNBC, but the frequency of EGFR protein expression as determined by IHC in TNBC, has been found to vary significantly depending on, e.g., evaluation technique and antibodies used [12]. EMMPRIN (CD147) is a transmembrane glycoprotein of the immunoglobulin superfamily, and overexpression has been found to be associated with an invasive phenotype as well as with metastasis in various types of cancers, including subtypes of breast cancer [13]. The expression has, however, been found to be heterogeneous [14]. The assessment of tumor proliferation through immunhistochemical detection of Ki-67 has been practiced for many years, but the clinical utility of this biomarker and its prognostic value is complicated by the lack of a standardized procedure for assessment [15]. Ki-67 has, nevertheless, become a key player, when discussing breast cancer prognosis.

The aim of this study was to examine the influence of intra-tumoral heterogeneity and inter-observer agreement on the evaluation of various IHC markers with potential prognostic impact in breast cancer (BCL2, E-cadherin, EGFR, EMMPRIN, and Ki-67). Intra-tumoral heterogeneity was evaluated as 'intra-block agreement' by comparing the information from a central, a peripheral TMA core and a WS within a single tumor-containing paraffin block, and as 'inter-block' agreement by comparing information from TMA cores (central/peripheral) and WS between two different tumor-containing paraffin blocks from the same primary tumor. Finally, inter-observer agreement was calculated for WS and TMA cores, independently evaluated by two observers.

Materials and methods

The study material encompassed tumor-containing, formalin-fixed, paraffin blocks (FFPE) from 27 patients diagnosed with breast cancer in 1992–1993, as described in detail elsewhere [5]. The histological subtypes included invasive ductal carcinomas ($N = 22$) as well as invasive lobular carcinomas ($N = 5$). From each patient, two FFPE blocks were sampled, sliced, and stained with hematoxylin and eosin. Invasive carcinoma in the block was verified and marked, and from the invasive areas one 1 mm core was taken from the tumors central and peripheral compartments, respectively, and transferred to recipient TMA blocks. Four micrometer thick sections were cut from the TMA recipient blocks and the original donor blocks (supplementary Figure 1). Sections were immunohistochemically stained for BCL2, E-cadherin, EGFR, EMMPRIN and Ki-67 (supplementary Table 1 includes information on antibodies and staining procedures). Sections from other breast tumors, previously defined as positive, when stained with the respective antibodies, were additionally stained with and without the primary antibody and served as positive and negative controls.

For the individual stainings, percentage of invasive tumor with cytoplasmic BCL2 staining, membranous E-cadherin, EGFR and EMMPRIN, and nuclear Ki-67 were recorded, as was intensity (on a scale from 0 to 3). All sections were scored semi-quantitatively by two observers (MK and TT or MK and FBS). Afterwards, scores from all six stainings were dichotomized into positive and negative. The cut off used for distinguishing between negative and positive samples was 10% (with any intensity) for BCL2, E-cadherin, EGFR, and EMMPRIN, according to previous publications [8,16–18]. An optimal cut off point for Ki-67 has been widely discussed, but in this study a tumor was defined as positive ('high expression'), if at least 20% of invasive tumor nuclei stained with any intensity according to the St. Gallen consensus 2013 [19]. The percentages were recorded semi-quantitatively as an average of invasive tumor cells with positive reaction in comparison to invasive tumor cells in total.

Statistics

Inter-observer agreements and intra-observer agreements within as well as between tumor containing blocks and TMA cores are expressed as Kappa coefficients. Kappa statistics is explained as the chance-corrected proportional agreement, and possible values range from +1 (perfect agreement) via 0 (no agreement above that expected by chance) to –1 (complete disagreement). A rough interpretation of Kappa values rates $< +0.2$ as poor, $+0.2$ to $+0.4$ as fair, $+0.4$ to $+0.6$ as moderate, $+0.6$ to $+0.8$ as substantial, and $+0.8$ to $+1.0$ as almost perfect agreement [20]. Calculations were performed using Stata 15.0.

Results

The frequency of positive tumors varied for the different markers (Figure 1). On average, including all three cores and sections for both blocks and for both observers for 27 patients ($N = 324$), 223 out of 306 (73%) of the samples were positive for BCL2. For the other markers, the frequencies of positive tumors were E-cadherin: 73% (219/301), EGFR 19% (58/302), EMMPRIN: 58% (165/286), and Ki67: 43% (133/311).

To evaluate intra- and inter-block agreements as well as inter-observer agreements, Kappa values were calculated for various comparisons as listed in Table 1. Comparisons were mainly performed between individual cores and sections. In some cases, a cumulated score was used for the TMA cores of a single block, scoring the sample as positive if either or both the central or the peripheral core was positive (listed as 'central + peripheral' in Table 1).

In general, BCL2, E-cadherin and EGFR were found to be homogeneously expressed, and showed little intra-tumoral variation within and between blocks. The Kappa values for

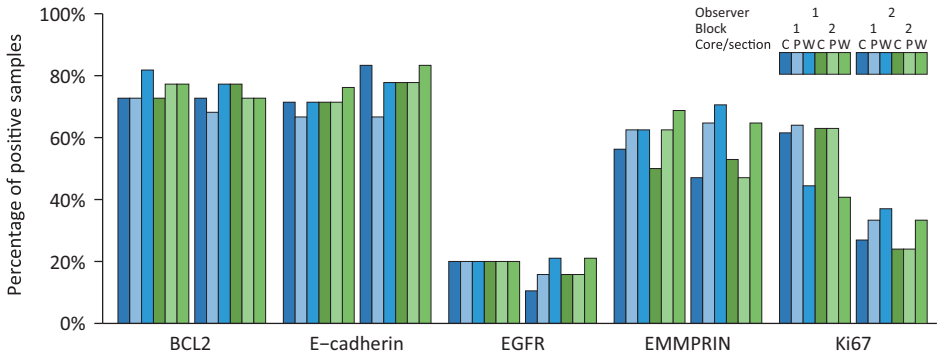


Figure 1. Frequency of positive tumors for markers BCL2, E-cadherin, EGFR, EMMPRIN, and Ki-67. Immunohistochemical analyzes of markers were interpreted from TMA cores, one central (C) and one peripheral (P), and whole slide sections (W) each from two blocks (1 and 2) from each tumor. All cases have been scored by two observers, 1 and 2, respectively. Only tumors that were interpretable on all cores and sections were included in the analysis.

Table 1. Kappa values describing comparisons between immunohistochemial stainings for BCL2, E-Cadherin, EGFR, EMMPRIN (10% cut offs), and Ki67 (20% cut offs) of 108 TMA biopsies and 54 whole slide sections (WS) from 27 breast carcinomas.

	BCL2		E-cadherin		EGFR		EMMPRIN		Ki67	
Observer	1	2	1	2	1	2	1	2	1	2
Intra-block agreement										
TMA central cores *vs* peripheral	0.95	0.89	0.95	0.82	1.00	0.81	0.55	0.50	0.66	0.85
TMA central cores *vs* WS	0.84	0.90	0.95	0.89	1.00	0.67	0.60	0.45	0.53	0.55
TMA peripheral cores *vs* WS	0.78	0.90	0.90	0.83	1.00	0.82	0.76	0.49	0.57	0.73
TMA central + peripheral cores *vs* WS	0.79	0.90	0.95	0.90	1.00	0.82	0.78	0.57	0.51	0.70
Inter-block agreement										
TMA central cores	1.00	0.90	1.00	0.89	1.00	0.78	0.90	0.50	0.67	0.68
TMA peripheral cores	0.89	0.89	0.89	0.48	0.88	0.86	0.59	0.27	0.43	0.90
TMA central + peripheral cores	0.90	0.90	1.00	0.90	0.88	0.86	0.55	0.38	0.74	0.82
Whole slide sections	0.90	0.91	0.90	0.91	0.79	0.79	0.69	0.55	0.77	0.76
Inter-observer agreement										
TMA cores	0.95		0.91		0.82		0.78		0.33	
Whole slide sections	0.90		0.90		1.00		0.63		0.54	

comparison between central and peripheral TMA cores within a single block exceeded 0.81 corresponding to almost perfect agreement, and agreement between cores (central or peripheral) and WS were very high for E-cadherin and substantial to almost perfect for BCL2 and EGFR. The agreements for the three stainings did not increase further, when cumulating the central and the peripheral TMA core and comparing to the corresponding WS from the same tumor-containing paraffin block. For BCL2 and E-cadherin, there was in general a very high inter-block agreement, using either TMA cores or WS, whereas the agreement between information from WS from two different blocks were slightly lower but still substantial for EGFR. A very high inter-observer agreement was further found for BCL2, E-cadherin and EGFR, using either TMAs or WS, with Kappa values in the almost perfect range.

For EMMPRIN, intra-tumoral heterogeneity did, however, affect the expression on IHC with considerable intra- as well as inter-block variation. Figure 1 indicates that the expression of EMMPRIN was higher in cores taken from the periphery of the tumor as compared to cores from the center, and the Kappa values for comparison between the central and the peripheral TMA cores within the blocks were in the moderate range. Figure 1 further indicates that a higher percentage of positive cases were found, when evaluating WS as compared to TMA cores. Agreement between cores (central or peripheral) and corresponding WS were, however, found to be substantial for observer 1, but only moderate for observer 2. When cumulating the central and the peripheral TMA core and comparing to a WS, the Kappa values increased slightly, but were still within the moderate to substantial range. The inter-block agreement for EMMPRIN, using either cores or WS, was found to be highly variable for the two observers with Kappa values ranging from fair to almost perfect and with higher Kappa values found for observer 1 than for observer 2. Despite the intra-tumoral heterogeneity, a substantial inter-observer agreement was found for EMMPRIN for both TMA cores and WS.

The intra-observer agreements for Ki-67 was substantial to almost perfect, when comparing central and peripheral TMA cores, and moderate to substantial when comparing cores (central or peripheral) and corresponding WS. The agreements did not increase, when cumulating the central and the peripheral TMA core and comparing to a WS. As for EMMPRIN, the inter-block agreements were highly variable for the two observers, when evaluating TMA cores, with Kappa values ranging from fair to almost perfect. For both observers, a substantial agreement was, however, found between WS from two different blocks with Kappa values exceeding 0.76. Figure 1 shows a difference in individual scaling for Ki-67 with a substantially higher number of positive cases, especially on TMA cores, for observer 1 as compared to observer 2. The inter-observer agreement for Ki67 was, accordingly, only fair, when using TMA cores, but increased to moderate, when using WS.

Discussion

The results of the present study show BCL2, E-cadherin and EGFR to be homogeneously expressed in breast cancer and indicate that TMA cores are representative for the tumor bulk for these three biomarkers. The findings are in line with previous findings regarding these markers in breast cancer [21,22]. Nassar et al. [22] examined three cores from different regions of breast carcinomas and normal tissue, and similarly found that E-cadherin and EGFR lacked heterogeneity. The general consensus is that two to four tissue cores are representative with 95–97% concordance rates [23,24]. For BCL2, E-cadherin and EGFR, the present results, however, indicate that it is sufficient to use TMAs constructed with only one 1 mm core to determine the IHC expression in a breast carcinoma. Furthermore, it does not seem to influence the results, whether the TMA core is taken from the center or from the invasive front of the tumor. The results further shows that the variation between different tumor-containing blocks is negligible, and that choosing material from a single representative block is sufficient.

On the contrary, the results for EMMPRIN indicated that intra-tumoral heterogeneity affects the interpretation of the staining. The information from TMA cores were found to be in better concordance with information from the corresponding WS, when combining information from both a central and a peripheral core. It is, therefore, advisable to include more cores, preferably from different areas of the tumor, when constructing TMAs for evaluating EMMPRIN. There was, however, still only moderate agreement between the data obtained from two different blocks, meaning that the intra-tumoral heterogeneity cannot be fully compensated for, if evaluating the expression of EMMPRIN from a WS instead of from TMA cores. We have not found any other studies having validated EMMPRIN staining of TMA cores.

For Ki-67, intra-tumoral heterogeneity was found to affect the interpretation, which is in accordance with the current knowledge that Ki-67 harbors both spatial and temporal heterogeneity [22,25]. Muftah et al. [26] have examined the concordance between a WS and a single peripheral core, and found a low concordance, when dichotomized ($\kappa = 0.30$), and a moderate concordance when using Ki-67 as a continuous variable (Intraclass coefficient = 0.61). On the contrary, Batistatou et al. [27] found a very high correlation between TMA cores and WS ($\kappa = 0.95$), using a 14% cut off, and only a single core from a non-specified area. In our study, however, the inter-observer variability seemed to be an issue of even more impact.

Previous publications have shown that the analytical validity is especially low in the intermediate range for Ki-67, whereas there may be evidence for a clinical utility of very low and high levels of Ki-67 [28], and estimation of Ki-67 has been found especially difficult to standardize in the intermediate range [25]. In our study, we only evaluated the agreement of dichotomized results on the basis of a 20% cut off. Evaluating agreements in categories of, e.g., very low, intermediate, and high may have shown higher agreements in the very low and very high categories. Actually, it may be that the observed poor inter-observer agreements are primarily due to variations in the intermediate range. It was, however, not within the scope of this study to test the agreement within different categories of expression using different cut off points. The present study was carried out before the international recommendations for Ki-67 assessment was published [29], and the expression was not evaluated in the invasive edge only or in hot spots. Counting a specific number of cells, e.g., 500 tumor cells was also not pre-specified, and the subjectively estimated, average percentage from WS and TMA cores were recorded. It is highly likely that the intra- and inter-block agreements would have been higher, if using a different assessment method for Ki-67. Denkert et al. [25] have, assisted by a mathematical model, shown that counting at least 500–1000 cells is necessary to achieve an acceptable error rate, when using a 15% cut off value for Ki-67. The present results did not support that Ki-67 expression was higher in the invasive edge of the tumor than in the center as could have been expected (Figure 1), and did not indicate that WS instead of TMA cores should be favored.

Intra-tumoral heterogeneity may apply to intrinsic as well as extrinsic factors, meaning that the heterogeneity may arise from subclones in the tumor or may be due to differences in differentiation in the tumor or variations in the tumor-microenvironment. The heterogeneity may, however, also be due to pre-analytical factors related to variation in pre-fixation time or fixation etc. [30]. The extrinsic factors are, nevertheless, not likely to have contributed much to the inter-block variability in this study, since the two blocks were processed from the same tumor and as such handled under the same conditions.

It has been anticipated that the TMA technology would become an important vehicle in defining predictive biomarkers for future biospecific therapies [31]. Ilyas et al. [30] provided guidelines for conducting experiments with TMAs and suggested that the guidelines should be used as a supplement to the REMARK criteria for reporting IHC studies [32]. This study supports the findings by Ilyas et al. [30], and emphasizes the importance of examining the intra-tumoral heterogeneity of IHC stainings applied to TMA cores individually in each type of cancer as part of securing the analytical validity of new biomarkers.

Acknowledgements

The authors thank Birthe Hermansen and Mogens J. Johannsen for excellent technical assistance.

Disclosure statement

TT has received royalties from Pfizer and Roche A/S for lectures given. The funding sources had no role in the manuscript production. No potential conflicts of interest were disclosed by the other authors.

Funding

This study was supported by grants from the Danish Cancer Society, the University of Aarhus, the Danish Medical Research Council, Danish Ministry of Health, and ML Jørgensen and Gunnar Hansen's Foundation.

ORCID

Jan Alsner http://orcid.org/0000-0002-5395-3193

References

[1] Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med. 1998;4:844–847.
[2] Bubendorf L, Nocito A, Moch H, et al. Tissue microarray (TMA) technology: miniaturized pathology archives for high-throughput in situ studies. J Pathol. 2001;195:72–79.
[3] Horvath L, Henshall S. The application of tissue microarrays to cancer research. Pathology. 2001;33:125–129.
[4] Simon R, Sauter G. Tissue microarrays for miniaturized high-throughput molecular profiling of tumors. Exp Hematol. 2002;30:1365–1372.
[5] Kyndi M, Sorensen FB, Knudsen H, et al. Tissue microarrays compared with whole sections and biochemical analyses. A subgroup analysis of DBCG 82 b&c. Acta Oncol. 2008;47:591–599.
[6] Kyndi M, Sorensen FB, Knudsen H, et al. Carbonic anhydrase IX and response to postmastectomy radiotherapy in high-risk breast cancer: a subgroup analysis of the DBCG82 b and c trials. Breast Cancer Res. 2008;10:R24.
[7] Callagy GM, Webber MJ, Pharoah PD, et al. Meta-analysis confirms BCL2 is an independent prognostic marker in breast cancer. BMC Cancer. 2008;8:153
[8] Eom YH, Kim HS, Lee A, et al. BCL2 as a subtype-specific prognostic marker for breast cancer. J Breast Cancer. 2016;19:252–260.
[9] Callagy GM, Pharoah PD, Pinder SE, et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Clin Cancer Res. 2006;12:2468–2475.
[10] Gould Rothberg BE, Bracken MB. E-cadherin immunohistochemical expression as a prognostic factor in infiltrating ductal carcinoma of the breast: a systematic review and meta-analysis. Breast Cancer Res Treat. 2006;100:139–148.
[11] Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10:5367–5374.
[12] Nakai K, Hung MC, Yamaguchi H. A perspective on anti-EGFR therapies targeting triple-negative breast cancer. Am J Cancer Res. 2016;6:1609–1623.
[13] Zhao S, Ma W, Zhang M, et al. High expression of CD147 and MMP-9 is correlated with poor prognosis of triple-negative breast cancer (TNBC) patients. Med Oncol. 2013;30:335–012-0335-4.
[14] Grass GD, Tolliver LB, Bratoeva M, et al. CD147, CD44, and the epidermal growth factor receptor (EGFR) signaling pathway cooperate to regulate breast epithelial cell invasiveness. J Biol Chem. 2013;288:26089–26104.
[15] Pathmanathan N, Balleine RL. Ki67 and proliferation in breast cancer. J Clin Pathol. 2013;66:512–516.
[16] Liu Y, Xin T, Jiang QY, et al. CD147, MMP9 expression and clinical significance of basal-like breast cancer. Med Oncol. 2013;30:366.
[17] Howat WJ, Blows FM, Provenzano E, et al. Performance of automated scoring of ER, PR, HER2, CK5/6 and EGFR in breast cancer tissue microarrays in the Breast Cancer Association Consortium. J Pathol Clin Res. 2014;1:18–32.
[18] Meijnen P, Peterse JL, Antonini N, et al. Immunohistochemical categorisation of ductal carcinoma in situ of the breast. Br J Cancer. 2008;98:137–142.
[19] Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2013. Ann Oncol. 2013;24:2206–2223.
[20] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–174.
[21] Chhieng DC, Frost AR, Niwas S, et al. Intratumor heterogeneity of biomarker expression in breast carcinomas. Biotech Histochem. 2004;79:25–36.
[22] Nassar A, Radhakrishnan A, Cabrero IA, et al. Intratumoral heterogeneity of immunohistochemical marker expression in breast carcinoma: a tissue microarray-based study. Appl Immunohistochem Mol Morphol. 2010;18:433–441.
[23] Simon R, Mirlacher M, Sauter G. Tissue microarrays. BioTechniques. 2004;36:98–105.
[24] Watanabe A, Cornelison R, Hostetter G. Tissue microarrays: applications in genomic research. Expert Rev Mol Diagn. 2005;5:171–181.
[25] Denkert C, Budczies J, von Minckwitz G, et al. Strategies for developing Ki67 as a useful biomarker in breast cancer. Breast. 2015;24 Suppl 2:S67–S72.
[26] Muftah AA, Aleskandarany MA, Al-Kaabi MM, et al. Ki67 expression in invasive breast cancer: the use of tissue microarrays compared with whole tissue sections. Breast Cancer Res Treat. 2017;164:341–348.
[27] Batistatou A, Televantou D, Bobos M, et al. Evaluation of current prognostic and predictive markers in breast cancer: a validation study of tissue microarrays. Anticancer Res. 2013;33:2139–2145.
[28] Varga Z, Diebold J, Dommann-Scherrer C, et al. How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists. PLoS One. 2012;7:e37379.
[29] Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103:1656–1664.
[30] Ilyas M, Grabsch H, Ellis IO, et al. Guidelines and considerations for conducting experiments using tissue microarrays. Histopathology. 2013;62:827–839.
[31] Camp RL, Neumeister V, Rimm DL. A decade of tissue microarrays: progress in the discovery and validation of cancer biomarkers. J Clin Oncol. 2008;26:5630–5637.
[32] Altman DG, McShane LM, Sauerbrei W, et al. Reporting Recommendations for tumor marker prognostic studies (REMARK): explanation and elaboration. PLoS Med. 2012;9:e1001216.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 107–112
https://doi.org/10.1080/0284186X.2017.1406140

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

DBCG hypo trial validation of radiotherapy parameters from a national data bank versus manual reporting

Carsten Brink[a,b], Ebbe L. Lorenzen[b], Simon Long Krogh[b], Jonas Westberg[b], Martin Berg[c], Ingelise Jensen[d], Mette Skovhus Thomsen[e], Esben Svitzer Yates[e] and Birgitte Vrou Offersen[f,g]

[a]Institute of Clinical Research, University of Southern Denmark, Odense, Denmark; [b]Laboratory of Radiation Physics, Odense University Hospital, Odense, Denmark; [c]Department of Medical Physics, Vejle Hospital, Vejle, Denmark; [d]Department of Medical Physics, Aalborg University Hospital, Aalborg, Denmark; [e]Department of Medical Physics, Aarhus University Hospital, Aarhus, Denmark; [f]Institute of Clinical Medicine, Aarhus University, Aarhus, Denmark; [g]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark

ABSTRACT

Introduction: The current study evaluates the data quality achievable using a national data bank for reporting radiotherapy parameters relative to the classical manual reporting method of selected parameters.

Methods: The data comparison is based on 1522 Danish patients of the DBCG hypo trial with data stored in the Danish national radiotherapy data bank. In line with standard DBCG trial practice selected parameters were also reported manually to the DBCG database. Categorical variables are compared using contingency tables, and comparison of continuous parameters is presented in scatter plots.

Results: For categorical variables 25 differences between the data bank and manual values were located. Of these 23 were related to mistakes in the manual reported value whilst the remaining two were a wrong classification in the data bank. The wrong classification in the data bank was related to lack of dose information, since the two patients had been treated with an electron boost based on a manual calculation, thus data was not exported to the data bank, and this was not detected prior to comparison with the manual data. For a few database fields in the manual data an ambiguity of the parameter definition of the specific field is seen in the data. This was not the case for the data bank, which extract all data consistently.

Conclusions: In terms of data quality the data bank is superior to manually reported values. However, there is a need to allocate resources for checking the validity of the available data as well as ensuring that all relevant data is present. The data bank contains more detailed information, and thus facilitates research related to the actual dose distribution in the patients.

ARTICLE HISTORY
Received 15 September 2017
Accepted 8 November 2017

Introduction

Clinical trials depend upon the ability to report treatment and outcome values. This can be done by manually reporting a few key values or by sending the entire data set to a data bank directly from the treatment planning system. It is often stated that a data bank approach will ensure better data quality, since mistakes occur during the manual reporting stage [1,2]. This might be true, but neglects possible mistakes in a data bank reporting system. Thus, such systems need to be verified in order to validate how the data quality compares to quality of a manual reporting system.

Using a data bank to record treatment data allows the possibility to collect detailed information on the radiotherapy as the entire treatment planning dataset can be submitted with a time cost comparable to or even less than that used to report selected parameters manually. Such an approach will make it possible to address more detailed treatment planning questions in clinical trials, but will also make it possible to access all data for retrospective analyzes. Storage of all the relevant planning data is also the basis for federated databases [3] facilitating international data mining. This will allow researchers to address questions which would be hard or impossible to address using standard randomized phase III trials. However, before starting such studies it is important to validate the data quality of the applied data bank.

There are a number of initiatives around the world to collect large amounts of radiotherapy treatment parameters [4–7]. In Denmark, a national storage facility of DICOM data has been established [8]. It is currently used by a number of clinical trials [9–13] and contains radiotherapy information from more than 5000 patients. This study focuses on the largest clinical trial in the data bank; a clinical randomized trial on hypo-fractionation from the Danish Breast Cancer Group (DBCG) with 1883 patients, of which 1550 patients were Danish. The aim of the current study is to compare the data quality within the data bank relative to the quality of manually reported values; such that this information can be used as a reference of data quality in future trials based on similar data bank systems.

CONTACT Carsten Brink carsten.brink@rsyd.dk Laboratory of Radiation Physics (afd R), Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense C, Denmark

Supplemental data for this article can be accessed here.

Method

Among 1883 randomized early breast cancer patients for the DBCG trial 'Hypofractionated Versus Standard Fractionated Whole Breast Irradiation to Node-negative Breast Cancer Patients' (clinicaltrials.gov NR NCT00909818), 1550 patients were from Denmark, and 1522 of these accepted submission of their treatment plan to the Danish radiotherapy data bank. These 1522 patients are all included in the current analysis between dose parameters from the dose plans in the data bank and the prospectively manually reported parameters. The patients were enrolled from four Danish centers.

The clinical trial is a randomized radiotherapy trial between two post-surgery treatment arms of either 50 Gy/25 fractions (2.0 Gy per fraction) or 40 Gy/15 fractions (2.67 Gy per fraction) with the primary endpoint late radiation morbidity. If indicated by the DBCG guidelines a sequential boost of either 10 or 16 Gy was delivered to the tumor bed (e.g., patients < 50 years or resection margin < 2mm). As part of the clinical trial both radiotherapy and follow-up data were reported to the DBCG database in line with previous DBCG trials. In parallel to the manual reporting all DICOM RT information of the treatment plans were also collected in the national DICOM data bank. The infrastructure of the national DICOM data bank has been described by Westberg et al. [8]. The database consists mainly of three parts (1) A DICOM server that receives data exported from the participating institutions (2) A set of servers to process the data (3) A web interface for accessing the data. Data are only available within the data bank after they are assigned to a specific clinical collaboration, which is done using the web interface. Permission to access the data are user specific and can range from viewing trial aggregated values only from the users own institution, to the ability to download the entire set of data available in the data bank. Institution specific rules for mapping local structure names to trial specific structure names are defined within a given clinical trial in the data bank. The web interface provides direct access to predefined trial specific dose values (e.g., mean dose, maximum dose, and near max dose) and the ability to export the raw DICOM data makes it possible to calculate more specific values not implemented in the web interface.

The current study compares values reported manually versus those obtained from the DICOM data bank. The following parameters were available in both systems and thus used for comparison:

1. Laterality, i.e., left or right sided breast cancer
2. Treatment arm (40/50 Gy)
3. Boost (none/10/16 Gy)
4. CTV (for non-boost plans):
   a. Minimum dose in percentage of prescribed dose of primary treatment (i.e., 40/50 Gy)
   b. Volume fraction of CTV receiving dose $< 95\%$ of prescribed dose
   c. Maximum dose in percentage of prescribed dose
   d. Volume fraction of CTV receiving doses $> 105\%$ and $\leq 107\%$ of prescribed dose
   e. Absolute volume of CTV receiving doses $> 107\%$ and $\leq 110\%$ of prescribed dose
5. Heart: V20 and V40 for the 50 Gy arm and V17 and V35 for the 40 Gy arm
6. Left anterior descending artery (LADCA): Maximum dose
7. Ipsilateral lung: V20 for 50 Gy arm and V17 for 40 Gy arm
8. Volume of the breast CTV
9. Date of treatment (the data bank value defined by date of treatment planning CT scan).

Not all of the above items are directly accessible from the submitted data in the data bank since the information is not stored in the DICOM header. For boost treatments one center provided the dose summed over the primary and boost treatment, while the other centers provided the same data as two separate treatment plans. The dose from centers providing separate plans were summed, which was possible since the two plans were planned using the same planning CT. For a few patients a manually calculated electron boost was used for treatment, thus the dose distribution was not available in the data bank. To determine if a boost was delivered and if so the delivered dose (item 2 and 3 in list above), the following was evaluated: (1) number of treatment plans available for the patient, (2) detection of a boost structure in the data, (3) automatic shape detection of DVH (detection of peaks in the differential CTV DVH of the combined treatment) and (4) Near max dose (D0.027 cm^3 – the dose related to the upper 0.027 cm^3 of the DVH) values for CTV for the combined treatment. Determination of treatment arm (i.e., 40 Gy or 50 Gy), and type of boost were performed by at least two of these methods. Only in a few cases where the determined values disagreed a manual inspection was needed. Laterality (item 1 in the list above) was identified by comparing the summed dose in the left and right half of the CT scan.

Prior to comparison with the manual data a number of automated self-consistency checks were performed on the DICOM data in the data bank. The checks included detection of possible lack of data (e.g., missing dose files or missing boost plan), duplicate data (e.g., two plans for same treatment) and the mapping of local structure names to structure names used in the data bank.

The manual data were obtained directly from the DBCG secretary according to standard procedure in clinical DBCG trials [14].

Comparisons of manual versus the data bank obtained parameters were performed using either x-y scatter plots or contingency tables for continuous or categorical variables respectively. Differences between the data bank and manual values were investigated to clarify which of the two were closest to the actual/true value based on information from the local record and verify systems and by comparing patient specific deviation for a set of dose parameters as described in the results.

Results

Table 1 shows contingency tables comparing results from the data bank with manually reported values. In 18 cases the trial

Table 1. Contingency tables between manual reporting and data submitted to the data bank.

	Manual			
A: Trial arm	40 Gy	50 Gy	Missing	Sum
Data bank				
40 Gy	745 (48.9%)	3 (0.2%)	7 (0.5%)	755 (49.6%)
50 Gy	3 (0.2%)	753 (49.5%)	11 (0.7%)	767 (50.4%)
Sum	748 (49.1%)	756 (49.7%)	18 (1.2%)	1522 (100.0%)

	Manual			
B: Laterality	Right	Left	Missing	Sum
Data bank				
Right	730 (48.0%)	4 (0.3%)	1 (0.1%)	735 (48.3%)
Left	11 (0.7%)	772 (50.7%)	4 (0.3%)	787 (51.7%)
Sum	741 (48.7%)	776 (51.0%)	5 (0.3%)	1522 (100.0%)

	Manual				
C: Boost	None	10 Gy	16 Gy	Missing	Sum
Data bank					
None	1297 (85.5%)	2 (0.1%)	0 (0.0%)	2 (0.1%)	1301 (85.8%)
10 Gy	1 (0.1%)	169 (11.1%)	0 (0.0%)	0 (0.0%)	170 (11.2%)
16 Gy	1 (0.1%)	0 (0.0%)	45 (3.0%)	0 (0.0%)	46 (3.0%)
Sum	1299 (85.6%)	171 (11.3%)	45 (3.0%)	2 (0.1%)	1517 (100.0%)

Tables are shown for A: trial arm, B: laterality of treatment, and C: type of boost. For boost type five patients were excluded since they were detected in the automatic system, prior to data comparison, to be boost treatments. The treatment plans related to these patients were not available in the data bank since they were electron boost based on a manual dose calculation. Since analysis was performed after collection of all data in the data bank there are no missing values in the data bank.

arm was not identified in the manual data. For six patients the manual and the data bank assignments disagreed. Manual validation of the six treatment plans in the data bank supported the assignment performed by the data bank. For laterality 15 cases were not classified consistently between the two systems. Manual validation of the 15 patient treatment plans found all to agree with the data bank laterality classification. Type of boost classification was not consistent for four patients. Two patients who were reported to have received a boost in the data bank were verified in the local record and verify system to be assigned correctly. The remaining two patients which in the data bank were assigned to have had no boost did actually have a boost treatment. The wrong classification in the data bank was related to a lack of dose information, since the two patients had been treated with an electron boost based on a manual calculation, thus no data were exported to the data bank. The combined error and missing rate for the manual method was 1.5%, 1.3%, and 0.4% for trial arm, laterality, and boost type, respectively. For the data bank no wrong identifications were found for laterality and treatment arm, but an error rate of 0.1% in assignment of boost type was seen.

The continuous parameters for which the largest degree of consistency exists between the data bank and manual reported value is shown in Figure 1(a,b), having the manual value on the *x*-axis and the data bank value on the *y*-axis. The difference between the third and the first quartile (interquartile range – IQ) of distribution of the differences between the data bank and manual value is shown in the figure. The differences between the two sets of values have been examined for outliers, similar to what is typically used in boxplots initially introduced by Tukey [15]. In line with common practice outliers were defined as data points where the difference between the manual and the data bank value is further outside the first or third quartile than 1.5 times the interquartile range [15]. Similarly, extreme outliers were defined as points further away than three times the interquartile range [15]. If the data were normal distributed the probability of observing an outlier would be 7×10^{-3} and an extreme outlier 2×10^{-6}. In the figure the extreme outliers are shown as red points and the remainder as blue points. The percentage of outliers (out) and extreme outliers (ext) are also shown in the figure. The number of data points missing related to the *x*- and *y*-axis are also shown in the figure. Finally, a black line of identity is included.

The date of radiotherapy data in Figure 1(a) demonstrates a close to perfect line except for a few extreme outliers shown as red points. The same tendency is seen for the CTV volume in Figure 1(b) with a larger spread of the red points. The red points could potentially indicate that the data bank did not contain the correct treatment plans. However, if the data bank did contain a wrong treatment plan it is unlikely that the reported CTV volume would agree with the manually reported value. Given that the CTV is manually delineated a volume difference between delineations on two separate CT scans of the same patient will likely differ more than 5 cm^3 knowing that the median CTV volume is 627 cm^3 (5 cm^3 volume difference corresponds to a radius difference of 0.14 mm for a spherical CTV; 5 cm^3 is also only twice the interquartile range seen in Figure 1(b)). Selection of all the patients with CTV volume deviations of more than 5 cm^3 results in a set of 152 patients. Of these patients the dates of the CT scan were between −21 to 0 days relative to the manual reported start of treatment except for four patients. Except for these four cases the plans in the data bank are thus at least representative for the dose distribution in the patient, even if a re-plan should have been performed. For the last four patients their reported V20/V17 ipsilateral lung value are at the maximum 0.2% different from the manually reported value, indicating that the data bank plans are

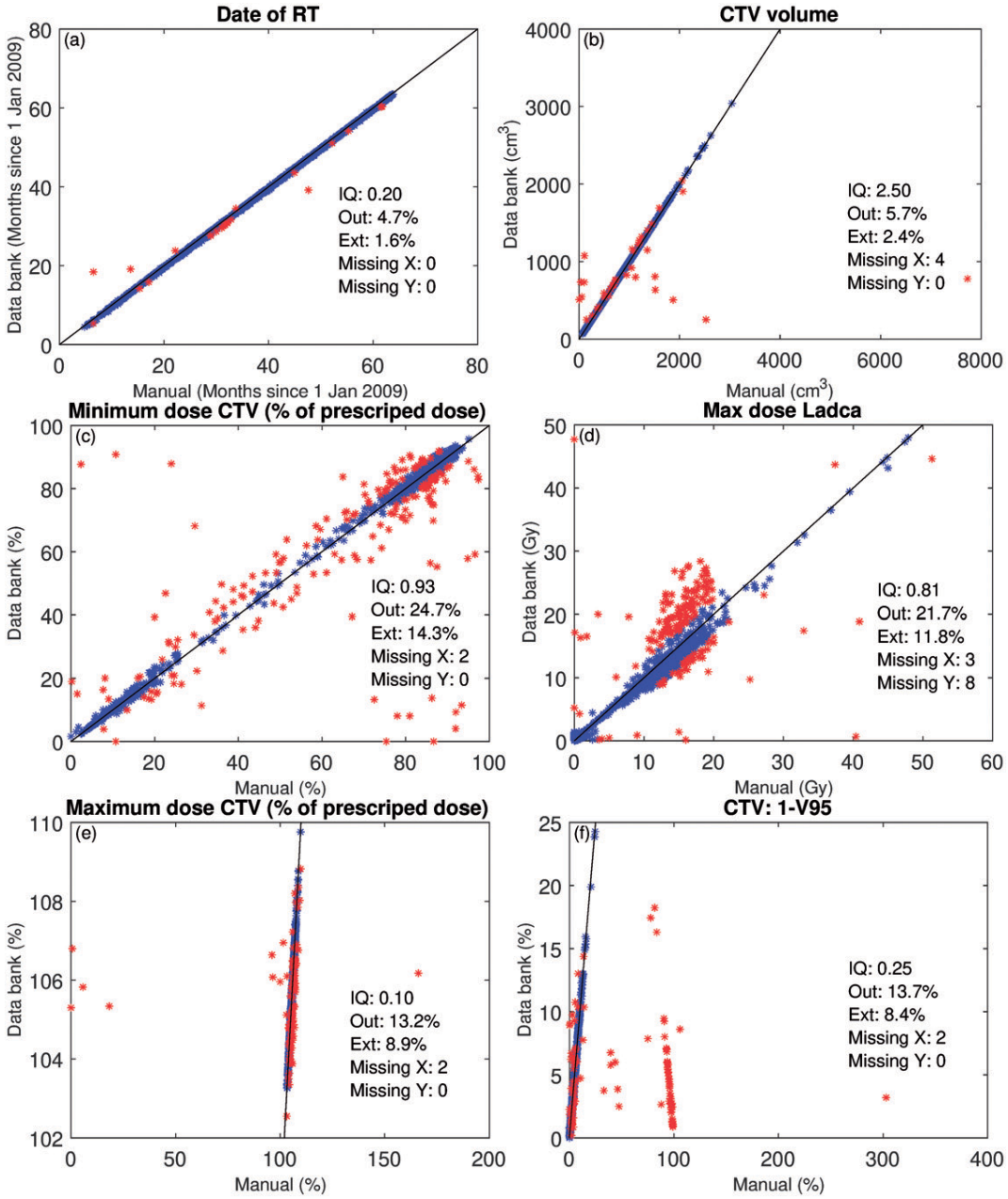


Figure 1. Comparison between values of manual and data bank for: date of RT, CTV volume, minimum dose to CTV, maximum dose to LADCA, CTV maximum, and 1-V95 values (Vx–x% of volume covered by the prescribed dose). IQ is the inter quartile range of the differences between the data bank and manual value. The percentage of outliers and extreme outliers (definition see text) is shown as well as the number of missing data on the *x*- and *y*-axis. Red points represent extreme outliers. Two points in subsection c with manual CTV values above 700% are outside the displayed area. Misunderstanding of the interpretation of the specific values in the manual reporting system is seen in subsection e–f, but most pronounced in subsection f.

identical to the plans used for manual reporting. The dominating effect for observed differences in Figure 1(a,b) is therefore uncertainties in the manual reporting process and not due to incorrect plans in the data bank.

Figure 1(c,d) is an example of data with a higher level of deviation between the two methods of reporting, and is an example of the problems related to reporting maximum and minimum values as opposed to near maximum and minimum values as recommended in the ICRU report 83 [16]. However, ICRU 83 was published after the DBCG hypo trial was initiated, which is part of the reason why no near max or min doses were reported manually. The large spread in data values for LADCA in Figure 1(d) is related to the presence of a steep dose gradient next to LADCA. Small differences in the interpretation of the structure position in different computer systems leads to large uncertainties in the maximum dose. The uncertainty will increase as the dose gradient next to LADCA increases. The largest dose gradient will be at doses close to half the prescribed dose which is also reflected in the figure as increased deviations in that dose area. The eight missing LADCA values in the data bank, is due to a local decision not to delineate LADCA on these eight right sided treatment plans.

Figure 1(e,f) illustrates some of the problems in manual reporting of more complicated dose parameters. Both for the CTV maximum dose and in particular for 1-V95 for the CTV,

it is clear that not all users have the same understanding of the individual parameters to report. For the CTV maximum some points are reported as percent over-dosage and others as percentage of prescribed dose. However, the problems are clearly more severe for the 1-V95 in which a large subset has been reported as V95, which is directly accessible in all planning systems. The wrong 1-V95 data are almost entirely from one of the contributing centers, but that center has only reported approximately 20% of their data in that format. Further plots of CTV dose coverage as well as dose to ipsilateral lung and heart is available in the Supplementary material.

In a large number of the presented figures the fraction of extreme outliers was approximately 10%. This value is a clear indication that quite long tails are present for the difference between the two set of reported values. Given the information from the data bank it is easy to see that a number of the manual values simply deviate by an order of magnitude (one zero too many or less during manual typing) or interchange of two digits.

Discussion

A national DICOM data bank has been clinically operating in Denmark since 2010 [17]. In the current study it is shown that the obtainable data quality of such a data bank exceeds the quality of manual reporting used previously. The number of detected assignment errors in the data bank was related to two patients who were given an electron boost, which was not documented in the data bank, since the boost dose was not calculated in the local treatment planning system but was based on a manual calculation. These two assignment errors correspond to an error rate of 0.1% which is much lower than the error rate observed for the manual reported values.

The fraction of extreme outliers was approximately 10% for a large number of the reported parameters. This might initially seem very high but a mistake in typing all the digits correctly is likely to result in an extreme outlier, in particular if it is one of the first digits which are mistyped. The value of 10% is thus probably a reflection of the error rate most people have in typing numbers into a computer system. Some of the obviously mistyped and in particular missing manual data could have been correct if a detailed inspection of the manual data had been performed by cross linking to other information such as randomization, pathology, size of resection margin and age of patient. However, most of the parameters related to delivered dose would probably be very difficult to locate during a validation based only on manual data.

The high level of data quality of the data bank is of course positive and provides a solid basis for future retrospective studies. But probably more importantly, data banks can be the basis for international data trials, either based on centralized data or by using distributed learning methods, as suggested by Skripcak et al. [3], which reduces the legal issues related to the exchange of data between different countries. The data bank can thus be used as the backbone in a number of international studies using, e.g., deep learning methods if the users choose to do so. Whether the current results can be extrapolated to other radiotherapy DICOM data banks can be debated. However, DBCG do have a long lasting tradition of clinical trials and the DICOM standard is an international accepted standard, thus it seems likely that comparisons between other data banks and manually data would not result in deviations significantly smaller than those in the current study.

All transmissions of DICOM data from the individual institutions to the data bank were performed on secure lines administered by the Danish national health system. One of the main problems in collecting treatment data is related to legal issues of privacy. It is therefore important that all transmissions of patient sensitive information are performed on secure lines, where all data transmissions are encrypted and the sender can validate the identity of the receiver. A common pitfall for people setting up quick data exchange between institutions is to use transmission protocols not design with security in mind (e.g., FTP), without encapsulating all network transmissions in a secure way. It should thus be stressed that it is important to spend quite some resources in designing a data bank such that confidentiality of the data is ensured at all times.

Even though the data quality of the data bank system seems superior, there are a number of challenges compared to the manual system – challenges which all data bank systems need to address. The first is related to exporting data from the participating institutions to the data bank, which is not a part of the clinical routine and often a manual step, thus data might not have been exported at all. Since the data bank does not have any additional knowledge about the number of expected patients, large numbers of treatment plans might be missing. The second challenge is that data has to be assigned to one or more trials in the data bank system, in order to allow access for other institutions. Such an assignment is needed in all data bank systems except if they are designed for a single clinical trial. However, this issue is to some degree a technical problem, and in the data bank in this study the users currently have to supply the additional information using the web interface. Unfortunately, many users find this additional step confusing when using the data bank. Also the naming of structures which may be performed manually in the local treatment planning system is an important issue. There are international recommendations for the naming of structures [18–20], and it can be strongly recommended to follow these, or maybe even more importantly to use 'scripts' in the local treatment planning systems to ensure local consistency of structure names, since that is central for a correct structure mapping in data banks. An additional approach could be to implement automatic structure recognition methods in data banks as proposed by McIntosh et al. [21], demonstrating an accuracy of 92% and therefore could be useful in pin-pointing incorrect structure mappings.

Due to the manual processes related to data banks, there are multiple causes that can result in the presence of incorrect data in data banks. Data validation is needed to ensure that all data has been submitted and to obtain a high level

of data quality. The current data bank uses data from the treatment planning systems which can differ from the data in the record and verify system. An example of this was detected during the initial validation of the data bank values where a nonstandard boost was detected - prescribed in the treatment planning system as 2 Gy in 10 fractions and not 5 fractions. The correct boost had been delivered, but the fractionation error was only corrected in the record and verify system. There is therefore a need to invest time to develop computer programs for the specific clinical trials to check the integrity of the data. The specific amount of time needed is difficult to estimate, but a data bank reporting system does not necessarily mean less manual work in the end, but it does provide a lot more opportunities to automate and apply new technologies to data and answer new questions. There is definitely a need for allocation of human resources if a data bank should be in clinical operation for a large number of clinical trials. The needed resources obvious depend on the actual data bank implementation, but even for the most automated systems it is likely that a least one full time position is needed to support customers, validate data quality, and perform bug-fix/development.

In summary the data bank was superior to manually reported values in terms of data quality. This study therefore provides a sound technical basis for transitioning future DBCG RT trials and studies to the national radiotherapy data bank. All the errors detected in this study regarding the manual reporting to the DBCG database in the DBCG hypo trial will obviously be corrected. But potentially more important data banks contain more detailed information. Thus, new relevant values may in the future be extracted from the submitted dose plan data compared to the limited number of predefined values reported manually.

Acknowledgments

This work is supported by CIRRO – The Lundbeck FoundationCenter for Interventional Research in Radiation Oncology and by The Danish Council for Strategic Research. BVO also acknowledge supported by the Danish Cancer Society. CB, ELL, SLK, and JW acknowledge support from AgeCare (Academy of Geriatric Cancer Research), an international research collaboration based at Odense University Hospital, Denmark.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Carsten Brink http://orcid.org/0000-0003-3906-1962

References

[1] Roelofs E, Persoon L, Nijsten S, et al. Benefits of a clinical data warehouse with data mining tools to collect data for a radiotherapy trial. Radiother Oncol. 2013;108:174–179.

[2] Roelofs E, Persoon L, Qamhiyeh S, et al. Design of and technical challenges involved in a framework for multicentric radiotherapy treatment planning studies. Radiother Oncol. 2010;97:567–571.

[3] Skripcak T, Belka C, Bosch W, et al. Creating a data exchange strategy for radiotherapy research: Towards federated databases and anonymised public datasets. Radiother Oncol. 2014;113:303–309.

[4] Fairchild A, Aird E, Fenton PA, et al. Eortc radiation oncology group quality assurance platform: establishment of a digital central review facility. Radiother Oncol. 2012;103:279–286.

[5] Källman HE, Halsius E, Olsson M, et al. Dicom metadata repository for technical information in digital medical images. Acta Oncol. 2009;48:285–288.

[6] Laprise NK, Hanusik R, Fitzgerald TJ, et al. Developing a multi-institutional PACS archive and designing processes to manage the shift from a film to a digital-based archive. J Digit Imaging. 2009;22:15–24.

[7] Roelofs E, Dekker A, Meldolesi E, et al. International data-sharing for radiotherapy research: an open-source based infrastructure for multicentric clinical data mining. Radiother Oncol. 2014;110:370–374.

[8] Westberg J, Krogh S, Brink C, et al. A DICOM based radiotherapy plan database for research collaboration and reporting. J Phys: Conf Ser. 2014;489:012100.

[9] Zukauskaite R, Hansen CR, Brink C, et al. Analysis of CT-verified loco-regional recurrences after definitive IMRT for HNSCC using site of origin estimation methods. Acta Oncol. 2017;56:1554–1561.

[10] Møller DS, Nielsen TB, Brink C, et al. Heterogeneous FDG-guided dose-escalation for locally advanced NSCLC (the NARLAL2 trial): design and early dosimetric results of a randomized, multi-centre phase-III study. Radiother Oncol. 2017;124:311–317.

[11] Hansen O, Knap MM, Khalil A, et al. A randomized phase II trial of concurrent chemoradiation with two doses of radiotherapy, 60Gy and 66Gy, concomitant with a fixed dose of oral vinorelbine in locally advanced NSCLC. Radiother Oncol. 2017;123:276–281.

[12] Pan Y, Brink C, Knap M, et al. Acute esophagitis for patients with local-regional advanced non small cell lung cancer treated with concurrent chemoradiotherapy. Radiother Oncol. 2016;118:465–470.

[13] Rønjom MF, Brink C, Bentzen SM, et al. External validation of a normal tissue complication probability model for radiation-induced hypothyroidism in an independent cohort. Acta Oncol. 2015;54:1301–1309.

[14] Mouridsen H, Christiansen P, Jensen MB, et al. Provision of data from the clinical database and of biological material from the tumour bank of the danish breast cancer cooperative group 2008–2017. Submitted for Acta Oncol. 2017.

[15] Dawson R. How significant is a boxplot outlier? J Stat Educ. 2011;19:1–13.

[16] ICRU Report 83. Prescribing, Recording, and Reporting Photon-Beam IMRT. Technical report, International commission on radiation units and measurements, 7010 Woodmont Avenue Bethesda, Maryland 20814 U.S.A, 2010.

[17] Westberg J, Jensen HR, Bertelsen A, et al. Reduction of Cone-beam CT scan time without compromising the accuracy of the image registration in IGRT. Acta Oncol. 2010;49:225–229.

[18] Santanam L, Hurkmans C, Mutic S, et al. Standardizing naming conventions in radiation oncology. Int J Radiat Oncol Biol Phys. 2012;83:1344–1349.

[19] Nyholm T, Olsson C, Agrup M, et al. A national approach for automated collection of standardized and population-based radiation therapy data in Sweden. Radiother Oncol. 2016;119:344–350.

[20] Denton TR, Shields LBE, Hahl M, et al. Guidelines for treatment naming in radiation oncology. J Appl Clin Med Phys. 2016;17:123–138.

[21] McIntosh C, Svistoun I, Purdie TG. Groupwise conditional random forests for automatic shape classification and contour quality assessment in radiotherapy planning. IEEE Trans Med Imaging. 2013;32:1043–1057.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 113–119
https://doi.org/10.1080/0284186X.2017.1406139

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The potential benefits from respiratory gating for breast cancer patients regarding target coverage and dose to organs at risk when applying strict dose limits to the heart: results from the DBCG HYPO trial

Martin Berg[a], Ebbe L. Lorenzen[b], Ingelise Jensen[c], Mette S. Thomsen[d], Christina Maria Lutz[d], Lasse Refsgaard[a], Henrik D. Nissen[a] and Birgitte V. Offersen[e]

[a]Department of Medical Physics, Vejle Hospital, Vejle, Denmark; [b]Laboratory of Radiation Physics, Odense University Hospital, Odense, Denmark; [c]Department of Medical Physics, Aalborg University Hospital, Aalborg, Denmark; [d]Department of Medical Physics, Aarhus University Hospital, Denmark Aarhus; [e]Department of Oncology, Aarhus University Hospital, Denmark

ABSTRACT

Purpose: The potential benefits from respiratory gating (RG) compared to free-breathing (FB) regarding target coverage and dose to organs at risk for breast cancer patients receiving post-operative radiotherapy (RT) in the DBCG HYPO multicentre trial are reported.

Material and methods: Patients included in the DBCG HYPO trial were randomized between 50 Gy in 25 fractions (normofractionated) versus 40 Gy in 15 fractions (hypofractionated). A tangential forward field-in-field dose planning technique was used to cover the clinical target volume (CTV) with the intent to limit dose to the left anterior descending coronary artery (LADCA) to 20 Gy and 17 Gy in the normo- and hypofractionated arms, respectively. Treatment plan data for 1327 patients from four Danish centres was retrospectively analyzed. FB right-sided patients served as control group for the left-sided patients regarding CTV $V_{95\%}$ (relative volume receiving at least 95% of the prescribed dose), mean heart dose (MHD) and mean lung dose (MLD).

Results: Median CTV $V_{95\%}$ was for FB right-sided, FB left-sided and RG left-sided patients 94.6, 92.6 and 94.7% for normofractionated therapy, respectively, and 94.6, 91.8 and 94.4% for hypofractionated therapy and did not differ significantly for RG left-sided plans compared to FB right-sided in either study arm. CTV $V_{95\%}$ was significantly lower for FB versus RG for left-sided plans in both arms. Median MHD was 0.7, 1.8 and 1.5 Gy (normofractionated therapy) versus 0.6, 1.5 and 1.2 Gy (hypofractionated therapy), respectively. The corresponding median MLD was 9.0, 8.3 and 7.3 Gy versus 7.3, 6.4 and 5.8 Gy, respectively.

Conclusions: RG for left-sided breast cancer patients ensured similar CTV $V_{95\%}$ as for FB right-sided patients. MLD was lower for RG due to the increased lung volume. MHD was generally low due to strict protocol-defined maximum dose to LADCA, but for left-sided patients RG led to significantly lower MHD.

ARTICLE HISTORY
Received 18 September 2017
Accepted 8 November 2017

Introduction

Adjuvant radiotherapy (RT) reduces loco-regional recurrence, distant failure and improves overall survival of early breast cancer patients [1–3]. On the other hand, adjuvant RT also increases the risk of heart- and lung-related toxicity compared to women not receiving RT [4,5]. A recent study also showed a further increase in risk among women who received anthracycline-containing chemoradiotherapy [6]. Inclusion of the internal mammary node (IMN) in the RT target in women with advanced disease increases mean heart dose (MHD) compared to patients having residual breast RT only [7–9]. However, the inclusion of the IMN is justified for high-risk patients according to recent studies showing reduced risk of breast cancer recurrence and improved survival after IMN RT [10–12]. Hence, careful decisions must be made on an individual patient level regarding target coverage versus dose to organs at risk (OAR). There has been quite some interest on reducing the dose to the left anterior descending coronary artery (LADCA) since reports have indicated an association between LADCA irradiation and the risk of ischemic heart disease [13–15]. In order to optimize target coverage while maintaining a low dose to OAR, techniques for RT with respiratory gating (RG), like enhanced inspiration gating and deep-inspiration breath hold (DIBH), have successfully been implemented in RT centres during the last decade [16–19]. These techniques exploit that during a deep inspiration the heart shifts in caudal and dorsal direction compared to the breast, moving the heart away from the high dose region of typical tangential fields. Simultaneously the lung is more inflated compared to normal breathing, reducing the relative volume of lung tissue in the high dose region. During the last decade RG radiotherapy for breast cancer patients has become standard in all Danish RT centres [20].

At the same time, the Danish Breast Cancer Group (DBCG) initiated the DBCG HYPO trial 'Hypofractionated

CONTACT Martin Berg martin.berg@rsyd.dk Department of Medical Physics, Beriderbakken 4, Vejle Hospital, 7100 Vejle, Denmark

Versus Standard Fractionated Whole Breast Irradiation to Node-negative Breast Cancer Patients' (clinicaltrials.gov NR NCT00909818). Women eligible for adjuvant whole breast RT after breast conserving surgery of early breast cancer were randomized between normofractionated RT (50 Gy in 25 fractions) versus moderately hypofractionated RT (40 Gy in 15 fractions).

The aim of the current work was to report the potential benefits regarding target coverage and dose to OAR for patients treated with RG compared to the free-breathing (FB) technique in the DBCG HYPO trial irrespective of fractionation scheme.

Material and methods

Patients

Patients receiving adjuvant RT after lumpectomy for early breast cancer were included in the clinically controlled randomized DBCG HYPO trial between May 2009 and March 2014 at four centres in Denmark. A total of 1883 patients were randomized, of these radiation treatment plans from 1522 patients were submitted to the Danish Treatment Plan Bank and used in the present study [21]. Detailed information on target coverage and dose to OAR was extracted on an individual patient basis. In order to avoid summation issues of dose from primary and boost treatment, only primary treatment plans were included in the current study. Whether a patient was treated using RG or not was retrieved from the DBCG trial database.

Clinical target volumes, OAR and dose planning

Patients were CT scanned on a breast board in an elevated position with either both arms or the ipsilateral arm above their head and with a CT slice thickness of 2–3 mm depending on institutional guidelines. The clinical target volume (CTVp_breast) defined as the residual breast tissue and OARs (heart, lung and LADCA) were delineated according to the national guidelines at time of start of inclusion in the trial [22,23]. The planning target volume (PTV) was created by expanding the CTVp_breast by typically 5 mm. Both CTVp_breast and PTV were cropped to 5 mm below the skin.

Treatment plans consisted of tangential medial and lateral field-in-field beams with energies between 6 MV and 18 MV and field edges around 5 mm from the PTV. A skin flash of minimum 2 cm was used in all centres. Centre 1 used a collapsed cone algorithm as implemented in Pinnacle (Philips Radiation Oncology Systems, Fitchburg, WI), Centre 2 the enhanced collapsed cone algorithm as implemented in Oncentra External Beam (Elekta AB, Stockholm, Sweden), Centre 3 an analytical anisotropic algorithm in Eclipse (AAA, Varian Medical Systems, Palo Alto, CA, USA), and Centre 4 initially used a pencil beam algorithm in Eclipse which was changed to the AAA in Eclipse in 2012.

Target coverage and dose to OARs were prioritized in the following order: tumor bed defined by surgical clips > LADCA > heart > lung > CTVp_breast > PTVp_breast > contralateral breast. The PTV should be covered by 95–107% and 95–105% of the prescribed dose in the normo- and hypofractionated arms, respectively. In the hypofractionated arm the CTVp_breast volume receiving between 105 and 107% of the prescribed dose should be kept below 2% of the CTVp_breast volume. For both treatment arms, the volume receiving 107–110% dose should be $<2\,cm^3$. In the normofractionated arm, the dose constraints for the OAR were: maximum LADCA dose $\leq$20 Gy, heart $V_{20Gy}\leq10\%$ and $V_{40Gy}\leq5\%$, ipsilateral lung $V_{20Gy}\leq25\%$ and mean lung dose (MLD) $\leq$ 18 Gy. Correspondingly in the hypofractionated arm: maximum LADCA dose $\leq$17 Gy, heart $V_{17Gy}\leq10\%$ and $V_{35Gy}\leq5\%$, ipsilateral lung $V_{17Gy}\leq25\%$ and MLD $\leq$16 Gy.

Gating techniques

Centre 1 and 2 used the Active Breathing Coordinator™ (ABC) system (Elekta AB, Stockholm, Sweden) and Centre 3 and 4 the Real-Time Position Management (RPM) system (Varian Medical Systems, Palo Alto, CA, USA) for gating. In all centres the patients were trained in using the equipment before the planning CT. When using RPM the treatment was given when the ventral chest wall was in a predefined window measured by an external marker block. When using the ABC system a valve in the mouth-piece made further inhalation impossible when the predefined amount of inhaled air was reached. In Centre 1–3 using DIBH, the patients were asked to take a 'comfortable deep inspiration' to ensure reproducible fixation. In Centre 4, using enhanced inspiration gating (EIG), the patients were encouraged to breathe as deeply as comfortable to achieve a higher inspiration level compared to FB. Exclusion criteria from gating in all four centres were: Not being able to maintain breath hold for at least 20 s (DIBH), or in case of EIG not being able to obtain reproducibly high amplitude. Furthermore, patients should be able to understand the necessary commands including audio guidance for RG. Patients unable to achieve an airtight seal around the mouth piece of the ABC system were also excluded from gating.

Statistical analysis

Data are presented in box-whisker plots showing first and third quartile, whiskers indicate range excluding outliers. Outliers are plotted as individual points and are defined as data further away from the quartiles than 1.5 times the interquartile range. The interpercentile range (0.16–0.84) corresponding to 68% of data is given. Group distributions are compared using Mann–Whitney *U*-test. *p* values below .05 are considered significant.

Results

Data from a total of 1522 Danish patients was available. At the time of data extraction, missing data on laterality and gating status and omission of summed primary and boost plans led to exclusion of 192 treatment plans from the present study. Three right sided patients were gated and hence these were omitted from the analysis as well. The remaining 1327 treatment plans were distributed among Centres 1–4 as

212 (57 FB left-sided and 52 with RG), 244 (22 FB left-sided and 102 with RG), 710 (203 FB left-sided and 161 with RG) and 161 (11 FB left-sided and 76 with RG), respectively. The treatment plans were divided into three groups: FB right-sided, FB left-sided and RG left-sided treatment plans and further separated by fractionation arm. Patient characteristics are given in Table 1. Target coverage for the CTVp_breast was available but not for the PTVp_breast. The present study is a dose planning study comparing potential benefits from RG irrespective of fractionation scheme and hence the physical doses were not transformed into biologically equivalent doses.

Median values, interpercentile range (0.16–0.84) and ranges for target coverage and dose to OARs are summarized in Table 2. The V_{20Gy} and V_{17Gy} constraints for the lung were violated for 5 and 1 treatment plans in the normo- and hypofractionated arms, respectively.

The FB right-sided treatment plans were considered a reference group for what could be achieved in terms of target coverage when heart dose was not limiting target coverage as for left-sided treatment plans. FB left-sided treatment plans showed lower CTVp_breast $V_{95\%}$ compared to the reference group, however, the use of RG improved CTVp_breast $V_{95\%}$ to the level of the reference group. For left-sided

Table 1. Patient characteristics in the normo- and hypofractionated arm.

	Normofractionated			Hypofractionated		
	Right-sided	Left-sided		Right-sided	Left-sided	
Characteristic	FB	FB	RG	FB	FB	RG
No. of patients	354	176	199	345	176	192
Age (years)	58	60	60	59	59	59
	50–67	53–68	51–58	50–68	51–68	51–67
	42–83	42–80	42–83	41–77	41–82	42–75
CTVp_breast volume (mL)	617	697	602	619	661	613
	328–1072	340–1105	339–997	313–1138	336–1163	305–1011
	86–3041	69–2501	74–2467	97–2454	98–2164	90–2624
Heart volume (mL)	625	582	594	628	610	601
	521–720	481–699	508–710	513–751	491–733	512–714
	333–980	376–1027	346–1132	341–932	396–1137	334–931
Ipsilateral lung volume (mL)	1542	1219	2170	1544	1226	2233
	1257–1906	961–1572	1756–2598	1255–1922	994–1559	1843–2564
	757–2790	703–2613	1026–3642	917–2890	626–2682	833–3316

Values presented are median, interpercentile range (0.16–0.84) and range.

Table 2. Median values, interpercentile range (0.16–0.84) and range for dose optimization parameters in the normo- and hypofractionated arm.

	Normofractionated			Hypofractionated		
	Right	Left		Right	Left	
Parameter	FB	FB	RG	FB	FB	RG
CTVp_breast $V_{95\%}$ (%)	94.6	92.6	94.7	94.6	91.8	94.4
	92.6–97.1	88.4–95.4	92.6–97.2	92.6–96.4	87.0–94.9	91.7–96.6
	70.6–99.1	72.0–99.3	81.4–99.7	79.4–99.4	80.0–98.7	76.8–99.0
	$p = .78$	$p < .001$	–	$p = .50$	$p < .001$	–
LADCA D_{max} (Gy)	0.6	112.9	12.6	0.4	11.8	11.0
	0.2–1.1	10.2–18.0	7.9–20.4	0.2–0.9	8.7–15.4	6.1–18.0
	0.1–26.3	0.5–48.0	2.2–29.5	0.0–19.7	0.18–39.3	2.4–32.0
	$p < .001$	$p = .56$	–	$p < .001$	$p = .22$	–
Lung MLD (Gy)	9.0	8.3	7.3	7.3	6.4	5.8
	7.0–10.9	6.2–10.3	5.7–9.0	5.6–9.0	4.9–8.3	4.6–7.2
	3.1–15.7	3.3–12.2	2.9–11.7	3.0–11.2	3.0–10.1	2.5–9.4
	$p < .001$	$p < .001$	–	$p < .001$	$p < .001$	–
Lung	16.7	15.3	13.6	16.4	14.6	12.7
V_{20Gy} (%) (normo)	12.2–21.0	10.7–19.8	9.9–17.2	12.0–21.1	10.4–19.5	9.7–16.4
V_{17Gy} (%) (hypo)	4.2–32.0	3.7–24.1	2.0–22.8	4.7–27.2	5.3–23.0	3.3–23.1
	$p < .001$	$p < .001$	–	$p < .001$	$p < .001$	–
Heart MHD (Gy)	0.7	1.8	1.5	0.6	1.5	1.2
	0.4–1.0	1.4–2.5	1.1–1.9	0.3–0.8	1.1–2.0	0.8–1.6
	0.2–2.4	0.4–9.2	0.5–3.5	0.2–2.9	0.3–4.2	0.4–4.7
	$p < .001$	$p < .001$	–	$p < .001$	$p < .001$	–
Heart	0.0	0.4	0.1	0.0	0.3	0.1
V_{20Gy} (%) (normo)	0.0–0.0	0.0–1.6	0.0–0.5	0.0–0.0	0.0–1.2	0.0–0.5
V_{17Gy} (%) (hypo)	0.0–0.8	0.0–8.4	0.0–4.1	0.0–1.1	0.0–7.0	0.0–8.9
	$p < .001$	$p < .001$	–	$p < .001$	$p < .001$	–
Heart	0.0	0.0	0.0	0.0	0.0	0.0
V_{40Gy} (%) (normo)	0–0	0.0–0.4	0.0–0.0	0.0–0.0	0.0–0.1	0.0–0.0
V_{35Gy} (%) (hypo)	0.0–0.1	0.0–4.6	0.0–1.4	0.0–0.1	0.0–2.7	0.0–4.6
	$p < .001$	$p < .001$	–	$p < .001$	$p < .001$	–

$V_{x\%}$ = volume (%) receiving x% of prescribed dose or higher. The p values are based on two-tailed Mann–Whitney U-tests and show whether group distributions differ from RG left-sided patient within each treatment arm.

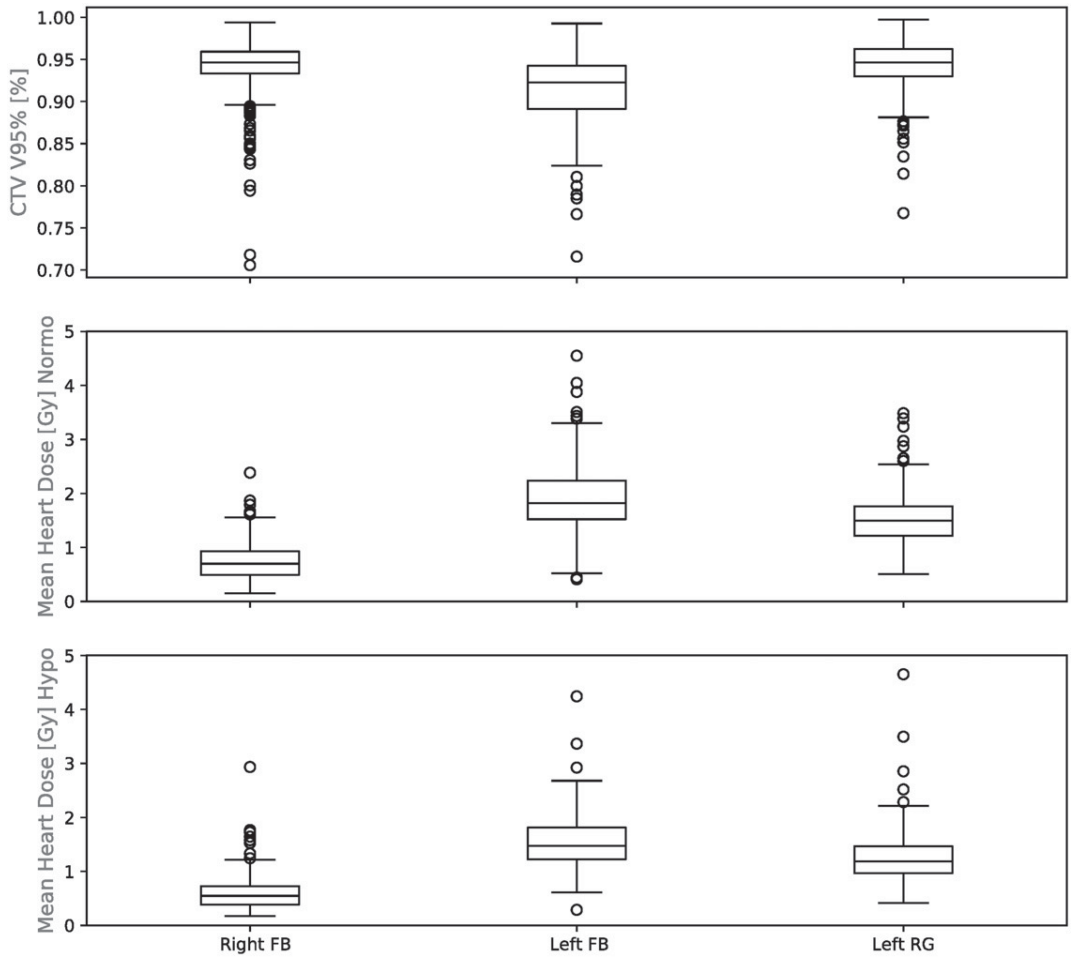


Figure 1. Box-whisker plots for (top) $V_{95\%}$ for the CTV for both normo- and hypofractionated arm, (middle) MHD for normofractionated arm, and (bottom) MHD for hypofractionated arm. $V_{x\%}$=volume (%) receiving x% of prescribed dose or higher.

treatment plans RG resulted in lower MLD and MHD compared to left-sided treatment plans treated with FB. Figure 1 shows the corresponding box-whisker plots for CTVp-breast $V_{95\%}$ and MHD for the normo- and hypofractionated arms. CTVp-breast $V_{95\%}$ is plotted against MHD in Figure 2 for left-sided treatment plans for the two arms. The solid line indicates the shift of group mean between FB and RG left-sided treatment plans.

A comparison between FB and RG for each of the four centres regarding $V_{95\%}$ for both arms and MHD for each arm is shown in Figure 3.

Discussion

The findings in the DBCG HYPO trial confirmed the expected advantages regarding target coverage of RG over FB for left-sided breast cancer patients receiving adjuvant RT in a clinical setting where low dose to the heart had high priority: RG ensured a target coverage similar to FB right-sided patients whereas FB left-sided patients had less optimal target coverage as can be seen in Figures (1,2). From Table 2, it is seen that compared to RG left-sided treatment only target coverage for FB right-sided treatment and maximum dose to LADCA for FB left-sided treatment were not statistically different irrespective of randomization arm. The findings between FB right-sided and FB left-sided versus RG left-sided treatments were identical in the two arms as expected. For right-sided treatment plans Essers et al. reported no gain from RG for breast only RT considering dose to OAR [24]. This supports the usefulness of considering FB right-sided treatment plans as the control group.

Regarding dose to lung, use of RG reduced the MLD significantly, since the irradiated volume of lung was relatively smaller in the inflated lung. The lung constraints were only violated in 6 treatment plans. However this may indicate that target coverage was compromised in other patients. Maximum dose to LADCA was exceeded in some cases but heart constraints were never violated. The MHD was lowest for FB right-sided treatment plans followed by RG left-sided treatment plans. For the whole-population MHD was significantly lower with RG compared to FB for left-sided treatment plans although not as low as for right-sided treatment plans. In general, the dose constraint for LADCA lead to low MHD as the heart was effectively shielded. The benefit from RG was also seen in better target coverage.

Taylor et al. [4] reported average MHD of about 4 Gy for left-sided breast only treatments in a review of published data for radiotherapy given between 2003 and 2013, and Lorenzen et al. reported MHD of 2.8 and 0.7 Gy for left-sided and right-sided treatment plans, respectively, treated in

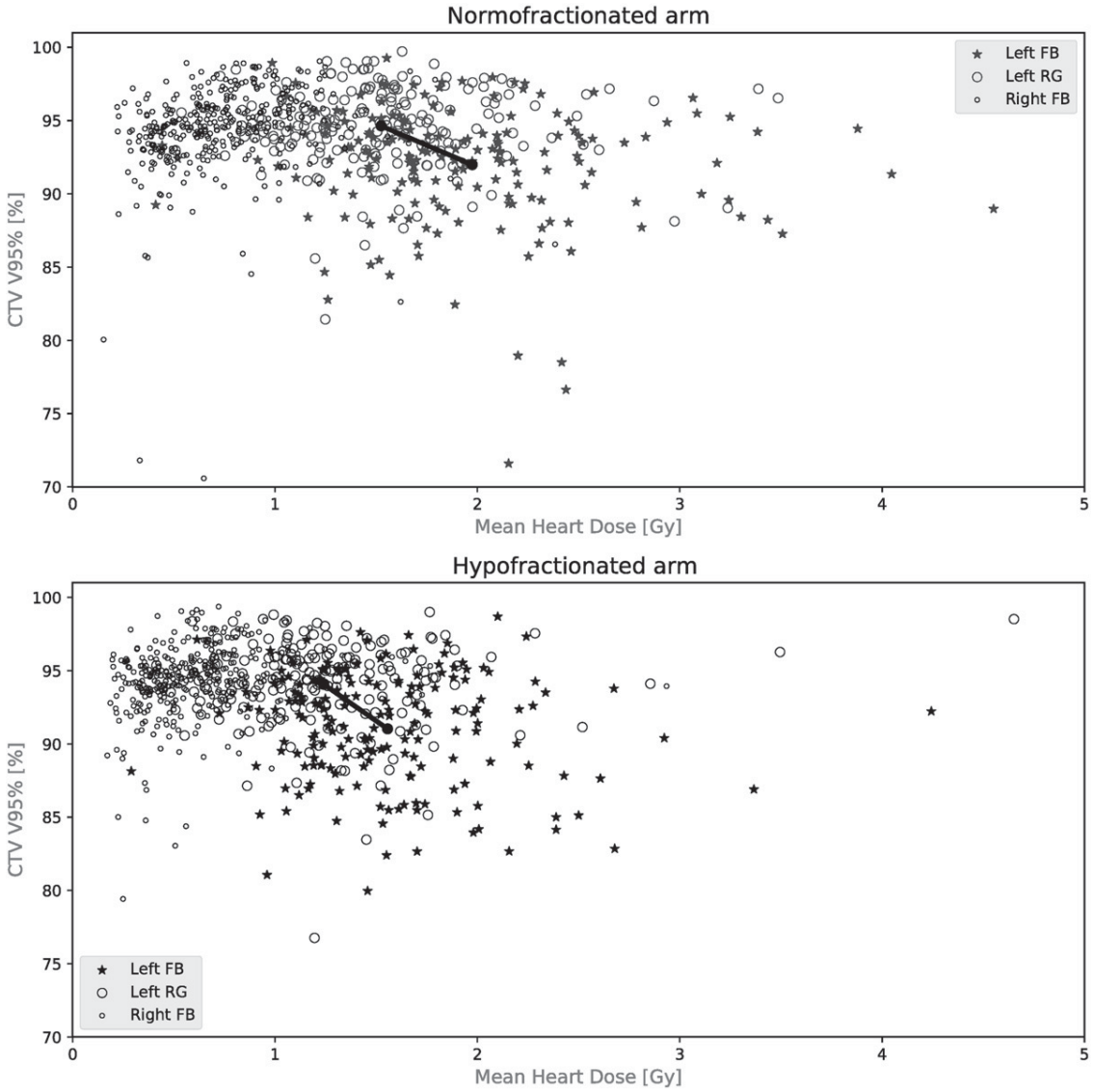


Figure 2. Target coverage of CTV against MHD for FB and RG left-sided patients for (top) normofractionated and (bottom) hypofractionated arm. The solid line indicates the shift of group mean. $V_{x\%}$=volume (%) receiving x% of prescribed dose or higher.

Denmark with 2D tangential radiotherapy (i.e., before ~2005) [4,25]. These findings, both the international and historical national doses, are above what is reported in the current study. This is a direct consequence of the prioritization of LADCA effectively shielding the heart from irradiation.

The risk of cardiac disease is increasing linearly with MHD with a rate of 4.1–7.4% per Gy MHD depending on endpoint [4,5]. Thus, due to the low values of MHD observed in this study the risk of cardiac disease is expected to be low.

The median $V_{95\%}$ of 94.8 and 94.7% for the CTVp_breast in the normo- and hypofractionated arms for RG left-sided treatment plans is somewhat lower than what was reported by Nissen and Appelt in a similar study [16]. In addition, the MHD dose was higher in their study which can be explained by the dose limits on LADCA in the present study. Schönecker et al. [26] reported for patients using DIBH a MHD of 1.3 Gy (based on 50 Gy in 25 fractions) which was very similar to 1.2 Gy presented in the current study [26].

Inherently different target delineation practices, algorithms and set-up guidelines exist among centres of which the consequences are not clear from Figure 1. Therefore a comparison between FB and RG for left-sided treatment plans within each centre was performed for target coverage and MHD as shown in Figure 3. Although the same constraints were applied in each centre differences are seen regarding target coverage and MHD. It is seen that the outliers in target coverage seen in Figure 1 can be explained by the data from Centre 2 showing inferior target coverage compared to the others. The origin is not clear but can have several causes: Differences in the medial and dorsal/lateral border of the CTVp_breast will lead to larger compromises regarding target coverage in order to fulfill the LADCA constraint. Depending on the centre-specific depth dose curves in the treatment planning system the target coverage in the superficial part of the CTVp-breast can be either over- or underestimated in the four dose planning systems.

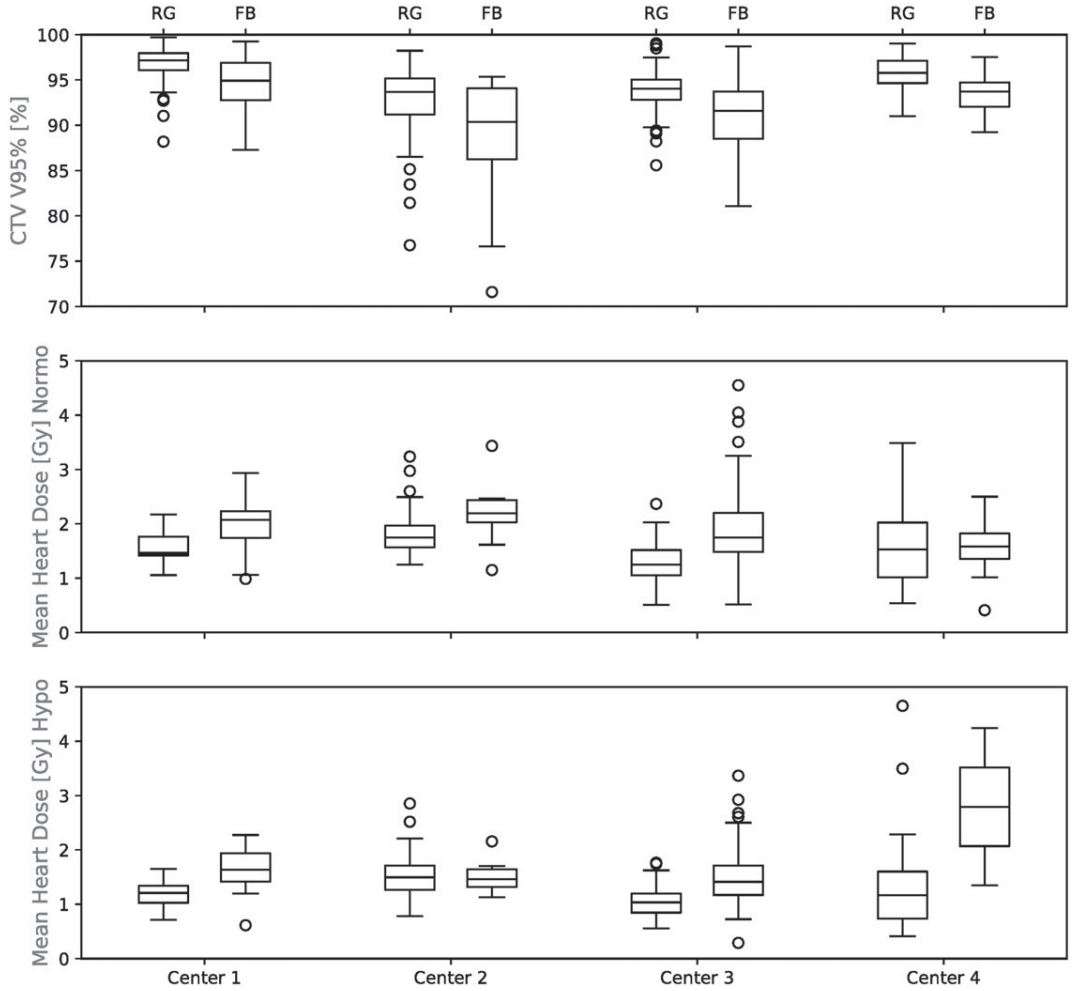


Figure 3. Box-whisker plots for each centre comparing RG versus FB for (top) target coverage for left-sided patients, (middle) MHD in the normofractionated arm, and (bottom) MHD in the hypofractionated arm. $V_{x\%}$=volume (%) receiving x% of prescribed dose or higher.

A limitation in the present multicentre study is the variations such as prioritization of target coverage over dose to OARs on an individual patient level, more strict local constraints for hotspots and more dose from high energy photons. However, all centres showed increased $V_{95\%}$ target coverage and similar or lower MHD when using RG irrespective of the system being used compared to FB in the same centre. For Centre 4 only two patients were treated in FB in the hypofractionated arm which can explain the large MHD and spread in the hypofractionated arm.

Conclusions

In this, to our knowledge, largest multicentre study on the effects of RG for left-sided breast cancer patients we have shown that separation of heart and target is feasible to an extent where target coverage is comparable to that of FB right-sided patients. MLD is lower using RG because of an absolute increase in lung volume causing a relative decrease in irradiated volume. MHD is reduced for RG compared to FB and lower than typically reported due to strict maximum dose to LADCA. Two RG techniques were used in the study and were found to be equal in terms of target coverage and sparing of the heart. Although differences were seen in target coverage and dose to OARs between centres improved target coverage was seen within each centre when RG is applied in all cases.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Center for Interventional Research in Radiation Oncology (CIRRO) and BVO was supported by the Danish Cancer Society.

References

[1] Overgaard M, Jensen M-B, Overgaard J, et al. Postoperative radiotherapy in high-risk postmenopausal breast-cancer patients given adjuvant tamoxifen: Danish Breast Cancer Cooperative Group DBCG 82c randomised trial. Lancet. 1999;353:1641–1648.

[2] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10 801 women in 17 randomised trials. Lancet. 2011;378:771–784.

[3] Bodilsen A, Offersen BV, Christiansen P, et al. Pattern of relapse after breast conserving therapy, a study of 1519 early breast cancer patients treated in the Central Region of Denmark 2000–2009. Acta Oncol. 2016;55:964–969.
[4] Taylor C, Candace C, Duane FK, et al. Estimating the risks of breast cancer radiotherapy: evidence from modern radiation doses to the lungs and heart and from previous randomized trials. JCO. 2017;35:1641–1649.
[5] Darby SC, Ewertz M, McGale P, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med. 2013;368:987–998.
[6] Rehammar JC, Jensen M-B, McGale P, et al. Risk of heart disease in relation to radiotherapy and chemotherapy with anthracyclines among 19,464 breast cancer patients in Denmark, 1977–2005. Radiother Oncol. 2017;123:229–305.
[7] Taylor CW, Wang Z, Macaulay E, et al. Exposure of the heart in breast cancer radiation therapy: a systematic review of heart doses published during 2003 to 2013. Int J Radiat Oncol Biol Phys. 2015;93:845–853.
[8] Thorsen LBJ, Thomsen MS, Berg M, et al. CT-planned internal mammary node radiotherapy in the DBCG-IMN study: benefit versus potentially harmful effects. Acta Oncol. 2014;53:1027–1034.
[9] Thorsen LBJ, Thomsen MS, Overgaard M, et al. Quality assurance of conventional non-CT-based internal mammary lymph node irradiation in a prospective Danish Breast Cancer Cooperative Group trial: the DBCG-IMN study. Acta Oncol. 2013;52:1526–1534.
[10] Thorsen LBJ, Offersen BV, Danø H, et al. DBCG-IMN: a population-based cohort study on the effect of internal mammary node irradiation in early node-positive breast cancer. J Clin Oncol. 2016;34:314–321.
[11] Whelan TJ, Olivotto IA, Parulekar WR, et al. Regional nodal irradiation in early-stage breast cancer. N Engl J Med. 2015; 373:307–316.
[12] Poortmans PM, Collette S, Kirkove C, et al. Internal mammary and medial supraclavicular irradiation in breast cancer. N Engl J Med. 2015;373:317–327.
[13] Nilsson G, Holmberg L, Garmo H, et al. Distribution of coronary artery stenosis after radiation for breast cancer. JCO. 2012; 30:380–386.
[14] Correa CR, Litt HI, Hwang W-T, et al. Coronary artery findings after left-sided compared with right-sided radiation treatment for early-stage breast cancer. JCO. 2007;25:3031–3037.
[15] Nilsson G, Witt Nyström P, Isacsson U, et al. Radiation dose distribution in coronary arteries in breast cancer radiotherapy. Acta Oncol. 2017;55:959–963.
[16] Nissen HN, Appelt A. Improved heart, lung and target dose with deep inspiration breath hold in a large clinical series of breast cancer patients. Radiother Oncol. 2013;106:28–32.
[17] Korreman SS, Pedersen AN, Nøttrup TJ, et al. Breathing adapted radiotherapy for breast cancer: Comparison of free breathing gating with the breath-hold technique. Radiother Oncol. 2005; 76:311–318.
[18] Rice L, Goldsmith C, Green MLM, et al. An effective deep-inspiration breath-hold radiotherapy technique for left-breast cancer: impact of post-mastectomy treatment, nodal coverage, and dose schedule on organs at risk. BCTT. 2017;9:437–446.
[19] Damkjaer SM, Aznar MC, Pedersen AN, et al. Reduced lung dose and improved inspiration level reproducibility in visually guided DIBH compared to audio coached EIG radiotherapy for breast cancer patients. Acta Oncol. 2013;52:1458–1463.
[20] Jensen M-B, Laenkholm A-V, Offersen BV, et al. The clinical database and implementation of treatment guidelines by the Danish Breast Cancer Cooperative Group in 2007–2016. Acta Oncol. Forthcoming. Available from: https://doi.org/10.1080/0284186X.2017.1404638
[21] Westberg J, Krogh S, Brink C, et al. A DICOM based radiotherapy plan database for research collaboration and reporting. J Phys: Conf Ser. 2014;489:012100.
[22] Nielsen MH, Berg M, Pedersen AN, et al. Delineation of target volumes and organs at risk in adjuvant radiotherapy of early breast cancer: national guidelines and contouring atlas by the Danish Breast Cancer Cooperative Group. Acta Oncol. 2013;52:703–710.
[23] Lorenzen EL, Taylow CW, Maraldo M, et al. Inter-observer variation in delineation of the heart and left anterior descending coronary artery in radiotherapy for breast cancer: a multi-centre study from Denmark and the UK. Radiother Oncol. 2013; 108:254–258.
[24] Essers M, Poortmans PM, Verschueren K, et al. Should breathing adapted radiotherapy also be applied for right-sided breast irradiation?. Acta Oncol. 2016;55:460–465.
[25] Lorenzen EL, Brink C, Taylor CW, et al. Uncertainties in estimating heart doses from 2D-tangential breast cancer radiotherapy. Radiother Oncol. 2016;119:71–76.
[26] Schönecker S, Walter F, Freislederer P, et al. Treatment planning and evaluation of gated radiotherapy in left-sided breast cancer patients using the Catalyst™/Sentinel™ system for deep inspiration breath-hold (DIBH). Radiat Oncol. 2016;11:143.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 120–128
https://doi.org/10.1080/0284186X.2017.1407040

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Concurrent new drug prescriptions and prognosis of early breast cancer: studies using the Danish Breast Cancer Group clinical database

Deirdre Cronin-Fenton[a], Timothy L. Lash[a,b], Thomas P. Ahern[c], Per Damkier[d,e], Peer Christiansen[f,g], Bent Ejlertsen[g,h] and Henrik T. Sørensen[a,i]

[a]Department of Clinical Epidemiology, Aarhus University, Aarhus, Denmark; [b]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA; [c]Departments of Surgery and Biochemistry, Larner College of Medicine, University of Vermont, Burlington, VT, USA; [d]Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark; [e]Department of Clinical Research, University of Southern Denmark, Odense, Denmark; [f]Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark; [g]Danish Breast Cancer Cooperative Group, Copenhagen, Denmark; [h]Department of Oncology, Rigshospitalet, Copenhagen, Denmark; [i]Department of Health Research & Policy (Epidemiology), Stanford University, Stanford, CA, USA

ABSTRACT

Background: Myriad reports suggest that frequently used prescription drugs alter the viability of breast cancer cells in pre-clinical studies. Routine use of these drugs, therefore, may impact breast cancer prognosis, and could have important implications for public health.

Methods: The Danish Breast Cancer Group (DBCG) clinical database provides high-quality prospectively collected data on breast cancer diagnosis, treatment, and routine follow-up for breast cancer recurrence. Individual-level linkage of DBCG data to other population-based and medical registries in Denmark, including the Danish National Prescription Registry, has facilitated large population-based pharmacoepidemiology studies. A unique advantage of using DBCG data for such studies is the ability to investigate the association of drugs with breast cancer recurrence rather than breast cancer mortality – which may be misclassified – or all-cause mortality. Here we summarize findings from pharmacoepidemiological studies, based on DBCG data, on the association between routinely used prescription drugs and risk of breast cancer recurrence.

Results: Our findings suggest that concurrent use of glucocorticoids, ACE inhibitors, aspirin, NSAIDs, selective COX-2 inhibitors, digoxin, and opioids has little impact on breast cancer recurrence. Similarly, patients who use SSRIs concurrently with tamoxifen treatment are not at increased risk of recurrence. In contrast, post-diagnostic use of simvastatin, a lipophilic statin, correlates with a decreased risk of breast cancer recurrence, providing a rationale for a prospective randomized clinical trial investigating simvastatin as an adjuvant therapy for breast cancer.

Conclusion: As a whole, findings of pharmacoepidemiological studies based on DBCG data provide reassurance to physicians and healthcare personnel who provide supportive care during and after cancer (including prescriptions for comedications) and to breast cancer survivors for whom the risk of breast cancer recurrence is a major concern.

ARTICLE HISTORY
Received 7 September 2017
Accepted 9 November 2017

Introduction

Breast cancer is the most common malignancy among women worldwide. In 2017, about 4900 women in Denmark will be diagnosed with breast cancer (http://www-dep.iarc.fr/NORDCAN/english/StatsFact.asp?cancer =200&country =208). The dissemination of increasingly effective adjuvant therapies has enlarged the pool of breast cancer survivors [1].

Denmark has a strong tradition and history of high quality registries with prospectively collected data. For 40 years, the Danish Breast Cancer Group (DBCG) clinical database has routinely registered data on breast cancer patients diagnosed in Denmark [2,3]. The DBCG data quality and validity are high [4]. The database records menopausal status, date and type of surgery, tumor characteristics, cancer treatment, and follow-up, including routine registration of breast cancer recurrence. For all patients who undergo breast cancer surgery, the DBCG registers data on follow-up examinations to detect recurrent disease. These examinations occur semiannually during the first 5 years after diagnosis and annually the next 5 years [5]. The DBCG records the civil personal registration (CPR) number, facilitating individual-level data linkage across Denmark's population-based registries (Figure 1), including the Danish National Prescription Registry [6].

Several frequently used prescription drugs alter breast cancer cell growth *in vitro*. The prospect of improving breast cancer prognosis through use of affordable drugs with relatively benign side effects has great appeal. Conversely, the potential for such medicines to worsen prognosis has critical implications for treatment of breast cancer and comorbidities, and for cancer-related healthcare costs.

Here, we present findings from selected pharmacoepidemiological studies that linked data from the DBCG to population-based prescription registries in Denmark. The studies aimed to investigate the impact of routinely prescribed drugs

CONTACT Deirdre Cronin-Fenton dc@clin.au.dk Department of Clinical Epidemiology, Aarhus University, Olof Palmes Alle 43-45, 8200 Aarhus N., Denmark

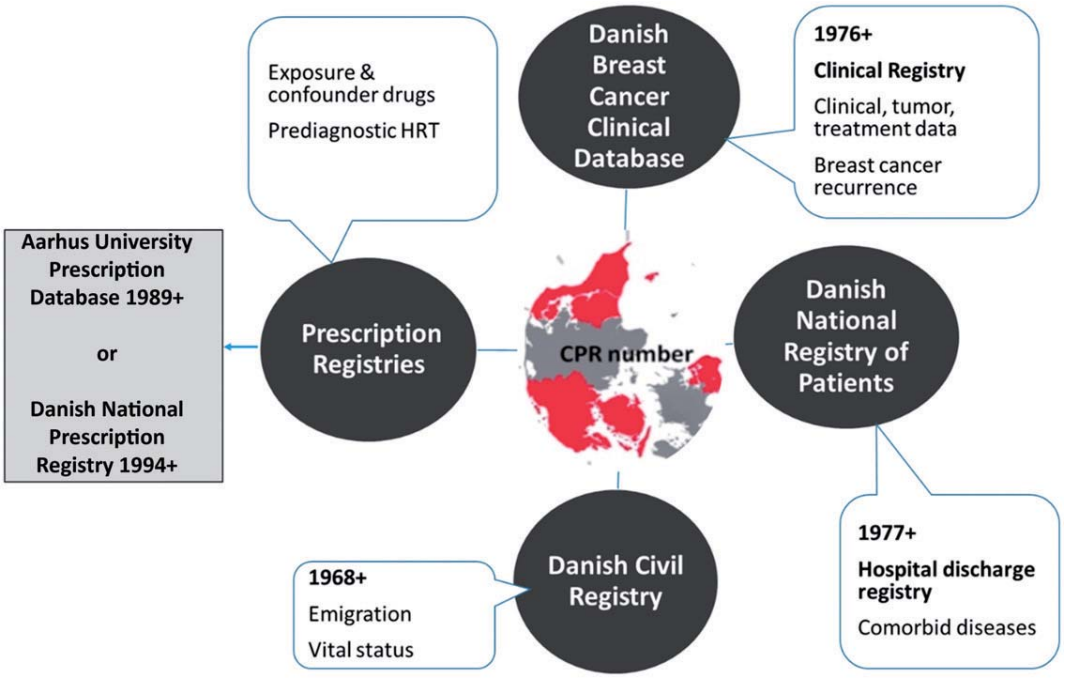


Figure 1. The Civil Personal Registration (CPR) number facilitates individual-level linkage of DBCG data to other population-based and medical registries in Denmark.

on the risk of breast cancer recurrence and mortality. We discuss findings for each drug in the context of the existing literature and highlight the clinical implications of observed associations.

Methods

Search strategy

We included observational studies that linked DBCG data to the following population-based prescription databases in Denmark: the Danish National Prescription Registry [7], the Aarhus University Prescription Database (AUPD) [8], and the Danish National Health Service Prescription Database [9]. Study data, design, and analytic strategy are described in the individual research papers. We provide brief synopses.

Study design and study population

Except for two studies, all used a cohort design, including patients diagnosed with breast cancer registered in DBCG. Three cohort studies included patients diagnosed between 1996 and 2003 with follow-up through 2008 [10–12]. Later the cohorts were expanded to include patients diagnosed between 1996 and 2008 with follow-up through July 2013 [13–15]. Patients diagnosed with metastatic breast cancer were excluded from all studies. Follow-up began on the date of breast cancer primary surgery, as recorded in DBCG, and ended on the date of breast cancer recurrence, death, 10 years, or end of follow-up.

Our studies of SSRIs, tamoxifen inhibition, and breast cancer recurrence used a case–control design, nested in the population of breast cancer patients diagnosed during 1985–2001, registered in the DBCG, who resided in the former Danish counties of North Jutland, Aarhus, Viborg, and Ringkøbing. We subsequently included women diagnosed during 1996-2001, registered in DBCG, who resided in Jutland. We included (1) ERα+/T+: ERα + patients treated with tamoxifen for >=1 year, and (2) ERα–/T–: ERα– patients not treated with tamoxifen, who survived >=1 year. '*Cases*' had recurrence within 10 years of diagnosis. "*Controls*" did not have a recurrence when their matched case recurred. We matched one control to each case based on ERα+/T + or ERα–/T–, menopausal status, breast cancer surgery date, county, and stage. We identified 541 ERα+/T + cases and 541 matched controls, and 300 ERα–/T– cases and 300 matched controls.

Prescription data

The Danish National Prescription Registry, maintained by Statistics Denmark, has registered all prescriptions dispensed at Danish pharmacies since 1995. Recorded data include the redemption date, prescribed drug [classified using Anatomical Therapeutic Chemical (ATC) codes], and fill quantity (see Appendix for list of ATC codes) [7]. For the cohort studies, prescription drug data were ascertained from the Danish National Prescription Registry. Via Statistics Denmark, the prescription data were linked from the Prescription Registry to the clinical cohort. For each drug, users were individuals who redeemed at least one prescription following breast cancer diagnosis. In all studies, medication use was modeled as a time-varying exposure, updated daily and lagged by 1 year, to avoid reverse causation and immortal person-time bias [16].

For one case–control study [17], we ascertained information on prescriptions from the AUPD [8]. The AUPD has

received and merged prescription data from the former Danish counties of North Jutland, Aarhus, Ringkjøbing, and Viborg (since 1989, 1996, 1998, and 1998, respectively). Since 2007, data in the AUPD are merged with data from the community pharmacies of the Central and North Denmark Region. The database records prescriptions dispensed at pharmacies for drugs that receive general or conditional reimbursement.

Outcomes

We obtained recurrence and vital status data from DBCG, which routinely follows patients for the development of recurrent disease up to ten years after primary diagnosis [4]. The DBCG defines breast cancer recurrence as any local, regional, or distant recurrent breast cancer, or contralateral breast cancer diagnosed during follow-up. Specific details on the site of recurrent breast cancer are also recorded.

Statistical analyses

Descriptive characteristics of the study populations were outlined. In the cohort studies, Cox regression models quantified the association of each prescription drug with rates of recurrence and all-cause mortality. In the case–control studies, conditional logistic regression estimated the association of SSRI use with recurrence.

Results

Statins

Statins (hydroxy-3-methylglutaryl coenzyme A – HMG-CoA reductase inhibitors) inhibit the rate-limiting step of cholesterol biosynthesis, and thereby lower serum cholesterol and prevent atherosclerotic disease. Although statins target lipid metabolism, they also exert pleiotropic effects including mevalonate inhibition, which impacts cell growth, signal transduction, differentiation, and apoptosis [18]. Statins thus modulate several physiological processes essential to cancer initiation and promotion. Although statins do not impact *breast cancer incidence*, observational data suggests the anti-cancer effects of statins in preclinical studies extend to modifying *cancer outcomes*.

We investigated the association of post-diagnostic statin use and breast cancer recurrence. Among over 18,000 Danish breast cancer patients registered in DBCG, we observed 20% lower rate of recurrence among users of lipophilic statins, primarily simvastatin [12]. In accordance with our *a priori* hypotheses, we observed no decrease in recurrence rates associated with hydrophilic statin use. The decreased rate of recurrence among users of lipophilic statins was observed for ipsilateral, contralateral, and regional lymph node recurrences, but not for bone metastases. The decreased rate of recurrence was robust to stratified analyses, although slightly stronger for ER + than ER– tumors, consistent with two previously published small studies [19,20]. Lower risks of recurrence or mortality in lipophilic statin users have been observed in a further four [21–24] out of five [25] observational studies.

The Breast International Group 1-98 (BIG 1-98) double-blind randomized clinical trial compared tamoxifen, letrozole, or a sequence of the two drugs in 8010 postmenopausal patients – 1396 patients were included via DBCG [26]. A *post-hoc* observational study within BIG 1-98 investigated the survival benefit of cholesterol-lowering drugs during endocrine therapy among 1700 breast cancer patients [27]. Findings suggested that patients with ER + breast tumors who used cholesterol-lowering medications had lower rates of recurrence compared with non-users.

Candidate biomarkers that may modify the effect of statins on tumor growth include HMG-CoA reductase expression [28], as well as several polymorphic genes encoding enzymes that metabolize statins [29]. Some believe that the cancer survival benefits of statins are attributable to selection and immortal person-time bias [30]. Using SEER-Medicare data, Emilsson et al. emulated a clinical trial investigating statin initiation up to 6 months after cancer diagnosis and cause-specific and all-cause mortality up to 3 years after colorectal, breast, prostate and bladder cancer diagnosis. Although the study had short follow-up, and no data on cancer recurrence, the paper highlights important limitations of the published studies of statins and cancer outcomes. Nonetheless, the analysis grouped all cancer sites together, thus allowing beneficial associations for one cancer site (breast cancer) [12,29] to be masked when averaged with null associations at other cancer sites (colorectal cancer) [31]. Despite the promising observational data, the hypothesis that statin therapy may reduce the risk of breast cancer recurrence has never been examined in a randomized clinical trial.

Aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), and selective COX-2 (sCOX-2) inhibitors

Aspirin, NSAIDs, and sCOX-2 inhibitors target the cyclooxygenase enzymes, COX-1 and COX-2, which promote angiogenesis and prevent apoptosis. COX-1 expression is ubiquitous; COX-2 is expressed during inflammation and in cancer [32]. Laboratory studies suggest that drugs targeting these enzymes inhibit breast cancer cell growth [33–35], but findings from observational studies are inconsistent [36–45]. For aspirin, three studies report lower mortality risks [36,38,46]; others show no association [37,42–44,47,48]. For NSAIDs, decreased mortality risks [38,39,49] and null associations have been reported [40,48,49]. Reasons underlying the inconsistent findings include variation in ascertainment of drug exposure (i.e., self-reported versus prescription-based) and confounder adjustment.

Low-dose aspirin reduces the risk of cardiovascular disease, so studies that assessed mortality [36–40,43], rather than the cancer-specific outcome recurrence [36,39], could not distinguish an effect of low-dose aspirin on cancer (via recurrence) from its direct effect on mortality. Importantly, several published aspirin studies did not adjust for statins [19,20,36,38–40,42,46], which are frequently prescribed with aspirin to prevent cardiovascular disease.

Our study of 34,188 breast cancer survivors showed no evidence of decreased recurrence rate associated with *post-diagnostic* aspirin, NSAIDs, or sCOX2 inhibitor use [13]. Our results were unchanged in stratified analyses and in analyses examining drug exposure and site-specific cancer recurrence.

Findings from preplanned analyses of pre-diagnostic aspirin use, and *post-hoc* analyses of pre-diagnostic use of NSAIDs and sCOX-2 inhibitors, suggested a slight decreased risk of recurrence. Our findings for pre-diagnostic aspirin use support a previous study by Barron and colleagues [41]. Pre-diagnostic use of these drugs may confer less aggressive tumor phenotypes [50], but these findings are unlikely to be of meaningful clinical relevance.

Despite inconsistent findings from observational studies, a randomized placebo-controlled clinical trial (NCT02804815) is underway to investigate the efficacy of adjuvant aspirin (100 mg or 300 mg versus placebo) in breast cancer patients, and in patients with colorectal, gastro-esophageal, and prostate cancers. The results of the trial will become available in 2026.

β-Blockers, ACE inhibitors, and ARBs

β-Blockers are indicated for hypertension and heart disease. They compete with epinephrine and norepinephrine to bind β-adrenergic receptors (β-AR) 1 and 2, thereby inhibiting the stress response. Physicians have increasingly prescribed selective β-blockers targeting β-AR1, such as atenolol, rather than non-selective β-blockers, such as propranolol, due to their cardioselective properties [51].

Breast tumors express β-ARs and preclinical studies suggest β-blockers prevent angiogenesis and metastasis [52]. Accordingly, drugs that inhibit β-ARs may favorably impact cancer survival. Epidemiologic studies note decreased risk of cause-specific mortality and breast cancer recurrence associated with β-blocker use [53,53–56]. Propranolol use has been correlated with an 80% decreased rate of breast cancer-specific mortality [55]. However, findings are inconsistent [22,52,57] and imprecise [58].

The indications for angiotensin-converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs) are like those for β-blockers, but also include prevention of renal insufficiency in type II diabetes and chronic kidney disease. These drugs inhibit the renin-angiotensin–aldosterone system (RAAS) [59]. Genetic polymorphisms that increase RAAS activity increase activation of several biomarkers and pathways essential to tumourigenesis. RAAS polymorphisms correlate with increased risk of breast cancer [60]. Thus ACEis and ARBs may prevent breast cancer progression. However, observational studies show decreased [61], null [62,63], and increased [22,64] risks of breast cancer progression or mortality associated with ACEis or ARBs.

In a cohort of 18,733 breast cancer patients registered in DBCG, we observed no evidence of a protective effect of β-blockers, ACEis, or ARBs on breast cancer recurrence [11]. The null associations remained robust regardless of selectivity or lipophilicity of β-blockers and the timing or intensity of drug use.

In a meta-analysis, Raimondi et al. [65] summarized the association of β-blockers, ACEis, and ARBs with progression in breast cancer patients. They concluded that β-blocker use correlated with longer disease-specific survival, while ACEis or ARBs had little impact on breast cancer survival. However, several of the included studies comprised small samples and some were prone to immortal person-time bias [20], which can inflate the magnitude of a protective association. Notably, the studies that reported null findings had the largest sample sizes and highest number of exposed patients who developed the outcome [11,22,56,66]. A recent study reported dramatically improved progression-free survival in patients with advanced HER-2 negative breast cancer who participated in the ROSE/TRIO-012 study [67]. The study has many limitations precluding any inference of a truly beneficial effect, most importantly the strong potential for the decreased risk of progression-free survival to have arisen from immortal person–time bias. These findings provide reassurance to cancer survivors and their physicians that use of β-blockers, ACEis, or ARBs is unlikely to exacerbate cancer progression.

Glucocorticoids

Synthetic glucocorticoids mediate immunosuppressive effects and are indicated for acute and chronic inflammatory diseases. In breast cancer cells, glucocorticoid treatment induces a less invasive phenotype in ER-negative cells compared with untreated ER-negative cells or ER-positive cells [68]. While glucocorticoid use does not correlate with breast cancer incidence [69], it may help tumor cells evade immune detection, thus aid cancer progression.

Using the DBCG database, we conducted the first and only large population-based study to investigate the association between prescriptions for glucocorticoids and risk of breast cancer recurrence [10]. We found no evidence of an association between prescriptions for systemic, inhaled, or intestinal-acting glucocorticoids and risk of recurrent breast cancer. These findings remained robust in stratified analyses – providing reassurance to patients and physicians about the safety of these drugs.

Opioids

Opioids are central to pain management, but they inhibit key elements of cell-mediated immunity – the primary innate defense against cancer [70]. Preclinical studies suggest that high-dose opioids inhibit angiogenesis, metastasis, and induce apoptosis. Morphine, a strong opioid, does not initiate tumourigenesis, but *in vitro* research suggests it promotes cancer progression. Tramadol has similar analgesic properties to morphine, and research in patients undergoing surgery for uterine carcinoma shows it can activate natural killer cells in the postoperative period [71]. Thus, opioids may modify cancer progression, but the direction of this association is not clear.

Opioid use is increasing [72], particularly in cancer survivors [73]. Studies have primarily investigated recurrence risk associated with perioperative opioid use. Most [74–76], but not all [77,78], have concluded that morphine-based systemic anesthesia correlates with increased recurrence risk compared with non-systemic anesthesia.

Our study linking DBCG data with population-based prescription data in Denmark is the first study to investigate the impact of opioid use on cancer recurrence [14]. Our study population included 34,188 patients with non-metastatic breast cancer followed for up to ten years after their primary diagnosis. We evaluated the impact of opioid strength, cumulative dose according to morphine equivalents, immunosuppressive effects, and chronicity of use, on recurrence risk. Except for the strongly immunosuppressive drugs, our findings show no evidence of an association between opioid use and breast cancer recurrence. Among patients who used strongly immunosuppressive drugs, we observed decreased recurrence, but increased mortality, likely attributable to channeling bias [79].

Thus, opioids do not appear to modify cancer progression. This is important to the increasing number of cancer survivors for whom management of post-cancer pain is a major concern.

Digoxin

Cardiac glycosides, including digoxin and digitoxin, inhibit the Na+/K+ATPase pump, and treat congestive heart failure and atrial fibrillation. Preclinical research highlights anti-cancer effects of cardiac glycosides, including pro-apoptotic effects and topoisomerase II inhibition, a target of several cancer therapies. Observational research suggests that prescription use of cardiac glycosides correlates with increased risk of breast cancer [80–82]. This result questions the safety of cardiac glycoside use by breast cancer survivors. Digoxin use correlates with better prognostic features in cancer [83]. However, prior to the publication of a study using DBCG data, only one study had reported on the impact of digoxin use on breast cancer prognosis. Among 175 patients followed for 22.3 years, Stenkvist observed a lower breast cancer-specific mortality rate (6%) associated with use of digitalis (digoxin) before breast cancer diagnosis compared with non-users (mortality rate =34%) [84]. However, in this small study, only 2 out of 32 patients died from breast cancer.

In 2013, Biggar et al. investigated the association between prescription use of digoxin and tumor characteristics, and breast cancer relapse among 34,085 breast cancer patients registered in DBCG [15]. Better prognostic features (higher frequency of ER+tumors, lower histologic grade, and less advanced stage at diagnosis) were observed among women who used digoxin, but overall digoxin use did not correlate with breast cancer relapse. These findings have since been replicated in a UK-based study by Karasneh et al. [85].

Selective serotonin reuptake inhibitors

Two-thirds of breast cancer patients have tumors that express estrogen receptor alpha (ERα), and are candidates for adjuvant endocrine therapy. Tamoxifen reduces the risk of breast cancer recurrence by about 50%. It is the only endocrine therapy recommended for ERα+premenopausal breast cancer patients, and an important alternative or sequential treatment to aromatase inhibitors for postmenopausal patients. Tamoxifen effectiveness is often tempered by the development of tamoxifen resistance, defined clinically as breast cancer recurrence. No biomarker of resistance beyond the absence of ERα has been identified [86]. Cytochrome P450 (CYP) enzymes catalyze tamoxifen metabolism to 4-hydroxy tamoxifen and 4-hydroxy-N-desmethyl tamoxifen (endoxifen) [87]. Tamoxifen metabolism is inhibited when women carry variant alleles leading to enzymatic impairment, or are concomitantly prescribed drugs that inhibit or compete for CYP2D6 [87].

SSRIs are used to treat depression and vasomotor symptoms due to menopause or side effects of tamoxifen. Women who use SSRIs and tamoxifen can have low serum endoxifen concentration, similar to women who carry no functional CYP2D6 allele [88,89]. Such women may have increased risk of breast cancer recurrence. Many studies have investigated the association between drug-induced inhibition of tamoxifen and breast cancer recurrence or mortality. Their findings are heterogeneous, with effect estimates ranging from a 0.3-fold decreased risk to a 3-fold increased risk of recurrence or death [88]. Reasons for the heterogeneity of the findings are not clear and are reviewed elsewhere [87,90–92]. Nonetheless, no single study characteristic can explain the inconsistency. A recent very large and methodologically strong pharmacoepidemiological study found a null-association between concomitant use of the SSRIs fluoxetine and paroxetine, both strong CYP2D6 inhibitors, and mortality among breast cancer patients receiving tamoxifen [93].

Consistent with this result, our nested case–control study of early-stage breast cancer patients registered in the DBCG showed no evidence that citalopram or other SSRIs diminish tamoxifen effectiveness in reducing breast cancer recurrence [17,94–96]. Furthermore, the use of SSRIs was not associated with recurrence risk in ER-negative breast cancer patients who received no tamoxifen, indicating no contraindication for use of these drugs after breast cancer diagnosis.

To be effective, tamoxifen and its metabolites must compete with estrogen for ER binding. Yet all existing clinical epidemiology studies of tamoxifen inhibition have included mostly post-menopausal women in whom estrogen concentrations are low. For this reason, in collaboration with DBCG, we have established a cohort of approximately 6000 premenopausal breast cancer patients, in whom estrogen concentrations are much. We will investigate tamoxifen metabolites, refining existing knowledge with comprehensive genotyping and incorporating comedications that inhibit the metabolism. Importantly, this design improves the current research paradigm, because non-null associations are most likely in premenopausal women (given higher endogenous estrogen) and because tamoxifen drug is the guideline anti-hormoanl therapy for premenopausal women.

Conclusions

Breast cancer accounts for a significant proportion of cancer deaths in women, and incurs extensive healthcare costs

worldwide. Identifying treatments with a beneficial role in breast cancer therapy and few side effects has huge public health implications. Findings from observational pharmacoepidemiology studies are unlikely to change clinical practice in the absence of a clinical trial. However, observational studies help identify patterns of association, pinpointing subcategories of patients likely to benefit from particular treatments, as well as those at risk of harmful treatment effects. Furthermore, adjuvant cancer-directed therapies may be difficult to implement in nations with a low Human Development Index, which incur a substantial proportion of the breast cancer burden [97].

Post-diagnosis statin use consistently correlates with lower recurrence and mortality risk in non-randomized studies. Statins are inexpensive, chemically stable without refrigeration, and have a well-understood safety profile. Their anticancer potential among breast cancer survivors in low-resource settings merits consideration, and may also improve cardiovascular health in breast cancer survivors in these nations.

Pharmacoepidemiology studies using DBCG data have noteworthy strengths. Individual-level linkage across Danish databases creates large cohorts nested in a nationwide source population. Tumor, treatment, and follow-up data in the DBCG registry are clinical trial quality [2,3]. Selection bias is negligible due to near-complete enrollment of breast cancer cases from the source population. Since Danish legislation does not require informed consent for registry-based studies, pharmacoepidemiology studies are not prone to bias due to self-selection. As well, linkage to the Danish National Prescription Registry provides information on prescriptions redeemed at pharmacies. In Denmark, patients pay a proportion of the cost of each prescription, so those who redeem a prescription are likely to consume the medication. The cohort studies coupling DBCG data to prescription data used lagged post-diagnostic drug exposures to avoid reverse causation, while capturing exposure during etiologically plausible time periods. This lag was generally 1 year – long enough to allow the drug to impact recurrence, but not so long as to reduce the likelihood of detecting a potential association. Sensitivity analyses altering the exposure lag yielded similar findings, justifying the lag duration.

A major advantage of the DBCG database for pharmacoepidemiology studies is the routine and valid recording of breast cancer recurrence during follow-up. Recurrence is a cancer-specific endpoint, so it highlights the direct effect of the drug on cancer, rather than on mortality. A study of statin use among Danish colorectal cancer patients showed a protective effect on cancer-specific and all-cause mortality, but not on recurrence – highlighting the importance of studying recurrence rather than mortality [31].

Several issues are relevant when interpreting the studies discussed above. All the studies ascertained comorbid diseases at the time of breast cancer diagnosis, but lacked information on the severity of these conditions, which may influence cancer-directed treatment. The comorbidity data relied on comorbidities sufficiently severe to warrant hospital admission or a visit to an outpatient clinic or emergency room. Thus, milder conditions, treated by primary care physicians, were unavailable. In several studies, the number of prescriptions was a proxy for cumulative dose, as the actual prescribed drug dose is not available in the Danish National Prescription Registry. Information on in-hospital drug use was also lacking. This may be particularly important for the studies on glucocorticoids, opioids, NSAIDs, and selective Cox-2 inhibitors, all of which are indicated for pain, and for glucocorticoids, used to treat emesis.

Thus, the pharmacoepidemiological studies coupling DBCG data with prescription registry data suggest that the use of aspirin, NSAIDs, sCOX-2 inhibitors, ACEis, beta-blockers, ARBs, glucocorticoids, digoxin, SSRIs, and opioids has little effect on breast cancer recurrence. Concerns about recurrence should not impact patient–physician decisions about the use of these drugs after breast cancer diagnosis. The lipophilic statin, simvastatin, may be beneficial in breast cancer survivors. Several subsequent observational studies have replicated the DBCG-based statin study findings. The large size of the DBCG-based study makes it unlikely that another observational study can substantially improve upon it. The convincing evidence from the accumulating observational data and *post-hoc* BIG 1-98 analyses provide strong justification for a trial. Such a trial also may provide impetus for research on statins and other cancers, several of which may also show a pleiotropic cancer-directed benefit.

Acknowledgments

The authors thank the Danish Breast Cancer Cooperative Group for preparation of the breast cancer datasets used in the referenced studies.

Disclosure statement

The authors declare no conflicts of interest. However, the Department of Clinical Epidemiology is involved in studies with funding from various companies as research grants to (and administered by) Aarhus University. None of these studies has any relation to the present study.

Funding

The study was supported by grants from the Danish Cancer Society (R73-A4284-13-S17) (HTS); the Aarhus University Research Foundation (HTS); Program for Clinical Research Infrastructure (PROCRIN) established by the Lundbeck Foundation and the Novo Nordisk Foundation (HTS); the Karen Elise Jensen Foundation (HTS); the Elvira & Rasmus Riisforts Fonden (DCF); the Lundbeck Foundation (R167-2013-15861) (DCF); Susan G. Komen for the Cure (CCR 13264024) (TPA); the Mary Kay Foundation (003-14) (TPA); the Clinical Institute of Aarhus University Hospital (TLL); and the US National Cancer Institute at the National Institutes of Health (R01CA118708 and R01CA166825) (TLL). The funding agencies had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the article; or the decision to submit the article for publication.

References

[1] Park JH, Anderson WF, Gail MH. Improvements in US Breast Cancer Survival and Proportion Explained by Tumor Size and Estrogen-Receptor Status. Jco. 2015;33:2870–2876.

[2] Jensen AR, Storm HH, Moller S, et al. Validity and representativity in the Danish Breast Cancer Cooperative Group – a study on

protocol allocation and data validity from one county to a multicentre database. Acta Oncol. 2003;42:179–185.
[3] Christiansen P, Ejlertsen B, Jensen MB, et al. Danish Breast Cancer Cooperative Group. Clin Epidemiol. 2016;8:445–449.
[4] Moller S, Jensen MB, Ejlertsen B, et al. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.
[5] Christiansen P, Al-Suliman N, Bjerre K, et al. Recurrence pattern and prognosis in low-risk breast cancer patients – data from the DBCG 89-A programme. Acta Oncol. 2008;47:691–703.
[6] Schmidt M, Pedersen L, Sorensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol. 2014;29:541–549.
[7] Pottegard A, Schmidt SAJ, Wallach-Kildemoes H, et al. Data Resource Profile: The Danish National Prescription Registry. Int J Epidemiol. 2017;46:798–798f.
[8] Ehrenstein V, Antonsen S, Pedersen L. Existing data sources for clinical epidemiology: Aarhus University Prescription Database. Clin Epidemiol. 2010;2:273–279.
[9] Johannesdottir SA, Horvath-Puho E, Ehrenstein V, et al. Existing data sources for clinical epidemiology: The Danish National Database of Reimbursed Prescriptions. Clin Epidemiol. 2012;4:303–313.
[10] Winther Lietzen L, Ahern T, Christiansen P, et al. Glucocorticoid prescriptions and breast cancer recurrence: a Danish nationwide prospective cohort study. Ann Oncol. 2014;25:2419–2425.
[11] Sørensen G, Ganz P, Cole S, et al. Use of beta blockers, ACE inhibitors, angiotensin II receptor blockers and risk of breast cancer recurrence: a Danish nationwide prospective cohort study. Jco. 2013;31:2265–2272.
[12] Ahern TP, Pedersen L, Tarp M, et al. Statin prescriptions and breast cancer recurrence risk: a Danish nationwide prospective cohort study. J Natl Cancer Inst. 2011;103:1461–1468.
[13] Cronin-Fenton DP, Heide-Jorgensen U, Ahern TP, et al. Low-dose aspirin, nonsteroidal anti-inflammatory drugs, selective COX-2 inhibitors and breast cancer recurrence. Epidemiology. 2016;27:586–593.
[14] Cronin-Fenton DP, Heide-Jorgensen U, Ahern TP, et al. Opioids and breast cancer recurrence: a Danish population-based cohort study. Cancer. 2015;121:3507–3514.
[15] Biggar RJ, Andersen EW, Kroman N, et al. Breast cancer in women using digoxin: tumor characteristics and relapse risk. Breast Cancer Res. 2013;15:R13.
[16] Suissa S. Immortal time bias in pharmaco-epidemiology. Am J Epidemiol. 2008;167:492–499.
[17] Lash TL, Pedersen L, Cronin-Fenton D, et al. Tamoxifen's protection against breast cancer recurrence is not reduced by concurrent use of the SSRI citalopram. Br J Cancer. 2008;99:616–621.
[18] Demierre MF, Higgins PD, Gruber SB, et al. Statins and cancer prevention. Nat Rev Cancer. 2005;5:930–942.
[19] Kwan ML, Habel LA, Flick ED, et al. Post-diagnosis statin use and breast cancer recurrence in a prospective cohort study of early stage breast cancer survivors. Breast Cancer Res Treat. 2008;109:573–579.
[20] Chae YK, Valsecchi ME, Kim J, et al. Reduced risk of breast cancer recurrence in patients using ACE inhibitors, ARBs, and/or statins. Cancer Invest. 2011;29:585–593.
[21] Cardwell CR, Hicks BM, Hughes C, et al. Statin use after diagnosis of breast cancer and survival: a population-based cohort study. Epidemiology. 2015;26:68–78.
[22] Boudreau DM, Yu O, Chubak J, et al. Comparative safety of cardiovascular medication use and breast cancer outcomes among women with early stage breast cancer. Breast Cancer Res Treat. 2014;144:405–416.
[23] Murtola TJ. Impact of statins on genitourinary cancer outcomes: the promise and the challenge. J Urol. 2014;191:889–890.
[24] Nickels S, Vrieling A, Seibold P, et al. Mortality and recurrence risk in relation to the use of lipid-lowering drugs in a prospective breast cancer patient cohort. PLoS One. 2013;8:e75088.
[25] Mc Menamin UC, Murray LJ, Hughes CM, et al. Statin use and breast cancer survival: a nationwide cohort study in Scotland. BMC Cancer. 2016;16:600.
[26] Regan MM, Neven P, Giobbie-Hurder A, et al. Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8.1 years median follow-up. Lancet Oncol. 2011;12:1101–1108.
[27] Borgquist S, Giobbie-Hurder A, Ahern TP, et al. Cholesterol, cholesterol-lowering medication use, and breast cancer outcome in the BIG 1-98 study. Jco. 2017;35:1179–1188.
[28] Borgquist S, Jogi A, Ponten F, et al. Prognostic impact of tumour-specific HMG-CoA reductase expression in primary breast cancer. Breast Cancer Res. 2008;10:R79.
[29] Ahern TP, Lash TL, Damkier P, et al. Statins and breast cancer prognosis: evidence and opportunities. Lancet Oncol. 2014;15:e461–e468.
[30] Emilsson L, Garcia-Albeniz X, Logan RW, et al. Examining bias in studies of statin treatment and survival in patients with cancer. JAMA Oncol. 2017 [Aug 20]. DOI:10.1001/jamaoncol.2017.2752
[31] Lash TL, Riis AH, Ostenfeld EB, et al. Associations of statin use with colorectal cancer recurrence and mortality in a Danish cohort. Am J Epidemiol. 2017;186:679–687.
[32] Elwood PC, Gallagher AM, Duthie GG, et al. Aspirin, salicylates, and cancer. Lancet. 2009;373:1301–1309.
[33] Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell. 1995;83:493–501.
[34] Allen JE, Patel AS, Prabhu VV, et al. COX-2 drives metastatic breast cells from brain lesions into the cerebrospinal fluid and systemic circulation. Cancer Res. 2014;74:2385–2390.
[35] Choi BH, Chakraborty G, Baek K, et al. Aspirin-induced Bcl-2 translocation and its phosphorylation in the nucleus trigger apoptosis in breast cancer cells. Exp Mol Med. 2013;45:e47.
[36] Holmes MD, Chen WY, Li L, et al. Aspirin intake and survival after breast cancer. J Clin Oncol. 2010;28:1467–1472.
[37] Murray LJ, Cooper JA, Hughes CM, et al. Post-diagnostic prescriptions for low-dose aspirin and breast cancer-specific survival: a nested case–control study in a breast cancer cohort from the UK Clinical Practice. Breast Cancer Res. 2014;16:R34.
[38] Blair CK, Sweeney C, Anderson KE, et al. NSAID use and survival after breast cancer diagnosis in post-menopausal women. Breast Cancer Res Treat. 2007;101:191–197.
[39] Kwan ML, Habel LA, Slattery ML, et al. NSAIDs and breast cancer recurrence in a prospective cohort study. Cancer Causes Control. 2007;18:613–620.
[40] Li Y, Brasky TM, Nie J, et al. Use of nonsteroidal anti-inflammatory drugs and survival following breast cancer diagnosis. Cancer Epidemiol Biomarkers Prev. 2012;21:239–242.
[41] Barron TI, Flahavan EM, Sharp L, et al. Recent prediagnostic aspirin use, lymph node involvement, and 5-year mortality in women with stage I-III breast cancer: a nationwide population-based cohort study. Cancer Res. 2014;74:4065–4077.
[42] Holmes MD, Olsson H, Pawitan Y, et al. Aspirin intake and breast cancer survival – a nation-wide study using prospectively recorded data in Sweden. BMC Cancer. 2014;14:391–2407.
[43] Barron TI, Murphy LM, Brown C, et al. De Novo post-diagnosis aspirin use and mortality in women with stage I–III breast cancer. Cancer Epidemiol Biomarkers Prev. 2015;24:898–904.
[44] Rothwell PM, Wilson M, Price JF, et al. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet. 2012;379:1591–1601.
[45] Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol. 2012;13:518–527.
[46] Fraser DM, Sullivan FM, Thompson AM, et al. Aspirin use and survival after the diagnosis of breast cancer: a population-based cohort study. Br J Cancer. 2014;111:623–627.

[47] Mc Menamin UC, Cardwell CR, Hughes CM, et al. Low-dose aspirin use and survival in breast cancer patients: a nationwide cohort study. Cancer Epidemiol. 2017;47:20–27.

[48] Wernli KJ, Hampton JM, Trentham-Dietz A, et al. Use of antidepressants and NSAIDs in relation to mortality in long-term breast cancer survivors. Pharmacoepidem Drug Safe. 2011;20:131–137.

[49] Bardia A, Ebbert JO, Vierkant RA, et al. Association of aspirin and nonaspirin nonsteroidal anti-inflammatory drugs with cancer incidence and mortality. J Natl Cancer Inst. 2007;99:881–889.

[50] Nishihara R, Lochhead P, Kuchiba A, et al. Aspirin use and risk of colorectal cancer according to BRAF mutation status. JAMA. 2013;309:2563–2571.

[51] Smith C, Teitler M. Beta-blocker selectivity at cloned human beta 1- and beta 2-adrenergic receptors. Cardiovasc Drugs Ther. 1999;13:123–126.

[52] Powe DG, Voss MJ, Habashy HO, et al. Alpha- and beta-adrenergic receptor (AR) protein expression is associated with poor clinical outcome in breast cancer: an immunohistochemical study. Breast Cancer Res Treat. 2011;130:457–463.

[53] Powe DG, Voss MJ, Zanker KS, et al. Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. Oncotarget. 2010;1:628–638.

[54] Melhem-Bertrandt A, Chavez-Macgregor M, Lei X, et al. Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. Jco. 2011; 29:2645–2652.

[55] Barron TI, Connolly RM, Sharp L, et al. Beta blockers and breast cancer mortality: a population-based study. J Clin Oncol. 2011; 29:2635–2644.

[56] Ganz PA, Habel LA, Weltzien EK, et al. Examining the influence of beta blockers and ACE inhibitors on the risk for breast cancer recurrence: results from the LACE cohort. Breast Cancer Res Treat. 2011;129:549–556.

[57] Cardwell CR, Coleman HG, Murray LJ, et al. Beta-blocker usage and breast cancer survival: a nested case–control study within a UK Clinical Practice Research Datalink cohort. Int J Epidemiol. 2013;42:1852–1861.

[58] Cardwell CR, Pottegard A, Vaes E, et al. Propranolol and survival from breast cancer: a pooled analysis of European breast cancer cohorts. Breast Cancer Res. 2016;18:119.

[59] Barron TI, Sharp L, Visvanathan K. Beta-adrenergic blocking drugs in breast cancer: a perspective review. Ther Adv Med Oncol. 2012;4:113–125.

[60] Koh WP, Yuan JM, Van Den BD, et al. Polymorphisms in angiotensin II type 1 receptor and angiotensin I-converting enzyme genes and breast cancer risk among Chinese women in Singapore. Carcinogenesis. 2005;26:459–464.

[61] Babacan T, Balakan O, Kuzan TY, et al. The effect of renin-angiotensin-system inhibition on survival and recurrence of N3+ breast cancer patients. J Buon. 2015;20:50–56.

[62] Chae YK, Brown EN, Lei X, et al. Use of ACE inhibitors and angiotensin receptor blockers and primary breast cancer outcomes. J Cancer. 2013;4:549–556.

[63] Holmes MD, Hankinson SE, Feskanich D, et al. Beta blockers and angiotensin-converting enzyme inhibitors' purported benefit on breast cancer survival may be explained by aspirin use. Breast Cancer Res Treat. 2013;139:507–513.

[64] Sendur MA, Aksoy S, Yaman S, et al. Efficacy of angiotensin-receptor blockers on demographic and clinico-pathological characteristics of breast cancer. Breast. 2012;21:419–420.

[65] Raimondi S, Botteri E, Munzone E, et al. Use of beta-blockers, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers and breast cancer survival: systematic review and meta-analysis. Int J Cancer. 2016;139:212–219.

[66] Cardwell CR, Mc Menamin UC, Hicks BM, et al. Drugs affecting the renin–angiotensin system and survival from cancer: a population based study of breast, colorectal and prostate cancer patient cohorts. BMC Med. 2014;12:28–7015-12-28.

[67] Spera G, Fresco R, Fung H, et al. Beta blockers and improved progression-free survival in patients with advanced HER2 negative breast cancer: a retrospective analysis of the ROSE/TRIO-012 study. Ann Oncol. 2017;28:1836–1841.

[68] Ferrand N, Stragier E, Redeuilh G, et al. Glucocorticoids induce CCN5/WISP-2 expression and attenuate invasion in oestrogen receptor-negative human breast cancer cells. Biochem J. 2012; 447:71–79.

[69] Sorensen GV, Cronin-Fenton DP, Sorensen HT, et al. Use of glucocorticoids and risk of breast cancer: a Danish population-based case–control study. Breast Cancer Res. 2012;14:R21.

[70] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–674.

[71] Sacerdote P, Bianchi M, Gaspani L, et al. The effects of tramadol and morphine on immune responses and pain after surgery in cancer patients. Anesth Analg. 2000;90:1411–1414.

[72] Humphreys K. Avoiding globalisation of the prescription opioid epidemic. Lancet. 2017;390:437–439.

[73] Sutradhar R, Lokku A, Barbera L. Cancer survivorship and opioid prescribing rates: a population-based matched cohort study among individuals with and without a history of cancer. Cancer. 2017;123:4286–4293.

[74] Exadaktylos AK, Buggy DJ, Moriarty DC, et al. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? Anesthesiology. 2006;105:660–664.

[75] Biki B, Mascha E, Moriarty DC, et al. Anesthetic technique for radical prostatectomy surgery affects cancer recurrence: a retrospective analysis. Anesthesiology. 2008;109:180–187.

[76] Smith TJ, Staats PS, Deer T, et al. Randomized clinical trial of an implantable drug delivery system compared with comprehensive medical management for refractory cancer pain: impact on pain, drug-related toxicity, and survival. Jco. 2002;20:4040–4049.

[77] Myles PS, Peyton P, Silbert B, et al. Perioperative epidural analgesia for major abdominal surgery for cancer and recurrence-free survival: randomised trial. BMJ. 2011;342:d1491.

[78] Tsui BC, Rashiq S, Schopflocher D, et al. Epidural anesthesia and cancer recurrence rates after radical prostatectomy. Can J Anesth/ J Can Anesth. 2010;57:107–112.

[79] Petri H, Urquhart J. Channeling bias in the interpretation of drug effects. Stat Med. 1991;10:577–581.

[80] Ahern TP, Lash TL, Sorensen HT, et al. Digoxin treatment is associated with an increased incidence of breast cancer: a population-based case–control study. Breast Cancer Res. 2008;10:R102.

[81] Ahern TP, Tamimi RM, Rosner BA, et al. Digoxin use and risk of invasive breast cancer: evidence from the Nurses' Health Study and meta-analysis. Breast Cancer Res Treat. 2014;144:427–435.

[82] Biggar RJ, Wohlfahrt J, Oudin A, et al. Digoxin use and the risk of breast cancer in women. J Clin Oncol. 2011;29:2165–2170.

[83] Flahavan EM, Sharp L, Bennett K, et al. A cohort study of digoxin exposure and mortality in men with prostate cancer. BJU Int. 2014;113:236–245.

[84] Stenkvist B. Is digitalis a therapy for breast carcinoma? Oncol Rep. 1999;6:493–496.

[85] Karasneh RA, Murray LJ, Mc Menamin UC, et al. Digoxin use after diagnosis of breast cancer and survival: a population-based cohort study. Breast Cancer Res Treat. 2015;151:661–669.

[86] Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. Jco. 2010;28:3784–3796.

[87] Cronin-Fenton DP, Damkier P, Lash TL. Metabolism and transport of tamoxifen in relation to its effectiveness: new perspectives on an ongoing controversy. Future Oncol. 2013;10:107–122.

[88] Lim YC, Li L, Desta Z, et al. Endoxifen, a secondary metabolite of tamoxifen, and 4-OH-tamoxifen induce similar changes in global gene expression patterns in MCF-7 breast cancer cells. J Pharmacol Exp Ther. 2006;318:503–512.

[89] Stearns V, Johnson MD, Rae JM, et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst. 2003;95:1758–1764.

[90] Ahern TP, Hertz DL, Damkier P, et al. Cytochrome P-450 2D6 (CYP2D6) genotype and breast cancer recurrence in tamoxifen-treated patients: evaluating the importance of loss of heterozygosity. Am J Epidemiol. 2017;185:75–85.

[91] Cronin-Fenton DP, Lash TL. Clinical epidemiology and pharmacology of CYP2D6 inhibition related to breast cancer outcomes. Expert Rev Clin Pharmacol. 2011;4:363–377.

[92] Lash TL, Lien EA, Sorensen HT, et al. Genotype-guided tamoxifen therapy: time to pause for reflection? Lancet Oncol. 2009;10:825–833.

[93] Donneyong MM, Bykov K, Bosco-Levy P, et al. Risk of mortality with concomitant use of tamoxifen and selective serotonin reuptake inhibitors: multi-database cohort study. BMJ. 2016;354:i5014.

[94] Lash TL, Cronin-Fenton D, Ahern TP, et al. CYP2D6 inhibition and breast cancer recurrence in a population-based study in Denmark. J Natl Cancer Inst. 2011;103:489–500.

[95] Ahern TP, Pedersen L, Cronin-Fenton DP, et al. No increase in breast cancer recurrence with concurrent use of tamoxifen and some CYP2D6-inhibiting medications. Cancer. Epidemiol Biomarkers Prev. 2009;18:2562–2564.

[96] Lash TL, Cronin-Fenton D, Ahern TP, et al. Breast cancer recurrence risk related to concurrent use of SSRI antidepressants and tamoxifen. Acta Oncol. 2010;49:305–312.

[97] Fidler MM, Bray F, Soerjomataram I. The global cancer burden and human development: a review. Scand J Public Health. 2017 [Jun 1]. DOI:10.1177/1403494817715400

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 129–134
https://doi.org/10.1080/0284186X.2017.1407496

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Influence of specific comorbidities on survival after early-stage breast cancer

Marianne Ewertz[a], Lotte Holm Land[a], Susanne Oksbjerg Dalton[b], Deirdre Cronin-Fenton[c] and Maj-Britt Jensen[d]

[a]Department of Oncology, Odense University Hospital, Institute of Clinical Research, University of Southern Denmark, Denmark; [b]Survivorship Unit, Danish Cancer Society Research Center, Copenhagen, Denmark; [c]Department of Clinical Epidemiology, Aarhus University, Aarhus, Denmark; [d]Danish Breast Cancer Cooperative Group Datacenter, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: While comorbidity indices are useful for describing trends in survival, information on specific comorbidities is needed for the clinician advising the individual breast cancer patient on her treatment. Here we present an analysis of overall survival, breast cancer-specific mortality, and effect of medical adjuvant treatment among breast cancer patients suffering from 12 major comorbidities compared with breast cancer patients without comorbidities.

Material and methods: The study population was identified from the Danish Breast Cancer Cooperative Group and included 59,673 women without prior cancer diagnosed with early-stage breast cancer in Denmark from 1990 to 2008 with an estimated median potential follow-up of 14 years and 10 months. Information on comorbidity and causes of death was derived from population-based registries. Multivariable proportional hazards regression models were used to assess the effect of comorbidities on mortality, all-cause and breast cancer specific, using patients without comorbidity as reference.

Results: At breast cancer diagnosis, 16% of patients had comorbidities and 84% did not. Compared with the latter, the risk of dying from all causes was significantly increased for all types of comorbidity, but the risk of dying from breast cancer was significantly increased only for peripheral vascular disease, dementia, chronic pulmonary disease, liver, and renal diseases. Comorbidities diagnosed within 5 years of breast cancer diagnosis correlated with a greater risk of dying than comorbidities diagnosed more than 5 years before breast cancer diagnosis. With a few exceptions, the effect of adjuvant treatment on breast cancer mortality was similar among patients with and without comorbidity.

Conclusion: Breast cancer mortality was not significantly elevated for patients with prior myocardial infarction, congestive heart failure, cerebrovascular disease, connective tissue disease, ulcer disease, and diabetes. The similar effect of adjuvant treatment in patients with and without comorbidity underlines the importance of adhering to guideline therapy.

ARTICLE HISTORY
Received 13 November 2017
Accepted 16 November 2017

Introduction

Preexisting diseases at breast cancer diagnosis, comorbidity, may influence the prognosis after breast cancer in several ways. If the comorbidity involves organ failure, like compromised respiratory, cardiac, or renal function, curative treatment may not be possible leading to an increased risk of dying from breast cancer due to insufficient treatment. On the other hand, the risk of dying from the comorbidity may be so high that the patients may not live for sufficiently long time to benefit from breast cancer treatment even if they receive guideline therapy [1].

Most published reports on comorbidity have combined the diseases into one summary measure like the Charlson Comorbidity Index [2]. Several studies demonstrate that a high comorbidity score is associated with a reduced likelihood of receiving guideline therapy and an increased risk of dying from breast cancer as well as from all causes [1,3–5]. Yet, few studies have addressed associations between specific comorbid conditions and breast cancer prognosis. Such information may be helpful for the clinician advising the individual patient on her treatment. In a study of 64,034 patients with breast cancer diagnosed at a median age of 75 years identified from the Surveillance, Epidemiology, and End Results – Medicare database, Patnaik et al. [6] found that each of the 13 comorbid conditions examined was associated with decreased overall survival and increased mortality. Apart from prior cancer, diabetes was the condition with the highest prevalence, present in 13% of the patients. Compared with nondiabetic women, patients with breast cancer and preexisting diabetes are reported to have a greater risk of death, to present at later stages, and receive altered treatment regimens [7,8].

Based on data from the Danish Breast Cancer Cooperative Group (DBCG), we have previously reported that comorbidity as measured by Charlson's Comorbidity Index at breast cancer diagnosis was an independent adverse prognostic factor for death after breast cancer [4]. The aim of this analysis was to examine the relationship between 12 major separate comorbidities derived from Charlson's Comorbidity Index and overall survival, breast cancer specific mortality, and effect of

CONTACT Marianne Ewertz Marianne.ewertz@rsyd.dk Department of Oncology, Odense University Hospital, Sdr. Boulevard 29, DK-5000 Odense, Denmark
Supplemental data for this article can be accessed here.

medical adjuvant treatment compared with breast cancer patients without comorbidities.

Material and methods

We performed a population-based cohort study by linking the following Danish registers using the unique personal identification number: the Danish Breast Cancer Cooperative Group (DBCG), the Danish National Patient Register (NPR), the Central Population Register (CPR), and the Danish Register of Causes of Death (RCD).

From the database of the DBCG we identified 62,591 women diagnosed with early-stage breast cancer in Denmark from 1990 to 2008. The DBCG is a nationwide multidisciplinary group, which since 1977 has registered women diagnosed with primary invasive non-metastatic breast cancer with a completeness gradually improving to more than 95% [9]. The DBCG provided information on tumor characteristics and treatment.

Information on comorbidity was obtained from the NPR, which has registered in-patient diagnoses since 1977 and outpatient diagnoses since 1995 [10]. The International Classification of Disease (ICD)-codes were grouped into 12 categories, modified from the Charlson Comorbidity Index: myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, liver disease (all grades), diabetes (all types), hemiplegia, and moderate to severe renal disease. We excluded 2910 patients with a prior cancer, leukemia or lymphoma, and eight patients with AIDS, leaving 59,673 patients available for analysis. Hospital contacts in a period from 10 years before and up to breast cancer diagnosis were included. ICD codes for identification of the comorbid diseases are listed in Supplementary Table I. Each patient can be registered with comorbidity in more than one category.

We grouped the diagnoses according to the time period in which the comorbidities were registered: within 5 years before the breast cancer diagnosis and from 10 to 5 years prior to the breast cancer diagnosis. Comorbidities can occur in both intervals for the same patient. The patients were followed from diagnosis to death, emigration or the end of December 2015 by linkage to the CPR. Information on cause of death was derived from RCD [11].

Statistical analysis

Endpoints were time to death, irrespective of cause (all-cause mortality and overall survival (OS)), and time to death from breast cancer (BC mortality), considering death from other causes as competing events. OS was calculated from time of breast cancer diagnosis using Kaplan–Meier estimates. BC mortality was calculated as cumulative incidence estimates. Follow-up time was quantified in terms of a Kaplan–Meier estimate of potential follow-up [12].

Univariate and multivariate regression analyses were performed, using Cox proportional hazards model for all-cause mortality and Fine-Gray proportional hazards subdistribution model for BC mortality. Comorbidities were included with one factor for each category, using patients with no comorbidity as reference. Factors included in the multivariable analyses were age (5-year categories), year of surgery, tumor size, lymph node status, lymphovascular invasion, invasion resection margin, histological type and grade, estrogen receptor (ER) status, menopausal status, and treatment (loco-regional and systemic). The assumption of proportional hazards was assessed by Schoenfeld residuals and by including time-dependent covariates in the model. Histology, ER status, and menopausal status did not fulfill the assumption and were included as stratification factors. Multivariate models were applied to investigate interaction between diabetes and vascular comorbidities in separate models, and to explore subgroups of comorbidity according to time and adjuvant treatment, respectively.

All p values are two-sided. Statistical analyses were done using SAS v9.4 (SAS Institute, Inc., Cary, NC, USA), and STATA IC 14 (StataCorp, College Station, TX, USA).

Table 1 Characteristics of 59,673 women diagnosed with early-stage breast cancer between 1990 and 2008 in Denmark.

	Characteristics of the study population		
		Number of deaths	
	Number of patients (%)	Breast cancer	Other causes
	59,673 (100)	20,381	10,648
Age at diagnosis			
<40	2808 (5)	943	73
40–49	9875 (17)	2695	494
50–59	15,213 (25)	4396	1277
60–69	15,198 (25)	4855	2790
70–79	10,655 (18)	4456	3594
80+	5924 (10)	3036	2420
Tumor size			
−10 mm	8257 (14)	1235	1454
11–20 mm	22,542 (38)	5638	4256
21–50 mm	22,267 (37)	9399	3874
51+ mm	2774 (5)	1797	346
Unknown	3833 (6)	2312	718
Histology and grade			
Ductal I	14,192 (24)	3391	2948
Ductal II	19,649 (33)	6826	3320
Ductal III	10,587 (18)	4421	1276
Ductal unknown grade	1430 (2)	492	281
Lobular	6587 (11)	2320	1279
Other	4293 (7)	1011	953
Unknown	2935 (5)	1920	591
ER-status			
Positive ≥10%	41,891 (70)	12581	7782
Poor <10%	11,734 (20)	4697	1547
Unknown	6048 (10)	3103	1319
Nodal status			
Positive	25,943 (43)	11382	3355
Negative	31,079 (52)	7190	6798
Unknown	2651 (4)	1809	495
Surgery			
Lumpectomy	19,047 (32)	3433	2389
Mastectomy	37,911 (64)	15042	7735
Biopsy only	2715 (6)	1906	524
Adjuvant therapy			
None	15,915 (27)	3678	3094
Chemotherapy	7871 (13)	2723	465
Chemo- and endocrine treatment	4692 (8)	644	128
Endocrine alone	13,943 (23)	4302	2033
Unknown	17,302 (29)	9034	4928

Table 2. Comorbidity among 59,673 Danish women 0–5 and 5–10 years before diagnosis of breast cancer (BC) in relation to 5- and 10-year overall survival (OS) and breast cancer specific mortality (BCM).

	No. of patients	Disease before BC		No of deaths		5-year		10-year	
		≤5 years	>5 years[a]	BC	Other causes	OS % (95% CI)	BCM % (95% CI)	OS % (95% CI)	BCM % (95% CI)
Total	59673								
None	49928			16616	7660	78.9 (78.5;79.2)	17.9 (17.6;18.2)	63.9 (63.5;64.3)	28.0 (27.6;28.4)
Myocardial infarction	831	562	322	351	305	56.7 (53.2;60.0)	28.6 (25.6;31.7)	34.3 (31.0;37.6)	39.2 (35.8;42.5)
Congestive heart failure	1168	958	302	502	511	42.4 (39.6;45.2)	33.5 (30.8;36.2)	18.3 (16.1;20.7)	42.5 (39.6;45.3)
Peripheral vascular disease	1076	837	365	430	397	54.6 (51.6;57.5)	28.9 (26.2;31.7)	30.1 (27.3;33.0)	38.2 (35.3;41.2)
Cerebrovascular disease	2154	1612	759	889	726	53.3 (51.2;55.4)	29.9 (27.9;31.8)	32.6 (30.5;34.6)	39.3 (37.2;41.4)
Dementia	369	325	64	211	132	28.1 (23.6;32.8)	48.0 (42.8;53.0)	09.3 (06.5;12.8)	56.8 (51.5;61.8)
Chronic pulmonary disease	2497	2018	888	901	764	62.0 (60.1;63.9)	24.4 (22.8;26.2)	40.4 (38.4;42.4)	34.2 (32.3;36.1)
Connective tissue disease	1236	928	563	428	329	66.7 (64.0;69.2)	22.5 (20.2;24.9)	46.1 (43.1;48.9)	33.0 (30.3;35.7)
Ulcer disease	1169	787	435	458	371	60.8 (58.0;63.6)	26.7 (24.2;29.3)	39.3 (36.4;42.2)	36.2 (33.4;39.0)
Liver disease	340	253	131	129	115	54.4 (49.0;59.5)	27.9 (23.3;32.8)	33.7 (28.5;39.0)	36.3 (31.1;41.5)
Diabetes	1843	1625	744	750	599	57.2 (54.9;59.4)	28.2 (26.2;30.3)	35.6 (33.3;37.8)	38.1 (35.8;40.3)
Hemiplegia	71	55	20	31	21	50.7 (38.6;61.6)	33.8 (23.0;44.9)	32.3 (21.5;43.7)	42.1 (30.0;53.7)
Renal disease	328	255	112	122	109	50.0 (44.5;55.3)	30.8 (25.9;35.8)	33.6 (28.4;38.8)	35.5 (30.4;40.7)

BC: breast cancer; OS: overall survival; BCM: breast cancer mortality; CI: confidence interval.
[a]≤10 years.

Table 3. Multivariable analysis of the hazard ratio (HR) of death from all causes and from breast cancer (BC) for 12 comorbidities with breast cancer patients without comorbidities as reference category.

	All cause mortality HR (95% CI)			BC mortality HR (95% CI)		
	Unadjusted	Age adjusted[a]	Fully adjusted[b]	Unadjusted	Age adjusted[a]	Fully adjusted[b]
Myocardial infarction	1.52 (1.40;1.65)	1.20 (1.11;1.30)	1.23 (1.14;1.33)	1.20 (1.07;1.35)	1.08 (0.96;1.21)	1.10 (0.98;1.24)
Congestive heart failure	2.22 (2.07;2.37)	1.42 (1.33;1.52)	1.42 (1.33;1.52)	1.28 (1.15;1.41)	0.99 (0.90;1.10)	0.99 (0.89;1.10)
Peripheral vascular disease	1.69 (1.57;1.81)	1.43 (1.33;1.53)	1.52 (1.42;1.64)	1.19 (1.07;1.32)	1.06 (0.96;1.18)	1.13 (1.01;1.27)
Cerebrovascular disease	1.81 (1.72;1.91)	1.30 (1.23;1.37)	1.30 (1.23;1.37)	1.26 (1.17;1.35)	1.05 (0.97;1.13)	1.05 (0.97;1.13)
Dementia	3.47 (3.12;3.87)	1.79 (1.61;2.00)	1.61 (1.44;1.80)	2.13 (1.82;2.50)	1.51 (1.28;1.78)	1.33 (1.13;1.58)
Chronic pulmonary disease	1.48 (1.40;1.55)	1.44 (1.37;1.52)	1.58 (1.50;1.66)	1.08 (1.00;1.16)	1.05 (0.97;1.12)	1.18 (1.10;1.28)
Connective tissue disease	1.28 (1.19;1.38)	1.14 (1.06;1.22)	1.22 (1.13;1.31)	1.02 (0.93;1.13)	0.95 (0.86;1.05)	1.01 (0.90;1.12)
Ulcer disease	1.49 (1.39;1.59)	1.21 (1.13;1.30)	1.28 (1.19;1.37)	1.15 (1.04;1.27)	1.03 (0.94;1.14)	1.09 (0.98;1.21)
Liver disease	1.76 (1.55;2.00)	2.01 (1.77;2.28)	2.25 (1.98;2.56)	1.15 (0.95;1.38)	1.23 (1.02;1.48)	1.41 (1.15;1.71)
Diabetes	1.58 (1.49;1.67)	1.39 (1.31;1.47)	1.33 (1.26;1.41)	1.22 (1.13;1.32)	1.13 (1.04;1.22)	1.05 (0.96;1.14)
Hemiplegia	1.59 (1.21;2.09)	1.52 (1.15;2.00)	1.42 (1.08;1.87)	1.33 (0.90;1.95)	1.27 (0.86;1.88)	1.27 (0.87;1.86)
Renal disease	1.42 (1.25;1.62)	1.46 (1.28;1.66)	1.69 (1.48;1.92)	1.07 (0.88;1.31)	1.07 (0.87;1.31)	1.26 (1.02;1.56)

HR: hazard ratio; BC: breast cancer; CI: confidence interval.
[a]In 5-year categories.
[b]Adjusted for age, year of surgery, tumor size, lymph node status, lymphovascular invasion, invasion resection margin, histological type and grade, hormone receptor status, menopausal status, treatment.

Table 4. Interaction between diabetes and vascular comorbidities on the hazard ratio of death from breast cancer with breast cancer patients without comorbidity as reference.

	Vascular morbidity No diabetes HR (95% CI)	Diabetes No vascular morbidity HR (95% CI)	Diabetes and Vascular morbidity HR (95% CI)	*p* test of interaction
Myocardial infarction	1.11 (0.97;1.26)	1.05 (0.96;1.15)	1.12 (0.82;1.51)	.82
Congestive heart failure	1.03 (0.92;1.16)	1.08 (0.99;1.18)	0.83 (0.64;1.08)	.05
Peripheral vascular disease	1.17 (1.04;1.33)	1.07 (0.98;1.17)	0.94 (0.68;1.30)	.11
Cerebrovascular disease	1.05 (0.96;1.14)	1.05 (0.96;1.14)	1.10 (0.88;1.38)	.97
Renal disease	1.21 (0.96;1.51)	1.04 (0.96;1.13)	1.59 (0.94;2.70)	.42

HR: hazard ratio; CI: confidence interval.

Results

The study population included 59,673 breast cancer patients with a median age of 61 years and with an estimated median potential follow-up of 14 years and 10 months. A total of 31,029 patients had died, 66% from breast cancer and 34% from other causes (Table 1). A third of the patients had a lumpectomy and about two thirds a mastectomy, while 5% had a biopsy only. For these, the histological diagnosis is often just stated as 'carcinoma' which explains why tumor size and histological type were unknown for about 6% of the patients. Seventy percent of the patients had ER positive tumors and 43% were node positive. Adjuvant medical treatment was not indicated for 27% of the patients because at the time of their diagnosis they were considered at low risk of recurrence, while 44% received chemotherapy or endocrine treatment alone or sequentially. For 29% the DBCG did not have information on given adjuvant therapy.

Table 2 gives an overview of the 12 categories of comorbidity in relation to 5- and 10-year OS and BC mortality. The OS was 79% after 5 years and 64% after 10 years among 49,928 patients (84%) with no comorbidity registered before the breast cancer diagnosis. The presence of any of the comorbidities reduced OS, most pronounced for dementia with a 5-year OS of 28% and a 10-year OS of 9%. Similarly for BC mortality it was 18% at 5 years and 28% at 10 years

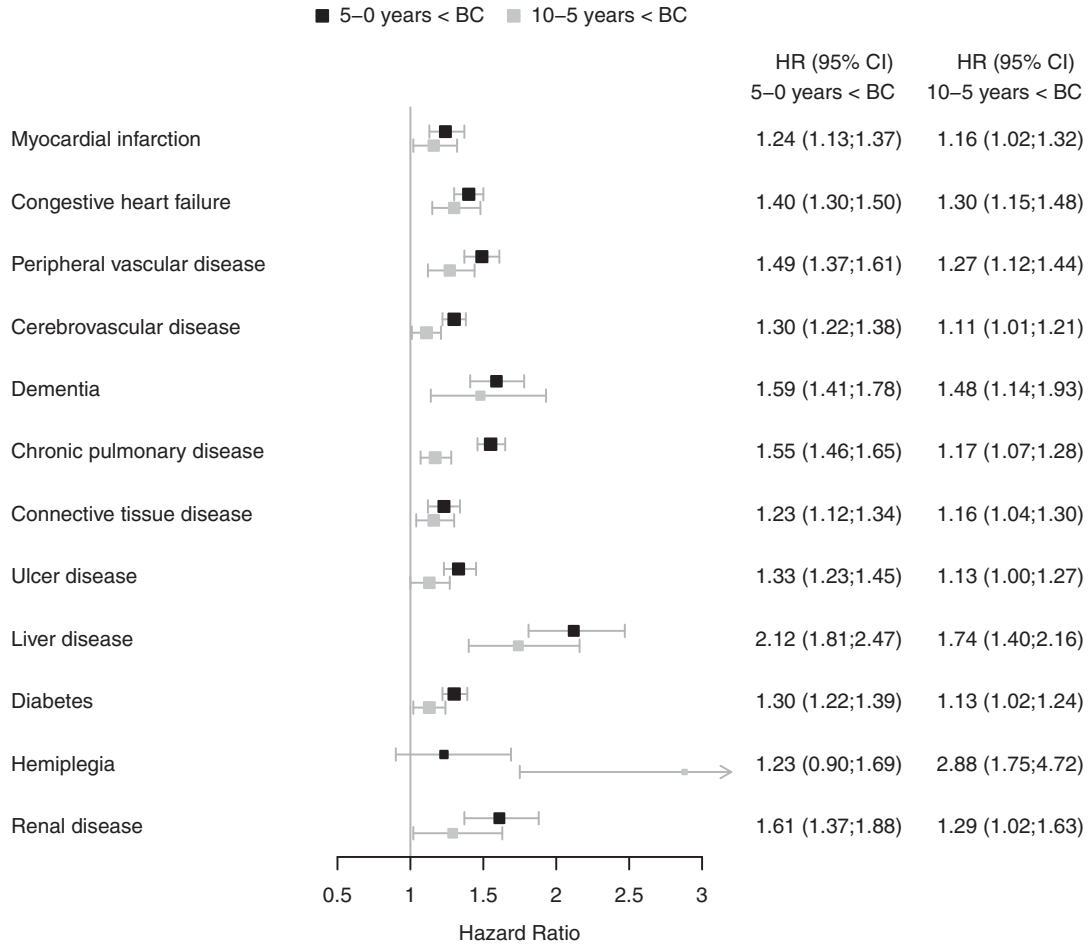


Figure 1. Hazard ratio of death from all causes in relation to time period of comorbidity with breast cancer patients without comorbidity as reference.

among patients without comorbidity compared with 48% and 57% among women with dementia. The most common comorbidities were chronic pulmonary disease, present in 4.2%, followed by cerebrovascular disease (3.6%) and diabetes (3.1%).

The results in Table 2 are as observed without any statistical modeling. Table 3 outlines the hazard ratio (HR) of dying from all causes and from breast cancer comparing patients with comorbidities with patients without comorbidities before breast cancer diagnosis. Adjustment for age at diagnosis reduced all HRs whereas adjustment for other factors (year of surgery, tumor size, lymph node status, lymphovascular invasion, resection margin, histological type and grade, ER status, menopausal status, treatment) resulted in minor changes. The HRs of dying from all causes remained statistically significantly increased for all 12 categories of comorbidity while the HR of dying from breast cancer also was significantly increased for peripheral vascular disease, dementia, chronic pulmonary disease, liver, and renal diseases, but not for myocardial infarction, congestive heart failure, cerebrovascular disease, connective tissue disease, ulcer disease, and diabetes.

Since most of the mortality associated with diabetes results from chronic vascular complications, we examined BC mortality in patients with diabetes only, vascular disease only, and diabetes with vascular complications (Table 4). BC mortality was not significantly increased in women with diabetes without complications but women with peripheral vascular disease without diabetes had a HR = 1.17 (95%CI 1.04–1.33). There was a borderline significant interaction ($p = .05$) between diabetes and congestive heart failure with HR = 0.83 (95% CI 0.64–1.08) in women with both conditions. A similar reduction of HR was seen for diabetes and peripheral vascular disease but the interaction did not reach statistical significance ($p = .11$).

We examined whether there was any difference in all-cause mortality between more recent comorbidity occurring within 5 years of the breast cancer diagnosis and past comorbidity occurring 5–10 years before the breast cancer diagnosis (Figure 1). Although all HR-estimates were significantly increased, there was a trend of higher HRs for recent comorbidity, except for hemiplegia with HR = 1.23 (95% CI 0.90–1.69) if registered within 5 years and HR = 2.88 (1.75–4.72) for years 5–10 (p for heterogeneity .006). Different estimates were also seen for cerebrovascular disease with HR = 1.30 (95% CI 1.22–1.38) for years 0–5 and HR = 1.11 (95% CI 1.01–1.21) for years 5–10 (p for heterogeneity .008), chronic pulmonary disease with HR= 1.55 (95% CI 1.46–1.65) for years 0–5, and HR= 1.17 (95% CI 1.07–1.28) for years 5–10 (p for heterogeneity <.0001) and for ulcer disease with HR = 1.33 (95% CI 1.23–1.45) for years 0–5 and HR = 1.13 (95% CI 1.00–1.27) for years 5–10 (p for heterogeneity .03). For diabetes, the p value for heterogeneity was .046. We also

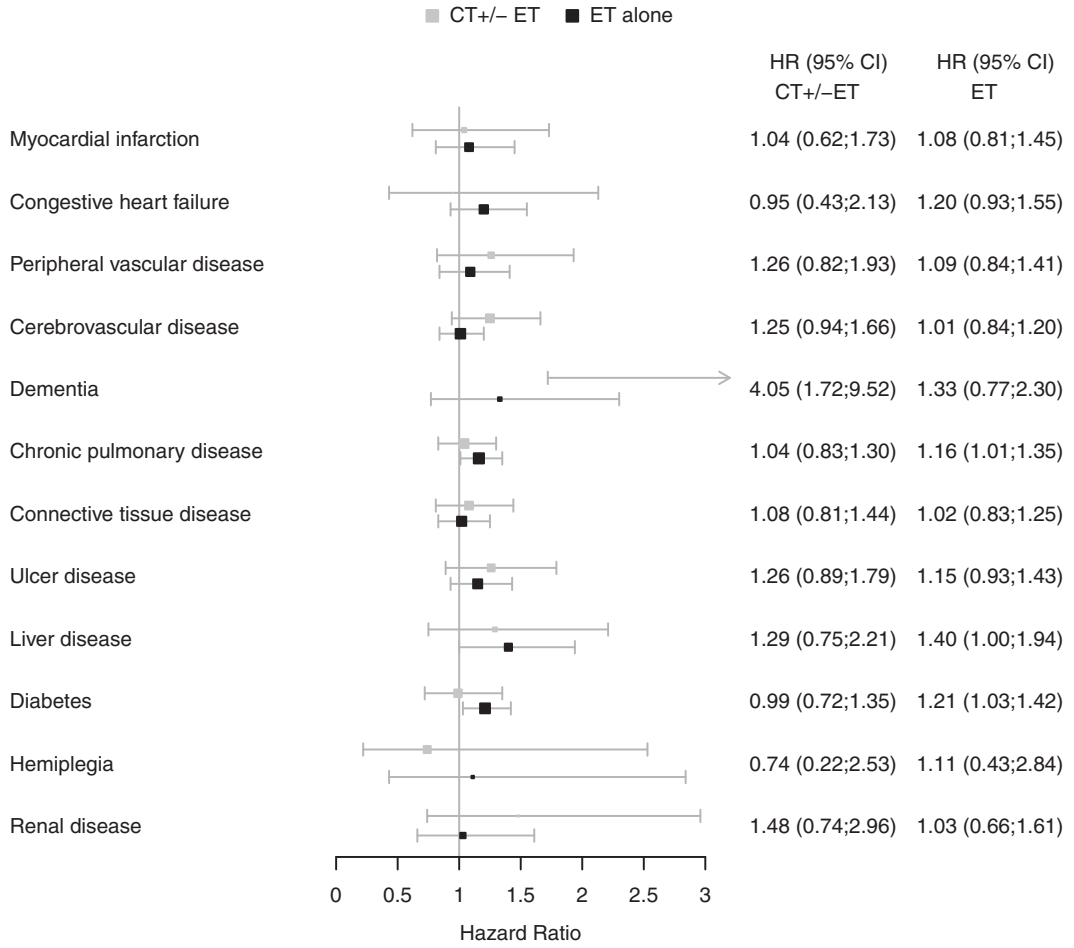


Figure 2. Hazard ratio of death from breast cancer in relation to receipt of adjuvant chemotherapy (CT) and adjuvant endocrine treatment (ET) with breast cancer patients without comorbidity as reference.

examined BC mortality but the results were largely similar to all-cause mortality (data not shown).

With a few exceptions, the effect of adjuvant treatment on BC mortality was similar among patients with and without comorbidity (Figure 2). Patients suffering from dementia receiving chemotherapy had a four-fold higher risk of dying (HR = 4.05, 95% CI 1.72–9.52), but this estimate was based on only eight patients. Among patients receiving endocrine treatment only, those with chronic pulmonary disease had HR = 1.16 (95% CI 1.01–1.35), liver disease HR = 1.40 (95% CI 1.00–1.94), and diabetes HR = 1.21 (95% CI 1.03–1.42).

Discussion

The prevalence of comorbidity increases markedly with age from less than 10% in breast cancer patients aged less than 50 years to 40% for those aged 80 years or more [4]. The relatively low prevalence of comorbidity (16%) in our study reflects that the median age was 61 years. In the US Medicare Population which included only individuals 65 years or older, the prevalence of comorbidity was similar among cancer-free Medicare beneficiaries (32%), breast cancer patients (32%), and prostate cancer patients (31%), but higher among patients with colorectal cancer (41%) and lung cancer (53%) [13].

The effect of age was also evident when adjustments for age were made in the multivariable analysis, e.g. for congestive heart failure, an unadjusted HR = 2.22 (95% CI 2.07–2.37) for death from all causes was reduced to HR = 1.42 (95%CI 1.33–1.52) after adjustment for age (Table 3). Despite adjustment for all available potential confounding factors, the all-cause mortality remained significantly increased for all the 12 comorbidities examined. This finding is in agreement with those of other studies [3,6,7]. However, it was reassuring that BC mortality was not significantly elevated for patients with prior myocardial infarction, congestive heart failure, cerebrovascular disease, connective tissue disease, ulcer disease, and diabetes. It was equally reassuring that with a few exceptions, the effect of adjuvant treatment was similar for patients with and without comorbidity. This finding is new and underlines the importance of adhering to guideline therapy even among patients who suffer from comorbidity.

Most register-based studies from Denmark have examined comorbidity over a period of 10 years preceding the breast cancer diagnosis [4,14], while others have collected the information at diagnosis without specifying when the comorbidity occurred [5]. Our results suggest that more recent comorbidity, i.e., within 5 years, carries a greater risk of dying than comorbidity occurring more than 5 years before breast cancer except for hemiplegia where the reverse was seen.

For diabetes, these findings confirm the observations by Goodwin et al. [15] of a pronounced effect on mortality of insulin-related variables during the first 5 years after diagnosis, but not thereafter. Such information is relevant for the clinician advising the individual patient on her treatment. Radiotherapy increases the risk of a myocardial infarction, particularly for left-sided breast cancers, and this risk is 3–5-fold increased for women with prior ischemic heart disease [16,17]. Chemotherapy with anthracyclines is also cardiotoxic [18] and the risk of heart disease may increase further by radiotherapy [19].

Information on diabetes relied in this material only on diagnoses reported to the NPR resulting in a prevalence of just over 3%. When other sources of information like measurements of blood glucose and diabetes medication were taken into account, the Danish Diabetes Registry reported prevalences of 3–6% for women aged 60 years between 1996 and 2010 with the prevalence peaking at 17% for women aged 80 years [20]. Diabetes is associated with microvascular complications such as kidney disease, which is a risk factor for macrovascular disease, i.e., atherosclerosis, myocardial infarction, heart failure, and cerebrovascular events [21]. We had expected that the presence of diabetes with such vascular complications would be associated with a higher BC mortality than diabetes without complications, but the data did not support this. On the contrary, HRs less than one were observed among diabetics with congestive heart failure and peripheral vascular disease although the estimates failed to reach statistical significance.

The strength of this study is that it was based on a prospective data collection in an entire population with valid information on tumor characteristics, given breast cancer-directed treatment, and all-cause mortality. Follow-up was 100% complete and the study population was followed for over 14 years. However, there are limitations. We had no information on potentially confounding factors, such as smoking or obesity. Data on given treatment were missing for 29% of the study population, partly explained by patients aged 75 years or older at diagnosis, who up to 2002 were not included in the national treatment guidelines [4]. Since the DBCG does not follow patients after a recurrence or a maximum of 10 years for recurrence-free patients [9], our only way to ascertain if deaths were due to breast cancer was to rely on information from death certificates. However, these are likely to be correct for patients dying with metastatic breast cancer.

Conclusion

This study confirmed that all-cause mortality was significantly increased for all the 12 comorbidities examined, but it also gave evidence that BC mortality was not significantly elevated for patients with prior myocardial infarction, congestive heart failure, cerebrovascular disease, connective tissue disease, ulcer disease, and diabetes. Our results suggest that more recent comorbidity, i.e., within 5 years, carries a greater risk of dying than comorbidity occurring more than 5 years before breast cancer. Finally, it was reassuring that with a few exceptions, the effectiveness of adjuvant treatment was similar for patients with and without comorbidity.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Land LH, Dalton SO, Jørgensen TL, et al. Comorbidity and survival after early breast cancer. A review. Crit Rev Oncol/Hematol. 2012;81:196–205.
[2] Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chron Dis. 1987;40:373–383.
[3] Hong CC, Ambrosone CB, Goodwin PJ. Comorbidities and their management: potential impact on breast cancer outcomes. Adv Exp Med Biol. 2015;862:155–175.
[4] Land LH, Dalton SO, Jensen M-B, et al. Influence of comorbidity on the effect of adjuvant treatment and age in patients with early-stage breast cancer. Br J Cancer. 2012;107:1901–1907.
[5] Wollschläger D, Meng X, Wöckel A, et al. Comorbidity-dependent adherence to guidelines and survival in breast cancer—is there a role for guideline adherence in comorbid breast cancer patients? A retrospective cohort study with 2137 patients. Breast J. 2017 [cited 2017 Jul 7]. DOI:10.1111/tbj.12855. [Epub ahead of print].
[6] Patnaik JL, Byers T, DiGuiseppi C, et al. The influence of comorbidities on overall survival among older women diagnosed with breast cancer. J Natl Cancer Inst. 2011;103:1101–1111.
[7] Peairs KS, Barone BB, Snyder CF, et al. Diabetes mellitus and breast cancer outcomes: a systematic review and meta-analysis. J Clin Oncol. 2011;29:40–46.
[8] Ranc K, Jørgensen ME, Friis S, et al. Mortality after cancer among patients with diabetes mellitus: effect of diabetes duration and treatment. Diabetologia. 2014;57:927–934.
[9] Christensen P, Ejlertsen B, Jensen M-B, et al. Danish Breast Cancer Cooperative Group. Clin Epidemiol. 2016;8:445–449.
[10] Lynge E, Sandegaard JL, Rebolj M. The Danish national patient register. Scand J Public Health. 2011;39:30–33.
[11] Helweg-Larsen K. The Danish register of causes of death. Scand J Public Health. 2011;39:26–29.
[12] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17:343–346.
[13] Edwards BK, Noone A-M, Mariotto AB, et al. Annual Report to the Nation on the Status of Cancer, 1975–2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast or prostate cancer. Cancer. 2014;120:1290–1314.
[14] Ording AG, Cronin-Fenton DP, Jacobsen JB, et al. Comorbidity and survival of Danish breast cancer patients from 2000 to 2011: a population-based cohort study. Clin Epidemiol. 2013;5:39–46.
[15] Goodwin PJ, Ennis M, Pritchard KI, et al. Insulin- and obesity-related variables in early-stage breast cancer: correlations and time course of prognostic associations. J Clin Oncol. 2011;30:164–171.
[16] Darby SC, Ewertz M, McGale P, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med. 2013;368:987–998.
[17] McGale P, Darby SC, Hall P, et al. Incidence of heart disease in 35,000 women treated with radiotherapy for breast cancer in Denmark and Sweden. Radiother Oncol. 2011;100:167–175.
[18] Sawyer DB. Anthracyclines and heart failure. N Engl J Med. 2013;368:1154–1156.
[19] Rehammar JC, Jensen M-B, McGale P, et al. Risk of heart disease in relation to radiotherapy and chemotherapy with anthracyclines among 19,464 breast cancer patients in Denmark, 1977–2005. Radiother Oncol. 2017;123:299–305.
[20] Carstensen B, Borch-Johnsen K. Register-based studies of diabetes. Scand J Public Health. 2011;39:175–179.
[21] Forbes JM, Fotheringham AK. Vascular complications in diabetes: old messages, new thoughts. Diabetologia. 2017;60:2129–2138.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 135–140
https://doi.org/10.1080/0284186X.2017.1400181

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Mortality and recurrence rates among systemically untreated high risk breast cancer patients included in the DBCG 77 trials

Maj-Britt Jensen[a], Torsten O. Nielsen[b], Ann S. Knoop[c], Anne-Vibeke Laenkholm[d], Eva Balslev[e] and Bent Ejlertsen[a,c]

[a]Danish Breast Cancer Cooperative Group (DBCG), Secretariat and Statistical Office, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [b]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; [c]Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [d]Department of Surgical Pathology, Zealand University Hospital, Slagelse, Denmark; [e]Department of Pathology, Herlev University Hospital, Herlev, Denmark

ABSTRACT

Background: Following loco-regional treatment for early breast cancer accurate prognostication is essential for communicating benefits of systemic treatment. The aim of this study was to determine time to recurrence and long-term mortality rates in high risk patients according to patient characteristics and subtypes as assigned by immunohistochemistry panels.

Patients and methods: In November 1977 through January 1983, 2862 patients with tumors larger than 5 cm or positive axillary nodes were included in the DBCG 77 trials. Archival tumor tissue from patients randomly assigned to no systemic treatment was analyzed for ER, PR, Ki67, EGFR and HER2. Intrinsic subtypes were defined as follows: Luminal A, ER or PR >0%, HER2-negative, PR >10% and Ki67 < 14%; Luminal B, ER or PR >0%, (PR ≤10% or HER2-positive or Ki67 ≥ 14%); HER2E, ER 0%, PR 0%, HER2 positive; Core basal, ER 0%, PR 0%, HER2 negative and EGFR positive. Multivariate categorical and fractional polynomials (MFP) models were used to construct prognostic subsets by clinicopathologic characteristics.

Results: In a multivariate model, mortality rate was significantly associated with age, tumor size, nodal status, invasion, histological type and grade, as well as subtype classification.

Conclusions: With 35 years of follow-up, in this population of high-risk patients with no systemic therapy, no subgroup based on a composite prognostic score and/or molecular subtypes could be identified without excess mortality as compared to the background population.

ARTICLE HISTORY
Received 14 September 2017
Accepted 30 October 2017

Introduction

Early breast cancer without recognizable distant metastases is potentially curable but long-term survival is extremely rare in entirely untreated patients [1]. Today, the general treatment principle is to obtain complete loco-regional eradication of cancer tissue with the addition of systemic treatment dependent on tumor biology and the risk of recurrence for the individual patient. Adjuvant tamoxifen and chemotherapy were widely introduced in the early eighties to those who at the time were considered high risk, i.e., those with large tumors, lymph node metastases or invasion of the skin or deep fascia. The evidence was provided by the overviews of Early Breast Cancer Trialists' Collaborative Group (EBCTCG) [2,3]. More recently the EBCTCG have demonstrated that modern chemotherapy reduces 10-year breast cancer mortality by about a third and importantly, the proportional benefits were similar in older and younger women and independent of age, nodal status, estrogen receptor status and type of chemotherapy regimen [4–6]. Systemic treatment has gradually been extended and will (depending on tumor characteristics) in most patients include endocrine therapy, HER2 targeting and/or chemotherapy [7].

Even in the absence of systemic treatment, about a third of high-risk breast cancer patients will, with efficient local treatment, be free of recurrence at 10 years [8,9]. A wide variation in the risk of recurrence has been demonstrated within different pathological stages [10]. Combining pathological stage with breast cancer subtypes may more accurately determine prognosis even in high risk patients. At least four clinically relevant subtypes – luminal A, luminal B, HER2-Enriched (HER2E) and basal-like – are now considered useful. Initially discovered on gene expression microarrays, these intrinsic molecular subtypes can be determined on clinical specimens using multigene assays such as PAM50 (Prosigna), but can also with reasonable accuracy be identified by inexpensive, widely-accessible immunohistochemistry panels [11,12].

In this study, we evaluated in systemically untreated high-risk breast cancer patients the ability of subtypes as designated by immunohistochemistry panels to predict long-term

CONTACT Maj-Britt Jensen mj@dbcg.dk Danish Breast Cancer Cooperative Group Secretariat, Building 2501 Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark

Supplemental data for this article can be accessed here.

survival and recurrence. Standard of care for high-risk patients has for a long time included adjuvant systemic treatment, and therefore this patient cohort with long-term, high quality detailed follow-up enables an exceptional study.

Patients and methods

In 1977, the Danish Breast Cancer Cooperative Group (DBCG) provided its first standard diagnostic and treatment protocols for early invasive breast cancer [13]. At the same time, the clinical DBCG database was established and has since prospectively accumulated diagnostic, therapeutic and follow-up data by the use of standardized forms nationwide [14]. The aim of the current study was among high-risk breast cancer patients to compare time to recurrence and long-term mortality according to intrinsic subtype.

Patients

Eligible for registration in the 77 protocol program was patients who were without previous or synchronous malignant disease, who were without evidence of advanced disease by physical examination, radiography of the chest and bone or bone scintigraphy, and who achieved a complete resection of a unilateral invasive adenocarcinoma of the breast by mastectomy with axillary sampling or clearance (level I and part of level II). Patients with axillary lymph node metastasis, tumors >5 cm, or invasion of the deep fascia without distant metastasis were, if premenopausal, eligible for the 77B trial, and if postmenopausal for the 77C trial.

Pathology

Surgical specimens were classified in a predetermined manner including tumor size, examination of tumor margins, invasion into skin or deep fascia, measurement of gross tumor size, total number of lymph nodes identified and number of metastatic nodes. Classification of histological type was performed according to WHO and grade (ductal carcinomas) according to Elston and Ellis [15,16].

Centralized collection of specimens and immunohistochemical classification have been described earlier [9,12]. In brief, from patients enrolled in 77B and 77C, formalin-fixed, paraffin-embedded blocks from primary excisional surgery specimens were retrospectively collected from the corresponding pathology departments. These were used to construct duplicate 0.6 mm core tissue microarrays, from which sections for immunohistochemistry were cut. The definition of subtypes used in prior publications [9,12] was maintained: luminal A = hormone receptor positive (i.e., ER or PR >0%), HER2-negative, PR >10% and Ki67 < 14%; luminal B = hormone receptor positive but (PR ≤10% or HER2-positive or Ki67 ≥ 14%); HER2E = ER negative, PR negative and HER2 positive; core basal = ER negative, PR negative, HER2 negative and EGFR positive.

Adjuvant treatment

Postoperatively, all patients received radiotherapy to the chest wall and regional nodes (40.92 Gy in 22 fractions, five fractions per week; or 36.60 Gy in 12 fractions, two fractions per week). In the 77B trial, patients were assigned randomly to one of four options: no systemic therapy, levamisole, oral cyclophosphamide, or oral cyclophosphamide plus methotrexate and fluorouracil [8]. Patients in the 77C trial were randomly assigned to no systemic therapy or tamoxifen [9].

None of the patients in the cohort analyzed in this current study received any adjuvant systemic treatment. Only patients randomly assigned to radiotherapy alone from the 77B trial and the 77C trial were included.

Follow-up

Treatment related adverse events and findings on clinical examination were recorded until a first event every third month during the first and second year, every six months during the third to fifth year, and thereafter annually to a total of 10 years. Biochemical tests and imaging were done when indicated by existing symptoms or signs. In addition, long-term follow-up was acquired on survival through linkage to the Danish Civil Registration System (CRS) [17].

Endpoints

The primary endpoint was standardized mortality ratio (SMR). Secondary endpoints included time to recurrence, and overall survival (OS). For SMR and OS, complete follow-up was achieved until 1 June 2017 by linkage to the CRS on individual level and retrieving mortality figures of the general Danish female population.

Statistical analysis

The DBCG Data Center undertook central review, query and analysis of data. Follow-up time was quantified in terms of a Kaplan–Meier estimate of potential follow-up [18]. Overall survival was calculated as the time elapsed from the date of definitive surgery until death from any cause, and was estimated using the Kaplan–Meier method. Time to recurrence was defined as the time from surgery to invasive locoregional recurrence or distant metastases. New contralateral breast cancer, another malignancy or death without prior recurrence were counted as competing events. Cumulative incidence of recurrence in the presence of competing risk was estimated. Univariate and multivariate regression analyses were performed, using the Cox proportional hazards model for OS and Fine-Gray proportional hazards subdistribution model for recurrence. The number of deaths observed was compared with the number of deaths expected, calculated by applying age and calendar year specific female mortality figures of the general Danish population and the corresponding person-years of the respective cohort members. Time at risk was defined as time from definitive surgery until date of death from any cause, emigration or end of follow-up (1 June 2017). The SMR, computed as the ratio of the observed to the expected number of deaths, served as an estimate of relative risk of death, and 95% confidence intervals (CIs) were computed based on the assumption that the

Table 1. Patient and tumor characteristics by subtype.

			Subtype									
	Total		Luminal A		Luminal B		HER2E		Core basal		NA	
Characteristics	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
No. of patients	1100		199		419		84		43		355	
Age												
<50	187	17	13	7	58	14	9	11	8	19	99	28
50–59	272	25	51	26	84	20	34	40	9	21	94	26
60–69	370	34	66	33	162	39	29	35	14	33	99	28
≥70	271	25	69	35	115	27	12	14	12	28	63	18
Lymph node status												
Negative	152	14	25	13	49	12	7	8	9	21	62	17
1–3 positive	591	54	118	59	220	53	44	52	19	44	190	54
4–9 positive	264	24	40	20	117	28	22	26	12	28	73	21
≥10 positive	39	4	6	3	17	4	8	10	1	2	7	2
Unknown	54	5	10	5	16	4	3	4	2	5	23	6
Total lymph nodes												
0 retrieved	54	5	10	5	16	4	3	4	2	5	23	6
1–3 retrieved	259	24	48	24	92	22	17	20	7	16	95	27
4–9 retrieved	610	55	113	57	240	57	44	52	25	58	188	53
≥10 retrieved	177	16	28	14	71	17	20	24	9	21	49	14
Tumor size												
0–20 mm	289	26	60	30	104	25	18	21	8	19	99	28
21–50 mm	587	53	106	53	233	56	47	56	25	58	176	50
>50 mm	213	19	33	17	78	19	19	23	10	23	73	21
Unknown	11	1	0	0	4	1	0	0	0	0	7	2
Deep fascia invasion												
Absent	818	74	168	84	307	73	60	71	30	70	253	71
Present	275	25	31	16	111	26	24	29	12	28	97	27
Unknown	7	1	0	0	1	0	0	0	1	2	5	1
Histologic type												
Ductal carcinoma	975	89	186	93	364	87	75	89	35	81	315	89
Lobular carcinoma	48	4	9	5	21	5	3	4	0	0	15	4
Other	77	7	4	2	34	8	6	7	8	19	25	7
Malignancy grade[a]												
Grade I	275	28	82	44	103	28	9	12	2	6	79	25
Grade II	534	55	94	51	205	56	47	63	16	46	172	55
Grade III	162	17	10	5	56	15	18	24	15	24	63	20
Unknown	4	0	0	0	0	0	1	1	2	6	1	0

HER2E: Her2-Enriched.
[a]Ductal carcinoma only.

observed number of deaths followed a Poisson distribution. The SMR was analyzed using univariate and multivariate Poisson regression models.

Multivariable fractional polynomials (MFPs) were used to assess the functional form of continuous prognostic variables building the multivariate regression models [19]. Factors included in the multivariable analyses were age, tumor size, number of positive lymph nodes, fraction of positive vs retrieved lymph nodes, histological type (ductal, lobular, other histological types), grade (1, 2, 3), and invasion of the tumor into deep fascia. Only ductal carcinomas were graded, and for multivariate models, grade was set to two for non-ductal carcinomas with separate estimates for histologic type. The assumption of proportional hazards was assessed by Schoenfeld residuals and by including time-dependent covariates in the model. To comply with the assumption of proportional hazards, time-dependent components were included in the models. All *p* values are two-sided. Statistical analyses were done using SAS v9.4 (SAS Institute, Inc., Cary, NC, USA), and STATA IC 14 (StataCorp, College Station, TX, USA).

Results

The DBCG 77B and 77C trials enrolled 2862 patients between November 1977 and January 1982. Following mastectomy for early high-risk breast cancer 1100 (38%) patients were followed without adjuvant systemic treatment after postoperative radiotherapy. Biomarker analyses were performed on archived FFPE tumor blocks in 820 (75%) and subtype classification was available in 745 (68%). Mean age at mastectomy was 61 years. Table 1 summarizes patient and tumor characteristics and shows a high proportion of patients with large tumors and heavy nodal involvement. Among the patients with subtype classification, 199 (27%) were assigned as luminal A, 419 (56%) luminal B, 84 (11%) HER2E and 43 (6%) core basal. The estimated potential median follow-up was 10.0 years for first event and 37.6 years for OS.

Analysis of recurrence

Within 2½ years, 20% of luminal A patients have a breast cancer recurrence increasing to 30% of luminal B and 40% or more in HER2E and core basal breast cancer patients. At 10 years, 526 patients (47.8%) had a breast cancer recurrence, including 450 with distant metastases, and 203 patients (18.5%) had a competing event. Factors significantly associated with recurrence were age, tumor size, nodal status, invasion and grade (all $p < .01$). Figure 1(A) shows the cumulative incidence of recurrence up to 10 years according to subtype. The corresponding hazard ratios are shown in Table 2, where

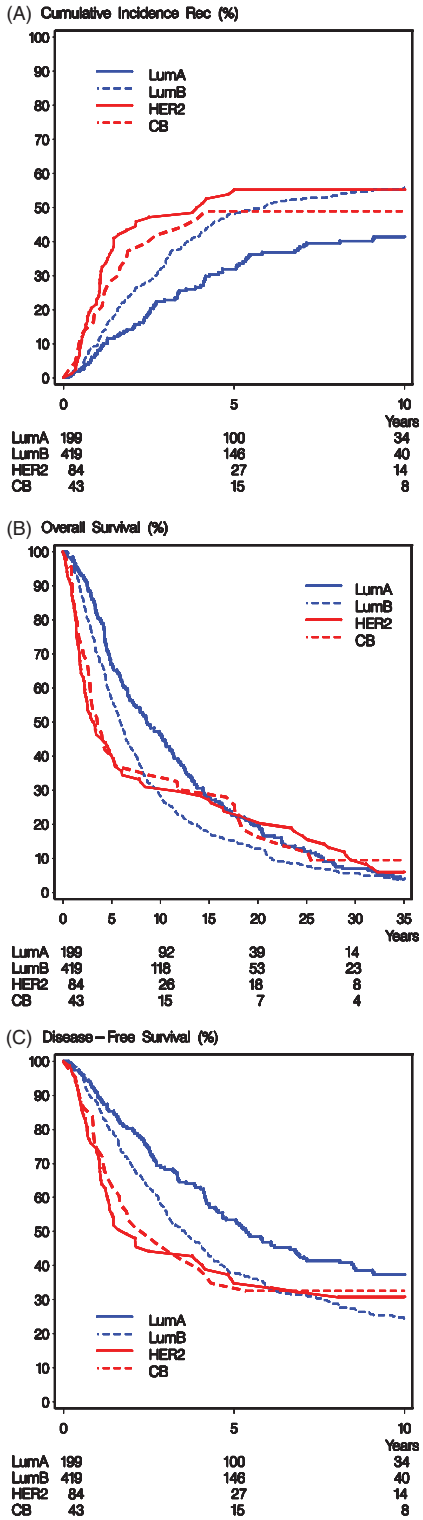


Figure 1. (Panel A) Cumulative Incidence estimates for recurrence (Rec) of systemically untreated patients with luminal A (Lum A), luminal B (Lum B), HER2-Enriched (HER2) and core basal (CB) breast cancer. (Panel B) Kaplan–Meier estimates of overall survival for systemically untreated patients with luminal A (Lum A), luminal B (Lum B), HER2-Enriched (HER2) and core basal (CB) breast cancer. Estimates for OS at 5, 10 and 20 years after surgery; Lum A 67% (60;73), 46% (39;53), 20% (14;25), Lum B 56% (51;61), 28% (24;33), 13% (10;16), HER2E 41% (30;51), 31% (21;41), 21% (13;31), core basal 42% (27;56), 35% (21;49), 16% (7;29). (Panel C) Kaplan–Meier estimates of disease-free survival (considering events of recurrence, contralateral breast cancer, other malignancy, and death as first event) for systemically untreated patients with luminal A (Lum A), luminal B (Lum B), HER2-Enriched (HER2) and core basal (CB) breast cancer. Patients at risk according to time after surgery listed below *x*-axis.

estimates are split according to time after surgery due to non-proportional hazards for HER2E and core basal as compared to luminal B. There is a highly significant effect of subtype ($p < .0001$), which is maintained in the multivariate analysis. Considering all first events, including recurrence, contralateral breast cancer, other malignancy, and death as first event, the pattern looks similar (Figure 1(C)).

Analysis of OS and SMR

Of the 1100 women in the study cohort, 1050 died (35-year OS 5.5%; 95% CI 4.3–7.0) while 372 deaths were expected (SMR 2.82; 95% CI 2.65–2.99, $p < .0001$).

Factors significantly associated with mortality (OS and SMR) were age, tumor size, nodal status and grade. Figure 1(B) shows the OS up to 35 years after surgery. Corresponding figures for OS at 5, 10 and 20 years after surgery are listed in the figure legend. Median survival was distinct across subtypes, with 8.6 years for patients in the luminal A group as opposed to 3.1 years in the HER2E group of patients. The relative risk according to follow-up time and subtype is shown in Table 2, both for univariate and multivariate analyses, with an overall statistically significant effect ($p < .0001$).

According to subtype, the number of observed vs expected deaths was 407 and 126.2 for luminal B, 194 and 89.1 for luminal A, 80 and 32.0 for HER2E and 39 vs 15 for core basal. The empirical SMR overall and divided by time of follow-up for each subtype are shown in Table 3.

Identifying patients free of excess mortality

By summing the estimated regression coefficients (β) of age, tumor size, nodal status, deep fascia invasion, and histological type and malignancy grade in the multivariate model for SMR (Supplementary Table 1), we constructed a clinical prognostic score index (cPSI). When patients were divided according to subtypes and cPSI, even those with the lowest decile cPSI combined with a luminal A subtype had an excess mortality as compared to the background population (data not shown), and with excess mortality progressively increasing with increasing score.

Discussion

The result from this historical follow-up clearly shows the appalling consequences of omitting systemic treatment in high-risk breast cancer patients and recalls the medical need of high-risk breast cancer patients not receiving adjuvant systemic treatment. The retrospective determination of breast cancer subtypes revealed a distinct pattern separating luminal A breast cancer from luminal B and both of them from HER2E and core-basal breast cancers. Two and a half years after primary surgery, recurrence rate was more than twice as high in patients with HER2E and core-basal breast cancers as compared to luminal A, while patients with luminal B breast cancers were in between. The diversity in mortality was even more separated among the four subtypes

Table 2. Proportional hazards models for recurrence and overall survival according to subtype.

		Years after surgery						
Subtype	*p*	HR	95% CI	HR	95% CI	HR	95% CI	$P_{heterogeneity}$
Recurrence		0–2½		2½–10				
Univariate	<.0001							
Luminal A		0.63	(0.43;0.90)	0.67	(0.47;0.96)			.78
Luminal B		Reference						
HER2E		1.97	(1.35;2.88)	0.39	(0.18;0.85)			.0002
Core basal		1.55	(0.93;1.60)	0.36	(0.13;1.02)			.01
Multivariate	.001							
Luminal A		0.79	(0.55;1.13)	0.69	(0.47;1.00)			.61
Luminal B		Reference						
HER2E		1.66	(1.11;2.49)	0.35	(0.16;0.76)			.0004
Core Basal		1.44	(0.84;2.47)	0.40	(0.14;1.15)			.03
OS		0–5		5–10		>10		
Univariate	<.0001							
Luminal A		0.67	(0.51;0.87)	0.57	(0.41;0.81)	1.09	(.84;1.42)	.03
Luminal B		Reference						
HER2E		1.75	(1.29;2.37)	0.44	(0.22;0.89)	0.61	(.39;.95)	<.0001
Core Basal		1.60	(1.06;2.42)	0.30	(0.10;0.94)	0.60	(.32;1.10)	.002
Multivariate	$p < .0001$							
Luminal A		0.79	(0.60;1.03)	0.60	(0.43;0.85)	0.97	(.75;1.26)	.08
Luminal B		Reference						
HER2E		1.72	(1.26;2.34)	0.46	(0.22;0.93)	0.48	(.31;.75)	<.0001
Core Basal		1.55	(1.01;2.37)	0.36	(0.11;1.11)	0.63	(.34;1.16)	.008

HER2E: Her2-Enriched; HR: hazard ratio; CI: confidence interval; OS: overall survival.
Hazard ratios for unadjusted and adjusted estimates. *p* Values for overall significance and for test of heterogeneity in relation to years after surgery (non-proportional hazard).

Table 3. Standardized mortality ratio according to subtype, overall and in relation to years after surgery.

	Years after surgery							
	0–5		5–10		>10		Overall	
Subtype	SMR	95% CI	SMR	95% CI	SMR	95% CI	SMR	95% CI
Lum A	3.20	(2.52;4.08)	2.14	(1.58;2.91)	1.76	(1.43;2.17)	2.18	(1.89;2.51)
Lum B	5.13	(4.43;5.92)	4.46	(3.73;5.35)	1.65	(1.37;2.00)	3.23	(2.93;3.55)
HER2E	13.0	(9.87;17.2)	2.53	(1.26;5.05)	0.88	(0.58;1.33)	2.50	(2.01;3.11)
Core Basal	10.1	(6.81;14.9)	1.35	(0.44;4.19)	1.07	(0.59;1.93)	2.60	(1.90;3.56)

Lum A: luminal A; Lum B: luminal B; HER2E: Her2-Enriched; CI: confidence interval; SMR: standardized mortality ratio.

after 5 years. In years 5–10, hazards reversed and mortality approximated to women in background population after 5 years in patients with core-basal cancers and after 10 years in patients with HER2E breast cancer. Patients with luminal breast cancer persistently had an excess mortality as described previously and only 20% were alive at 20 years [20–22]. We were, within this group of high-risk patients, unable to identify a subgroup without major excess mortality, even when combining subtypes with other known prognostic factors. Overall, our data reinforce that all clinically high-risk patients should be recommended some form of systemic treatment even with a luminal A subtype.

The strengths of our study include a prospectively defined and identified study cohort; use of standardized breast cancer management including diagnostic procedures, standardized loco-regional treatment with mastectomy and radiotherapy; and long-term, high quality detailed follow-up. In contrast, treatment information in the SEER registry has been inaccurate [23,24]. The access to complementing registries furthermore allowed us to calculate SMR, which compared to OS is less sensitive to competing mortality occurring over time from deaths from other causes, e.g., ischemic heart disease, stroke and non-breast cancer [25]. Our ability to analyze SMR according to subtype was however hampered by the considerable excess mortality in the first 10 years after diagnosis of breast cancer.

The group of long-term survivors in our study were very small, and clearly a limitation that we were not able to characterize this subset. One possible explanation was that we use immunohistochemistry to determine intrinsic subtype, as opposed to a gene based set-up as, for example the PAM50 or the OncotypeDx. In years 2½–10, patients with luminal subtypes had a higher rate of recurrence as compared to patients with core basal and HER2E subtypes, but unfortunately patients were followed for 10 years only and we are unable to describe the pattern of recurrence beyond 10 years. Immunohistochemical panels provide less prognostic information and do not contain the same level of analytical reproducibility as genomic-based nucleic acid tests [26,27]. In particular, immunohistochemical assessment of Ki67 and PR has analytic variability issues [28,29], and the predefined cut-off used for Ki-67 may have been suboptimal [30]. Other limitations include lack of availability of some tumor blocks and of preanalytical handling guidelines for older specimens. While our study suggests that adjuvant systemic treatment is required in high-risk breast cancer patients, it does not

elucidate the possible benefits [12]. Furthermore, our data do not in any way contradict the growing evidence supporting de-escalation of systemic treatment to many low- or moderate-risk breast cancers [31].

In conclusion, if adjuvant systemic treatment is omitted the vast majority of high-risk breast cancer patients are affected by excess mortality and it is not possible at the time of diagnosis to identify the relatively few good prognosis patients.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Shimkin MB, Griswold MH, Cutler SJ. Classics in oncology. Survival in untreated and treated cancer. CA Cancer J Clin. 1984; 34:282–294.

[2] Early Breast Cancer Trialists' Collaborative Group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. N Engl J Med. 1988;319:1681–1692.

[3] Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Lancet. 1992;339:71–85.

[4] Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. Lancet. 1998;352:930–942.

[5] Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005;365:1687–1717.

[6] Peto R, Davies C, Godwin J, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012;379:432–444.

[7] Harbeck N, Gnant M. Breast cancer. Lancet. 2017;389:1134–1150.

[8] Ejlertsen B, Mouridsen HT, Jensen MB, et al. Cyclophosphamide, methotrexate, and fluorouracil; oral cyclophosphamide; levamisole; or no adjuvant therapy for patients with high-risk, premenopausal breast cancer. Cancer. 2010;116:2081–2089.

[9] Knoop AS, Laenkholm AV, Jensen MB, et al. Estrogen receptor, progesterone receptor, HER2 status and Ki67 index and responsiveness to adjuvant tamoxifen in postmenopausal high-risk breast cancer patients enrolled in the DBCG 77C trial. Eur J Cancer. 2014;50:1412–1421.

[10] Yi M, Mittendorf EA, Cormier JN, et al. Novel staging system for predicting disease-specific survival in patients with breast cancer treated with surgery as the first intervention: time to modify the current American Joint Committee on Cancer Staging System. J Clin Oncol. 2011;29:4654–4661.

[11] Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10:5367–5374.

[12] Nielsen TO, Jensen MB, Burugu S, et al. High-risk premenopausal luminal a breast cancer patients derive no benefit from adjuvant cyclophosphamide-based chemotherapy: results from the DBCG77B clinical trial. Clin Cancer Res. 2017;23:946–953.

[13] Fischerman K, Mouridsen HT. Danish breast cancer cooperative group (DBCG). Present status and experience. Acta Chir Scand Suppl. 1984;519:55–59.

[14] Moller S, Jensen MB, Ejlertsen B, et al. The clinical database and the treatment guidelines of the Danish breast cancer cooperative group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.

[15] Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19:403–410.

[16] World Health Organization. International histological classification of tumours. 2nd ed. Geneva: World Health Organization; 1969–1981; Berlin: Springer-Verlag; 1988–present.

[17] Schmidt M, Pedersen L, Sørensen HT. The Danish civil registration system as a tool in epidemiology. Eur J Epidemiol. 2014;29: 541–549.

[18] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17:343–346.

[19] Royston P, Sauerbrei W. A new approach to modelling interactions between treatment and continuous covariates in clinical trials by using fractional polynomials. Stat Med. 2004;23:2509–2525.

[20] Hähnel R, Woodings T, Vivian AB. Prognostic value of estrogen receptors in primary breast cancer. Cancer. 1979;44:671–675.

[21] Aamdal S, Børmer O, Jørgensen O, et al. Estrogen receptors and long-term prognosis in breast cancer. Cancer. 1984;53:2525–2529.

[22] Bentzon N, Düring M, Rasmussen BB, et al. Prognostic effect of estrogen receptor status across age in primary breast cancer. Int J Cancer. 2008;122:1089–1094.

[23] Cooper GS, Yuan Z, Stange KC, et al. Agreement of Medicare claims and tumor registry data for assessment of cancer-related treatment. Med Care. 2000;38:411–421.

[24] Noone AM, Lund JL, Mariotto A, et al. Comparison of SEER treatment data with medicare claims. Med Care. 2016;54:e55–e64.

[25] Mell LK, Jeong JH, Nichols MA, et al. Predictors of competing mortality in early breast cancer. Cancer. 2010;116:5365–5373.

[26] Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res. 2010;16:5222–5232.

[27] Gluz O, Liedtke C, Huober J, et al. EC-Doc investigators (West German Study Group/AGO-B). Comparison of prognostic and predictive impact of genomic or central grade and immunohistochemical subtypes or IHC4 in HR+/HER2− early breast cancer: WSG-AGO EC-Doc Trial. Ann Oncol. 2016;27:1035–1040.

[28] Hefti MM, Hu R, Knoblauch NW, et al. Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. Breast Cancer Res. 2013;15:R68.

[29] Leung SCY, Nielsen TO, Zabaglo L, et al. Analytical validation of a standardized scoring protocol for Ki67: phase 3 of an international multicenter collaboration. NPJ Breast Cancer. 2016;2:16014.

[30] Laenkholm AV, Grabau D, Talman ML, et al. An inter-observer Ki67 reproducibility study applying two different assessment methods. On behalf of the Danish scientific committee of pathology, Danish breast cancer group (DBCG). Acta Oncol. 2018; in press.

[31] Curigliano G, Burstein HJ, P Winer E, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen international expert consensus conference on the primary therapy of early breast cancer 2017. Ann Oncol. 2017;28:1700–1712.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 141–145
https://doi.org/10.1080/0284186X.2017.1403043

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The occurrence of fractures after adjuvant treatment of breast cancer: a DBCG register study

Bent Kristensen[a], Bent Ejlertsen[b], Maj-Britt Jensen[b] and Henning T. Mouridsen[b]; for the Danish Breast Cancer Cooperative Group

[a]Department of Clinical Physiology and Nuclear Medicine, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark; [b]Danish Breast Cancer Cooperative Group (DBCG) Secretariat and Statistical Office, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

ABSTRACT

Background: Adjuvant treatment in breast cancer patients especially with aromatase inhibitors (AIs) has adverse effects on bone metabolism resulting in an increased occurrence of fractures. In order to demonstrate this occurrence, long-term follow-up studies are necessary. From several national registries in Denmark, it is possible to link data from different sources and analyze this issue.

Methods: A study cohort of 68,842 breast cancer patients prospectively diagnosed and registered in the Danish Breast Cancer Cooperative Group's database during the period 1995–2012 formed the basis of the analysis. These data were matched with data on all types of fractures from the Danish National Patient Register and vital data from the Danish Civil Registration System.

Results: After data cleaning 66,502 patients were available for analysis and 16,360 of these had incurred 20,341 fractures with 13,182 patients having just one fracture. These fractures were distributed over 214 specific fracture sites. An extended multivariable Cox regression model revealed significant association between the occurrence of fractures and age, menopause, Charlson comorbidity index (CCI) and endocrine therapy such that late menopause and tamoxifen treatment were associated with a lower occurrence and AI treatment, age and CCI were associated with a higher occurrence of fractures.

Conclusion: Before advising adjuvant therapy with AIs fragile patients with chronic diseases should receive special attention in order to reduce the incidence of fractures in this vulnerable group of patients.

ARTICLE HISTORY
Received 25 September 2017
Accepted 3 November 2017

Introduction

Breast cancer is the most common cancer among Danish women with a steadily increasing incidence during the last 60 years. Breast cancer mortality has declined as a result of successive improvements in loco-regional and systemic treatment in the same period [1]. In estrogen receptor positive early breast cancer adjuvant endocrine therapy with tamoxifen (TAM), an aromatase inhibitor (AI), or a sequence of these two drugs for 5 to 10 years is considered standard therapy [2]. These drugs inhibit the effects of estrogens on breast tissue: TAM being a partial nonsteroidal estrogen agonist and AIs significantly lowering the plasma estradiol concentration [3,4]. Since the introduction of TAM and AIs the treatment duration with these drugs has increased in favor of their long-term antineoplastic effects, but at the expense of potential long-term side effects, especially on bone metabolism resulting in fractures, especially for the AIs [5] as TAM seems to lower the risk of fractures [6]. Irrespectively of bone mineral density, the addition of adjuvant bisphosphonate, 6-monthly zoledronic acid or daily oral clodronate, prolongs disease-free survival in postmenopausal patients [7].

The aim of this study was to investigate the occurrence of fractures in a cohort of Danish breast cancer patients and possible associations with some patient characteristics, comorbidity and adjuvant, antineoplastic treatment in particular endocrine therapy before the general introduction of adjuvant bisphosphonate treatment.

Methods

Study cohort

The study cohort originated from the Danish Breast Cancer Cooperative Group (DBCG) register in which data on patient and tumor characteristics are collected nationwide and prospectively along with data on given therapy and disease recurrence. Data from all patients registered during the period 1 January 1995 to 31 December 2012 constitute the basis for the present analysis. These data were supplemented and combined with data from the Danish National Patient Register (NPR) [8] and data from the Danish Civil Registration System (CPS) [9]. Since 1995 the NPR contains all somatic diagnoses from hospital admissions and outpatient contacts for each patient together with dates on admission and

CONTACT Bent Kristensen bent.kristensen.01@regionh.dk Klinisk Fysiologisk & Nuklearmedicinsk Afdeling, Herlev Universitetshospital, Herlev Ringvej 75, 2730 Herlev, Denmark

discharge. Vital and emigration status for each patient were collected from the CPS.

Fracture diagnoses

All fracture diagnoses registered in the NPR were collected for the study cohort. No attempt was made to elucidate the cause of the fractures, that is, whether caused by a high- or low-energy impact. As the same diagnoses in the NPR may be registered more than once only one fracture type was allowed for each patient, that is, even if the same patient had two femoral fractures registered three years apart only the first fracture counted, but the patient could also have had a distal radius fracture that was counted. If the patients had fractures after recurrence of the breast cancer, these fractures were not counted as they might have been pathological and fractures before the breast cancer diagnosis were also excluded.

Comorbidity

Information on comorbidity for each patient was extracted from the NPR and used to construct the Charlson Comorbidity Index (CCI) [10]. For the present study, the CCI was obtained using ICD-8 and ICD-10 diagnoses up to 10 years before the date for the breast cancer diagnosis.

Statistical analyses

The cumulative incidence of new fractures after the breast cancer diagnosis was estimated, considering recurrence and deaths as competing events. Patients were censored (a) at date of death, (b) at recurrence regardless of the location, (c) at last registered visit date, or (d) when followed for the whole observation period which ended 31 December 2016.

As many patients in the study cohort had several fractures and because patients with a prior fracture are at an increased risk of new fractures, the data were analyzed using regression models for recurrent events [11]. The extended Cox regression model development followed the suggestions described as the purposeful selection method along with the assessment of model adequacy [12].

The following covariates were analyzed: age and menopause (women >55 years at diagnosis and all patients treated with AI alone were defined as postmenopausal), CCI, chemotherapy and endocrine therapy. Antineoplastic treatments were coded as categorical covariates, for example, TAM: no/yes, without regard to the total treatment duration.

All analyses were done using R, version 3.4.1 [13] and the survival package [14]. The study was given file number: 2012-58-0004 by the Danish Data Protection Authority.

Results

The initial study cohort comprised 68,842 patients. These patients have been described meticulously in a previous publication [1]. The number of patients with missing follow-up dates was 2340 leaving 66,502 (96.6%) patients for the final analyses. Of these 47,302 (71.1%), patients were classified as postmenopausal. During the follow-up period, 27,561 (41.4%) patients died. Median follow-up time was 5.9 years with an interquartile range of 3.7–9.2 years and range 0.01–21.9 years. Table 1 shows information about the patients receiving adjuvant endocrine therapy or no endocrine therapy.

The NPR contained 56,759 fracture codes corresponding to the study cohort. After removal of patients with duplicated identification number, contact date and coded diagnosis 20,651 different fracture diagnoses remained. Further 310 patients had a fracture diagnosis before the breast cancer diagnosis leaving 20,341 fractures for analysis. These fractures were distributed over 214 specific fracture sites as coded in the NPR. Table 2 shows the number of fractures classified in 10 major groups. It is seen that most fractures were located at typical sites for osteoporotic fractures: femur, forearm, and upper arm. Recurrent fractures were frequent: 2541 patients had two fractures, 504 patients had three, 119 patients had four, 13 patients had five and four patients had six fractures.

During 564,977 person-years (p-y) of observation (range, 0.01 to 21.9 years per subject), 16,363 women experienced 20,341 different fractures (crude incidence, 36 fractures per 1000 p-y; 95% confidence interval, 34–39). In the premenopausal group, 4513 fractures were observed compared to 15,624 fractures in postmenopausal women.

Figure 1 shows the cumulative fracture incidence curves for patients receiving endocrine treatment or no endocrine treatment. Initially, the curves follow each other closely, but after about 5 years the curve for the AI-treated patients

Table 1. Baseline information on endocrine treatment, age and CCI.

Treatment	None	Tamoxifen and aromatase inhibitor	Aromatase inhibitor	Tamoxifen	Other	Total
Number of patients	36,412	11,454	9875	8466	295	66,502
Age (mean; sd) years	64.0; 14.3	59.57; 10.3	65.6; 9.0	55.1; 12.3	53.0; 10.3	
CCI						
0	27,655	9666	7388	7235	271	52,215
1	4683	1196	1519	781	20	8199
2	2420	419	591	287	3	3720
3+	1654	173	377	163	1	2368

Table 2. The distribution of fractures in 10 major groups.

Location	Face	Cervical spine	Thoracic spine	Lumbar spine	Upper arm	Forearm	Hand	Femur	Lower leg	Foot	Total
Num-ber of patients	557	99	625	1219	3059	5226	1825	4060	2212	1459	20341

tends to increase above the non-treated patients. The curve for the TAM + AI-treated patients coincides with the curve for the TAM-treated patients until about 8 years when this curve starts to increase and crosses the curve for non-treated patients after about 12 years of observation. The curve for the TAM-treated patients remains well below the non-treated patients along with the curve for patients treated with other endocrine modalities for the whole observation period.

The extended Cox model chosen was the Prentice, Williams and Peterson (PWP) model which analyzes ordered multiple events by stratification, based on the prior number of events during the follow-up period [11], because it was reasonable to assume that the occurrence of the first fracture increases the likelihood of a new fracture [15].

Table 3 shows separate analyses for pre- and postmenopausal women. In premenopausal women, only age and CCI were significantly associated with fracture occurrence compared to postmenopausal women where age, CCI and endocrine treatment were significantly associated with fracture occurrence.

Analyzing all patients together the following covariates remained in the final model: age, menopause, CCI, and endocrine therapy, (Table 4). Chemotherapy either coded as a multinomial or dichotomized covariate was not associated with the occurrence of fractures in any of the analyses. Statistically the proportional hazards assumption was violated for covariates age and endocrine therapy, specifically for patients treated with AIs and for patients in the combined group TAM + AI. This was also assumed from the cumulative incidence plot. However, when assessed graphically the violations were not severe. As a consequence of this and due to the very large sample size with many events, it was surmised

Table 3. Summary of the multivariable PWP regression analyzes for pre- and postmenopausal women.

Covariate	Relative risk (95% confidence interval)	*p* value
Premenopausal women ($n = 19,200$)		
Age (years)	1.015 (1.010–1.021)	<.0001
CCI		
No comorbidity	1.0	
Category 1	1.40 (1.25–1.56)	<.0001
Category 2	1.45 (1.18–1.77)	<.0001
Category 3+	1.93 (1.46–2.56)	<.0001
Endocrine therapy		
None	1.0	
TAM + AI	1.00 (0.93–1.08)	.99
TAM	0.95 (0.88–1.03)	.22
Other	1.06 (0.86–1.31)	.59
Postmenopausal women (n = 47,302)		
Age (years)	1.031 (1.029–1.033)	<.0001
CCI		
No comorbidity	1.0	
Category 1	1.25 (1.20–1.31)	<.0001
Category 2	1.26 (1.18–1.34)	<.0001
Category 3+	1.49 (1.38–1.62)	<.0001
Endocrine therapy		
None	1.0	
AI	1.07 (1.02–1.12)	.0066
TAM + AI	0.89 (0.85–0.93)	<.0001
TAM	0.88 (0.83–0.93)	<.0001
Other	0.72 (0.54–0.96)	.025

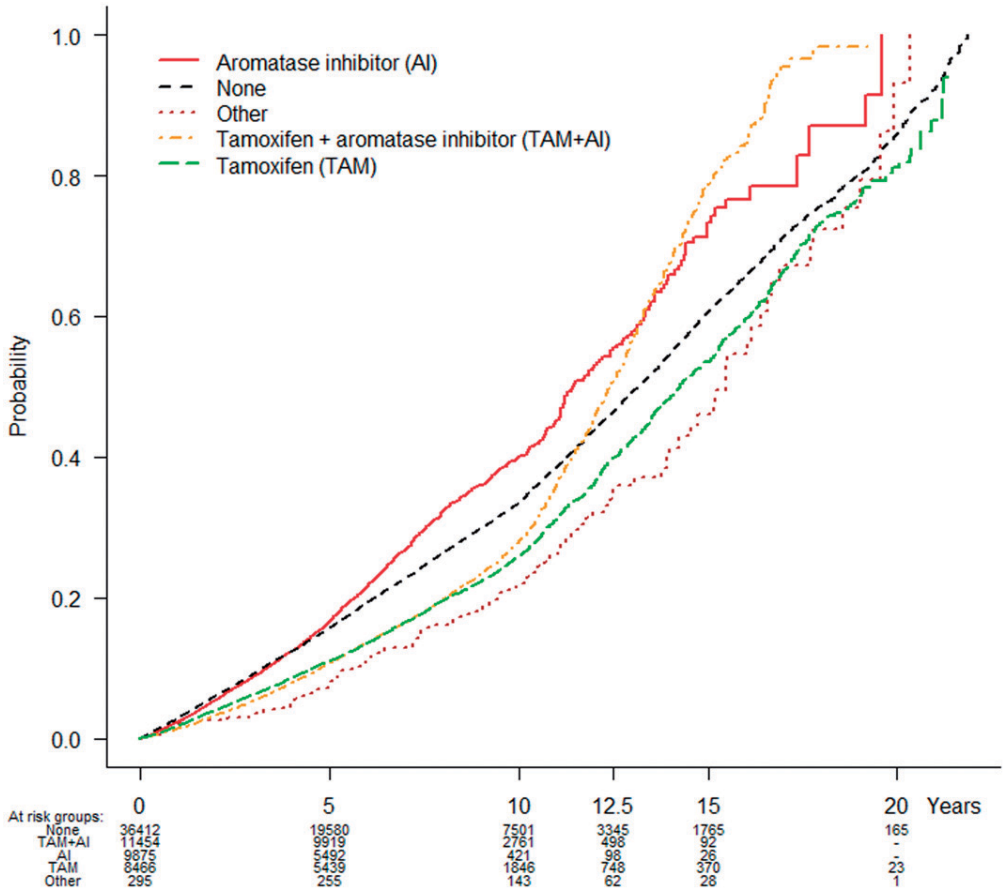


Figure 1. The cumulative fracture incidence curves for endocrine treated breast cancer patients.

Table 4. Summary of the final multivariable PWP regression analysis, all patients ($n = 66,502$).

Covariate	Relative risk (95% confidence interval)	p value
Age (years)	1.031 (1.028–1.032)	<.0001
Menopause		
Premenopause	1.0	
Postmenopause	0.82 (0.78–0.87)	<.0001
CCI		
No comorbidity	1.0	
Category 1	1.28 (1.23–1.34)	<.0001
Category 2	1.30 (1.22–1.37)	<.0001
Category 3+	1.55 (1.43–1.68)	<.0001
Endocrine therapy		
None	1.0	
AI	1.10 (1.05–1.15)	<.0001
TAM + AI	0.90 (0.87–0.94)	<.0001
TAM	0.88 (0.84–0.92)	<.0001
Other	0.84 (0.70–1.01)	.060

that the nonproportionality would make no difference to the interpretation of the data [14].

Discussion

In a nationwide population-based study, we have demonstrated a highly significant and clinically important association between adjuvant AI treatment and an increased risk of fractures. In contrast, the risk of fractures was decreased in patients treated with TAM alone as compared to endocrine untreated patients, suggesting a protective effect regarding fractures. These results are in accordance with other studies reporting on the occurrence of fractures in breast cancer patients treated with adjuvant TAM and AIs [5,6,16,17].

The course for the TAM + AI-treated patients is particularly interesting, because the patients in this group were first treated with TAM for an intended 5-year period followed by intended 5-year treatment with AI. This suggests an initial osteoprotective effect of TAM that is later dissolved due to the succeeding treatment with AI. The curve for the TAM-treated patients suggests that TAM provides a long-lasting effect on bone metabolism similar to the suggested long-lasting antineoplastic effect [18]. The curve for the last 'other' group similarly suggests an osteoprotective effect. However, the sample size for this group was small and so the curve shape should be interpreted with caution. This reservation is supported by the nonsignificant association in the multivariable model. Endocrine therapy was entered in the statistical models as a categorical covariate. However, as the endocrine therapy has been extended considerably during the study period it would be highly relevant to include the treatment duration as a covariate to clarify a possible dose response association.

The incidence of osteoporosis and the risk of later fractures increase with aging thus making age a known risk factor. In the present study, menopause at 55 years seemed to have a protective potential. This complies with early menopause being a known risk factor for osteoporosis, sustaining fragility fractures and increased mortality [19]. A late menopause postpones the period with accelerated bone loss associated with the menopause preceding the unavoidable bone loss associated with aging. An age of 55 years as a cut point for menopause was selected for comparability with other studies [20].

The study demonstrated a very strong association with increasing comorbidity as assessed by the CCI. This emphasizes the importance of taking into consideration other chronic diseases as for example diabetes mellitus [21] besides the breast cancer when an adjuvant treatment is advised. As an aid, an online service for health care professionals for calculating a fracture risk score, FRAX exists [22]. However, only some of the chronic disorders incorporated in CCI are included in FRAX such as diseases causing secondary osteoporosis.

Nevertheless, our study combined data from three registers and the available data were not intended for analyzing the occurrence of fractures. Other limitations include lack of reliable information on possibly important covariates like falling tendency, height, weight, smoking habits, alcohol use, osteoporosis, drug use, vitamin D status and bone mineral density. Other important confounders may not have been identified, measured and registered and may thus have had an unaccountable impact on the results.

It was not possible to clarify the causes of each fracture due to the large number and some surely have been caused by factors, for example, high-energy impact or focal bone pathology unrelated to the main question in this study. Fractures that occurred early in the study period were counted. Some of these were probably also unrelated to the main question in the study. On the other hand, it is well known that many fractures in the spine are not diagnosed [23], so the number of fractures may actually have been larger. Fractures in the forearm, femur, upper arm, lower leg, and hand were the most frequent in the study. These fractures are likely to cause pain and disability necessitating professional health care. For this reason, they are probably not underdiagnosed to the same extent as vertebral fractures. Counting just one different fracture location in each patient may also have underestimated the true number of fractures in the study cohort.

The concomitant use of antiosteoporotic treatment in the study period may have had an impact on the number of registered fractures possibly lowering the occurrence. Even so, it was already known during the study period that AIs had unwanted side effects [24] and TAM had beneficial effects on bone metabolism [25]. As a consequence, patients planed for AI treatment are recommended a bone density measurement as a routine. In light of such knowledge, one would expect the AI-treated patients to be treated with anti-osteoporotic drugs with a higher probability than TAM-treated patients and this would tend to reduce the occurrence of fractures in the AI-treated patients. However, judged by the study outcome this does not seem to be the case thus favoring our interpretation of the results.

In conclusion, our study supports previous studies that treatment with AIs in postmenopausal women is accompanied by an increased occurrence of fractures and that TAM has an osteoprotective effect resulting in a decrease in fracture occurrence in postmenopausal women. When advising adjuvant therapy with AIs especially fragile patients with chronic diseases should receive special attention including

regular bone density measurements in order to reduce the occurrence of fractures in this particularly vulnerable group of patients.

Disclosure statement

The authors have no conflicts of interest to report. The authors alone are completely responsible for the content, analysis, and writing of the paper.

References

[1] Jensen M-B, Ejlertsen B, Mouridsen HT, for the Danish Breast Cancer Cooperative Group, et al. Improvements in breast cancer survival between 1995 and 2012 in Denmark: the importance of earlier diagnosis and adjuvant treatment. Acta Oncol. 2016;55:24–35.

[2] Curigliano G, Burstein HJ, Winer EP, on behalf of the Panel Members of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2017, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol. 2017;28: 1700–1712.

[3] Lumachi F, Luisetto G, Basso SMM, et al. Endocrine therapy of breast cancer. Curr Med Chem. 2011;18:513–522.

[4] Dixon JM, Renshaw L, Young O, et al. Letrozole suppresses plasma estradiol and estrone sulphate more completely than anastrozole in postmenopausal women with breast cancer. J Clin Oncol. 2008;26:1671–1676.

[5] Rabaglio M, Sun Z, Price KN, et al. Bone fractures among postmenopausal patients with endocrine-responsive early breast cancer treated with 5 years of letrozole or tamoxifen in the BIG 1-98 trial. Ann Oncol. 2009;20:1489–1498.

[6] Cooke AL, Metge C, Lix L, et al. Tamoxifen use and osteoporotic fracture risk: a population-based analysis. J Clin Oncol. 2008;26: 5227–5232.

[7] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet. 2015;386:1353–1361.

[8] Schmidt M, Schmidt SAJ, Sandegaard JL, et al. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol. 2015;7:449–490.

[9] Pedersen CB. Description of Danish Registers. The Danish Civil Registration System. Scand J Public Health. 2011;39:22–25.

[10] Charlson ME, Pompei P, Ales KL, et al. new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–383.

[11] Amorim LD, Cai J. Modelling recurrent events: a tutorial for analysis in epidemiology. Int J Epidemiol. 2015;44:324–333.

[12] Hosmer DW, Lemeshow S, May S. Applied survival analysis. Regression modeling of time-to-event data. 2nd ed. New York: Wiley; 2008.

[13] R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL Available from: https://www.R-project.org/

[14] Therneau TM, Grambsch PM. Modelling survival data: extending the cox model. 2nd ed. New York, NY: Springer; 2000.

[15] Klotzbuecher M, Ross PD, Landsman PB, et al. Patients with prior fractures have an increased risk of future fractures: a summary of the literature and statistical synthesis. J Bone Miner Res. 2000;15:721–739.

[16] Vestergaard P, Rejnmark L, Mosekilde L. Effect of tamoxifen and aromatase inhibitors on the risk of fractures in women with breast cancer. Calcif Tissue Int. 2008;82:334–340.

[17] Neuner JM1, Yen TW, Sparapani RA, et al. Fracture risk and adjuvant hormonal therapy among a population-based cohort of older female breast cancer patients. Osteoporosis Int. 2011;22:2847–2855.

[18] Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15- year survival: an overview of the randomised trials. Lancet. 2005;365:1687–1717.

[19] Svejme O, Ahlborg HG, Nilsson JÅ, et al. Early menopause and risk of osteoporosis, fracture and mortality: a 34-year prospective observational study in 390 women. BJOG. 2012;119:810–816.

[20] Koopal C, Janssen-Heijnen ML, van de Wouw AJ, et al. Fracture incidence in pre- and postmenopausal women after completion of adjuvant hormonal therapy for breast cancer. Breast. 2015;24:153–158.

[21] Majumdar SR, Leslie WD, Lix LM, et al. Longer Duration of Diabetes Strongly Impacts Fracture Risk Assessment: The Manitoba BMD Cohort. J Clin Endocrinol Metab. 2016;101: 4489–4496.

[22] FRAX. Fracture Risk Assessment Tool. Available from: https://www.sheffield.ac.uk/FRAX/index.aspx

[23] Delmas PD, van de Langerijt L, Watts NB, et al. Underdiagnosis of Vertebral Fractures Is a Worldwide Problem: The IMPACT Study. J Bone Miner Res. 2005;20:557–563.

[24] Lester J, Coleman R. Bone loss and the aromatase inhibitors. Br J Cancer. 2005;93 (Suppl 1):S16–S22.

[25] Kristensen B, Ejlertsen B, Dalgaard P, et al. Tamoxifen and bone metabolism in postmenopausal low risk breast cancer patients: a randomized study. J Clin Oncol. 1994;12:992–997.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 146–151
https://doi.org/10.1080/0284186X.2017.1406141

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Quality of life and care needs in women with estrogen positive metastatic breast cancer: a qualitative study

Gitte Lee Mortensen[a], Ivan Bredbjerg Madsen[a], Randi Krogsgaard[b] and Bent Ejlertsen[c]

[a]AnthroConsult, Aarhus C, Denmark; [b]Danish Breast Cancer Organization, Holte, Denmark; [c]Department of Oncology, DBCG Secretariat, Rigshospitalet, Copenhagen Ø, Denmark

ABSTRACT

Background: In recent years, the prognosis of metastatic breast cancer (MBC) has improved with more effective therapies applicable to a wider range of patients. To many patients, a MBC diagnosis thus initiates a prolonged course of illness and treatment. This qualitative study aimed to explore the long-term health-related quality of life (HRQoL) and support needs in MBC patients of all ages in the Danish context.

Material and methods: Eighteen MBC patients participated in five qualitative focus group interviews that were analyzed using content analysis and a constructivist approach.

Results: The participants described how MBC severely reduced their physical and psychosocial functioning and required a constant adaptation of their quality of life (QoL) standards in relation to their changing life situation and disease progression. Overall, they felt medically well-treated but lacked a multidisciplinary approach to care including psychological support, in particular, but also manual physiotherapy, health care coordination and social counseling. The participants called for continuity of care with the same health care professionals as this facilitated communication and flexibility in planning treatment and controls. They requested a reduction of precious time spend on treatment to enable them to focus on their most meaningful relations and activities.

Conclusion: With the MBC diagnosis, the focus of treatment switches from disease eradication to prolonging survival, alleviating symptoms and improving QoL. To patients, MBC marks a shift in expectations from quantity to quality of life and a perpetual adaptation of their QoL standards. To sustain patients' HRQoL, it is important that along with improvements in life-prolonging treatment, comprehensive care also supports their main psycho-social needs. These patients needed support in maintaining normality and role functioning enabling them to focus on living, not merely surviving, through this prolonged disease phase.

ARTICLE HISTORY
Received 23 September 2017
Accepted 9 November 2017

Introduction

Breast cancer (BC) is the most common cancer in women worldwide. In developing countries it is the second cause of death after lung cancer. The prevalence of distant metastasis is high in patients with locally advanced cancer (LBC) including those with large tumors (>5 cm) and noticeable nodal disease (> 3 cm), even if asymptomatic [1]. About 5% of BC patients in Western countries are diagnosed with LBC or metastatic breast cancer (MBC) while around 30% of the patients with early breast cancer (EBC) eventually develop MBC [2–4]. While breast cancer mortality overall has decreased as a result of earlier detection and adjuvant treatment, only minor survival gains have been achieved in MBC though individual patients may obtain prolonged survival, predominantly following endocrine or HER2 targeted treatment. With no cure yet available for MBC, palliation and prolongation of survival are the main treatment goals. Median survival is 2–3 years but as long as 15 years in indolent disease [2,5] implying that a diagnosis with MBC often initiates a prolonged course of illness and treatment rather than immediate end-of-life care [6]. This augments the importance of examining MBC patients' health-related quality of life (HRQoL), i.e. the disease's impact on their physical and psycho-social well-being and everyday lives, and needs to provide optimal care and support in this phase of disease.

The complex nature of metastatic disease including the patient's subtype of BC, menopausal status, age, comorbidity and previous treatment experiences must be taken into account when discussing treatment options [3,7–9]. Emphasis will predominantly be on molecular targets such as estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). Common side effects of treatment (targeted treatment, chemo- and radiotherapy) are fatigue, nausea, vomiting, hair loss, diarrhea, peripheral neuropathy, menopausal symptoms, poor sleep and skin toxicity [3,10–15]. Palliation may also include bone modifying agents, pain medication, antidepressants or anti-emetics, for instance [7,10].

CONTACT Gitte Lee Mortensen glm@anthroconsult.dk AnthroConsult, Aarhus C, Denmark

Supplemental data for this article can be accessed here.

MBC patients' quality of life (QoL) has been shown to be severely reduced with symptoms and side effects having a detrimental impact on psychosocial and physical functioning, working ability, family life and couple relations [2,6,10–13,16–19]. Fatigue is dominant but depending on the extension and localization of metastasis, physical symptoms such as pain, dyspnea, and lymphedema are frequent. Emotionally, MBC patients are much affected by treatment response and recurrences, and uncertainty, grief, lack of control, fear of death and loss of identity and future life often lead to depression and anxiety [2,10,11,16,20]. Physical and psychological symptoms tend to exacerbate one another [10,11,21]. It has been suggested that younger women and mothers may have lower HRQoL and functioning as compared to older women [6,12]. Despite such serious QoL impairment, MBC patients often have an unmet need for information about treatment options and prognosis, as well as management of symptoms, side effects and psychological problems [2,5,6,10,12,14,22].

While quantitative studies have described factors reducing QoL in MBC, studies exploring the relations between these issues, i.e. providing explanations of *why* certain symptoms are important to patients and *how* they affect their QoL and support needs, are lacking. This is particularly the case in the present context of improved treatment options and prolonged life expectancy. As most QoL studies have been linked to clinical trials, there is also shortage of studies examining HRQoL and care needs in MBC patients >60 years of age albeit the median age at BC diagnosis is above that [3,9]. To our knowledge, studies of Danish MBC patient perspectives are absent. The present qualitative study therefore aimed to explore in depth the HRQoL of Danish MBC patients of all ages and how this might be related to their support needs.

Material and methods

An initial search for literature was carried out in PubMed, Embase, PsycInfo and Artikelbasen yielding 635 abstracts of articles published in English 2010–2015 of which 74 were selected for full-text analysis. The literature study informed the qualitative study design including a question guide used in focus group interviews with 18 MBC patients. Focus groups were chosen to elicit a broad variety of patient perspectives on the women's experiences and needs. Participants were invited by The Danish Breast Cancer Organization (DBO) encouraging interested patients to contact the researchers directly for further information and screening. Eligible patients were diagnosed with estrogen receptor-positive MBC, i.e. the most common type of BC, to enhance comparability. Due to the focus on subjective patient perspectives and experiences, no medical records were collected. Informed written consent was obtained, participation was anonymous and ethical committee approval was therefore not required. Five small focus groups – representing all regions of Denmark – were held with 3–4 participants in each, to create confidential settings for discussions among peers about their HRQoL and supporting care needs.

The focus groups were set in regional health centers or conference rooms and lasted two hours. They were moderated by G Lee Mortensen, assisted by IB Madsen. When introducing the focus group discussions, the participants were told of the aim to explore all experiences and encouraged to voice converging as well as diverging perspectives, provide examples and elaborate on their own and others' experiences and expressed needs. The questions in the semi-structured interview guide were open-ended [23,24] and began with questions to the participants' disease trajectory: time of first diagnosis with BC, time of diagnosis with MBC, subsequent disease development and current disease status. The women then described their reactions to the MBC diagnosis followed by its physical, cognitive, psychosocial and professional impact on their QoL and functioning. They then described their treatment experiences, and finally, their medical and non-medical care needs (Supplementary file: Question guide).

The interviews were transcribed verbatim and analyzed using Nvivo 8 software (QSR International) and a combination of inductive content analysis and a constructivist approach, i.e. focusing on how the language used in the focus groups unveiled how patterns of meaning were created in social interaction among peers and in relation to each patients' personal situation [25,26]. First, the transcripts were read through numerous times (independently, by three researchers) to get familiar with the data. Second, the data was coded (categorized) into moderator- and participant-generated topics that were raised during the discussions. Third, main themes within each topic were identified, and finally, recurrent connections between topics and themes were analyzed. This generated a pattern of the relative significance that the topics and themes had for the participants, i.e. the main QoL impact of MBC and the participants' most important care needs. All methodological and analytical steps were discussed among GLM, IBM and an independent qualitative researcher (SLM) until agreement was reached.

Results

The participants resided in 17 different towns and attended 11 hospitals across Denmark; they were aged between 41 and 72 years old, and had been diagnosed with MBC for 1–11 years. At the time of MBC diagnosis they were aged between 35 and 65 years reflecting great variation of life situations with respect to family, e.g. age of children, and work status. 16 participants had been well for 2–16 years following treatment of EBC; two had MBC at diagnosis (Supplementary file: Table 1).

Two main topics were discussed in the focus groups: The quality of life impact of living with MBC and patient needs for treatment and care, both including a number of subthemes to be described below (Supplementary file: Figure 1).

Quality of life impact of metastatic breast cancer

The first main topic included several subthemes: (a) reactions to the MBC diagnosis, (b) cognitive, (c) physical, (d) psychological, and (e) social/relational QoL aspects of MBC, and (f) strategies to cope with MBC.

(a) The participants described reacting to the MBC diagnosis with shock and fear of imminent death. This was aggravated by hearing that only 'life-prolonging treatment' remained which they interpreted as implying very short life expectancy. Once new treatments were initiated, the women's anxiety was somewhat reduced by learning that they might have more treatment options and time than expected. Still, the MBC diagnosis was depicted as marking an end to 'ordinary everyday life' with family life, work, social and leisure activities.

(b) Many participants described *cognitive* problems with memory and concentration but none felt this significantly reduced their QoL. (c) More importantly, their *physical QoL and functioning* was severely impaired by symptoms such as pain, gastrointestinal upsets, flu symptoms, nausea, cardiovascular dysfunction, edema, stomatitis, neuropathy, stiffness of muscles and joints, dyspnea, dizziness, problems with sleep and gait, hair loss and menopausal symptoms. Fatigue was particularly limiting the women's role functioning and participation in activities bringing meaning and joy to life. (d) Yet the main hardship of MBC was said to be *emotional*. Most participants had recurrent spurs of depression and anxiety, especially when feeling particularly poorly and with the occurrence of new metastases. Metastasis to vital organs and the brain were the most dreaded sites. Living on borrowed time was stressful and included alertness to possible symptoms of relapse, hypersensitivity or propensity to anger in some.

> When I relapsed, I just sat down in my couch for half a year and thought "this is the end" because he said it was life-prolonging treatment now. It was extremely stressful. You never know how long "life-prolonging" is, they cannot tell you that. But just when you hear it, you think it's half a year… It is so stressful not knowing if you're here in half a year. For instance, when my daughter said she was getting married in a year. In one year?! If only she had said "next week"! As it turns out, that's a year ago now, but you don't know that (FG2B).

> That's right, you always feel the guillotine hanging above your neck (FG2A).

(e) Regarding the women's relational QoL, children's welfare was the main concern, especially to mothers of young children. Some had written letters or speeches for children's future communions and weddings or recorded stories from their own lives for them. The women had become acutely aware of prioritizing meaningful activities and relations. Overall, they tired easily with many people and those with children tended to focus on the close family, while older patients were more active, socially and with leisure activities. Having a supporting partner and family was crucial to cope with the disease, but MBC was a heavy burden on partner relations and spouses who often reacted with depression and anxiety. While most felt their marriages had been strengthened after the MBC diagnosis, four had chosen to divorce despite the solitude of missing intimacy and daily support.

(f) Finally, the participants described their strategies to cope with living with MBC. Although distressing, many found that 'getting things sorted' – e.g. making arrangements for the funeral – enabled a feeling of control and to focus on living: 'It allowed me to put the disease on a separate shelf and then live life on the other shelves' (FG2B). Coping with MBC required a constant adaptation of individual QoL standards and changed roles vis-à-vis the patients' self-perceptions and social relations. For instance, 'while you have children at home it is either sink or swim; when they are fledged and move out, you have to adapt to a new situation' (FG4D). Maintaining normality – especially upholding one's role functioning as a mother, grandmother, partner or professional – was of major importance. Half of the women found that even a few hours of work per week allowed them to still feel useful, to get out of the house and keep their minds busy 'to stay sane'. Others felt that stopping work had been a great loss. Setting milestones like turning 40 years old or attending a baptism, was described as a crucial means to stay strong. Fortunately, many milestones were reached and new ones set along the way. Many expressed surprise at how long they had survived with MBC and that the disease course had not merely been downhill.

Treatment and support needs

The second major topic concerning the participants' treatment and supporting care needs included the subthemes: (a) treatment needs, (b) worries related to treatment and controls, (c) minimizing time spend on treatment, (d) socio-economical clarification, (e) psychological counseling, and (f) information needs.

(a) The women mostly felt they received adequate symptom treatment but some called for improved options of receiving manual physiotherapy to alleviate the physical pain and discomfort. Although dealing with symptoms and treatments was challenging, they 'grew with the task' and would accept almost any medication that might keep them alive.

(b) Controls and waiting for test results tended to reactivate concerns about whether the cancer had spread, about running out of treatment options and the QoL impact of the next treatment.

> I'm thinking more and more about how long this treatment will work. How long will my luck last? It won't last forever, you know? I have lasted ten years now, but it is getting increasingly difficult to get through those scans (FG1B).

> The main concern is whether the treatment you are receiving continues to work and if the side effects are getting worse. The worries arise: 'what if you have to stop this, what will you then have to go through? Do they have more options in store – and for how long do they have more? And will the side effects be worse than the ones I have now?' (FG1A).

(c) The limited time perspective was closely related to the women's care needs. They felt that treatment and controls were time consuming and requested short waiting times for test results at all hospitals and the assemblage of as many medical issues as possible at the specialized clinic. Acting themselves as coordinators between health professionals – some seemingly unfamiliar with MBC related issues – was challenging. All preferred seeing the same or very few oncologists as this ensured a feeling of continuity and confidence. This again facilitated flexibility in planning treatment and

discussing test results or concerns over the telephone – all with an end to minimize the disruption of everyday life.

> After the examination of my stomach – cause that's where they thought something was wrong – the doctor said 'there is some cancer going on in L2'. I asked what would happen next. 'Why, he didn't know that; he didn't even know if there was anything that could be done at all'. So I asked for my medical record so that I could go see an oncologist and ask myself. Then he told me to take it easy. I became so angry and said that he shouldn't bloody hell tell me to take it easy! 'I have two small children. I do not have time for this nonsense! (FG2D).

> Our time is important. That is the thing with all the waiting, well, 'I have a job for God's sake, I could have worked during those hours'! … It's like, when you're a patient, that's all you are, and you're available when they have time. That needs to be turned around (FG4A).

(d) The participants called for a more comprehensive approach to MBC care including professional social support. Women with cognitive problems particularly wished for a health care coordinator helping with the various medical and social aspects of living with MBC, e.g. getting rapid clarification of their financial support options.

> I really appreciate the 'stand-by plan' according to which they [the job centre] don't have to follow up on you all the time … Because I just try to live my life every single day without the disease taking up too much space and that is such a struggle if social workers call you up constantly to ask how you're doing … And then you get to sit there afterwards and think 'God, yes, I do actually feel awful and there are all these things I can't do'. The option of staying on sickness benefit [with a flex job in this case] rather than receiving disability pension is really good' (FG4D).

> I've tried to get that but, unfortunately, I didn't succeed yet. It would give me some peace of mind (FG4A).

(e) Lacking psychological support was the greatest unmet need. Participants attending one hospital much appreciated being offered psychological counseling at the hospital; all strongly believed this should be offered everywhere.

> When I was told there was nothing they could do, that it had spread so much, I thought to myself 'when is a psychologist entering that door?' It seemed absolutely insane that this doctor and nurse just sat there – and they do say things quite directly when something's wrong. After that, you are supposed to just … go home? You are devastated. They should have offered something there … They should ask if you need to talk to someone. You can always say no. They do ask you how you're doing and if you have any questions, so I guess you could bring it up yourself. But I've got the impression that it's easier to get morphine than a psychologist (FG5B).

> Yes, and a crisis can also appear later on, when you get home and it starts to dawn on you. Then too it would be nice if you could contact someone. I've heard of many who get a depression when they're actually over it [the first shock], after half a year. Surely, they must know that out there … and be able to tell you (FG5D).

Participants living alone particularly needed professional psychological support to cope with MBC being 'like a rollercoaster switching between anxiety and relief' (FG4D).

(f) The final needs-related subtheme pertained to the participants' information needs. One hospital was commended for offering patient education courses for new MBC patients, including information on treatment, physical and emotional disease aspects and a visit to relevant hospital departments. This too was requested at all hospitals. While most felt adequately informed about standard treatment options, a few called for early genomic testing to prepare for targeted or experimental treatment and information about treatment options abroad. Others requested independent professional advice regarding non-medical options of symptom management and fortification, issues that are much discussed in patient networks. The Danish Cancer Society and DBO organizes highly valued services such as meetings, mindfulness courses and support groups for children. An unmet need was expressed for individual counseling of spouses who were often severely troubled but uneasy with group support.

Just as importantly – and reflecting the prolonged life expectancy in MBC – some participants underlined a need to hear more 'good stories' about MBC patients. They had heard much about the hardships of MBC and prepared themselves for dying, but had lacked complementary help to focus on living – on *'how to grab life'* (FG4A). Especially at the time of diagnosis, the patients needed stories about how women live with MBC and may indeed lead good lives for several years. This prospect led some to prefer coining MBC as a 'chronic' disease while cringing at terms like 'incurable' cancer and 'life-prolonging treatment'.

Discussion

Improved treatment options imply that, today, a MBC diagnosis often marks the beginning of a prolonged course of illness and treatment with ensuring optimal QoL being a major aim. The present study explored how Danish MBC patients' QoL was related to their main support needs during this particular disease phase.

Patient experiences with disease are related to their sociocultural context. Our participants' expectations, needs and priorities were based on their personal situation as well as a societal context with public health care and various financial support options. Danish MBC patients are thus not representative of MBC patients worldwide. A selection bias should also be considered as our participants were relatively high-functioning MBC patients that may not reflect the experiences or lower functioning patients. We believe, however, that the care needs expressed by our participants may well be even more pronounced in patients with poorer function and caregiver support. With these reservations, our qualitative methodology allowed for an in-depth understanding of MBC patient perspectives, i.e. analytical generalization, but they do not support statistical generalization. Our analysis, however, has the strength of representing patient perspectives unrelated to a clinical trial.

MBC severely reduced the participants' HRQoL and functioning. This was one of the two main topics and the women's descriptions of its various subthemes revealed that despite substantial physical symptoms, the psychological burden was the hardest and socio-relational QoL aspects of key importance. This ordering of QoL aspects is likely affected by the participants' satisfaction with medical treatment as well

as a health care system providing financial security. The participants described a dynamic perception of their QoL as requiring constant adjustment to their changing circumstances and disease progression. For instance, the younger women focused much on their children's present and future welfare and struggled with maternal role functioning. Many also felt a loss of feminine and professional identity. Such altered roles are well-known to challenge patients and their relations [2,6,11,13,15]. Yet while patients' physical well-being has been shown to decrease with older age, social and functional well-being tends to increase [6]. In our analysis, this may be due to impaired physical functioning interfering relatively less with the *roles, self-perception and expectations* of older women. Still, they too prioritized the potential benefits of treatment over the risk of side effects and would accept almost any treatment giving them extra time – a priority that has previously been shown in younger MBC patients [3,5,18,19,27].

Others have shown that goal adjustment is indeed crucial to coping, i.e. disengaging from unattainable goals and reengaging in more attainable goals that are often more immediately rewarding and emotionally meaningful [28]. Our participants had become keenly aware of prioritizing meaningful relations and activities and set milestones for events they hoped to live to experience. They described their course of disease as fluctuating between periods of relative well-being and tougher patches – 'a rollercoaster' – which is in line with what Sarenmalm et al. described as a dialectal process of suffering and easing distress and constantly 'making sense of living under the shadow of death' [11]. Following the improved life expectancy, some of our participants preferred coining MBC as a *chronic* condition and called for an increased support in how to *continue* life rather than ending it. They especially needed support in upholding identity and normality with respect to work and role functioning as much as possible. Continuity was thus a key aspect of their QoL priorities and coping with MBC.

Following these main QoL concerns with psychosocial issues being vital, and pertaining to the second main topic, the participants requested a more comprehensive approach to the care of MBC patients. This supports that, seen from a patient perspective, cancer is a biopsychosocial illness, yet cancer care is mostly bio-medically focused [11]. Our participants lacked a multidisciplinary approach to care, primarily including ongoing management of psychological issues, possibly intensified at diagnosis, in patients living alone and with the occurrence of new metastases. Psychological support of spouses, who can be principal sources of support but are often destabilized by anxiety and depression [17], also appeared lacking. Finally, our participants requested manual physiotherapy, health care coordination and social counseling to clarify their options of financial support and perhaps maintain some professional activity, e.g. via a flexible work position. Our study supports previous findings that work and keeping busy are crucial to maintain normality and QoL [13,16].

Living a medicalised lifestyle is stressful and time consuming [19]. To MBC patients, time is sparse and precious. The less time spend on treatment related activities, the more time for meaningful activities – i.e. *living*. Minimising medical disruptions of everyday life, e.g. by reducing waiting times and pooling medical issues, are thus ways to maintain normality and support QoL as conveyed by the patients. Continuity of care with the same health care professionals enabled this and was thus of great importance to our participants. Overall, they felt adequately informed, but just like other MBC patients, some requested more knowledge about clinical trials, treatment options abroad and non-medical symptom alleviation and fortification [5].

With the MBC diagnosis, the focus of treatment switches from disease eradication to prolonging survival, alleviating symptoms and improving QoL [2,5]. To patients, MBC marks a shift in expectations from quantity to quality of life and a perpetual adaptation of their QoL standards. To enhance patients' QoL, it is important that along with improvements in life-prolonging treatment, comprehensive care supports their changing physical and psycho-social support needs. Finally, we suggest that more attention should be paid to the needs of older women who also want a full life as long as possible but have received less attention despite constituting the majority of MBC patients [8].

Acknowledgments

The authors wish to thank all the participants as well as the local members of the Danish Breast Cancer Organization (DBO) who helped to include the participants in the study. We also thank social anthropologist Steen Lee Mortensen for providing independent methodological and analytical counseling as well as Pfizer, Denmark, for the research grant funding the study.

Disclosure statement

AnthroConsult, which is owned by GLM, received an unrestricted research grant from Pfizer Denmark to carry out the present study. However, Pfizer had no involvement in the methodological design, analysis, manuscript writing or choice of journal. AnthroConsult has previously received research grants from Pfizer for other studies, though none related to breast cancer. IBM and RK have no conflicting interests. Institutionally, Rigshospitalet has received research funding from Roche, Novartis and NanoString for other projects, but BE has no conflicting interests in relation to the present project.

Funding

The study was funded by an unrestricted research grant from Pfizer, Denmark. Pfizer had no involvement in the methodological design, analysis, manuscript writing or choice of journal.

ORCID

Gitte Lee Mortensen http://orcid.org/0000-0001-9750-2493

References

[1] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:359–386.

[2] Kriegel S, Myers J, Befort C, et al. 'Cancer changes everything!'. Exploring the lived experiences of women with metastatic breast cancer. Int J Palliat Nurs. 2014;20:334–342.
[3] Gupta S, Zhang J, Jerusalem G. The association of chemotherapy versus hormonal therapy and health outcomes among patients with hormone receptor-positive, HER2-negative metastatic breast cancer: experience from the patient perspective. Expert Rev Phamacoecon Outcomes Res. 2014;6: 929–940.
[4] Gerber B, Seitz E, Müller H, et al. Perioperative screening for metastatic disease is not indicated in patients with primary breast cancer and no clinical signs of tumor spread. Breast Cancer Res Treat. 2003;82:29–37.
[5] Harding V, Afshar M, Krell J, et al. 'Being there' for women with metastatic breast cancer: a pan-European patient survey. Br J Cancer. 2013;109:1543–1548.
[6] Reed E, Simmonds P, Haviland J, et al. Quality of life and experience of care in women with metastatic breast cancer: a cross-sectional survey. J Pain Symptom Manage. 2012;43:747–758.
[7] Cardoso F, Costa A, Norton L, et al. ESO-ESMO 2nd international consensus guidelines for advanced breast cancer (ABC2). Ann Oncol. 2014;25:1871–1888.
[8] Glück S, von Minckwitz G, Untch M. Aromatase inhibitors in the treatment of elderly women with metastatic breast cancer. Breast. 2013;22:142–149.
[9] Jolly T, Williams GR, Jones E, et al. Treatment of metastatic breast cancer in women aged 65 years and older. Womens Health (Lond Engl). 2012;8:455–471.
[10] Irvin W, Jr, Muss HB, Mayer DK. Symptom management in metastatic breast cancer. Oncologist. 2011;16:1203–1214.
[11] Sarenmalm EK, Thorén-Jönsson AL, Gaston-Johansson F, et al. Making sense of living under the shadow of death: adjusting to a recurrent breast cancer illness. Qual Health Res. 2009;19: 1116–1130.
[12] Willis K, Lewis S, Ng F, et al. The experience of living with metastatic breast cancer-a review of the literature. Health Care Women Int. 2014;36:514–542.
[13] Vilhauer RP. A qualitative study of the experiences of women with metastatic breast cancer. Palliat Support Care. 2008;6: 249–258.
[14] McClelland SI, Holland KJ, Griggs JJ. Vaginal dryness and beyond: the sexual health needs of women diagnosed with metastatic breast cancer. J Sex Res. 2015;52:604–616.
[15] Denieffe S, Cowman S, Gooney M. Symptoms, clusters and quality of life prior to surgery for breast cancer. J Clin Nurs. 2014;23:17–18.
[16] Warren M. Uncertainty, lack of control and emotional functioning in women with metastatic breast cancer: a review and secondary analysis of the literature using the critical appraisal technique. Eur J Cancer Care. 2010;19:564–574.
[17] Badr H, Shen MJ. Pain catastrophizing, pain intensity, and dyadic adjustment influence patient and partner depression in metastatic breast cancer. Clin J Pain. 2014;30:923–933.
[18] White CB, Smith ML, Abidoye OO, et al. Impacts of toxicity on patient treatment choices for metastatic breast cancer. J Clin Oncol. 2012;30:18.
[19] Ghislain I, Zikos E, Coens C, et al. Review. Health-related quality of life in locally advanced and metastatic breast cancer: methodological and clinical issues in randomised controlled trials. On behalf of the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Group the Breast Cancer Group EORTC Headquarters. Lancet Oncol. 2016;17:294–304.
[20] Hurvitz SA, Lalla D, Crosby RD, et al. Use of the metastatic breast cancer progression (MBC-P) questionnaire to assess the value of progression-free survival for women with metastatic breast cancer. Breast Cancer Res Treat. 2013;142:603–609.
[21] Faller H, Strahl A, Richard M, et al. Symptoms of depression and anxiety as predictors of physical functioning in breast cancer patients. A prospective study using path analysis. Acta Oncol 2017;56:1677–1681.
[22] Grimsbø GH, Finset A, Ruland CM. Left hanging in the air: experiences of living with cancer as expressed through E-mail communications with oncology nurses. Cancer Nurs. 2011;34:107–116.
[23] Halkier B. Fokusgrupper [Focus groups]. Roskilde, Denmark: Roskilde University Press; 2003.
[24] Kvale S. Interviews. An introduction to qualitative research interviewing. Thousand Oaks [CA]: Sage; 1996.
[25] Winther JM, Phillips L. Diskursanalyse som teori og metode [Discourse analysis: a theory and methodology]. Roskilde, Denmark: Roskilde University Press; 1999.
[26] Elo S, Kyngäs H. The qualitative content analysis process. J Adv Nurs. 2008;62:107–115.
[27] Smith ML, White CB, Railey E, et al. Examining patient choices for metastatic breast cancer drugs. J Clin Oncol. 2012;30:6053.
[28] Thompson E, Stanton AL, Bower JE. Situational and dispositional goal adjustment in the context of metastatic cancer. J Pers. 2013;81:441–451.

ACTA ONCOLOGICA, 2018
VOL. 57, NO. 1, 152–173

Taylor & Francis
Taylor & Francis Group

LETTER TO THE EDITOR

Breast cancer patient advocacy in Denmark

Eva Bundesen

Danish Breast Cancer Organisation, Hesselager, Denmark

Introduction

A woman diagnosed with breast cancer (BC) is receiving good and professional treatment in Denmark. Every health care professional is doing their best to give as much information as possible. Family and friends try to give help and to be understanding and caring. Shared decision making is regarded an important issue, to ensure that the women feel acknowledged and understood. But even though we receive a lot of oral information and printed information materials, it feels like being sucked into a vacuum, where everything is decided from the 'cancer point of view' – an expression often heard when talking to BC survivors. Survival is the first and foremost purpose.

After completing the treatment and an endless number of visits to the hospital, the BC patient is expected to return to being the same person as before treatment. Often with a box of medicine to be taken daily for the next 5–10 years. This is where the realization process takes its beginning and where we, as a patient organization, has our primary purpose. This is where patient advocacy begins, this is where our members contact us, this is where they join in and begin the journey of learning to live with the fact that cancer hit me this time – and not my neighbor. The current paper addresses the work we do in DBO, the Danish Breast Cancer Organization (www.brystkraeft.dk), and the importance of participating in different national and international fora.

The journey into patient advocacy in Denmark normally takes its beginning with a breast cancer diagnosis and treatment. According to DBCG's annual report more than 46,000 Danish women were diagnosed with BC in 2014, and sadly 1115 women died from BC. The same year my own journey into the BC world took its beginning. Many of us, treated for BC, are living with the results of surgery and side effects of systemic treatments and radiotherapy. We feel the need to understand what has happened, why we never will be the same person again and how to cope with the risk of relapse. Hence, we look for information and participate in meetings and discussions with other BC survivors to get knowledge and learn to understand. As a consequence, some of us became volunteers. Often, in The Danish Cancer Society or as a member of our organization for BC women, Danish Breast Cancer Organization (DBO).

The Danish Breast Cancer Organization (DBO)

DBO was established in January 1999 as a nonprofit organization founded and run by volunteers. Presently DBO has 2500 members, all being breast cancer patients, organized in eight groups working all around Denmark. DBO is supported by The Danish Cancer Society. The members pay a moderate annual fee. In addition, DBO applies for financial support from a broad range of foundations.

The main goals of DBO are

- To increase the knowledge of breast cancer through information and education to our members
- To work towards a uniform and optimal BC treatment in Denmark
- To support local activities in the groups
- To give guidance and support to BC patients

Organization and activities

DBO organizes a general assembly and an annual member meeting. At the meeting, a variety of presentations are given, mainly regarding BC treatment and survivorship management. Especially the interest in the latter has increased, partly due to more survivors with long term adverse events from treatment.

The DBO groups are spread all over Denmark. In each group, there are normally 3–7 active members, who take responsibility to arrange local meetings and other events. These members meet once a year with the board to discuss past events, information and news, and share ideas for the upcoming period. The groups are the ones talking to members and they know what the important issues are.

Our main and most important work is to share information and support. This happens through seminars and meetings all over the country. Mostly organized by the local groups alone or in cooperation with the board.

Another important task for the board is the contact to health care professionals, journalists, politicians, etc. We attend meetings, seminars, and conferences, both in Denmark and in Europe. In this way, we meet with professionals as well as other BC organizations, and are kept up to date regarding new treatments and other relevant issues for our members.

CONTACT Eva Bundesen eva.bundesen@brystkraeft.dk Danish Breast Cancer Organisation, Degnelodden 3, 5874 Hesselager, Denmark

https://doi.org/10.1080/0284186X.2017.1407497

DBO has an active homepage and distribute a magazine to our members 3–4 times a year. It is delivered to many hospitals as well. The board members take care of the daily work in the organization, from registering new members, taking telephone calls, e-mail correspondence, bookkeeping, planning of seminars, and events, to keeping contact to the groups and participating in relevant meetings in Denmark and abroad. This work is time consuming, but also very giving and inspiring.

Seminars and events planned by board members, alone or together with *the* group*s* include: seminars for the groups; the annual meeting; seminars for women with metastatic BC; seminars for young women with breast cancer; and seminar for women with side effects from treatment. The typical themes for the seminars range from new cancer treatments, physiotherapy methods, lymphoedema care, and treatment, to presentations from BC survivors on how to cope or other survivorship issues. The seminars are well attended, and we have waiting lists every time. The women appreciate the information they get, and enjoy being with other with BC diagnosis. The women *enjoy being with other BC survivors and feel* the information they get, both regarding treatment and survivorship are important for their wellbeing and help them to cope with their situation.

The local groups organize approximately each eight arrangements yearly amounting to a total of approximately 60–65 arrangements a year. This include informative meetings, evenings where they meet and talk, dance events, walks in nature, Pink Saturday arrangements, information about breast reconstruction or lymphoedema, training events, and many more.

BC survivors often feel the need to talking to others and to exchange knowledge around survivorship in every aspect of the word. Personally, DBO provided me with the main tools for understanding and knowledge of my own situation. Especially, the area of side effects is on a short and long term an issue amongst us and something we discuss at almost every meeting.

To deal with some of these issues in practical terms, the volunteers also produce and distribute 'heart pillows' (heart formed pillows to protect the axilla after surgery). The heart pillow is for instance supportive when traveling, knitting, sleeping, or just when we need to rest.

National and international collaboration

At the national level, the collaboration with The Danish Cancer Society and Danish Breast Cancer Cooperative Group (DBCG) is very important. It supplies us with relevant information, help, and support. As an example of collaboration with the health authorities, DBO participated as patient representatives in the review of the National Cancer Plan IV and is currently involved in work to prevent bad practice in the screening programs.

Internationally DBO participates in the Nordic coalition of BC organizations which every 2 years organize a Nordic conference. In 2016, DBO joined the Europe Donna network as the Danish representative. Europe Donna is a European network with national groups in 47 countries and is our door to collaborate on an international level. We work together to increase the awareness of BC, including better treatment, early detection, acknowledgement of good practice in the health care system, and many other issues. We get advocacy training in Milan on such topics as BC screening, diagnosis, surgery, adjuvant therapy, as well as advocacy training to learn to talk to politicians, media, health care professionals and so forth.

In summary, the advocacy work by DBO is important to our members. We collect and spread information, who help BC survivors to get a better knowledge of different aspects of diagnosis, treatment and follow-up of breast cancer.

Our members are often told 'you are cured' and 'your risk of relapse is very low'. They hear the words – but the understanding takes time. Due to the fact, that many of our members must go to screening or must visit the hospital for follow-up, they meet the cancer fear repeatedly for the rest of their life. That is where advocacy matters. They find comfort in the talk over a cup of coffee, listening to the story of others, talk about side effects of the 'little pill that keeps me safe' and how to cope for 5–10 years with it. Our seminars are important as well. Both the ones held for our members and the board participate in. We enjoy every presentation given by health care professionals. It is giving us and our members so much knowledge and understanding.

Although my journey into the breast cancer world closed some doors, it also opened many new ones. Especially, the advocacy work has grown into being one of the most important things I do. Even though I must live with side effects for the rest of my life I can say the very important sentence. 'I am alive, I am scarred but I am not scared' and as many of our members I can thank the health care professionals that helped saving us.

Disclosure statement

No potential conflict of interest was reported by the author.

LETTER TO THE EDITOR

Provision of data from the clinical database and of biological material from the tumor bank of the Danish Breast Cancer Cooperative Group 2008–2017

Henning Mouridsen[a], Peer Christiansen[b], Maj-Britt Jensen[a], Anne-Vibeke Laenkholm[c], Henrik Flyger[d], Birgitte Offersen[e], Ilse Vejborg[f] and Bent Ejlertsen[a]

[a]Danish Breast Cancer Cooperative Group (DBCG) Secretariat and Statistical Office, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [b]Breast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark; [c]Department of surgical pathology, Sjaelland University Hospital, Slagelse, Denmark; [d]Department Breast Surgery, Herlev University Hospital, Herlev, Denmark; [e]Department of oncology, Aarhus University Hospital, Aarhus, Denmark; [f]Department of Radiology, Copenhagen University Hospital, Copenhagen, Denmark

Introduction

The capability to link health care information across various health care providers (institutions, clinics and professions) is increasingly recognized as an important source for clinical decision making and improved patient care [1]. A multi-institutional and -disciplinary records linkage system will potentially make information on treatments and events available to treating physicians and reduce repeat testing [2]. A comprehensive linkage of medical records also allows to access patient outcomes individually and for quality assurance and research if applied to a sufficiently large group over extended time. A clinical database becomes particularly informative if it is population-based, e.g., covers all residents in a well-defined area. The ability to improve quality of care will further increase when data in multiple databases can be coupled on an individual patient level, and in Denmark this is enabled by the unique civic registration number assigned to all inhabitants. Finally, the research opportunities will expand considerably by access to tumor tissue if linked to clinical data.

The clinical database of the Danish Breast Cancer Cooperative Group (DBCG) was established in 1977 in conjunction with the foundation of the multidisciplinary group [3]. Since then relevant data of diagnostic aspects, of loco-regional and systemic therapies and follow-up has been collected nationwide by the DBCG from all women newly diagnosed in Denmark with breast cancer [4]. The clinical database has collected data from about 130,000 breast cancer cases and the database has provided an instrument for monitoring community breast cancer standards and for the conduct of large series of randomized trials [5], and data from these trials have successively modified the current guidelines. These initiatives have significantly contributed to an improvement in the prognosis of breast cancer [6,7].

This paper will provide information on how data from the clinical database and of how biological material from the DBCG tumor bank has been utilized for correlative research studies.

The DBCG clinical database and access to the data

All Danish units involved in the diagnosis, treatment, and follow-up of breast cancer patients have contributed to the database in reporting data of histopathology, treatment, and follow-up [8]. Data from a total number of about 130,000 women have now been reported . A total of about 30,000 patients are currently in treatment or in follow-up, and 65,000 are still alive. Early on the data was reported by paper forms to the secretariat with subsequent transfer of data to the database, but from 2007 a web system was developed to enable on-line reporting from the departments.

From 2006 data concerning demographics, diagnostics, surgery, and oncologic treatment strategies, defined as quality data, have been extracted from the database to be used for the DBCG quality indicators [www.dbcg.dk/kvalitetsdatabase] according to the program of the Danish Clinical Registries (RKKP) [www.rkkp.dk].

The data of the database are unique. They are individual based, and longitudinal with successive dates of therapeutic interventions and events. And the database, following improvement over time, is now close to have a complete coverage of the Danish breast cancer population. This has been achieved by the development of an effective system of reminders, based partly on identification of gabs in the reporting and by linkage to the Danish Pathology Registry, which registers data from every pathology report performed by the Danish departments of pathology. Thus patients not registered from the departments can be identified and enquiries sent to the departments. And finally, the database is constructed to give advice to the clinicians, based on the reported data of the clinical, histopathological and genetic characteristics about the recommended oncological treatment according to current evidence based guidelines.

In addition link to other public registries by use of the unique 10-digit Danish Civil Registration Number (CPR), assigned to all Danish residents, offers an excellent possibility to run comparative research studies such as link to The Danish National Board of Health Registry (LPR) which

CONTACT Henning Mouridsen mou@dbcg.dk DBCG, Copenhagen University Hospital, Rigshospitalet, 9, Blegdamsvej, DK-2100 Copenhagen, Denmark
Supplemental data for this article can be accessed here.

http://dx.doi.org/10.1080/0284186X.2017.1403039

registers all diagnoses from admission to hospital, such as other diseases, co-morbidity and long-term adverse events, link to the Danish National Prescription Registry which registers drugs prescribed, link to the Cancer Registry for other malignant diseases and link to the CPR for vital status.

Access of data from the database to be used for research by members of the DBCG-organization or by other groups or institutions with expertise in breast cancer research required an application sent to the DBCG secretariat and to be evaluated by members of the DBCG executive committee, possibly following advice from one of the relevant scientific committees [3]. The applications are approved provided certain scientific criteria, if necessary following correspondence with the applicant, and current legal requirements are met.

The DBCG tumor bank and access to biological material

The biological material from Danish breast cancer patients is stored at three locations. In the departments of pathology responsible for the diagnostic procedures as well as the molecular biological analyses required for allocation of the patient to the proper treatment. The material is stored as paraffin-embedded tissue, fresh frozen tissue and to a lesser extent extracted RNA/DNA. Since 2009 all Danish cancer patients have been asked for permission to collect biological material including paraffin embedded tissue and fresh frozen tissue, when available, as well as a blood sample to the Danish Cancer Biobank [www.cancerbiobank.dk]. Finally in the DBCG tumor bank, which was established in 1991, in connection with the initiation of the centralized biochemical ER analysis. Since then the DBCG tumor bank has stored biological material as paraffin-embedded tissue, fresh frozen tissue and extracted RNA/DNA, and excess TMA's collected in relation to translational research projects.

Access to biological material requires approval of the project by the DBCG executive committee as described for access to clinical data complemented by a Material Transfer Agreement signed by the medical leader of the DBCG secretariat and the chairman of the project in question. If the material is used to produce TMA's the residuals are to be returned to the DBCG tumor bank while residual RNA/DNA can be stored with the pathologist responsible for the specific study. The residual TMA's or RNA/DNA is registered for potential future studies.

Requests for access to data from the clinical database

Since the establishment of the database DBCG has received 350 requests for extracts from the database to be used in research studies. This analysis covers the time since January 2008 during that period DBCG received 167 requests. These are summarized in Supplementary Table 1. Applications concerning update of previously accepted studies are not included in the table. As it appears the topics cover almost all aspects of breast cancer: diagnostic aspects, including mammography, histopathology including potential prognostic and predictive factors, surgery, radiotherapy, systemic therapy, epidemiology, adverse events, importance of concomitant drug administration, genetics, and rehabilitation.

Since mid-2013, we prospectively recorded certain characteristics of the research projects ($n = 82$). During this period 16 of the studies was run as single institution studies but 64 (78%) were multicenter or nation-wide studies. In 24 of the cases the requested data included quality data only, but the majority of cases (70%) requested additional data from the database. Access to biological material, this was requested in 13 (=16%) of the applications.

Publications from these studies are included in the DBCG bibliography which as of ultimo 2016 counts 452 peer reviewed publications [www.dbcg.dk/publications].

Discussion

The data presented demonstrate how a well-organized clinical database in conjunction with a multidisciplinary organization can be efficiently utilized to answer a large variety of research questions related to the total course of the disease.

Several factors have probably been important in making the clinical database of the DBCG a major research resource. First, for 40 years DBCG has provided easily accessible application forms and guidelines for requesting data and the board of directors, the responsible authority, is appointed by the scientific societies. Second, the clinical database contains four decades of comprehensive and population-based data on breast cancer patients in Denmark. Third, linkage to information from a variety of other database has been possible on an individual patient basis. Finally, tumor-tissue has been available on around 80% of patients enabling biological research.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Henrik Flyger http://orcid.org/0000-0001-8441-0805

References

[1] Visvanathan K, Levit LA, Raghaven D, et al. Unlapped potential of observational research to inform clinical decision making: American Society of Clinical Research Statement. J Clin Oncol. 2017;35:1845–1854.

[2] Sauver JL, Grossardt BR, Yawn BP, et al. Use of medical records linkage system to enumerate dynamic population over time: the rochester epidemiology project. Am J Epidemiol. 2011;173:1059–1068.

[3] Blichert-Toft M, Christiansen P, Mouridsen HT. Danish Breast cancer Cooperative Group – DBCG: history, organization, and status of scientific achievements at 30-year anniversary. Acta Oncol. 2008;47:497–505.

[4] Møller S, Jensen MB, Ejlertsen B, et al. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DCG); its 30-years anniversary and future promise. Acta Oncol. 2008;47:506–525.

[5] Ejlertsen B, Offersen B, Jensen MB, et al. Forty years of landmark trials undertaken by the Danish Breast Cancer Cooperative Group (DBCG) nationwide or in international collaboration. Acta Oncol. 2018; in press.
[6] Mouridsen H, Bjerre KD, Christiansen P, et al. Improvement of the prognosis in breast cancer in Denmark 1977–2006, based on the nationwide reporting to the DBCG Registry. Acta Oncol. 2008;47:525–537.
[7] Jensen MB, Ejlertsen B, Mouridsen H, for the Danish Breast Cancer Cooperative Group, et al. Improvements in breast cancer survival between 1995 and 2012 in Denmark: the importance of earlier diagnosis and adjuvant treatment. Acta Oncol. 2016;55:24–25.
[8] Jensen MB, Laenkholm AV, Kroman N, et al. The clinical database and implementation of treatment guidelines by the Danish Breast Cancer Cooperative Group in 2007-2016. Acta Oncol. 2018; in press.

LETTER TO THE EDITOR

Palliative treatment with carboplatin as late line therapy to patients with metastatic breast cancer

Lena H. Rosvig[a], Sven T. Langkjer[a], Ann Knoop[b] and Anders B. Jensen[a]

[a]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Oncology, Odense University Hospital, Odense, Denmark

Introduction

Among patients with breast cancer, 20–25% will develop metastatic disease, and the treatment will be palliative [1]. In this situation, the purpose of treatment will be to ease symptoms in order to maintain or improve quality of life and if possible, to prolong life [2,3].

When the disease no longer respond to standard treatment regimes, the patient is either treated symptomatic or with experimental chemotherapy. The optimal cytostatic treatment of heavily pretreated breast cancer patients is not standardized, often of low efficacy, and limited by comorbidity and performance status. As the treatment is palliative, the benefits of tumor response and improvement in disease-related symptoms as a result of chemotherapy must be weighed against treatment-induced toxicity and its impact on quality of life [4]. Still more patients are receiving chemotherapy near end of life [5]. This is due to constant improvement in breast cancer treatment and an increasing patient wish for further treatment [6]. For several patients, even the smallest possibility of benefit appears to be worth fighting for and they prefer to do something actively [6,7].

Carboplatin has been proved effective in the treatment of early-stage breast cancer, both as monotherapy [8,9] and as combination therapy [10–12]. The efficacy of carboplatin in the treatment of pretreated metastatic breast cancer is more equivocal [13–19]. Furthermore, the efficacy in treating heavily pretreated metastatic breast cancer is still debatable and only few studies exist [20,21]. Recent studies have indicated effect of platinum-containing regimes in treatment of triple negative breast cancer [14,20,22,23], and international guidelines have included platinum-containing regimes in the treatment of BRCA1/2 associated triple-negative metastatic breast cancer [2]. Most of the studies involving carboplatin, show an advantageous side effect profile [8,9,15–17], making the drug suitable for palliative care. The aim of the current study was to evaluate whether a carboplatin regime could be suitable for late line treatment of heavily pretreated patients with disseminated breast cancer, and to evaluate whether a carboplatin regime could be an option for those patients, who desire additional treatment, but with a reasonable balance between chance of effect and amount of side effects.

Patients and treatments

The study was performed at two oncological departments in Denmark, Aarhus (cohort 1) and Odense (cohort 2). The databases were searched to find all patients ever treated with a carboplatin regime. All files were accessible from October 1990, where the first patient, later treated with carboplatin, was diagnosed with breast cancer.

The patients included women with advanced breast cancer, treated with a carboplatin regime between July 2004 and February 2012. Inclusion criteria for this retrospective study were histological verified breast cancer, advanced disease proven by biopsy or radiological investigations, and a minimum of one treatment course with a carboplatin-regime. In addition, the patients should not have been exposed to carboplatin (cohorts 1 and 2) or gemcitabine (cohort 2) as part of prior treatment. As carboplatin was not a part of the standard treatment, the use of carboplatin was different in the two hospitals.

Treatments

In cohort 1, the treatment consisted of carboplatin AUC 5 monotherapy or carboplatin and trastuzumab 6 mg/kg (loading dose 8 mg/kg) in case of HER2-positive disease. Both treatments were given on day 1 in a 3 week cycle. In cohort

CONTACT Lena H. Rosvig lenahede@rm.dk Department of Oncology, Aarhus University Hospital, Nørrebrogade 44, Bld. 5, DK-8000 Aarhus C, Denmark
Supplemental data for this article can be accessed here.

http://dx.doi.org/10.1080/0284186X.2017.1407495

2, the treatment regime was carboplatin AUC 5 on day 1 plus gemcitabine 1000 mg/m^2 on day 1 + 8 in a 3-week cycle. Before every treatment, side effects were evaluated. The treatment was continued until disease progression or unacceptable side effects. Dose reduction or postponement of treatment followed local instructions. Evaluation of treatment efficiency and side effects was performed at every third treatment cycle and included CT- and/or MRI scans, biochemistry, and physical examination.

Data collection and statistical analysis

Data were collected through patient records, treatment charts, laboratory rapports, imaging examination, and pathology reports. Baseline was first treatment day with carboplatin.

Performance status (PS) was evaluated according to WHO score, based on medical records. The side effect profile of carboplatin was evaluated by biochemical markers and the patient's subjective perception according to CTCAE 3.0 (GFR and creatinine from MedDRA Version 12.0, SPSS Inc., Chicago, IL, USA), need for hospital admissions and dose reduction, as well as postponement of treatment due to side effects. Tumor response was evaluated according to RECIST criteria (version 1.0/1.1) based on CT and MRI. In addition, the response was based on the clinical examinations described in the medical record. Stable disease was defined as no change for a minimum of 3 months.

The primary endpoint was time to progression (TTP). TTP takes stable disease (SD) into account, which is a desirable and achievable goal in palliation. Secondary end-points included side effect profile, objective response rate (ORR), clinical benefit rate, time to response, and time to death (TTD). Hypothesis generating analysis was performed to identify possible subgroups (hormone receptor (HR)-positive, HER2-positive and triple negative status) with different responses to treatment. All descriptive statistics and analytical statistics were performed in Stata/IC 12.0 (StataCorp LLC, College Station, TX, USA). Estimates for time-to-event data with censoring, were determined from Kaplan–Meier curves. Due to the number of patients in the study, hypothesis generating analysis of time-to-event data were performed as differences in median TTP.

Results

The patient population initially consisted of 27 women in cohort 1 and 26 women in cohort 2. One patient in cohort 1 was excluded from the study because of possible primary lung cancer. Five patients in cohort 2 were excluded, because carboplatin was given as neoadjuvant/adjuvant therapy. For final analysis, in cohort 1, 12 women were treated with carboplatin monotherapy and 14 women were treated with carboplatin and trastuzumab. In cohort 2, 21 women were treated with carboplatin-gemcitabine combination therapy (Supplementary material). Trastuzumab was available in the entire period. Patient characterization and previous courses of treatment are summarized in Table 1.

The median number of treatments was 4 series with a range of 1–21 series. In 40 (85%) women, termination of treatment was due to disease progression and only in three (6%) women treatment terminated was due to side effects (Supplementary material).

Table 1. Patient characterization.

	Cohort 1	Cohort 2	All	*p* Value
Number of patients, *n*	26	21	47	
Age (year)				.9
Median	49	48	47	
Range	31–63	31–66	31–66	
Histopathology				.1
Ductal	19 (73)[a]	18 (86)	37 (79)	
Lobular	3 (12)	1 (5)	4 (9)	
Other	4 (15)	0 (0)	4 (9)	
Unknown	0 (0)	2 (9)	2 (4)	
ER status				.2
Pos	10 (38)	12 (57)	22 (47)	
Neg	16 (62)	9 (43)	25 (53)	
HER-2 status				.02
Normal	11 (42)	16 (76)	27 (58)	
Pos	14 (54)	3 (14)	17 (36)	
Unknown	1 (4)	2 (10)	3 (6)	
Triple negative status				.6
Yes	7 (27)	7 (33)	14 (30)	
No	19 (73)	14 (67)	33 (70)	
Grade of anaplasia				.4
1	3 (12)	1 (5)	4 (9)	
2	4 (15)	7 (33)	11 (23)	
3	14 (54)	8 (38)	22 (47)	
Unknown	5 (19)	5 (24)	10 (21)	
Lines of palliative treatments				.2
0–1	2 (8)	5 (24)	7 (15)	
2–4	13 (50)	12 (57)	25 (53)	
5–7	7 (27)	2 (10)	9 (19)	
8–10	4 (15)	2 (10)	6 (13)	
Overall relapse duration (month)				.01
Median (CI)	28.3 (18.4;43.7)	9.5 (4.2;21.4)	17.4 (11.2;27.2)	
Range	0.9–129.2	0.3–114.8	0.3–129.2	
PS (WHO)				.1
0	12 (46)	6 (29)	18 (38)	
1–2	12 (46)	15 (71)	27 (58)	
3–4	2 (8)	0 (0)	2 (4)	
Metastatic sites				.8
1	1(4)	2 (10)	3 (6)	
2–3	15 (58)	12 (57)	27 (58)	
4–5	10 (38)	7 (33)	17 (36)	

Age (year): age at the time of primary diagnosis. Lines of previous treatments: the number of lines of systemic antineoplastic treatment (chemotherapy and/or anti-hormonal therapy) the patient has been exposed to before carboplatin treatment. Overall relapse duration (month): describes the patients' overall duration of time of the disease from first relapse to the start of carboplatin treatment. PS: Performance status at the start of carboplatin treatment. Metastatic sites: metastatic sites at the start of carboplatin treatment.
[a]Percent values in brackets.

The response to carboplatin containing-treatment is summarized in Table 2. A separate analysis was made for the most heavily pretreated women, i.e., those treated with 5–7 lines and 8–10 lines of systemic antineoplastic treatment respectively (Table 2).

Exploratory analysis showed no correlation between TTP and ER status (difference in TTP −1.0; 95% CI −2.5 to 0.5; $p = .2$ for cohort 1 and 0.7; 95% CI −2.7 to 4.1; $p = .7$ for cohort 2), HER2 status (difference in TTP −0.4; 95% CI −1.7 to 1.0; $p = .6$ for cohort 1 and −0.1; 95% CI −4.0 to 3.8; $p = 1.0$ for cohort 2) or those described as triple negative (difference in TTP 0.5; 95% CI −2.0 to 3.0; $p = .7$ for cohort 1 and 0.4; 95% CI −3.6 to 4.4; $p = .8$ for cohort 2).

The main reason for termination of treatment was progressive disease. Side effects resulted only in few dose reductions, treatment postponements, hospitalizations, and/or cessations of treatments. The dominant side effects were hematological

Table 2. Response to therapy.

	Cohort 1	Cohort 2	All
Time to progression (month)			
All			
Median (CI)	2.3 (1.7;3.2)	4.1 (2.4;4.7)	2.8 (1.9;3.9)
Range	0.4–19.3	1.2–14.5	0.4–19.3
5–7 lines			
Median (CI)	3.1 (1.9;9.6)	5.0 (5.0; .)	4.4 (1.9;9.6)
Range	1.9;19.3	5.0;9.1	1.9;19.3
8–10 lines			
Median (CI)	7.0(1.8; .)	2.8(2.8; .)	4.4(1.8; .)
Range	1.8;8.5	2.8;4.4	1.8;8.5
Time to response (weeks)			
Median (CI)	6.1 (3.0;7.6)	8.9 (6.1;11.6)	7.6 (4.1;8.9)
Range	3.0–8.4	6.1–12.9	3.0–12.9
Best tumor response, *n* (%)			
CR	1 (4)[a]	2 (10)	3 (6)
PR	6 (23)	6 (29)	12 (26)
SD	5 (19)	4 (19)	9 (19)
PD	11 (42)	7 (33)	18 (38)
Unknown	3 (12)	2 (10)	5 (11)
Objective response rate % (CI)			
All	26.9 (11.6;47.8)	38.1 (18.1;61.6)	31.9 (19.1;47.1)
5–7 lines	28.6 (3.7;71.0)	50.0 (1.3;98.7)	33.3 (7.5;70.1)
8–10 lines	50.0(6.8;93.2)	0.0(0.0;84.2)	33.3 (4.3;77.7)
Clinical benefit rate % (CI)	46.2 (26.6;66.6)	57.1 (34.0;78.2)	51.1 (36.1;65.9)
Time to death (month)			
Median (CI)	8.8 (5.3;13.7)	8.2 (6.2;13.4)	8.8 (6.8;11.0)
Range	0.4–54.2	1.7–26.0	0.4–54.2

5–7 lines: women pretreated with 5–7 lines of systemic antineoplastic treatment.
8–10 lines: women pretreated with 8–10 lines of systemic antineoplastic treatment.
[a]Percent values in brackets.

or nausea/vomiting and fatigue. Myelosuppression, in particular neutropenia and thrombocytopenia was of medium severity, with only few grades 3 and 4 events. Non-hematologic side effects were generally mild and mainly of grads 0–2 and with only one grade 3 event in fatigue and non-grade 4 events at all (Supplementary material).

Discussion

The study included women treated with several lines of antineoplastic treatments prior to the carboplatin containing-regimes. Despite this, the women in general had a good PS prior to initiation of treatment, meaning that their quality of life could make additional treatment relevant.

The carboplatin regimes in our study were administered considerably later in the disease course than in the previously mentioned studies. Nevertheless, the treatment effect was comparable with what has been observed in previous studies [8,9,12–21,23]. Our study demonstrates that carboplatin regimes were effective in treatment of heavily pretreated patients with up to ten lines of previous treatments, and both as monotherapy and combination therapy with trastuzumab or gemcitabine. Despite being heavily pretreated the women in our study showed a TTP of 2.3–4.1 months, and a relatively high ORR of 26.9–38.1%. The response to carboplatin containing treatment was independent of the number of previous lines of relapse treatments. Neither did our results indicate a difference in treatment response depending on tumor subgroups and triple negative status. However, the number of patients was small and the results of the subgroup analysis should, therefore, be taken with caution.

As the study is retrospective, there is a risk of selection bias, such as the fact that the women in our study in general responded well to previous treatment lines (data not showed), which could indicate a population with more chemo-sensitive disease compared to the average patient.

The treatment was generally well tolerated. Still the degree of myelosuppression was not insignificant. Especially, neutropenia was frequent and of higher grades, but only few cases of hospitalization due to febrile neutropenia were recorded. The women treated with carboplatin-gemcitabine combination therapy showed a tendency towards more severe myelosuppression, indicating a more burdensome treatment.

The extend of side effects was consistent with what has been observed in previous studies of carboplatin monotherapy [8,9,15,16] and in studies of carboplatin in combination with gemcitabine [13,14,19,21], and less than in some studies where more frequent and treatment limiting side effects have been observed [12,17,18].

Cytostatic treatment of heavily pretreated patients with metastatic breast cancer near the end of life should be considered carefully [24]. Often patients and their families want to continue anti-neoplastic therapy and value even small benefits greatly, and consider side effects as less important [5]. Thus, a number of studies have shown that chemotherapy can improve the quality of life of cancer patients and result in better performance status, despite low objective responses [3,4,24].

In conclusion, treatments with carboplatin regimes showed an encouraging effect and were well tolerated and may be candidates for late line treatment of heavily pretreated patients with metastatic breast cancer, where the primary treatment goal is to minimize the negative impact from the disease upon the quality of life of the patient. However,

it is also clear that more studies concerning treatment in heavily pretreated patients are needed.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Kræftens Bekæmpelse, [10.13039/100008363].

References

[1] Perez EA. Carboplatin in combination therapy for metastatic breast cancer. Oncologist. 2004;9:518–527.
[2] Cardoso F, Costa A, Norton L, et al. ESO-ESMO 2nd international consensus guidelines for advanced breast cancer (ABC2). Ann Oncol. 2014;25:1871–1888.
[3] Bang SM, Park SH, Kang HG, et al. Changes in quality of life during palliative chemotherapy for solid cancer. Support Care Cancer. 2005;13:515–521.
[4] Park YH, Jung KH, Im SA, et al. Quality of life (QoL) in metastatic breast cancer patients with maintenance paclitaxel plus gemcitabine (PG) chemotherapy: results from phase III, multicenter, randomized trial of maintenance chemotherapy versus observation (KCSG-BR07-02). Breast Cancer Res Treat. 2015;152:77–85.
[5] Matsuyama R, Reddy S, Smith TJ. Why do patients choose chemotherapy near the end of life? A review of the perspective of those facing death from cancer. J Clin Oncol. 2006;24:3490–3496.
[6] Balmer CE, Thomas P, Osborne RJ. Who wants second-line, palliative chemotherapy? Psychooncology. 2001;10:410–418.
[7] de Haes H, Koedoot N. Patient centered decision making in palliative cancer treatment: a world of paradoxes. Patient Educ Couns. 2003;50:43–49.
[8] Martin M, Diaz-Rubio E, Casado A, et al. Carboplatin: an active drug in metastatic breast cancer. J Clin Oncol. 1992;10:433–437.
[9] Kolaric K, Vukas D. Carboplatin activity in untreated metastatic breast cancer patients-results of a phase II study. Cancer Chemother Pharmacol. 1991;27:409–412.
[10] Liu J, Li Q, Zhang P, et al. Paclitaxel plus carboplatin for women with advanced breast cancer. Chin Med Sci J. 2007;22:93–97.
[11] Ruiz M, Salvador J, Bayo J, et al. Phase-II study of weekly schedule of trastuzumab, paclitaxel, and carboplatin followed by a week off every 28 days for HER2+ metastatic breast cancer. Cancer Chemother Pharmacol. 2008;62:1085–1090.
[12] Yardley DA, Burris HA, III Simons L, et al. A phase II trial of gemcitabine/carboplatin with or without trastuzumab in the first-line treatment of patients with metastatic breast cancer. Clin Breast Cancer. 2008;8:425–431.
[13] Loesch D, Asmar L, McIntyre K, et al. Phase II trial of gemcitabine/carboplatin (plus trastuzumab in HER2-positive disease) in patients with metastatic breast cancer. Clin Breast Cancer. 2008;8:178–186.
[14] Maisano R, Zavettieri M, Azzarello D, et al. Carboplatin and gemcitabine combination in metastatic triple-negative anthracycline- and taxane- pretreated breast cancer patients: a phase II study. Cancer. 2011;23:40–43.
[15] O'Brien ME, Talbot DC, Smith IE. Carboplatin in the treatment of advanced breast cancer: a phase II study using a pharmacokinetically guided dose schedule. J Clin Oncol. 1993;11:2112–2117.
[16] Vermorken JB, Gundersen S, Clavel M, et al. Randomized phase II trial of iproplatin and carboplatin in advanced breast cancer. The EORTC Early Clinical Trials Group and the EORTC Data Center. Ann Oncol. 1993;4:303–306.
[17] Chan D, Yeo WL, Tiemsim Cordero M, et al. Phase II study of gemcitabine and carboplatin in metastatic breast cancers with prior exposure to anthracyclines and taxanes. Invest New Drugs. 2010;28:859–865.
[18] Nasr FL, Chahine GY, Kattan JG, et al. Gemcitabine plus carboplatin combination therapy as second-line treatment in patients with relapsed breast cancer. Clin Breast Cancer. 2004;5:117–122. discussion 123–4.
[19] Nagourney RA, Flam M, Link J, et al. Carboplatin plus gemcitabine repeating doublet therapy in recurrent breast cancer. Clin Breast Cancer. 2008;8:432–435.
[20] Nelli F, Moscetti L, Natoli G, et al. Gemcitabine and carboplatin for pretreated metastatic breast cancer: the predictive value of immunohistochemically defined subtypes. Int J Clin Oncol. 2013;18:343–349.
[21] Laessig D, Stemmler HJ, Vehling-Kaiser U, et al. Gemcitabine and carboplatin in intensively pretreated patients with metastatic breast cancer. Oncology. 2007;73:407–414.
[22] Koshy N, Quispe D, Shi R, et al. Cisplatin-gemcitabine therapy in metastatic breast cancer: Improved outcome in triple negative breast cancer patients compared to non-triple negative patients. Breast. 2010;19:246–248.
[23] Isakoff SJ, Mayer EL, He L, et al. TBCRC009: a multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol. 2015;33:1902–1909.
[24] Goncalves JF, Goyanes C. Use of chemotherapy at the end of life in a Portuguese oncology center. Support Care Cancer. 2008;16:321–327.

LETTER TO THE EDITOR

A Danish national effort of *BRCA1/2* variant classification

Inge Søkilde Pedersen[a,b*], Ane Y. Schmidt[c*], Birgitte Bertelsen[c], Anja Ernst[d], Christian Liebst Toft Andersen[d], Torben Kruse[e], Maria Rossing[f*] and Mads Thomassen[g*]

[a]Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark; [b]Clinical Cancer Research Center, Aalborg University Hospital, Aalborg, Denmark; [c]Centre for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Denmark; [d]Section of Molecular Diagnostics, Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark; [e]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark; [f]Centre for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Denmark; [g]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark

CONTACT Mads Thomassen mads.thomassen@rsyd.dk Department of Clinical Genetics, Odense University Hospital, Odense, Denmark
Supplemental data for this article can be accessed here.

http://dx.doi.org/10.1080/0284186X.2017.1400693

Introduction

With the technological development for sequencing and automation of sample handling, interpretation of data and classification of variants are becoming the more labor intensive part of genetic screening. By 2008, 107 unique pathogenic *BRCA1/2* variants had been identified in Danish hereditary breast and/or ovarian cancer families [1]. Identification of pathogenic *BRCA* variants affects not only choice of preventive measures but also affects the effect of treatment in cancer patients. The latter has most recently been shown in a Danish cohort of breast cancer *BRCA* carriers [2]. Since then, the number of identified pathogenic variants has almost tripled. All though the methods for variant classification have improved, the number of variants of unknown clinical significance has increased even more rapidly.

The five tier International Agency for Research on Cancer (IARC) classification system [3] is the classification system generally used in Denmark. All three participating laboratories (Rigshospitalet, Odense and Aalborg University Hospital) are longstanding members of Evidenced-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) [4] actively working towards classification of *BRCA* variants using a multifactorial likelihood model first described in 2004 [5] and subsequently revised and refined by incorporation of additional data [6–11]. Current ENIGMA classification rules can be found at https://enigmaconsortium.org/library/general-documents/.

For counseling and clinical decision-making, individuals with C5 (definitely pathogenic) and C4 (likely pathogenic) variants are treated equally and offered full high-risk surveillance programs. Likewise individuals with C1 (not pathogenic/low clinical significance) and C2 (likely not pathogenic/little clinical significance) variants are counseled based on family history and other risk factors and treated as 'no pathogenic *BRCA* variant detected'. Hence, misclassification between groups C1/C2 or C4/C5 will not have any clinical consequences, whereas misclassification between the group with no/little clinical significance (C1/C2) and the group of likely/definitely pathogenic variants (C4/C5) obviously would be severe. C3 is the group in between, representing variants of uncertain significance. This is a large group of variants with a probability of pathogenicity of 5–95%. However, an overly conservative and cautious approach leading to an overuse of C3 classifications could also be problematic and cause an unclear risk prediction. Thereby, leading to subjectivity in conveying and perceiving cancer risk.

Variant classification is not static. Obviously, reclassification of C3 variants is a natural consequence of growing information from new variant carriers, segregation and/or functional analyses. However, with the continuous gain of knowledge of protein function and particularly importance of naturally occurring isoforms there are examples of variant reclassification from C4/C5 to C1/C2. The most well-known example is *BRCA1*, LRG292t1:c.594-2A > C originally considered pathogenic due to exon10 skipping. However, further analysis showed when this variant occurs in *cis* with LRG292t1:c.641A > G it also produces 20–30% in-frame naturally occurring isoform Δ9,10 which retains the tumor suppressive function of BRCA1 [12]. In addition, there are reports of synonymous variants and deep-intronic variants originally deemed benign or likely benign subsequently showing an effect on splicing [13].

Here we present the concerted effort of our national Danish breast cancer variant classification group (DBKG) on streamlining *BRCA* variant classification.

Material and methods

Mutation screening of the *BRCA1* and *BRCA2* genes and variant classification were performed in three different laboratories (Rigshospitalet, Odense and Aalborg University Hospital). Variant lists from the three laboratories were collated from the uptake of *BRCA* screening (1999 Rigshospitalet, 2000 Odense and 2003 Aalborg) until the end of 2016. Nomenclature was revised according to current HGVS guidelines [14] and checked for consistency using https://mutalyzer.nl/.

Classification was updated using a batch search for ENIGMA approved classifications in ClinVar [15] on the 16 August 2017. Remaining variants were classified according to current ENIGMA rules by the representatives from the three laboratories. In addition, information on mRNA splicing analyses and functional studies previously published or carried out in the participating laboratories were taken into account.

Design of splicing assays is based on recommendations from ENIGMA [16] and when possible allele-specific in nature.

Results

A total of 945 unique variants have been detected by the three Danish laboratories carrying out *BRCA1* and *BRCA2* screening in a diagnostic setting (Supplementary Table 1). Searching ClinVar for ENIGMA validated variant classification 164, 61, and 199 variants classified as C1, C2, and C5, respectively, resulting in 521 unclassified variants (Table 1). After classifying the remaining variants according to ENIGMA

Table 1. Proportion of variant classes.

Variant classification	ClinVar (ENIGMA)		DBKG	
Number	Percentage	Number	Percentage	
C1-benign	164	17%	167	18%
C2-likely benign	61	6.5%	211	22%
C3-uncertain significance	–	–	268	28%
C4-likely pathogenic	–	–	17	1.8%
C5-likely pathogenic	199	21%	282	30%
Not Classified by ENIGMA by 29 June 2017.	521	55%	–	–

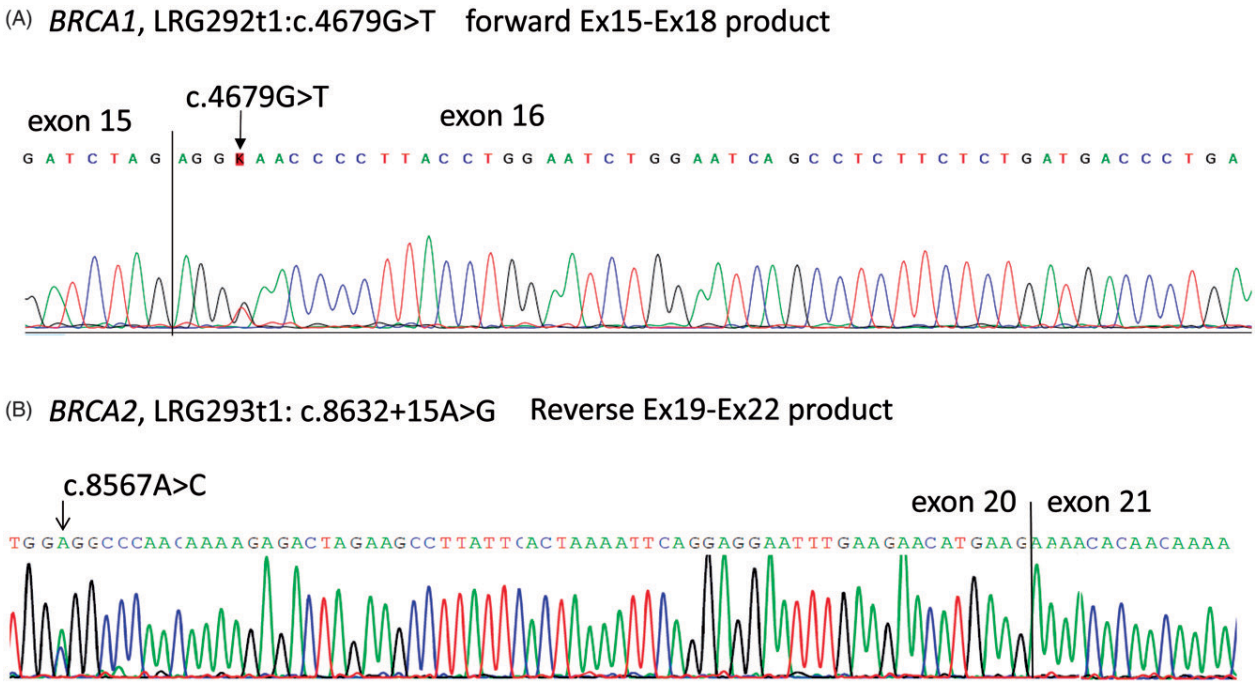


Figure 1. Allele specific mRNA splicing assays. (A) Direct Sanger sequencing electropherogram of RNA from a *BRCA1*, LRG292t1: c.4679G > T carrier showing equal representation of wildtype and variant alleles and no cryptic splicing in the RT-PCR products generated with primers located in exon 15 and 18. Albeit, the variant does not affect splicing, it is a missense variant and the effect on protein function has not been established. Hence it is still classified as C3. (B) Direct Sanger sequencing electropherogram of RNA from a *BRCA2*: LRG293t1: c.8633 + 15A > G carrier showing equal representation of wildtype and variant alleles as evident from exonic variant c.8567A > C in the RT-PCR products generated with primers located in exon 19 and 22. Furthermore, no cryptic splicing is observed at the exon 20–21 junction. Based on this c.8633 + 15A > G is classified as C2.

recommendations and based on additional available data 167, 211, 17, and 282 variants were classified as C1, C2, C4, and C5, respectively (Table 1), reducing the number of C3 to 268.

Examples of mRNA splicing assays with information on allelic usage, as recommended by ENIGMA are shown in Figure 1. Variants with equal contribution to assumed full-length transcript from both alleles and no usage of cryptic splice sites are considered likely benign (C2) if they do not cause direct changes to the protein sequence. Therefore, the intronic mutation in *BRCA2*, LRG293t1:c.8632 + 15A > G (Figure 1(B)) is classified C2, whereas *BRCA1* LRG292t1:c.4679G > T, although not causing aberrant splicing, is classified C3 because of the resulting rare missense variant p.(Gly1560Val). Variants producing no full-length transcript or no naturally occurring isoforms from the variant allele are deemed likely pathogenic (C4).

Discussion

As evident from the collection of variants classified across Denmark, a large proportion of variants detected in a routine diagnostic setting have not been formally classified by ENIGMA in ClinVar. Additional variants may be classified by searching the literature or carrying out functional and splicing assays. However, to ensure consistency in variant classification the efforts must be concerted, which is a major priority of the national initiative DBKG.

The example in Figure 1 illustrates the results of splicing assays performed according to ENIGMA's rules. *BRCA2*, LRG293t1:c.8632 + 15A > G is classified as C2 by splice assay. In order to reach a final classification as C1, support from multifactorial analysis using co-segregation, pathology information etc. is necessary. Likewise, the C3 variant *BRCA1*, LRG292t1:c.4679G > T requires multifactorial analysis possibly supported by functional data to be classified further. This calls for a collaborative approach among clinicians and molecular genetic laboratories. This approach have already been applied for many variants in ENIGMA and a large number of additional variants, likely counting the variants presented here, will be included in coming analyses.

The classification of variants collected for this study is updated and presented in the Supplementary material. However, classifications are not static and therefore the listed results should not be used for clinical purposes in the current form. DBKG will ensure continuous revision of classifications kept in an updated national database and eventually upon formal ENIGMA validation will be posted in ClinVar.

Acknowledgements

Thomas van Overeem Hansen is acknowledged for invaluable insight and work of importance for the article.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Thomassen M, Hansen TVO, Borg Å, et al. BRCA1 and BRCA2 mutations in Danish families with hereditary breast and/or ovarian cancer. Acta Oncol. 2008;47:772–777.

[2] Sønderstrup IMH, Laenkholm AV, Jensen MB, et al. Clinical and molecular characterization of BRCA-associated breast cancer: results from the DBCG. Acta Oncol. 2018.
[3] Plon SE, Eccles DM, Easton D, et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat. 2008;29:1282–1291.
[4] Spurdle AB, Healey S, Devereau A, et al. ENIGMA-evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. Hum Mutat. 2012;33:2–7.
[5] Goldgar DE, Easton DF, Deffenbaugh AM, et al. Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. Am J Hum Genet. 2004;75:535–544.
[6] Walker LC, Whiley PJ, Couch FJ, et al. Detection of splicing aberrations caused by BRCA1 and BRCA2 sequence variants encoding missense substitutions: implications for prediction of pathogenicity. Hum Mutat. 2010;31:E1484–E1505.
[7] Spurdle AB, Lakhani SR, Healey S, et al. Clinical classification of BRCA1 and BRCA2 DNA sequence variants: the value of cytokeratin profiles and evolutionary analysis–a report from the kConFab Investigators. J Clin Oncol. 2008;26:1657–1663.
[8] Spearman AD, Sweet K, Zhou XP, et al. Clinically applicable models to characterize BRCA1 and BRCA2 variants of uncertain significance. J Clin Oncol. 2008;26:5393–5400.
[9] Osorio A, Milne RL, Honrado E, et al. Classification of missense variants of unknown significance in *BRCA1* based on clinical and tumor information. Hum Mutat. 2007;28:477–485.
[10] Easton DF, Deffenbaugh AM, Pruss D, et al. A systematic genetic assessment of 1433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. Am J Hum Genet. 2007;81:873–883.
[11] Gómez García EB, Oosterwijk JC, Timmermans M, et al. A method to assess the clinical significance of unclassified variants in the BRCA1 and BRCA2 genes based on cancer family history. Breast Cancer Res. 2009;11:R8.
[12] de la Hoya M, Soukarieh O, López-Perolio I, et al. Combined genetic and splicing analysis of BRCA1 c.[594-2A > C; 641A > G] highlights the relevance of naturally occurring in-frame transcripts for developing disease gene variant classification algorithms. Hum Mol Genet. 2016;25:2256–2268.
[13] Anczuków O, Buisson M, Léoné M, et al. BRCA2 deep intronic mutation causing activation of a cryptic exon: opening toward a new preventive therapeutic strategy. Clin Cancer Res. 2012;18:4903–4909.
[14] den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat. 2016;37:564–569.
[15] ClinVar [Internet].USA: NCBI;[cited 2017 August 16]. 2013. Available from: https://www.ncbi.nlm.nih.gov/clinvar/
[16] Whiley PJ, de la Hoya M, Thomassen M, et al. Comparison of mRNA splicing assay protocols across multiple laboratories: recommendations for best practice in standardized clinical testing. Clin Chem. 2014;60:341–352.

LETTER TO THE EDITOR

The accuracy of preoperative staging of the axilla in primary breast cancer: a national register based study on behalf of Danish Breast Cancer Group (DBCG)

Kristine Hamran[a], Linnea Langhans[a], Ilse Vejborg[b], Tove Filtenborg Tvedskov[a] and Niels Kroman[c]

[a]Department of Breast Surgery, Copenhagen University Hospital Herlev, Copenhagen, Denmark; [b]Department of Radiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [c]Danish Breast Cancer Group, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Introduction

Staging of axillary lymph nodes in women with breast cancer is an important guide for treatment decisions. For decades, axillary lymph node dissection (ALND) was the standard procedure in staging the axilla, but today, sentinel lymph node biopsy (SLNB) is the standard of care in clinically node negative women.

In Denmark, patients suspicious of breast cancer are referred for a triple test assessment which consists of clinical examination, mammography, whole-breast ultrasonography (US) and needle biopsy of suspicious lesions. The preoperative examination also includes US of the axillary lymph nodes and fine needle aspiration cytology (FNAC) if enlarged or suspicious lymph nodes are present [1]. This examination is an important tool in the preoperative staging of patients with primary breast cancer [2,3].

Patients are classified as clinically node negative if no suspicious axillary lymph nodes are seen on US. These patients will be offered a SLNB, followed by ALND if macrometastases (tumor deposits >2 mm) are found in the sentinel lymph node(s). SLNB was completely implemented in Denmark by the end of 2004 [4].

Patients with preoperatively verified axillary metastases will have ALND performed immediately or receive neoadjuvant treatment. Accurate preoperative axillary lymph node status can reduce the numbers of patients having unnecessary SLNB performed. This reduces the time of the surgical procedure and it has been shown to lower healthcare costs [5,6].

The sensitivity of the preoperative staging of the axilla has been shown to vary between studies from [2,5]. In the meta-analysis by Diepstraten et al. [5] the sensitivity were found to be 50%.

CONTACT Niels Kroman Niels.Kroman@regionh.dk Breast Surgery Section, Rigshospitalet, Blegdamsvej 9, 3014, DK-2100 Copenhagen, Denmark

http://dx.doi.org/10.1080/0284186X.2017.1406138

The primary aim of this study was to evaluate the accuracy of preoperative staging of axillary lymph node involvement in patients with primary breast cancer based on preoperative US with or without FNAC. It was done in a large population-based Danish breast cancer cohort in a period with routine use of diagnostic procedures and mammographic screening.

Material and methods

The patient cohort was retrieved from the Danish Breast Cancer Group (DBCG) database. All departments in Denmark involved in the treatment of breast cancer report information regarding diagnosis, individual patient data, type of surgery, and adjuvant treatment to the DBCG database [7].

Study design and study population

The study is a retrospective register-based study which includes women diagnosed with unilateral primary breast cancer between 1 January 2010 and 31 December 2015. Only patients, in whom staging of the axillary lymph nodes was performed, were included. The exclusion criteria were patients treated with neoadjuvant therapy, patients not receiving any surgical treatment, patients with previous surgery in the breast or axillary region and patients with occult breast cancer. Furthermore, patients with inflammatory, multifocal or ulcerating breast cancer were excluded since ALND was standard of care in these patients regardless of the result of the axillary US. Patients with lymph node metastases found using other imaging methods, such as MRI and PET scanning were excluded.

More than 2500 patients were manually cross checked using the Danish Pathology Data Bank, due to missing data, and were either excluded or included according to listed criteria. See flowcart and Appendix I.

The study was approved the Danish Data Protection Agency and the Danish Patient Safety Authority.

Specificity and sensitivity

The proportion of patients who were preoperatively clinically node-negative and subsequently had macrometastases identified on SLNB were calculated. Additionally, we identified the number of patients with preoperatively verified axillary involvement on US and FNAC and afterwards confirmed on ALND. Sensitivity of US and FNAC was defined as the total number of patients with a positive preoperative US + FNAC examination among the patients with macrometastases in the axilla. Furthermore, we looked at the specificity, defined as the number of patients with a negative US and FNAC examination among the patients without macrometastases in the axilla. The positive predictive value (PPV) for predicting the risk of having axillary macrometastases with a positive US + FNAC, and the negative predictive value (NPV) for predicting the probability of not having axillary macrometastases with a negative US + FNAC was calculated. The sensitivity and specificity of preoperative axillary US and FNAC was calculated accordingly.

The difference between the mean numbers of macrometastases in the SLNB group compared to the group who had ALND performed initially was tested with a Wilcoxon rank test. $p < .05$ was considered statistically significant. A Chi-squared test was used to test whether histologic subtype was a risk of overseeing macrometastases on the preoperative examination. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. All statistical analyzes were performed using SAS statistical software, version 5.1 (SAS Institute Inc., Cary, NC).

Results

A total of 20,498 patients were retrieved from the DBCG database during the 5-year study period. After exclusion according to the listed criteria 18,968 patients remained for further analysis (Figure 1).

The prevalence of macrometastases in the axillary lymph nodes in the present study cohort was 29.1% (Table 1). Of these, 17,265 (91.0%) were clinically node negative and had SLNB performed. The remaining 1703 patients (9%) did not have SLNB performed as a consequence of a positive preoperative US and FNAC. A positive preoperative examination was defined as a suspicious US and malignant cells or cells suspicious of malignancy in the axillary lymph nodes on FNAC leading to an immediate ALND. In a few patients, ALND was performed based only on a suspicious US without FNAC.

In all, 13,469 patients (78%) did not have macrometastases in the sentinel node, but 194 of these patients had macrometastases in non-sentinel nodes, and 242 patients had non-detection of SN and had ALND performed. Out of these, 150 did not have any macrometastases. In total, 13,425 patients did not have macrometastases and were defined as true node-negative. Of the 17,265 clinically node negative patients, 3554 patients had macrometastases in the sentinel node, 194 patients had macrometastases in non-sentinel nodes and 92 patients in the non-detection group had macrometastases on the subsequent ALND (Figure 1). This resulted in 3840 (22.2%) patients who were false negative on the preoperative examination.

Out of the 1703 patients who did not have a SNLB performed, 1691 patients had macrometastases on ALND and were true positive. The remaining 21 patients did not have macrometastases on ALND, but eight of the patients had micrometastases and one patient had isolated tumor cells. These nine patients were not considered false positive. In total, 12 patients did not have any malignant cells on ALND and were defined as false positive. These 12 patients had suspicious malignant cells on FNAC ($n = 10$) or a suspicious US ($n = 2$).

The sensitivity of the combined preoperative examination including US and FNAC was 30.6%. The risk of overlooking macrometastases on the preoperative examination was associated to lobular histologic subtype compared to ductal histologic subtype, OR = 2.54 (95% CI: 2.03–3.17). There was a significant difference between the mean number of

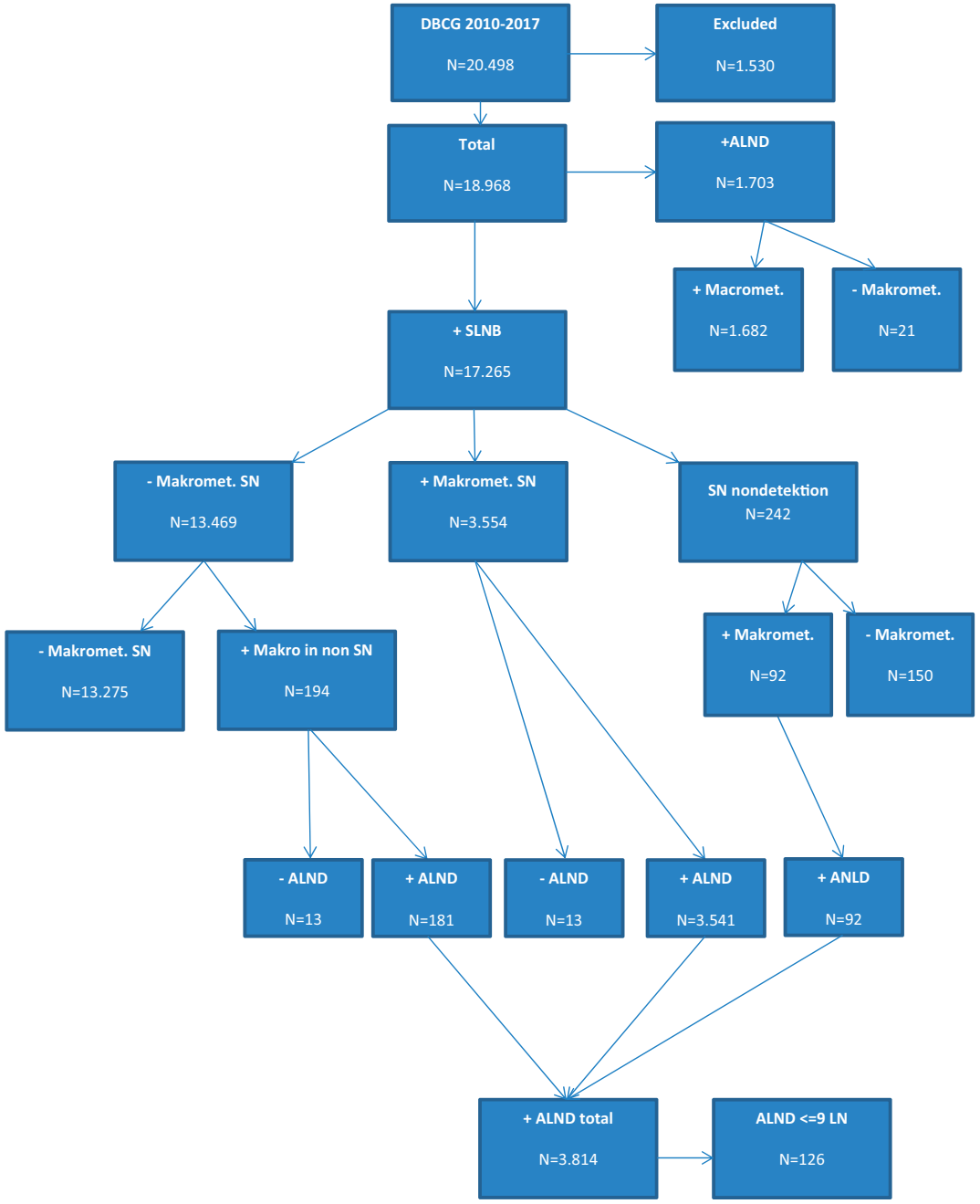


Figure 1. Flowchart 20,498 Danish womem with primary breast cancer treated 2010–2015. Macromet: Macrometastasis; LN: Lymphnode.

macrometastases in the group who has SLNB performed (2.8) compared to those who have ALND performed initially (5.8), $p < .0001$. The specificity of the combined preoperative examination was 99.9%. PPV was 99.3% based on the 1691 patients who were true positive and the total number of lymph node positive patients. Hence, the NPV was 77.8%, calculated from the number of 13,425 patients being true negative and the total number of negative (13,425 + 3840 patients).

Discussion

The implementation of SLNB is the largest breakthrough in breast cancer surgery since the introduction of breast conserving surgery in the eighties. Today, about 65% of breast cancer patients are spared an ALND without impairment of prognosis and local control, but gaining much less arm morbidity and increasing quality of life [8]. However, as long as ALND is the standard of care in patients with positive SN, the SLNB is basically, in case of macrometastatic spread, only waste of time, money and induction of false hope for the patient.

The present study indicates that only about one-third of women with macrometastases are identified prior to surgery. Not surprisingly the chance of finding metastatic lymph nodes preoperatively was associated with high metastatic burden in the axilla. During recent years, international randomized trials have indicated that that omission of ALND is safe in women with minor spread to the axillary lymph nodes [9,10]. This increases the need for a precise

Table 1. Distribution according to histologic subtype of 5496 Danish women with primary breast cancer and macrometastasis in axillary lymph nodes treated in the period of 2010–2015.

	Procedure SLNB	Procedure ALND	Total
Diagnosis			
Ductal	3126 (82.0%)	1479 (87.9%)	4605
Lobular	536 (14.1%)	100 (6.0%)	636
Other	152 (4.0%)	103 (6.1%)	255
Total	3814	1682	5496

preoperative staging of the axilla in order to identify patients with major metastatic spread, where ALND may still be indicated.

An increasing proportion of patients with verified metastases in the axilla by axillary US and FNAC are offered neoadjuvant treatment. Around 30% of these patients experience pathologic complete response (PCR) in the axilla [11,12]. Recent studies have shown that ALND can safely be omitted in patients with PCR if no metastases or even isolated tumor cells are found in the SN or in a FNAC proven metastatic lymph node, marked before treatment [13,14]. Again, this increases the need for a more accurate preoperative staging of the axilla.

The study shows that the Danish diagnostic set-up is very predictive concerning positive axillary status since only on average two women in Denmark of the about 4700 new cases of breast cancer every year, undergo direct ALND based on a false-positive preoperative diagnosis of axillary spread. However, two-thirds of the patients with macroscopic positive lymph nodes were diagnosed on SLNB, which prolonged the surgical procedure, and excluded axillary status as indication for neoadjuvant treatment. Data from DBCG shows that the sensitivity varies from 24 to 48% between the different centers in Denmark [15]. Hence, it is possible to optimize the present technology. The need for new diagnostic tools to identify nodal status in women with breast cancer is obvious. At present a new PET-tracer is tested at Rigshospitalet with particular focus on sensitivity of axillary status.

Acknowledgment

We would like to thank Maj-Britt Jensen for statistical support.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was funded by Danish Cancer Society.

ORCID

Tove Filtenborg Tvedskov http://orcid.org/0000-0002-7568-1790
Niels Kroman http://orcid.org/0000-0002-6631-2993

References

[1] DBCG Guidelines [Internet]. Copenhagen: Danish Breast Cancer Group; 2017 [cited 2017 Oct 30]. Available from: http:/www.dbcg.dk

[2] Houssami N, Ciatto S, Turner RM, et al. Preoperative ultrasound-guided needle biopsy of axillary nodes in invasive breast cancer: meta-analysis of its accuracy and utility in staging the axilla. Ann Surg. 2011;254:243–251.

[3] Houssami N, Turner RM. Staging the axilla in women with breast cancer: the utility of preoperative ultrasound-guided needle biopsy. Cancer Biol Med. 2014;11:69–77.

[4] Christiansen P, Friis E, Balslev E, et al. Sentinel node biopsy in breast cancer: five years experience from Denmark. Acta Oncol. 2008;47:561–568.

[5] Diepstraten SC, Sever AR, Buckens CF, et al. Value of preoperative ultrasound-guided axillary lymph node biopsy for preventing completion axillary lymph node dissection in breast cancer: a systematic review and meta-analysis. Ann Surg Oncol. 2014;21:51–59.

[6] Boughey JC, Moriarty JP, Degnim AC, et al. Cost modeling of preoperative axillary ultrasound and fine-needle aspiration to guide surgery for invasive breast cancer. Ann Surg Oncol. 2010;17:953–958.

[7] Moller S, Jensen MB, Ejlertsen B, et al. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.

[8] Gartner R, Jensen MB, Nielsen J, et al. Prevalence of and factors associated with persistent pain following breast cancer surgery. JAMA. 2009;302:1985–1992.

[9] Giuliano AE, Ballman K, McCall L, et al. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: long-term follow-up from the American College of Surgeons Oncology Group (Alliance) ACOSOG Z0011 randomized trial. Ann Surg. 2016;264:413–420.

[10] Donker M, van TG, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. Lancet Oncol. 2014;15:1303–1310.

[11] Donker M, Straver ME, Wesseling J, et al. Marking axillary lymph nodes with radioactive iodine seeds for axillary staging after neoadjuvant systemic treatment in breast cancer patients: the MARI procedure. Ann Surg. 2015;261:378–382.

[12] Boughey JC, Ballman KV, Hunt KK, et al. Axillary ultrasound after neoadjuvant chemotherapy and its impact on sentinel lymph node surgery: results from the American College of Surgeons Oncology Group Z1071 trial (alliance). JCO. 2015;33:3386–3393.

[13] Boileau JF, Poirier B, Basik M, et al. Sentinel node biopsy after neoadjuvant chemotherapy in biopsy-proven node-positive breast cancer: the SN FNAC study. JCO. 2015;33:258–264.

[14] Caudle AS, Yang WT, Krishnamurthy S, et al. Improved axillary evaluation following neoadjuvant therapy for patients with node-positive breast cancer using selective evaluation of clipped node-s:implementation of targeted axillary dissection. JCO. 2016;34:1072–1078.

[15] DBCG Kvalitetsindikatorrapport [Internet]. Copenhagen: Danish Breast Cancer Group; 2016 [cited 2017 Oct 30]. Available from: http:/www.dbcg.dk/PDF%Filer/aarsrapport_2016_final.pdf

Appendix 1: Postop. axilstatus.

Reasons for exclusion of patients:

- Neoadjuvant treatment (n = 1201)
- No surgical treatment ($n = 120$)
- No axillary dissection ($n = 60$)
- Multifocal or ulcerating tumor (primary ALND) ($n = 27$)
- Relapse of previous cancer or DCIS ($n = 25$)
- DCIS/LCIS ($n = 23$)
- Primary ALND with no reason noted ($n = 16$)
- Previous surgery in breast/axil, therefore no SLNB (primary ALND) ($n = 14$)

- Diagnostic excision biopsy or excision of DCIS which proved cancer, therefore primary ALND ($n = 12$)
- Occult breast cancer ($n = 5$)
- LN found with other imaging modality than US ($n = 5$)
- Inflammatory breast cancer (primary ALND) ($n = 4$)
- Other cancer in same breast/axil ($n = 4$)
- Clinical metastasis to axil, nothing noted about US ($n = 4$)
- Breast cancer found in relation to breast reducing surgery ($n = 2$)
- No SLNB because of breast implants (primary ALND) ($n = 1$)
- 2 macrometastases found in lumpectomy, nothing noted about SLNB ($n = 1$)
- Patient from Greenland, no SLNB ($n = 1$)
- Normal axil at time of diagnosis, alternative treatment for 1 year, clinical metastases at time of operation ($n = 1$)
- Patient refrains from SLNB (primary ALND) ($n = 1$)
- Tumor excision in other department showed breast cancer, hereafter unsuccessful fine needle aspiration x 2, primary ALND ($n = 1$)
- Patient diagnosed in 2007, surgery in 2012 for unknown reasons ($n = 1$)

LETTER TO THE EDITOR

Axillary lymph node dissection in breast cancer patients after sentinel node biopsy*

Christina Jessing[a], Linnea Langhans[a], Maj-Britt Jensen[b], Maj-Lis Talman[c], Tove Filtenborg Tvedskov[a] and Niels Kroman[a]

[a]Department of Breast Surgery, Copenhagen University Hospital Herlev, Denmark; [b]Danish Breast Cancer Cooperative Group Secretariat, Rigshospitalet, Copenhagen University Hospital, Denmark; [c]Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Denmark

Introduction

Axillary lymph node status has for long been the most important prognostic factor in patients with primary breast cancer [1]. Axillary lymph node dissection (ALND) provides the most accurate staging of axillary lymph nodes and the primary objective of ALND is, besides staging, local disease control [2]. Furthermore, axillary lymph node status determines adjuvant treatment protocols based on the number of macro metastases in the axillary lymph nodes, and survival is improved and recurrence reduced when patients receive adjuvant treatment corresponding to their axillary status [2,3]. Advancements in adjuvant systemic therapy and radiotherapy have improved loco-regional control of breast cancer and the therapeutic value of axillary surgery has been diminished as well [4]. Besides this an increasing number of patients are treated with neoadjuvant therapy, which may result in complete pathologic response with remission of axillary metastases in about 30% of patients [5–8]. Thus, axillary dissection can be spared in these patients.

In Denmark, breast cancer patients are treated according to national guidelines from the DBCG. The preoperative examination includes ultrasonography (US) of the axillary lymph nodes and fine needle aspiration if suspicious lymph nodes are present. A systematic review and meta-analysis from 2014 found that 50% of women with axillary involvement can be identified preoperatively by axillary US-guided biopsy [9]. Staging of patients who are clinically node negative on US is done by sentinel lymph node biopsy (SLNB). SLNB is followed by ALND if metastases are found in the sentinel node (SN). In 2013, ALND was restricted to patient with macro metastases (>2 mm) [10].

ALND can cause significant morbidity such as impaired arm-/shoulder mobility, sensory disturbances, lymphedema and persistent pain. The prevalence of sequelae is influenced by the extent of the axillary surgery. ALND is associated with an increased prevalence and intensity of pain as well as an increased number and sensory disturbances compared with SLNB [11–13].

In a randomized trial (ACOSOG Z0011), the American College of Surgeons Oncology Group studied disease-free survival and mortality in selected patients with positive SNs treated with or without ALND [14]. The trial showed that ALND can be omitted in a patient group with one or two positive SNs, treated with breast conserving surgery (BCS) and adjuvant radiotherapy without compromising loco-regional control or survival. However this study did not reach planned inclusion of patients and more than hundred institutions contributed some of which only included few patients. Surgical treatment of the axilla is still the gold standard in patients with extensive nodal involvement. It is therefore important to identify patients with a high risk of extensive axillary involvement besides SN.

The aim of this study is to further characterize patients with extensive nodal involvement among women with early stage breast cancer, who are preoperatively clinically node negative, based on preoperative US with or without fine

CONTACT Niels Kroman Niels.Kroman@regionh.dk Breast Surgery Section, Rigshospitalet, Blegdamsvej 9, 3014, DK-2100 Copenhagen, Denmark
*A national register based study on behalf of Danish Breast Cancer Group, DBCG.
Supplemental data for this article can be accessed here.

http://dx.doi.org/10.1080/0284186X.2017.1401227

needle aspiration cytology, who were found with macro metastases in SN. The results may be used to select high-risk patients who might benefit from axillary surgery or maybe neoadjuvant treatment for down-staging the axilla. We chose to include patients treated after the implementation nationwide mammography screening in 2010 in order to base the study on a data material where clinical practice is similar of today.

Material and methods

Patients

Data on patients diagnosed with primary unilateral breast cancer, in 2010 and through 2015, were retrieved from the DBCG database. In Denmark, clinical and histopathological data and information on treatment and follow-up status on patients with breast cancer are registered in the DBCG national database, which was established in 1977. Data are prospectively entered into the database from all Danish departments involved in the diagnosis and treatment of breast cancer. The database is considered to have a close to complete reporting of all cases [15]. Missing data were obtained from the Danish Pathology Data Bank.

In the analysis, we included patients who were preoperatively clinically node negative but were found to have macro metastases on SLNB. The exclusion criteria were age $\geq$80 years and, neoadjuvant treatment. Patients who did not receive SLNB were not included in the analysis, as well as patients who did not receive ALND after SLNB with macro metastases. We set a minimum of 10 lymph nodes as standard for a sufficient ALND and excluded women with $<$10 lymph nodes removed.

Statistical analysis

Univariate and multivariate logistic regression were used to estimate odds ratios (OR) with 95% confidence intervals (CI) for ten or more nodes with macrometastases, and a supplementary analysis considering 4 + macrometastases an event. The Wald test was used to test hypotheses on parameters. Included in the models were age, histological type and grade, tumor size, ER and HER2 status. Unknowns were included in largest categories. $P < .05$ was considered statistically significant. All statistical analyses were performed using SAS statistical software, version 5.1 (SAS Institute Inc, NC, USA).

Results

A total of 20498 patients were included in the study. Based on the listed criteria 1530 patients were excluded due to neoadjuvant treatment, misclassification and diagnosis with only DCIS/LCIS after reexamination of the pathology reports or because they met other exclusion criteria (Supplementary material). Of the remaining patients 1,703 did not have SLNB performed as they immediately underwent ALND because of axillary metastases found by US with or without US-guided fine needle aspiration. The remaining 17265 patients were clinically node negative and underwent SLNB. A total of 3554 patients had macro metastases in one or more SN and 3,541 subsequently had ALND. In 13,469 patients, no macro metastases were found in SN but 194 patients had macro metastases in non SNs removed during the SLNB procedure. No matter of the macro metastases were identified as a radioactive lymph node, a blue dye–stained lymph node or another lymph node resected during the SLNB procedure they were considered SN positive. A total of 181 of them went on to have an ALND. In 242 cases SN could not be identified and these patients were treated with ALND. Among these, 92 patients had macro metastases.

Altogether 3814 clinically node negative patients had macrometastases in the axilla and an ALND. 126 were excluded because less than 10 lymph nodes had been removed during SLNB and ALND. The final cohort consisted of 3,688 patients of which only 6% (215 patients) had $\geq$10 lymph nodes with macrometastases (Table 1). The majority (3473 patients) had $\leq$9 lymph nodes with macrometastases and among these, 2946 (80%) had only 1–3 macrometastasis. (Table 2).

Age more than 50 years, lobular subtype, increasing tumor size and histologic grade II + III, were significantly associated with more than 10 positive nodes, when adjusting for age, histological type, tumor size, grade, ER and HER2 status (Table 2). The most profound risk factors was lobular subtype compared to ductal subtype (OR: 3.09; 95% CI: 2.20–4.34) and tumor size $>$50 mm. compared to size 11–20 mm. (OR: 6.47; 3.97–10.5).

We subsequently performed an exploratory analysis with a cutpoint of four or more positive nodes revealing similar results as mentioned above (data not shown).

Discussion

This large population-based study based on close to 4000 women with macrometastases in SN showed that only 15% harbor in all more than three macrometastases in the rest of the axilla. Currently, it is standard treatment to perform completion ALND removing on average more than 15 lymph nodes in patients with macrometastases in the SN, which means that many lymph nodes are unnecessarily removed, with increasing risk of arm morbidity but without therapeutic gain [16,17]. It is obvious that adherence to this guideline often leads to overtreatment.

Of the 17,265 clinically node negative patients, 3688 had macrometastases at SLNB and/or ALND, which equals 21.4%. Of these patients 215 (1.3%) had $\geq$10 lymph nodes with macrometastases.

The role of ALND in the treatment of SN-positive patients is currently being questioned and the ACOZOG Z0011 study by Guiliano et. al. argues that in a group of women ALND can be omitted when only 1 or 2 SN are with macrometastases [14]. Currently, the SENOMAC trial is including patients with macrometastases in 1-2 SN for randomization to either ALND or no ALND [18]. However, as the trend of de-escalation of axillary surgery becomes more widely implemented,

Table 1. Characteristics of 3688 Danish women where macrometastases were found in SLNB and subsequent ALND subdivided according to the number of macrometastases.

	Total (3688) No (%)	1–3 Macro (2946) No (%)	4–9 Macro (527) No (%)	≥10 Macro (215) No (%)
Age, y				
<50	963 (26.1)	785 (26.6)	139 (26.4)	39 (18.1)
50–59	1095 (29.7)	869 (29.5)	163 (30.9)	63 (29.3)
60–69	1242 (33.7)	994 (33.7)	162 (30.7)	86 (40.0)
70–79	388 (10.5)	298 (10.1)	63 (12.0)	27 (12.6)
Diagnosis				
Ductal	3016 (81.8)	2473 (83.9)	407 (77.2)	136 (63.3)
Lobular	526 (14.3)	355 (12.1)	97 (18.4)	74 (34.4)
Other	146 (4,0)	118 (4.0)	23 (4.4)	5 (2.3)
Tumor size, mm				
≤10	390 (10.6)	355 (12.1)	24 (4.6)	11 (5.1)
11–20	1686 (45.7)	1417 (48.1)	207 (39.3)	62 (28.8)
21–50	1462 (39.6)	1093 (37.1)	261 (49.5)	108 (50.2)
>50	145 (3.9)	76 (2.6)	35 (6.6)	34 (15.8)
Unknown	5 (0.1)	5 (0.2)	0 (0.0)	0 (0.0)
Grade				
I	857 (23.2)	738 (25.1)	96 (18.2)	23 (10.7)
II	1784 (48.4)	1384 (47.0)	275 (52.2)	125 (58.1)
III	872 (23.6)	682 (23.2)	130 (24.7)	60 (27.9)
Unknown	175 (4.7)	142 (4.8)	26 (4.9)	7 (3.3)
ER status				
Positive	3324 (90.1)	2658 (90.2)	471 (89.4)	195 (90.7)
Negative	350 (9.5)	275 (9.3)	55 (10.4)	20 (9.3)
Unknown	14 (0.4)	13 (0.4)	1 (0.2)	0 (0.0)
HER2 status				
Normal	3113 (84.4)	2523 (85.6)	420 (79.7)	170 (79.1)
Positive	499 (13.5)	367 (12.5)	95 (18.0)	37 (17.2)
Unknown	76 (2.1)	56 (1.9)	12 (2.3)	8 (3.7)

Table 2. Univariate and multivariable analysis of risk for ≥10 macrometastases compared to 1–9 macrometastases in the 3688 Danish patients with macrometastases SLNB and subsequent ALND.

	Unadjusted OR (95%CI)	*p* value	Adjusted OR (95%CI)	*p* value
Age, y		.029		.0031
<50	1.00 (ref)		1.00 (ref)	
50–59	1.45 (0.96–2.18)		1.86 (1.222.86)	
60–69	1.76 (1.20–2.60)		2.14 (1.42–3.21)	
≥70	1.77 (1.07–2.94)		2.06 (1.22–3.47)	
Diagnosis		<.0001		<.0001
Ductal	1.00 (ref)		1.00 (ref)	
Lobular	3.47 (2.57–4.68)		3.09 (2.20–4.34)	
Other	0.75 (0.30–1.86)		1.46 (0.53–3.57)	
Tumor size, mm		<.0001		<.0001
≤10	0.76 (0.40–1.46)		0.84 (0.44–1.63)	
11–20	1.00 (ref)		1.00 (ref)	
21–50	2.10 (1.52–2.89)		1.78 (1.29–2.50)	
>50	8.05 (5.08–12.8)		6.47(3.97–10.5)	
Grade[a]		<.0001		.0005
I	1.00 (ref)		1.00 (ref)	
II	2.73 (1.74–4.29)		2.24 (1.41–3.57)	
III	2.68 (1.64–4.37)		2.85 (1.66–4.89)	
ER status		0.91		0.81
Positive	1.00 (ref)		1.00 (ref)	
Negative	0.97 (0.61–1.56)		0.94 (0.56–1.58)	
HER2 status		.083		0.06
Normal	1.00 (ref)		1.00 (ref)	
Positive	1.39 (0.96–2.01)		1.47 (0.98–2.21)	

[a]If diagnosis is not ductal or lobular the reference is ductal grade I.
CI: Confidence interval; OR: odds ratio.

it may be important to identify women who have a high risk of having many axillary metastases since this group could be at increased risk of axillary relapse if not treated with ALND. Furthermore, these women may befit from neoadjuvant therapy with chance of complete pathologic response in the axilla and hence omission of ALND.

Several other studies have previously tried to identify clinical and pathological characteristics of the primary tumor and SN significant for metastatic spread to other axillary lymph nodes, but these studies have not focused on the extent of metastatic spread [17,19]. Studies on prediction of extensive

nodal involvement in the axilla exist but most of these studies include patients diagnosed as node positive on axillary US [20,21]. One previous European multicenter study has tried to identify risk factors for four or more positive axillary lymph nodes [22]. Like us, this study identified size of the primary tumor as a risk factor, as well as extracapsular extension and number of positive SN. To our knowledge, no previous study has focused on the risk of more than 10 positive axillary lymph nodes. In our study, only 6% of the SN-positive patients had metastases in >10 axillary nodes. These patients are rather candidates for neoadjuvant treatment than primary surgery.

The omission of ALND in SN-positive patients represents a shift in approach for surgical practice as this leaves a group of patients with residual disease in the axilla to be targeted by adjuvant therapy. This changing approach to patients with a positive SN will benefit women with limited axillary involvement as morbidity following axillary surgery will be reduced. It has been showed that ALND is associated with a higher prevalence of persistent pain, lymphedema and sensory disturbances than SLNB. It is out most important to continue to limit the number of women undergoing ALND without therapeutic benefit, but on the other hand not withhold ALND among women with high risk of axillary recurrence.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was funded by Kräftens Bekämpelse.

ORCID

Niels Kroman http://orcid.org/0000-0002-6631-2993

References

[1] Goldhirsch A, Ingle JN, Gelber RD, et al. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol. 2009;20:1319–1329.
[2] Blichert-Toft M. Axillary surgery in breast cancer management–-background, incidence and extent of nodal spread, extent of surgery and accurate axillary staging, surgical procedures. Acta Oncol. 2000;39:269–275.
[3] Axelsson CK, Mouridsen HT, Zedeler K. Axillary dissection of level I and II lymph nodes is important in breast cancer classification. The Danish Breast Cancer Cooperative Group (DBCG). Eur J Cancer. 1992;28A:1415–1418.
[4] Jatoi I, Benson JR, Toi M. De-escalation of axillary surgery in early breast cancer. Lancet Oncol. 2016;17:e430–e441.
[5] Donker M, Straver ME, Wesseling J, et al. Marking axillary lymph nodes with radioactive iodine seeds for axillary staging after neoadjuvant systemic treatment in breast cancer patients: the MARI procedure. Ann Surg. 2015;261:378–382.
[6] Boughey JC, Ballman KV, Hunt KK, et al. Axillary ultrasound after neoadjuvant chemotherapy and Its impact on sentinel lymph node surgery: results From the American College of Surgeons Oncology Group Z1071 Trial (Alliance). JCO. 2015;33:3386–3393.
[7] Rubio IT. Sentinel lymph node biopsy after neoadjuvant treatment in breast cancer: Work in progress. Eur J Surg Oncol. 2016;42:326–332.
[8] Caudle AS, Yang WT, Krishnamurthy S, et al. Improved axillary evaluation following neoadjuvant therapy for patients with node-positive breast cancer using selective evaluation of clipped nodes: implementation of targeted axillary dissection. JCO. 2016;34:1072–1078.
[9] Diepstraten SC, Sever AR, Buckens CF, et al. Value of preoperative ultrasound-guided axillary lymph node biopsy for preventing completion axillary lymph node dissection in breast cancer: a systematic review and meta-analysis. Ann Surg Oncol. 2014;21:51–59.
[10] Tvedskov TF. The evolution of the sentinel node procedure in the treatment of breast cancer. Dan Med J. 2017;64:B5402.
[11] Husted MA, Haugaard K, Soerensen J, et al. Arm morbidity following sentinel lymph node biopsy or axillary lymph node dissection: a study from the Danish Breast Cancer Cooperative Group. Breast. 2008;17:138–147.
[12] Gartner R, Jensen MB, Nielsen J, et al. Prevalence of and factors associated with persistent pain following breast cancer surgery. JAMA. 2009;302:1985–1992.
[13] Mejdahl MK, Andersen KG, Gartner R, et al. Persistent pain and sensory disturbances after treatment for breast cancer: six year nationwide follow-up study. BMJ. 2013;346:f1865.
[14] Giuliano AE, Ballman K, McCall L, et al. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: long-term follow-up from the American College of Surgeons Oncology Group (Alliance) ACOSOG Z0011 Randomized Trial. Ann Surg. 2016;264:413–420.
[15] Moller S, Jensen MB, Ejlertsen B. The clinical database and the treatment guidelines of the Danish Breast Cancer Cooperative Group (DBCG); its 30-years experience and future promise. Acta Oncol. 2008;47:506–524.
[16] Damgaard OE, Jensen MB, Kroman N, et al. Quantifying the number of lymph nodes identified in one-stage versus two-stage axillary dissection in breast cancer. Breast. 2013;22:44–46.
[17] Degnim AC, Griffith KA, Sabel MS, et al. Clinicopathologic features of metastasis in nonsentinel lymph nodes of breast carcinoma patients. Cancer. 2003;98:2307–2315.
[18] de BJ, Frisell J, Andersson Y, et al. Survival and axillary recurrence following sentinel node-positive breast cancer without completion axillary lymph node dissection: the randomized controlled SENOMAC trial. BMC Cancer. 2017;17:379.
[19] Canavese G, Bruzzi P, Catturich A, et al. A risk score model predictive of the presence of additional disease in the axilla in early-breast cancer patients with one or two metastatic sentinel lymph nodes. Eur J Surg Oncol. 2014;40:835–842.
[20] Verheuvel NC, Ooms HW, Tjan-Heijnen VC, et al. Predictors for extensive nodal involvement in breast cancer patients with axillary lymph node metastases. Breast. 2016;27:175–181.
[21] Yun SJ, Sohn YM, Seo M. Risk stratification for axillary lymph node metastases in breast cancer patients: what clinicopathological and radiological factors of primary breast cancer can predict preoperatively axillary lymph node metastases?. Ultrasound Q. 2017;33:15–22.
[22] Meretoja TJ, Audisio RA, Heikkila PS, et al. International multicenter tool to predict the risk of four or more tumor-positive axillary lymph nodes in breast cancer patients with sentinel node macrometastases. Breast Cancer Res Treat. 2013;138:817–827.

LETTER TO THE EDITOR

Occult breast cancer in breast reduction specimens

Lotte Meyer[a], Camilla Bille[b], Thomas Foged[c] and Anne Marie Bak Jylling[d]

[a]Department of Pathology, Odense University Hospital, University of Southern Denmark, Odense, Denmark; [b]Department of Plastic Surgery Z, Odense University Hospital, Odense, Denmark; [c]Department of Plastic Surgery, Aarhus University Hospital, Aarhus, Denmark; [d]Department of Pathology, Odense University Hospital, Odense, Denmark

Introduction

In Denmark, the risk of developing invasive breast cancer before the age of 75 is 11% [1]. Screening for breast cancer is offered routinely from age 50-69 [2]. Patients undergoing breast reduction are in risk of having occult invasive breast cancer or ductal carcinoma in situ at the time of surgery. Detection of such malignancies can be made pre- or postoperatively. In order to detect malignancies preoperatively the Danish guidelines suggest that women 40 + years of age ought to have a clinical mammography prior to breast reduction surgery [3].

Postoperative diagnosis can be verified at the pathologic examination. Two different principles in this exist: (1) consistent microscopic examination of all specimens or (2) macroscopic examination, only followed by microscopy in case of focal abnormalities.

This study aimed to evaluate if macroscopic examination of breast reduction specimens is a sufficient procedure for detection of cancer. Second, we intended to map the methods for specimen examination in Denmark.

Method

Two sub-studies were undertaken:

1. A study regarding quality of specimen examination method.
   Here we quantified the subsequent occurrence of breast cancer in women who had earlier had breast reduction surgery. Furthermore, every cancer case was thoroughly evaluated in order to decide if the cancer might have been present and overlooked at the time of breast reduction surgery.
2. A mapping of current specimen examination method used in Departments of Pathology in Denmark.

Study 1 – quality of specimen examination method

Study population and data processing

The Danish National Pathology Register comprises information on all pathoanatomical analysis of specimens performed by Danish pathology departments. Information regarding all tissue specimens is registered with the international coding system "Systematized Nomenclature of Medicine" (SNOMED) [4]. Using this register it was possible to identify 1120 women who have had breast reduction surgery at Odense University Hospital from January 2005 until December 2010. Of these 363 fulfilled the inclusion criteria; female sex, bilateral breast reduction surgery and no previous breast cancer.

All specimens from the included 363 women were examined at the Pathology department at Odense University Hospital. At this department macroscopic examination is manual slicing of the tissue and examination of the tissue slices by palpation and vision. Microscopic examination is only performed when suspicious areas with focal abnormalities are found.

Information on possible diagnoses of invasive breast cancer and ductal carcinoma in situ registered after breast reduction surgery were ascertained from the Danish National Pathology Register by searching for relevant SNOMED codes.

The follow-up period was calculated from the day of breast reduction surgery until a registered diagnosis of breast cancer, death or September 1. 2015 occurred. Histological details for women diagnosed with invasive breast cancer or ductal carcinoma in situ can be seen in Table 1.

Data on expected numbers of invasive breast cancers was obtained from NORDCAN. NORDCAN comprise information on incidence, mortality, prevalence and survival statistics on more than 50 major cancers in the Nordic countries [1]. No information on ductal carcinoma in situ is retrievable from this database. In order to calculate the expected number of breast cancers in our population, data for age specific incidence rates from 2006 to 2014 from NORDCAN was used.

Statistics

Statistical calculations were made using Stata 14. Statistical significance was defined as a two-sided p-value $<.05$. Risk ratio for the examination procedures was calculated using Fishers exact test.

Study 2 – mapping of specimen examination method in Denmark

Information on clinical practice regarding examination method of tissue from breast reduction surgery was obtained through e-mails to all 12 Departments of Pathology in Denmark.

CONTACT Lotte Meyer ceautiful@hotmail.com University of Southern Denmark, Odense, Denmark

Supplemental data for this article can be accessed here.

http://dx.doi.org/10.1080/0284186X.2017.1400178

Results

Study 1 – quality of specimen examination method

For 84.3% ($n = 306$) of the women, only macroscopic examination was found to be relevant. Further microscopic examination was performed in the remaining 15.7% ($n = 57$) of the women. In two of the 306 and three of the 57 women breast cancer was found. One case was identified during the surgery and was excluded. The others were identified during the follow-up period.

Risk ratio for being diagnosed with breast cancer in the follow-up period showed a significantly increased risk for women whose tissue was initially examined microscopically. Risk ratio was 0.12 (95%CI 0.03–0.55), $p = .0292$.

Prevalence of invasive breast cancer was 0.3% ($n = 4$) and the incidence rate was 1.1% per 2868 person years. One patient was diagnosed solely with ductal carcinoma in situ. Incidence rate of ductal carcinoma in situ was 0.3% ($n = 1$) per 2868 person years. Mean follow-up was 7.9 years (95%CI 7.7–8.1).

The expected number of invasive breast cancers in the population at 8 years of follow-up was 3.47. No statistical significant difference between observed and expected incidence rates was found. Rate ratio was 1.17 ($p = .734$, 95%CI .28–7.04). The average age of the women were 38.8 years (95%CI 37.4–40.1).

We found no statistical significant difference in incidence rate of breast cancers based on the examination method ($p = .1147$).

Study 2 – mapping of specimen examination method in Denmark

The response rate was 83%, as 10 out of 12 departments answered the questionnaire. One of these did not received breast reduction specimens and was excluded. Six departments systematically conducted a microscopic examination of their specimens. The remaining three performed a microscopic examination only when considered relevant after the macroscopic examination.

Discussion

Our data suggest that the risk of missing a cancer at the examination of breast reduction specimens by macroscopic examination is low. The prevalence of invasive breast cancer in our study was in line with previous reported prevalence ranging from 0% to 0.99% [5–14]. Overlooked breast cancer is therefore very rare in breast reduction specimens. Invasive breast cancer is even rarer in studies that, like ours, subtracted data for women with prior breast cancer [5,7,8,10,11,14].

A former Danish autopsy study of 110 younger to middle aged women found a much higher prevalence of invasive breast cancer (2%) and ductal carcinoma in situ (14%). A total of 275 tissue samples were here thoroughly microscopically examined suggesting a correlation between the number of tissue samples and probability of finding histologic abnormalities on a microscopic level.

Many international studies recommend consistent microscopic examination based on findings of high abnormality prevalence in breast reduction specimens alone [6,10,11,14]. Detection of other abnormalities than invasive breast cancer or ductal carcinoma in situ do not have the same clinical consequences in Denmark, therefore these recommendations cannot be applied uncritically. Lobular carcinoma in situ for example is still regarded an incidental finding, although increasing the subsequent risk of carcinoma [15]. Comparison with international studies and application of their recommendations are therefore difficult.

Women with benign breast abnormalities, typically found in breast reduction specimens, generally have more precursor prone tissue that can lead to breast cancer [6,14]. Second, invasive breast cancer found at the time of operation immediately gets histologically diagnosed by microscopic analysis. Excluding the one case in our study that was diagnosed at the time of the operation from the statistical analysis results in no difference between the incidence rates of breast cancers based on the examination method.

We can't exclude that a small breast cancer has been removed at the breast reduction without diagnosing it in the subsequent pathology examination. A further limitation is the relatively few numbers of women with invasive breast cancer and ductal carcinoma in situ in this study. Several large international retrospective studies have found fewer observed invasive breast cancer than expected in cohorts of women who underwent breast reduction surgery [13,16,17].

Identified cases

Evaluation of each identified breast cancer case is shown in Table 2. The timespan between the operations and the succeeding cancers, combined with tumor characteristics and clinic, makes it unlikely that any of the breast cancers were overlooked at the initial pathology examination (Supplementary material).

Evaluating the examination method

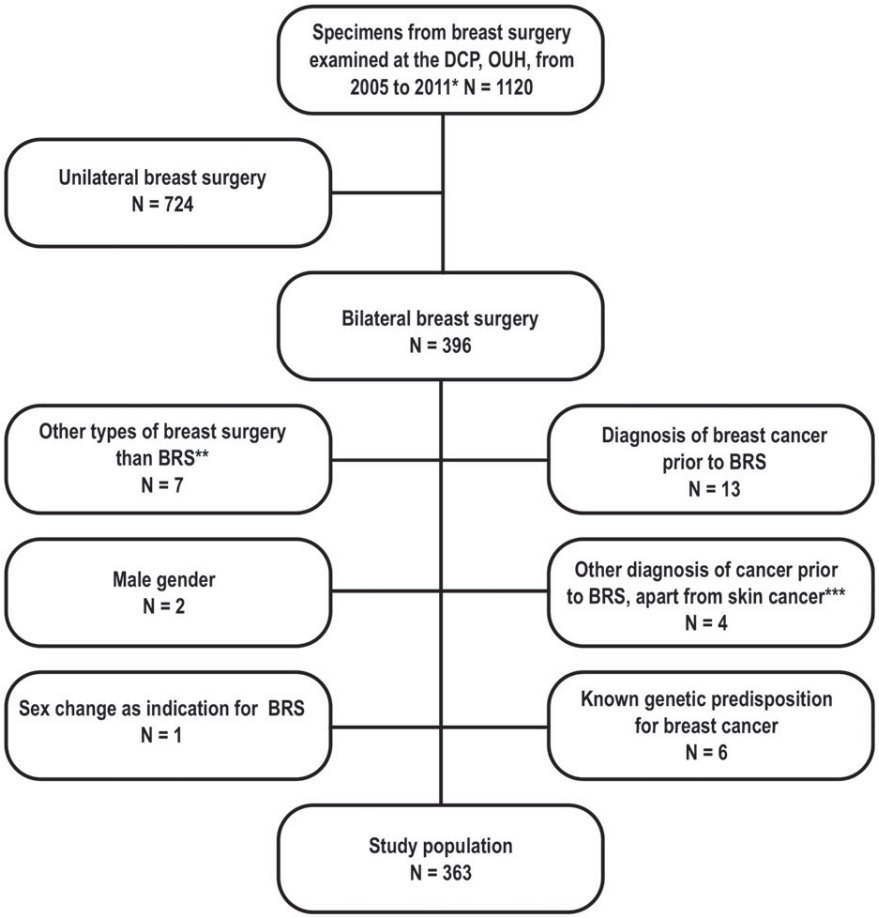
The examination procedure chosen by pathology departments should represent a method not only oncological safe but also one that makes economic sense.

The pathologists in this study found macroscopic abnormalities in 16% of the specimens, which is high when compared to Cook et al. [12]. This could reflect that macroscopic examination is performed more thoroughly when subsequent microscopic examination is not standard procedure. Despite this, breast tissue initially not considered suspicious of occult cancer by macroscopic examination can contain abnormalities [7,12,18].

Conclusion

Breast carcinoma is rare in specimens from breast reduction surgery. This study examined the quality of a time-efficient

Table 1. Flowchart of selection of the study population.



*Specimens from patients were registered and examined at the Department of Clinical Pathology (DCP), Odense University Hospital (OUH). Patients were identified in Patobank using the following codes: T04030, T04020, T04800, T04010 and T04000 from January 1. 2005 to December 31. 2010. Exclusion by the following codes: P306Y3, P306X4, P30990, P32940 and P31060.
**Unilateral removal of a lipoma in two patients, a fibroadenoma in one patient, an abscess in two patients, a fistula in one patient and a nonosseous extramedullary plasmacytoma in one patient.
***except malignant melanoma.

Table 2. Characteristics of the 5 cases of invasive breast cancer (IBC) and ductal carcinoma in situ (DCIS) in the cohort.

Patient (age at surgery)	Follow-up (years)	Diagnosis	Tumour characteristics	Pathology examination method
1 (38,5)	0	Multifocal invasive ductal carcinoma, DCIS AL metastasis: 1/13	19 mm diameter (29 mm including DCIS areas) Grade II. ER pos, PR neg, HER2 normal	Macro and Micro
2 (43,5)	3,6	Invasive ductal carcinoma. AL metastasis: 1/17	63 mm diameter Grade II. ER pos, HER2 normal.	Macro and Micro
3 (38,8)	4,9	Invasive ductal carcinoma with satellites, DCIS. AL metastasis: 0/15	30 mm diameter Grade III. ER pos, HER2 normal, Ki67-PI >80%	Macro
4 (48,4)	5,2	Invasive ductal carcinoma, DCIS AL metastasis: 9/15	35 mm diameter Grade III. ER neg, HER2 normal Ki67-PI = 70%	Macro and Micro
5 (44,3)	7,0	DCIS. Sentinel node: 0/2	Area >60 mm diameter Van Nuys group 2	Macro

AL: axillary lymph node; ER: estrogen receptor; PR: progesterone receptor; HER2: HER2 receptor expression; Ki67-PI: Ki67 proliferation index.

method where microscopic examination was only done when macroscopically suspect areas were found. We did not find evidence of undiagnosed breast cancers at the time of surgery. Therefore, the method seems appropriate. The method is, however, dependent on a careful macroscopic examination and we encourage the results to be replicated in other populations so national guidelines for macroscopic examination can be established.

Disclosure statement

The authors report no conflicts of interest

Trial registration/ethics

This study was approved by the Danish Data Protection Agency and Danish Patient Safety Authority.

References

[1] Engholm G, Ferlay J, Christensen N, et al. NORDCAN: Cancer Incidence, Mortality, Prevalence and Survival in the Nordic Countries, Version 7.1 (09.07.2015). Association of the Nordic Cancer Registries. Danish Cancer Society. [Internet]; 2015. Available from: http://www.ancr.nu

[2] sst.dk[internet]. København: Sundhedsstyrelsen;c1928–2016. [updated 2014 oct 2; cited 2015 jan 12]. 2015. Available from: https://www.cancer.dk/fagfolk/screening/brystkraeft/

[3] Foged T, Sørensen JA, Søe KL, et al. Lack of nationwide Danish guidelines on mammography before non-oncological breast surgery. Dan Med J. 2015;62:A5076.

[4] patobank.dk. København: Danske regioner;c1999–2017[updated 2017; cited 2017 july 5]. 2017. Available from: http://www.patobank.dk/

[5] Viana GAP, Pitanguy I, Torres E. Histopathological findings in surgical specimens obtained from reduction mammaplasties. Breast. 2005;14:242–248.

[6] Clark CJ, Whang S, Paige KT. Incidence of precancerous lesions in breast reduction tissue: a pathologic review of 562 consecutive patients. Plast Reconstr Surg. 2009;124:1033–1039.

[7] Goyal A, Coulson SG, Wu JM, et al. Occult breast carcinoma in breast reduction specimens in European women. Breast Cancer Res Treat. 2011;128:749–753.

[8] Hassan FE, Pacifico MD. Should we be analyzing breast reduction specimens? A systematic analysis of over 1000 consecutive cases. Aesth Plast Surg. 2012;36:1105–1113.

[9] Ambaye AB, MacLennan SE, Goodwin AJ, et al. Carcinoma and atypical hyperplasia in reduction mammaplasty: increased sampling leads to increased detection. A prospective study. Plast Reconstr Surg. 2009;124:1386–1392.

[10] Colwell AS, Kukreja J, Breuing KH, et al. Occult breast carcinoma in reduction mammaplasty specimens: 14-year experience. Plast Reconstr Surg. 2004;113:1984–1988.

[11] Desouki MM, Li Z, Hameed O, et al. Incidental atypical proliferative lesions in reduction mammoplasty specimens: analysis of 2498 cases from 2 tertiary women's health centers. Hum Pathol. 2013;44:1877–1881.

[12] Cook IS, Fuller CE. Does histopathological examination of breast reduction specimens affect patient management and clinical follow up? J Clin Pathol. 2004;57:286–289.

[13] Boice JD, Friis S, McLaughlin JK, et al. Cancer following breast reduction surgery in Denmark. Cancer Causes Control. 1997; 8:253–258.

[14] Schantz PAM, Jahkola TA, Krogerus LA, et al. Should we routinely analyse reduction mammaplasty specimens? J Plast Recon Aesth Surg. 2017;70:196–202.

[15] dbcg.dk[internet]. København: Danish Breast Cancer Cooperative Group; c1976-2016[updated 2015 nov 30; cited 2015 dec 10]. 2006. Available from: http://www.dbcg.dk/DBCG%20Retningslinier.htm

[16] Fryzek JP, Ye W, Nyrén O, et al. A nationwide epidemiologic study of breast cancer incidence following breast reduction surgery in a large cohort of Swedish women. Breast Cancer Res Treat. 2006;97:131–134.

[17] Brown MH, Weinberg M, Chong N, et al. A cohort study of breast cancer risk in breast reduction patients. Plast Reconstr Surg. 1999; 103:1674–1681.

[18] Nielsen M, Thomsen JL, Primdahl S, et al. Breast cancer and atypia among young and middle-aged women: a study of 110 medicolegal autopsies. Br J Cancer. 1987;56:814–819.