Optimizing breast cancer survival models based on conventional biomarkers and stromal parameters

Dekker, T.J.A.; Dekker T.J.A.

Citation

Cover Page

The handle http://hdl.handle.net/1887/55957 holds various files of this Leiden University dissertation

Author: Dekker T.J.A.
Title: Optimizing breast cancer survival models based on conventional biomarkers and stromal parameters
Date: 2017-09-26

1

Reproducibility and optimization of prognostic and predictive factors in breast cancer

Background

Positive developments regarding the survival of breast cancer patients have been observed in the past decade. These developments are thought to be primarily due to the screening for early malignant disease and the effects of adjuvant endocrine-, HER2-inhibiting- and cytotoxic therapies [1]. The advantages of these treatment modalities have initially been observed in clinical trials and have also translated to improved survival for patients treated as part of routine clinical care [2, 3]. Careful patient selection is essential for determining which patients are eligible to receive additional systemic treatments. Recent guidelines regarding these treatment decisions recommend to determine intrinsic breast cancer subtypes based on the expression of hormone receptors (HR), human epidermal growth factor receptor 2 (HER2) and Ki-67 [4] in order to select patients for systemic treatment (table 1). Optimal assessment of all these factors in routine testing is essential and must be assured as much as possible.

The first part of this thesis concerns the detection of potential irreproducibility of the established parameters (hormone receptors, human epidermal growth factor receptor 2, Ki-67 and lymph vascular space invasion) that are assessed during the pathological analysis of breast cancer specimens. The current standard of care and points of discussion are addressed in this introduction.

Hormone receptors

The presence of estrogen receptor (ER) or progesterone receptors (PR) is reflective of a distinct biology compared to HR-negative tumors [5] and is associated with beneficial outcome and differential clinical behavior [6]. ER-negative tumors generally show early relapse with subsequent plateau, whereas ER-positive tumors follow a slower, more constant rate of relapse [7]. ER is currently assessed both because of its prognostic power and because it is the greatest determinant of response to estrogen-modulating therapies [4].

The presence of ER and PR positive cells is currently established via immuno-histochemistry (IHC) [8]. Compared to the previously used ligand-binding assays (LBA), IHC assays are less expensive and correlate more strongly to survival in patients treated with adjuvant hormonal therapy [9]. IHC also allows for the evaluation of the morphology of the ER-positive cells. LBAs are capable of providing continuous levels of either ER or PR, whereas IHC is strictly speaking not a quantitative technique. The threshold for ER positivity was initially established via LBAs, which has since been

correlated to IHC cut-off values [10, 11]. Zhang et al. published the ER test results from 1700 breast carcinomas and showed that ER-negative tumors (< 1% ER-positive cells) occur in about 24% cases and highly positive tumors (> 70% ER-positive cells) in 64% percent, with the remaining cases to be in between these extremes [7]. A publication by Collins et al. suggested that when using modern-day IHC methods, almost all tumors display either predominately positive or negative expression of the estrogen receptor [12].

Unfortunately, IHC procedures are subject to several variables that can potentially endanger testing reliability (table 2). One comparative study of 172 laboratories has shown that only 43% of participating laboratories were able to identify ER-low expressing tumors [13]. Only 36% of labs that participated in the UK NEQAS-ICC assessment in 2001 achieved acceptable assay performance [14]. Retesting of HR-tested tumors entered in the Eastern Cooperative Oncology Group (ECOG) study E2197 showed a concordance of 90% and 84%, respectively for locally tested and centrally tested ER and PR results [15]. Central review of the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization (ALTTO) demonstrated that ER-results could not be reproduced for 4.3% of ER-positive cases and for 21.6% of ER-negative cases [16]. A Danish study published in 2012 showed that the reproducibility of locally performed ER results was in the same range, with positive predictive value and negative predictive values of 94% and 75% respectively [17]. All of these studies indicate (i) poor reproducibility of ER-negative test results, (ii) average reproducibility of ER testing below 95%, (iii) an even lower reproducibility for PR testing. A panel organized by the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) has addressed the need for improving ER and PR testing and published a set of guidelines concerning this matter in 2010 [8].

Although determining HR-status has been common practice for quite some time, much work remains to be done on improving testing quality. Quality assessment studies have been performed by assessing the reproducibility of ER and PR testing. Some studies (such as the NordiQC and UK NEQAS) have sent unstained whole sections or TMAs including several different tissue cores to assess the efficacy of local IHC protocols and observer performance. Although this provides valuable information, this does not necessarily reflect the performance of the participating laboratory in the everyday practice and as such does not evaluate the consequences for the individual patient (i.e. withholding anti-hormonal treatment strategies when the patient was in fact eligible for these agents). Another possibility has been assessing the reproducibility of local IHC results of patients that were entered into clinical trials by performing central retesting. However, not all centers provide ample

cases for inclusion in clinical trials to allow for a reliable evaluation of their local IHC results and such retesting is not routinely performed. Participation in quality control schemes should be considered mandatory for all HR-testing laboratories and novel platforms for such assessments should be developed. In the Netherlands, quality of HR (and HER2) tests has recently been assessed with a TMA-based method, the results of which are described in this thesis.

Human epidermal growth factor receptor 2

The human epidermal growth factor receptor 2 (HER2, Her2/neu, ErbB-2) is a member of the family of tyrosine-kinase growth factor receptors [18]. This proto-oncogene can undergo gene amplification, leading to overexpression of the receptor at the cellular membrane [19]. This in turn can lead to ligand-independent receptor dimerization, resulting in activation of proliferative and anti-apoptotic signaling pathways. The HER2 gene was originally thought to be amplified in 25-30% of breast tumors. More recent patient cohorts have revealed that this percentage is likely around 10% [20], which is possibly reflective of increased reliability of HER2 assays. Trastuzumab is a humanized monoclonal antibody directed at the extracellular domain of the HER2 receptor. Trastuzumab and other HER2-targeting agents such as pertuzumab and trastuzumab-emtansine (T-DM1) have been shown to increase survival of HER2-positive patients [21-25]. Due to the considerable costs of these drugs and the potential toxicity associated with HER2-inhibition, accurate HER2 testing prior to drug administration is essential.

In the early trastuzumab trials, participants were entered upon displaying complete membranous staining (irrespective of staining intensity) in at least 10% of tumor cells on IHC [24]. Subsequent analyses have shown that patients with strong membranous staining (so-called 3+ staining pattern) are most likely to benefit from HER2-inhibiting therapies and that amplification is found in a high number of these 3+ staining tumors [26, 27]. Protein overexpression without HER2 gene amplification has been shown to occur in previous publications on this matter [27], but with modern IHC procedures, these tumors are thought to be exceedingly rare [20]. In cases of 2+ staining (defined as weak membranous staining), HER2 gene amplification is found in a far smaller proportion of cases [26, 27].

Standard HER2 testing was first recommended by Bast et al. in 2000 [28]. Unfortunately, the reproducibility of the locally performed HER2 IHC tests performed as part of the NSABP-31 trial was disappointing, as 18% of locally positive tests were irreproducible [29]. Similarly, Perez et al. found that reproducibility of HER2

tests varied from 75-88.1% [30]. A 2007 publication by an expert panel initiated by the ASCO/CAP estimated that 20% of all HER2 testing is unreliable [31]. This panel recommended increasing the threshold of HER2 positivity (3+) to 30% of tumors displaying strong membranous staining. The equivocal (2+) category was expanded to include tumors with complete membranous staining in less than 30% of tumor cells. Concerning in situ hybridization assays, HER2 to CEP17 ratios exceeding 2.2 were considered to be convincing for HER2 amplification (table 3). All these changes to the HER2 scoring algorithm were made to increase the reliability of HER2 testing and subsequent studies have shown such a development [32].

Regardless, in 2013 a revised guideline was published by the ASCO/CAP panel with additional changes to this testing algorithm [33]. The threshold for HER2-positivity was brought back down to 10% from the 30% invasive tumor cells with strong membranous staining and changes were made to the definitions of the 0 and 1+ categories (table 3). For the everyday practice, this means that the number of equivocal and the so-called "double-equivocal" test results will increase [34]. What effect these changes will have on tumors that were considered 2+ and 3+ according to the 2007 guidelines and HER2 testing quality in general is unknown, but is believed to be modest.

Lymph vascular space invasion

Although many breast cancer patients present with hematogenous dissemination of tumor cells at some point during the course of their disease, the most common initial metastatic event is thought to be entry into regional peritumoral lymph vessels [35]. Studies have almost invariably found a correlation between this initial metastatic event (locoregional lymph vascular space invasion, LVSI) and clinical outcome. These studies have either used H&E-stained slides to identify LVSI [36-42] or employed IHC staining to highlight the lymphatic endothelium, thereby hypothetically improving the detection of lymphatic spaces and tumor emboli [43-46] (table 4).

When using H&E stains for LVSI scoring, clear definitions should be applied to the scoring process in order to minimalize interobserver irreproducibility. Retraction artefacts can give a false impression of LVSI and thereby hinder the reliable identification of these lesions. An identical shape between the tumor embolus and that of the suspected lymph vessel or obvious retraction artefacts in its immediate surroundings should raise suspicion of a retraction artefact. The presence of a clear endothelial lining has been named as one of the prerequisites for scoring a suspected lesion as LVSI [37, 47]. The presence of a nearby blood vessel or nervous tissue has

also been considered as confirmation of lymphatic vessels [37, 47, 48]. Lastly, multiple areas can be considered for scoring LVSI, i.e. the intratumoral and peritumoral space and this should be sharply defined [48]. None of these criteria have found consistent application in subsequent literature on the subject.

The downside of using IHC for lymph vessel detection is the specificity of the staining pattern of these markers. Although D2-40 is considered a marker for the endothelium lining the lymphatic vessels [49], myoepithelial cells in terminal ductolobular units also show D2-40-positivity [50]. On top of that, myoepithelial cells surrounding cases of ductal carcinoma in situ can also be D2-40-positive, albeit with a weaker staining intensity. As these lesions can look very similar to LVSI in cases of retraction artefacts, additional staining in the form of p63 is required in order to resolve whether the D2-40-positive cells are in fact the lymphatic endothelial cells or myoepithelial cells [51].

The 2005 St. Gallen guidelines stated that the presence of peritumoral vascular invasion is reason to classify patients as intermediate risk as opposed to low risk [52] based on three studies [53-55]. Colleoni et al. investigated the extent of vascular invasion observed using H&E-stained breast carcinoma slides [56]. Multivariate analyses revealed that only tumors with extensive vascular invasion were associated with significantly decreased disease free- and overall survival compared to tumors without vascular invasion [56]. The subsequently published 2007 St. Gallen guidelines rephrased their previous statement of 'peritumoral vascular invasion' with 'extensive peritumoral vascular invasion' based on this study [57]. A 2011 study published by Mohammed et al. employed D2-40, CD31 and CD34 IHC stains in order to assess the presence of vascular invasion in a series of 1005 lymph node negative breast [43]. This study found no statistical differences regarding the extent of LVSI [43]. In 2013, the St. Gallen panel concluded that LVSI is not an indication for adjuvant chemotherapy [4], while other guidelines have continued to endorse the role of vascular invasion in clinical decision making [58].

Although studies on the prognostic significance of LVSI have been published since the 1980's, major questions remain regarding the implementation of this parameter. Firstly, the contrasting results of the studies performed by Mohammed et al. and Colleoni et al. leave the matter of (semi-)quantitative LVSI scoring undecided. Secondly, the question still remains on how this parameter should be implemented in clinical decision making. The crucial question in this regard is, does the presence of LVSI justify "upgrading" a patient that is otherwise low risk to intermediate- or high risk? Eljertsen et al. found no significant survival difference between LVSI-positive

and LVSI-negative tumors within the low risk group, nor was survival of otherwise low-risk, LVSI-positive tumors comparable to high risk tumors [38]. This data indicates that there is no benefit for assessing LVSI for low-risk tumors. Unfortunately, no quantification was performed as part of this latter study. Thirdly, these studies have not addressed the interobserver discordance for LVSI assessments. To our knowledge, only two studies have assessed the interobserver variation of LVSI scoring in breast cancer and both showed significant discordance between observers [59, 60].

In order to resolve the place of LVSI in the clinical decision making process, all these issues should be addressed in further studies. Considering the demonstrated prognostic power of this parameter in multiple studies, it would be regrettable if it would be excluded from the decision making process especially considering the fact that no costs are required for determining this parameter.

Ki-67

Ki-67 is a nuclear antigen that is expressed in all phases of cell division [61]. Two meta-analyses have investigated the prognostic influence of Ki-67, including studies that used multiple different antibodies and scoring cut-offs. Regardless, both meta-analyses found increased hazard for disease relapse and death for tumors with a high Ki-67 labelling index [62, 63]. Ki-67 has also been described as part of an IHC panel to classify ER-positive breast tumors into either luminal A or luminal B subtypes [64]. The predictive value of Ki-67 for long-term survival after short-term endocrine therapy has also led to a renewed interest for this parameter as a marker for benefit from endocrine therapies [65].

Cut-off values for Ki-67 positivity employed in the literature vary from 0% to 34% and multiple different values in between [62, 63]. The cut-off for intrinsic subtype determination has been validated with a clear objective (identifying luminal B tumors), but does not necessarily translate to an optimal cut-off point for prognostic and/or predictive studies. To complicate matters, considerable staining heterogeneity may exist in the form of so-called 'hot spots'. The International Ki-67 in Breast Cancer working group could not provide an evidence- based cut-off, stating that: "Currently, in the absence of harmonized methodology, the International Ki67 in Breast Cancer Working Group was unable, therefore, to come to consensus regarding the ideal cut point(s) that might be used in clinical practice" [66]. The St. Gallen guidelines have recommended the use of Ki-67 staining with a cut-off at 20% Ki-67-positive cells to identify ER-positive tumors that might benefit from additional cytotoxic therapies [67].

Two studies that investigated the reproducibility of Ki-67 IHC assays found good intraobserver and intralaboratory concordance but relatively high interlaboratory variability [68, 69]. Varga et al. examined the intra- and interobserver variability of Ki-67 IHC scoring in grade 2 breast tumors and found significant interobserver discordance [70]. Remarkably, even thorough counting of cells as opposed to 'eyeballing' did not improve this concordance. The selection of the area used for scoring was also not the main determinant of variation, considering the fact that scoring pre-determined fields did not improve interobserver agreement. Gudlaugsson et al. compared multiple for methods for the assessment of Ki-67-positive nuclei and found that digital analysis provides the most reliable Ki-67 assessment [71]. The feasibility of such automated analyses by a central laboratory has been demonstrated in the GeparTrio trial [72].

The decades' worth of data regarding Ki-67 has several serious flaws that hamper the implementation of this parameter into routine diagnostics. Due to interlaboratory and interobserver variation, prognostic data obtained by one center cannot be extrapolated to other testing laboratories. The implementation of stringent IHC protocols and automated digital analysis might improve this situation, even though the implementation of the latter has not found widespread use in pathology practice to this day [71].

Conclusion

The assessments of ER, PR, HER2, Ki-67 are currently recommended to aid the clinical decision making process regarding neoadjuvant, adjuvant and metastatic treatment. The current St. Gallen guidelines emphasize the role of intrinsic subtypes for guiding systemic therapy choices [4]. This assessment is almost completely reliant on accurate pathological specimen handling and IHC procedures. Stringent quality control is emphasized in every guideline, without clarification on how this is to be performed. Despite the fact that these markers have been assessed via immunohistochemistry for years, the current situation is far from optimal practice. Secondly, despite almost decades of research, no consensus has been reached on the optimal implementation and determination of these parameters. Research efforts should focus on increasing testing reliability and determining which markers complement the decision making process for which patients.

Outline of part I of this thesis

The first part of the thesis discusses the optimization of breast cancer risk stratification along established parameters. Although testing hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status is considered standard-of-care, no consensus has been reached on the reliability of testing these receptors on pre-operative core needle biopsies (CNBs).

Whether and how CNBs can be used to determine HR and HER2 status was investigated in **chapter 2**. For a series of patients treated in the LUMC, ER and HER2 testing results from CNBs and resection specimens were compared. These cases were combined with published case series. For this study, patients treated with neoadjuvant chemotherapy were excluded, as this might have an influence on biomarker expression. The extent to which ER, PR or HER conversion occurs following neoadjuvant chemotherapy remains unknown. This is investigated as part of a systemic review in **chapter 3**.

Quality control is essential for determining ER and HER2 testing and should be considered mandatory for testing laboratories. A tissue microarray(TMA)-approach for assessing ER, PR and HER2 testing quality is investigated in **chapters 4 and 5**. The results of such a TMA-based method for assessing ER and PR testing reproducibility of 9 different Dutch pathology laboratories is described in **chapter 4**. Previously HR-tested breast carcinomas were retested using TMAs to the determine reproducibility of local results. Additionally, the consequence of the ER-positivity thresholds on testing reproducibility was examined. A similar TMA-approach for assessing HER2 testing reproducibility among 6 HER2-testing laboratories was examined in **chapter 5**. The optimal testing methods for such a TMA assessment were investigated. HER2 scores on TMA were then compared to original HER2 test results and whole slides to assess testing reliability.

Lymph vascular space invasion (LVSI) has been frequently reported to be of prognostic importance in node-negative breast cancer. However, if and how this parameter should be implemented in clinical decision making is unknown. Whether this parameter should be assessed in a quantitative manner for optimal prognostic power is also unclear. Both these issues are investigated in **chapter 6.** Whether a quantitative assessment can be reliably determined by different pathologists is investigated in **chapter 7.** In this study, 60 slides were scored by 4 pathologists for LVSI foci. Concordance between these observers for individual foci was assessed and sensitivity and specificity for quantitative LVSI scoring was determined.

In **chapter 8**, several aspects that hamper the reliability of Ki-67 assays were investigated in a cohort of patients tested in the MicroarRAy PrognoSTics in Breast CancER (RASTER) study. The potential impact of tumor heterogeneity was investigated and related to interobserver variation. Secondly, reproducibility of Ki-67 testing among two different pathology laboratories was tested.

References

1. Berry DA et al.: Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med 2005, 353:1784-1792

2. Peto R 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

3. Giordano SH et al.: Is breast cancer survival improving? Cancer 2004, 100:44-52

4. Goldhirsch A 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

5. Perou CM et al.: Molecular portraits of human breast tumours. Nature 2000, 406:747-752

6. Osborne CK et al.: The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980, 46:2884-2888

7. Zhang Z et al.: Pathological features and clinical outcomes of breast cancer according to levels of oestrogen receptor expression. Histopathology 2014, 65:508-516

8. Hammond ME et al.: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med 2010, 134:e48-e72

9. Harvey JM et al.: Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999, 17:1474-1481

10. Dowsett M et al.: Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. Ann Oncol 2006, 17:818-826

11. Fisher B et al.: Influence of tumor estrogen and progesterone receptor levels on the response to tamoxifen and chemotherapy in primary breast cancer. J Clin Oncol 1983, 1:227-241

12. Collins LC et al.: Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases. Am J Clin Pathol 2005, 123:16-20

13. Wasielewski et al.: Tissue array technology for testing interlaboratory and interobserver reproducibility of immunohistochemical estrogen receptor analysis in a large multicenter trial. Am J Clin Pathol 2002, 118:675-682

14. Rhodes A et al.: Study of interlaboratory reliability and reproducibility of estrogen and progesterone receptor assays in Europe. Documentation of poor reliability and identification of insufficient microwave antigen retrieval time as a major contributory element of unreliable assays. Am J Clin Pathol 2001, 115:44-58

15. Badve SS et al.: Estrogen- and progesterone-receptor status in ECOG 2197: comparison of immunohistochemistry by local and central laboratories and quantitative reverse transcription polymerase chain reaction by central laboratory. J Clin Oncol 2008, 26:2473-2481

16. Gelber RD, Gelber S: Facilitating consensus by examining patterns of treatment effects. Breast 2009, 18 Suppl 3:S2-S8

17. Cronin-Fenton DP et al.: Factors associated with concordant estrogen receptor expression at diagnosis and centralized re-assay in a Danish population-based breast cancer study. Acta Oncol 2012, 51:254-261

18. Hynes NE, Stern DF: The biology of erbB-2/neu/HER-2 and its role in cancer. Biochim Biophys Acta 1994, 1198:165-184

19. Slamon DJ et al.: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989, 244:707-712

20. Dekker TJ et al.: Determining sensitivity and specificity of HER2 testing in breast cancer using a tissue micro-array approach. Breast Cancer Res 2012, 14:R93

21. Baselga J et al.: Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012, 366:109-119

22. Piccart-Gebhart MJ et al.: Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005, 353:1659-1672

23. Romond EH et al.: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005, 353:1673-1684

24. Slamon DJ et al.: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001, 344:783-792

25. Verma S et al.: Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012, 367:1783-1791

26. Roche PC et al.: Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. J Natl Cancer Inst 2002, 94:855-857

27. Reddy JC et al.: Concordance between central and local laboratory HER2 testing from a community-based clinical study. Clin Breast Cancer 2006, 7:153-157

28. Bast RC, Jr. et al.: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 2001, 19:1865-1878

29. Paik S et al.: Real-world performance of HER2 testing – National Surgical Adjuvant Breast and Bowel Project experience. J Natl Cancer Inst 2002, 94:852-854

30. Perez EA et al.: HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol 2006, 24:3032-3038

31. Wolff AC 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

32. Middleton LP et al.: Implementation of American Society of Clinical Oncology/College of American Pathologists HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence in situ hybridization concordance and decreases the number of inconclusive cases. Arch Pathol Lab Med 2009, 133:775-780

33. Wolff AC 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

34. Lambein K et al.: impact on immunohistochemistry-negative breast cancers. J Clin Oncol 2014, 32:1856-1857

35. Mohammed RA et al.: Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. Am J Surg Pathol 2007, 31:1825-1833

36. Colleoni M et al.: Prognostic role of the extent of peritumoral vascular invasion in operable breast cancer. Ann Oncol 2007, 18:1632-1640

37. De Mascarel I et al.: Obvious peritumoral emboli: an elusive prognostic factor reappraised. Multivariate analysis of 1320 node-negative breast cancers. Eur J Cancer 1998, 34:58-65

38. Ejlertsen B et al.: Population-based study of peritumoral lymphovascular invasion and outcome among patients with operable breast cancer. J Natl Cancer Inst 2009, 101:729-735

39. Kato T et al.: The combination of angiogenesis and blood vessel invasion as a prognostic indicator in primary breast cancer. Br J Cancer 2003, 88:1900-1908

40. Lauria R et al.: The prognostic value of lymphatic and blood vessel invasion in operable breast cancer. Cancer 1995, 76:1772-1778

41. Lee AH et al.: Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. Eur J Cancer 2006, 42:357-362

42. Ragage F et al.: Is it useful to detect lymphovascular invasion in lymph node-positive patients with primary operable breast cancer? Cancer 2010, 116:3093-3101

43. Mohammed RA et al.: Objective assessment of lymphatic and blood vascular invasion in lymph node-negative breast carcinoma: findings from a large case series with long-term follow-up. J Pathol 2011, 223:358-365

44. Sabatier R et al.: Peritumoural vascular invasion: a major determinant of triple-negative breast cancer outcome. Eur J Cancer 2011, 47:1537-1545

45. Schoppmann SF, Bayer G, Aumayr K, Taucher S, Geleff S, Rudas M, Kubista E, Hausmaninger H, Samonigg H, Gnant M, Jakesz R, Horvat R: Prognostic value of lymphangiogenesis and lymphovascular invasion in invasive breast cancer. Ann Surg 2004, 240:306-312

46. Yamauchi C et al.: Accurate assessment of lymph vessel tumor emboli in invasive ductal carcinoma of the breast according to tumor areas, and their prognostic significance. Hum Pathol 2007, 38:247-259

47. Rosen PP: Tumor emboli in intramammary lymphatics in breast carcinoma: pathologic criteria for diagnosis and clinical significance. Pathol Annu 1983, 18 Pt 2:215-232

48. Orbo A et al.: Topographic criteria in the diagnosis of tumor emboli in intramammary lymphatics. Cancer 1990, 66:972-977

49. Kahn HJ, Marks A: A new monoclonal antibody, D2-40, for detection of lymphatic invasion in primary tumors. Lab Invest 2002, 82:1255-1257

50. Kanner WA et al.: Podoplanin expression in basal and myoepithelial cells: utility and potential pitfalls. Appl Immunohistochem Mol Morphol 2010, 18:226-230

51. Ren S et al.: D2-40: an additional marker for myoepithelial cells of breast and the precaution in interpreting tumor lymphovascular invasion. Int J Clin Exp Pathol 2011, 4:175-182

52. Goldhirsch A et al.: Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. Ann Oncol 2005, 16:1569-1583

53. Hasebe T et al.: Histological characteristics of tumor in vessels and lymph nodes are significant predictors of progression of invasive ductal carcinoma of the breast: a prospective study. Hum Pathol 2004, 35:298-308

54. Kato T et al.: The combination of angiogenesis and blood vessel invasion as a prognostic indicator in primary breast cancer. Br J Cancer 2003, 88:1900-1908

55. Pinder et al: Pathological prognostic factors in breast cancer. III. Vascular invasion: relationship with recurrence and survival in a large study with long-term follow-up. Histopathology 1994, 24:41-47

56. Colleoni M et al.: Prognostic role of the extent of peritumoral vascular invasion in operable breast cancer. Ann Oncol 2007, 18:1632-1640

57. Goldhirsch A et al.: Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Ann Oncol 2007, 18:1133-1144

58. Senkus E et al.: Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013, 24 Suppl 6:vi7-23

59. Gilchrist KW et al.: Interobserver variation in the identification of breast carcinoma in intramammary lymphatics. Hum Pathol 1982, 13:170-172

60. Orbo A et al.:Topographic criteria in the diagnosis of tumor emboli in intramammary lymphatics. Cancer 1990, 66:972-977

61. van Dierendonck JH et al.: Cell-cycle-related staining patterns of anti-proliferating cell nuclear antigen monoclonal antibodies. Comparison with BrdUrd labeling and Ki-67 staining. Am J Pathol 1991, 138:1165-1172

62. De Azambuja E 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

63. Stuart-Harris R et al.: Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 2008, 17:323-334

64. Cheang MC et al.: Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009, 101:736-750

65. Dowsett M et al.: Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst 2007, 99:167-170

66. Dowsett M 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

67. Goldhirsch A 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

68. Mengel M et al.: Inter-laboratory and inter-observer reproducibility of immunohistochemical assessment of the Ki-67 labelling index in a large multi-centre trial. J Pathol 2002, 198:292-299

69. Polley MY et al.: An international Ki67 reproducibility study. J Natl Cancer Inst 2013, 105:1897-1906

70. Varga Z 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

71. Gudlaugsson E et al.: Comparison of the effect of different techniques for measurement of Ki67 proliferation on reproducibility and prognosis prediction accuracy in breast cancer. Histopathology 2012, 61:1134-1144

72. Klauschen F et al.: Standardized Ki67 diagnostics using automated scoring – clinical validation in the GeparTrio breast cancer study. Clin Cancer Res 2014

73. Vogel CL et al.: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002, 20:719-726

1

Table 1. Systemic treatment recommendations, adapted and modified from Goldhirsch et al. (4).

Subtype	Type of therapy	Notes
Luminal A-like ER and PR positive, HER2 negative, Ki-67 low*	Endocrine therapy is the most critical intervention and is often used alone	Cytotoxics may be added in case of high 21-gene RS score, high 70-gene risk status, grade 3 disease, 1-4 positive lymph nodes, possibly young age (< 35)
Luminal B-like (HER2-negative) ER positive, HER2 negative and at least one of: Ki-67 high* or PR negative or low	Endocrine therapy for all patients, cytotoxic therapy for some	
Luminal B-like (HER2-positive) ER positive, HER2 over-expressed or amplified, any Ki-67, any PR	Cytotoxics + anti-HER2 + endocrine therapy	
HER2-positive (non-luminal) HER2 over-expressed or amplified and ER and PR absent	Cytotoxics + anti-HER2	Threshold for use of anti-HER2 therapies was defined as pT1b or larger tumor or node-positivity
Triple negative (ductal) ER and PR absent and HER2 negative	Cytotoxics	
Special histological subtypes Endocrine responsive Endocrine non-responsive	Endocrine therapy	

* Threshold for Ki-67 is generally considered to be 20% positive tumor cells, although this varies over various guidelines and is more based on expert-opinion than evidence.

Table 2. Several factors that influence the reproducibility of IHC-based parameters.

Pre-analytical variables

Selection and availability of tissues

Tissue transport

Warm and cold ischemic intervalsA

Specimen type (core needle biopsy or resection specimen)

Fixation (duration, type)

Analytical variables

Antigen retrieval procedure

Antibody

Selection of control tissues

Procedure type (automated or manual)

Post-analytical variables

Scoring methodologies

Observer skill and experience

Evaluation of internal and external controls

Reporting

Table 3. Changes in human epidermal growth factor receptor 2 (HER2) testing recommendations, according to the inclusion criteria of the original trastuzumab trials, the 2007 ASCO/CAP guidelines and the revised 2013 ASCO/CAP guidelines.

	Trastuzumab trials (24;73)	2007 ASCO/CAP guidelines (31)	2013 ASCO/CAP guidelines (33)
Unequivocally HER2 positive	- Strong complete, membranous staining in at least 10% of tumor cells (3+) - HER2 to Chromosome17 ratio > 2.0	- Strong complete, membranous staining in at least 30% of tumor cells (3+) - HER2 to Chromosome17 ratio > 2.2 - HER2 copy numbers ≥ 6.0/cell	- Strong complete, membranous staining in at least 10% of tumor cells (3+) - HER2 to chromosome 17 ratio > 2.0 - HER2 copy numbers ≥ 6.0/cell
Equivocal HER2 test result	- Weak to moderate complete membranous staining in at least 10% of tumor cells (2+)	- Weak to moderate complete membranous staining in at least 10% of tumor cells (2+) - HER2 to chromosome17 ratio between 1.8 and 2.2	- Weak to moderate complete membranous staining in at least 10% of tumor cells or complete strong staining in < 10% of tumor cells - HER2 to chromosome17 ratio < 2.0 with HER2 copy number > 4.0 but less than 6.0/cell - HER2 copy number > 4.0/cell but less than 6.0/cell
Unequivocally HER2 negative	- No membranous staining (0) - Incomplete membranous staining, regardless of intensity or cell percentage (1+) - HER2 to chromosome17 ratio	- No membranous staining (0) - Weak to moderate complete membranous staining in < 10% of tumor cells. - HER2 to chromosome17 ratio < 1.7	- No membranous staining or weak incomplete membrane staining in < 10% (0) - Incomplete membranous staining in> 10% (1+) - HER2 copy number < 4.0/cell

Human epidermal growth factor receptor 2 (HER2), American society for Clinical Oncology/College of American Pathologists (ASCO/CAP).

Table 4. Studies that have investigated the relationship between lymphvascular space invasion (LVSI) with clinical outcome.

Study	N	Subpopulation	Methods	Interobserver analysis	Location	Strict criteria described	Quantification	Outcome measure	Hazard ratio (95% CI)	Ref
Eljertsen	15659	No	H&E	No	Peritumoral	+	No	OS	2.48 (2.32-2.66)	(38)
Lee	2760	LN-negative	H&E	No	Peritumoral	+	No	DFS	1.66 (1.28-2.09)	(41)
Mohammed	1005	LN-negative	IHC	No	Intra- and peritumoral	-	Yes	DFS	1.640 (1.278-2.105)	(43)
								OS	1.903 (1.393-2.601)	
Sabatier	344	Triple-negative	IHC	No	Peritumoral	+	No	MFS	2.41 (1.10-5.26)	(44)
Ragage	931	LN-positive, HER2-negative	H&E	No	Peritumoral	++	No	MFS	1.71 (1.12-2.40)	(42)
Mascarel	1320	LN-negative	H&E	No	Peritumoral	++	No	MFS	2.18 (1.6-2.9)	(37)
								OS	1.65 (1.19-2.28)	
Colleoni	2606	No	H&E	No	Peritumoral	+	Yes	DFS	2.11 (1.02-4.34)	(36)
								OS	4.51 (1.96-10.4)	
Yamauchi	151	No	IHC	No	Intra- and peritumoral	++	No	DFS	3.9 (1.8-8.6)	(46)
Schoppmann	374	No	IHC	No	Intra- and peritumoral	-	No	DFS	2.015 (1.311-3.04)	(45)
								OS	1.810 (1.181-2.773)	
Lauria	1408	No	H&E	No	Peritumoral	+	No	OS	1.92 (1.51-2.45)	(40)
Kato	509	No	H&E	No	Peritumoral	-	No	DFS	5.1 (3.3-7.8)	(39)

Lymph node negative (LN-negative), hematoxylin and eosin (H&E), overall survival (OS), DFS (disease-free survival),
MFS (metastases-free survival), human epidermal growth factor receptor (HER2), 95% confidence interval (95% CI).