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Inter-observer variation in pathological review and its impact on clinicopathological risk assessment and patient selection for adjuvant systemic treatment in node-negative breast cancer patients

J.M. Bueno-de-Mesquita[1], D.S.A. Nuyten[2], J. Wesseling[1], H. Van Tinteren[3], S.C. Linn[4], M.J. Van de Vijver[5]. *[1]Netherlands Cancer Institute, Pathology, Amsterdam, The Netherlands; [2]Netherlands Cancer Institute, Radiotherapy, Amsterdam, The Netherlands; [3]Netherlands Cancer Institute, Biometric Department, Amsterdam, The Netherlands; [4]Netherlands Cancer Institute, Medical Oncology, Amsterdam, The Netherlands; [5]Amsterdam Medical Center, Pathology, Amsterdam, The Netherlands*

Introduction: The aim of this study was to assess the effect of inter-observer variation in pathological examination of node-negative breast carcinomas on clinical risk assessment and the selection of patients for adjuvant systemic treatment.

Methods: In a retrospective multicenter study, the clinical and pathological data of 708 patients of 2 consecutive patient series were analysed: 123 patients of a validation study (Bueno-de-Mesquita, EurJCancerSuppl 2006) and 585 patients of the RASTER study (Bueno-de-Mesquita, Lancet Oncology 2007). Patients (<61 years) had primary unilateral breast cancer (T1−4N0M0); patients with prior malignancies were excluded. Tumour size and histologic grade were assessed locally; central review was performed at the Netherlands Cancer Institute (Amsterdam, the Netherlands). Clinicopathological low or high risk was assessed using national Dutch (CBO) guidelines and the Adjuvant Online program (www.adjuvantonline.com). Patients with a 'low' risk were deemed to have a good prognosis and, therefore, could be spared adjuvant systemic treatment. A low clinical risk based on the Adjuvant Online program was defined as patients with a 10-years survival probability of at least 90%.

Results: The original pathological examination was discordant with the central review for histologic grade in 28% (196/690) of patients (kappa 0.56; missing 18) and for oestrogen receptor in 5% (33/686) patients (kappa 0.85; missing 22). Translation into clinical Dutch CBO risk based on these pathological evaluations was discordant in 15% (102/690) of patients (kappa 0.70; missing 18). Clinical CBO risk based on centrally reviewed data would change high risk into low risk in 9% (61/690) of patients and no adjuvant systemic treatment would have been advised in these patients. Vice versa, 6% (41/690) of these patients would change from clinical CBO low risk to high risk based on the reviewed data and adjuvant systemic treatment would be advised. If Adjuvant Online was used, 8% (55/691) of patients (kappa 0.82; missing 17) would have been given a different clinical risk and adjuvant systemic treatment advice (5% (32/691) high to low risk, and 3% (23/691) low to high risk).

Conclusion: Inter-observer variation in pathological examination of breast carcinomas results in significant differences in clinicopathological risk assessment and adjuvant systemic treatment advice. Dutch CBO guidelines were more sensitive to inter-observer variation than Adjuvant Online.

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Interlaboratory variation in the results of Her2 testing in a population-based series of breast cancer patients

P.J. Westenend[1], A.M.J. Reedijk[2], R.A.M. Damhuis[2]. *[1]Laboratory for Pathology, Dordrecht, The Netherlands; [2]Rotterdam Cancer Registry, Rotterdam, The Netherlands*

Introduction: Accurate determination of Her2 receptor status is critical in the selection of breast cancer patients for Her2 targeted therapy. Quality control can be achieved by adhering to good laboratory practice and participating in external quality control programs. The proportion of patients with HER2 overexpression can be used as an additional quality indicator, provided that a suitable benchmark is available to control for differences in patient profiles. In this study, we investigated interlaboratory variation in the prevalence of Her2 positive breast cancers and developed a multivariable model to control for differences in patient-mix.

Patients and Methods: The Rotterdam Cancer Registry covers the southwestern part of the Netherlands, a region with 2.4 million inhabitants, 16 hospitals and 8 Pathology Laboratories. From the registry, we selected patients diagnosed with invasive breast cancer in 2005, in whom Her2 receptor status was determined. Other relevant clinical and pathologic findings were abstracted from the medical files. Determinants of the prevalence of positive HER2 results were analyzed both univariable (chi-square statistics) and multivariable (logistic regression analysis) and significant variables ($p < 0.05$) were used to develop a case-mix model. This model was then applied to analyze results by Pathology Laboratory, controlling for variation in case-mix. Confidence intervals (95% CI) were calculated as exact intervals for binomial proportions.

Results: The study population consisted of 894 patients of whom 168 (19%) were diagnosed with Her2 overexpression. Correlates of Her2 overexpression were non lobular tumour type, younger age at diagnosis, higher grade tumours, negative hormonal receptor status, local and regional metastasis at diagnosis and being diagnosed in the second half of 2005. Standardized prevalence rates varied between laboratories from 6% to 40%, which is equivalent to risk ratios ranging from 0.4 to 1.6, as compared to the mean.

Conclusion: Due to the association between traditional prognostic factors and Her2 overexpression, the actual prevalence is dependent on selection criteria for testing. Because selection criteria may differ between physicians, hospitals and laboratories, multivariable analysis is needed to study variation between Pathology Laboratories. A case-mix model can be used to derive a standardized prevalence. Even after controlling for case-mix, considerable variation between Laboratories was observed. This observation highlights the importance of quality control measures to improve the performance of staining and scoring of tumour material.

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Prediction of prognosis by lymph node ratio of involved axillary lymph nodes to the total number of removed lymph nodes in early stage breast cancer

N. El Saghir[1], H.A. Hatoum[1], W. Shamseddeen[1], E. Sbaity[1], A.I. Shamseddine[1]. *[1]American University of Beirut, Medicine, Beirut, Lebanon*

Background: Axillary lymph node involvement is a major determinant of stage, prognosis, and adjuvant systemic and radiation therapy in primary breast cancer. The absolute number of positive lymph nodes affects the prognosis and choice of systemic therapy and radiation therapy. However, the total number of LN removed is variable. We investigated the role of lymph node ratio (LNR, the ratio of the number of lymph nodes involved by tumor to the total number of removed lymph nodes) for predicting prognosis in early stage breast cancer.

Materials and Methods: We reviewed the medical records and pathology of patients with early stages (I and II) breast cancer treated at the American University of Beirut Medical Center (AUBMC) between 1990 and 2003.

Results: Out of a total of 1,254 patients, 877 were stages I and II. 52% were below age 50 years and 48% were above 50 years. 73% had modified radical mastectomy (MRM) and 27% had breast conserving surgery (BCS). 182 patients were stage I and 754 patients were stage II. 73.5% of stage II patients had positive axillary lymph nodes (LN). 51% of those patients had 1−3 positive LN, 33% had 4−9 LN and 16% had 10 or more positive LN. Of 877 patients who had lymph nodes examined (includes those who had sentinel lymph node biopsy (SLNB)), the median number of LN removed was 17 (Range 1−46). The average ratio of LN positive to LN removed was 0.6. 83.5% of patients had less than 0.6 LNR positive and 16.5% had more than 0.6 LNR positive. Factors that were associated with negative impact on survival included positive lymph nodes, negative hormone receptor status, and presence of lymphatic invasion. Kaplan–Meier analysis showed that patients with LN ratio over 0.6 versus patients with LN ratio below 0.6 had overall survival of 70% vs 88% at 5 years, 22% versus 77% at 10 years, and 22% versus 77% at 150 months, respectively. We assessed LNR using smaller ratio of 0.2 and this LNR of 0.2 was also predictive of survival. The 10-year survival of patients with LNR $\leqslant 0.2$ was 72% compared to 51% for those with LNR > 0.2.

Conclusions: LNR of number of positive axillary lymph nodes over total number of removed lymph nodes over 0.6, as well as LNR over 0.2, predicts worse survival in early stage breast cancer. LNR may be an important tool in patients with lower numbers of dissected axillary LN.

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Microtubule-associated protein tau is a marker of pathological complete response in Her-2/neu positive neoadjuvant treated breast cancer patients

S. Rueckert[1], R.M. Wirtz[2], M.S. Lenhard[1], S. Hasmueller[1], N. Ditsch[1], I. Ruehl[1], S. Kahlert[1], I. Bauerfeind[1], P.A. Fasching[3], M. Untch[4]. *[1]Frauenklinik der LMU München, Obstetrics and Gynecology, München, Germany; [2]Siemens Medical Solutions Diagnostics GmbH, Siemens Medical Solutions Diagnostics GmbH, Leverkusen, Germany; [3]Frauenklinik Universitätsklinikum Erlangen, Obstetrics and Gynecology, Erlangen, Germany; [4]Helios Klinik Berlin-Buch, Obstetrics and Gynecology, Berlin, Germany*

Background: Tau protein promotes tubulin polymerization and stabilizes microtubules. Microarray analysis of fresh breast cancer tissues revealed,

that low expression of Microtubule-associated protein tau (MAPT) is a predictive marker for response to anthracycline based chemotherapy with or without taxane. In contrast, it has recently been shown, that high expression of MAPT may indicate response to endocrine therapies. Here, we have analyzed the RNA expression of MAPT in formalin fixed paraffin embedded breast cancer tumour tissues of patients treated within two neoadjuvant chemotherapy trials (TECHNO/PREPARE trial) to evaluate the prognostic value in this setting.

Patients and Methods: Breast cancer patients (?cT2, N0-N3, M0) received neoadjuvant chemotherapy of 4 cycles of epirubicin and cyclophosphamide followed by 4 cycles paclitaxel (PREPARE trial). In Her-2/neu positive breast cancer patients trastuzumab was additionally administered (TECHNO trial). Samples of an initial group of 57 patients (34 TECHNO/23 PREPARE) were prepared for first analysis. RNA was successfully isolated from the tissue samples by an automated system based on magnetic beads (Siemens Medical Solutions Diagnostics GmbH). RNA expression of ESR1, Her-2/neu and MAPT expression was determined by quantitative RT-PCR. MAPT expression was correlated to histopathological findings.

Results: RNA expression of MAPT significantly correlated with ESR1 expression (Spearman $r = 0.677$; $p < 0.0001$), but not with Her-2/neu expression. Median expression of MAPT was 4 fold higher ESR1 positive tumors. Of the 57 patients included in the first analysis, 10 patients showed a pathological complete response (pCR), 4 in the PREPARE group and 6 in the Her-2/neu positive TECHNO group. There was a significant difference of MAPT-RNA expression between those patients with histopathologically complete response after neoadjuvant chemotherapy and those with no or partial response in the first cohort (Mann-Whitney-U, $p = 0.019$). Patients with a complete response showed significantly lower levels of MAPT-RNA. Interestingly, this difference was prominent in the group of Her-2/neu positive tumors (TECHNO trial) treated in addition with trastuzumab with all responding tumors exhibiting a 4 fold lower median MAPT expression (Mann-Whitney-U, $p = 0.009$). In contrast, no statistically difference was seen in the PREPARE group (Mann-Whitney-U, $p = 0.59$) with only half of the tumors being below the median MAPT expression.

Conclusion: These results validated the initial hypothesis that low expression of MAPT indicates sensitivity to chemotherapy on RNA level in clinical routine paraffin tissue. Moreover, low MAPT-expression was particularly informative in Her-2/neu positive breast cancer patients treated with anthracyclin-based chemotherapy containing paclitaxel and trastuzumab.

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External and internal assurance of the determination of the prognostic factors uPA and PAI-1 for the ongoing NNBC 3-Europe trial

M. Vetter[1], A. Geurts-Moespot[2], E.J. Kantelhardt[1], F. Sweep[2], G. von Minckwitz[3], C. Thomssen[1], J. Dittmer[1], N. Harbeck[4]. *[1]Martin Luther University, Gynecology, Halle/Saale, Germany; [2]Radboud University, Chemical Endocrinology, Nijmegen, The Netherlands; [3]German Breast Group, Science Research Organisation, Neu Isenburg, Germany; [4]Technical University München, Gynecology, München, Germany*

Background: The biomarkers urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 (plasminogen activator inhibitor-1) are suitable tools to predict which patient with node-negative breast cancer would benefit from chemotherapy and which not. Based on this finding, a still ongoing multi-center trial, the NNBC 3-Europe trial, is conducted, which is supported by the German AGO, the German Breast Group and the European EORTC PathoBiology Group. Recruitment centers are allowed to select risk assessment criteria either by using established clinical and histomorphological criteria or by using uPA/PAI-1 measurements in primary tumor tissue extracts.

External Quality Assessment (EQA) of total protein and the uPA and PAI-1 determination in the cytosol of the primary tumors are performed by the Department of Chemical Endocrinology of the University Hospital Nijmegen, The Netherlands.

Material and Methods: Quality assessment samples are being prepared centrally as a lyophilised cytosol. In the nine participating laboratories the vial contents are reconstituted as instructed and assayed as part of the next routine batch. After an initial pilot phase the qualtity control included the monitoring of the between- and the within-laboratory variations.

In order to get some more information on the homogeneity of the uPA/PAI-1 expression within the tumor tissue, 30 different tumor samples were cut up to nine pieces and each piece analyzed individual for the level of these biomarkers.

Results: The median values of the coefficient of variation (CV) between labs are for uPA and PAI-1 15%. The median of the within-lab between-run CVs are for uPA 10%, for PAI-1 12% ranging from 0.0 and 18%. For quality assessment of total protein measurement, all CVs values are acceptable.The analyses of different pieces of the same tumor revealed homogenous and heterogenous distribution of uPA and PAI-1 levels within the tumor.

Conclusions: In order to get a representative value of the invasion factors, a minimum of 100 mg tumor weight preferably from different areas of the tumor periphery are recommended. The NNBC 3-Europe trial shows that inclusion of patients based on biological testing of fresh frozen tumor material is feasible. The quality assurance showed acceptable performance.

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Increasing Her2/Centromere17(CEP17) ratio predicts for greater sensitivity to trastuzumab based therapy in metastatic breast cancer (MBC)

S. Howell[1], A. Sawyerr[1], A. Cramer[2], D. Ryder[3], A.M. Wardley[1]. *[1]Christie Hospital NHS Foundation Trust, Medical Oncology, Manchester, United Kingdom; [2]Christie Hospital NHS Foundation Trust, Pathology, Manchester, United Kingdom; [3]Christie Hospital NHS Foundation Trust, Medical Statistics, Manchester, United Kingdom*

Background: Recent preliminary results suggest that a higher Her2/CEP17 ratio (Her2/CEP17R) predicts for improved response to trastuzumab (T) and that some H/17R non-amplified cancers with polysomy CEP17 may respond to T. We sought to examine the relationships between primary tumour Her2 gene copy number (Her2GCN), Her2/CEP17R and polysomy CEP17 and time to progression (TTP) with T based therapy in MBC.

Methods: Cases of Her2 positive MBC treated with T were identified from our databases. Data were extracted from case notes on previous treatments for MBC, TTP on T and primary tumour immunohistochemistry for ER, PgR, Ki67, EGFR and Her2 expression. FISH analysis was performed on freshly cut sections using Her2/CEP17 probe mix (Abbott-Vysis PathVysion®). Statistical analysis of TTP by categorical variables was by Kaplan–Meier log rank test. Cox regression with continuous covariates log(Her2GCN) and log(CEP17) was used to explore their combined prognostic content.

Results: Data were available on 169 patients with MBC treated with T between 1999 and 2007. 61/169 (36%) had T monotherapy (Tm) and 108 (64%) combination T+chemotherapy (Tc). Prior chemotherapy regimens for MBC: 0 = 117/169 (69%), 1 = 41 (24%) and >1 = 11 (7%). No differences were seen for TTP between Tm vs Tc ($p = 0.78$) or by line of treatment ($p = 0.88$; logrank). Her2/CEP17R and to a lesser degree Her2GCN had positive relationship with TTP when categorised as borderline/low ($\leqslant 2.2$ and $\leqslant 6$), moderate ($2.2–\leqslant 7$ and $6–\leqslant 20$) and high amplification (>7 and >20; $p = 0.004$ and $p = 0.14$ respectively by logrank). Polysomy 17 (CEP $\geqslant 3$; 64/169 (38%)) predicted for shorter TTP versus tumours with CEP17 < 3 ($p = 0.056$; logrank). Polysomy 17 (CEP $\geqslant 3$) did not influence TTP in cancers with Her2/CEP17R $\leqslant 2.2$, but numbers were small. Cox regression showed no evidence of an interaction between log(Her2GCN) and log(CEP17) ($p = 0.60$). Their combined prognostic information was best carried in a single variable: log(Her2/CEP17R). Fitting this single variable yielded a hazard ratio for TTP of 0.68 for each unit increase in log(Her2/CEP17) ($p = 0.001$).

Conclusion: Her2/CEP17R is positively associated with the duration of benefit from T containing regimens for MBC. We found no convincing evidence for benefit from T in patients with Her2 non amplified tumours and polysomy CEP17. These data on the relative benefits of T based therapy may help to guide patient information, treatment choices and follow up strategies in patients with Her2+ MBC.