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Agreement Between Visual and Automated Assessment of HER2 Overexpression and Ki67 Labeling Index in Breast Cancer − Comparison of Core Biopsy and Surgical Specimens

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Background: Correct immunohistochemical assessment of HER2 and Ki67 expression is a crucial diagnostic procedure to select the appropriate treatment for breast cancer patients. Although several image analysis systems have been developed for scoring prognostic and predictive breast cancer markers, most of these focused on evaluation of ER and PgR expression. Therefore, the present study investigated agreement between visual and automated HER2 and Ki-67 assessment. Additionally we analyzed the impact of sample types (core biopsy versus surgical specimen) on the reliability of the two assessment methods.

Materials and Methods: In the present study we evaluated matched pairs of core biopsy samples and surgical specimens of 96 breast cancer patients that were immunohistochemically stained for expression of HER2 and Ki67. Visual assessment was performed in accordance with recent guidelines by two experienced pathologists. Automated analysis was carried out using Ventata Image Analysis System (VIAS). For Ki-67, a cutoff of 10% between low and high proliferative cases was chosen. Cohen's kappa coefficients (κ) were calculated using SPSS version 17 for agreement between different analytical methods and core biopsy vs. surgical specimen as well. Results were further evaluated according to the method of Bland and Altman.

Results: For HER2, agreement between visual and VIAS assessment was higher for surgical specimens than for core biopsy samples, reaching κ-values of 0.927 and 0.795, respectively. Agreement between core biopsy and surgical specimens was comparable for both methods with κ-values of 0.897 for visual and 0.865 for VIAS-assisted determination.

Agreement between Ki-67 assessments for the two methods was nearly identical with rather low κ-values of 0.595 and 0.578 for core biopsy and surgical samples, respectively. However, 95% limits of agreement according to Bland and Altman were higher for VIAS (40%) than for visual determination (21%). All strongly divergent cases represented invasive ductal carcinomas of intermediate or low grade of differentiation (G2-G3) with Ki67 values ranging from 6−75%. Agreement between core biopsy and surgical specimens was low in general, but higher for visual than for automated determination with κ-values of 0.537 and 0.418, respectively.

Conclusions: Visual and VIAS-assisted determination are both accurate methods for determination of HER2, whereas agreement between visual and automated assessment was higher in surgical specimens than in core biopsy samples. Ki67 assessment could be optimized upon assessment of a greater proportion of the primary tumor as core biopsy samples or selected areas might not be representative of the entire tumor sample.

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The EORTC 10041/BIG 03−04 MINDACT Trial Quality Assurance Program − Results of the Questionnaire for Pathologists

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Background: MINDACT investigates the added clinical value of Mammaprint™ to standard Clinicopathological criteria for the accurate selection of breast cancer (BC) pts for adjuvant chemotherapy. The trial logistics involve especially local pathologists for tissue collection, preparation of frozen samples and histopathological workup. As part of the MINDACT Quality Assurance (QA) program a questionnaire (Q) was created aiming to describe the characteristics and practices of participating pathologists and laboratories (labs).

Material and Methods: A non-compulsory, web-based Q was submitted to the pathologists of each participant centre between Sep. 2011 and Oct. 2012. The Q comprises 40 questions on 4 main topics: demographic and accreditation, MINDACT logistics, techniques for hormone (HR) and HER-2 receptors and integration of pathologists in clinical trials.

Results: A hundred five centres in 9 European countries enrolled a total of 6694 pts in the trial. Of them 83 (79%) replied the Q, which represents 85.9% (5752) of total pts enrolled. Nearly 71% of pathologists work at a tertiary high complexity hospitals, with 58% big-sized (>450 beds) facilities. About 69% of labs are affiliated to a university, 70% have a pathology residence program and 75% serve a cancer centre. Around 88% of labs have some accreditation (i.e. health authority, professional organization, societies, ISO). In 89% of centres the lab staff has knowledge of the trial tissue handling standard operation procedures. For 93% of pathologists, the 1 h timeline for freezing the tissue samples is easily achieved. HER-2 IHC and FISH/CISH tests are performed in house in 92% and 73% of labs respectively. HR results are reported by percentage in 64% of labs and 22% use Allred score. HR positive is defined as $\geqslant$ 1% stained cells in 42% of labs and $\geqslant$ 10% for another 42%. In about 95% of centres, pathologists participate in BC multidisciplinary meetings. For 78% of the pathologists, the logistics and timelines of MINDACT are feasible in clinical practice. Finally, 78% of pathologists actively participate in clinical trials but 10% don't feel integrated.

Conclusions: Pathologists and corresponding labs have an important role in biomarkers-based clinical trials. We reported the current state of practice in BC pathology of 83 European labs participants in MINDACT trial. QA programs that focus on the variability between participating centres/labs are of the most value for current clinical trials.

Friday, 23 March 2012 10:30–11:30

PROFFERED PAPER

Patient Related Factors and Responsive Therapy

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Relationship Between Body Mass Index (BMI) and Outcomes in Node-positive Breast Cancer Patients Receiving Chemotherapy − Results From CALGB/Intergroup 9741

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Background: Observational studies suggest that obesity at diagnosis is associated with poor prognosis in women with breast cancer, but there is debate regarding potential confounding effects of treatment-related factors, particularly with regard to chemotherapy dosing of obese individuals. We sought to examine the relationship between BMI at diagnosis and outcomes in CALGB 9741, an adjuvant study which mandated that patients receive chemotherapy dosed according to actual body weight.

Methods: CALGB 9741 evaluated dose-density and sequence in a 2×2 factorial design in lymph-node-positive breast cancer. All patients received doxorubicin, cyclophosphamide and paclitaxel based on body surface area without a cap or adjustment. Height and weight at diagnosis were abstracted from patient records. The relationship of BMI with relapse-free (RFS) and overall survival (OS) was evaluated using multivariate proportional hazards regression after adjusting for number of involved nodes, estrogen receptor (ER) status, tumor size, menopausal status, drug sequence and dose density. Additionally, we graphically displayed the univariate relation of BMI with each outcome. Due to the variability of BMI data, BMI was smoothed using a moving average of 200 patients.

Results: 2005 women were enrolled in CALGB 9741 between September 1997 and March 1999. Baseline height and weight were available for 1909 patients, with median follow-up of 11 years. Overall, 49% of patients were premenopausal, 65% had ER positive cancers, and 70% received tamoxifen. Mean baseline BMI was 28.5 kg/m^2; 1.2% of patients were underweight (BMI <18.5 kg/m^2), 32.6% normal weight (18.5–24.9 kg/m^2), 32.9% overweight (BMI 25–29.9 kg/m^2), and 33.3% obese (BMI $\geqslant$30 kg/m^2). The graphics of smoothed BMI with RFS and OS each described a linear relationship with approximately the same slope. In adjusted analyses, BMI was significantly related to both RFS (P = 0.010) and OS (P = 0.022). Table 1 shows the 5- and 10-year RFS and OS by BMI

category. The 10-year RFS of a patient with a BMI of 25 kg/m^2 vs. one with a BMI of 35 kg/m^2 was approximately 70% vs. 65%.

Table 1. Relapse Free and Overall Survival by BMI

BMI (kg/m^2)	N	RFS (%)		OS (%)	
		5 year	10 year	5 year	10 year
<25	642	80.9	71.4	87.7	76.9
25.0–29.9	628	75.5	66.5	84.1	70.6
⩾30	636	74.9	65.0	82.7	69.8

Conclusions: We found a modest linear relationship between BMI and outcome in node-positive breast cancer patients receiving chemotherapy. Obesity, an increasing public health concern, is a modifiable factor; additional research is needed to determine the impact of weight loss on breast cancer outcomes.

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Variations in the Prevalence of Risk Factors for Breast Cancer in Different Ethnic Groups in the Million Women Study

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Background: The Million Women Study is a large prospective study in the United Kingdom (UK), designed to investigate the health of women, with a focus on breast cancer. There are known differences in the incidence of breast cancer in different ethnic groups in the UK, but information about the risk factors for the disease in these ethnic groups is limited. The aim of this study is to describe the distribution of known risk factors for breast cancer by ethnic origin in this cohort.

Materials and Methods: UK women aged 56 years, on average, were recruited into the Million Women Study between 1996 and 2001. Information about risk factors and potential confounders for breast cancer and other diseases were collected using self administered questionnaires. Participants of the study are linked to routinely-collected national databases, such that information on incident cancers and hospital admissions are notified automatically to the study investigators.

Results: Of the 1.1 million women in the study with a recorded ethnicity, almost 8000 women were Asian and almost 5000 women were Black. On average, Black women had 3.1 children, compared to 2.9 children for Asian women and 2.4 children for white women. The prevalence of having ever breastfed amongst parous women was lower for white women (69%) than for Asian (83%) or Black (83%) women. The mean body mass index was higher for Black women (28.1 kg/m^2) compared to Asian (26.0 kg/m^2) and white (26.2 kg/m^2) women. Never use of alcohol was much more common amongst Asian (70%) than Black (38%) or white women (23%). Current HRT use was higher for white women (35%), compared to Black (29%) and Asian (24%) women. 10% of white women had a first degree relative with breast cancer, compared to 8% of Black women and 6% of Asian women. A much higher prevalence of social deprivation was found in Black and Asian women than in white women with 55% of Blacks, 43% of Asians and 19% of whites in the lowest socio-economic quintile. All these differences were highly statistically significant ($P < 0.001$).

Conclusion: The Million Women Study provides a unique opportunity to compare the health of women of different ethnic origins in the UK. These results show substantial and significant differences in the risk factors for breast cancer between middle-aged Black, Asian and white women in the UK. Further analyses will be done comparing differences in the incidence and management of breast cancer in women by ethnic origin, allowing for their large differences in risk factors for the disease.

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Genetic Variability in Multi Drug Resistance Protein 1 (ABCC1/MRP1) and UDP-Glucuronosyltransferase-2B7 (UGT2B7) Are Highly Correlated with Severe Haematological Toxicity of Adjuvant FEC in Breast Cancer

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Background: We assessed the impact on hematological chemotherapy toxicity of single nucleotide polymorphisms (SNP) in germline DNA in a panel of potential genes of interest through high throughput sequencing. First aim was to validate the predictive value of certain SNP that have previously been shown to correlate with toxicity/outcome in small patient groups receiving at least one of the FEC compounds (ABCBI/MDRI, ABCC1/MRP1, ABCC2/MRP2, ABCG2, ALDH3A1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A5, DPYD, GSTP1, MTHFR, NQ01, TYMS, XPD/ERCC2, XRCC1). Secondly we investigated previously not studied genes known to be involved in epirubicin metabolisation (UGT1A1, UGT1A6, UGT2B7).

Material and Methods: We identified 1089 breast cancer patients treated in a single centre with 3 to 6 cycles of (neo-)adjuvant FEC (fluorouracil 500, epirubicin 100, cyclophosphamide 500 mg/m^2) from 2000–2010 for whom germline DNA is available. All patients were retrospectively evaluated through electronic chart review for febrile neutropenia (primary endpoint), febrile neutropenia first cycle, prolonged grade 4 or deep (<100/μl) neutropenia, anemia grade 3−4 and thrombocytopenia grade 3-4. For statistical evaluation, correction was made for number of planned cycles, primary growth factor use, age and body mass index. Because of multiple testing the false discovery rate (FDR) was calculated.

Results: Variant genotypes for rs45511401 (GT/TT, 12%) in the Multi Drug Resistance Protein 1 gene (MRP1/ABCC1), compared to the wild-type (GG, 88%) were associated with febrile neutropenia, febrile neutropenia in first cycle and thrombocytopenia (respectively 26.5 vs 15.8%, 17.1 vs 9.7% and 3.4 vs 0.3%; p-value 0.007, 0.027 and 0.005, FDR 0.3, 0.79 and 0.19). Variant genotypes for rs7668282 (CC/CT, 1.5%) in the UDP-Glucuronosyltransferase 2B7 gene (UGT2B7) compared to the wild-type (TT, 98.5%) genotype were associated with febrile neutropenia and prolonged or deep neutropenia (respectively 6.7 vs 17.2% and 6.7 vs 35.3%, p value 0.024 and 0.001, FDR 0.53 and 0.04). More details on other endpoints and other SNP will be presented, although in general no important association was found for other SNP mentioned.

Conclusions: Genetic variation in the MRP1 and UGT2B7 gene was highly associated with severe haematologic toxicity of FEC, while other previously described SNP were not validated. This is by far the largest breast cancer cohort in which the impact of genetic variability on toxicity was investigated.

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An Investigation of Interactions Between Genetic Variants and Established Risk Factors for Breast Cancer in the NCI Breast and Prostate Cancer Cohort Consortium (BPC3)

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Background: Recently various breast cancer susceptibility loci have been identified by genome wide association studies (GWAS). Relatively little is known about the possible interplay between these loci and established risk factors for breast cancer risk. Prospectively collected data from large populations are needed to test reliably for such gene-environment interactions.

Methods: We studied 8,576 women with breast cancer and 11,892 controls from the NCI Breast and Prostate Cancer Cohort Consortium (BPC3). We assessed whether 17 single nucleotide polymorphisms (SNPs) previously associated with breast cancer risk,
(*FGFR2*-rs2981582, *FGFR2*-rs3750817, *TNRC9*-rs3803662, 2q35-rs13387042, *MAP3K1*-rs889312, 8q24-rs13281615, *CASP8*-rs1045485, *LSP1*-rs3817189, *COL1A1*-rs2075555, *COX11*-rs6504950, *RNF146*-rs2180341, 6q25-rs2046210, *SLC4A7*-rs4973768, *NOTCH2*-rs11249433, 5p12-rs4415084, 5p12-rs10941679, *RAD51L1*-rs999737),
modified the odds ratios for established risk factors (age at menarche, parity, age at menopause, use of hormone replacement therapy, family history, height, body mass index, smoking status, and alcohol consumption). We also studied the possible differential effect of the polymorphisms by subgroups of tumor stage, estrogen receptor, progesterone receptor status and age at diagnosis.

Results: We confirmed the association of all but three SNPs (in *LSP1*, *COL1A1* and *RNF146*) with breast cancer risk. After correction for multiple testing, we did not find any significant interactions between SNPs and the established risk factors. We confirmed previously reported reports of differential effects of SNPs in *FGFR2* and *TNRC9* with estrogen and progesterone receptor status.

Conclusions: Our study provides evidence against the hypothesis that known common breast cancer loci strongly modify the associations