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Special histologic subtypes of estrogen receptor positive breast cancer by quantitative RT-PCR

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Background: Invasive estrogen receptor positive (ER+) breast carcinoma is morphologically characterized by many histologic subtypes. Some special histologic subtypes have been reported to be prognostically significant (Rosen, 2009). We report here the histologic subtypes of estrogen receptor positive breast carcinoma and their associated patterns of observed gene expression as measured by the 21 quantitative gene Oncotype DX[®] assay.

Material and Methods: 100,000 tumor specimens successfully examined in the Genomic Health laboratory from July 2005 through May 2009 were included in these analyses. Academic surgical pathologists reviewed all specimens for invasive carcinoma and categorized them by histologic subtype using World Health Organization criteria (IARC 2003). Quantitative expression of 16 individual cancer related genes was measured on a scale from 0 to 15 (relative to reference genes), where a one unit increment is associated with a approximate 2-fold change in expression. Recurrence Score (RS) was calculated using the published equation (Paik et al, NEJM 2004). Descriptive statistics for the RS and the individual genes [estrogen receptor (ER), progesterone receptor (PR), HER2, invasion gene expression (IGE) and proliferation gene group (PGG): ki-67, cyclin b1, survivin, STK15, MYBL2] among the different subtypes were obtained.

Results: The vast majority of the cancers (94.3%) were ductal, lobular, or mixed. Using ductal carcinoma as the comparator, papillary carcinoma had the highest ER (10.9) and PR (9.2) with low IGE and lowest RS. Medullary-like tumors (4.6 per 1000 cases) had the lowest ER, negative PR, higher PGG and highest RS which may make them more responsive to chemotherapy. The RS, on average, was lower for the classic lobular, mixed ductal/lobular, solid/alveolar lobular, tubular, cribriform, mucinous, and papillary subtypes. The special histologic subtypes (tubular, cribriform, mucinous) were characterized by higher PR, lower IGE, lower PGG and higher ER (except for tubular carcinoma that may reflect bias in submission for RS testing).

Conclusion: Histologic subtypes are characterized by differential gene expression profiles. The special subtypes of invasive breast cancer tend to have higher ER, PR and lower proliferation and invasion gene expression; however, outlier cases are not infrequent within each of the special subtypes of invasive breast cancer in this large observational cohort of estrogen receptor positive tumors.

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Frequency of low ER-positive tumors by RT-PCR in patients with low recurrence scores in Europe and Middle East

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Background: The Recurrence Score (RS), derived from the quantitative RT-PCR analysis of 21 individual genes, has been shown to quantify the likelihood of distant recurrence and of chemotherapy benefit in hormone-treated patients with estrogen receptor (ER)-positive breast cancer. We examined the frequency of low Recurrence Scores (<18) in the lowest quartile of ER-positive expressing samples submitted for Oncotype DX[®] testing in Europe and Middle East (EME).

Material and Methods: Since January 2008, a quantitative ER value has been provided with all RS results. Tumor specimens submitted by EME physicians that were successfully examined in the Genomic Health laboratory from February 2008 through October 2009 were eligible. Quantitative expression of 16 individual cancer related genes was measured by the pre-specified 21 gene Recurrence Score assay on a scale from 2 to 15 (relative to reference genes), where a one unit increment is associated with an approximate 2-fold change in expression. ER status by RT-PCR (with ER positivity prespecified as ≥ 6.5) has been shown to be highly concordant with ER by Central IHC (93% in Badve et al, JCO, 2009; 96% in Habel, ASCO Breast 2007). For the ER pos tumors by RT-PCR, the RS by risk group was analyzed by quantitative ER by quartiles.

Results: 2,645 tumors were eligible for analysis. 29 cases were excluded from the analysis because they were ER-neg by RT-PCR, leaving 2,616 evaluable tumors. The distribution of quantitative ER by quartiles for the low (<18), intermediate (18–30), and high (≥ 31) RS groups is shown in the Table. Of the 652 samples in the lowest ER-pos quartile, ER values ranged from 6.5–9.1 and 31.9% of these low ER-pos expressing tumors had RS < 18.

| Er | | | | | RS group | | |
|----------|------|------|---------|--------|----------|--------------|-------|
| Quartile | Min | Max | Average | Number | Low | Intermediate | High |
| 1 | 6.5 | 9.1 | 8.5 | 652 | 31.9% | 54.3% | 13.8% |
| 2 | 9.2 | 9.9 | 9.6 | 653 | 55.4% | 35.4% | 9.2% |
| 3 | 10 | 10.7 | 10.3 | 684 | 65.1% | 26.6% | 8.3% |
| 4 | 10.8 | 12.5 | 11.4 | 627 | 69.4% | 21.5% | 9.1% |

Conclusion: Many low ER-pos expressing tumors have low Recurrence Scores. However, patients with a RS < 18 have been shown to have a good prognosis and minimal, if any, chemotherapy benefit. (Paik et al., JCO, 2006; Albain et al., SABCS 2007). The Recurrence Score incorporates other important biological pathways beyond ER that provide additional clinically meaningful information.

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Ductal carcinoma in situ of the breast: interobserver reproducibility of three classification schemes

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Background: Several systems have been proposed for the classification of ductal carcinoma in situ of the breast (DCIS). The objective of the study is to determine the degree of diagnostic agreement among pathologists between three DCIS classification systems.

Material and Methods: 43 cases with a diagnosis of DCIS were reviewed by two pathologists and selected for interobserver analysis. Thirteen pathologists, one of them a specialist in breast pathology, received the same set of digitized images of microscopy of the DCIS cases in JPG format, and answered a questionnaire containing the criteria to compose the three classification systems studied – Holland, modified Lagios and Van Nuys. For this purpose a computer program was created, which organizes the information collected from each pathologist, supplying the histological grading of the cases for the three classification systems. The results were analyzed using percentage-wise agreement and the Kappa test.

Results: Diagnostic agreement for the three DCIS of the breast classification systems presented K values that varied from 0.27 to 0.37. Among the three classifications studied the best agreement was for Van Nuys, showing a Kappa index of 0.37. Analysis of subgroups of pathologists showed that there was greater diagnostic reproducibility for the group of specialists compared to the group of pathology residents for the Van Nuys and modified Lagios systems ($p=0.005$ and 0.023 , respectively). The accuracy similarly accompanied the results of the interobserver agreements, with Kappa indices varying from 0.13 to 0.64 for the Holland classification, 0.23 to 0.61 for the modified Lagios classification, and 0.23 to 0.74 for the Van Nuys classification.

Conclusion: Comparing the three classification systems, better agreement was obtained for the histological grade with the Van Nuys scheme. Pathologists specialized in breast pathology showed greater reproducibility for all the criteria evaluated. Diagnostic accuracy was superior for the classification of Van Nuys compared to modified Lagios and Holland.

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Reasons for over- and underestimation of lymphatic vessel invasion in breast cancer

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Background: Lymph vessel invasion (LVI) can be decisive for treatment and prognosis of breast cancer patients but often it is over- or underestimated in the routine practice.

Aim of our study was to establish the reasons leading to over- or underestimation of LVI in breast cancer, which occur in the routine practice and to create a reliable and applicable protocol for its appropriate evaluation.

Materials and Methods: Archival formalin-fixed, paraffin embedded tissue materials from 40 cases of invasive breast cancer were randomly selected and revealed from the archives of our department. The breast cancer specimen had been processed according to standard laboratory protocols. Tumors had been classified and staged according to the WHO criteria. Immunohistochemical staining with D2-40, according to protocol of the manufacturer DAKO® was used to selectively mark lymphatic vessels. Statistical analysis was performed using the chi-square test, calculated by Statgraphics Plus®.

Results: The possibility for peri-tumor lymphatic evaluation depended significantly on the amount of peri-tumor tissues analyzed $\chi^2=14.48$; $p=0.0007$ ($n=40$). In general, the presence of LVI correlated with axillary lymph node (LN) status $\chi^2=6.37$; $p=0.0116$ ($n=40$). When analysed in more than 3 mm of peri-tumor tissues, LVI matched significantly better with axillary LN status than when analyzed in peri-tumor tissues less than 3 mm wide $\chi^2=8.65$ $p=0.0343$; ($n=35$). D2-40 played decisive role in the differentiation of LVI from post-fixation tumor tissue shrinkage.

Discussion: We believe that underestimation of lymphatic invasion in breast cancer can be reduced by evaluation of at least 3 mm of peri-tumor tissues. Overestimation can be practically avoided by the application of specific endothelial marker. The application of large section technique may be the most appropriate approach for LVI evaluation in breast cancer.

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Turnaround-time reduction of pathological examination

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Background: Reduction of waiting time is very important for patient service because short turnaround time (TAT) of pathological examination allows for rapid response including medical decision-making by clinicians that may increase patient satisfaction. Pathological examination of breast specimens tends to take longer TAT because breast tissue contains abundant fat and its diagnosis routinely requires results of IHC staining. To shorten the TAT of breast cancer cases, improvements of routine work and rescheduling of procedures have been performed in our hospital.

Material and Methods: 202 breast cancer specimens, performed surgical resection (preservation resection or mastectomy) from 2007 November to 2008 May, were typically examined according to the protocol as follows. Specimens were fixed with formalin injection immediately after tissue removal. On the second day, tissue specimens were cut for pathological samples, and samples for IHC (ER, PgR, HER2) were selected at the same time. The whole of the cancer areas including intraductal spread was cut for pathological examination. After cutting sections, fixation and delipidation were accelerated by supersonic wave and thermostat bath. On the third day, pathological reports with results of IHC and digital images (demarcated cancer area with separate colors accordingly invasive or non-invasive lesions) were submitted to clinicians through electronic-pathology-data-record system (Dr. Helper, FUJITSU, Japan). In our pathology section, there are two full-time pathologists and six technicians.

Results: Average number of slides was 36.2/case (excluded LN samples). In 202 cases, TATs (after-operation days, excluding holidays) were consumed to complete each diagnosis as follows, 2 days for 78 (38.6%); 3 days for 89 (44.0%); 4 days for 25 (12.4%); 5 or more days for 10 (5.0%) cases. Less than 20% of all cases required 4 or more days mainly because of necessity of additional samples and staining.

Conclusions: In more than 80% of the surgical cases, final pathological reports including result of IHC and digital images were completed within three days after operation. To compare with previous period before practice of this protocol, average TAT of breast cancer cases was obviously shortened. These procedures do not need special equipment or extra manpower and thus this protocol for breast cancer specimens is available in other laboratories.

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Characteristics of invasive micropapillary carcinoma of the breast: Is it related to the triple negative breast cancer?

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Background: Invasive micropapillary carcinoma (IMPC) of the breast is a rare and associated with high incidence of lymph node metastasis and

poor outcome. The aims of this study were to provide a comprehensive analysis of clinicopathologic and immunohistochemical characteristics of IMPC and to elucidate the differences between IMPC and invasive ductal carcinoma (IDC).

Methods: Sixty-one patients of IMPC were identified by retrospective review of database from Jan 2004 to December 2008. 221 patients were randomly selected among the IDC patients who received operation during the same period. Two groups were compared with uni- and multi-variate analysis.

Results: We observed significant differences in mean number of metastatic lymph nodes (6.1 vs. 1.9, $p=0.001$), positivity of lymph node (70.5% vs. 45.2%, $p<0.001$), and presence of lymphatic vessel invasion (75.4% vs. 34.8%, $p<0.001$) between IMPC and IDC patients. Although it has been known that triple negative breast cancer (TNBC) have lymphotropic tendency in their early T stage, 11.8% (26/221) of IDC and 3.3% (2/61) of IMPC patients were TNBC in this study ($p=0.050$). In multivariate analysis, IMPC histology showed no correlation with disease-free survival (DFS) and the lymphatic vessel invasion was a significant predictor of DFS.

Conclusions: The results of this study confirm that IMPC is unique subtype of breast cancer that is commonly accompanied by axillary lymph node metastasis and shows poor outcome, although it rarely presents the pattern of TNBC. Lymphatic vessel invasion rather than histology of IMPC seems to be more closely related to DFS.

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Basal-phenotypes in breast carcinoma - morphological, immunohistochemical and clinical analysis

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Background: The basal-like breast cancer sub-type represents probably a new treatment challenge for oncologists. The aim of the study was to analyse morphological, immunohistochemical and clinical features in breast invasive carcinomas which diagnosed as basal-like type of invasive ductal carcinoma (BLIDC) from needle core biopsies (NCB).

Methods: We reviewed 21 (11.7%) breast carcinomas :20 primary ones (T1-T4) and one after 7 years recurrence carcinoma (r T1) for hematoxylin-eosin slides, immunohistochemistry for ER, PR, HER-2, CK 5/6, CK17, CK 18, p53, BRCA1 testing, clinical information. Morphological aspects as architectural features (sheets, nests, tubular formation, ribbon-like formation), intensity of lymphocytic infiltration, necrosis, fibrous and/or hyalinised stroma, nuclear pleomorphism, mitoses has been studied. Semiquantitative evaluation of basal cytokeratine positive cancer cells has been performed. Overexpression of p53 cases has been examined.

Results: BRCA1 cases of BLIDC (3/21) were triple negative and just a case overexpressed p53. All sporadic BLIDC (18/21) were ER, PR negative but 3 cases were 3+ scored and 4 cases 2+ scored for HER-2. Overexpression of p53 was noted in 11/18 carcinomas. At least one basal cytokeratin CK 5/6 or CK17 was positive in all cases. 5-30% cancer cells expressed CK 5/6 in 53% BLIDC and there were more than 30% positive cells in 29% BLIDC. CK 17 was expressed in 10-30% neoplastic cells in 36% BLIDC and more than 30% positive cancer cells were noted in 56% cases. CK 18 was positive in all BRCA1 cases and 75% sporadic cases carcinoma cells.

Conclusions: CK 17 is recommended as first basal marker in NCB for immunohistochemical confirmation of basal-like type when amount of cancer cells is small.

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The prognostic factors of breast cancer related with axillary lymph node metastasis in T1 breast cancer

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Background: Axillary lymph node metastasis can occur even in breast cancer sized less than 2 cm. There are several prognostic factors related to the lymph node metastasis in breast cancer. We aimed to investigate the clinicopathologic factors that affect the node metastasis in T1 breast cancer. **Methods:** We reviewed the medical records and pathologic reports of all breast cancer patients who had undergone surgical procedure from 2001 to 2006. Among these patients, we retrieved 230 T1 breast cancer patients divided them into two groups according to the presence or absence