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ABSTRACTS

ESP Abstracts 2013

Oral Free Paper Sessions

Sunday, 1 September 2013, 08.30–12.00, Room 5C
OFP-01 Oral Free Paper Session Breast Pathology

OFP-01-001
Fully automated FISH staining and digital analysis of HER2 in breast cancer: A validation study
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Objective: To validate the detection of human epidermal growth factor receptor-2 (HER2) status using the Leica HER2 fluorescent in situ hybridization (FISH) system and subsequent semi-quantitative analysis with the Menarini Benelux D-Sight digital imaging platform, in order to predict HER2-directed therapy in patients with invasive breast cancer.
Method: HER2 assessment was performed on 328 formalin-fixed/paraffine-embedded invasive breast cancer tumours on tissue microarrays (TMA) and 100 (50 selected IHC++ and 50 random IHC scores) full-sized slides of breast cancer resections biopsies obtained for diagnostic purposes. For digital analysis slides were pre-screened at 20× and 100× magnification for all fluorescent signals and semi-automated scoring was performed on at least two pictures with the D-Sight HER2 FISH analysis module. Results were compared to data obtained previously with the manual Abbott FISH test.
Results: The overall agreement with Abbott FISH data among TMA samples and in 50 selected IHC++ cases was 98.8 % (kappa = 0.94) and 93.8 % (kappa = 0.88), respectively. The results of 50 unselected IHC cases were concordant with previously obtained IHC and/or FISH data.
Conclusion: The combination of the Leica FISH system with the Menarini Benelux D-Sight digital imaging platform is feasible for the assessment of HER2 status in routine clinical practice in patients with invasive breast cancer.

OFP-01-002
A new quantitative in-situ immunohistochemistry method
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Objective: There is a general need to make pathologic examinations less subjective and to support the accuracy required for companion diagnostics. The aim of this study was to develop a new IHC method based on bright field technology combining morphological information with quantitative assessment.
Method: By chemical manipulation of a visualization system, single antibodies are visualized as dots instead of a conventional stain. The dots can easily be counted by image analysis, and the number of dot reflects protein expression levels. Initial assay performance was evaluated using Her2 as a test system; breast cancer cell lines as well as breast cancer tissue specimen were included.
Results: The assay generates highly reproducible results and gives a linear assay with a larger dynamic range than the conventional assays. No overlap was seen between the different breast cancer cell lines. Moreover, evaluation of HER2 status in a number of breast cancers by the use of this new method showed a strong correlation with established methods.
Conclusion: The data suggest enumeration of dots can be direct related to protein expression levels. This represents a new and standardized way of objectively determining protein amounts in cells and tissue in situ.

OFP-01-003
Automated image analysis enables accurate enumeration of the Ki-67 labelling index of breast cancer
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Objective: Immunohistochemical Ki67 evaluation reflects proliferative activity and is regarded as important prognostic/predictive marker of breast cancer. However, its potential is hindered by lack of standardized and efficient methodologies to measure the Ki67 expression. Besides many other aspects, key element of the methodology remains accurate enumeration of Ki67-labelling index (LI). We investigated the accuracy aspect of automated image analysis (IA) approach.
Method: TMA (1 mm diameter spot per patient, $n=140$) from invasive ductal breast carcinoma, stained for Ki67 and digitized by Aperio scanner, were used for the study. Reference values (RV) were obtained by counting the LI using stereological frame. IA was performed with Aperio Genie/Nuclear algorithms enabling automated selection of tumour tissue. The images were semi-quantitatively evaluated by 3 pathologists (P1,2,3).
Results: RV correlated strongly with IA ($r=0.95$) and P1,2,3 ($r=0.86$, $r=0.89$, $r=0.93$, respectively), $p<0.0001$. Analysis of variance revealed no significant pairwise differences of the LI means of RV(40 %) versus IA(36 %), P2(43 %), or P3(44 %); however, the IA versus P2,3 differed, and P1(24 %) was significantly lower ($p<0.05$). Regression analysis to predict RV revealed best performance for the IA results.
Conclusion: IA provided most accurate enumeration of the Ki67 LI. Validation against proper RV is a crucial step in setting up an IA tool.