Prediction of distant recurrence in low risk early breast cancer by RT-qPCR based subtyping using MammaTyper®

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Background and Objective

Estrogen receptor (ER)/HER2+ progestin receptor (PR)/HER2− and human epidermal growth factor receptor-2 (HER2/ERBB2) are routinely assessed by immunohistochemistry (IHC) during setup of breast cancer samples. The routine use of IHC (MMI)/IHC in the assessment of the context of breast cancer subtyping however, remains controversial, due to poor reproducibility and lack of standardization.

The MammaTyper® test is a CE-marked in vitro diagnostic (IVD) test which measures the expression status of the four breast cancer biomarkers ER, PR, HER2, and Ki67 on the mRNA level via reverse transcription quantitative PCR (RT-qPCR) and has demonstrated a high degree of reproducibility in the assessment of the four markers.

In this retrospective analysis we assessed the prognostic power of molecular subtyping by MammaTyper® in archived samples from low risk early breast cancers treated with adjuvant endocrine therapy only.

Patient samples

Inclusion criteria for this retrospective analysis were 1) FFPE samples from female invasive breast cancer, 2) ER positive and HER2 negative according to initial diagnosis by IHC/ISH, 3) No distant metastasis at time of diagnosis, 4) Treatment with adjuvant endocrine therapy alone (no use of chemotherapy).

Samples and data were obtained through RT-qPCR. The MammaTyper® (MammaTyper®, Bochum/Herne) and it’s network of six different breast cancer centers in Germany, were included in this sample. In BioNTech Diagnostics, FFPE sections were made from routine patient diagnostic material, collected between 2003 and 2011. Clinical approval of the performance of MammaTyper® was done by ethics committee of the medical faculty of the University of Bonn (255/06). All patients had provided individual written informed consent for the storage of samples and data, follow-up contact, and further use of samples and data for research purposes.

Methods

Histology: Tumor cell content [TCC] was determined on a 24x H&E stained slide by counting the surface covered by tumor cells with the surface of the complete tissue. TCC was defined as the ratio of areas covered by invasive carcinoma in relation to the area covered by DSD and or non-epithelial neoformations (stroma, necrotic-, vasc., edema tissue).

RNA extraction: For samples with a minimum TCC of 20% RNA was extracted from one 10μm section per block using the bead-based RNA purification kit (RNeasy®) according to the manufacturer’s instructions.

RT-qPCR: MammaTyper® RT-qPCR was carried out according to manufacturers’ instructions on a COBAS® system [Roche Diagnostics], using total RNA from FFPE tissue as input. Expression values were classified as positive or negative for each marker based on pre-defined cutoff values. Tumor subtypes were defined to each sample based on the combination of IHC/ISH single marker expression status according to the St Gallen surrogate subtype definition (TABLE 1).

Staging: Breast cancers were staged by a single dedicated breast surgeon, using standard criteria for direct staging. Metastatic spread and recurrence were documented and classified according to standard criteria for staging.

Conclusion and Perspectives

Determination of ER, PR, and/or HER2 mRNA levels allows tumor subtyping according to the St Gallen surrogate subtype definition. Low risk of distant recurrence could be confirmed for the MammaTyper® Luminal A-like subtypes suggesting that for this patient group targeted endocrine therapy alone could be sufficient. The high degree of standardization and robustness of the RT-qPCR measurement may drive the use of the ER/PR/Ki67 biomarker in routine breast cancer pathology.

Patient samples were prospectively assigned to the luminal A-like group (ER+PR+HER2−), the luminal B-like group (ER+PR−HER2−), the HER2 positive group (ER−PR−HER2+), or the remainder group (ER−PR−HER2−).

Coexpression and log rank analysis confirmed MammaTyper® Luminal A-like subtype as significant predictor of distant recurrence (TABLE 4). Luminal A-like subtype remained an independent prognostic factor in multivariate analysis (TABLE 5).

TABLE 3: Translation of MammaTyper® single marker values into molecular subtypes according to factor receptor 2 (HER2/ERBB2) was routinely assessed by immunohistochemistry (IHC) during setup of breast cancer samples. The routine use of IHC (MMI/IHC) in the assessment of the context of breast cancer subtyping however, remains controversial, due to poor reproducibility and lack of standardization.

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