Oncotype DX® recurrence score (RS) has emerged as an established risk classifier for patients with ER+/HER2- early-stage breast cancer. While RS is one of the most rigorously studied risk scores, it is also one of the most expensive tests, beyond reach for many patients.

The necessity for an affordable method for estimating risk of recurrence has motivated investigations into the correlation between RS and traditional parameters such as IHC for ER, PR, and Ki-67. However, semi-quantitative IHC lacks standardization across different laboratories, especially for Ki-67.

In the present study we investigated whether a highly standardized assessment of HER2, ER, PR, and Ki-67 in breast carcinomas on mRNA level using the RT-qPCR-based CE-marked in-vitro diagnostic device (IVD) MammaTyper® has the potential to serve as an affordable and decentrally performed predictor of the Oncotype DX® RS.

**Background**

**Samples and Methods**

**Samples**: A total of 219 formalin-fixed, paraffin-embedded (FFPE) invasive breast carcinoma samples were used for the purposes of the present study. Ten samples were excluded due to low tumor cellularity (<20%) and 2 samples were excluded due to low RNA content. Complete data for RS, IHC, grading and mRNA measurement was available in 196 samples. RT-qPCR: RNA was extracted from one 10μm thick section per sample using bead based kit RNAscript® (R883, E85L, P62) and mRNA expression was measured by RT-qPCR in extracts from FFPE breast cancer samples using the MammaTyper test. Tumor subtypes were assigned according to the 2013 St Gallen surrogate definition based on binary mRNA marker classifications (pos/neg) according to predefined cut-offs.

**Statistical**: Correlation of quantitative single marker results from MammaTyper® and Oncotype DX® was assessed. MammaTyper® subtype results were compared to RS risk classes based on commercial and TAILORx® trial cut-offs. The prediction of continuous RS values by mRNA or semi-quantitative IHC measurement was compared by linear regression and subsequent correlation and ROC analyses of prediction models. Finally, a two step method was proposed for filtering out Oncotype DX® low risk cases (RS ≤25) based on IHC based subtyping or estimation of Oncotype DX risk classes (using the online tool breastcancerriskcalculator.onc.fhms.eu) and a subsequent RT-qPCR based subtyping of IHC luminal A-like / low risk cases.

**Results**

**Table 1**: Result of linear regression analysis of four markers against Oncotype DX® RS

<table>
<thead>
<tr>
<th>Marker</th>
<th>Intercept</th>
<th>Coefficient</th>
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<tr>
<td>Ki-67</td>
<td>0.34</td>
<td>0.87</td>
<td>0.04</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Figure 1**: Distribution of Oncotype DX® values and single marker scores in the set of 219 invasive breast carcinomas.

**Figure 2**: Correlation of single marker assessments determined by MammaTyper® and Oncotype DX®. Dashed red lines are pre-specified single marker cut-offs.

**Figure 3**: Correlation of MammaTyper® single marker assessments (ER, ESR1, HER2, and Ki-67) with Oncotype DX® RS.

**Figure 4**: Comparison of MammaTyper® subtype results with Oncotype DX® commercial and TAILORx® trial risk classes. Left: relative frequencies, Right: absolute frequencies.

**Figure 5**: ROC analysis of mRNA and IHC based prediction models for prediction of a high risk (<0.05) Oncotype DX® result.

**Conclusions**

In conclusion, this study documents the possibility of using standardized and reproducible gene expression measurements for establishing a clinically informative method for risk estimation for breast cancer patients with early-stage disease. A major advantage of the test is that it can be performed decentrally and at affordable costs in peripheral pathology services. On economic grounds, the two step approach for identifying patients who can forego costly multigene testing, results in a financial benefit for the entire study population if the costs for the MammaTyper® were set at approximately half (47.5%), the proportion of samples which are low risk/Luminal A-like according to IHC and mRNA measurement) of the price of Oncotype DX®.