Comparison of the RNA-based EndoPredict multigene test between core biopsies and corresponding surgical breast cancer sections

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ABSTRACT

Aim This study compared the perfomance of the RNA-based EndoPredict multigene test on core biopsies and surgical breast cancer specimens and analysed the influence of biopsy-induced tissue injuries on the test result.

Methods 80 formalin-fixed paraffin-embedded samples comprising paired biopsies and surgical specimens from 40 ER-positive, HER2-negative patients were evaluated. Total RNA was extracted and the EndoPredict score was determined.

Results RNA yield was considerably lower in core biopsies, but sufficient to measure the assay in all samples. The EndoPredict score was highly correlated between paired samples (Pearson r=0.92), with an excellent concordance of classification into a low or high risk of metastasis (overall agreement 95%).

Conclusions The measurements are comparable between core biopsies and surgical sections, which suggest that the EndoPredict assay can be performed on core biopsy tissue. Inflammatory changes induced by presurgical biopsies had no significant effect on the RNA-based risk assessment in surgical specimens.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer-related death in women.[1] The use of validated multigene assays might assist clinical treatment decisions. To this end, several prognostic and predictive tests have been developed to allow a more tailored treatment strategy in breast cancer.[2–6] The EndoPredict test has recently been introduced as a novel multigene classifier to predict the likelihood of distant recurrence in ER-positive, HER2-negative breast cancer patients treated with adjuvant endocrine therapy.[4] Two large randomised phase III trials (ABCSG6 and ABSCG8) involving endocrine therapy only (n=1702) demonstrated additional prognostic information of the EndoPredict test beyond all common clinicopathological parameters.[4]

Formalin-fixed, paraffin-embedded (FFPE) tumour tissue sections from surgical specimens are commonly used as the source material for the quantitative PCR-based expression analysis of multigene assays such as the EndoPredict test. However, the comparability of multigene assays between core biopsies and surgical specimens is largely unknown. In addition, the surgical specimens may contain inflammatory changes that are caused by preoperative core biopsy sampling. As a consequence, wound healing and inflammation processes could potentially lead to relevant changes for the established molecular tests. For instance, the protein levels of the prognostic breast cancer markers urokinase-type plasminogen activator (uPA) and the plasminogene activator inhibitor (PAI-1) are considerably altered in the tissue area surrounding the biopsy channels leading to unreliable results of the ELISA test.[7] So far it is not clear whether RNA levels of prognostic multigene assays are also altered by the presence of tissue injuries in the tumour sections analysed.

In this study, we compared the EndoPredict score between core biopsies and corresponding surgical specimens of breast cancer patients. Second, we analysed the influence of tissue injuries caused by a preceding biopsy on the quantification of the EndoPredict score in the paired breast cancer samples.

METHODS

Study population and histopathological examination

Eighty FFPE tumour blocks comprising 40 paired samples (core biopsies and corresponding surgery specimens) from patients with ER-positive, HER2-negative breast carcinomas were retrospectively evaluated. The cases were selected based on the presence of typical changes of biopsy-induced inflammation, such as inflammatory infiltrates, localised fibroblast proliferation or focal residual haemorrhage in the H&E sections from surgical specimens. There were four cores (median) per FFPE tissue block. The majority of the cores were taken under ultrasound control with 14G needles. The median time between biopsy and resection was 14 days.

Patient characteristics are shown in table 1. Two consecutive 5 μm FFPE tissue sections from each pretherapeutic core biopsy and the corresponding surgical specimen were used for this study, respectively. One section was routinely stained by H&E. The second slide was used for molecular analyses. The hormone receptor and the HER2 status as well as the proliferation activity were extracted from pathology reports. The receptor status was determined during routine diagnosis by immunohistochemistry, in the case of HER2 2+ (Dako score) followed by a silver enhanced in-situ hybridisation.[8]

Table 1 Patient characteristics

Characteristic	No of patients	%
All cases*	40	100
Histological type		
Ductal carcinoma	31	77.5
Lobular carcinoma	5	12.5
Other carcinoma	4	10
Tumour size (mm)		
≤20	23	57.5
>20	17	42.5
Nodal status		
pN0	27	67.5
pN1a	10	25
pN2a	3	7.5
Histological grade		
G1	8	20
G2	26	65
G3	6	15
Tumour proliferation (surgery specimen)†		
MIB-1 <14%	21	52.5
MIB-1≥14%	16	37.5

*All cases were hormone receptor positive (ER ≥60% positive tumour cells) and HER-2 negative (Dako score 0–2; in the case of Dako score 2+ without amplification using silver-enhanced in-situ hybridisation).
†Data were not available for all samples.

RNA extraction and assessment of the EndoPredict score

Total RNA was extracted using a fully automated isolation method, as described previously.[9–11] DNA-free total RNA from the FFPE sections was finally eluted with 100 µl elution buffer and stored at −80°C.

The EndoPredict assay was carried out to determine the expression levels of three reference genes and the relative expression levels of eight genes of interest. To obtain reliable results, all genes were assessed in triplicate by quantitative one-step reverse transcription PCR using the SuperScript III PLATINUM (Invitrogen, Karlsruhe, Germany) one-step quantitative reverse transcription PCR system with ROX (final concentration 50 nM). Measurements were carried out with the VERSANT kPCR system (Siemens Healthcare Diagnostics, Tarrytown, USA). Samples were classified as low or high risk of distant metastasis according to the predefined cut-off value of 5.[4]

Statistical analysis

Absolute expression levels (Ct values) were calculated using VERSANT software. Relative expression levels of the eight prognostic genes (*AZGP1*, *BIRC5*, *DHCR7*, *IL6ST*, *MGP*, *RBBP8*, *STC2*, *UBE2C*) were assessed as delta cycle threshold (ΔCt) values with respect to the three reference genes (*CALM2*, *OAZ1* and *RPL37A*). The EndoPredict score was calculated for the biopsy and tumour sections, as described.[4]

RESULTS

Forty FFPE tumour sections and 40 corresponding core biopsies were analysed and used for comparison. RNA yield was compared between biopsy and tissue sections by using the mean Ct value of the three reference genes as a surrogate marker. With a median difference of two Ct units, RNA yield was considerably lower in core biopsies. However, the extracted RNA amount was still sufficient to measure the EndoPredict score in all biopsies and tumour tissue areas, respectively.

Gene expression levels of the eight EndoPredict genes (*AZGP1*, *BIRC5*, *DHCR7*, *IL6ST*, *MGP*, *RBBP8*, *STC2*, *UBE2C*) were compared between the biopsy and the corresponding surgical specimen. Relative RNA levels of each of the eight prognostic genes were significantly correlated between core biopsies and surgical specimens with Pearson correlation coefficients ranging from 0.65 to 0.89 (table 2). The EndoPredict score was also highly correlated between biopsies and tumour sections (r=0.92; table 2, Figure 1), and the concordance for the EndoPredict-based risk classification was excellent (overall agreement 95%, κ=0.89). Only two disagreements were found in terms of risk categorisation; however, both samples exhibited an EndoPredict score that was close to the predefined cut-off level.

Table 2 Analysed genes and correlation between core biopsy and surgical specimens

Gene name	Correlation (Pearson r)
AZGP1	0.82
BIRC5	0.72
DHCR7	0.77
IL6ST	0.89
MGP	0.69
RBBP8	0.84
STC2	0.65
UBE2C	0.76
EndoPredict score	0.92

DISCUSSION

The results indicate that the EndoPredict score between core biopsy and corresponding surgical specimens is identical. In the evaluated samples, a biopsy-induced injury seems to have no influence on the EndoPredict score. This might be due to the selected genes in the EndoPredict assay, which are not directly linked to inflammation and wound repair processes. Haas and colleagues[7] recently showed that prognostic markers such as uPA/PAI-1, which are associated with wound healing and

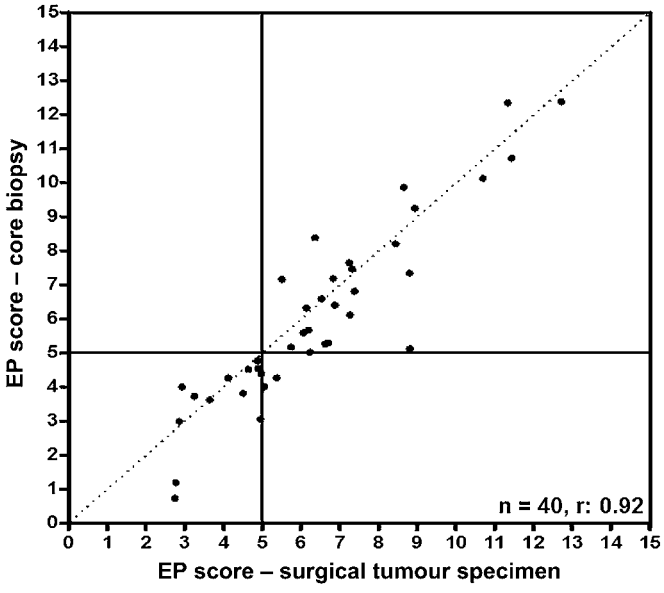

Figure 1 Correlation between the EndoPredict (EP) scores from biopsies and corresponding surrounding tumour tissue (n=40). Samples with an EndoPredict score below 5 were considered 'low risk', whereas samples with an EndoPredict score above 5 were considered 'high-risk'. Pearson correlation coefficient 0.92.

Take-home messages

- The EndoPredict score was highly correlated between core biopsies and corresponding surgical tissue specimen; core biopsies appear to be a biomaterial to run the EndoPredict.
- Tissue injury induced by preoperative biopsy sampling in this cohort seems to have no considerable effect on risk categorization by the EP.

inflammation, can be considerably altered by the fibroblastic inflammatory reaction around the biopsy channel. In addition, expression levels of 60 genes were compared between core biopsies and surgical tumour tissue specimens by Zanetti-Dällenbach *et al*.[12] All analysed genes were selected due to their relevance in breast cancer and their association with biological functions such as proliferation, survival, invasiveness and wound healing. Among the genes, *uPAR*, *PAI-1*, *COX2* and *MMP1* showed an increased expression level in surgical tissue compared with the core biopsy. The data also indicate that the expression changes could be influenced by tissue injuries from preoperative core biopsy sampling.[12]

Despite the marked differences in RNA yield between core biopsies and corresponding surgical specimens, correlations between the EndoPredict scores were very high. The data suggest that the EndoPredict score can be reliably determined in core biopsy specimens. Inflammatory changes induced by presurgical core biopsies had no significant effect on the RNA-based risk assessment in the surgical specimens evaluated in this study. This suggests that tumour areas with biopsy-induced changes might be used for molecular testing.

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Competing interests KEW, CP, MD, RK and CD are shareholders in Sividon Diagnostics. The remaining authors report no competing interests.

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