Introduction

The ability to detect breast cancer early is essential for the treatment and prognosis of patients. In many countries this early detection is based on a screening programme which includes mammography. However, the presence of dense tissue, as often seen in pre-menopausal women, is a confounding factor in the interpretation of mammographic images. The problem of reading mammographic images with increasing breast density is seen with all age groups. In the US and many parts of Europe the follow-up procedure for an uncertain diagnosis is enhanced magnetic resonance imaging (CE-MRI). CE-MRI has high sensitivity for invasive breast cancer but is a costly procedure and suffers from poor specificity.

The DiaGenic BCtect® Test: Basic Principles

- The primary diseased part of the body is not the only part responding to a disease
- The disease also leaves a unique "signature" (subtle systemic changes in gene expression) in other parts of the body
- This signature can be identified using gene expression technology in peripheral blood

The development of the BCtect® test (see Box 2) was based on whole genome screening of more than 32000 genes transcripts. During these developmental studies it was clearly shown that patients with breast cancer could be distinguished from women with benign lesions or women without breast abnormalities using a blood-based gene expression test.5,6

Development of BCtect®

The BCtect® gene expression based test has now been designed using a commercially available real-time PCR platform that utilizes micro-fluidic cards (MFC). An outline of the test procedure is shown below in Box 3.

Sample Preparation

Whole blood is collected from individuals in PAXgene® Blood RNA tubes and processed according to the manufacturer’s instructions. Total RNA is extracted from blood samples using PAXgene® Blood RNA kit and quality assessed by NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer. Samples are prepared using the high-capacity cDNA reverse transcriptase kit and the Universal PCR Master Mix reagent from Applied Biosystems. Up to 4 individual samples are applied to the BCtect® MFC. Gene sets and expression analysis

Gene expression analysis is performed using the ABI Prism 7900HT Fast System with the BCtect® multi-fluidic card (MFC) with a 96-assay format, containing a BC-specific gene signature in a custom format, such that 4 individual samples can be run in parallel on each MFC (see Box 3).

The dedicated BCtect® software performs quality control on the gene expression data and implements data integrity checks. The software applies a disease specific algorithm to the expression data results which in turn results in a test score. A positive score is consistent with a gene expression pattern seen in BC and a negative is consistent with a gene expression pattern seen in non-BC.

Standard of Truth

The standard of truth was determined at each site based on mammographic findings and histology/cytology.

Results

The BCtect® algorithm was developed using partial least square regression on a balanced set of samples from a total of 223 breast cancer patients, benign and healthy controls (non-BC) recruited from Norwegian, Swedish and North American hospitals. Leave-one-out cross-validation showed a 72% agreement of the BCtect® test with the standard truth. The final algorithm was built into the BCtect® software for patient reporting. A separate validation of the BCtect® test was performed in a multi-centre Scandinavian study including 5 collection sites in Norway and Sweden, wherein 2 of the recruitment sites were independent from the calibration study. All samples (N=108: 55 BC, 54 non-BC) were independent from those used in the calibration study. The demographics of all subjects recruited to the calibration and validation studies are presented in Box 4. Overall, the BCtect® test showed 72% agreement with the standard truth. The data was similar for the validation and combined data sets. Data are presented in Box 5 with ROC curve in Box 6.

Performance characteristics for BCtect®

<table>
<thead>
<tr>
<th>Calibration (N=223)</th>
<th>Validation (N=109)</th>
<th>Combined (N=332)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>73% (5.8)</td>
<td>72% (8.0)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>72% (7.8)</td>
<td>69% (12.2)</td>
</tr>
<tr>
<td>Specificity</td>
<td>73% (8.7)</td>
<td>74% (11.7)</td>
</tr>
</tbody>
</table>

Menopausal status

Menopausal status was recorded for each subject by self-declaration. A subject was classified as pre-menopausal if more than 1 year had passed since the last menstrual period. The number of pre and post-menopausal women in the calibration and validation populations was balanced as far as possible. Accuracy of the BCtect® was similar in both pre- and post-menopausal cohorts. Accuracies for both the validation set and combined validation and calibration set are presented in Box 7.

Lobular cancer

Pilot data for detection of pure lobular cancers (ic without any recorded ductal component) shows 16 of 21 lobular cancers were detected with the blood based gene expression test. Of the 21 cases lobular cancers, 6 subjects had stage 1 cancer, 13 subjects had stage 2 cancer, and 2 subjects had stage 3 cancer. Nodal involvement was seen in 11 subjects. See Box 8

Discussion

Mammaphy has high performance characteristics with palpable breast lesions, however, sensitivity falls with decreasing lesion size and with increasing breast density. BCtect® has equal characteristics in pre- and post-menopausal women. Pre-menopausal women often have dense breast tissue making mammaphy difficult to interpret (approx 59% of pre-menopausal women have dense breast tissue). BCtect® may provide a useful complement to mammaphy in cases where there are inconclusive results, incomplete data, or the image is difficult to interpret.

Conclusions

- A novel blood test has been developed that can aid in the early detection of breast cancer using a peripheral blood sample.
- The test has been developed in accordance with the In Vitro Diagnostic Medical Device Directive IVD 98/79/EC.
- The test is a relevant biomarker for early detection of breast cancer and can be used as a complement to mammaphy for all women, and with particular relevance to pre-menopausal women who often have high breast density.

References


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