Validation of a blood based gene expression test, BCtect®, for the detection of breast cancer

Introduction

Early detection of breast cancer is essential for improved survival of this disease. The presence of mammographically dense tissue, as often seen in pre-menopausal women, is a confounding factor in the interpretation of mammographic images and increasing density is associated with decreased performance of mammography (see Box 1).

We report the detection of breast cancer using a blood based gene expression test. Such tests provide a patient-friendly adjunct to mammography in cases where additional testing is required.

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 2.

Methods

Sample Preparation

Whole blood is collected in PAXGene® Blood RNA tubes and processed according to the manufacturer’s instructions. Total RNA is extracted using PAXGene® Blood RNA kit and quality assessed by NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer. lDNA is prepared using the high-capacity cDNA reverse transcription 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 cards (MFC) with a life-assay format, containing a BC-specific gene signature in a custom format, such that 4 individual samples can be run on each MFC (see Box 3).

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

Patient selection and standard of Truth

Subjects were selected from screening centres and from a non-screening population. All subjects underwent mammography. Those with suspicious lesions underwent histopathology/cytology, which was used as the standard of truth. In the case of cysts, ultrasound and mammography was acceptable as the standard of truth. For women without mammographic abnormalities, mammography was considered the standard of truth. Donors were recruited from Norwegian and Swedish hospitals. All blood samples were collected after the donor had provided written informed consent.

Biological activity of selected assays

The biological processes for the assays used in BCtect® are shown in Box 3. The largest categories represent nucleoside, nucleotide and acid metabolism, protein metabolism and modification, signal transduction, immunity defence and as yet unclassified biological processes.

Results

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 and all donors were independent from the calibration set (N=109; 55 BC, 54 non-BC). The demographics of all subjects recruited to the calibration and validation studies are presented in Box 4. Leave-one-out cross-validation for the calibration cohort showed a 72% agreement of the BCtect® test with the standard of truth with similar results in the validation and combined data sets. The data for the validation, calibration and combined data sets are presented in Box 5.

Performance characteristics for BCtect®

<table>
<thead>
<tr>
<th></th>
<th>Calibration (N=222)</th>
<th>Validation (N=109)</th>
<th>Combined (N=332)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>73% (5.5)</td>
<td>72% (5.5)</td>
<td>72% (4.8)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>72% (7.0)</td>
<td>69% (12.3)</td>
<td>72% (6.7)</td>
</tr>
<tr>
<td>Specificity</td>
<td>76% (6.7)</td>
<td>74% (11.7)</td>
<td>73% (7.0)</td>
</tr>
</tbody>
</table>

Menopausal status

Menopausal status was recorded for each subject by self-declaration. A subject was classified as post-menopausal if more than 1 year had passed since the last regular menstruation. 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, calibration and combined data sets are presented in Box 6.

Additional findings

Lobular cancer: 16 of 21 lobular cancers were detected with BCtect®. Of the 21 lobular cancers, 6 subjects had stage 1 cancer, 13 subjects had stage 2 cancer, and 2 subjects had stage 3 cancer.

Medications: Co-medications were recorded in 49% of the combined population. Common medications were for hyperlipidaemia, hypertension, cardiac conditions, asthma, diabetes and depression. No correlation between co-medications and incorrect test score was noted.

Pregnancy: 16 of 20 pregnant women tested in a separate study showed a positive test result. Similar results were seen with the whole genome array data.

Exogenous hormones: Hormone replacement therapy or hormonally based contraceptives were registered in 22% of the combined population. No correlation between exogenous hormone use and incorrect test score was noted.

Menstrual cycle: In a separate study, samples were collected weekly from 5 women. No correlation in test score was seen with menstrual cycle.

Discussion

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Mammography sensitivity falls with decreasing lesion size and with increasing breast density.

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BCtect® has equal sensitivity in pre- and post-menopausal women.

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BCtect® detects lobular cancers with equal efficacy as ductal cancers

The test has been developed in accordance with the In Vitro Diagnostic Medical Device Directive IVDD 98/79/EC.

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The test is a relevant biomarker for early detection of breast cancer with particular relevance in cases where there are inconclusive mammography results, incomplete data, or imaging is difficult to interpret.

References

3. Aare J. et al. (2006) 97th AACR Annual Meeting, Washington DC, USA