Application of a 70-Gene Expression Profile to Japanese Breast Cancer Patients

Hideo Shimizu a Yoshiya Horimoto a Atsushi Arakawa b Hiroshi Sonoue b Mami Kurata a Taijirō Kosaka a Katsuya Nakaj a Takanori Himuro a Emi Tokuda a Yuka Takahashi a Fumi Taira a Mayuko Ito a Ikuko Abe a Koji Senuma a Lisette Stork-Sloots c Femke de Snoo c Mitsue Saito a

a Department of Breast Oncology, Juntendo University School of Medicine, Tokyo, Japan; b Department of Human Pathology, Juntendo University School of Medicine, Tokyo, Japan; c Agendia N.V., Amsterdam, The Netherlands

Introduction

The decision to perform adjuvant chemotherapy in breast cancer patients has usually been based on risk factors for recurrence, including tumor size and lymph node metastasis [1]. However, the indications for chemotherapy are sometimes uncertain, especially in patients without lymph node involvement. Thus, the ability to identify patients who would not benefit from chemotherapy has long been a key issue. With recent advancements in genomic microarray technology, it has become possible to perform gene profiling and thereby obtain clinically useful information. van de Vijver et al. [2] developed the MammaPrint® system to evaluate the risk of breast cancer recurrence by profiling the expression levels of 70 specific genes. Using 10-year outcome data from an untreated breast cancer patient population, these 70 critical genes, which showed the highest correlation with the likelihood of distant recurrence, were chosen from among 25,000 genes for the MammaPrint profile. The ability of the 70-gene profile to predict disease relapse has been supported by a number of retrospective studies [2–5]. The TRANSBIG Consortium showed the 70-gene profile data to be equivalent to tumor size, grade and estrogen receptor (ER) status for predicting the outcomes of node-negative patients [3]. The 70-gene profile was included in the 2009 St Gallen International Expert Consensus, together with OncotypeDX, another gene profiling kit [6]. Since obtaining approval from the Food and Drug Administration in the USA, the 70-gene profile has been widely used in early breast cancer patients.

Since the concept of intrinsic subtype was introduced at the 2011 St Gallen Consensus meeting [6–9], the application of ‘classical’ risk factors has become increasingly rare and there has been a shift toward consideration of the response to drug therapy. While chemotherapy is generally recommended for patients who have...
Table 1. Clinicopathological features of the 50 patients

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>MammaPrint</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk (n = 36)</td>
<td>High risk (n = 14)</td>
<td></td>
</tr>
<tr>
<td>Mean age, years (± SD)</td>
<td>53 (9.8)</td>
<td>58 (8.7)</td>
</tr>
<tr>
<td>Mean tumor size, mm (± SD)</td>
<td>24 (7.7)</td>
<td>21 (7.4)</td>
</tr>
<tr>
<td>Tumor structure</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillotubular</td>
<td>8 (22%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Solid-tubular</td>
<td>7 (19%)</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>Scirrhoust</td>
<td>14 (39%)</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>7 (19%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Nuclear grade</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>34 (94%)</td>
<td>12 (86%)</td>
</tr>
<tr>
<td>High</td>
<td>0 (0%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ER positive, %</td>
<td>97</td>
<td>71</td>
</tr>
<tr>
<td>PR positive, %</td>
<td>81</td>
<td>64</td>
</tr>
<tr>
<td>Ki-67&lt;sup&gt;a&lt;/sup&gt;, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 14%</td>
<td>5 (14%)</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>≥ 14%</td>
<td>31 (86%)</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>&gt; 14%</td>
<td>26 (72%)</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>≤ 14%</td>
<td>10 (28%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Mean ± SD = Standard deviation, ER = estrogen receptor, PR = progesterone receptor.

<sup>a</sup>Mean ± SD = 26.6 ± 18.7%.

Application of a 70-Gene Expression Profile to Japanese Breast Cancer Patients

Results

Among the 65 patients enrolled in this study, 5 were found to have sentinel lymph node metastasis during surgery. In another 10 samples, the 70-gene profile failed due to an inadequate volume, despite our efforts to carefully choose patients and obtain samples of adequate size. Samples from the remaining 50 cases were used for the 70-gene profile analysis. The 70-gene profile classified 36 patients as low-risk and 14 as high-risk patients. All 50 cases in this study were HER2 negative. The absence of HER2-positive tumors was a consequence of registering patients in sequence according to our criteria.

Relationships between the 70-Gene Profile and Tumor Grade, Ki-67 and Other Markers

When the low- and high-risk groups according to the 70-gene profile results were compared, there were no significant differences in patient age or tumor size (table 1). In this study, we chose tumors that were clinically > 15 mm in diameter. Moreover, most patients with large tumors (e.g. > 50 mm) were given chemotherapy before surgery. These background factors could have affected the observation that there was no difference between the low- and high-risk groups in tumor size. In the high-risk group, there were significantly more ER-negative (< 0.05) and/or highly Ki-67-expressing (< 0.01) tumors. When 14% was set as the cut-off value for distinguishing between luminal A-like and B-like tumors, the same trend was observed. Tumors with low Ki-67 expression were all in the low-risk group. In contrast, all tumors in the high-risk group showed a high level of Ki-67 expression.
Comparison with the St Gallen 2007 Risk Categorization

We classified our 50 cases using the 2007 version of the St. Gallen risk categorization (SG 2007) [1] and compared the 70-gene profile results between women at high and low risk of recurrence (table 2). Of the 38 cases judged to be at intermediate risk based on the SG 2007, 27 (71%) were in the low-risk group and 11 (29%) in the high-risk group based on the 70-gene profile.

Comparison with Intrinsic Subtype Categorization Results

Next, the results were reanalyzed using the intrinsic subtype classification [9] (table 3). In this study, luminal B-like was defined as a luminal tumor that was PR negative and/or showed high Ki-67 expression (> 30%). The patients were categorized into 3 groups, luminal A-like, luminal B-like, and triple negative, as no patients had a HER2-type tumor. 1 of the 30 luminal A-like tumors (3%) was judged to be in the high-risk group based on the 70-gene profile, while 7 of 20 tumors (35%) categorized as luminal B-like or triple negative were in the low-risk group.

Discussion

Comparison of the 70-Gene Profile and the St Gallen 2007 Consensus

Among the 38 cases with intermediate risk according to the SG 2007, 27 (71%) were in the low-risk group based on the 70-gene profile results (table 2). These patients thus might not obtain a relevant benefit from adjuvant chemotherapy [15, 19]. In contrast, 3 patients who were judged to be at low risk based on the SG 2007 but at high risk by the 70-gene profile might have benefited from chemotherapy.

Table 2. Comparison of the 70-gene profile and the St. Gallen 2007 risk categorization [1]

<table>
<thead>
<tr>
<th>St. Gallen 2007 risk categorization</th>
<th>MammaPrint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk (n = 36)</td>
<td></td>
</tr>
<tr>
<td>High risk (n = 14)</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>9</td>
</tr>
<tr>
<td>Intermediate risk*</td>
<td>27</td>
</tr>
</tbody>
</table>

*Large tumor (> 20 mm), high grade, vascular involvement, ER and PR negative or young age (< 35 years).

Table 3. Comparison of the 70-gene profile and the intrinsic subtype classification

<table>
<thead>
<tr>
<th>Intrinsic subtype</th>
<th>MammaPrint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low risk (n = 36)</td>
</tr>
<tr>
<td>Luminal A-like</td>
<td>29</td>
</tr>
<tr>
<td>Luminal B-like*</td>
<td>6</td>
</tr>
<tr>
<td>Triple negative</td>
<td>1</td>
</tr>
</tbody>
</table>

*Defined as PR < 10% and/or Ki-67 > 30%.

The 70-Gene Profile and Ki-67 Expression

In our study, being categorized as high risk by the 70-gene profile correlated well with high Ki-67 expression. We found 60% of the luminal B-like tumors to be in the high-risk group, while only 3% of the luminal A-like tumors were considered to carry a high risk for recurrence (table 3). Focusing only on the cases with Ki-67 staining in > 50% of the cells in order to ensure that we were examining only luminal B-like tumors, there were 4 such cases and just 2 were categorized into the high-risk group. Taken together, these observations allow us to conclude that the 70-gene profile cannot be replaced by Ki-67 staining results.

Contribution of the 70-Gene Profile to Patient Outcomes

No patient in the 70-gene profile group has developed recurrent disease, to date, during a median follow-up period of 31 months. We also compared patients assessed by the 70-gene profile with those diagnosed and treated according to the SG 2007 criteria (SG 2007 control group), i.e. before the 70-gene profile was introduced at our institution. 197 patients with invasive breast cancer meeting the same requirements for the 70-gene profile as those undergoing surgery between April 2007 and August 2009 served as a control group. HER2-positive cases were excluded from the SG 2007 control group as there were no such cases in the 70-gene profile group. The results are shown in table 4 and fig. 1. 9 patients in the SG 2007 control group developed recurrent disease during a median follow-up period of 58 months. Significantly more patients (71%) in the high-risk than in the low-risk 70-gene profile and SG 2007 control groups received adjuvant chemotherapy. This is presumably because doctors actively recommended these treatments, after obtaining the 70-gene profile results. Table 4 shows how many of the patients to whom chemotherapy should have been recommended according to SG 2007 actually received these treatments. In the SG...
Table 4. Comparison of clinicopathological features between the 70-gene profile and the SG 2007 control patients

<table>
<thead>
<tr>
<th></th>
<th>MammaPrint</th>
<th>SG 2007 control&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>50</td>
<td>14</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>55 (± 9.5)</td>
<td>58 (± 8.7)</td>
</tr>
<tr>
<td>Mean size, mm</td>
<td>24 (± 7.5)</td>
<td>21 (± 7.4)</td>
</tr>
<tr>
<td>Structure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>41</td>
<td>12</td>
</tr>
<tr>
<td>Lobular</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Nuclear grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>46</td>
<td>12</td>
</tr>
<tr>
<td>High</td>
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<td>2</td>
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<td>Subtype</td>
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<tr>
<td>Luminal type</td>
<td>45</td>
<td>10</td>
</tr>
<tr>
<td>Triple negative</td>
<td>5</td>
<td>4</td>
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<tr>
<td>HER2 type</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adjuvant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chemotherapy regimens, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEF only</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Taxane only</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CEF + taxane</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Patients who underwent surgery between April 2007 and August 2009 and met the same requirements for the 70-gene profile. CEF = Cyclophosphamide, epirubicin, and 5-fluorouracil.

2007 control group, 38% of patients who were at intermediate risk received chemotherapy, while 61% of those in the 70-gene profile group at intermediate risk according to the SG 2007 received such agents, indicating the impact of the 70-gene profile on treatment decision. It is possible that management strategies reflecting the 70-gene profile results have contributed to the absence of recurrences in the 70-gene profile group, although a larger number of subjects and longer observation periods are clearly needed to make a definitive conclusion.

The 70-Gene Profile in Japanese Patients

Since most of the 70-gene profile evidence has been established in Europe [2–5, 14–16], there is little information pertaining to Asian patients [17, 18]. Ishitobi et al. [17] retrospectively examined 102 Japanese women using the 70-gene profile. After a median of 7.2 years of follow-up, 12 of 82 high-risk patients developed recurrent disease, while all 20 low-risk group patients remained recurrence free. Na et al. [18] argued against employing criteria from Western countries for Asian patients because of racial differences. However, we believe that there is no valid reason for not using the current gene profiling methods based on possible racial differences. The only major difference in breast cancers between Asian and Westerners has been reported to be the peak age of onset, while other epidemiologic and clinical outcome data showed significant similarities [20]. In fact, treatments for our patients are determined based on the St Gallen Consensus and National Comprehensive Cancer Network guidelines. To our knowledge, this study is the first prospective investigation of the 70-gene profile in Japan. We believe that our results will allow us to confirm the validity of using the 70-gene profile in Asian patients and we plan to follow up our study subjects for 10 years.

**Adaptation of the 70-Gene Profile to Current Management Strategies**

The 70-gene profile was developed during the period when risk categorizations such as the SG 2007 were still in widespread use. Now, its significance is tested under the current subtype classification. As to how reliably the 70-gene profile identifies patients who would not benefit from chemotherapy, 71% (27 patients) in the intermediate group based on the SG 2007 criteria were categorized as low-risk patients based on the 70-gene profile results (table 2), while 35% (7 cases) of luminal B-like or triple-negative subtype tumors were in the low-risk group (table 3). The decrease from 71% to 35% is presumably attributable to the fact that many patients who were classified as intermediate by the SG 2007 but who did not need chemotherapy actually had luminal A-like tumors.

Recently, other gene classifiers have been developed [21, 22]. PAM50 is expected to predict late recurrence in patients with ER-positive breast cancer [21]. The 70-gene profile results were previously regarded as predicting patient outcomes, i.e. as a prognostic factor, rather than responses to treatment. However, based on the results from the TRANSBIG Consortium [3], the 70-gene profile results, in combination with subtype classification, could now serve as a factor predicting treatment responsiveness. Although final conclusions cannot be drawn until further investigations have documented long-term patient outcomes, we believe the significance of the 70-gene profile to actually be increasing. The results of ongoing trials (e.g. MINDACT) [23, 24] are eagerly awaited and we will continue to follow up the patients in our current study.

As to the relatively high rate of sampling failure (17%, 10 out of 60 cases), this is mainly because samples must be taken during/just after surgery in a short time, with a narrow vision at that time. This problem has been overcome owing to technological development as, at present, samples can be retrospectively carefully taken from formalin-fixed, paraffin-embedded surgical specimens.

Our results indicate the applicability of the 70-gene profile to Japanese patients and its potential value in current clinical practice for devising individualized treatments, although further follow-up is required and additional confirmatory studies using a larger sample size are needed to evaluate the significance of our results.

**Acknowledgments**

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Disclosure Statement

L.S.-S. and F.d.S. are employed by Agendia N.V., the manufacturer of MammaPrint. All other authors have no conflict of interest associated with this study.

References


