Oesophagogastric cancer: exploring the way to an individual approach

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Citation for published version (APA):

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PROGNOSTICATION AND PREDICTION
USING GENE EXPRESSION PROFILING IN OESOPHAGEAL CANCER
ABSTRACT

Aim
To evaluate current literature on gene expression profiling in oesophageal cancer.

Methods
We performed a review of the literature (2000-2010) on prognostication and prediction using gene expression analysis in oesophageal cancer.

Results
Seventeen papers comprising 638 patients were included. Gene expression profiles studied in relation to survival, lymph node metastasis and response to neoadjuvant therapy. Most studies included a limited number of patients. Several prognostic and predictive gene signatures were identified with different accuracies. In only one study, the gene signature was validated in a large, independent patient cohort.

Conclusion
Gene expression profiling has potential clinical applications in oesophageal cancer. Especially a signature which is predictive for response to neoadjuvant treatment could be of great clinical value. To date, most published studies suffer from an underpowered training cohort or lack adequate validation. Clinicians should put effort in the collection of high quality tissue samples and should participate in biobank initiatives, considering the increasing availability and possibilities of sequencing technology.
INTRODUCTION

The incidence of oesophageal cancer is rising. Surgery is as yet the most important potentially curative treatment modality, but the role of other therapeutic options is being evaluated increasingly. The value of neoadjuvant treatment has been investigated in several randomized trials. In three meta-analyses, a survival benefit of neoadjuvant chemoradiotherapy (CRT) plus surgery compared to surgery alone has been shown. The outcome of patients treated with neoadjuvant therapy is significantly related to the degree of pathological response. For patients with neoadjuvant CRT, five-year survival rates for patients with pathological complete response (pCR) vary between 34 and 62 percent, compared to 16 and 59 percent for all patients. On average, pCR after neoadjuvant CRT is seen in about 25 percent of patients. In patients who respond poorly, the risks associated with neoadjuvant therapy are not compensated for by its benefits. Therefore, identifying patients with a poor prognosis or predicting which patients will respond to neoadjuvant therapy could be of great value in optimizing individualized treatment of oesophageal cancer.

In other types of cancer, gene expression data have been shown to be prognostic, predictive, or both. For example, in breast cancer, a 70-gene signature has been found to predict patients’ survival. This so-called ‘MammaPrint’ is currently under evaluation to determine whether it can be used to select patients for adjuvant chemotherapy after surgical treatment of breast cancer. The ‘ColoPrint’ is a recently published 42-gene signature predictive for disease relapse in early stage colon cancer. And, in head and neck squamous cell carcinoma, a 102-gene signature that is predictive for lymph node metastasis has been described.

In this review of the literature, we have summarized published results on gene expression profiling in oesophageal cancer patients with a focus on its utility in predicting clinical outcome and response to neoadjuvant therapy.

METHODS

Search strategy

We performed a search of the Pubmed and Embase databases, using the medical subject headings (MeSH) terms ‘Oesophageal Cancer AND (Gene Expression Profiling OR Microarray Analysis) AND ((Survival OR Prognosis) OR (Radiosensitivity OR Chemosensitivity) OR (Neoplasm Metastasis OR Lymphatic Metastasis))’. In both Pubmed and Embase, the search was carried out using the limits ‘human’, ‘English language’ and ‘publication date 2000 – 2010’. A professional librarian from the Netherlands Cancer Institute has supervised this search. Predefined inclusion and exclusion criteria were used to select articles (supplemental Table 1). A cross-reference search was performed manually in the bibliographies of all eligible papers.
RESULTS

The result of our search strategy is summarized in Figure 1.

Included papers
In the 17 eligible papers, comprising 638 patients, the number of study patients used for gene expression analysis varied from 10 to 89 (Table 1). Gene expression profiles were studied in relation to survival in five papers, and lymph node metastasis in eight papers and response to neoadjuvant therapy in five papers. There were no papers in which gene expression profiles were evaluated in relation to the occurrence of haematogenous metastasis.

Gene expression and survival
Table 2 shows a summary of studies in which gene expression profiles were studied in association with survival in oesophageal cancer patients. A gene signature with a significant association with (relapse-free) survival was constructed in all but one study. In only two studies, the gene signature was tested in an independent patient series. For this, Peters et al. examined their 4-gene signature on a protein level using immunohistochemistry in a cohort of 371 patients. In this validation series, the signature could significantly discriminate three groups of patients with a 5-year survival rate of 58%, 26% and 14% encompassing 6%, 77% and 17% of patients in this validation cohort.
Gene expression and lymph node metastasis

In eight articles, an association between gene expression profiles and lymph node metastasis was investigated (Table 3). There were four studies in which a gene signature predictive of lymph node metastasis was found.\textsuperscript{12, 13, 17, 20} Different sets of genes were identified. In two of these four studies, the gene signature was tested in a validation cohort. An independent patient series was used in both studies.\textsuperscript{12, 13} In the study by Yamabuki et al., 34 genes were found to be associated with lymph node metastasis, of which a set of 20 genes was the most accurate in discriminating between lymph node negative and lymph node positive patients.\textsuperscript{17} Lagarde et al. found a signature with an accuracy of 64\%, which did not outperform current clinical practice.\textsuperscript{20} Therefore, the authors did not test this signature on an independent patient cohort.

Table 1. Selected papers on gene expression profiling in oesophageal cancer

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year of publication</th>
<th>Number of patients used for microarray analysis</th>
<th>Histological tumour type</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ishibashi et al.\textsuperscript{11}</td>
<td>2003</td>
<td>12</td>
<td>SCC</td>
<td>Survival</td>
</tr>
<tr>
<td>Tamoto et al.\textsuperscript{12}</td>
<td>2004</td>
<td>36</td>
<td>SCC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Kan et al.\textsuperscript{13}</td>
<td>2004</td>
<td>15</td>
<td>SCC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Luthra et al.\textsuperscript{14}</td>
<td>2006</td>
<td>19</td>
<td>SCC/AC</td>
<td>Response to neoadjuvant CRT</td>
</tr>
<tr>
<td>Sato et al.\textsuperscript{15}</td>
<td>2006</td>
<td>54</td>
<td>SCC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Ashida et al.\textsuperscript{16}</td>
<td>2006</td>
<td>33</td>
<td>SCC</td>
<td>Survival</td>
</tr>
<tr>
<td>Yamabuki et al.\textsuperscript{17}</td>
<td>2006</td>
<td>19</td>
<td>SCC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Uchikado et al.\textsuperscript{18}</td>
<td>2006</td>
<td>16</td>
<td>SCC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Duong et al.\textsuperscript{19}</td>
<td>2007</td>
<td>46</td>
<td>SCC/AC</td>
<td>Response to neoadjuvant CRT</td>
</tr>
<tr>
<td>Lagarde et al.\textsuperscript{20}</td>
<td>2008</td>
<td>77</td>
<td>AC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Hammoud et al.\textsuperscript{21}</td>
<td>2009</td>
<td>89</td>
<td>AC</td>
<td>Lymph node metastasis and survival</td>
</tr>
<tr>
<td>Maher et al.\textsuperscript{22}</td>
<td>2009</td>
<td>13</td>
<td>SCC/AC</td>
<td>Response to neoadjuvant CRT</td>
</tr>
<tr>
<td>Schauer et al.\textsuperscript{23}</td>
<td>2010</td>
<td>10</td>
<td>AC</td>
<td>Response to neoadjuvant CT</td>
</tr>
<tr>
<td>Sano et al.\textsuperscript{24}</td>
<td>2010</td>
<td>35</td>
<td>SCC</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>Motoori et al.\textsuperscript{25}</td>
<td>2010</td>
<td>25</td>
<td>SCC</td>
<td>Response to neoadjuvant CT</td>
</tr>
<tr>
<td>Kim et al.\textsuperscript{26}</td>
<td>2010</td>
<td>64</td>
<td>AC</td>
<td>Survival</td>
</tr>
<tr>
<td>Peters et al.\textsuperscript{27}</td>
<td>2010</td>
<td>75</td>
<td>AC</td>
<td>Survival</td>
</tr>
</tbody>
</table>

SCC, squamous cell carcinoma; AC, adenocarcinoma; CRT, chemoradiotherapy; CT, chemotherapy

In the remaining four articles reporting on gene expression profiles in relation to lymph node metastasis, various genes were identified to be significantly related to the presence of lymph node metastasis, but a predictive gene signature was not constructed.\textsuperscript{15, 18, 21, 24}

Gene expression and response to neoadjuvant therapy

Neoadjuvant treatment regimes in the five selected articles consisted of chemoradiotherapy or chemotherapy alone (Table 4). Response to neoadjuvant treatment was evaluated by the histological examination of the surgical resection specimen,\textsuperscript{14, 22, 23} by computed tomography\textsuperscript{25} or by a combination of Positron Emission Tomography (PET), computed tomography and endoscopy.\textsuperscript{19} In all five studies either a single gene or a set of genes was found with differential expression levels regarding the response to neoadjuvant treatment. Two studies included both histological subtypes.\textsuperscript{19, 22} In the study by Duong...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients (training cohort)</th>
<th>Treatment</th>
<th>Study material (method of conservation)</th>
<th>Microarray</th>
<th>Statistical Analysis (signature performance assessment on training set)</th>
<th>Signature identified (number of genes)</th>
<th>Validation cohort (number of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ishibashi et al.</td>
<td>12</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>Affymetrix 12,6K microarray</td>
<td>Unsupervised clustering</td>
<td>Yes (NR)</td>
<td>No</td>
</tr>
<tr>
<td>Ashida et al.</td>
<td>33</td>
<td>Definitive CRT</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>Affymetrix 12,6K microarray</td>
<td>Unsupervised clustering</td>
<td>Yes (177 genes)</td>
<td>No</td>
</tr>
<tr>
<td>Hammoud et al.</td>
<td>89</td>
<td>Surgery</td>
<td>Resection specimens (FFPE)</td>
<td>DASL 502 cancer genes</td>
<td>Unsupervised clustering</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Kim et al.</td>
<td>64</td>
<td>CRT and surgery</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>48K Oligonucleotide microarray</td>
<td>Supervised analysis (not performed)</td>
<td>Yes (2 genes)</td>
<td>Yes (52)</td>
</tr>
<tr>
<td>Peters et al.</td>
<td>75</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>44K Oligonucleotide microarray</td>
<td>Supervised analysis (not performed)</td>
<td>Yes (4 genes)</td>
<td>Yes (371)</td>
</tr>
</tbody>
</table>

NR, not reported; CRT, Chemoradiotherapy; FFPE, Formalin Fixed Paraffin Embedded; DASL, cDNA-mediated Annealing, Selection, extension and Ligation
et al., only in patients with squamous cell cancer a 32-gene signature was predictive for response.\textsuperscript{19} This predictive model had a sensitivity of 100%, i.e. no patient with a benefit from CRT would be missed. A five-gene signature found by Maher et al. could only make a prediction in 20 out of 27 patients (74%) in the validation set, because of large variances in the gene expression profiles.\textsuperscript{22}

**DISCUSSION**

In this review of the literature on gene expression profiling in oesophageal cancer patients, 17 papers were included in which gene expression data were studied in relation to survival,\textsuperscript{11, 16, 21, 26, 27} lymph node metastasis,\textsuperscript{12, 13, 15, 17, 18, 20, 21, 24} or response to neoadjuvant therapy.\textsuperscript{14, 19, 22, 23, 25} Until 2007, selected studies almost invariably involved patients with squamous cell cancer (Table 1). Thereafter, a shift is seen towards patients with adenocarcinoma. This observation is compatible with the rising incidence of oesophageal adenocarcinoma in Western industrialised countries.\textsuperscript{28} The value of a gene signature largely depends on the clinical endpoints for which it is prognostic or predictive. In current clinical practice, the TNM system is used to predict patients’ survival. In case of resectable disease and in the absence of distant metastasis, a potentially curative treatment can be installed. If the patient is fit enough, this treatment will consist of neoadjuvant treatment followed by surgery.\textsuperscript{4-6} Despite elaborate diagnostic work-up, it occurs that patients present with metastatic disease just after finishing local aggressive therapy. A gene signature that predicts such a dismal prognosis could prevent that patients undergo multimodality treatment with no benefit. In the study by Peters et al. a prognostic signature was found and validated in a large patient cohort (N = 371).\textsuperscript{27} This signature awaits prospective validation and it would be interesting to see if it maintains its prognostic power in a patient cohort treated with neoadjuvant CRT.

The clinical application of a gene signature related to lymph node metastasis in oesophageal cancer is questionable. It should have an extremely high negative predictive value to justify a limited lymph node dissection based on a gene expression profile only. Also, the prognostic value of lymph node metastasis has become less important due to the prognostic value of response to neoadjuvant therapy.\textsuperscript{29-31} Of potentially great clinical value is a gene signature that predicts response to (neoadjuvant) CRT or CT in oesophageal cancer. Current diagnostic modalities fail to accurately predict response to neoadjuvant treatment.\textsuperscript{32, 33} Other results on response prediction with early PET await validation.\textsuperscript{34} Results with gene signatures predictive for response to neoadjuvant therapy are promising, although available studies have their limitations.\textsuperscript{14, 19, 22, 23, 25} The reported gene signature was not validated\textsuperscript{14, 19} or it was only found to be predictive in a subgroup of patients despite a very high sensitivity (Table 4).\textsuperscript{19, 22} Response measurement by computed tomography, as was done in the study by Motoori et al.,\textsuperscript{25} is suboptimal. In three studies\textsuperscript{14, 22, 23} response was graded on surgical specimens, which is the preferred method.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients (training cohort)</th>
<th>Treatment</th>
<th>Study material (method of conservation)</th>
<th>Microarray</th>
<th>Statistical Analysis (signature performance assessment on training set)</th>
<th>Signature identified (number of genes)</th>
<th>Validation cohort (Number of patients)</th>
<th>Sens</th>
<th>Spec</th>
<th>Acc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoto et al.12</td>
<td>36</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>13K cDNA microarray</td>
<td>Supervised analysis (Leave-one-out cross validation)</td>
<td>Yes (44 genes)</td>
<td>Yes (18)</td>
<td>89%</td>
<td>89%</td>
<td>89%</td>
</tr>
<tr>
<td>Kan et al.13</td>
<td>15</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>8,1K cDNA microarray</td>
<td>Supervised analysis (not performed)</td>
<td>Yes (60 genes)</td>
<td>Yes (13)</td>
<td>NR</td>
<td>NR</td>
<td>77%</td>
</tr>
<tr>
<td>Sato et al.15</td>
<td>54</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>Affymetrix 22K microarray</td>
<td>Supervised analysis</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Yamabuki et al.17</td>
<td>19</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>32K cDNA microarray</td>
<td>Supervised analysis (Leave-one-out cross validation)</td>
<td>Yes (20 genes)</td>
<td>No</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Uchikado et al.16</td>
<td>16</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>17K oligonucleotide microarray</td>
<td>Supervised analysis</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lagarde et al.20</td>
<td>77</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>41K oligonucleotide microarray</td>
<td>Supervised analysis (Repeated random sampling)</td>
<td>Yes (NR)</td>
<td>No</td>
<td>75%</td>
<td>41%</td>
<td>64%</td>
</tr>
<tr>
<td>Hammoud et al.21</td>
<td>89</td>
<td>Surgery</td>
<td>Resection specimens (FFPE)</td>
<td>DASL 502 cancer genes</td>
<td>Unsupervised clustering</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sano et al.24</td>
<td>35</td>
<td>Surgery</td>
<td>Resection specimens (fresh frozen)</td>
<td>Affymetrix 12.6K microarray</td>
<td>Unsupervised clustering</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Sens, sensitivity; Spec, specificity; Acc, accuracy; cDNA, complementary-DNA; NR, not reported; FFPE, Formalin Fixed Paraffin Embedded; DASL, cDNA-mediated Annealing, Selection, extension and Ligation;
The inclusion of two different histological subtypes has lead to various observations and conclusions. The signature found by Duong et al.\textsuperscript{19} was predictive in squamous cell cancer patients only, and not in adenocarcinoma patients. The authors contributed this finding to genetic differences between the two subgroups.\textsuperscript{13, 35} In contrast, the signature found by Maher et al.\textsuperscript{22} was predictive in both histological subtypes and the authors hypothesized that the genes responsible for therapy resistance are likely to be part of the tumours’ general make-up. However, in 26% of cases their model was unable to make a prediction due to large variances in the gene expression profiles, which could be the result of using both histological subtypes to train their classifier. The above findings suggest that it is best to analyze different histological subgroups separately.

An interesting observation is that the identified gene signatures from different studies only have a few genes in common. This was also seen in breast cancer studies: a 70-gene signature\textsuperscript{8} and a 76-gene signature\textsuperscript{36} had only three similar genes. However, both signatures were validated in a large independent patient cohort. This has led to the hypothesis that different gene-signatures with predictive or prognostic value for the same endpoints use different genes to monitor the same biological processes.\textsuperscript{37} This hypothesis is supported by a study in which five different gene-signatures were tested in the same dataset, with four signatures resulting in similar predictions.\textsuperscript{38}

In gene expression studies, due to large inter-individual variability in a (genetically heterogeneous) patient group, an adequate sample size is a necessity.\textsuperscript{39, 40} In previous studies on prognostic and predictive signatures in head and neck, breast and colon cancer patients,\textsuperscript{8-10, 36} sample sizes ranging from 82 to 188 were used for the development of gene signatures and even more cases were used for the subsequent validation step. Since oesophageal cancer is a relatively uncommon disease, it is difficult to obtain samples from a large patient cohort. In only three studies in the present review, 75 or more patients were included.\textsuperscript{20, 21, 27} Furthermore, adequate sample handling consists of snap freezing each tumour specimen at -80 °C.\textsuperscript{41, 42} In all studies -except one\textsuperscript{21}- fresh frozen tumour samples were used, either from endoscopic biopsies or from surgical resection specimens.\textsuperscript{11-20, 22-27} The importance of sample quality is increasingly being acknowledged.\textsuperscript{43, 44} For instance, in one study significant RNA degradation already occurred after 30 minutes of ischemia.\textsuperscript{45} In daily practice, snap freezing is a logistic challenge: it requires a meticulous work-flow from endoscopic department or operating theatre to the pathology lab. One way to ease this process is by using RNA storage solutions such as RNALater. However, reports are conflicting on RNA integrity compared to snap freezing.\textsuperscript{45-47} Several initiatives are focussing on the availability of high quality tissue samples in nationwide collaborations of ‘biobanks’ using stringent criteria, with the appropriate standards for future research (http://biospecimens.cancer.gov/about/default.asp, http://www.bbmri.nl/).

Another strategy to overcome the sample size issue, was employed by Hammoud et al.\textsuperscript{21} They used formalin-fixed, paraffin-embedded (FFPE) samples on a DASL-assay, which is a microarray platform specifically designed for use with FFPE tissue. In this study,
Table 4. Summary of studies evaluating gene expression profiles as a predictor of response to neoadjuvant therapy in oesophageal cancer patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients (training cohort)</th>
<th>Neoadjuvant therapy</th>
<th>Study material (method of conservation)</th>
<th>Microarray</th>
<th>Statistical Analysis (signature performance assessment on training set)</th>
<th>Signature identified (number of genes)</th>
<th>Validation cohort (Number of patients)</th>
<th>Sens</th>
<th>Spec</th>
<th>Acc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luthra et al.14</td>
<td>19</td>
<td>CRT (Docetaxel/ Irinotecan/5-FU/50.4 Gray)</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>Affymetrix 22K microarray</td>
<td>Unsupervised clustering</td>
<td>Yes (3 genes)</td>
<td>No</td>
<td>89%</td>
<td>86%</td>
<td>NR</td>
</tr>
<tr>
<td>Duong et al.10</td>
<td>46</td>
<td>CRT (Cisplatin/5-FU 35 - 50 Gray)</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>10,5K cDNA microarray</td>
<td>Supervised analysis (Leave-one-out cross validation)</td>
<td>Yes, in SCC (32 genes)</td>
<td>No</td>
<td>100%</td>
<td>67%</td>
<td>76%</td>
</tr>
<tr>
<td>Maher et al.22</td>
<td>13</td>
<td>CRT (Cisplatin/5-FU 40.5 - 44 Gray)</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>32K Oligonucleotide microarray</td>
<td>Supervised analysis (Leave-one-out cross validation)</td>
<td>Yes (5 genes)</td>
<td>Yes (27)</td>
<td>100%</td>
<td>91%</td>
<td>95%</td>
</tr>
<tr>
<td>Schauer et al.23</td>
<td>10</td>
<td>CT (Cisplatin/5-FU/ Leucovorin)</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>Affymetrix 55K Microarray</td>
<td>Supervised analysis (not performed)</td>
<td>Yes (1 gene)</td>
<td>Yes (47)</td>
<td>89%</td>
<td>84%</td>
<td>NR</td>
</tr>
<tr>
<td>Motoori et al.25</td>
<td>25</td>
<td>CT (Cisplatin/Doxorubicin/5-FU)</td>
<td>Endoscopic biopsies (fresh frozen)</td>
<td>30K Oligonucleotide microarray</td>
<td>Supervised analysis (Leave-one-out cross validation)</td>
<td>Yes (199 genes)</td>
<td>Yes (10)</td>
<td>79%</td>
<td>83%</td>
<td>82%</td>
</tr>
</tbody>
</table>

Sens, sensitivity; Spec, specificity; CRT, chemoradiotherapy; 5-FU, 5-fluorouracil; SCC, squamous cell carcinoma; CT, chemotherapy
underexpression of one gene correlated with lymph node metastasis, and underexpression
of nine other genes correlated with prolonged survival. No gene signature with predictive
or prognostic value was reported. A limitation to their study design could be the
‘knowledge-driven’ approach. Since only 502 known cancer genes were present on the
DASL-assay, prior assumptions were made regarding which genes might be associated
with the endpoints. Alternatively, when a ‘data-driven’ approach is chosen for, a genome-
wide analysis of gene expression is carried out. Then the ratio of the number of samples
(patients) to the number of features (genes) decreases as compared to the ‘knowledge-
driven’ approach. This increases the chance of over-training, i.e. resulting in a classifier
with high predictive power on the training set, but failing when tested on an independent
validation cohort. Consequently, large sample sizes are needed. In a recent report, 20
FFPE and 20 fresh frozen matched breast cancer samples were analysed with a whole
genome DASL-assay and the MammaPrint, respectively. The results of the two analyses
were highly concordant. Validation of these results would considerably increase available
tissue resources and make the DASL-assay a promising alternative.

An even more exciting development is the increasing availability of next-generation
sequencing, including RNA-sequencing. Several advantages include less background
noise, a higher accuracy, an increased sensitivity for genes expressed at very low or high
levels, and this technique requires less RNA sample. Furthermore, RNA-sequencing
can give additional information about alternative splicing, gene fusions and small RNA
profiling (e.g. microRNA).

The final validation on an independent cohort is vital before a gene signature can be used
in clinical practice. The study by Peters et al. is the only one included in this review in
which the gene signature was validated, albeit retrospectively, on a large independent
patient cohort. Ideally, validation of a gene signature is carried out prospectively on
a large independent patient cohort at different centres, like the ‘MammaPrint’ in the
MINDACT trial.

**CONCLUDING REMARKS**

Due to last decades’ developments, “personalized medicine” including gene expression
profiling is increasingly likely to be introduced in the near future. Microarray analysis was
the first affordable technique that enabled genome-wide RNA analysis thereby providing
invaluable insights in tumour behaviour. In various types of cancer, clinical applications
are emerging. In oesophageal cancer, only one prognostic signature has been validated
in a large independent patient cohort.

Recent advances in sequencing technologies, e.g. RNA-sequencing, will revolutionize
transcriptome studies. To fuel this revolution, clinicians will have to participate in
‘biobank’ initiatives and invariably incorporate translational research in clinical trials.
Only by an adequate number of high quality samples for future studies, the development
of clinically useful prognostic and predictive signatures can be realized.
REFERENCES