Optimizing treatment of low risk breast cancer patients

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Chapter 2

Predictive factors for local recurrence in breast cancer

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Abstract

Risk factors for local recurrence in breast cancer after breast conserving therapy (BCT) differ from those for local recurrence after mastectomy. To better guide optimal treatment of individual patients, it is desirable to identify patients at high risk for local recurrence. Several clinical and histopathological factors, such as young age and presence of ductal carcinoma in situ, are known to be predictors for local recurrence after BCT. After mastectomy, lymph node status and tumor size are dominant risk factors for local recurrence. The results of recent expression profiling studies have explained differences in prognosis and risk for local recurrence and also explained response to different therapies (adjuvant systemic therapy and radiotherapy). Because of the variation in different subtypes of breast cancer and the difference in amount of tumor burden remaining after surgery, finding robust predictive profiles is complex. In this review, we describe the predictive and prognostic factors for local recurrence after mastectomy and BCT and also describe the role of radiosensitivity in local recurrence.
Introduction

Radiotherapy (RT) after mastectomy and breast conserving therapy (BCT) leads to a significant reduction in the local recurrence rate and breast cancer mortality, as was shown by meta-analysis of the Early Breast Cancer Trialists Collaborative Group at 15 years of follow-up. It was stated that for every 4 local recurrences avoided in the first 5 years 1 breast cancer death could be prevented. Predicting the chance of a local recurrence will allow treatment adaptation in individual patients, leading therefore to better treatment outcome. This adaptation is already possible, for example, by changing the radiation dose. After whole breast irradiation (WBI) to a dose of 45 to 50 gray (Gy), the local recurrence rate is reduced by 60-70%. When an additional boost of 16 Gy is added to the treatment, local recurrence is further reduced with a relative risk reduction of about 50%2. These relative reduction percentages are independent of patient, tumor and treatment factors. Adding (neo)adjuvant hormonal therapy and/or chemotherapy to the treatment leads to a 50% relative risk reduction of local recurrence1.

It is desirable to identify patients at high risk for local recurrence to better guide optimal treatment of individual patients. Conversely, identification of low-risk patients can help to reduce or omit a radiation dose where appropriate. Several clinical and histopathological factors are known to be predictors for local recurrence. Especially, young age has been associated with a high risk of local recurrence after BCT; at present, it is poorly understood what the biological mechanism for this association is (mentioned later in the text). More recently, gene expression profiling studies using DNA microarrays have identified prognostic gene expression profiles to predict outcome in breast cancer patients3-6. Molecular tests based on assessment of the expression of prognostic gene sets are being developed, and some are commercially available. In breast cancer, several studies have shown the existence of gene expression profiles predictive for distant-metastasis-free survival. Some of these studies have led to clinical studies in which the gene-expression profiles are used to guide treatment decisions, especially for adjuvant systemic therapy. An example is the 70-gene expression profile for predicting early distant metastases in oestrogen receptor (ER)-positive and ER-negative lymph-node-negative patients. This profile is derived from the analysis of 78 frozen samples from lymph node-negative breast cancers smaller than 5 cm, from patients younger than 55 years treated at the Netherlands Cancer Institute. By comparing the gene expression profiles of tumors from patients who developed a distant metastasis within 5 years with those who did not, a prognostic signature comprising 70 genes was identified and validated in several studies3,7,8. The test classifies tumors into ‘good’ and ‘poor’ prognosis groups. The signature was superior to clinicopathologic assessment in predicting distant metastases and overall survival7. Chemotherapy might be safely avoided in patients identified with a good prognosis3,9. This 70-gene profile is now commercially available as the MammaPrint (Agendia, Amsterdam, the Netherlands) and has been approved by the US Food and Drug Administration. The recently closed MINDACT trial (Mircoarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy)
is a prospective trial to validate the MammaPrint for the adjuvant treatment in early breast cancer with 0 to 3 positive nodes \(^{10}\). A similar trial is the also recently closed TAILORx trial (Trial Assigning Individualized Options for Treatment) \(^{11}\), which is based on the Oncotype DX assay (Oncotype DX; Genomic Health Inc, Redwood City, CA). This reverse-transcriptase polymerase chain reaction assay measures the expression of 21 genes (16 cancer-related genes and 5 reference genes) in RNA extracted from paraffin-embedded tumor samples from primary breast cancer. The levels of expression of the 21 genes are manipulated by an empirically derived, prospectively defined mathematical algorithm to calculate a recurrence score (RS), which is then used to assign a patient to 1 of 3 groups by estimated risk of distant recurrence: low, intermediate and high \(^{4}\). Both trials are aiming at reducing the number of patients receiving adjuvant chemotherapy.

Prediction of response to treatment, including chemotherapy, hormonal therapy and radiation, is promising and has potential clinical use. Although several studies have attempted to identify a gene expression profile predictive for local recurrence, leading to more personalized treatments \(^{12-14}\), the risk assessment for local recurrence is still primarily based on traditional clinical and histopathologic factors. In this review we describe the studies predicting local recurrence in breast cancer. High risk patients are all considered to benefit from (more) RT, although even in this group, there may be considerable differences in their response to radiation. Thus, their sensitivity to radiotherapy might be a risk factor in itself. Therefore, in addition to predictors for local recurrence after breast conserving therapy and mastectomy, we also focus on the role of intrinsic radiosensitivity in determining outcome.

**Predicting local recurrences after BCT**

**Risk factors: clinical and histopathologic**

Young age, < 50 years, is a major independent risk factor for local recurrence after BCT, as found in several studies \(^2,^{15-18}\). Patients younger than 35 years are at especially high risk for local recurrence. In these patients, 10-year local recurrence rates of 30% or higher have been reported \(^{15,}\)\(^{17}\). Young age was one of the most important risk factors for local recurrence in the boost-no boost study \(^1\). The relative risk reduction of local recurrence by the boost in addition to WBI was significant in all age-groups, although the absolute risk reduction at 10 years was larger in younger patients. Local recurrence was reduced from 23.9% to 13.5% in those aged ≤ 40 years, from 12.5% to 8.7% in the 41- to 50-year age-group, from 7.8% to 4.9% in the 51-60 year age-group, and from 7.3% to 3.8% in those older than 60 years \(^2\).

Tumor recurrences are assumed to arise from microscopic tumor foci left behind after surgery. The presence of positive margins has been found to be of predictive value for local recurrence after BCT \(^{15,}\)\(^{19,}\)\(^{20}\), although the incidence of local recurrence varied among the different studies. The significance of a close tumor margin, variably defined as tumor within 1-2 millimetres of the margin, remains controversial. The definition of a negative margin has ranged from no cancer cells at the margin to no tumor cells at a distance of greater than 1-2 millimetres. Park et al \(^{21}\)
showed that there was no difference in local recurrence rate in patients with negative margins compared to those with close margins (≤ 1 mm); for both, local recurrence rates were 7% at 8 years after BCT. The highest rate of local recurrence (27%) was observed among patients with extensively involved positive margins. An intermediate rate of local recurrence (14%) was observed among patients with focally (≤ 3 low-power microscopic fields) positive margins. In a subgroup analysis of the previously mentioned boost-no boost trial, Jones et al reviewed the role of margins for invasive breast carcinoma and ductal carcinoma in situ (DCIS): close (≤ 2 mm) or positive margins (defined as invasive carcinoma/DCIS at an inked resection edge) 22. In this study, margin status of both the invasive breast carcinoma and the DCIS component had no significant influence on local relapse. In a recent analysis of the boost-no boost trial by Werkhoven et al 23, a nomogram to predict local recurrence after BCT is presented (http://research.nki.nl/ibr/). The most important factors in this nomogram influencing the local recurrence rate after BCT are young age, the presence of any DCIS, and a radiation boost. Systemic treatment, tumor size, and high-grade malignancy are other factors contributing to the nomogram 23.

Invasive breast carcinoma is accompanied by an extensive component of DCIS (EDCIS) in 15%-30% of patients. DCIS grows along the ducts in the breast without invasion of the underlying tissue, which results in a nonpalpable lesion and is difficult to remove with tumor-free margins. EDCIS is found to be a risk factor for local recurrence in several older studies 15, 20, but when EDCIS is completely removed with negative tumor margins, it loses its predictive value for local recurrence 20, 24.

**Immunohistochemical markers**

Three markers, ER, progesterone-receptor (PR), and human epidermal growth factor (HER2) status, are important prognostic factors as well as predictive factors for response to hormonal treatment and to HER2-targeted therapy in both BCT and mastectomy 25-27.

Ki-67 is a nuclear marker of cell proliferation. Several studies showed that a high Ki-67 proliferation index is associated with worse distant disease-free survival and/or overall survival in node-positive and node-negative patients 28-30. In a meta-analysis, de Azambuja et al 31 showed that high Ki-67 levels confer a worse prognosis. In contrast, it is also a predictor of better response to chemotherapy 29. The cut-off of Ki-67 can be predefined at the seventh decile, corresponding to a high Ki-67 proliferation index of > 20% positively stained tumor cells 28. Elkhuizen et al 32 found high Ki-67 as a risk factor for local recurrence after BCT. Several other markers such as p53, bcl-2 and cyclin D1, have been studied as predictive factors for local recurrence and survival in breast cancer, although results have been inconsistent.

**Molecular subtypes**

There are five molecular subtypes of breast cancer identified by gene expression studies, which have been shown to be of prognostic value in breast cancer. The classification in these subtypes
shows the heterogeneity of breast cancer and may help to select systemic treatment and to evaluate the risk for a recurrence. The subtypes are ER-positive luminal A (luminal A), ER-positive luminal B (luminal B), HER2 enriched, basal like, and normal breast like. HER2-enriched and basal-like subtypes are hormone receptor negative and have poor prognosis. HER2-enriched subtypes have high expression of the HER2 gene and other genes of the HER2 amplicon. Basal-like subtypes tend to have high expression of proliferation genes. The luminal breast cancers are characterized by the expression of ER-associated genes, with luminal B tumors having poorer outcomes than luminal A tumors. The major biological distinction between luminal A and B is the proliferation signature. This includes genes such as CCNB1, MKI67 (encoding Ki67), and MYBL2, which are expressed higher in luminal B tumors than in luminal A tumors. In a recent study by Dawood et al, the categories luminal A, luminal B, HER2-enriched, basal-like, and unclassified were used to focus on survival outcomes. In a multivariable model, luminal B, HER2-type, basal-like, and unclassified tumors had a higher hazard of breast cancer death compared with luminal A tumors. Similar trends were observed for both overall and recurrence-free survival.

Several studies have investigated the association of the molecular subtypes with rates of local recurrence. In a study by Nguyen et al, receptor status of breast tumors of 793 patients was used to approximate the molecular subtypes; ER+/PR+/HER2- represented luminal A, ER+/PR+/HER2+ represented luminal B, ER-/PR-/HER2 + represented HER2 subtype and ER-/PR-/HER2- represented the basal subtype. After a median follow-up of 70 months the 5-year local recurrence rate was 1.8%. Local recurrence varied according to subtype, ranging from 0.8% (95% confidence interval [CI], 0.3%-2.2%) for luminal A and 1.5% (95% CI, 0.2%-10%) for luminal B, to 8.4% (95% CI, 2.2%-30%) for HER2 and 7.1% (95% CI, 3.0%-16%) for basal subtypes. In a study of Voduc et al, 2985 patients were classified into luminal A, luminal B, luminal-HER2, HER2-enriched, basal-like, or nonbasal triple-negative phenotype categories, determined by ER, PR, Ki-67, HER2, epidermal growth factor receptor and cytokeratin 5/6. With a median follow-up of 12 years, HER2-enriched and basal subtypes showed a significantly higher risk of regional relapse after BCT. In these studies no patient received adjuvant trastuzumab, although with appropriate anti-HER2 treatment local control may be better in the HER2 subtype group.

Gene expression profiling

Microarray-based gene expression profiling techniques make it possible to study the association of the expression of thousands of genes with local recurrence after breast cancer treatment. Kreike et al studied the relationship of gene expression profiles of primary invasive breast carcinomas and a local recurrence after BCT in a series of 165 patients. Fifty-six patients developed a local recurrence after a median interval of 3 years, and 109 remained free of local recurrence for at least 10 years. They showed a difference in gene expression profile between the primary tumors that recurred after BCT compared with those that did not. Seventy-seven percent of all local recurrences were correctly predicted using a local recurrence classifier. Genes
associated with cell proliferation were expressed at higher levels in tumors that recur after BCT. However, in multivariate analysis in this study, age was again the only independent predictor of local recurrence. In addition, a significant relationship between molecular breast carcinoma subclasses and local recurrence was found. The tumors with a gene expression profile resembling normal breast-like subtype and the majority of luminal A-like tumors remained free from local recurrence, in contrast to luminal B-like and HER2-like tumors which were associated with a high local recurrence risk. These data are from the pre-trastuzumab era, and may not completely apply to current patient cohorts.

Nuyten et al. evaluated microarray-based gene expression profiles previously shown to predict metastasis-free and overall survival as predictors of local recurrence in patients treated with breast conserving surgery and RT. They used the wound-response gene expression signature, the 70-gene prognosis profile, and the hypoxia profile. Of these 3 signatures, only the wound-response signature showed a significant association with local recurrence.

The RS assay mentioned earlier in the text quantifies risk of distant recurrence in patients with node-negative, ER-positive, tamoxifen-treated breast cancer and has been validated in independent data sets. In a recent study of Mamounas et al., the association between RS and risk for locoregional recurrence (LRR) in patients with node-negative, ER-positive breast cancer was investigated in 2 National Surgical Adjuvant Breast and Bowel Project (NSABP) trials (NSABP B-14 and B-20). They showed that in tamoxifen-treated patients, LRR was significantly associated with RS risk groups. In a multivariate analysis, RS was an independent significant predictor of LRR along with age and type of initial treatment (mastectomy or breast conserving surgery plus RT).

A limitation of the aforementioned studies is the fact that they concern retrospective analyses. The results, therefore, need to be validated in other datasets and preferably confirmed in prospective randomized trials, such as the Young Boost Trial (http://clinicaltrials.gov/show/NCT00212121). In this ongoing trial, patients under age of 51 years are randomized between a standard RT dose after BCT (50 Gy WBI and 16 Gy boost) and an additional boost dose (50 Gy WBI and 26 Gy boost). Fresh frozen tumor samples are being collected, which will allow prospective validation of previously identified gene expression profiles.

Predicting local recurrences after mastectomy

Risk factors: clinical and histopathological factors

After mastectomy, several risk factors for local recurrence have been identified. As described earlier in the text, the number of tumor-positive axillary lymph nodes and tumor size are well-established risk factors for local recurrence. In an analysis of patients in the Danish Breast Cancer Cooperative Group randomized trials 82b and c, large primary tumor size, histologic ductal carcinoma, high malignancy grade, fascia invasion, low number of removed nodes, many positive nodes and extracapsular invasion were all risk factors for developing LRR in univariate analyses. In a study by Jagdi et al., a retrospective analysis of a cohort of 877 cases of node-negative
breast cancer treated with mastectomy, without adjuvant radiation, was performed. They showed that tumor size > 2 cm, margin < 2 mm, premenopausal status and lymphovascular invasion were independent prognostic factors for LRR. Ten-year LRR was 1.2% for those with no risk factors, 10% for those with 1 risk factor, 17.9% for those with 2 risk factors, and 40.6% for those with 3 risk factors. The chest wall was the site of failure in 80% of patients. Young age, a major predictive risk factor for local recurrence after BCT, was also found to be of importance for local recurrence after mastectomy, although not in all studies.

**Immunohistochemical markers and molecular subtypes**

ER, PR and HER2 status are important predictive factors for response to hormonal treatment and to HER2-targeted therapy, as described earlier in the text. In an analysis of the Danish 82b&c trials, a higher risk of local recurrence was found in postmastectomy patients who were ER/PR/HER2 negative, ER/PR negative, and HER2 positive, compared with other receptor status combinations. The overall survival benefit after postmastectomy RT in this analysis was most evident in more favorable luminal subtypes. In the study by Voduc et al, luminal B, luminal-HER2, HER2-enriched and basal subtypes were all associated with an increased risk of local and regional recurrence after mastectomy. The analyses in these studies are of patients treated in the 1980s, with inadequate systemic therapy according to current standards. Whether the factors such as HER2 positivity, are still predictive for local control with current systemic therapy is one of the questions to be answered by the Selective Use of Postoperative Radiotherapy after Mastectomy (SUPREMO) trial from the Medical Research Council/European Organisation for Research and Treatment of Cancer (MRC/EORTC).

**Gene expression profiling**

A predictive profile for local recurrence in mastectomy patients who did not receive adjuvant RT treatment has been studied by Cheng et al. In this study, 94 patients were analyzed; 27 had a LRR and 67 patients were free of local recurrence after a follow-up of at least 3 years. They identified 2 sets of gene expression profiles that were independent predictors of LRR, one consisting of 258 genes and another of 34 genes. Using the 258-gene model, the local recurrence control probability at 3 years for patients with a predictive index > 0.8 was 95% and a predictive index ≤ 0.8 was 46%. Using the 34-gene model, the difference of 3 year local recurrence control probability in patients with predictive index > 0.8 was 91% and a predictive index ≤ 0.8 was 40%. The pathways represented in these signatures are cell death, cell cycle and proliferation, DNA replication and repair, and immune response.

**Predicting local recurrence in BCT versus mastectomy**

DCIS is a risk factor for local recurrence after BCT, but not after mastectomy. Young age is an unfavourable predictive factor for local recurrence in BCT; however, in mastectomy, this differs...
between studies. The number of positive lymph nodes is a clearer risk factor in mastectomy. Lobular carcinoma is identified as a risk factor for local recurrence after mastectomy and not after BCT.  

The discovery of different breast cancer subtypes, classified by immuno-histochemistry and microarrays, has shown that each type has a different prognosis. After validation in prospective trials, new predictive assays are likely to be more personalized indicate an appropriate treatment.

RT sensitivity

After breast conserving surgery nearly all patients are irradiated, and after mastectomy there is an indication for RT in selected intermediate- and high-risk patients. RT is known to reduce the local recurrence in 60-70% of all patients, although it is very likely that different types of breast cancer respond differently to irradiation. RT sensitivity might therefore be a risk factor in itself. Local recurrence is the result of tumor cells left behind after surgery, combined with resistance of these tumor cells to the radiation therapy. It is therefore useful to understand mechanisms of sensitivity and resistance to radiation therapy.

DNA double-strand break repair

One important determinant of radiation sensitivity is the efficiency of DNA double-strand break repair. BRCA1 and BRCA2 proteins are involved in the repair of DNA damage, including double-strand breaks induced by RT. BRCA1/2 germ-line mutations are associated with impaired DNA double-strand break repair. Several retrospective studies have been published on the risk of local recurrence in BRCA 1/2 mutation carriers. The results of these studies are contradictory, possibly because of differences in adjuvant systemic treatment and whether the patients underwent oophorectomy. For example, in a study by Pierce et al., a total of 160 BRCA1/2 mutation carriers with breast cancer were matched with 445 controls with sporadic breast cancer, all treated with BCT. There was no significant different in breast tumor recurrence in this study between carriers and controls at 10 and 15 years (12% and 24% for carriers and 9% and 17% for controls, respectively; P = 0.19). In multivariate analysis, BRCA1/2 status was not predictive for an ipsilateral recurrence. Although when patients who had undergone oophorectomy were removed from the analysis, BRCA1/2 status was an independent predictor for recurrence (hazard ratio, 1.99, P = 0.04). It has to be stated that it can be difficult to obtain the distinction between second primary breast tumors and true recurrences in a conserved breast.

Two studies investigated radiosensitivity in BRCA1/2-associated cancer by investigating radiation-associated complications in women with a BRCA1/2 mutation treated with RT after BCT compared with sporadic controls. Both studies, with 202 BRCA1/2 patients included in total, showed comparable acute and late effects of RT in the mutation carriers and the sporadic group. The normal somatic cells are heterozygous for the affected gene, whereas tumors are more likely to have loss of heterozygosity and should be more radiosensitive.
**Chapter 2**

**In vitro studies**
Several studies have been performed to investigate gene expression signatures in RT sensitivity in cell lines. In a study of Amundson et al \(^\text{58}\), global gene expression and radiation survival parameters were compared. Sixty cell lines of the US National Cancer Institute anticancer drug screen (NCI60) were used, and 5 of these were breast cancer cell lines. In this study, it was shown that some radiation-induced changes in gene expression were associated with radiation survival parameters, and that the basal gene expression pattern before irradiation may be a better predictor of radiation sensitivity. Torres-Roca et al \(^\text{59}\) developed a radiation classifier predicting the inherent radiosensitivity of 35 tumor cell lines, of which 6 breast cancer cell lines, as measured by surviving fraction at 2 Gy. Some of the genes involved were mechanistically involved in radiation response. Gene expression classifiers predicting normal tissue reactions have also been developed. Rodninghen et al \(^\text{60}\) generated gene expression profiles from fibroblast cell lines developed from breast cancer patients with and without severe radiation-induced fibrosis. The fibroblasts were treated in vitro with different radiation schemes. RNA was isolated 24 hours after the last fraction. An 18-gene classifier was found to be predictive for the risk of developing radiation-induced fibrosis. However, in 2 large studies, radiation sensitivity of skin fibroblasts has not been confirmed as predictive for breast fibrosis after RT \(^\text{61,62}\).

**In vivo studies**
In a study by Helland et al \(^\text{63}\), tumor material of 19 breast cancer patients was investigated to study the molecular basis underlying response to RT. Tumor biopsies were sampled before radiation and after 10 treatments of 2 Gy. Gene expression microarray analysis was performed to identify in vivo radiation-responsive genes involved in these tumors. Several genes involved in cell cycle regulation and DNA repair were significantly found to be induced by radiation treatment. In the study by Kreike et al \(^\text{12}\), it was found that the gene expression profile of the primary tumor and the (irradiated) recurrence were very similar. No prediction profile for RT sensitivity in breast cancer patients has yet been developed. At the Netherlands Cancer Institute in association with the Institut Gustave Roussy and the Karolinska Institute, there is a currently ongoing trial in which patients are irradiated preoperatively (Preoperative Accelerated Partial Breast Irradiation trial, [http://clinicaltrials.gov/ct2/show/NCT01024582](http://clinicaltrials.gov/ct2/show/NCT01024582)). Response to RT will be studied in association with the gene expression profiles of the tumors. Hopefully, an in vivo predictive profile for radiosensitivity will be found that can be useful in the clinic. For the development of a robust radiosensitivity profile, it is needed to link the in vitro data described earlier in the text to more in vivo studies in which gene expression data involved in RT treatment and treatment outcome are studied.
Conclusions

Risk factors for local recurrence play an important role in the decisions for the treatment of breast cancer. This decision-making can be optimized if patients at high risk for a local recurrence can be identified. At present, this is mainly done based on clinical factors, histopathologic factors, and several immunohistochemical markers. Expression profiling studies could provide explanations for differences in prognosis and risk for local recurrence and response to different therapies (adjuvant systemic therapy and RT). However, because of the variation in different subtypes in breast cancer and variations in the amount of tumor burden remaining after surgery, finding robust predictive profiles is complex. Only when such robust predictors for local recurrence and sensitivity for systemic treatment and RT are found will better personalized treatment for breast cancer patients be accomplished.
References


Predictive factors for local recurrence


