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HIGH-THROUGHPUT TECHNIQUES IN BREAST CANCER: HAS THEIR TIME COME?

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REVIEW

ABSTRACT. IN BREAST CANCER PATIENTS, high-throughput technologies such as DNA-microarrays or RT-PCR can improve the prognostic and predictive information that we now get from classical parameters. Classical factors do not accurately inform about which patients need adjuvant therapy or which tumours will resist the effect of that therapy. Studies performed in breast cancer with high-throughput techniques have focused on tumour biology, prognosis and prediction of response, but further refinement is needed before these techniques become part of the clinical routine. In the meantime, they will be used in clinical investigation, particularly in the adjuvant setting, where modest improvements in the capacity of prediction can benefit many women. Close cooperation among clinicians, pathologists and basic investigators is essential to take high-through techniques to daily practice. New diagnostic tools will be complex but they will provide valuable information for our patients.

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1. INTRODUCTION

Breast cancer is one of the most common malignancies in humans. Surgery can cure a significant proportion of cases, but in many women threaten of relapse remains for prolonged periods of time. Adjuvant therapies are those given after surgery with the aim of decreasing the likelihood of recurrence. These include radiotherapy, chemotherapy, hormonal therapy and the antibody therapy trastuzumab (Herceptin®). Risk of recurrence in a given patient determines whether she will need adjuvant treatment as well as the kind of treatment: if the risk is very low, surgery will suffice, whereas intensive adjuvant therapy will be needed if the risk is very high. Thanks to the widespread use of adjuvant therapies, the outcome of these patients has improved along the last decades.

Outcome can be predicted with the help of clinical and pathological parameters, the so-called prognostic factors, but these factors have serious limitations. High-throughput technologies may be the way to increase accuracy in prognosis estimation. We shall deal with the limitations of classical prognostic factors and what high-throughput techniques should offer before being incorporated to daily practice. DNA microarrays and polymerase chain reaction (PCR) will be addressed in our review because they have been used in many studies of breast cancer prognosis.

2. PROGNOSIS AND ADJUVANT TREATMENT

2.1. LOCALISED DISEASE

Surgery remains the cornerstone of therapy for localised breast cancer. Unfortunately, even small tumours may have disseminated beyond the breast by the time they are excised and, in such cases, relapse will eventually happen. Adjuvant treatments are administered after surgery and contribute to decrease the chance of relapse. Nowadays, most patients receive some kind of adjuvant therapy, but at the cost of considerable toxicity. The likelihood that a patient will benefit from adjuvant therapy depends on prognostic factors: number of affected lymph nodes, size of the tumour, grade of differentiation and expression of hormonal receptors and ERBB2 [1-6]. However, many patients do not benefit from adjuvant therapy, either because they will be cured just with local treatments or because they will relapse anyway (FIG. 1).

In the case of big tumours, treatment is usually initiated with chemotherapy in an attempt to shrink the mass before surgery. If a complete response is obtained with chemotherapy (in 10–20% of cases), survival will also be increased [7-9], but some tumours may be primarily resistant, a situation associated with very poor prognosis. Unfortunately, we cannot presently identify who will have a resistant tumour [10].

2.2. DISSEMINATED DISEASE

Women with metastatic disease have a life expectancy of two to three years, as an average. Breast cancer being so heterogeneous, the clinical evolution is very different among patients: some of them survive for almost a decade with only bone disease, whereas others die within few months due to liver metastases unresponsive to therapy [11,12]. It is not currently possible to predict what will happen once metastases appear. Of course, dissemination to the liver or brain is associated with a poor outcome, but even in these cases the evolution varies from one patient to another. Whereas some tumours are very responsive to hormones or chemotherapy, others do not respond from the very beginning. No pathological feature helps in this regard.

In summary, clinical and pathological factors do not accurately indicate which patients will require adjuvant therapy or which tumours will hold resistance to anticancer drugs. New factors are obviously needed to optimise cancer therapy and high-throughput techniques could be of help in this regard.

3. STUDIES WITH HIGH-THROUGPUT TECHNIQUES IN BREAST CANCER

High-throughput techniques have been used in

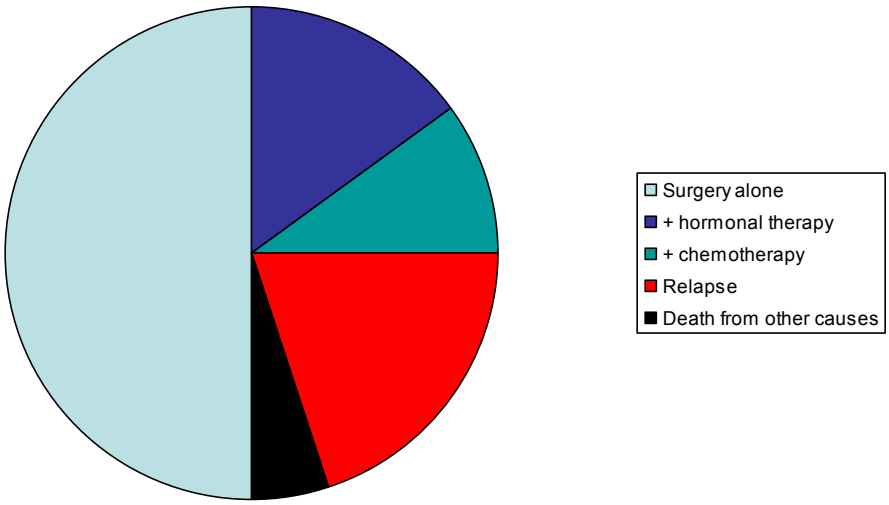

FIGURE 1. BENEFIT OBTAINED FROM ADJUVANT THERAPY. In this example, the risk of relapse after 10 years is 45% with surgery alone. This percentage is reduced by 25% with the administration of hormonal therapy and chemotherapy. The numbers vary from patient to patient depending on prognostic factors such as lymph node status, size of the tumour, grade of differentiation, expression of hormonal receptors and patient's age. In some cases, benefit from adjuvant therapy is as small as 2-3%, whereas in others it can reach 50%.

normal breast tissue and breast tumours with different clinical or pathological features. The major findings in these studies are: (1) there is a marked difference in the gene profile between normal and neoplastic tissue; (2) even invasive tumours are quite heterogeneous and several subclasses may be distinguished attending to the gene profile; and (3) some sets of genes may determine prognosis or drug sensitivity.

3.1. NORMAL TISSUE VS TUMOUR

Ma et al. used a 12,000-gene cDNA microarray to analyze normal samples from mammoplasty reduction, normal samples from patients with cancer and the tumours of these patients [13]. Normal samples were all very similar, whether they came from mammoplasty of from patients' normal tissue. A dramatic change in expression appeared between normal tissue and all neoplastic lesions, regardless of their stage. Even an early lesion such as atypical ductal hyperplasia was similar to overt invasive disease.

Several studies have demonstrated high concordance between tumoral markers in *in situ* versus invasive tumours, for instance, in the expression of ERBB2, Ki67, P53 and angiogenesis markers [14]. Weigelt et al. compared the gene expression of primary tumours and their metastases in a series of patients [15]. These results confirm that the capacity to metastasize appears soon in the natural history of breast cancer, so that the gene expression profile of early stage disease could reflect the metastatic potential of the lesion.

3.2. SUBCLASSES

The expression of either hormonal receptors or ERBB2 drive the expression of many other genes, so that these tumours posses characteristic profiles [16,17]. This may explain why oestrogen-receptor positive breast cancer usually behaves in a more indolent way and has a better prognosis, whereas ERBB2 positive primaries tend to recur more often. There is even a proposal for categorisation of breast cancer into five subclasses: normal breast-like, basal-like, ERBB2 and luminal types A and B [18]. The basal-like and ERBB2 subclasses are associated with shortest survival times, as opposite to the luminal A type. Tumours in carriers of BRCA-1

mutations usually correspond to the basal-like subclass [19]. Although the prognostic value of this classification has been challenged, most investigators now admit that gene profiling can identify different entities in breast cancer. A genetic taxonomy supports the observation that the clinical evolution of breast cancer is extremely heterogeneous.

3.3. PROGNOSIS

Lymph node status is the most powerful predictor of outcome but, as we have already indicated, it is far from accurate, even when combined with other factors such as size of the tumour or grade of differentiation. High-throughput techniques may offer an advantage because they allow the simultaneous analysis of many genes, each being a potential prognostic marker. We shall comment on some of the most important studies performed so far in this area.

van de Vijver et al. evaluated a 70-gene prognosis profile in 295 patients: those with a poor-prognosis signature had an overall 10-year survival of 55%, as compared to 95% for those with a favourable signature [20]. Differences remained significant when the groups were analysed according to lymph node status, and the Cox analysis revealed that the profile was an independent prognostic factor. Interestingly, patients were 53 years-old or younger. The authors used the microarray platform from Agilent™ and we have reproduced these results with quantitative reverse-transcriptase PCR [21].

Paik et al. used a set of 16 genes plus 5 control genes in a prospectively defined algorithm to calculate a recurrence score in low-risk patients [22]. The technique was reverse-transcriptase PCR. Over 600 samples from patients who had received tamoxifen for node-negative disease were analysed. The profile identified up to 50% of patients whose prognosis with hormonal therapy was excellent. In this group chemotherapy could be avoided [22,23]. The set of genes has been marketed in some countries under the name OncoType™ and is used to make decisions about adjuvant chemotherapy in low-risk disease.

Wang et al. reported a 76-gene signature consisting of 60 genes for patients positive for oestrogen receptors and 16 genes for oestrogen receptor-negative patients [24]. This time the microarray platform was Affymetrix™ and the study included 280 patients. Patients with a relapse score above a defined threshold had an odds ratio of 12 to develop metastasis within 5 years. The profile remained valid after correction for traditional prognostic factors in multivariate analysis. This is a purely prognostic signature because patients had not received adjuvant systemic therapy, unlike those in the two other studies.

3.4. PREDICTION OF RESPONSE

Some studies have found gene expression profiles predicting response to taxanes [25-27] or tamoxifen [28-30]. A study of cDNA microarrays in poor-prognosis tumours treated with anthracycline-based adjuvant chemotherapy found a 23-gene set that was associated with different survival [31]. On the other hand, single-nucleotide polymorphisms can contribute to individual drug response [32, 33]. This is an area of paramount importance. Current therapeutic strategies are planned with no information about the susceptibility of the tumour to anticancer drugs in a given patient, so that this patient has to face side effects with no guarantee of success. We must also consider the huge economic cost of useless drugs.

4. INCORPORATION OF MOLECULAR PROFILES INTO THE CLINIC

Should clinicians rush to incorporate high-throughput techniques in their daily practice? Results from the mentioned studies are very appealing, but more investigation is needed and some practical issues must be addressed before that. Requirements to include a new technique in the clinic may be summarized as follows:

GENERAL AVAILABILITY. The reason why traditional prognostic factors succeeded lies in their availability. Any pathologist can determine the size of the tumour and the presence of infiltrated lymph

nodes, and is also able to perform immunohistochemistry. However, microarray technology can be afforded only by a few. PCR is more familiar to pathologists, but not a routine technique. The need for frozen samples is another problem, although PCR can be performed from paraffin-embedded samples.

EASY INTERPRETATION. Traditional factors are straightforward: a given patient either does or does not have affected lymph nodes, the tumour is either positive or negative for hormonal receptors or ERBB2. The software Adjuvant!™ [34] and prognostic indexes such as the NCI's [35] or St. Gallen's [36] can be used to combine several of these factors and are very easy to use. However, high-throughput techniques need a statistician to interpret the results and statistics for this technology is still under development. One study demonstrated that molecular signatures strongly depend on the selection of patients in the training sets, so that validation studies should be performed with all candidate profiles [37].

AGREEMENT ON WHAT IS STANDARD. Measuring the primary tumour or counting the number of lymph nodes is simple, immunohistochemistry for hormonal receptors and even ERBB2 is standardised. This means that prognosis is determined in the same way throughout the world. On the contrary, gene profiling can be performed using different platforms and universal profiles have not been agreed yet. This is one of the most complex areas of development in this regard, because direct comparisons between platforms or profiles have not been performed.

SOLID SUPPORT FROM CLINICAL TRIALS. Oncologists base their decisions about adjuvant therapy in comparative trials that have included thousands of patients and also in meta-analysis of trials with prolonged follow-up of patients. Classical prognostic factors were also accepted after meticulous analysis of data coming from those trials or from retrospective studies with huge samples.

In summary, high-throughput techniques do not fulfil any of the requirements to become part of the clinical routine. Further development is obviously needed.

5. DESIGN OF CLINICAL TRIALS USING GENE PROFILES

In spite of their problems, molecular markers hold great promise for refining our ability to establish early diagnosis and prognosis, and to predict response to therapy. We can say that new technologies are here to stay, but the question is which trials will be needed to support their clinical use. Trials using this technology could be divided into two main groups, according to the main objective:

1. The main objective is the characterisation or the validation of a gene profile;
2. Gene profiles are included in clinical trials evaluating drugs.

5.1. STUDIES TO FIND GENE PROFILES

In the first case, we can study who will benefit most from hormones or chemotherapy, for instance. Recent reports have identified gene profiles that can predict either response to the antioestrogen tamoxifen in metastatic breast cancer [28] or resistance in the adjuvant setting [22]. Similar studies should be performed with aromatase inhibitors. The same applies to chemotherapy. The EORTC (European Organisation for the Research and Treatment of Cancer) will shortly begin a trial to validate a 70-gene profile in women with node-negative breast cancer. Patients' risk will be defined with either the St. Gallen prognostic index (based on classical factors) or the gene profile: the purpose of the trial is to compare the outcome of patients in both low risk groups. In the United States the Intergroup will initiate another clinical trial using the 21-gene profile, as part of the Program for Assessment of Clinical Cancer Tests (PACCT). Women with intermediate-risk recurrence score will be randomized to receive adjuvant hormonal therapy with or without chemotherapy.

Of course, the profiles we have reviewed are not perfect. We are very far from 100% accuracy in predicting outcome. Further studies will be needed to refine current profiles. For instance, specific signatures for non-ductal histologies (lobular or comedo, for instance) have not been developed, con-

sidering that these tumours have a different clinical behaviour. On the other hand, patient's ability to mount an immune response or to metabolize drugs has not been studied so far. In essence, we can predict that future profiles will be a lot more complex and sophisticated.

Apart from more accurate profiles, we need cheap and standard techniques, so further refinement is needed in this regard. Pathologists will have to incorporate them into their training and practice, thinking that new equipments and procedures should be useful not only for the study of breast cancer but also of other tumours. In the meanwhile, investigators will have to get a consensus on platforms and profiles. As this technology is evolving very fast at the moment, it will take time to reach such a consensus.

5.2. STUDIES IN THE CONTEXT OF DRUG TRIALS

In the other kind of trials, a clinical study would include one or more gene profiles. The main objective will not be the characterisation of these profiles, but rather a comparison of two therapies (phase III trial). Let's say that therapy A turns out to be superior to therapy B, but at the cost of more toxicity; a gene profile could identify who obtains most benefit from therapy A, so that it is worth facing side effects or, alternatively, who may harbour a tumour resistant to this therapy, in which case, B could be better than A.

Whereas in the first case nothing is added to the efficacy of treatment, in the second there is a possibility to set a new and more active standard. Considering the growing difficulty to include patients in big trials, the second option could be more promising for the introduction of high-throughput techniques in the clinic. The reason is that only profiles supported by good clinical trials have a chance of being widely accepted in this setting.

As a conclusion, cDNA microarrays, RT-PCR and other high-throughput techniques will improve our ability to predict prognosis in breast cancer patients. However, they are not still ready for routine use and future studies should be designed carefully to make them useful and widely available.

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