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Review

Assessment of HER2 status in breast cancer: why, when and how?

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Abstract

Human epidermal growth factor receptor 2 (HER2) is overexpressed, usually as a result of *HER2* proto-oncogene amplification, in 20–30% of breast cancers. A HER2-positive status is generally associated with more aggressive disease and a worse prognosis. Furthermore, a positive HER2 status may predict the likelihood of resistance to some conventional therapies, as well as probably being predictive of sensitivity to anthracycline dose intensification. In addition to this prognostic/predictive value, HER2 is a target for specific therapy, with anti-HER2 monoclonal antibody therapy available in the USA. This article reviews the different assays used to determine HER2 status, discussing their relative advantages/disadvantages and the need for their standardisation before integration alongside other pathological indices into the clinical management of breast cancer.

Keywords: Breast cancer; HER2; Pathology; Tumour markers

1. Introduction

The human epidermal growth factor receptor-2 (*HER-2*) proto-oncogene encodes a 185-kDa glycoprotein, often simply called the HER2 or c-erbB2 protein or receptor. HER2 plays a key role in one of the best studied growth factor receptor systems in breast cancer, the HER (or erbB, or Type 1) tyrosine kinase receptor family. This family comprises four homologous epidermal growth factor (EGF) receptors: HER1 (EGFR/erbB1), HER2 (erbB2), HER3 (erbB3) and HER4 (erbB4) [1]. Each of these receptors comprises an extracellular ligand-binding domain, a transmembrane lipophilic segment and an intracellular protein kinase domain with a carboxyl terminal segment containing sites of phosphorylation or tyrosine residues [2].

The HER family plays an important role in regulating cell growth, survival and differentiation in a complex manner. Various ligands have been identified that activate individual HER receptors, although no ligand has been identified that binds directly to the HER2 receptor. However, cell surface HER receptor monomers form homodimers with the same receptor or heterodimers with other members of the HER family in response to ligand binding [3]. HER2 is the preferred heterodimerisation partner within the family [4] and can be stabilised and transactivated in heterodimers by ligands for the partner HER monomer, such as HER1 or HER3 [1]. This heterodimerisation between HER2 and the other receptors of the family allows the participation of HER2 in signal transduction, even in the absence of a cognate ligand. In fact, heterodimers containing HER2 appear to show particularly high signalling potency compared with other homodimer or heterodimer combinations [5], which may explain the particularly significant role of HER2 in the oncogenic phenotype [6].

In vitro and animal studies have indicated that *HER2* gene amplification and protein overexpression play a pivotal role in oncogenic transformation, tumorigenesis

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and metastasis [7–9]. *HER2* gene amplification and/or HER2 protein overexpression can occur in a relatively high percentage of various tumour types [10], for example 20–30% of breast and ovarian carcinomas [11,12]. Furthermore, the growth of tumours and human breast cancer cell lines overexpressing the HER2 receptor is inhibited by anti-HER2 monoclonal antibodies (MAbs), opening a new avenue for targeted cancer therapy [13,14].

The normal epithelial cell possesses two copies of the *HER2* gene and expresses low levels of HER2 protein on the cell surface, equivalent to some tens of thousands of receptors per cell. With oncogenic transformation, *HER2* gene amplification generating more than the normal two gene copies and/or increased mRNA transcription leads to 10- to 100-fold increases in HER2 receptor monomers on the cell surface, i.e. HER2 overexpression equivalent to millions of receptors. The extracellular domain of the HER2 receptor (ECD^{HER2}) may be shed from the cell surface and detected in the circulation in some cases [15].

2. Why measure HER2 status?

The pathologist plays a central role in the accurate diagnosis of breast cancer and in the subsequent assessment of prognostic and predictive factors that assist in treatment and management decisions. The primary prognostic factors in breast cancer remain those determined by clinical or standard pathological approaches: axillary lymph node status, tumour size, histological or nuclear grade, and histological subtype. Although predictive factors are more valuable clinically, the only clearly validated predictive factors are oestrogen receptor (ER) and progesterone receptor (PgR) status, which predict response to hormonal therapy. Considerable attention has been directed at identification of additional prognostic, and particularly, predictive factors that might guide more appropriate individualisation of therapy. In this regard, HER2 has been the focus of intensive study over the past decade.

2.1. Prognostic significance of HER2

The pivotal publication in 1987 by Slamon and colleagues [12] revealed that *HER2* gene amplification independently predicted overall survival (OS) and disease-free survival (DFS) in a multivariate analysis in node-positive patients. Since then, most large studies have confirmed HER2 status, generally measured by gene amplification or receptor protein overexpression, as a significant independent predictor of prognosis in multivariate analysis in node-positive patients (see [16] for a recent analysis of a large number of trials). Interestingly, it has recently been reported that HER2 mRNA underexpression might also predict poor prognosis in breast cancer [17]. This confirms earlier studies in which HER2 protein had been detected using radioimmunochemistry [18,19]. Using this technique, 85% of breast cancers were shown to express levels of HER2 protein above that seen in normal breast cells. There was bimodal distribution, with 25% of cancers expressing very high levels of protein and all these cancers had gene amplification. HER2 was undetectable in 15% of cancers, and in the remaining 60% there was overexpression but at a level significantly below that for amplified tumours. Patients with cancers with amplified levels of HER2 protein or the absence of protein expression had significantly worse prognosis when compared with the remainder [20].

Detection of small, node-negative breast lesions is becoming more frequent with the introduction of breast cancer screening programmes. Although such node-negative patients have better prognosis following surgery than node-positive patients, approximately one-third still develop distant metastases. In this setting, it is of great clinical importance to identify those node-negative patients at higher risk of progressing to metastatic disease, so that this specific group can be treated with appropriate systemic adjuvant therapy. Studies of HER2 status and prognosis in node-negative patients have yielded conflicting results [16], which might possibly be explained by the low numbers of patients evaluated in some studies, the low event rate and the diversity of methods used to determine and define HER2 status. Thus, further larger studies are required to establish whether HER2-positive, node-negative patients have a clinically meaningful increased risk and whether this provides valuable information in selection of patients for systemic adjuvant therapy. At present, the measurement of HER2 purely for prognostication in node-negative patients is not justified in a routine setting, but more research is required to assess its ultimate value.

There is also a possibility that HER2 status may be useful in determining invasive potential in patients with ductal carcinoma *in situ* (DCIS), since HER2 overexpression is very common in high-grade, comedo-type DCIS [21,22]. Breast cancer mortality for DCIS is very low if treated with mastectomy, but increasing numbers of patients are being treated with breast-conserving treatment. Future prognostic studies may help to identify whether HER2 status can be integrated into optimal treatment planning in DCIS by stratifying patients according to their risk of local recurrence and progression to invasive cancer.

2.2. Prediction of response to therapy

There have been numerous studies evaluating the predictive value of HER2 status in relation to response

to various therapies. However, results have not been consistent and interpretation of these data is complicated and open to discussion [23].

2.2.1. Hormonal therapy

In vitro evidence suggests an association between a HER2-positive status and hormone resistance: transfection of normal breast cancer cells with the HER2 gene to produce HER2-overexpressing cells results in acquisition of oestrogen-independent growth that is insensitive to tamoxifen [7,24]. A range of studies have reported that a HER2-positive status is associated with resistance to hormonal therapy [25–32]. Of particular note, the recently reported 10-year update of the Naples GUN Trial [26] found that HER2 overexpression not only predicted resistance to tamoxifen, but that node-negative, HER2-positive patients had a worse outcome on adjuvant tamoxifen therapy than those who were untreated. Substantial reductions in response rates to hormonal therapy have been seen in metastatic breast cancer patients with high plasma levels of ECD^{HER2} [27,32].

However, it is important to note that some other studies, which generally analysed large numbers of patients, found no significant association or even a trend towards an association between HER2 status and response to hormonal therapy [33–35]. Elledge and colleagues [34] examined the response to tamoxifen in 205 patients with ER-positive disease, and found no significant evidence for a poorer response or a more aggressive clinical course in HER2-positive compared with HER2-negative patients. These authors suggested earlier contrary results may have been related to failure to rigorously exclude ER-negative patients, who are much less likely to respond to tamoxifen and more likely to be HER2-positive. In a more recently presented report [35], the relationship between HER2 overexpression and response to tamoxifen was analysed in the adjuvant setting in 741 patients (650 ER-positive, 91 ER-negative/PgR-positive) of a total 1572 patients in the CALGB 8541 Trial who had HER2 status measured. Again, tamoxifen significantly improved DFS and OS to the same degree irrespective of HER2 status, but these patients also received CAF chemotherapy which may have affected any interaction.

Thus, whilst there is some evidence that HER2 status is a possible indicator of hormone resistance, the clinical data are contradictory. The answer will only be resolved with additional large-scale, long-term prospective studies, ideally comparing hormonal therapy versus no therapy and looking at the response in relation to HER2 status. Overview analysis may be required for sufficient power: there is an inverse correlation between HER2 and ER [12] such that in an unselected population only approximately 10% of patients will be positive for both ER and HER2 status and, therefore, approximately 15% of an ER-positive population are HER2 positive. Even in a total dataset of 1000 ER-positive patients from a two-arm trial, only approximately 75 would be HER2-positive in each arm, giving very little power to the estimation of an effect on clinical outcome.

2.2.2. Chemotherapy

Some of the first studies that examined the role of HER2 in predicting response to chemotherapy looked at regimens containing cyclophosphamide, methotrexate and 5-fluorouracil (5-FU) (CMF). Most of these studies have indicated a reduced benefit from CMF therapy in HER2-positive versus HER2-negative patients [25,36–39], although no such association was found in recent studies [40,41]. Thus, HER2 status may possibly be predictive of response to CMF therapy, but it is important to bear in mind that most of these studies were based on retrospective analyses and have not been validated in prospective trials involving sufficient numbers of patients to determine a statistically significant interaction.

The potential relationship between HER2 status and response to anthracycline-based chemotherapeutic regimens, usually doxorubicin combined with cyclophosphamide and 5-FU (CAF), has been examined in a number of studies. Muss and colleagues [42] published the first analysis of an interaction between expression of HER2 and adjuvant therapy with doxorubicin-containing regimens using results from a Cancer and Leukemia Group B (CALGB) study. The finding that tumours with high expression of HER2 responded well to dose-intensive CAF treatment stimulated additional analyses of the original study [43,44] and new studies. In essence, the first paper revealed an apparent dose-related sensitivity to doxorubicin in HER2-positive patients which was not present in HER2-negative patients. The second larger cohort of samples failed to show the same significant result, but the trend was similar. The second set was a substantially different prognostic group than the first, and a complex adjustment for prognostic factors revealed a significant result which was also present when the two data sets were combined. Recently, at least two additional large studies have also reported results suggesting anthracycline sensitivity in HER2-positive patients [45,46]. The National Surgical Adjuvant Breast and Bowel Project (NSABP) study B-11 [45] was a trial originally designed to compare regimens of L-phenylalanine mustard plus 5-fluorouracil 5-FU with and without doxorubicin. In this trial the addition of doxorubicin improved outcomes in HER2-positive patients to the extent that they were equivalent to those experienced by patients with HER2-negative tumours. The South West Oncology Group (SWOG) study [46] compared regimens of tamoxifen either with or without CAF. Again, the results were similar to the prior studies. Patients whose tumours expressed low levels of

HER2 did not benefit from the addition of CAF, but CAF improved the DFS of patients with HER2-positive tumours such that their outcome became similar to that of HER2-negative patients. Taken together, these data suggest a significant interaction between HER2 overexpression and chemosensitivity to anthracyclines.

A number of other studies indicated that HER2 status has no predictive value in determining response to anthracycline-based chemotherapy [47–50]. It is important to note, however, that most of these studies had lower numbers of patients than those studies which indicated positive prediction, thus reducing their statistical power. Also, in the study by Clahsen and colleagues [47] the patients only received one cycle of perioperative chemotherapy rather than the standard of four or more cycles. Importantly, none of the studies suggested that HER2 status was a predictor of increased resistance to anthracycline-based chemotherapy.

HER2 overexpression may, therefore, indicate a relative sensitivity to optimal versus suboptimal anthracycline dosage. Stronger, direct evidence would be helpful, but the current data indicate that patients scheduled for CMF therapy who are HER2 positive might be advantageously treated instead with an anthracycline-based treatment. Such treatment selection, however, requires a consensus about the most reliable, most reproducible and most predictive assay to determine HER2 status.

Increasing attention is being given to the HER2 receptor as a direct target for therapy in breast cancer overexpressing HER2, for which HER2 overexpression is logically a prerequisite for prediction of the need for, and probability of response to, therapy. One such approach, anti-HER2 monoclonal antibody therapy with trastuzumab (Herceptin®), is licensed for the treatment of HER2-positive metastatic breast cancer in the USA. For the present, this is the only circumstance in which there is an absolute requirement that HER2 overexpression is determined prior to the instigation of therapy. The availability of trastuzumab has, however, invigorated and increased the importance of the debate about when and how to measure HER2 status.

3. When to measure HER2 status?

In most studies to date, HER2 status has been determined retrospectively using fresh frozen or paraffin-embedded tissue employing immunohistochemical (IHC) assays, which detect HER2 protein overexpression. However, it is becoming more important that HER2 status is determined prospectively so that it can be used as a prognostic/predictive factor and to guide therapeutic possibilities. If the determination of HER2 status is to guide the application of adjuvant therapy, the analysis will clearly be required as soon as possible after diagnosis. The absence of HER2 status determination at diagnosis does not preclude its later measurement on archival specimens for treatment decisions in the metastatic setting. This will, however, require histological block retrieval, sometimes involving the contact of other hospitals. As HER2-directed therapy becomes more widespread, as seems likely, it may be expected that establishing HER2 status at diagnosis as part of the routine work-up of breast carcinoma will be more convenient and cheaper.

In general, HER2 status in a primary lesion predicts the status of metastatic lesions such that biopsy of metastatic patients is unnecessary if archival tissues can be accessed. The availability of a serum assay for ECD^{HER2} which reliably predicted outcome would be highly advantageous. However, for the moment, further clinical trial data are required to establish that utility. There are no data to suggest that it is helpful to monitor serum ECD^{HER2} levels during therapy.

4. How to measure HER2 status?

Numerous techniques have been used and are being developed to determine HER2 status, although some of them are only suitable for research purposes rather than routine screening of patients. Various target molecules related to HER2 amplification/overexpression, i.e. DNA, mRNA and receptor protein, can be used in different assays. Each technique has advantages and disadvantages which need to be weighed against each other in any assessment of the most appropriate technique for routine laboratory assessment of HER2 status.

For assessment of *HER2* gene amplification, fluorescent *in situ* hybridisation (FISH) is the most convenient current assay. Southern blotting has been used in research studies but is infrequently applied in routine use. FISH has a number of advantages, such as being highly sensitive and specific, standardised (but not automated), and able to be used on small tissue samples. With FISH, it is easy to provide a positive or negative result, although there needs to be standardisation of the cut-off point (number of gene copies per nucleus used to define amplification). This has varied in prognostic/predictive studies of HER2 status and is a confounding factor in interpretation of apparently conflicting results between studies. However, a minority of pathology laboratories are set up to assay HER2 status using FISH and it is also relatively time consuming and complicated to perform. FISH has been approved in the USA to measure *HER2* gene amplification for prognostic assessment of node-negative breast cancer patients who are at risk of recurrent disease.

Assays measuring HER2 mRNA have only rarely been used, mainly for research purposes. There may be a potential future in the development of polymerase chain reaction (PCR) assays of either DNA or mRNA

as such techniques are likely to be cost-effective, highly sensitive and specific, and able to analyse large numbers of samples rapidly in an automated manner. However, for the moment IHC is likely to continue to dominate analytical practice.

IHC is the most widely used and practical assay available at present for assessment of HER2 status. It can be specific and rapid, requires few reagents and steps to perform compared with FISH, and can be used with equipment found commonly in most laboratories. The sensitivity of IHC can vary considerably depending on the particular MAb used in the assay, and a wide range of detection rates can be found when using different antibodies on the same specimens [51,52].

Many laboratories may only be able to perform IHC on referred specimens and have no control on the time and nature of tissue fixation, the method used to process tissue, or the temperature of paraffin embedding. All of these procedures may affect HER2 protein antigen loss or intensity of immunostaining in the specimens [53]. Prolonged storage of unstained slides can also result in significant loss of immunostaining intensity for some antigens/antibodies, but this has not been widely described for HER2 [54]. The duration and type of fixative used may mask antigenic sites in tissue sections. Antigen retrieval can be achieved by protease digestion or heat treatment in citrate buffer by the use of microwave or pressure cooking. However, antigen unmasking can result in an increased incidence of detection of HER2 which does not relate to gene amplification, and the significance of this is less clear [55]. It would therefore be preferable to use a standardised method. Commercially available immunohistochemistry (IHC) tests (e.g. HercepTest^{TM}, DAKO) have become available. These are a step forward in the world of immunohistochemistry in their provision of a detailed standardised procedure and inclusion of a set of controls against which scoring can be judged. However, even with these tests there has been recent discussion on sensitivity and/or interlaboratory standardisation of results [56–59], although this might be the simple consequence of the introduction of a new technique which can be overcome with adequate training to ensure quality control. Adoption of one such standard method of IHC assessment across laboratories would eventually improve the consistency of results between centres. Such kit technology is, however, inevitably more expensive than locally developed assays.

The thickness of sections for staining needs to be standard for IHC testing and FISH assay, and this may differ between these tests. If a marginally positive IHC test needs to be subsequently verified by FISH testing, a serial sample of the appropriate thickness needs to be taken. In a small percentage of cases, a positive IHC test representing HER2 protein overexpression can occur with a truly negative FISH test, since amplification of the *HER2* gene is not an absolute prerequisite of HER2 receptor overexpression [55,60–62]. In their review of different prognostic studies of HER2 status, Ross and Fletcher [16] found that assays based on DNA amplification, such as FISH, were more likely to be indicative than IHC assays based on receptor overexpression. If FISH testing becomes automated in the near future, it may ultimately supercede IHC, but for the foreseeable future IHC is likely to dominate testing for practical reasons.

All these and many other factors need to be taken into consideration when choosing an appropriate laboratory test for determination of HER2 status. As HER2 status becomes widely utilised for clinical management, standardisation of techniques with quality control between laboratories will become essential.

5. Conclusions

HER2 is a proto-oncogene that encodes a human epidermal cell growth factor receptor-like protein. Overexpression of HER2 protein, which is usually the consequence of *HER2* gene amplification, results in oncogenic transformation. *HER2* gene amplification occurs with a frequency of up to 20–30% in breast cancer. Clear HER2 protein overexpression is generally only detected in tumours that show *HER2* gene amplification. A HER2-positive status generally appears to be a good indicator of poor prognosis, with more aggressive disease leading to shortened DFS and OS when compared with patients bearing HER2-negative tumours. A positive HER2 status in breast cancer may predict the likelihood of resistance to some conventional therapies, as well as probably being predictive of an increased chemosensitivity to anthracyclines. Thus, determination of HER2 status may be of value for the patient and physician, and can be readily performed in many hospitals as part of the routine clinical assessment for breast cancer patients, alongside other pathological indices such as ER or PgR status. The development of a HER2 assay that is simple to use and can be integrated into routine testing should help in determining the rationalisation of the most appropriate therapy for the individual. This will become even more imperative when anti-HER2 targeted therapy becomes widely available. IHC is the easiest assay at present but some groups contend that it may be superceded by FISH, if the latter becomes automated. An enzyme-linked immunosorbent assay (ELISA) test on plasma would be ideal, as it is convenient and economical and may be applied in patients with no archival or accessible tissue for biopsy.

HER2 status is one of the first examples of a predictive/prognostic factor which was discovered as a result of studying oncogene/tumour suppressor gene

alterations in breast cancer. It is to be expected that, in the future, assessment of tumour suppressor genes and oncogene alterations in many tumour types will play a critical role in guiding diagnosis and treatment. Standardisation of the assays to assess these gene alterations (or their effects on protein expression) will be necessary before these assays can be used for routine clinical evaluation. Assessment of HER2 status can serve as a model for the standardisation of such assays and their interpretation.

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