Symposium article

HER2 status: A statistician’s view

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Summary

The human epidermal growth factor receptor-2 (HER2) has been investigated as a prognostic and predictive factor in breast cancer. The studies undertaken have included retrospective subset analyses and this had led to problems with bias such that only marginal statistical significance can be given to the results obtained. Overall, the majority of studies have found that a HER2-positive status may predict a poor patient outcome, although some studies have contradicted this finding. As a predictor of response to treatment, the results on the value of HER2 status are less well defined. Many studies suggest that a HER2-positive status correlates with resistance to hormonal therapy. Furthermore, a number of studies have found that a HER2-positive status is associated with a relative resistance to CMF (cyclophosphamide, methotrexate, 5-fluorouracil) and chemosensitivity to anthracycline-based therapies. However, there are enough discrepancies between studies for the predictive value of HER2 status to be unproven at the present time. Large, well-defined and accurately reported prospective studies are required to determine with any statistical clarity the value of HER2 status as a prognostic or predictive factor in breast cancer. In the meantime, no therapeutic option should be overlooked based solely on the HER2 status of a patient.

Key words: HER2, predictive factor, prognostic factor, statistical significance

Introduction

The prognosis of breast cancer can be determined by a large number of morphologic-based variables including tumor type, size and grade, and lymph-node status. Over the last few decades the development of molecular genetic techniques has allowed the identification and analysis of molecular factors that have an important role in normal cell growth and differentiation. Such molecular factors have also been shown to influence the behavior of tumors with respect to cellular differentiation, growth rate, metastatic pattern and response to therapy. Many of these factors have been examined in order to determine whether they can be used to predict disease prognosis and to stratify patients to the most appropriate treatment regimen.

The human epidermal growth factor receptor-2 (HER2) is a member of the growth factor receptor family of tyrosine kinases and is involved in the regulation and differentiation of normal cell growth [1–3]. In 1987, Slamon et. al. first demonstrated that amplification of HER2 occurred in 20%–30% of breast tumors and that this was associated with malignant transformation [4]. It was also suggested that HER2 gene amplification, and concurrent HER2 receptor overexpression, was an indicator of poor disease prognosis [4, 5]. Since then many studies have investigated the value of HER2 as a prognostic and predictive factor in breast cancer.

The results obtained to date on the relationship between HER2 status and clinical outcome and response to treatment have been somewhat controversial with many conflicting conclusions being drawn (reviewed in Ross and Fletcher [6]). This can be explained to a large degree by the low number of patients evaluated in some studies and the multitude of different methods used to determine and define HER2 status [6, 7]. For example, the use of different antibodies in immunohistochemistry (IHC) results in considerable variation in HER2 overexpression rates [7, 8]. In addition, the use of paraffin-embedded or frozen tissue samples can have an effect on the HER2 expression rates detected by IHC. This is because the fixation process used in paraffin-embedding can destroy cell-surface HER2 receptors [7]. Conversely, fluorescence in situ hybridization (FISH) can be particularly sensitive at identifying HER2 status but standardization of the technique is vital for consistency between results.

Effective reporting of patient characteristics, procedures and results are an integral part of effective analyses. The ideal protocol to assess prognostic and predictive relevance of a given factor is a prospective study with well-defined inclusion/exclusion criteria. In addition, it is essential to define locoregional and systemic therapies in relation to the main aim of the study and to describe the timing and method of follow-up examinations. Furthermore, specimen assessment and statistical considerations need to be reported fully in order to determine the significance of small or moderate differences between sample groups.

Unfortunately, numerous reviews have indicated that the quality of reporting clinical trial results is poor [9–12].
All patients with the condition of interest

Patients with specific characteristics

Measurement confounding chance

External validity?

Generalizability?

Samples

Sampling

? ?

Figure 1. Subset analysis of patient groups. (Reproduced with permission from Fletcher et al. [13].)

The analysis of particular variables and endpoints are generally determined by subset analyses in which a patient sample with specific characteristics is selected from a large group of patients with the condition of interest [13]. From this subset, a further patient sample is identified on which the analysis will be carried out (Figure 1). Such analyses have a certain amount of bias and chance, particularly if inadequate patient samples are used, because they reduce the power to detect significant differences [14, 15]. Therefore, the validity of any conclusions drawn from a subgroup analysis must be assessed.

To date, the studies used to determine the value of HER2 status as a prognostic or a predictive marker have been retrospective analyses. Adjusting for variables such as prognostic/predictive factors after the study has finished results in a degree of subjectivity that could be avoided if the factors are incorporated as stratification variables in the initial randomization [16]. In addition, these retrospective analyses are associated with difficulties due to lack of definition of patient characteristics and different methods of determining HER2 status. In this review we examine the available literature and assess the statistical significance of HER2 status as a prognostic factor and a predictor of treatment response.

HER2 and prognosis in breast cancer

In order to determine the significance of HER2 as a prognostic factor in breast cancer, a systematic review of the literature was undertaken. However, in order to do this it was necessary to disregard the technicalities of individual studies, including discounting specimen type, method of HER2 analysis, in situ components, and the definition of HER2 amplification/overexpression. Despite this, it has proved almost impossible to generate a systematic analysis of all the published papers.

Since the initial report by Slamon et al. [4], there have been more than 50 reports, on more than 16,000 samples assayed, published with sufficient detail to allow an analysis on the value of HER2 status as a prognostic factor. The majority of the reports used paraffin-embedded tissue samples (> 70%) and analyzed HER2 status by IHC (> 60%). Several problems have occurred with the analysis of these non-randomized trials such as lack of definition of the recruitment period in individual trials, where patients were recruited over a long period of time but no indication was given as to whether the case history was consecutive or not. In addition, ratios of analyzed samples to total number of specimens available for analysis were generally not available and there was non-uniformity between locoregional and systemic therapies. Furthermore, many of the studies involved inadequate patient numbers to determine small or moderate differences between different sample groups. Here we describe three case studies that have found an association between HER2 status and poor clinical outcome and the problems associated with the statistical analysis of these results.

Study 1: Prognostic significance of HER2 gene amplification in node-positive breast cancer

As previously mentioned, the first study to address the potential prognostic significance of HER2-gene amplification was by Slamon et al. [4]. This study examined the four-year relapse-free survival (RFS) (median follow-up 46 months) in a small case study of 86 node-positive breast cancer patients (Figure 2). Patients with two or more copies of the HER2 gene (n = 34) had a marginally shorter RFS compared with patients with no HER2-gene amplification (n = 52) (56% vs. 60%, respectively). Patients whose tumors amplified HER2 with five or more copies (n = 11) had an even shorter RFS of 37%
difference in survival curves between HER2-positive and -negative patients was evident from 12 months on-
patients after a median follow-up of 30 years [18]. The factor in 209 node-positive and -negative breast cancer
A further study examined HER2 status as a prognostic
in breast cancer - a 30-year follow-up
subset analysis only 8% of the specimens were classified
HER2-negative patients (P < 0.0001). However, in this
(n - 179). The five-year disease-free survival (DFS) for
patients and the relationship between previous systemic therapy and RFS was not reported.
This study was important as the first report to associate HER2-positive status with a poor prognosis, and
it set the precedent for further investigation into the significance of HER2 status as a prognostic factor.

Study 2: Prognostic significance of HER2 overexpression in node-negative breast cancer
The second study looked at 453 node-negative patients
who had not received previous systemic therapy [17].
The patients in this study were defined as being low risk
if they had small (< 3 cm) estrogen receptor (ER)-positive
tumors or high risk if they had ER-negative or large
(≥ 3 cm) tumors. Analysis of the relationship between
HER2 status and six-year RFS (median follow-up of 5.1
years) showed no significant difference between HER2-
positive and -negative patients (approximately 70% in
both groups). To investigate the possible influence of
the histologic composition of the tumor, an additional
analysis was carried out on a subset of the patient group
who were low risk with predominantly invasive tumors
(n = 179). The five-year disease-free survival (DFS) for
HER2-positive patients was 41% compared with 80% in
HER2-negative patients (P < 0.0001). However, in this
subset analysis only 8% of the specimens were classified
as HER2 positive.

Study 3: Prognostic significance of HER2 overexpression in breast cancer – a 30-year follow-up
A further study examined HER2 status as a prognostic
factor in 209 node-positive and -negative breast cancer
patients after a median follow-up of 30 years [18]. The
difference in survival curves between HER2-positive
and -negative patients was evident from 12 months on-
wards. The 25-year, cause-specific survival was 31% vs.
39% for HER2-positive and -negative patients, respect-
ively (P = 0.004). Patients were also stratified according
to axillary node status, and at eight-year follow-up node-
positive, HER2-positive patients had a worse outcome
than node-positive, HER2-negative patients (9% vs.
25%, P = 0.003). However, the small number of patients
at risk in this subgroup prevents any sound conclusion
being drawn from a clinical point of view.

Taken together, the results from these studies suggest
that a HER2-positive status can predict poor prognosis.
However, the retrospective subgroup analyses from these
studies do not allow unequivocal statistical conclusions
to be made.

HER2 and prediction of response to therapy
Between 1992 and 1999 more than 20 analyses (corre-
sponding to more than 5,500 tumor samples) of HER2
status as a predictor of treatment response have been
carried out. The tumor samples were taken from random-
ized trials and were retrospectively analyzed for HER2
status, in the majority of cases by IHC (> 50%) on
paraffin-embedded specimens (approximately 60%).
While the use of randomized trials helps reduce bias,
problems with regard to the statistical analysis of the
trials were still encountered. For example, the ratio of
analyzed samples to total number of specimens available
for analysis was generally not described. In addition, the
distribution of tumor and patient characteristics were
different in the subgroups devised for the predictive
studies compared with those used for the original studies
from which the tumor samples were obtained. Problems
were also encountered due to a lack of definition of
treated and control groups and with regard to inadequate
patient numbers in the patient subsets, making it difficult
to test small or moderate differences or interactions.

HER2 status and hormonal therapy in advanced
breast cancer
Several studies have investigated the relationship between
HER2 status and response to hormonal therapy. Many
reviews in the literature have indicated that a HER2-
positive status correlates with resistance to hormonal
therapy in advanced breast cancer [19–21]. However,
some of these studies have been too small to have any
statistical meaning. Although the findings generally
concur that a HER2-positive status is a marker of
resistance to hormonal therapy, contradictory results
have been described. For example, a study was under-
taken on 205 paraffin-embedded tumor samples from
patients enlisted into the Southwest Oncology Group
(SWOG) phase II trial, which examined the efficacy of
tamoxifen as front-line therapy for metastatic breast
cancer [22]. When a HER2-positive status was defined
as IHC ≥ 3+, the difference in response rates between
HER2-negative and -positive patients (57% vs. 47%,
The results of the reports in advanced breast cancer seem conflicting. While many studies indicate that a HER2-positive status is associated with strong resistance to tamoxifen, a number of those trials included too few patient numbers to be statistically significant. Large-scale, prospective studies may clarify the role of HER2 status and the effectiveness of tamoxifen therapy, but at the present time it seems unwise to deny any patients hormonal therapy. This is particularly true in light of the benefit that tamoxifen therapy can have for many breast cancer patients.

**HER2 status and hormonal therapy in adjuvant breast cancer**

In the adjuvant setting the Naples GUN trial has examined HER2 status and response to tamoxifen [23]. Node-negative patients (n = 145) were randomly assigned to two-year adjuvant tamoxifen (30 mg/day) or no further therapy. HER2 overexpression was detected in 43 patients and at 12-year follow-up these patients were found not to benefit from tamoxifen therapy compared with HER2-negative patients. However, this study was relatively small and did not take into consideration the benefit of tamoxifen in ER-positive, HER2-positive patients. It will require several large, prospective analyses before the role of HER2 status and response to tamoxifen in the adjuvant setting can be determined with some degree of statistical accuracy.

**HER2 status and CMF therapy**

The relationship between HER2 status and response to CMF (cyclophosphamide, methotrexate, 5-fluorouracil (5-FU)) has been investigated in a number of studies. The general trend of these reports suggests that a HER2-positive status may predict a relative resistance to CMF [17, 24, 25], however, these results cannot be considered definitive. This is in part due to differing trial conditions with regard to axillary node status, use of prednisone and/or tamoxifen therapy alongside CMF therapy, and the use of small subset analyses. For example, in one case series 152 node-negative (tumors > 3 cm) or node-positive patients were randomized to receive 12 cycles of CMF or radiotherapy [25]. Of these patients, 22% were identified as HER2 positive and at eight-year median follow-up HER2-positive patients receiving CMF had a poorer seven-year distant DFS compared with HER2-negative patients (15% vs. 65%). However, these patients represented a subset of only 28% of the original patient population. This leaves doubts as to the validity of generalizing results from this report and, consequently, on the real predictive relevance of HER2 in this context. Only one study has demonstrated a significantly lower benefit for HER2-positive patients receiving CMF compared with HER2-negative patients [24], although 66% of patients also received low-dose prednisone and 40% of patients who were post-menopausal received tamoxifen.

Recently, we carried out a retrospective analysis on 337 node-positive breast cancer patients randomized to receive adjuvant CMF (n = 207) or no further treatment (n = 179) [26]. HER2 was overexpressed in 16% of patients. At 20-year follow-up, there was no evidence for a statistically different effect of CMF for HER2-positive or -negative patients (Figure 3). Therefore, the data indicating that HER2 can predict resistance to CMF therapy is less than compelling at the present time.

**HER2 status and anthracycline-based therapy**

The value of HER2 status as a predictor of response to anthracycline-based therapy is also intriguing. The first study suggesting that HER2 overexpression might correlate with response to anthracycline-based therapies was by Muss et al. [27]. Patients (n = 337) were randomized to receive six treatment cycles of either high-, moderate- or low-dose CAF (cyclophosphamide, doxorubicin, 5-FU). Treatment with high-dose CAF was found to be superior in HER2-positive patients, suggesting that HER2 status could be predictive of CAF sensitivity.

A further study also provides evidence that HER2 status correlates with sensitivity to anthracycline-based therapies [28]. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-11 trial was originally designed to compare regimens of L-phenylalanine mustard plus 5-FU with or without doxorubicin. The addition of doxorubicin to the regimen improved outcomes in HER2-positive patients such that the 10-year DFS for HER2-negative and -positive patients was comparable.

Taken together, the results from these two studies do suggest a strong interaction between HER2 overexpression and chemosensitivity to anthracyclines. However,
weaknesses within the trials preclude any definitive conclusions being drawn. In particular, while in the initial study the predictive ability of HER2 was supported by a dose-related interaction, the NSABP trial found that a low dose of doxorubicin also had therapeutic benefit.

Conclusions

At this time, one of the greatest problems in identifying the role of HER2 status as a prognostic factor and as a predictor of response to therapy has been the use of retrospective analyses. This has resulted in small, under-defined patient subsets being assessed, reducing the power to detect significant differences between patient groups, while increasing the likelihood of finding a significant result by chance alone.

The value of HER2 status as a prognostic factor or predictor of response to therapy has not been determined unequivocally because the evidence available has been somewhat contradictory. While the majority of reports indicate that HER2-positive tumors are resistant to hormonal therapy, the consensus on response to chemotherapy treatment is less clear. There are several explanations for this, including differences in type and dose of therapy, the presence or absence of endocrine components in the treatment regimen, differences in patient age (post- or pre-menopausal), treatment of adjuvant or advanced disease, and whether the treatment is first or second line. The ideal protocol to assess the relevance of HER2 status would be a large, well-defined, prospective study with adequate descriptions of study protocols and patient demographics. In addition, due to the effect that different methods of HER2 testing can have on the reported incidence of HER2 overexpression, it may be necessary to have a standard preferred method for determining HER2 status. This will produce a higher degree of accuracy when directly comparing the results from different studies.

In summary, while HER2 is promising as an indicator of prognosis and responsiveness, the statistical significance of this is marginal at the present time. Appropriate prospective studies and analyses are required to shed light on the complex interactions between different cytotoxic therapies and the relationship of this to HER2 status. It is important to note that until the prognostic/predictive value of HER2 amplification/overexpression has been determined conclusively, no therapeutic regimen should be overlooked based solely on a patient's HER2 status.

Note

The author has not reported any financial relationships with companies whose products are mentioned in the text.

References


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