Predictability of Adjuvant Trastuzumab Benefit in N9831 Patients Using the ASCO/CAP HER2-Positivity Criteria

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The 2007 American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) joint guidelines defined criteria for HER2 positivity of tumors that modified those of the US Food and Drug Administration (FDA), causing some confusion and uncertainty among clinicians. Using data from the HER2-positive breast cancer adjuvant trial N9831, we compared eligibility for patients who met both criteria, and disease-free survival (DFS) was assessed by Cox proportional hazards regression. The number of patients in the N9831 trial retrospectively eligible for trastuzumab therapy was decreased when ASCO/CAP criteria vs FDA criteria were applied to immunohistochemistry and/or fluorescence in situ hybridization results (107 [3.7%] of 2904 patients with immunohistochemistry results, 37 [1.3%] of 2809 patients with fluorescence in situ hybridization results, and 47 [1.7%] of 2809 patients with both results). Improvement in DFS was similar among patients treated with trastuzumab under either set of criteria (concurrence trastuzumab and chemotherapy compared with chemotherapy alone: by ASCO/CAP criteria, hazard ratio of DFS = 0.59, 95% confidence interval = 0.48 to 0.73; by FDA criteria but not ASCO/CAP criteria, hazard ratio = 0.60, 95% confidence interval = 0.12 to 3.13; number needed to treat to prevent one additional DFS event at 5 years: 10 and 11.2 patients, respectively). Following the 2007 ASCO/CAP criteria for HER2 positivity would negate the option of potentially life-saving trastuzumab therapy for a small but meaningful group of patients. We recommend using FDA-approved HER2 criteria for therapeutic decision making.

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Accurate assessment of HER2 status became fairly controversial after the 1998 approval of trastuzumab for HER2-positive metastatic breast cancer by the US Food and Drug Administration (FDA) and the approval as adjuvant therapy for patients with HER2-positive disease granted in 2005 (1–6). The original, and still current, definition for HER2 positivity approved by the FDA is a score of 3+ by immunohistochemistry (IHC), defined as complete intense membrane staining of more than 10% of tumor cells. HER2 gene amplification is also a component of the criteria and was defined as a HER2 gene to chromosome 17 (HER2/CEP17) ratio of at least 2.0 by fluorescence in situ hybridization (FISH) (7–10). These definitions of HER2 positivity were used for patient enrollment into prospective randomized adjuvant trials of trastuzumab (6,11–16).

The 2007 American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) guidelines (17) modified the FDA criteria for HER2 positivity, which have been used in pivotal adjuvant trastuzumab trials (7–10), and defined HER2-positive results according to one of the following guidelines: HER2 positivity by measuring protein expression was defined as an IHC score of 3+ with more than 30% of the cell membranes staining intensely, and gene amplification was defined as more than six gene copies per nucleus or a HER2/CEP17 ratio greater than 2.2. Equivocal results are defined as tissues with an IHC score of 2+ (complete but weak or weak to moderate cell membrane staining in >10% of cells), 4.0–6.0 HER2 gene copies per nucleus determined by FISH, or a HER2/CEP17 ratio of 1.8–2.2 determined by FISH. The ASCO/CAP guidelines recommend that equivocal IHC samples be reassessed using a validated assay for HER2 gene amplification and that equivocal FISH samples have additional nuclei counted or be retested using IHC (17).

Differences in the criteria used in clinical trials, HER2 testing methods, and HER2-positivity criteria create uncertainty among clinicians and investigators in their accepted definitions of HER2 positivity. Little is known about how the updated criteria for HER2 positivity in the ASCO/CAP guidelines affect patient eligibility for treatment or clinical outcomes associated with HER2-targeted therapy. These new definitions have led to confusion among practitioners about which criteria should be used for consideration of anti-HER2 treatment for breast cancer patients, although that was not the intention of the ASCO/CAP committee.

To investigate these issues, we conducted an analysis of breast tumor samples from 2904 patients enrolled in the randomized phase 3 N9831 (NCT00005970) (18) trial that investigated trastuzumab as an adjuvant therapy for patients who had HER2-positive resected early breast cancer. The three-armed trial randomized patients to receive chemotherapy alone (Control; arm A), or with trastuzumab given sequentially (arm B) or concurrently (arm C) with chemotherapy. The endpoints of the trial included disease-free survival (DFS) and overall survival (Supplementary Materials, available online). The study was approved by the institutional review boards of the participating institutions (see Supplementary Methods, available online), and all patients provided written informed consent. Details of the trial protocol, including detailed accrual characteristics and clinical outcomes,
CONTEXTS AND CAVEATS

Prior knowledge

In the year 2007, the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) outlined criteria for clinicians to use to determine HER2 positivity of tumors for patient inclusion in clinical trials. The ASCO/CAP guidelines were a modified version of the US Food and Drug Administration (FDA) criteria that have been in use for trastuzumab trials, such as N9831. Two sets of HER2-positivity criteria with overlap have created confusion among clinicians on which should be used to determine patient eligibility in clinical trials.

Study design

Data on HER2 status from immunohistochemistry of paraffin-embedded tissues and/or fluorescence in situ hybridization of deparaffinized tissue sections from patients enrolled in the N9831 trial were analyzed. The number of HER2-positive patients retrospectively eligible for the trial under either the ASCO/CAP or FDA-only criteria was investigated. The improvement in disease-free survival observed among patients meeting either set of criteria who were treated with trastuzumab was also compared.

Contribution

The number of patients eligible for trastuzumab therapy was decreased under the ASCO/CAP criteria compared with those eligible under the FDA criteria. This created a group of patients who may benefit from trastuzumab therapy who would not be eligible under the ASCO/CAP criteria. Also, disease-free survival observed among patients treated with trastuzumab was similar when either set of criteria were applied to determine patient eligibility.

Implication

The FDA HER2-positivity guidelines should be used to determine HER2 status for clinical trial eligibility.

Limitations

The number of patients who were HER2 positive under the FDA but not the ASCO/CAP criteria was relatively small, and analyses to determine the statistical significance of the relationship between improvements in disease-free survival and administration of trastuzumab therapy in HER2-positive patients under either set of criteria could not be assessed.

From the Editors

have been previously published (6,19,20) and are briefly described in the Supplementary Materials (available online). Furthermore, a detailed patient flow diagram (Supplementary Figure 1, available online), inclusion and exclusion criteria, and accrual information are also included in the Supplementary Materials (Supplementary Methods, available online). We retrospectively applied the original FDA criteria used for enrollment into the study or the ASCO/CAP criteria to these samples to determine HER2 positivity under both criteria to investigate the effect of each on the number of patients eligible for the trial and the relationship between DFS and trastuzumab therapy in the resulting cohorts.

The original HER2-stained slides from the N9831 trial were reread, documenting the percent of cells with positive staining by our pathologists blinded to patient outcome. The percent of tumor cells with 0, 1+, 2+, and 3+ IHC staining intensities were determined, and these results were used for comparisons of the two criteria and for additional statistical analysis. FISH slides were not scored as the exact HER2/CEP17 ratios for gene amplification were documented at the time of the initial reading. The IHC 3+ interpretation guidelines determined by ASCO/CAP were set at a revised threshold of greater than 30% membrane staining of tumor cells to reduce the risk of false-positive results. The revised threshold reflected the cumulative experience of panel members and published reports using cutoff values higher than the FDA recommended 10% (17,21).

Cox proportional hazards regression was used to compare DFS between patients randomly assigned to receive standard chemotherapy alone or chemotherapy with trastuzumab in different IHC (0 and 1+, 2+, 3+ and >10% to 30% strong membrane staining, or 3+ and >30% strong membrane staining)/FISH (ratios of HER2 to CEP17 of <1.8, 1.8–<2.0, 2.0–2.2, >2.2, and unknown) subgroups who met either the FDA but not ASCO/CAP or ASCO/CAP criteria. Patients were also stratified by hormone receptor status (estrogen or progesterone receptor positive vs other) and nodal involvement (axillary nodal dissection with 1–3 positive nodes vs axillary nodal dissection with 4–9 positive nodes vs axillary nodal dissection with ≥10 positive nodes vs positive sentinel node with no or negative axillary nodal dissection performed vs negative sentinel node with no axillary nodal dissection performed vs axillary nodal dissection with no positive nodes). Assumptions of proportionality were confirmed using the method of Lin et al. (22). Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. DFS, as defined in the study protocol, at specific time points was determined using the Kaplan–Meier method. All statistical tests were two-sided, and a P value of less than .05 was considered statistically significant. The absolute risk reductions were calculated as the difference between arms in the Kaplan–Meier 5-year rates of DFS events. The numbers needed to treat were calculated as the reciprocal of the absolute risk reductions.

The results of our analyses showed that, when applying the ASCO/CAP criteria retrospectively, 107 (3.7%) of 2904 patients identified as HER2 positive by IHC and enrolled in N9831 would not have been eligible for enrollment. It has been estimated that a false-negative rate of this magnitude could negatively affect the care of 3000–5000 women annually in the United States alone. If these patients were retested using FISH, the number of ineligible patients would have been reduced to 29 (1.0%). According to the ASCO/CAP criteria for HER2 positivity by FISH, 37 (1.3%) of 2809 patients would have been ineligible. If these patients were retested using IHC, the number of ineligible patients would have been reduced to 31 (1.1%). Of the 2809 patients with both IHC and FISH results, 47 (1.7%) patients were HER2 positive by FDA criteria but not by ASCO/CAP criteria for IHC or FISH (Table 1). Among these 47 patients, 22 (47%) and 31 (66%) were HER2 positive by FDA criteria for IHC and FISH, respectively. These results indicate that the number of patients in the N9831 trial retrospectively eligible for trastuzumab therapy was decreased when ASCO/CAP criteria vs FDA criteria were applied. Furthermore, determining HER2 positivity on the basis of both IHC and FISH results vs either IHC or FISH alone decreases the number of ineligible patients and decreases the number of false-negative results.

Among patients HER2 positive by ASCO/CAP criteria for IHC or FISH, concurrent trastuzumab and chemotherapy
Table 1. Analysis of FISH HER2 gene amplification results by IHC subgroup (n = 2904) in patients from the N9831 breast cancer trial*

<table>
<thead>
<tr>
<th>HER2 IHC staining level</th>
<th>&lt;1.8</th>
<th>1.8 to &lt;2.0</th>
<th>2.0 to 2.2†</th>
<th>&gt;2.2‡</th>
<th>ND</th>
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<tr>
<td>0, 1+</td>
<td>137</td>
<td>66 (48.2)</td>
<td>3 (2.2)</td>
<td>9 (6.6)§</td>
<td>49 (35.8)</td>
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<td>2+</td>
<td>221</td>
<td>69 (31.2)</td>
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<td>3+ and &gt;10% to 30% strong membrane staining of tumor cells†</td>
<td>107</td>
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<tr>
<td>3+ and &gt;30% strong membrane staining of tumor cells‡</td>
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* CEP17 = chromosome 17; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; ND = not done. HER2 was assessed by IHC and FISH using whole tissue sections from patients enrolled in the N9831 trial. HER2 staining by IHC was graded on a scale of weak (0, 1+) to strong (3+), and the percentage of tumor cells having strong membrane staining for HER2 was determined when a score of 3+ was given. Data of the HER2/CEP17 ratio was used as a measure of HER2 gene amplification.
† Includes patients identified as HER2 positive by US Food and Drug Administration criteria.
‡ The cutoff for HER2 positivity by American Society of Clinical Oncology and College of American Pathologists joint criteria.
§ Values indicate the number and percentage of patients who would be HER2 positive by US Food and Drug Administration criteria but not by criteria set forth jointly by the American Society of Clinical Oncology and College of American Pathologists.

Figure 1. Disease-free survival (DFS) by treatment group in patients with tumors defined as HER2 positive by either immunohistochemistry (IHC) or fluorescence in situ hybridization meeting American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines or meeting US Food and Drug Administration (FDA) guidelines but not ASCO/CAP guidelines. HER2-positive status by ASCO/CAP criteria is IHC 3+ with greater than 30% tumor cell membranes strongly stained or a HER2/CEP17 ratio of greater than 2.2. FDA criteria define HER2 positivity as IHC staining of 3+ with greater than 10% tumor cell membranes strongly stained or a HER2/CEP17 ratio of 2.0 or greater. Cox proportional hazards regression was used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) and two-sided P-values. An asterisk indicates that the hazard ratio was calculated without stratifying for hormone receptor status or nodal involvement. AC = doxorubicin plus cyclophosphamide; H = trastuzumab; Ref = reference; T = paclitaxel.

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statistically significantly improved DFS relative to chemotherapy alone (HR of DFS = 0.59, 95% CI = 0.48 to 0.73; P < .001) (Figure 1). For the 47 patients who met FDA criteria but not ASCO/CAP criteria, the hazard ratio for DFS (HR = 0.60, 95% CI = 0.12 to 3.13) was similar to that of the patients deemed positive by ASCO/CAP criteria. This number did not reach statistical significance (P = .55), probably because of the small number of patients (Figure 1). Results from exploratory analyses also showed descriptive improvement in DFS with concurrent trastuzumab for patients with greater than 10%–30% 3+ tumor cell staining by IHC (n = 67; HR = 0.40, 95% CI = 0.09 to 1.75; P = .18; data not shown), which was not statistically significant.

On the basis of estimated 5-year DFS rates in the cohort with disease meeting the ASCO/CAP criteria and in the cohort with disease meeting the FDA criteria but not ASCO/CAP criteria, the absolute risk reduction of a DFS event at 5 years associated with concurrent trastuzumab was 10% and 8.9%, respectively. This translates to a number needed to treat to prevent one additional DFS event at 5 years of 10 and 11.2 patients, respectively (Supplementary Table 1, available online). Consistent with other descriptive presentations in this communication, the absolute risk reduction and number needed to treat describe similar trastuzumab effectiveness in these two cohorts of patients. These findings raise the possibility that adoption of the ASCO/CAP criteria, which are now mandated as part of HER2 testing laboratory accreditation, may be too restrictive, with the potential to miss patients with early breast cancer who may benefit from the addition of adjuvant trastuzumab.

Figure 1: Disease-free survival (DFS) by treatment group in patients with tumors defined as HER2 positive by either immunohistochemistry (IHC) or fluorescence in situ hybridization meeting American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines or meeting US Food and Drug Administration (FDA) guidelines but not ASCO/CAP guidelines. HER2-positive status by ASCO/CAP criteria is IHC 3+ with greater than 30% tumor cell membranes strongly stained or a HER2/CEP17 ratio of greater than 2.2. FDA criteria define HER2 positivity as IHC staining of 3+ with greater than 10% tumor cell membranes strongly stained or a HER2/CEP17 ratio of 2.0 or greater. Cox proportional hazards regression was used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) and two-sided P-values. An asterisk indicates that the hazard ratio was calculated without stratifying for hormone receptor status or nodal involvement. AC = doxorubicin plus cyclophosphamide; H = trastuzumab; Ref = reference; T = paclitaxel.
Our study was not without limitations. The relatively small number of patients identified in the “window” between the different guidelines defining HER2 positivity is a limitation to this study, making it difficult to derive definitive statistical conclusions about the improvement in DFS associated with concurrent trastuzumab for patients with tumors that are HER2 positive by FDA criteria but not HER2 positive by ASCO/CAP criteria. Also, our analysis revealed that up to approximately 4% of patients may miss receiving a recommendation to receive adjuvant trastuzumab, depending on the testing done, if the ASCO/CAP vs the FDA cutoffs for HER2 positivity are used. These cancers would be “false negatives” using ASCO/CAP criteria. Our study could not address the so-called false-positive results using ASCO/CAP cutoffs, as all positives by ASCO/CAP criteria were also positive by the FDA criteria used in the N9831 trial. Moreover, our study could not assess a potential false-positive rate associated with either guideline because the clinical trial enrolled only patients with HER2-positive disease.

In our study, a greater number of patients eligible for trastuzumab therapy were identified by HER2 positivity under the FDA criteria vs the ASCO/CAP criteria. Extrapolating these numbers to the estimated 50930 patients diagnosed with HER2-positive breast cancer in 2010 suggests that these data could potentially affect management of 662–1884 patients per year in the United States (23). Our data support a recommendation that the FDA HER2-positivity criteria be used for patient selection for adjuvant trastuzumab therapy.

References


