Fixation Time Does Not Affect Expression of HER2/neu

A Pilot Study

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Key Words: Fixation time; HER2/neu; Immunohistochemistry; Breast cancer

DOI: 10.1309/AJCPAIJPSN4A9MJI

Abstract

It is said that HER2/neu expression by immunohistochemical analysis varies with the time of fixation. The purpose of this pilot study was to determine the impact of the length of fixation in 10% buffered formalin on the expression of HER2/neu by immunohistochemical analysis.

We studied tissue samples from 10 invasive breast cancer cases after fixation for 3, 48, 72, 96, and 120 hours. The tissue was processed immediately after fixation, resembling routine practice. The 50 resulting blocks were then batch stained with PATHWAY HER2/neu clone 4B5 rabbit monoclonal antibody using the Ventana Ultraview DAB detection kit in a Ventana BenchMark XT processor (Ventana, Tucson, AZ). The stained slides were reviewed and scored.

We found no significant difference in the intensity of the stain or the percentage of cells stained regardless of the time of fixation. Fixation times between 3 and 120 hours in 10% buffered formalin do not appear to have an impact on the expression of HER2/neu by immunohistochemical analysis.
All cases, except cases 5, 7, and 10, were processed using a short cycle for 2 hours, 45 minutes in a Shandon Excelsior Tissue Processor (Thermo Scientific, Waltham, MA). Case 5 at 48 hours and case 10 at 3 hours were processed in a 4-hour cycle in a Leica Peloris tissue processor (Leica Microsystems, Bannockburn, IL). The remainder of the blocks from cases 5 and 10 and the entire case 7 were processed overnight for 11 hours in a Shandon Excelsior Tissue Processor. The difference in processing times was a logistic issue determined by processor availability at the conclusion of the specified fixation interval. All 3-hour blocks were processed after exactly 3 hours of fixation. The other blocks (fixation times 48, 72, 96, and 120 hours) were processed within the time defined or within ± 1 hour of the defined time. For example, some of the 48-hour blocks may have been in fixative for 47 or 49 hours instead of the defined 48 hours.

All blocks were then batch stained with PATHWAY HER2/neu clone 4B5 rabbit monoclonal antibody using a Ventana Ultraview DAB detection kit in a Ventana BenchMark XT processor (Ventana, Tucson, AZ). Antigen retrieval was a standard automated process on the Ventana BenchMark XT at 37°C for 16 minutes. The antibody was used as received from Ventana prediluted at a stated final concentration of 6 μg/mL of specific antibody. We reviewed every slide independently following the ASCO/CAP guidelines for HER2/neu scoring.1

Results

We analyzed 50 blocks from 10 cases of invasive breast carcinoma. All samples, regardless of whether they were fixed for 3, 48, 72, 96, or 120 hours, had diffuse membranous staining with HER2/neu; most of the case samples had strong staining intensity. No significant staining difference was noted among the various fixation times. The block from case 3 at 48 hours did not have tumor for evaluation. Because all of our blocks had similar staining results, we include only 2 representative cases, which are shown in Image 1. These cases demonstrate that the intensity of staining does not change between 3 and 120 hours of fixation.

Discussion

Much debate has taken place regarding the “gold standard” testing method for determining patient eligibility for treatment with trastuzumab. The most frequently used testing methods for HER2/neu include immunohistochemical analysis, fluorescence in situ hybridization, and chromogenic in situ hybridization. Because of technical and interpretation issues with the immunohistochemical technique, central laboratories in charge of reviewing cases for different research protocols have found significant variation in the results submitted by community pathologists and their own. In an attempt to standardize the technical processes and the interpretation of HER2/neu, the ASCO and CAP created a task force. This group generated a special article with recommendations for human epidermal growth factor receptor 2 testing in breast cancer.1 While this document has several good recommendations, some of the imposed guidelines are not evidence-based. This is the case for the required formalin fixation interval of 6 to 48 hours. This imposed time limit has created logistical problems in the processing of specimens for many laboratories across the United States. Moreover, the CAP has added another standard in its inspection checklist to this effect.2 Standard ANP.22998 asks “If the laboratory assesses Her2...
protein over-expression by immunohistochemistry (IHC) or Her2 gene amplification by in situ hybridization, or estrogen/progesterone receptor expression by immunohistochemistry, there is a documented procedure to ensure appropriate fixation time.”

Our studies have suggested that formalin fixation time over wide intervals has little or no impact on the expression of routine prognostic markers. We demonstrated in our recently published series that estrogen receptor expression is essentially unaffected by short fixation times. HER2/neu expression was shown in this study to be essentially unaffected by brief and extended periods of buffered formalin fixation. This pilot study examined only cases known from core biopsy testing to exhibit 3+ overexpression of HER2/neu. Also, our estrogen receptor study included only high expressors (or strongly positive cases). It would be of considerable interest to also study the effects of fixation time, if any, in cases with intermediate levels of estrogen receptor and HER2 expression. Nevertheless, in this study, the failure of prolonged or very short fixation intervals to measurably diminish HER2 expression suggests that other factors than fixation times may be more important in producing variable results in known overexpressors.

Recently, Khoury et al reported a study in which they determined that delayed time to fixation has a negative impact on the expression of prognostic markers. Our study supports the findings of Khoury et al and suggests that preanalytic factors other than the absolute time in formalin may have an impact on the quality of HER2/neu immunostaining. All tissues in this study were delivered to the laboratory from the operating suite immediately after excision. At that point, tissues were sliced thinly (0.5-1.0 cm). A 3-mm core was obtained from one of the slices with tumor and immediately placed in neutral buffered formalin for the specified intervals before processing to paraffin. As a consequence, this study controlled for many other potential preanalytic sources or causes of antigenic degradation. Ambient temperature effects on unfixed tissue and delay in placing thinly sliced tissue in fixative may be major factors in the variability of HER2/neu staining of concern to the breast cancer treatment community. For example, a mastectomy specimen obtained Friday morning could remain all day at room temperature in the operating room. Next, late Friday in the laboratory, the specimen might be incompletely sliced at 2-cm intervals and placed in formalin, but the slices might not be adequately separated to allow exposure to fixative. Monday, tumor tissue chosen for histologic examination may originate from areas with incomplete formalin penetration. On paper, the tissue was in formalin for more than 48 hours, but in fact, the tissue may have been unfixed for more than 72 hours. Dintzis and Allison recently studied the effect of the length of formalin fixation in cell culture material and concluded that the expression of HER2/neu as detected by immunohistochemical analysis was unaffected by prolonged fixation times.

Perhaps the recommendations emanating from ASCO/CAP should focus more on time before fixation of thinly sliced specimens rather than time tissue remains in formalin. Fixation times in 10% buffered formalin between 3 and 120 hours do not affect HER2/neu expression. Further studies are needed to confirm this finding.

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Supported by a grant from the Foundation at Orange Coast Memorial Medical Center.

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References


