

Breast Cancer Predictive Factor Testing: the Challenges and Importance of Standardizing Tissue Handling

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Introduction:

Breast cancer is a disease with significant biologic and clinical diversity requiring an individualized approach to management and treatment for each patient (1). A major clinical challenge is identifying patients who are more likely to develop recurrence and would potentially benefit from adjuvant therapy. In current clinical practice, breast cancer biomarkers, including the estrogen receptor and HER2, are routinely assessed to identify subsets of patients who are appropriate candidates for specific treatments that target these major molecular drivers of disease progression (2,3). However, the selection of patients who are likely to benefit from these targeted approaches remains challenging and requires that the assays for these biomarkers be as accurate as possible, given their role in determining optimal treatment (4,5).

Breast cancer predictive factor testing and adjuvant therapy:

The introduction of biomarkers into breast cancer diagnosis and their role in adjuvant treatment decisions represents a paradigm shift for pathology. These tissue samples are no longer used only for microscopic interpretation, but must also be of sufficient quality to preserve macromolecules that may have clinical relevance. The tissue sample has become an analyte and the quality of the tissue becomes an important element of validated assays that are performed on these samples (6,7). A key question is how variability in tissue handling affects the validity of these assays.

Tissue handling, warm and cold ischemic time and the accuracy of predictive factor testing:

The current clinical practice of tissue handling and specimen preparation is diverse and lacks strict standardization and results in significant variability in the quality of formalin fixed paraffin embedded clinical samples (7). The surgical disruption of blood flow to an excised clinical sample leads to progressive tissue ischemia, hypoxia and the degradation of macromolecules that are of potential clinical importance. The time between arterial ligation and tissue removal from the patient has been termed the warm ischemic time and can vary considerably, depending upon the complexity of the surgical procedure. During this interval the tissue remains alive, is reactive and will undergo progressive metabolic stress due to hypoxia. The cold ischemic time is the interval between removal of the tissue sample from the surgical field until incision of the tissue and placement in a suitable tissue fixative. Studies have shown that significant nucleic acid and protein changes occur during these ischemic time intervals (7,8,9). These changes stop when the tissue sample is placed into formalin and chemical fixation begins. Currently, the magnitude of these changes and their potential influence on clinically relevant target molecules such as ER and HER2 are poorly understood. Depending on the institution, and the processes and procedures in place, the time interval from disruption of blood flow and sample collection to the start of fixation can range from minutes to hours. For many institutions, both the time interval and the extent of variability are virtually unknown. Efforts to minimize and document both the warm and cold ischemic time will be important and will allow investigators to determine which genes and proteins are potentially valid and reliable as biomarkers for clinical decision-making (7). The warm

ischemic time is dependent on the surgical procedure and is harder to control. The cold ischemic time is more dependent on institutional procedures involving tissue transport, and is an area where improvement can more readily be achieved.

Cold ischemic time and the potential impact on breast predictive factor testing:

Estrogen and progesterone receptors are thermolabile proteins whose levels of expression are altered by prolonged cold ischemic time (10). Recent reports suggest that delays from tissue collection to the initiation of formalin fixation may adversely affect both hormone receptor assays (10,11) and HER2 analysis (11), and that some tumors with excessive cold ischemic times may be falsely classified as negative. The consequences of invalid breast predictive factor testing could be catastrophic to the patient and has the potential to change the type of adjuvant therapeutic regimen offered, which in turn could adversely affect outcome.

New ASCO/CAP guidelines for ER and PR testing:

New ER and PR testing guidelines from the ASCO/CAP task force recommend that breast biopsies and excised breast tissue samples be placed in formalin within 1 hour from excision (5). This will require that the operating rooms and clinics record the collection time for each sample, and likewise that the laboratories record the fixation start times, so that the cold ischemic times for each clinical sample can be calculated and monitored. Upon removal, some excised breast specimens undergo specimen radiographs to help insure that the

targeted lesion has been excised. These specimens need to be transported to the laboratory where they are oriented, painted with ink for margin assessment and then carefully “bread-loafed” before being placed in fixative. Given these complexities and the need for coordination between surgery, radiology and the laboratory; we became interested in how difficult it would be for different institutions, including ours, to achieve the one hour time window and comply with this guideline recommendation.

URMC: a single institution experience with standardizing tissue handling:

At URMC, we have implemented a rapid tissue acquisition program in which technical personnel from pathology are stationed in the operating rooms and equipped with cell phones. They are notified when a specimen has been removed from a patient and then transport the tissue sample to the laboratory. The collection time from the operating room, the laboratory receipt time and the fixation start time are all recorded for each specimen. Data collection began in 2008 and we have recently audited cases with grossly visible tumors to see how well we are meeting the ASCO/CAP tissue handling requirements. A summary of our findings is shown in Figure 1.

Median cold ischemic time for breast cancer resection specimens at

URMC:

Figure 1 displays the median time to fixation for breast cancer excisions between 2008 and 2010, and the interquartile range of fixation times about the median. The overall median time for all years (n=137 cases) was 55 minutes, which just meets the 1 hour recommended ASCO/CAP standard. The median

time in 2008 was 61 minutes (n=37), 54 minutes in 2009 (n=79) and 49 minutes in 2010 (n=21) (Chi-Square test for differences in medians was significant at $p=0.0174$, using “Analyse-it” software for Excel). The median interval was significantly longer in 2008 than in both 2009 ($p=0.0456$, Fisher Exact Test) and 2010 ($p=0.0277$, Fisher Exact Test). Collection to formalin times were not significantly different between 2009 and 2010 ($p=0.3262$, Fisher Exact Test). Our data suggests that the one hour window proposed by the ASCO/CAP task force is achievable but it required a system change in our institution in the way tissue was transferred between the OR and the laboratory, and a commitment of personnel and resources to achieve this goal.

Conclusions:

The use of breast predictive factor assays to identify subsets of breast cancer patients suitable for targeted therapeutics requires that such assays be as accurate and reliable as possible (3). The growing importance of molecular pathology and the use of biomarkers in clinical medicine have led to an increased emphasis on optimal tissue preparation for these assays (12). It has become increasingly clear that every effort must be made to avoid delays from tissue collection to the initiation of formalin fixation. The emerging data for breast cancer specimens suggest that delays as short as 1 to 2 hours can begin to compromise assay validity (11). In addition, the new ER and PR testing guidelines from the ASCO/CAP task force state that breast biopsies and excised breast tissue samples need to be incised to expose the tumor and placed in formalin within 1 hour of removal (5). The experience at our institution suggests

that this goal is achievable, but not without a commitment of resources and personnel and a significant change in practice, specifically an emphasis on standardizing tissue handling.

Awareness of these new developments should prompt every institution involved in the care of breast cancer patients to review their procedures related to tissue collection and determine if this goal is currently being met. Going forward, a greater emphasis must be placed on developing standardized methods of tissue procurement and processing for all types of tumor specimens, accompanied with documentation regarding how these variables affect the quality of clinical samples for molecular analysis. This is an area requiring further research on biospecimen quality, so that evidence based guidelines for best practices in surgical pathology can be developed and disseminated. Such standards must be practical, realistic and achievable in both academic medical centers and community practice settings. The ultimate goal for pathology laboratories, in collaboration with surgeons, is to adopt standard operating procedures that will, if possible, reduce ischemic times to less than 30 minutes (13). The issue of ischemic time is important not only for breast cancer, but also is becoming increasingly important for all solid tumors that are resected. Having a better understanding of how specimen handling requirements affect diagnostic testing, and approaching excised tissues as an analyte for molecular analysis, will greatly improve the quality of clinical samples and thus provide more accurate information on which to base adjuvant treatment decisions. These

efforts also will improve the quality of archival tissue samples for future biomarker discoveries and translational research.

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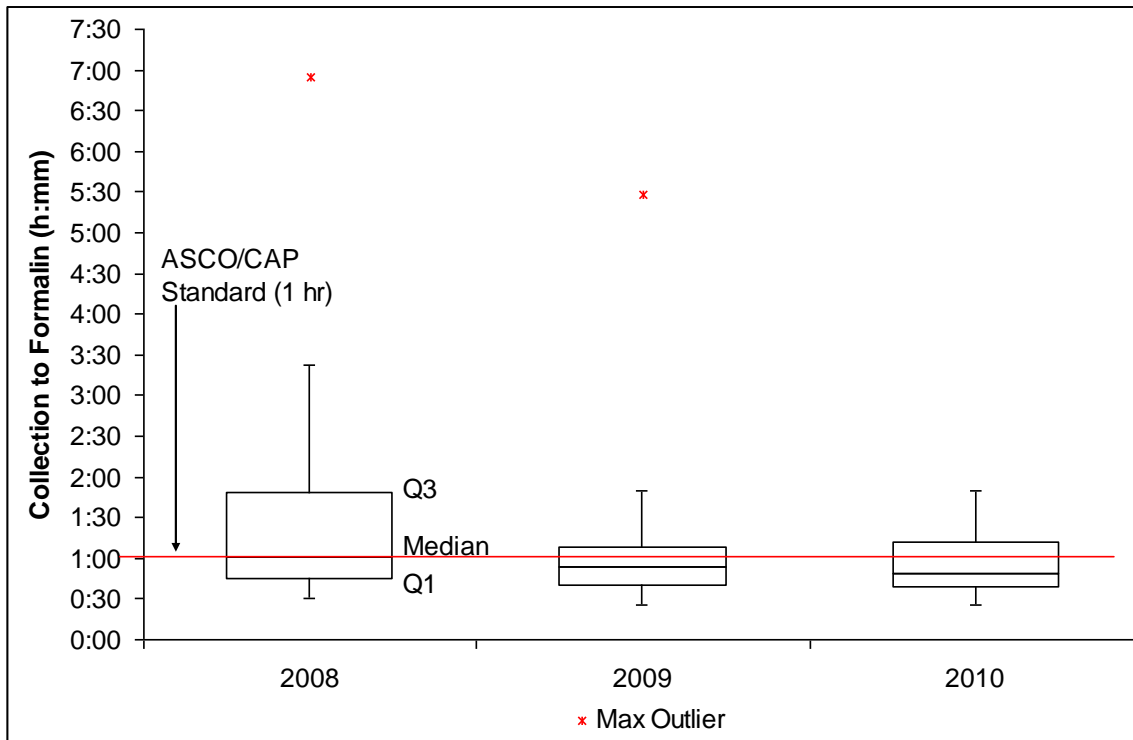


Figure 1: This figure shows the median collection time to the time in formalin for tumor specimens collected at Strong Hospital from 2008-2010 (This figure was generated using the Vertex42 template in Excel). Histograms of each of the years demonstrated a positive skew, and non-normality was confirmed with Normal Quantile Plots (NormQuant.xls, Dr. Scott Guth at Mt. San Antonio College). Because of this, the median was taken over the mean as a more reliable estimate of central tendency.

	2008	2009	2010
Min	0:30	0:25	0:25
Q₁	0:45	0:40	0:39
Median	1:01	0:54	0:49
Q₃	1:48	1:08	1:12
Max	6:55	5:28	1:50
IQR	1:03	0:28	0:33

This table presents the data used to generate Figure 1 above.