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A rapid triage protocol to optimize cold ischemic time for breast resection specimens☆

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ABSTRACT

Prolonged time from specimen excision to adequate formalin exposure, or cold ischemic time (CIT), negatively impacts estrogen receptor (ER), progesterone receptor (PR) and HER-2 biomarker studies routinely performed on breast specimens. Current guidelines recommend CIT of ≤1 h. Since formalin penetrates resections slowly, optimal fixation requires incision. We evaluated the efficacy of a rapid triage protocol developed to optimize CIT. We identified 2821 specimens: 650 (23.0%) excisional biopsies (EB), 1051 (37.3%) lumpectomies, and 1120 (39.7%) mastectomies. CIT was available for 2362 (83.7%), with 1845 (78.1%) ≤1 h and 2323 (98.3%) ≤4 h. IHC was performed in 533/2821 (18.9%) and was associated with lumpectomy and mastectomy procedures when compared to EB. However, IHC was also performed on 11.1% (72/650) of EB specimens despite EB being significantly less likely to have CIT recorded (468/650; 72% for EB vs. 1894/2171; 87.2% for lumpectomies/mastectomies).

Our study highlights the need for rapid triage of breast resections with known or suspected malignant diagnoses and outlines our procedure for optimizing CIT. Additionally, we advocate treating ALL breast resections as having the potential of being malignant and requiring biomarker studies for which optimal CIT is of great importance.

1. Introduction

Prolonged cold ischemic time (CIT), or time from tissue removal until *adequate* formalin exposure, negatively impacts estrogen receptor (ER), progesterone receptor (PR) and/or HER-2 biomarker studies routinely performed on breast specimens [1–4] and reduces RNA integrity of fresh breast specimens [5,6]. According to 2010 College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guidelines, CIT of ≤1 h is recommended [7]. Additionally, some publications support that CIT of up to 2–3 or even 4 h (if the specimen is refrigerated) may be acceptable [4,8,9]. However, since formalin does not rapidly penetrate resections, optimal formalin exposure of tumor within these specimens does not occur unless it is incised.

Immunohistochemical (IHC) expression of ER and PR provide insight into tumor prognosis as hormone receptor positive tumors are frequently well-differentiated, have low proliferative indices, and respond to endocrine therapy [10,11]. Similarly, overexpression/amplification of HER-2 is associated with high histologic grade, lower responsiveness to endocrine therapy, and response to trastuzumab therapy [12].

Repeat evaluation of ER, PR and/or HER-2 on resection specimens is performed in selected cases per CAP/ASCO guidelines. In general, a previous biopsy with indeterminate/equivocal results, small tumor size, lack of adequate control staining or immunoprofile discordant with tumor histology or grade are scenarios in which repeat analysis is recommended [7,13]. Due to concerns for tumor heterogeneity, most recent (2013) CAP/ASCO guidelines for HER-2 testing add recommendation for repeat analysis if the tumor is grade 3 and previous testing was negative [13]. With this, and other recommendations, repeat testing on breast resections is increasing; thus, optimizing CIT for these specimens is of growing importance. We sought to examine the efficacy of a rapid triage protocol to reduce CIT for breast resections at a large academic institution.

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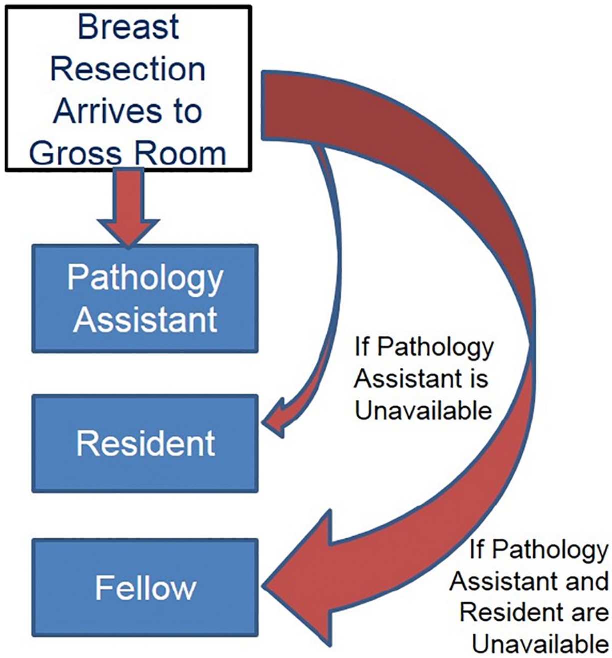

Fig. 1. Daytime procedure for rapid triage of breast resections.

2. Methods

2.1. Triage protocol

We developed a triage protocol for surgically-excised breast resections, including excisional biopsy (EB), lumpectomy and mastectomy specimens, which was implemented in January 2014. New specimen labels with space for operating room staff to record excision time (ET) were used.

During daytime weekday hours (8:00–17:00), breast specimens were delivered to the frozen section laboratory, where the specimen was treated with the same immediacy as a frozen section specimen (Fig. 1). After accessioning, the specimen followed a triage protocol, which consisted of weighing, measuring, inking and incising the specimen before placing in formalin. The pertinent information, including time the incised specimen was placed in formalin, was recorded on the specimen requisition or specimen container for reference for the individual who would later perform an in-depth gross evaluation (Fig. 2). Time in formalin (TIF) and/or calculated CIT were included in the gross description, and therefore included in the final pathology report. During non-business hours triage was performed by the first available of a night shift pathology assistant, on-call resident or fellow.

2.2. Case selection and review

Following institutional board review approval, we identified breast resection specimens from 2014 to 2016 (after triage protocol implementation) using key word searches. Pathology reports were reviewed for clinicopathologic features including patient age, general diagnostic category (benign, atypia, or malignant), availability of CIT, absolute and categorical (≤1 h, > 1 but ≤4 h, > 4 h) CIT, missing data (ET and/or TIF) when CIT was unavailable, and the performance of ER, PR and/or HER-2 studies.

2.3. Statistical methods

Descriptive statistics presented include continuous data (mean and range) and categorical data (frequency and percentage). Chi-square and *t*-tests were used to test differences in proportions and means. Following simple logistic regression, multivariable logistic regression models were used to associate procedure (lumpectomy, mastectomy, and excisional biopsy), completion of IHC, diagnosis and age to the CIT outcome (recording of CIT or categorical CIT dichotomized using a threshold of 4 h). A multivariable logistic regression model was used to associate procedure (lumpectomy, mastectomy and excisional biopsy), age, CIT availability and CIT categories with completion of IHC. For multivariable regression with the IHC outcome, a stepwise variable selection procedure was used to select important variables to be included in the final logistic regression model (a significance level of 0.35 was used to allow a variable into the model, and a significance level of 0.20 was used for a variable to stay in the model). A $p \leq 0.05$ was considered to be statistically significant. All analyses were conducted using SAS (version 9.4, SAS Institute, Cary, NC).

3. Results

2821 specimens consisting of 650 (23.0%) EB, 1051 (37.3%) lumpectomy, and 1120 (39.7%) mastectomy specimens from 2344 patients were identified. The mean age at the time of surgery was 53.5 years (range 11–96) and 99.6% of patients were female. Final histologic diagnoses were benign in 1077 (38.2%), atypical in 208 (7.4%) and malignant in 1536 (54.4%) (Table 1).

CIT was available for 2362 of 2821 (83.7%) specimens. Five had nonnumeric (approximate) values for CIT, but had categorical CIT recorded. Of the 2362 specimens with available CIT, 1845 (78.1%) had CIT ≤1 h, 478 (20.2%) between 1 and 4 h and 39 (1.7%) > 4 h. 459 of 2821 (16.3%) specimens had no CIT available, of which 143 (31.2%) were missing both ET and TIF, 87 (18.9%) ET only and 229 (49.9%) TIF only. Of cases without CIT available, 182 (39.7%) were EB, 136 (29.6%) lumpectomies and 141 (30.7%) mastectomies. Additionally, of 459 cases without CIT available, 61 (13.3%) had IHC performed.

On multivariable analysis, availability of CIT was significantly associated with lumpectomy (OR = 2.670, 95% CI 1.926–3.703) and mastectomy procedure (OR = 2.734, 95% CI 2.097, 3.564) versus EB and the performance of IHC (OR = 1.534, 95% CI 1.097, 2.117), adjusting for age and diagnosis.

CIT ≤4 h (vs. > 4 h) was significantly associated with lumpectomy procedure versus EB (OR = 5.485, 95% CI 1.543, 19.502), adjusting for procedure, dx, age and performance of IHC, via multivariable analysis.

IHC was performed in 533 of 2821 (18.9%) specimens, all of which had malignant diagnoses. ER and/or PR were done in 64 (12%), HER-2 in 280 (52.5%) and ER and/or PR and HER-2 in 189 (35.5%). On multivariable analysis, performance of IHC was significantly associated with lumpectomy (OR 2.105, 95% CI 1.565, 2.830) and mastectomy (OR 1.490, 95% CI 1.10, 2.000) procedures when compared to EB, as well as age (OR = 1.018, 95% CI 1.010, 1.025) and CIT availability (OR = 1.449 95% CI 1.080, 1.944) (Table 2).

4. Discussion

In most cases of breast carcinoma undergoing surgical resection, a prior core biopsy yields adequate tumor for testing of ER, PR and HER-2. However, there are scenarios in which these studies are routinely repeated on breast carcinomas in resection (EB, lumpectomy and mastectomy) tissue [7,13]. Additionally, EB is performed in cases in which a lesion is not amenable to core biopsy such as diffuse microcalcifications, dense breast tissue and difficult location to access, or when a previous core biopsy suggests a benign or atypical diagnosis that does not correlate with more worrisome clinical and/or radiologic features. When subsequent EB yields a malignant diagnosis, biomarker studies must be performed for prognostic and therapeutic purposes.

In this study, over one-third (533/1536; 34.7%) of patients with malignant diagnoses had ER, PR and/or HER-2 performed on resection material, highlighting the need for optimal CIT and rapid triaging of resection specimens with known or suspected malignant diagnoses.