Frozen Section versus Gross Examination for Bone Marrow Margin Assessment During Sarcoma Resection

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Abstract

Background Complete resection is critical for local control of primary bone sarcomas. Intraoperative consultation, including frozen section of bone marrow margins, frequently is used to aid in this goal.

Questions/purposes We therefore sought to determine (1) how often intraoperative frozen section of a bone marrow margin correlates with inspection of the gross split specimen and, in cases of a discrepancy, what clinical decision is made; and (2) how well each of these assessments agrees with the final pathologic assessment of a marrow margin.

Methods One hundred ninety-five bone marrow margins from 142 patients (74 males, 68 females; mean age, 12.8 years) with primary sarcomas who underwent resection and had frozen section(s) performed on a bone marrow

margin were analyzed. Agreement between frozen section interpretation and inspection of the split gross specimen was analyzed in their application to determine adequacy of the bone marrow margin intraoperatively.

Results In 179 margins, the frozen section agreed with the gross inspection decision (95.6% negative agreement, 38.5% positive agreement). Decisions regarding further surgical action in all 16 instances of disagreement were based on inspection of the split gross specimen, and the frozen section was disregarded. In 195 of 195 margins, intraoperative decisions were made based on gross specimen inspection. Full pathologic examination confirmed negative final bone marrow margins in all patients.

Conclusions Frozen section is commonly redundant or disregarded for intraoperative surgical decisions, and it may be omitted, saving operative time and cost. Examination of split gross specimens appears an adequate adjunct to clinicoradiographic assessment to achieve negative margins in the current era of modern imaging and surgical techniques.

Level of Evidence Level II, diagnostic study. See Guidelines for Authors for a complete description of levels of evidence.

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Introduction

Primary sarcomas of bone affect roughly 2800 people in the United States every year [9]. Whether limb salvage or amputation is attempted, the universal goal is wide local resection with negative margins. Many centers use pathologic consultation for intraoperative margin assessment to ensure that margins are clear before proceeding with reconstruction. However, published protocols on the pathologic handling of bone tumor resection specimens provide

little or no guidance on specific methods for intraoperative assessment of margins [3, 6, 10, 11].

Emerging data have shown that imaging modalities, particularly MRI, have improved our ability to determine the local extent of bone sarcomas more exactly [4]. New techniques such as image navigation have improved our ability to make carefully directed osteotomies [2, 5]. Because of surgeons' increasingly better foreknowledge of tumor extent, the prevailing trend has been a decrease in acceptable margin size while maintaining good oncologic outcomes [1, 7], yet there are currently no published benchmark data on the rates of positive intraoperative margins requiring recuts and the rates of positive final margins in bone sarcoma resection specimens.

At our institution, the typical protocol has been to conduct intraoperative assessment of margin status by a combination of inspection of the split gross specimen (Fig. 1) and frozen section of the bone marrow margin from the residual bone. Assessment of a bone marrow margin with frozen section is technically challenging as a result of the fatty and bony nature of the specimen making it difficult to freeze and cut. We have found that inspection of the gross split specimen often shows the margin clearly with evident areas of tumor replacement and adjacent normal fatty bone marrow. We hypothesize that in the current era, many frozen section assessments of bone marrow margin are perfunctory and constitute a component of intraoperative evaluation that may not impact intraoperative management. Critical evaluation of the diagnostic use of bone marrow margin frozen section could lead to improved evidence-based practice algorithms that may save operative time and cost, resulting in improved patient care.

We therefore sought to determine (1) how often intraoperative frozen section of a bone marrow margin correlates with inspection of the gross split specimen and, in cases of a discrepancy, what clinical decision is made; and (2) how well each of these assessments agrees with the final pathologic assessment of a marrow margin.

Patients and Methods

Institutional review board approval was granted for the study of all patients undergoing bone resection for sarcoma between 1997 and 2011 at a single institution (Boston Children's Hospital, Boston, MA, USA). Cases were ascertained by a review of all pathology reports containing the words "bone marrow" and "margin". Inclusion criteria were (1) pathologic diagnosis of a bone sarcoma or soft tissue sarcoma with bone involvement necessitating en bloc bone resection at Boston Children's Hospital; and (2) intraoperative frozen section of at least one bone marrow margin was performed. Review of pathology archives returned 191 bone resections in 188 patients, of which 47 resections in 46 patients were excluded because the bone marrow margins were not assessed with frozen section. In 28 of these excluded patients, the bone was split intraoperatively and thus decisions about margin status were based on that alone. For the remaining 18 patients, intraoperative decisions about margin status were based on external inspection of the bone.

The standard institutional practice over the study period was to initiate intraoperative pathologic consultation at the time of bone sarcoma resection. Inspection of the gross split specimen was carried out immediately on resection.

Fig. 1A–B Example of a split gross specimen of a resected distal femur osteosarcoma. (A) View of whole split specimen. (B) Detailed view of gross marrow margin. Note fatty marrow apparent proximal to tumor edge indicating a negative bone marrow margin on gross inspection.

Table 1. Tumor histologic subtypes

Tumor	Number of patients
Osteosarcoma	106
Ewing's sarcoma	28
Bone sarcoma not otherwise specified	3
Osteoliposarcoma	1
Synovial sarcoma	1
Clear cell sarcoma	1
Alveolar soft part sarcoma	1
Angiosarcoma	1

The surgeon and pathologist discussed the best plane to cut the bone based on preoperative imaging, multidisciplinary conference discussion, and/or any limitations imposed by the specimen size or shape. The bone was cut using a band saw and then the distance from the tumor to the marrow margin was assessed with the naked eye and/or with the assistance of a dissecting microscope, usually supplemented by palpation. Generally, the distance from the margin was determined by the pathologist, whereas the decision as to whether that distance represented an adequate margin was left to the operating surgeon.

There were 142 patients with 144 bone sarcoma resection procedures during the study period who met the inclusion criteria (one patient had a second procedure for local recurrence 5 years after her initial resection and another had wide resection of a late rib metastasis). This included 74 (52%) males and 68 (48%) females. Age at resection ranged from 2 to 22 years (mean, 12.8 years; SD, 3.98). Types of sarcoma (summarized in Table 1) included osteosarcoma (75%), Ewing's sarcoma (20%), and rare other sarcomas (5%). Anatomic sites of resection included the femur (56%), tibia (23%), humerus (9%), fibula (5%), and less common other sites (Table 2).

Each intraoperative pathologic frozen section assessment of a bone marrow margin was counted as the number of margin diagnoses per single operation. The number of bone marrow frozen section assessments ranged from zero to four per case, including 104 cases in which one was made, 31 in which two were made, seven in which three were made, and two in which four were made. Each was a separate area of bone marrow margin assessment except in one case in which a second frozen section was performed on the same bone marrow margin after the bone was recut. The total number of frozen section assessments was 195. Frozen section diagnoses of marrow margins were classified as negative, positive, or indeterminate/suspicious. Final pathologic diagnoses of bone marrow margins were classified as negative or positive. Intraoperative gross findings were correlated with histologic findings. Additional data such as

Table 2. Location of tumor

Primary site of tumor	Number of cases
Femur	80
Tibia	34
Humerus	13
Fibula	7
Rib	3
Scapula	2
Elbow	2
Pelvis	2
Talus	1

age, sex, anatomic site, and pathologic diagnoses were also recorded.

The use of frozen section diagnosis to determine margin adequacy intraoperatively was assessed through agreement analysis. Based on the correlation between intraoperative assessment methods and subsequent surgical action, intraoperative gross assessment and frozen section diagnosis were classified as (1) in agreement: there was agreement between the gross assessment and the frozen section (leading to an additional recut if the margin was positive and no further recut if negative); or (2) not in agreement: there was lack of agreement between the gross assessment and the frozen section. Note was made as to which was weighted more heavily in intraoperative decision-making to perform an additional recut or not.

Calculations included the overall proportion of agreement between gross assessment and frozen section diagnosis as well as estimates of positive and negative agreement with 95% confidence intervals. For the purpose of this analysis, positive agreement refers to agreement on decisions where further surgical action was recommended and negative agreement refers to agreement on decisions where no further surgical action was recommended.

Because analysis of the permanent section is a reliable gold standard for the accuracy of frozen section assessments, the positive predictive value (PPV) and the negative predictive value (NPV) with exact 95% confidence intervals were estimated for the frozen section diagnoses with respect to the permanent section diagnoses. In addition, the PPV and NPV were estimated for the gross specimen diagnoses with respect to the permanent section outcome. For the latter analysis, specimens with a positive final margin included only those in which tumor touched the inked marrow margin of the resection specimen, because pathologic interpretation did not seek to set any arbitrary distance criteria for an adequate margin. Thus, the clinical and therapeutic implications of this final pathology finding were subjectively interpreted in conjunction with other variables. The NPV can be equitably estimated and

interpreted for gross findings, but the PPV is clinically negligible given the differing definitions of positive that were applied to a positive intraoperative gross split specimen (ie, prompting a recut) versus a positive final margin (ie, tumor directly at the margin). Determining whether or not a recut that consisted entirely of nonneoplastic bone (providing a greater final margin distance) was necessary was not a focus of this study.

Results

Frozen section diagnosis showed four bone marrow margins positive for tumor in three cases, three indeterminate or suspicious for tumor in two cases, and 188 negative for tumor. There were 21 margins in 18 patients in which the bone was recut. Five were recut based on an assessment that there was tumor present or close to the margin on gross examination of the specimen with correlative frozen section findings. Fourteen were recut based on the gross impression of a positive or close margin with a negative frozen section. In two cases, additional bone and surrounding soft tissue were taken over concern about a positive or close margin in the periosteum, not the marrow. In five of 195 margins, both inspection of the split gross specimen and frozen section of the bone marrow indicated the need for further surgical action (Table 3). In 14 margins, the frozen section was negative, suggesting no further surgical action was required, but inspection of the split gross specimen directed the surgeon to recut the bone at that margin. Conversely, there were two of 195 margins in which the frozen section was suspected to possibly be positive (called suspicious), yet inspection of the split gross specimen was reassuring and so no recut was performed. In the remaining 174 margins, there was agreement between both the frozen section and examination of the split gross specimen that no further surgical action was required. Intraoperative decisions based on the frozen section agreed with inspection of the split gross specimen in 179 of 195 margins, yielding an overall percent of agreement of 91.8% with estimated 95.6% negative agreement (95% confidence

Table 3. Agreement by assessment type and intraoperative decision

Frozen section	Gross split specimen	Agreement	Number of margins	Decision
Negative	Negative	Yes	174	None recut
Positive/suspicious	Positive	Yes	5	All recut
Positive/suspicious	Negative	No	2	None recut
Negative	Positive	No	14	All recut

Table 4. Agreement analysis

Agreement	Estimate	95% confidence interval
Overall percent agreement	91.8%	
Positive percent agreement	38.5%	26.4%–50.6%
Negative percent agreement	95.6%	94.5%–96.7%

Table 5. Bone marrow margin results

Margin result	Marrow frozen section	Marrow permanent
Positive	4	All confirmed positive
Suspicious	3	All confirmed negative
Negative	188	All confirmed negative

interval [CI], 94.5%–96.7%) and estimated 35.8% positive agreement (95% CI, 26.4%–50.6%) (Table 4).

Permanent sections of these margins confirmed the frozen section findings in all except the three suspicious margins (Table 5). These three were determined to be negative on final pathology.

Final pathology reports documented margins were negative in all 195 bone marrow margins. In seven patients, there was a positive soft tissue ($n = 6$) or cortical bone ($n = 1$) margin. When compared with the findings of the permanent section, the frozen section diagnosis yielded a PPV of 57.1% (95% CI, 18%–90%) and a NPV of 100% (95% CI, 98%–100%). Using the same standard, the gross split specimen yielded a PPV of 19.1% (95% CI, 5%–42%) and a NPV of 100% (95% CI, 98%–100%).

Discussion

Frozen section of bone marrow at a margin and inspection of the gross split specimen are two methods commonly used to assess margin status intraoperatively in resection of bone sarcomas. However, there are little published data on which surgeons and pathologists can base intraoperative assessment and decision-making algorithms regarding accurate margin assessment and the need to take more tissue or not. The primary aim of this study was to evaluate agreement between frozen section of bone marrow at a margin and inspection of the gross split specimen and to compare their practical use. Intraoperative frozen section was in agreement with inspection of the gross split specimen in the majority of cases, especially when negative. In all discrepant cases, the decision whether or not to take more bone was based on inspection of the gross split specimen. The results from both intraoperative assessment methods were in accord with the final pathologic analysis more often in the case of gross inspection over frozen section.

Our study has some limitations. We assessed and made practice recommendations for bone marrow margins only. As noted, at our institution, bone marrow margins are commonly assessed with intraoperative frozen section. Soft tissue and cortical margins are rarely assessed with this technique and so there were not enough numbers to include them in this analysis. Second, splitting and examining the gross split specimen proved to be a reliable method of intraoperative margin assessment in this study in a practice with significant experience with bone sarcoma specimens. The bone must be split in a proper plane to allow for the type of examination we describe. Typically the bone marrow margin on the gross specimen can be adequately assessed in only one plane and surgeons and pathologists rely on invaluable prior experience, multidisciplinary conference discussion, and areas of concern during intraoperative dissection (such as regions near critical neurovascular structures) to determine the most crucial plane to evaluate. Also, not all institutions have access to the equipment necessary to split a bone specimen intraoperatively, and so these results would not be applicable.

Another limitation is that there is no universally accepted definition of what amount of bone at a marrow margin is necessary to be considered negative. Thus, in this analysis, the surgeon's decision to recut the bone was always considered to be correct. One could argue that several bone recuts were unnecessary because there was no tumor at the margin with the final pathologic analysis. However, the goal of the intraoperative decision is to achieve final negative margins. At times, this is at the expense of taking extra bone margin as a precaution, but if there is no consequence to limb salvage, this is an accepted practice. The decision-making process intraoperatively is challenging, and analyzing these data in this manner was determined to be the most accurate assessment of this complex process. A final limitation of this study is that the outcome measurement (final pathology margin status), although a reasonable surrogate for clinical outcome, may not be as robust a measure as more clinically oriented long-term followup data such as local recurrence. Local recurrence is uncommon in sarcoma of bone, and so a multiinstitutional study would be necessary to fully answer that question.

The agreement analysis of frozen section and inspection of the gross split specimen demonstrated some key findings. The high percentage of negative agreement indicates that both methodologies produced similar conclusions when there was no evidence of residual tumor and thus a recut was not required. The low positive percent agreement indicates that discrepancies emerged when either the gross specimen suggested a recut (14 margins for which the concurrent frozen section assessment was read as negative) or the frozen section was interpreted as

indeterminate/suspicious (which we conservatively chose to define as positive in our statistical analysis; two frozen section assessments for which the concurrent gross inspection suggested no recut). In these 16 margins of disagreement, the final intraoperative action was based on the assessment of the split gross specimen in all cases, disregarding the frozen section result.

When compared against the final pathology result, the two intraoperative methods of margin assessment yielded the same NPV estimate but different PPV estimates. The NPV of 100% for each method indicates that both frozen assessment and gross inspection were effective at limiting the occurrence of false-negatives. This is reassuring in an operative setting where a specimen that is falsely negative for tumor at the margin would generally be more problematic than one that is falsely positive. The discrepancy in PPVs for the two methods correlates to the discrepancy in positive agreement. The CIs for the PPV for the frozen section and the gross specimen overlapped, indicating that there was no statistically significant difference in the positive predictability of the two decision methods. Therefore, although the estimated PPV for the frozen section assessment was higher than that of the gross specimen, we cannot conclude that one method is any more accurate or reliable than the other with regard to positive decisions. We also remind the reader that the PPV of the gross specimen is negligible in the clinical setting because there is no clinical false-positive with respect to decisions to recut based on the gross specimen. Given that all marrow margins were negative on final pathology, we conclude that inspection of the gross split specimen led to reliable surgical decisions and that the information from the frozen section analysis was noncontributory in each of these instances. The results of this study confirm that there is no evidence that frozen section assessment is a superior diagnostic method compared with a split gross specimen, and vice versa, for intraoperative decisions. These results, in conjunction with clinical findings, suggest that inspection of the gross specimen is as reliable a method as frozen section assessment to make intraoperative decisions regarding primary resection of the bone when the goal is negative bone marrow margins. Moreover, the assessment of frozen specimens may be an unnecessary intraoperative procedure when gross split specimen assessment is used. These results are supported by the work from Meyer et al. [8], the only other evaluation of this practice in the literature. In this study, there were no positive frozen sections in patients after the era of preoperative evaluation of tumor extent with MRI, but there were three in the group that did not have MRI, one of which was a false-positive. There was one false-negative in the pre-MRI group as well. The focus of this study was the reliability of modern imaging techniques in determining placement of bone osteotomies

along with intraoperative assessment of the split specimen to achieve resection with negative margins.

The findings from Meyer et al.'s study [8] hold true in the current study. What has changed in the time since that study, however, is the acceptance of smaller margins. The definition of a negative margin is difficult, but several studies have demonstrated that narrow but negative margins can result in good outcomes with comparable rates of local recurrence and distant disease as cases with wider margins [1, 7]. The continued improvement in imaging techniques, new guidance techniques for osteotomies, and reliance/assessment of chemotherapy response are what enable surgical resection with marrow margins and limb salvage surgery to result in acceptable oncologic results. The current study thus allows reassessment of frozen section analysis of bone marrow margins in light of this emerging practice.

Elimination of unnecessary or redundant practices without added value is mandatory in health care today. Based on the results from the current study and the study from Meyer et al. [8], a reasonable approach is for surgeons to rely on intraoperative pathologic inspection of the gross specimen alone for assessment of bone marrow margins in cases in which this examination is feasible and provides a straightforward result. If a particular margin is questionable, then certainly specific frozen sections of that area may be performed, but the routine use of bone marrow frozen section analysis can be eliminated when a gross split specimen is being examined. Future efforts to confirm our findings in a larger study might be helpful to define the rate of rare events (such as a false-negative bone marrow margin in a gross split specimen) not captured in the current study.

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