Frozen Section of Placental Membranes and Umbilical Cord

An Aid to Early Postpartum Diagnosis of Intra-Amniotic Infection

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Key Words: Placenta; Chorioamnionitis; Funisitis; Intra-amniotic infection; Frozen section; Maternal and fetal inflammatory response

ABSTRACT

Objectives: We devised a rapid frozen section (FS) assessment technique of placental tissues and performed the first rigorous assessment of FS relative to conventional workup.

Methods: We evaluated 49 placentas with clinical/gross suspicion of intra-amniotic infection by FS. Relative to formalin-fixed and paraffin-embedded tissues, we compared the grading, staging, and interobserver variability.

Results: FS assessment demonstrated a sensitivity of 0.91 (95% CI, 0.77-0.97) and a specificity of 0.60 (95% CI, 0.36-0.80) for the presence of chorioamnionitis and a sensitivity of 0.89 (95% CI, 0.75-0.96) and a specificity of 0.69 (95% CI, 0.42-0.87) for the presence of funisitis. The χ2 goodness of fit for grade and stage in both placental membrane and umbilical cord sections was significant (P < .001). There was no significant difference in interobserver variability in comparison with permanent section results (P = 0.06).

Conclusions: We conclude that FS is a reasonably sensitive screening technique, correlating well with conventional assessment, without significantly different interobserver variability.

Chorioamnionitis and funisitis are pathologic entities characterized by neutrophilic infiltration of the fetal membranes or umbilical cord, respectively.1 These two entities are frequently encountered in pediatric pathology and represent histopathologic manifestations of intra-amniotic infection (either as part of a maternal and/or fetal inflammatory response, respectively).2 The most frequent infectious sources are bacterial organisms originating from the maternal genital tract that are thought to subsequently ascend the reproductive tract.3-5

Intra-amniotic infections complicate up to 10% of pregnancies1,6,7 and are particularly common in preterm labor.3,8 Although modern maternal mortality is relatively low in comparison to ages past, neonatal mortality ranges from 1% to 4% in term and up to 10% in preterm babies.6 Intra-amniotic infections also have significant associated morbidity:
from the maternal perspective, intra-amniotic infection has been associated with abnormal subsequent uterine function; very serious neonatal morbidities include sepsis, meningitis, and pneumonia. A variety of chronic illnesses have been recently strongly associated with intra-amniotic infection: these include developmental delay, neurologic sequelae, and pulmonary disease, among others.

The diagnosis of intra-amniotic infections is typically presumptive and made clinically, including a combination of maternal pyrexia (temperature >37.8°C) and two or more of the following: maternal tachycardia (maternal heart rate >100 beats/min), fetal tachycardia (fetal heart rate >160 beats/min), uterine tenderness to palpation, foul odor, or appearance of effluent amniotic fluid or maternal peripheral blood leukocytosis (>15,000 neutrophils/µL).

Ideally, the diagnosis of intra-amniotic infection is made early in the peripartum period in order that maternal antibiotic treatment might be instituted quickly. Laboratory testing of the effluent amniotic fluid has proven useful in the many cases of clinically equivocal intra-amniotic infection and may help confirm otherwise clear-cut cases. Ancillary laboratory tests, beyond clinical acumen alone, are of use when the concern for overtreatment is considered. Empirical overuse of antibiotic therapy is attributed as the major cause of antibiotic resistance, a pervasive problem in neonatal intensive care units. Furthermore, the overuse of antibiotics portends the risk of neonatal morbidity, a problem most frequently seen in the context of widely used aminoglycosides. Finally, the availability of ancillary/confirmatory laboratory tests is warranted, especially in light of a recent survey suggesting wide variation in the clinical management of chorioamnionitis.

Although the traditional confirmatory laboratory test has been culture of the effluent amniotic fluid, recent polymerase chain reaction (PCR) studies have noted a disheartening lack of sensitivity of standard culture techniques for the detection of potentially infectious microorganisms; the use of amniotic fluid culture, furthermore, is often limited given the long turnaround time required for its completion. Other culture techniques of the placenta have been fraught with low clinical sensitivity and lengthy turnaround times. Many biomarkers have been studied in the hopes of developing a rapid and sensitive test that might be used early in the peripartum period, occasionally, these have been found to be unreliable. PCR for the nucleic acids of microorganisms, although highly sensitive, also raises challenges when the potential for contamination by nonpathogenic sources of DNA is considered. Furthermore, the detection of microorganisms by PCR does not necessarily imply pathogenicity, nor is PCR able to provide any measure of the degree of either maternal or fetal inflammatory response.

Examination of the fetal tissues (membranes and umbilical cord) has proven to be a reliable and clinically useful undertaking in cases of intra-amniotic infection. Experience has suggested that gross examination of the placental tissues alone is insufficient in screening for maternal/fetal inflammatory syndrome since most cases of chorioamnionitis appear macroscopically unremarkable. In contrast, some authors believe histologic examination of placental tissues to be the “gold-standard” modality in the diagnosis of intra-amniotic infection. The histopathologic evaluation of fetal membranes and umbilical cord using the Redline system is currently recommended; this system recognizes both the degree of inflammation as well as the anatomic extent of inflammation. The Redline system reflects the biologic mechanisms of the maternal and fetal inflammatory responses and, most important, has been proven to provide prognostic value.

In many clinical scenarios, rapid tissue diagnosis by means of frozen section is requested; this is especially common in surgical pathology in the context of arthroplasty. Occasional reference has been made to the potential utility of frozen section for the early diagnosis of chorioamnionitis/funisitis; the technique has not been studied rigorously in the context of placental tissue evaluation, but to our knowledge, no published evidence supports the contention that frozen section diagnosis of chorioamnionitis/funisitis is reasonably sensitive, specific, or reliable as a diagnosis made by routine tissue-processing methods. The following study evaluates an approach to the rapid diagnosis of maternal and fetal inflammatory response by frozen section of fetal membranes and umbilical cord.

Materials and Methods

Institutional ethical approval from the Hamilton Integrated Research Ethics Board was granted. Consecutive cases of clinically or grossly suspect chorioamnionitis/funisitis received for processing by the pediatric pathology department were considered for inclusion in the study. After routine gross examination and processing was performed in keeping with standard laboratory procedures, extra samples of umbilical cord and fetal membranes were individually flash-frozen in optimal cutting temperature and stored at −70°C. Unlike the grossing process normally performed in our laboratory, in which a tight roll of the amniotic membranes is first formalin fixed and later sectioned and embedded, we employed an alternate placental membrane grossing procedure due to the mucoid nature of fresh placental membranes; the entire grossing process is illustrated in Image II. The frozen tissue blocks were assigned a random study number to ensure blinding from the original case, with umbilical cord and fetal membranes from the same case enumerated identically.
**Image 1** Examination and preparation of placental tissues: 
A. Grossing of fresh placental membrane strip (taken from point of rupture back toward the placental disk). B. Producing placental membrane roll in optimal cutting temperature (OCT) medium block mold. The highly mucoid nature of the placental membrane strips made it challenging to produce a placental membrane roll to then transfer into OCT. We therefore employed a “membrane curl” technique as illustrated. C. Sectioning of umbilical cord was performed with one section taken from each of the most proximal and distal ends. D. Embedding of sections of proximal and distal umbilical cord in OCT. E. Labeling of OCT blocks. F. Liquid nitrogen freezing of OCT blocks.
Frozen sections were then cut at a 5-μm thickness and stained routinely with H&E. The remaining tissue was then formalin fixed and paraffin embedded by routine methods. The paraffin-embedded tissue blocks were then assigned a second study number to ensure blinding to the frozen section results, with umbilical cord and fetal membranes from the same case enumerated identically. Permanent sections of each frozen block were then generated and stained by routine H&E staining methods.

Three pathologists (J.W.J., J.B., and J.A.-M.) then evaluated the frozen and permanent slides, both independently and by panel consensus after an interval delay of several weeks. Each umbilical cord and fetal membrane slide was assigned a grade and stage of inflammation, as pertaining to maternal or fetal inflammatory response, based on the Redline grading and staging system. Given research ethics limitations (and in keeping with the standard of care), frozen section results were used only for the purposes of this study and were not incorporated into the clinical record or communicated to the attending clinicians.

The frozen section slides were evaluated relative to the permanent slides (assumed for our purposes to be the gold standard) by panel consensus. Sensitivity, specificity, and positive and negative likelihood ratios for frozen section diagnosis of chorioamnionitis and funisitis, relative to the corresponding permanent sections, were then calculated. Scoring results were also tested with the chi-square goodness-of-fit statistic. Interobserver variability (κ statistic) scores were then calculated and compared between frozen and permanent section diagnoses by χ² testing. All statistical calculations were performed using SPSS version 20 (SPSS, Chicago, IL) and P values of less than .05 were considered statistically significant.

Results

During the period allotted for the study, 52 cases of clinically or grossly suspect chorioamnionitis/funisitis were considered for inclusion in the study sample, all originating from singleton pregnancies. Three cases were rejected due to insufficient tissue or labeling inconsistencies. Two-fifths of the final sample consisted of cases from preterm births. The median gestational age in the final sample was 39.5 weeks, the mean gestational age was 36.4 weeks, and the range of gestational ages was 24 to 42 weeks. One case of single umbilical artery was noted.

Of the 49 cases assessed, 41 had either chorioamnionitis or funisitis of varying grades and stages by consensus review of the permanent sections. Of these, 34 demonstrated chorioamnionitis, while 36 demonstrated funisitis. Furthermore, of those cases with chorioamnionitis, 12 were grade 1 and 22 were grade 2; two cases had stage 1 inflammation, 15 cases had stage 2 inflammation, and 17 cases had stage 3 inflammation. Of those cases with funisitis, 21 were grade 1 and 15 were grade 2; 15 cases had stage 1 inflammation, 17 cases had stage 2 inflammation, and four had stage 3 inflammation.

In comparison, frozen section assessment identified 37 cases with chorioamnionitis and 36 cases with funisitis. Furthermore, of those cases noted to have chorioamnionitis by frozen section assessment, 16 were grade 1 and 21 were grade 2; zero were stage 1, 24 were stage 2, and 13 were stage 3. Of those cases noted to have funisitis by frozen section assessment, 22 were grade 1 and 14 were grade 2; 14 were stage 1, 20 were stage 2, and two were stage 3. Frozen sections and permanent sections were of sufficient technical quality by all pathologists. Image 2 presents photomicrographs comparing the H&E appearance of frozen and permanent sections of placental membranes and umbilical cord sections both with and without inflammation.

A breakdown of the sensitivity, specificity, and positive and negative likelihood ratios of frozen sections for the detection of either chorioamnionitis or funisitis by panel consensus is outlined in Table 1. The χ² goodness-of-fit testing demonstrated a significant correlation between the frozen section and permanent section diagnoses for interpretation of placental membranes and umbilical cord (P < .001 for both). The calculated interobserver variability scores ranged from 0.60 to 0.82 on evaluation of the frozen sections and from 0.71 to 0.87 on evaluation of the resulting permanent sections. These data did not indicate a statistically significant difference by χ² testing (P = .06).

Discussion

We have performed, to our knowledge, the first rigorous study of frozen section for the early postpartum diagnosis of intra-amniotic infection. We have demonstrated that frozen sections of fetal membranes and umbilical cord are reasonably accurate when used as a screening tool for the estimation of the grade and stage of maternal and fetal inflammatory

<table>
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<tr>
<th>Metric</th>
<th>Chorioamnionitis</th>
<th>Funisitis</th>
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<tbody>
<tr>
<td>Sensitivity (95% CI)</td>
<td>0.91 (0.77-0.97)</td>
<td>0.89 (0.75-0.96)</td>
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<tr>
<td>Specificity (95% CI)</td>
<td>0.60 (0.36-0.80)</td>
<td>0.69 (0.42-0.87)</td>
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<tr>
<td>Positive likelihood ratio</td>
<td>2.28 (1.22-4.27)</td>
<td>2.89 (1.27-6.58)</td>
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<tr>
<td>Negative likelihood ratio</td>
<td>0.15 (0.046-0.47)</td>
<td>0.16 (0.059-0.43)</td>
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* Values in parentheses are 95% CIs.
Comparative photomicrographs of frozen and permanent H&E sections. 

A, B, Photomicrographs of frozen sections of placental membranes and umbilical cord without inflammation, respectively. 

C, D, Corresponding permanent section slides. 

E, Photomicrograph demonstrating frozen section interpreted as stage 2, grade 2 chorioamnionitis; inset demonstrates focal involvement of the chorion. 

F, Photomicrograph demonstrating frozen section interpreted as grade 2 funisitis; inset demonstrates linear infiltration of neutrophils beyond the vessel wall into Wharton’s jelly. (A–D, x20; E–F, x40.)
responses. In our institutional experience, placental pathology evaluation by traditional means may take a week or more. By contrast, the rapidity by which frozen sections can be performed (with 90% of frozen sections performed in less than 20 minutes) portends a valuable and rapid aid in the diagnosis of intra-amniotic infection, especially during the vital immediate postpartum period. Early screening diagnosis by frozen section may be a valuable ancillary test, perhaps even aiding in better outlining early postpartum treatment regimens, in births complicated by intra-amniotic infection. As has been noted in the context of arthroplasty, we anticipate that future intervention trials will highlight the utility of frozen section evaluation for fetal inflammatory response in the critical early neonatal period.

As is a common observation in frozen sections, freezing artifact was identified in many of the samples in this study. Interestingly, the effect of this artifact on the Wharton’s jelly of the umbilical cord resulted in a relative dispersion of the spindle cells, in many cases resulting in easier identification of neutrophilic infiltration. In some cases, it was also noted that the delicate amnion was less well preserved in cuts taken from the frozen section blocks than in the permanent sections. This observation made frozen section interpretation difficult in rare cases, especially in light of the fact that high-stage chorioamnionitis is notable for subchorionic abscess formation and potential sloughing of the delicate chorionic membrane.

A number of potential limitations to this study should be noted. We felt it necessary to choose permanent sections produced from the original frozen section tissues as our gold standard in order that the inflammation noted on the frozen section blocks than in the permanent sections. As it happens, this approach is also the approach most frequently employed for frozen-permanent section quality assurance. We also relied on panel consensus for gold-standard diagnoses; future studies exploring less artificial clinical end points are warranted. The distribution of cases in this study was also skewed toward more severe cases of chorioamnionitis and less severe cases of funisitis; our results should therefore be interpreted with caution in any population with a different distribution of severity of chorioamnionitis and/or funisitis. Finally, we did not explore any specific clinical factors potentially influencing intra-amniotic infection such as gestational age, twinning, or other prenatal risk factors; a larger, more appropriately powered study would be needed to explore these potential factors.

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References


Table 2

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<tr>
<th>Parameter</th>
<th>Frozen Sections</th>
<th>Permanent Sections</th>
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<tbody>
<tr>
<td>Cord grade</td>
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<tr>
<td>Cord stage</td>
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<td>Membrane stage</td>
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