Immediate Evaluation of Endomyocardial Biopsies for Clinically Suspected Rejection After Heart Transplantation

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Background Acute rejection may be suspected in heart transplant recipients in the setting of new onset of clinical symptoms or alterations in cardiac function. Immediate diagnosis may be obtained by performing a frozen section on endomyocardial biopsy (EMB) specimens. However, little is known about the indications for, and the diagnostic reliability of, this procedure.

Methods and Results EMBs with frozen section (n=98) from 65 of 214 consecutive orthotopic heart transplant recipients were reviewed and divided into early (≤45 days; n=47) and late (>45 days; n=51) posttransplant periods. Frozen section diagnoses (x̄=1.5 EMB samples) were compared with corresponding permanent section diagnoses (x̄=4.4 EMB samples), and clinical indications were analyzed. Comparison of frozen and permanent section interpretation revealed concordant pathological processes—rejection (n=31) versus no rejection (n=37) versus ischemic injury (n=20)—in 88 of 98 (90%) cases. Discordant pathological processes on frozen versus permanent section in 10 of 98 (10%) cases could be attributed to ischemic injury (n=5), sampling (n=4), and infection (n=1). In the 92 cases with defined clinical indications, the indication and number of EMBs positive for rejection early and late after transplantation were arrhythmia: 2 of 12 early, 4 of 10 late; congestive heart failure: 1 of 2 early, 5 of 12 late; fever: 0 of 2 early, 1 of 4 late; echo abnormality: 0 of 5 early, 0 of 1 late; syncope: 1 of 5 early, 0 of 1 late; hypotension: 1 of 3 early, 1 of 2 late; noncompliance: 0 of 0 early, 4 of 5 late; more than one of the above: 3 of 7 early, 2 of 5 late; other: 1 of 7 early, 1 of 9 late; total: 9 of 43 early, 15 of 49 late.

Conclusions Frozen section on EMB specimens accurately reflected the permanent section diagnosis in 90% of cases. No specific clinical indication predicted EMB rejection positivity with high sensitivity in either the early or late posttransplant periods. (Circulation. 1994;89:2079-2084.)

Key Words • arrhythmia • ischemia • transplantation • biopsy

Endomyocardial biopsy (EMB) is widely used to diagnose acute allograft rejection after heart transplantation. Because histopathological changes frequently precede clinical symptoms of rejection, surveillance EMBs are performed at regular intervals after transplantation. When EMB specimens are routinely processed for light microscopy using paraffin embedding techniques, results are available approximately 24 hours after the biopsy procedure.

Occasionally, acute rejection may be suspected in the setting of new onset of clinical symptoms or alterations in cardiac function. In these situations, immediate biopsy diagnosis may be warranted. One method of obtaining an immediate diagnosis is by performing a frozen section on fresh, unfixed EMB samples. Preparation of frozen sections involves rapid solidification of tissue by freezing that permits sectioning by a cryomicrotome for histological examination. Results of frozen sections can be available approximately 15 to 20 minutes after specimen acquisition; thus, the results can immediately affect patient management. However, little is known about the appropriate clinical indications for, and the diagnostic reliability of, frozen section diagnosis of EMB specimens.

The purpose of this study was to review our institution's experience with EMB frozen sections for immediate diagnosis over a 9-year period and to assess the reliability of frozen section diagnosis compared with permanent section diagnosis.

Methods

Study Population Of the 214 orthotopic heart transplant recipients at Brigham and Women's Hospital, 65 underwent 98 endomyocardial biopsy procedures (mean, 1.5; range, 1 to 4) with frozen sections during the 9-year period from February 1984 to March 1993. Patient charts were reviewed to determine the clinical indication for seeking an immediate EMB diagnosis.

Patient Characteristics The mean age of the study population was 46±12 years (x̄±SD; range, 18 to 64). There were 49 men and 16 women. Pretransplant diagnosis was ischemic heart disease in 26, idiopathic dilated cardiomyopathy in 35, and valvular heart disease in 4 patients. Maintenance immunosuppression consisted of cyclosporine and prednisone before 1988 and triple-drug immunosuppression of cyclosporine, azathioprine, and prednisone beginning in 1988. Patient demographics and immunosuppression protocols were similar in the study group and in the total recipient population.

Biopsy Protocol After heart transplantation, EMBs were routinely performed according to the following schedule: first posttrans-
plant year, weekly for 4 weeks, biweekly for 2 weeks, monthly for 6 months, then every other month to 1 year; second posttransplant year, every 3 months twice, then every 6 months; third and subsequent posttransplant years, every 6 months. Conventional processing of these biopsies consisted of fixation in 10% neutral buffered formalin followed by paraffin embedding, sectioning at 4-µm thickness, and staining with hematoxylin and eosin. Special stains (most frequently Masson’s trichrome) and special studies (such as immunohistochemistry) were done when indicated based on morphological findings.

Urgent interpretation of EMBs was necessitated by adverse change in the recipient’s clinical condition or worrisome new findings on objective testing. These parameters included supraventricular arrhythmia (paroxysmal atrial tachycardia, atrial fibrillation, atrial flutter), bradyarrhythmia, congestive heart failure/shortness of breath, fever, isolated echocardiographic abnormality (usually decreased left ventricular function), hypotension, noncompliance, syncope/cardiac arrest, myocardial infarction/chest pain, early graft failure, multifactorial (more than one of the above listed indications), and other (ie, nausea, fatigue).

Frozen Section Preparation
EMBs submitted for frozen section were placed in OCT embedding medium (Miles Laboratories, Inc) and frozen at −20°C. Multiple sections were cut at 6 µm, stained with hematoxylin and eosin, and examined immediately. After interpretation, the frozen section residue as well as the nonfrozen samples were submitted for routine processing as described above.

Biopsy Interpretation and Treatment of Rejection
Acute rejection is characterized by a perivascular and/or interstitial lymphocytic infiltrate, which may be accompanied by myocyte damage. In more severe forms of rejection, the inflammatory infiltrate is extensive and may be polymorphic; widespread myocyte necrosis, hemorrhage, and vasculitis may be present. Ischemic injury is characterized by coagulative myocyte necrosis, which may be accompanied by polymorphonuclear leukocytes during the early stages. As healing ensues, a mononuclear infiltrate of histiocytes, lymphocytes, and plasma cells may be present.

EMB frozen sections were performed to assess the presence or absence of a pathological process (ie, rejection versus no rejection versus other processes) rather than to provide precise grading of any detected rejection. Thus, biopsy findings were described but not graded at the time of actual frozen section. EMB samples routinely processed for permanent section were assigned a histological grade of rejection according to the International Society for Heart and Lung Transplantation (ISHLT) working formulation.¹ Permanent sections of EMBs obtained before 1991 were retrospectively graded for the purpose of this study. The pathological process and extent of rejection, if present on permanent sections, were compared with the pathological findings present on frozen section. EMBs with an ISHLT rejection grade of IIA or higher were routinely treated with increased immunosuppression and were considered positive for rejection.

Definitions
For the purpose of this study, concordance between the frozen section diagnosis and the permanent section diagnosis was used when the same pathological process was identified. Discordance was used when a different pathological process was diagnosed on frozen and permanent sections. EMBs performed early were defined as those occurring 45 days or less after transplantation. EMBs performed late were defined as those occurring greater than 45 days after transplantation. The distinction between early and late was set at 45 days because most rejection occurs within the first 6 to 8 weeks of transplantation.²

Results
Reliability of Frozen Section Diagnosis
The mean number of tissue samples from each biopsy procedure on which a frozen section was performed was 1.5 (range, 1 to 4) compared with 4.4 (range, 1 to 10) submitted for permanent sections. Of note, in 89 of the 98 EMBs (91%), only one (n=55) or two (n=34) tissue samples were submitted for frozen section.

The most common pathological processes diagnosed on frozen section were acute rejection and ischemic injury to the myocardium (Fig 1). Histological concordance between the pathological processes diagnosed on frozen section and permanent section occurred in 88 of 98 (90%) EMBs (Fig 2). The concordant findings consisted of acute rejection in 31 cases, ischemic injury in 20 cases, and no rejection or other pathological processes identified in 37 cases. The 10 discordant cases could be divided into those in which the frozen section was diagnosed as no rejection and permanent section was diagnosed as rejection (n=7); those in which the frozen section was diagnosed as rejection and permanent section was diagnosed as no rejection (n=3). Of the 7 cases diagnosed as rejection on permanent section, 3 had been diagnosed as ischemic injury on frozen section. These cases occurred 7, 15, and 27 days after transplantation. In the other 4 cases, the areas of lymphocytic infiltrate seen on permanent section had not been present on the tissue submitted for frozen section. Of the cases diagnosed as rejection on frozen section but no rejection on permanent section, 2 had ischemic injury on permanent section and 1 had cytomegalovirus (CMV) infection of the myocardium. The 2 cases with ischemic injury occurred 11 and 18 days after transplantation.

Although no attempt was made to grade the rejection detected on frozen section, an estimate of severity was conveyed to the clinician so that immediate treatment with augmented immunosuppression could be instituted if indicated. In 28 of the 31 concordant rejection cases, the rejection identified on frozen and permanent sections would have elicited similar clinical response. Either minimal rejection was detected on frozen and permanent sections so that treatment was not indicated or significant rejection warranting treatment was detected on both. Each of the 3 cases with potentially different treatment in response to the frozen compared with the permanent section contained a single focus of rejection with myocyte damage on frozen section and multiple foci of rejection on the more extensive sample submitted for permanent sections.

Overall, in 13 of 98 (13%) frozen section cases, consisting of the 10 discordant cases plus 3 rejection cases described above, permanent section diagnosis would have dictated different clinical management when compared with the frozen section interpretation.

Clinical Indications for Immediate EMB Diagnosis
Adequate clinical data were available for 92 of the 98 EMBs submitted for frozen section diagnosis. The mean time interval between transplantation and EMB submitted for frozen section diagnosis was 313 days (range, 1 to 1934). However, 43 of 92 (47%) frozen sections were
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FIG 1. Micrographs: Comparison between frozen section and corresponding permanent section for acute rejection and ischemic injury. Acute rejection (A, frozen section; B, permanent section) is characterized by a dense lymphocytic infiltrate with associated myocyte damage. Ischemic injury (C, frozen section; D, permanent section) consists of coagulative myocyte necrosis with an associated polymorphous infiltrate containing numerous neutrophils. Frozen section artifacts including myofiber separation and increased thickness of the histological section are evident in A and C. Hematoxylin and eosin stain; magnification ×400.

performed within 45 days of transplantation (Fig 3). The clinical indications prompting immediate diagnosis are listed in the Table. The total number of EMBs for each clinical indication are then subdivided into early (≤45 days) or late (>45 days) after transplantation and positive (≥ISHLT grade IIIA) or negative (<ISHLT grade IIIA) for rejection based on the permanent section diagnosis.

Three clinical indications accounted for 48% of EMBs submitted for immediate diagnosis: supraventricular arrhythmia in 18 (20%), shortness of breath/congestive heart failure in 14 (15%), and multifactorial in 12 (13%). Supraventricular arrhythmia was the most common early indication for immediate diagnosis.

When all presentations involving early supraventricular arrhythmia were considered, including those presenting as a component of multifactorial indications, only 1 of 13 EMBs was positive for rejection. Shortness of breath/congestive heart failure was the most common late indication for immediate diagnosis. When any presentation involving late shortness of breath/congestive heart failure was considered, including those presenting as a component of multifactorial indications, EMBs were positive for rejection in 5 of 14 cases.

Overall, rejection was diagnosed in 9 of 43 (21%) patients in the early posttransplant period and in 18 of 49 (37%) patients in the late posttransplant period. No specific clinical indication predicted EMB rejection.
positivity with a high degree of sensitivity in either the early or late posttransplant periods. Of the most common indications for immediate diagnosis, the biopsy results were either predominantly negative for rejection or evenly divided between positive and negative results.

Discussion

EMB has proven to be a reliable and clinically useful method of assessing cardiac allograft rejection and allows for sampling multiple areas of right ventricular endomyocardium. Mathematical analysis has shown that obtaining three negative samples yields a false-negative rate of 5%; four negative samples reduce this chance of error to 2%. As a result, most transplant programs recommend obtaining a minimum of three and preferably four or five tissue samples to adequately assess the rejection status of the allograft.

Frozen Sections Reflect the Pathological Process Present on Permanent Sections But Have Limitations

In this study, frozen section diagnosis was usually performed on one to two myocardial fragments with the understanding of clinicians and pathologists that this represents very limited sampling and cannot be used for the purpose of grading rejection. Because rejection may be focal or multifocal, limited sampling on frozen section may miss or underestimate the extent of rejection. The rationale behind using frozen section diagnosis on to two myocardial samples was to attempt to identify clinically significant rejection (that which warrants increased immunosuppression) or to identify another pathological process responsible for the patient's clinical symptoms (ie, ischemic injury) rather than to provide precise histological grading of rejection. Submitting all of the myocardial samples for frozen section runs the risk of introducing artifact, which could potentially impede interpretation of the permanent sections.

In 88 of 98 cases (90%), there was concordance between frozen and permanent section diagnosis in assessing the presence or absence of rejection or the presence of another pathological process such as ischemic injury. Considering the extremely limited sampling on most frozen sections, it was surprising that, in as many as 28 of 31 (90%) cases identified as having rejection on frozen section, the extent of rejection was judged to be comparable to that on the permanent section, warranting similar treatment for both. Each of the three cases in which the permanent compared with the frozen section may have elicited a different clinical response were diagnosed as a single focus of rejection on frozen section and multiple foci of rejection on additional samples available for permanent section. These three cases provide a good example of the importance of more extensive sampling and illustrate why rendering a grade of rejection should not be attempted with inadequate sampling on permanent as well as on frozen section.

In some situations, however, EMBs that are negative for rejection or that demonstrate processes other than rejection may be as clinically useful as those demonstrating rejection. Ischemic myocyte injury after heart transplantation has been reported to occur in 20% to 89% of EMBs obtained during the first 4 to 6 posttransplant weeks and must be differentiated from rejection. Early ischemic injury is characterized by coagulative myocyte necrosis. The accompanying inflammation consists of polymorphonuclear leukocytes, which are replaced during the healing phase by a histiocytic infiltrate admixed with lymphocytes and plasma cells. Frozen section artifact, however, may confound this diagnosis, as was the case in 5 of 10 cases with discordance between frozen section and permanent section diagnoses. Artifactual separation of the myofibers and the appearance of increased interstitial cellularity in combination with the increased thickness of the frozen section preparation all contribute to the difficulty in interpreting frozen sections. Each of the five cases in which ischemic injury resulted in discordant diagnoses between frozen and permanent sections occurred within the first month after transplantation, when ischemic injury is most prevalent. Increased awareness of the high frequency of ischemic injury, especially during the early posttransplant period, may be helpful in interpreting these EMBs and avoiding unnecessary augmentation of immunosuppression.

One of the discordant cases, diagnosed as rejection on frozen section, was found to have CMV infection of the myocardium on permanent sections. CMV infection of the myocardium may elicit an inflammatory response.
Clinical Indications and Results of Rapid Endomyocardial Biopsy Diagnosis

<table>
<thead>
<tr>
<th>Clinical Indication</th>
<th>Total EMB</th>
<th>Early, ≤45 d (n=43)</th>
<th>Late, &gt;45 d (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>REJ+</td>
<td>REJ-</td>
</tr>
<tr>
<td>Supraventricular arrhythmia</td>
<td>18</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CHF/shortness of breath</td>
<td>14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fever</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Echo abnormality</td>
<td>6</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Hypotension</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Noncompliance</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Syncope/cardiac arrest</td>
<td>6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Ml/chest pain</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early graft failure</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Multifactorial (&gt;1 of above)</td>
<td>12</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>9</td>
<td>34</td>
</tr>
</tbody>
</table>

*Includes any combination of supraventricular arrhythmia, bradycardia, CHF/shortness of breath, echo abnormality, and hypotension.

EMB indicates endomyocardial biopsy; REJ, acute rejection; CHF, congestive heart failure; and MI, myocardial infarction.

Reminiscent of localized rejection.8,9 Typical inclusions may be difficult to find on permanent as well as frozen sections. Additional studies such as immunoperoxidase staining for CMV may be necessary to confirm the diagnosis.

An alternative to frozen section for immediate EMB diagnosis is available in some laboratories. Rapid routine processing of EMB specimens for paraffin embedding may be done on a shortened processing cycle (ie, 3 hours). While allowing high quality and expeditious preparation of the total number of EMB samples, it requires the availability of specially assigned histology laboratory personnel and equipment.

Clinical Indications for Immediate Evaluation Did Not Reliably Predict Biopsy Findings

Since frozen section diagnosis was found to accurately reflect the final permanent section diagnosis so that treatment is unchanged in 87% of EMBs, it appears to be a valid option for immediate diagnosis in selected clinical situations. However, no specific clinical indication prompting frozen section diagnosis reliably predicted biopsy findings of rejection.

Two of 18 cases biopsied on an emergent basis for a diagnosis of supraventricular arrhythmia had associated rejection.10,11 When all presentations with supraventricular arrhythmias were considered, including multifactorial cases, rejection was present in 4 of 23 cases (17%) but in only 1 of 13 during the early posttransplant period. Prior studies support a possible association between atrial arrhythmia and rejection. For example, in the precycoisolopine era, the Stanford group, using clinical criteria for the diagnosis of rejection and serial ECGs, found rejection in 41% of patients with an arrhythmia, most of whom had supraventricular tachycardia.12 A subsequent study did not show an association.13 The lack of a correlation in our study, especially in the early period after cardiac transplantation, also suggests a low yield for EMB. The mobilization of resources for immediate diagnosis for this indication does not appear to be warranted.

The finding of bradycardia, with or without other clinical findings, was associated with rejection in a higher proportion of patients (5 of 9 for any presentation with bradycardia). These numbers are small and represent only a small fraction of patients who developed a bradycardia at some point in the posttransplant period. Prior studies report an incidence of bradycardia ranging from 4.4% to 18%, without a clear association with rejection.13-16 Nevertheless, a diagnosis of rejection should be considered in patients presenting with bradycardias, especially late in the posttransplant course.

Overall, rejection was diagnosed in 9 of 43 (21%) patients in the early posttransplant period when clinical suspicion may be heightened and in 18 of 49 (37%) patients in the late posttransplant period. The greatest yield for rapid EMB diagnosis occurred in patients with congestive heart failure symptoms or shortness of breath, noncompliance in taking immunosuppressive medication, and bradycardia. It is interesting to note that in the 6 cases where a new echo abnormality was the sole clinical indication for immediate evaluation, none of the EMBs showed rejection. Thus, no one clinical finding or constellation of findings correlated well with EMBs positive for rejection in either the early or the late posttransplant period.

The uncertainty of the clinical diagnosis and the potential for profoundly devastating sequelae of rejection have led to the practice of emergency myocardial biopsy. Alternative noninvasive modalities have not proved sufficiently sensitive or specific to supplant EMB.
as the standard for diagnosing rejection.\textsuperscript{17-22} Empiric intensification of immunosuppressive therapy, even in the short term, carries defined risks, which are heightened if sepsis is suspected.\textsuperscript{23} Nevertheless, from the standpoint of clinical practice, intensification of immunosuppression remains an option for the truly unstable patient until the results of permanent section evaluation become available.

Conclusions

We have demonstrated that performing frozen sections on EMB specimens accurately reflects the permanent section diagnosis so that treatment based on permanent versus frozen section interpretation is unchanged in 87\% of cases. However, the limitations of frozen section, including sampling and the presence of artifacts, must be acknowledged. The identification of pathological processes other than rejection, such as ischemic damage to the myocardium, may be equally important in explaining clinical symptoms or alterations in cardiac function. Even if rejection is detected on frozen section, it is most important to correlate whether the extent and severity of rejection present adequately explain the observed change in clinical condition. Clinical parameters that prompted immediate EMB interpretation could not reliably predict EMB positivity for rejection so that overall yield of immediate processing is low.

References

Immediate evaluation of endomyocardial biopsies for clinically suspected rejection after heart transplantation.
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