# Under the Microscope

# Utility of Heart Biopsy in Transplant Patients

**Edwin N. Beckman, MD[1]; Mandeep R. Mehra, MD; Myung H. Park, MD; Robert L. Scott, MD**

*Department of Pathology[1] and Cardiomyopathy and Heart Transplantation Center, Department of Cardiology, Ochsner Clinic and Alton Ochsner Medical Foundation, New Orleans, LA*

Following heart transplantation, rejection of the transplanted tissue may occur by cellular- or vascular-oriented mechanisms, leading to graft failure, cardiac insufficiency, and death. To prevent allograft rejection, patients require immunosuppression therapy. Immunoprophylaxis application is based on a fine balance between exploiting the immunosuppressive properties, yet avoiding infection, malignancy, hyperlipidemia, and diabetes mellitus. Optimizing immunosuppression is based on ongoing surveillance for rejection. Unfortunately, noninvasive methods of monitoring heart transplant patients have not developed to an adequate extent to determine incipient rejection, and invasive endomyocardial biopsy remains the effective gold standard of monitoring (1). Therefore, at periodic intervals following transplantation, patients undergo biopsy of the right ventricular myocardium via intravenous catheterization. The biopsy findings may detect incipient rejection and lead to additional therapy and, in some cases, the biopsy will detect other cardiac pathology such as infection.

## Developments

To standardize the description of observed morphologic findings, the International Society for Heart Transplantation developed a grading scale for heart transplant biopsies (2). The initial description of the grades included ambiguous phraseology. Twenty-five pathologists discussed problems with the phraseology at a January 8, 1994, meeting in San Francisco. Ambiguities were explained by the developers of the grading scale (Table), but the clarifications have never been published.

In the evaluation of myocardial biopsies, inflammation must be actually within the myocardium, while subendocardial collections of lymphocytes (named the Quilty phenomenon after the first patient in whom they were recognized) are ignored. These subendocardial collections of B lymphocytes do not indicate the presence of rejection, even when there is some direct extension into adjacent myocardium, termed the Quilty B phenomenon (2). Furthermore, the lymphocytes of the Quilty phenomenon are B cells, in contrast to the T lymphocytes of cellular rejection (3). Immunophenotyping is not a standard part of the biopsy evaluation, which consists of light microscopic evaluation of routinely processed, hematoxylin and eosin stained slides.

The biopsy procedure has low morbidity, but complications have been known to occur, such as infection, pneumothorax, tamponade, or thrombosis of the right internal jugular vein. Yet, adverse sequelae are rare, even if the biopsy includes the full thickness of the right ventricle.

The biopsy findings of Grades 0 and 1 (both 1A and 2B) do not indicate a need for additional treatment beyond normal baseline therapy. Patients with Grade 3 changes may or may not have clinical features of hemodynamic compromise, but the findings of Grades 3A and B are usually taken to indicate, on morphologic grounds alone, the presence of active cellular rejection, and the patient will usually receive additional therapy. When morphologic features indicate the presence of rejection, decisions are made on an individual basis taking various clinical factors into account, such as duration after transplantation and grade of cellular rejection in relation to the presence or absence of hemodynamic compromise.

Grade 2 findings pose a challenge and must, especially, be considered in the context of the patient's total picture. A Grade 2 focus might actually be one part of unrecognized Grade 3A changes.

**Table.** International Society of Heart-Lung Transplantation Standardized Cardiac Biopsy Grading: Descriptive features (slight condensation) (2) and subsequent clarifications.

| GRADE | FEATURES | CLARIFICATIONS |
|---|---|---|
| 0 | No evidence of acute rejection or myocyte damage. Equivocal findings of rejection should also be graded zero. | No lymphocytic infiltrate. Myocyte damage acceptable, if that damage is interpreted as being of ischemic origin. |
| 1A | Focal perivascular or interstitial infiltrates of large lymphocytes that cause no myocyte damage. One or more pieces may be involved. | Lymphocytes tightly packed around vessels. Lymphocytes do not have to be large. (Figure 1) |
| 1B | A more diffuse, perivascular and/or interstitial infiltrate of large lymphocytes with no myocyte damage. One or more pieces may be involved. | Lymphocytes not only around vessels, but dispersed out into the vicinity in a fine "chicken-wire" pattern. Dispersion no more than two lymphocytes wide. Lymphocytes do not have to be large. |
| 2 | Only one focus of inflammatory infiltrate of large aggressive lymphocytes with or without eosinophils; the focus is sharply circumscribed. Architectural distortion with myocyte damage should be present in the solitary focus. | Aggressive lymphocytic extension into the interstitium with infiltrating border, rather than pushing border. There may be damaged myocytes. The architecture may be distorted; this distortion implies destroyed myocytes. Lymphocytes do not have to be large. (Figure 2) |
| 3A | Multifocal inflammatory infiltrates consisting of large aggressive lymphocytes with or without eosinophils. | Multiple of the grade 2 foci; typically there will be damaged myocytes. Lymphocytes do not have to be large. (Figure 2) |
| 3B | Diffuse inflammatory process within several pieces of biopsy tissue. Myocyte damage is present as well as an aggressive inflammatory infiltrate of large lymphocytes and eosinophils with an occasional neutrophil. | A lymphocytic infiltrate which is global, as opposed to the multifocal infiltrate of Grade 3A. Lymphocytes do not have to be large. Other types of inflammatory cells do not have to be present. (Figure 3) |
| 4 | Diffuse aggressive, polymorphous inflammatory infiltrate that includes aggressive lymphocytes, eosinophils, and neutrophils. Myocyte necrosis and damage is (sic) always seen. Edema, hemorrhage and vasculitis are usually present. | No clarifications. |

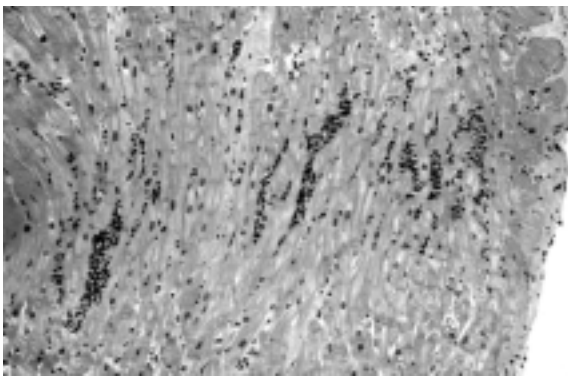

**Figure 1.** Grade 1A changes. Perivascular lymphocytic collections. Hematoxylin and eosin.

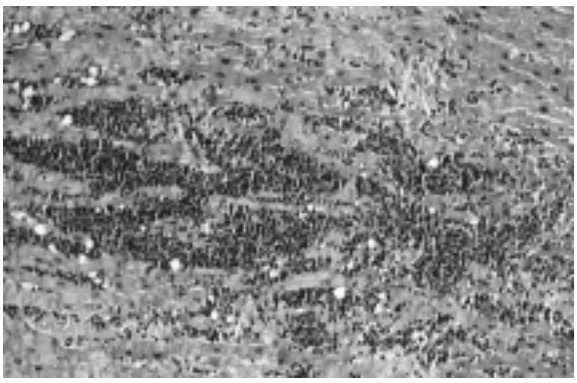

**Figure 2.** Interstitial lymphocytic infiltrate with obscured myocytes. This region is interpreted as having two adjacent foci, and it therefore graded as Grade 3A. If there were only a single focus in all the biopsy specimens, the picture would be Grade 2. Hematoxylin and eosin. 100X

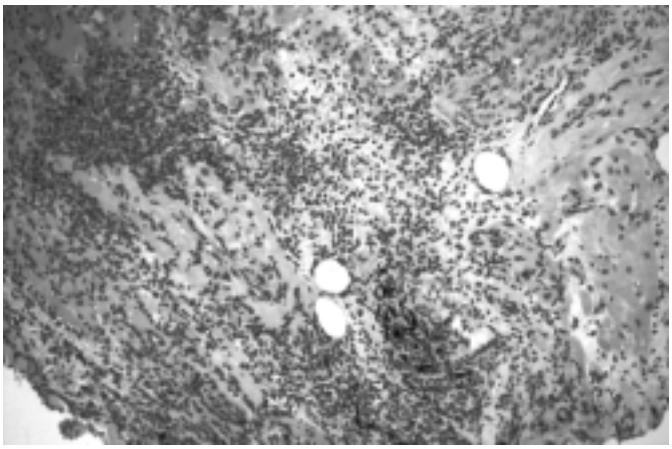

**Figure 3.** Grade 3B. Global lymphocytic infiltrate between myocytes. Hematoxylin and eosin. 100X

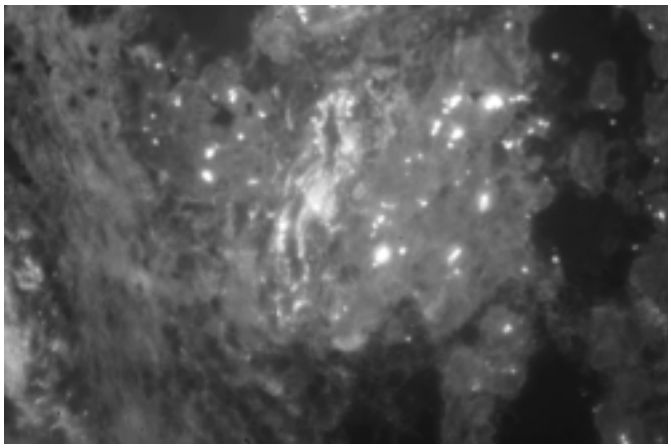

**Figure 4.** Humoral rejection. Positive immunofluorescence for compliment (C-3) along intima of vessel; this specimen was also had vascular positivity for IgM. Immunofluorescence stain. 400X

Because of the effect of sampling, there is always the possibility of missing evidence of rejection in heart biopsies. Four or more biopsy fragments are needed in order to decrease the likelihood of missing rejection from inadequate sampling (3). Usually, a patient with Grade 2 changes will require no additional therapy unless hemodynamic compromise is evident (4). Grade 4 changes are, fortunately, uncommon, but indicate the critical status of severe rejection and impending severe transplant malfunction.

The greatest likelihood of transplant rejection is during the first post-transplant year, especially during the first 6 months (1). The frequency of monitoring biopsies decreases with time after transplantation. After a year following transplantation, rejection is distinctly uncommon and would usually be the result of the patient not taking the prescribed level of maintenance therapy or of there being an unsuccessful attempt to lower the level of baseline therapy. More rarely, events that stimulate the immune system, such as viral infections, can also result in late allograft rejection.

## Myocardial Biopsies in Evaluating Possible Rejection

Transplant cardiologists may obtain myocardial biopsies as part of the evaluation of a patient displaying clinical evidence of possible rejection. Clinical features pointing towards rejection include shortness of breath, fatigue, leg edema, and relative hypotension. When rejection is suspected, biopsy findings of rejection would be good supporting evidence. On the other hand, if there is strong clinical evidence of rejection without evidence in the biopsies, the prudent clinical course may still be for the patient to receive additional immunosuppression. The transplant cardiologists must employ the heart biopsy findings in the context of the overall case.

In addition to cellular rejection, a patient could have vascular-oriented rejection on a presumed humoral basis. When such a rejection process is suspected, myocardial biopsies are also evaluated with immunofluorescent techniques. Positive staining of the endothelium by complement in conjunction with either IgM or IgG is taken as evidence of vascular-oriented humoral rejection (Figure 4) (5). Vascular-oriented rejection therapy may include plasmapheresis.

The cardiologist may also suspect another basis for the patient's picture, and absence of rejection on the biopsy will support the clinician's diagnosis. Less commonly, myocardial biopsies have evidence of other disease processes than rejection. Biopsies can provide evidence of disease such as toxoplasmosis infection (Figure 5) or recurrent amyloidosis (Figure 6).

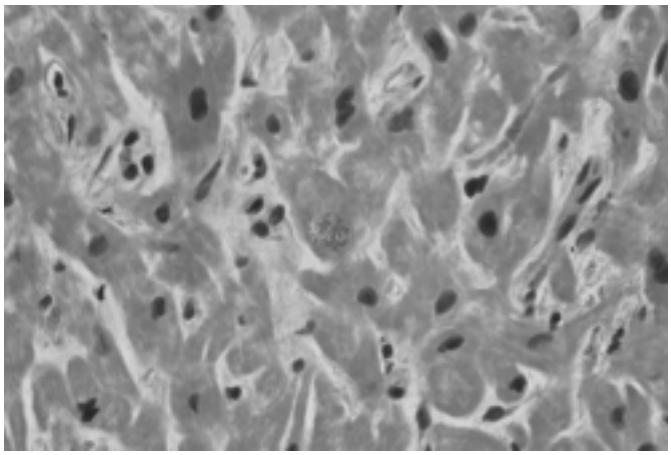

Figure 5. Toxoplasmosis. Cyst of toxoplasmosis within a myocyte. Hematoxylin and eosin. 400X

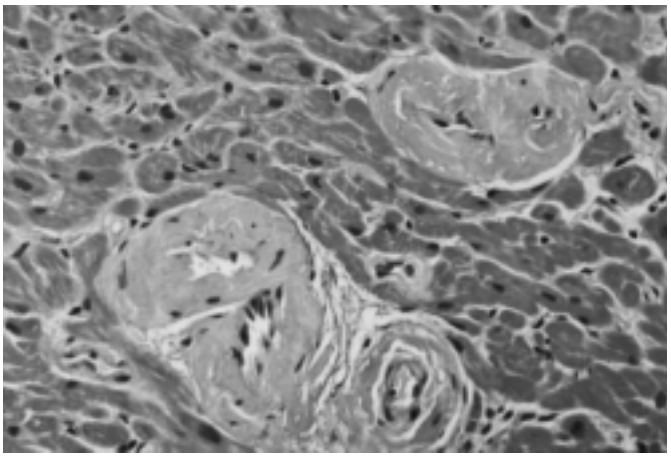

**Figure 6.** Amyloidosis. Amorphous pink deposits around vessels and within the interstitium. Hematoxylin and eosin. 250X

## Summary

Heart biopsies have proved extremely useful in the care of patients following heart transplantation, but the findings must be correlated with the clinical situation. Non-invasive methods for monitoring patients are highly desirable, but adequate, less intrusive methods have yet to be developed to the extent that they can replace the need for myocardial biopsies.

## References

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*Dr. Beckman has been a pathologist at the Ochsner Clinic and Ochsner Foundation Hospital for 25 years, performing general anatomic pathology but specializing in cardiovascular and genitourinary pathology. He is Clinical Associate Professor in Pathology at Tulane Medical School.*