Acute Rejection After Heart Transplantation: Noninvasive Echocardiographic Evaluation

GUGLIELMA RITA CILIBERTO, MD, MARGHERITA MASCARELLO, MD, EDOARDO GRONDA, MD, EDGARDO BONACINA, MD, MARIA CLEMENCIA ANJOS, MD, GIANBATTISTA DANZI, MD, PAOLA COLOMBO, MARIA FRIGERIO, MD, ANTONIA ALBERTI, MD, CLAUDIO DE VITA, MD

Objective. The purpose of this study was to assess the reliability of echocardiography in the noninvasive diagnosis of acute rejection in heart transplant recipients.

Background. Although echocardiographic results seem to correlate well with allograft rejection, published data are limited and contradictory.

Methods. In 130 transplant recipients, 1,400 serial echocardiograms were recorded within 24 h of endomyocardial biopsy. Increased wall thickness, myocardial echogenicity, pericardial effusion, shorter pressure half-time, isovolumetric relaxation time and a decrease in left ventricular ejection fraction were considered markers of rejection.

Results. The distribution of echocardiographic markers revealed highly significant differences between biotically graded moderate, mild and no rejection and between untreated and treated rejection episodes (chi-square test, p < 0.0001). Specificity was 98.4% for two markers, but sensitivity was good (80%) for only moderate rejection because of the large number of false negatives in untreated patients with mild rejection. In untreated patients, there was a highly significant difference in the number of echocardiographic criteria between a benign and nonbenign outcome (chi-square test, p < 0.0001). In treated patients, the significant difference in the variation in echocardiographic criteria between favorable and unfavorable responses after 1 week was more pronounced after 2 weeks (t test, p = 0.01 vs. < 0.001). Diastolic indices and pericardial effusion at 2 weeks seemed to be predictive of therapeutic response.

Conclusions. Poor sensitivity to mild rejection indicates that serial echocardiography cannot supplant endomyocardial biopsy in the early diagnosis of acute rejection, but it seems to be a reliable noninvasive means of identifying acute rejection requiring intensified immunosuppressive therapy and of evaluating outcome.

(J Am Coll Cardiol 1994;23:1156-61)
frequently when necessary). The study protocol was approved by our institutional ethics committee, and all patients gave informed consent.

Endomyocardial biopsy. Monitoring for acute rejection was based on the results of serial endomyocardial biopsies performed weekly during the 1st month, at fortnightly intervals during the 2nd and 3rd month, monthly up to the 5th month and then at the end of the 9th and 12th month (or more frequently when necessary).

Although the biopsy specimens taken after 1991 were actually classified according to modifiedBillingham criteria (21), all biopsy acute rejection grades reported in this study are based on Billingham's original classification (22): no rejection; mild (lymphoyctic infiltrate without myocyte necrosis); moderate (cellular with necrosis) or severe (myocyte necrosis with hemorrhage); resolving or resolved (the reduction or disappearance of cellular infiltrate after treatment) or ongoing (if the observed changes were the IT or worse than the previous biopsy); inadequate.

All episodes of moderate rejection were treated with high dose methylprednisolone, as were all cases of mild rejection with diffuse or multifocal lymphocytic infiltrate. Patients who had mild monofocal rejection were not treated unless rejection was accompanied by clinical signs or symptoms, or unless it occurred during the 1st postoperative month.

Echocardiography. M-mode, two-dimensional and Doppler echocardiographic examinations were performed using an Interean Cardioscan and Vingmed 700 with a 3.5-MHz combined imaging and Doppler transducer. All echocardiographic examinations were carried out by the same three operators who used a strictly standardized method. The same equipment was used for every patient. The position of the patient and the transducer, the echocardiographic approaches and all of the settings (gain setting, depth compensa- tion, contrast and brightness, etc.) were maintained the same for all examinations of each individual patient. Blood pressure and heart rate were recorded at the beginning of each examination.

M-mode, two-dimensional echocardiograms were obtained in parasternal long- and short-axis and apical two- and four-chamber views. Left ventricular wall thickness and dimensions were measured according to the criteria of the American Society of Echocardiography (23).

Left ventricular ejection fraction was derived from end-diastolic and end-systolic volumes calculated by the single plane area-length method. Myocardial echogenicity was qualitatively evaluated and classified as normal or increased in comparison with the preceding echocardiograms, with only the myocardium of the interventricular septum and left ventricular posterior wall perpendicular to the ultrasound beam being considered. Pericardial effusion was graded using the criteria described by Martin et al. (24).

Left ventricular diastolic studies were performed with pulsed wave Doppler ultrasound. The transducer was positioned at the cardiac apex, with the pulsed Doppler sample volume near the tip of the mitral leaflets (to obtain pressure half-time) or along the basal segment of the septum where the closing of the aortic valve and the opening of the mitral valve could be clearly identified (to obtain the isovolumetric relaxation time) (25,26). Simultaneous strip chart recordings were obtained at a paper speed of 100 mm/s. Ten consecutive cycles were analyzed at each examination, and the mean values of the diastolic indexes were computed. The systolic beats occurring during recipient atrial contraction were carefully excluded, as suggested by Valentine et al. (27). Pressure half-time was not measured in patients with moderate mitral regurgitation, pacer makers, arrhythmias, widespread pericardial effusion, important variations in arterial pressure or a reduced left ventricular ejection fraction. The images were all recorded on videotape and subsequently analyzed by two independent observers who had no knowledge of the biopsy findings.

Changes in comparison with the previous examination in each individual patient provided the index for the echocardiographic monitoring of rejection for that particular patient. The following variations in echocardiographic examination results were considered suggestive of the onset of acute rejection: 1) increase in wall thickness >4 mm (interventricular septum plus left ventricular posterior wall); 2) appearance or increase in pericardial effusion; 3) increase in myocardial echogenicity; 4) >10 decrease in left ventricular ejection fraction; 5) >20 ms decrease in pressure half-time; 6) >20 ms decrease in isovolumetric relaxation time.

The first four criteria were chosen on the basis of our previous experience (18); the last two as a result of an analysis of the pressure half-time and isovolumetric relaxation time values reported in previous studies (9,10,14) and those revealed by 300 examinations of the patients in this series who did not experience rejection.

Clinical course. Histologic features were examined by dividing the acute rejection episodes into those with a benign course (improvement and rapid resolution of acute rejection without treatment in the group of untreated patients and after treatment in the group of treated patients); and those with a nonbenign course (when the evolution of acute rejection was slow after two or more treatments or when there was a recurrence during the month after the onset of acute rejection).

Statistical analysis. Contingency tables (chi-square test using the Yates uniformity correction where necessary) were used to analyze frequency data. Sensitivity (true positives divided by true positives plus false negatives) and specificity (true negatives divided by true negatives plus false positives) were computed by constructing tables of frequencies. The Student t test was used for analysis of unpaired data (two-tailed test), and p < 0.05 was considered statistically significant.

Results

Endomyocardial biopsy. A total of 1,400 biopsy-correlated echocardiographic examinations were consid-
Biopsies showed no rejection in 1,009 patients but showed the onset of mild rejection in 95, the onset of moderate rejection in 30 and resolved, resolving or ongoing rejection in 185. The biopsy results were considered inadequate in 81 patients. On the basis of the histologic and clinical criteria described previously, 49 of the 59 mild rejection episodes were treated with high-dose corticosteroids; the remaining 46 were not treated.

Diagnostic reliability of echocardiography. The echocardiographic criteria had a very high level of specificity (range 98.3% to 99.8%) but a low level of sensitivity (reduced pressure half-time 44%, increased wall thickness 45%, increased echogenicity 40%, pericardial effusion 28%, decreased isovolumetric relaxation time 28%, reduced left ventricular ejection fraction <5%). None of them were sufficiently sensitive for diagnostic purposes.

The distribution of one, two and three or more associated echocardiographic criteria in patients with biopsy-graded no rejection and mild and moderate rejection is shown in Table 1. The number of echocardiographic criteria increased in line with the biopsy grade of acute rejection, the chi-square test revealing a very highly significant difference (p < 0.0001). The presence of two or more associated criteria was highly specific (98.6% for two, and 100% for three or more criteria). Of the 14 false positives with two associated criteria, occurred in patients who developed viral myocarditis or pericarditis within a few days. One patient had a low blood level of cyclosporine, and one developed coronary artery disease (the remaining eight patients had no problems).

Sensitivity was good only for moderate rejection (60.8%). It is worth noting that all six false negative moderate rejections were of the focal type (one focus only) and were resolved within 1 week, and all of the true positive moderate rejections showed two or more associated criteria. The poor sensitivity in patients with mild rejection was mainly due to very low sensitivity in untreated patients. Sensitivity was fair in treated patients (69% for two associated criteria, 79.1% for one criterion only). If the subgroup of patients with mild rejection evaluated after 1990 according to the new classification is considered (21), sensitivity for two or more associated criteria was 24% in patients with rejections of the focal type and 68.8% in those with a diffuse type (for only one criterion, the percents were 34% and 86%, respectively).

Figure 1 shows the distribution of one, two and three or more echocardiographic-associated criteria in treated patients experiencing the onset of mild or moderate rejection and untreated patients with mild rejection, together with their contingency table values. Once again, the chi-square test showed a very highly significant difference (p < 0.0001). Thus, the number of echocardiographic-associated criteria seems to correlate very closely with severity (grading and treatment).

Similarly, the same analysis (the distribution of one, two and three or more echocardiographic criteria) showed a statistically significant difference between treated and untreated patients with mild rejection (p < 0.005) and between treated patients with mild and moderate rejection (p < 0.03).

Figure 2 shows the distribution of the number of echocardiographic criteria in 46 untreated patients with mild rejection according to evolution of rejection. There was a highly significant difference in the number of echocardiographic...
criteria at onset between benign and nonbenign evolution (chi-square test, p < 0.0001). The resolution of acute rejection was spontaneous and rapid in all 21 false negatives and in 6 of 9 with one echocardiographic criterion (the other three, all with pericardial effusion alone, were subsequently treated because of a recurrence of mild rejection, after which the pericardial effusion decreased and disappeared). All three patients with three or more echocardiographic criteria and three of the six patients with two associated criteria were subsequently treated because they developed moderate or persistent diffuse mild rejection (Fig. 3).

Value of echocardiography after therapy. In the group of treated patients, the Student t-test showed a significant difference in terms of histologic evolution and changes in the number of echocardiographic criteria between responding and nonresponding patients. The ratio between the number of echocardiographic criteria 1 and 2 weeks after treatment and the number at onset of acute rejection showed that the difference between a benign and unfavorable course after treatment was statistically significant after 1 week (p < 0.01) and even more so after 2 weeks (p < 0.001). Patients with a benign course showed an improved echocardiographic picture as early as the 1st week after treatment (mean value 58% of initial values) and a further improvement after 2 weeks (75%). Those with an unfavorable course had further worsening echocardiographically after 1 week (~11%) and only a minimal improvement after 2 weeks (20%).

Of the different variables, pressure half-time at 1 and 2 weeks and isovolumetric relaxation time and pericardial effusion at 2 weeks after treatment showed a significant difference (Table 2).

**Discussion**

Acute rejection and infection remain the most frequent causes of death in the 1st 2 years after transplantation (28). The use of cyclosporine has dramatically improved survival in transplant recipients but has modified the clinical and pathologic features of acute rejection.

Endomyocardial biopsy represents the standard for monitoring transplanted hearts but has not eliminated all problems, mainly because of invasiveness (29,30), sampling error, misinterpretation of biopsy specimens and atypical rejections in the absence of lymphocytic infiltrates. It is
known that the histologic changes in acute rejection often have an asymmetric pattern (31) and are sometimes missed by biopsic sampling. When repeated endomyocardial biopsies are performed, the histology can also depend on the interval between biopsies (32,33). Furthermore, vascular rejection may occur in the absence of the characteristic histologic features of rejection (34,35).

The decision to give additional acute rejection therapy only on the basis of the histologic information provided by endomyocardial biopsy is often doubtful. Several studies have shown that successive biopsies do not show progression for most mild rejection episodes, but mild rejection may sometimes be the beginning of a significant episode leading to moderate or severe acute rejection (33,36). Additional therapy in mild acute rejection can prevent the progression to more severe forms and could probably reduce the incidence of coronary artery disease (37-39), but it may be dangerous because of its infective complications. There is no doubt that reliable, repeatable and reproducible noninvasive methods are useful in monitoring acute rejection.

Correlation of echocardiographic and biopic findings. A number of different studies (4-20) have shown that echocardiography appears to be very helpful in monitoring graft function as well as morphologic or functional changes in the rejecting heart, but there is little agreement among them, and some are based on only a limited number of selected cases. In our study, although all echocardiographic criteria proved to be very specific, their low level of sensitivity meant that none of them was reliable in the early diagnosis of acute rejection.

Global evaluation of the various morphologic and functional changes between serial examinations seems to be more reliable (19,20). In the present study, the presence of two or more associated criteria provided excellent specificity and good sensitivity for significant rejection. In fact, the sensitivity in moderate rejection was very good (86%), considering that all of the false negatives were cases of monofocal rejection. The poorer sensitivity in mild rejection seems to be due to the particularly low sensitivity observed in untreated patients.

The distribution of the number of echocardiographic criteria correlated well with the histologic grading of acute rejection and seemed to identify those patients requiring additional treatment. This observation was confirmed by the analysis of untreated mild rejection episodes: All of the false negative and most of the positive cases for one echocardiographic variable (with the exception of pericardial effusion) resolved rapidly and spontaneously. In agreement with Valentine et al. (39), our experience suggests that an increase in pericardial effusion is a warning of the need for closer monitoring.

Independent of histologic features, most of the untreated mild rejection episodes with two or more echocardiographic criteria at onset required one or more additional treatments because of progression to moderate or ongoing rejection. Furthermore, echocardiography shows the morphologic and functional impact of rejection on the heart (particularly on the left ventricle), whereas endomyocardial biopsy provides precise information relating only to small samples taken from the right ventricle.

Echocardiography in the assessment of the response to treatment. Few data are available in published reports concerning the results of treatment. In our study, the global changes in the different echocardiographic variables allowed the response to treatment to be assessed after 1 week and, more significantly, at 2 weeks. These data are in agreement with the histologic features of resolving or resolved acute rejection that occur within 2 weeks in most patients treated with cyclosporine-based immunosuppressive protocols (21,40).

In agreement with other investigators (14), the persistence of impaired diastolic function or pericardial effusion seemed to be a reliable sign of an unfavorable response to additional therapy, whereas variations in myocardial echogenicity and wall thickness seemed to be nonpredictive. As reported by Masuyama et al. (13) for integrated ultrasound backscatter, altered echoreactivity continues long after the resolution of a rejection, and the lack of significance of a reduction in wall thickness is perhaps an expression of a cortisone-induced reduction of edema.

Conclusions. Although echocardiography cannot replace endomyocardial biopsy in the early diagnosis of acute rejection because of its poor sensitivity in cases of mild rejection, its excellent specificity and good sensitivity in detecting significant rejection led to a reduction in the number of biopsies necessary, particularly after the first phases.

Echocardiography can provide reliable, noninvasive surveillance of transplant recipients in the period between biopsies, and the presence of more than one of the echocardiographic criteria should be taken as an indication of the need for closer monitoring. Echocardiography can be considered a valuable adjunct to endomyocardial biopsy in evaluating the severity of rejection and treatment response.

References

2. Sagar KB, Hanillo A, Wolking TC, Lower RL, Hess ML. Left ventricu-
lar mass in M-mode echocardiography in cardiac transplant patients 


mass in cyclosporine-treated patients after cardiac transplantation: deter-
rmination by two-dimensional echocardiography. Surg Forum 1985;26: 
371-3.

5. Pustain W, Hingel N, Sagar K. Left ventricular function of heart 
allografts during acute rejection: an echocardiographic assessment. Heart 


function as a noninvasive marker of cardiac allograft rejection. Heart 

indices of diastolic function as markers of acute cardiac rejection. 

diographic indices of left ventricular function as potential markers of 

for the diagnosis of acute cardiac allograft rejection. J Am Coll Cardiol 


12. Foster T, McElhinny I, Ritterborough H, et al. Can we assess the changes 
of ventricular filling resulting from acute allograft rejection with Doppler 

measurement of integrated ultrasonic backscatter from human cardiac al-

14. Valantine HA, Yentch TW, Gibbons R. Sensitivity and specificity of 
diastolic indices for rejection surveillance temporal correlation with 

15. Liedberg E, Niholay B, Mark H, et al. Diagnostic abilities of echocar-
diographic tissue characterization for differentiation between rejection 
and viral infection after heart transplantation by means of a combination 
of mathematical morphology and texture analysis. Circulation 1992;86 


surveillance in children, prospective comparison with endomyocardial 

18. Gibbert GI, Cataldo O, Cipriani M. Echocardiographic assessment of 

Echocardiography in Diagnosis of ABMR After Cardiac Transplantation 
with heart transplanton. Patient under Cyclosporine treatment. Z Kardiol 

echocardiographie Doppler dans la transplantation cardiaque orthotopique. 
Etude prospective comparative avec la biopsie endomy-

standardization of nomenclature in the diagnosis of heart and lung 

22. Billingham ME. Diagnosis of cardiac rejection by endomyocardial biopsy. 