New Methods for Noninvasive Monitoring of Rejection after Heart Transplantation

Hermann Reichenspurner, MD
Ralph Haberl, MD
Christiane Angermann, MD
Matthias Anthuber, MD
Georg Osterholzer, MD
Bernhard M. Kemkes, MD
Clas Hammer, MD
Joachim M. Gokel, MD
Bruno Reichert, MD

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From: The Departments of Cardiac Surgery (Drs. Reichenspurner, Angermann, Anthuber, Osterholzer, Kemkes, and Reichert), Cardiology (Dr. Haberl), Experimental Surgery (Dr. Hammer), and Pathology (Dr. Gokel), University of Munich, Klinikum Grosshadern, D-8000 Munich 70, FRG; and the Department of Cardiothoracic Surgery, University of Cape Town Medical School, Observatory 7925, Cape Town, Republic of South Africa.

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Address for reprints: H. Reichenspurner, MD, Department of Cardiothoracic Surgery, University of Cape Town Medical School, Observatory 7925, Cape Town, Republic of South Africa.

Between August 1981 and February 1987, 67 orthotopic heart transplants and three heart-lung transplants were performed in 69 patients at the University of Munich Hospital. The immunosuppressive regimen consisted of cyclosporine, azathioprine, and prednisone. The diagnosis of acute rejection was based on cytoimmunologic monitoring, frequency analysis of fast Fourier transformed surface electrocardiograms (FFT-ECGs), and two-dimensional echocardiography. The results of these diagnostic methods were compared to the findings provided by endomyocardial biopsies, which were performed simultaneously with the noninvasive studies.

Seventy patients underwent cytoimmunologic monitoring. In 88% of all rejection episodes, this technique revealed activated lymphocytes and lymphoblasts in the mononuclear concentrate of the peripheral blood samples; the presence of such cells is known to be an extremely early sign of acute rejection.

Twenty-six patients were monitored by means of FFT-ECG. In 20 of the 21 cases of rejection, this method disclosed significant changes in the frequency spectrum of the QRS complex in the 70- to 110-Hz range; in 12 cases, these changes were the earliest sign of acute rejection. Therefore, FFT-ECG had a sensitivity of 95%. All of the QRS changes were reversible with rejection therapy.

Forty-five patients were subjected to two-dimensional echocardiography. In 31 of the 35 cases of rejection, the echocardiogram showed a significant increase in the left ventricular wall thickness and a decrease in the left ventricular cross-sectional area during mild rejection. Moderate or severe rejection was characterized by an increase in the diastolic area, as well as a decrease in the systolic area change and in the diastolic maximum velocity of area change. Thus, two-dimensional echocardiography had a sensitivity of 89%.

In the recent cases, the diagnosis of rejection was based on noninvasive methods alone. After rejection therapy had been instituted, endomyocardial biopsies were performed to assess the effectiveness of the treatment. With noninvasive rejection monitoring, the number of endomyocardial biopsies performed during the first three postoperative months was only 2.8 per patient; in comparison with invasive rejection monitoring, noninvasive follow-up was associated with a 75% reduction in the need for biopsy. (Texas Heart Institute Journal 1988;15:7-11)

Since the first heart transplant, in December 1967, the results of this procedure have steadily improved until the 1-year actuarial survival rate now averages between 80% and 90%. This success is mainly due to improved immunosuppressive therapy, more precise donor/recipient selection criteria, and more sophisticated methods of rejection monitoring. Although routine endomyocardial biopsies have been the mainstay of rejection monitoring, several noninvasive techniques for diagnosing rejection have recently been developed. The present study was undertaken to evaluate three of these methods, and to determine their effect on the outcome of cardiac transplantation.

Patients and Methods

Between August 1981 and February 1987, 67 orthotopic heart transplants were performed in 66 patients at the University of Munich Hospital. The preoperative diagnosis was congestive cardiomyopathy in 47 cases, coronary artery disease in 15 cases, valvular heart disease in three cases, and morbus...
Uhl in one case. Three additional patients (two with primary pulmonary hypertension, and one with pulmonary hypertension in association with Eisenmenger syndrome) underwent combined heart and lung transplantation.

**Immunosuppressive Regimen**
The immunosuppressive regimen consisted of cyclosporine A (3 to 10 mg/kg/day, based on a serum trough level between 80 and 200 g/ml), azathioprine (1 to 2 mg/kg/day, based on a white blood cell count of 5000 cells/mm³), and prednisone (1.0 mg/kg/day; this dosage was reduced to 0.15 mg/kg/day within 6 weeks). A broad-spectrum antibiotic (cefoxamide, 2 g, tid) was administered on the day of the transplant and on the first postoperative day. Cytomegalovirus hyperimmunoglobulin, 1 ml/kg (Biotest, Inc., FRG) was given on the first and twentieth postoperative days for prophylaxis against cytomegalovirus infection.

**Rejection Monitoring**

**Cytoimmunologic Monitoring**
Seventy patients were subjected to cytoimmunologic monitoring, a technique based on the evaluation of peripheral lymphocyte subpopulations and their precursors, as isolated by Ficoll-Hypaque separation. The details of this procedure have previously been published in this journal.³

**Fast Fourier Transformed Surface Electrocardiography**
Twenty-six transplant recipients were monitored by means of fast Fourier transformed surface electrocardiography (FFT-ECG), a method that is quite sensitive to changes in the frequency spectrum and the amplitude of the QRS complex. In these patients, two well-defined surface ECG recordings were analyzed daily for 4 weeks by means of fast Fourier transformation (which includes low noise and high gain amplification). The procedure was performed under sterile conditions in the intensive care unit, and the preamplifier was placed close to the patient’s chest in order to minimize environmental noise. A long, shielded cable connected the preamplifier to the main amplifier, which was located outside the sterile area. The filter setting was opened to 3 to 300 Hz. Analysis and storage of the signals were done with a computer. Single beats, as well as signal averages of 100 consecutive beats, were recorded.⁴

**Two-Dimensional Echocardiography**
Forty-five transplant recipients underwent daily two-dimensional echocardiography. Short-axis cross-sections of the left ventricle were evaluated for area change calculations. A computer was used to follow the endocardial borders of the complete cardiac cycle; graphs of the left ventricular cross-sectional area during the complete cardiac cycle were then designed. These graphs showed a decrease in the cross-sectional area during systole, as well as a rapid increase during diastolic filling.

**Endomyocardial Biopsy**
In the recent cases, only when the foregoing methods yielded inconclusive results was a transvenous right ventricular endomyocardial biopsy performed to diagnose acute rejection. Further biopsies were then routinely performed to monitor the effectiveness of rejection therapy.

**Results**

Of the 66 heart transplant recipients in this study, 41 are currently alive up to 5½ years after transplantation. The actuarial 1-year survival rate is 78%. Of the three heart-lung recipients, one remains alive after 3½ years.
TABLE 1. Results of Postoperative Fast Fourier Transformed Surface Electrocardiography (n = 26)

<table>
<thead>
<tr>
<th>Method</th>
<th>Episodes of Rejection (n)</th>
<th>Absence of Rejection (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endomyocardial biopsy</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Fast Fourier Transformed Electrocardiography</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Cytoimmunologic monitoring</td>
<td>21</td>
<td>5</td>
</tr>
</tbody>
</table>

Cytoimmunologic Monitoring

Ninety percent of the patients subjected to cytoimmunologic monitoring showed signs of acute rejection. In 88% of these cases, activated lymphocytes or lymphoblasts were observed in the mononuclear concentrate; the presence of such cells has proved to be an extremely early sign of acute rejection. Therefore, cytoimmunologic monitoring was shown to have a sensitivity of 88%. Moreover, this method allows rejection to be differentiated from various infectious processes; the specificity was 86%.5

Fast Fourier Transformed Surface Electrocardiography

From one day to the next, no fluctuation in frequency content was seen in the 0- to 45-Hz range. In the tenfold extended frequency plot from 45 to 200 Hz, daily fluctuations were minimal for the first few postoperative days. To confirm this finding, we compared data obtained in cases of cardiac transplantation with those obtained in cases of routine cardiac operations. During acute rejection, an increase in frequency content was observed in the 70- to 110-Hz range (Fig. 1). Endomyocardial biopsies, performed simultaneously with FFT-ECG, showed moderate or severe acute rejection. In these cases, the frequency spectrum of the QRS complex and the corresponding areas returned to control levels within 1 week after treatment with three pulses of methylprednisolone (1 g/day) (Fig. 2).

In 21 of the 26 patients, endomyocardial biopsy indicated acute rejection (Table 1). In 20 of these cases, a change in frequency content was noted on the FFT-ECG. There was one false positive result, owing to acute mediastinitis and pericardial empyema. The correlation between QRS voltage and rejection was nonsignificant. The QRS duration did not reflect changes characteristic of heart transplantation or rejection. Therefore, with regard to acute rejection, FFT-ECG had a sensitivity of 95%, based on the frequency spectrum of the QRS complex.

![Fig. 1](image1.png)

**Fig. 1** Changes in left ventricular cross-sectional area during acute rejection. The diastolic cross-sectional area was noted to increase, but the systolic area change and the diastolic maximum velocity of area change underwent a decrease. t₀ = end of diastole; t₁ = end of systole; t₂ = maximum velocity of systolic area change; t₃ = maximum velocity of diastolic area change

![Fig. 3](image2.png)

**Fig. 3** Changes in left ventricular cross-sectional area during a complete cardiac cycle were evaluated by means of two-dimensional echocardiography. This graph is based on results obtained 5 days after cardiac transplantation. t₀ = end of diastole; t₁ = end of systole; t₂ = maximum velocity of systolic area change; t₃ = maximum velocity of diastolic area change

![Fig. 4](image3.png)

**Fig. 4** Changes in left ventricular cross-sectional area during acute rejection. The diastolic cross-sectional area was noted to increase, but the systolic area change and the diastolic maximum velocity of area change underwent a decrease. t₀ = end of diastole; t₁ = end of systole; t₂ = maximum velocity of systolic area change; t₃ = maximum velocity of diastolic area change

Two-Dimensional Echocardiography

The changes in calculated left ventricular cross-sectional area seen in transplant recipients (Fig. 3) did not differ from those observed in normal donor-matched control patients. Mild rejection was associated with a decrease in the diastolic cross-sectional area, owing to edema and increased ventricular wall thickness. This finding constituted an extremely early sign of acute rejection. Moderate or severe rejection was associated with a significant decrease in left ventricular cross-sectional area change and in the diastolic maximum velocity of this area change. This corresponded to impairment of the systolic function and of the diastolic relaxation period (Fig. 4).

Thirty-five of the 45 patients subjected to this method showed signs of acute rejection, as detected
by endomyocardial biopsy (Table II). In 31 of these cases, two-dimensional echocardiography showed changes typical of such rejection, i.e., increased ventricular wall thickness in 24 cases and changes in cross-sectional area and/or functional shortening in 26 cases. Therefore, with regard to acute rejection, two-dimensional echocardiography had a sensitivity of 89%.

**Endomyocardial Biopsy**

All 69 patients were examined by means of endomyocardial biopsy. Three of 60 acute rejection episodes were missed with this method. Thus, endomyocardial biopsy had a sensitivity of 95%.

**Discussion**

After heart transplantation, infection and acute rejection are extremely serious threats. In managing these complications, close postoperative follow-up is the key to success. So far, endomyocardial biopsy has been considered the only valid method for diagnosing acute rejection in patients on cyclosporine therapy. Because of its invasive nature, however, this technique cannot be performed on a daily basis. Therefore, several new methods have been developed for the noninvasive monitoring of rejection.

In detecting acute rejection, cytoimmunologic monitoring of peripheral blood has a sensitivity of 88% and allows not only the diagnosis of rejection but also the differentiation of infection from rejection. Frequency analysis of surface ECG by FFT-ECG has a sensitivity of 95%; moreover, since this technique is highly specific, it may be satisfactorily combined with cytoimmunologic monitoring. Echocardiography has also proved helpful: when M-Mode echocardiography was used to diagnose acute rejection in patients being treated with cyclosporine A at Stanford University Hospital, the diastolic relaxation period was noted to be prolonged during acute rejection. Two-dimensional echocardiography allows extremely early detection of such rejection; an increase in left ventricular wall thickness is noted during mild episodes, whereas an additional change in the diastolic and systolic function is observed during moderate and severe episodes. Furthermore, two-dimensional echocardiography is quite useful for the long-term follow-up of cardiac function in transplant recipients.

All three of the above named noninvasive methods are extremely sensitive and highly specific diagnostic tools for diagnosing acute rejection before clinical symptoms become apparent (Fig. 5). At our institution, the diagnosis of rejection is now based primarily on these methods, which also allow us to monitor the effectiveness of rejection therapy. In the presence of a doubtful result, a diagnostic endomyocardial biopsy is still performed, but the main indication for biopsy is to monitor the success of rejection therapy. As a result of this reliance on noninvasive procedures, the number of endomyocardial biopsies performed at our institution has been reduced by 75%, to only two to three biopsies per patient during the first 3 postoperative months.

Endomyocardial biopsies are still important for confirming doubtful results, and such biopsies should be used routinely at centers that are still new at heart transplantation. Once experience has been gained, however, the use of noninvasive techniques will allow a reduction in the need for endomyocardial biopsies.

### Table II. Results of Two-Dimensional Echocardiography during Acute Rejection (n = 45)

<table>
<thead>
<tr>
<th>Pathological Results in:</th>
<th>Episodes of Rejection (n)</th>
<th>Absence of Rejection (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endomyocardial biopsy</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Two-dimensional echocardiography (total)</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Left ventricular wall thickness</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Area and/or functional change</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

**Rejection Diagnosis after Cardiac Transplantation**

Dept. of Cardiac Surgery, University of Munich

- Cytoimmunologic monitoring
- Fast Fourier transformation electrocardiography
- Two-dimensional echocardiography

**Fig. 5** Combining all three noninvasive techniques facilitates the diagnosis of acute rejection.
References