METHODS

Signal-Averaged Electrocardiography and Detection of Heart Transplant Rejection: Comparison of Time and Frequency Domain Analyses

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To evaluate the role of the signal-averaged electrocardiogram (ECG) in the detection of heart transplant rejection, findings on 277 ECGs were compared with those in 218 endomyocardial biopsy specimens in 25 patients followed up for a median duration of 5.2 months (range 7 days to 17.5 months). Signal-averaged ECGs obtained at intervals of 16.4 ± 22.3 days were analyzed in the time domain before and after high pass filtering at 25 and 70 Hz. Frequency domain analysis was performed with use of a fast Fourier transform algorithm.

Sixteen severe rejection episodes requiring treatment were observed. These episodes induced significant decreases in peak and root-mean-square voltages of both filtered and unfiltered QRS complexes, as well as in the total spectral area. Conversely, QRS duration and 50- to 250-Hz or 70- to 110-Hz spectral areas were not significantly altered. In 14 cases mild rejection episodes were observed that did not significantly alter any of the variables studied.

The root-mean-square voltage of the 70-Hz high pass filtered QRS complex was found to be the most accurate variable in detecting rejection. Moreover, this variable was also the most reproducible in 10 healthy control subjects. The optimal rejection criterion was defined as an 11% decrease in voltage between two consecutive recordings. It provided 87.5% sensitivity with 78.4% specificity.

In conclusion, the signal-averaged ECG is helpful in the management of heart transplant rejection. Frequency domain analysis of the QRS complex does not increase the accuracy of the technique compared with the time domain approach.

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Since the introduction of cyclosporine A in the management of patients undergoing heart transplantation, the 12-lead electrocardiogram (ECG) has been reported (1-3) to be relatively insensitive in assessing graft rejection. Consequently, other noninvasive methods have been designed to obviate the need for endomyocardial biopsy, such as cytotoxic immunologic monitoring (4), echocardiography (5,6), nuclear magnetic resonance imaging (7), precordial ECG mapping (8) and signal-averaged or high resolution electrocardiography (9,10). Regarding the latter technique, only limited but promising results have been obtained. Time domain analysis of the averaged QRS complex and ST-T segment has also been advocated by other investigators (10) as more sensitive in detecting rejection during the early postoperative period.

Our purpose was to evaluate the respective accuracy of the signal-averaged and standard ECG in detecting rejection. We also compared frequency domain and time domain analyses of the signal-averaged ECG. The latter analysis was performed using a filter that was specially designed for measurement of the entire QRS complex.

Methods

Study patients. Twenty-five patients receiving cyclosporine A after orthotopic cardiac grafting were selected for the study. The group comprised 21 men, 3 women and 1 child; the age range was 2.6 to 64.2 years (mean 44.2 ± 12.4). The patients were enrolled after a median postoperative delay of 8 days (range 1 day to 25 months); 20 of the 25 patients were included within the 1st postoperative month. The preoperative diagnosis was congestive ischemic cardiomyopathy in 8 patients, idiopathic dilated cardiomyopathy in 16 and a complex congenital heart defect in 1 patient. The median duration of the follow-up period was 5.2 months (range 7 days to 17.5 months). In all but one patient (the 2.6-year old
child), routine endomyocardial biopsy was performed for diagnosis of rejection episodes. Pertinent clinical data and the signal-averaged ECG were obtained within 24 h of each biopsy.

Thus, a total of 218 biopsy specimens and 277 signal-averaged ECGs taken at intervals of 16.4 ± 22.3 days were obtained. The number of signal-averaged ECGs exceeded the number of biopsy specimens because the signal-averaged ECG was repeated at closer intervals in the case of overt rejection crisis. However, the value of the method in detecting rejection did not depend on these additional recordings.

A group of 10 healthy men (mean age 28.3 ± 2.03 years) was used to analyze the hour to hour and week to week reproducibility of the ECG variables. These men underwent two signal-averaged ECGs on the same day, one in the morning and the other in the afternoon. Two further acquisitions were performed 7 days later under the same conditions.

All patients gave informed consent for the study. The protocol was approved by our Institutional Committee on Clinical Research.

Diagnostic techniques. Endomyocardial biopsy was performed with a conventional percutaneous internal jugular approach, weekly for the first 6 weeks, every 2 weeks for the next 4 months, then monthly or every 2 months. The biopsy fragments were classified into five stages according to the criteria of Billingham (12).

From the standard ECG, a summated voltage was calculated by using the algebraic sum of the QRS amplitude in leads I, II, III, V1, and V6. This index was selected on the basis of previously published studies (9,13).

Signal-averaged ECGs were obtained with use of an ART 1200 EPX recorder (Arrhythmia Research Technology) from orthogonal bipolar X, Y and Z leads: X from V6 to V6, Y from the upper sternum to the left thigh and Z from V1 back to V2. The signals were recorded using silver-silver chloride electrodes positioned according to precise anatomic landmarks with the patient in a supine position. After preamplification, the signals were sampled at 1,000 Hz and digitized with a 16-bit resolution. After elimination of ectopic complexes, 150 to 200 beats were time averaged until a background noise <0.4 μV of amplitude was obtained. Averaged data were stored on the hard disk of a personal computer for further processing.

Signal processing. Time domain analysis was performed before and after high pass filtering of the X, Y and Z leads at 25 and 70 Hz. These corner frequencies were suggested by previous studies in either the time (9) or the frequency domain (10). The duration, peak voltage amplitude and ground noise <0.4 μV of amplitude were measured. The bandwidth corresponding to 99% of the amplitude was also calculated.

Definitions. Severe rejection was defined as large cellular infiltrates without myocyte necrosis (stage 2) or myocytolysis with or without hemorrhage (stages 3 to 5). Mild rejection episodes corresponding to limited cellular infiltrates (stage 1) were considered nonsignificant and were therefore not treated.

The reference tracing was defined as the last tracing obtained with a concomitant normal biopsy result for analysis of mild rejection crisis. For a severe episode, the reference was the last recording obtained with concomitant biopsy results indicating normal findings or mild stage 1 rejection. This method of analysis was chosen rather than use of a control tracing taken when the patient entered the study for two reasons. First, we hypothesized that a shift in the values of the ECG variables may occur over long periods of time as a result of chronic rejection (15), scars from preceding acute rejection episodes (16) or myocarditis. Second, the ECG data were supposed to be unstable during the early postoperative period (9), which could lead to the selection of aberrant variables as baseline values.

Statistics. Data are expressed as mean values ± 1 SD. The reproducibility of the measurements within the control group was evaluated with use of the Spearman rank coefficients. A Student t test was used to analyze paired or unpaired observations as necessary. When the variances were nonhomogeneous, the Wilcoxon rank-sum test was used. Discrete variables were compared using the chi-square statistic with the Yates continuity correction or the Fisher exact probability test.

Results

Reproducibility analysis in control subjects (Table 1). The most reproducible variable was the root-mean-square voltage of the 70-Hz high pass filtered QRS complex. The worst
Rejection episodes. In all, 30 rejection episodes were documented: 14 mild episodes in 14 patients and 16 severe episodes in 15 patients. These severe crises were diagnosed on the basis of endomyocardial biopsy in 13 cases, clinical findings (congestive heart failure) in 2 cases (including the 2.6-year-old child) and cardiac autopsy after a normal biopsy result in 1 case.

Results of standard ECG (Tables 2 and 3). The values of the summated voltage (Table 2) and their relative changes observed between ECGs obtained during normal periods and ECGs accompanying severe rejection. These same variables were not significantly altered from normal during mild rejection crises. Figures 1 and 2 depict a typical severe rejection episode with time and frequency domain results, respectively. A substantial decrease appeared before and after filtering in the initial forces of the QRS complex. This phenomenon, predominantly involving the first half of the QRS complex, was observed in four additional episodes. In the frequency domain, rejection induced overall spectral changes. The analysis performed in comparison with a reference tracing provided similar results. All time domain variables and one frequency domain variable (the total spectral plot) were significantly altered in the presence of severe rejection (Table 3, Fig. 3).

Marked changes in the ECG variables were frequently observed: 14 mild episodes in 14 patients and 16 severe episodes in 15 patients. These severe crises were diagnosed on the basis of endomyocardial biopsy in 13 cases, clinical findings (congestive heart failure) in 2 cases (including the 2.6-year-old child) and cardiac autopsy after a normal biopsy result in 1 case.

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Overall reproducibility was observed with the 99% bandwidth of the amplitude spectrum.

### Table 2. Absolute Measurements in the Absence and Presence of Rejection in 25 Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Results (n = 120)</th>
<th>Mild Rejection (n = 14)</th>
<th>Severe Rejection (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Sigma V ) (mV) (I, II, III, V_1, V_6)</td>
<td>4.92 ± 1.34</td>
<td>4.45 ± 1.27*</td>
<td>4.27 ± 1.05*</td>
</tr>
<tr>
<td>PVA (unfilt) (( \mu V ))</td>
<td>2,626.4 ± 708.8</td>
<td>2,560.9 ± 731.8*</td>
<td>1,771.2 ± 661.2t</td>
</tr>
<tr>
<td>RMSA (unfilt) (( \mu V ))</td>
<td>1,228.2 ± 344.8</td>
<td>1,133.0 ± 350.7*</td>
<td>849.1 ± 294.3t</td>
</tr>
<tr>
<td>DUR (unfilt) (ms)</td>
<td>94.7 ± 10.4</td>
<td>94.5 ± 9.6*</td>
<td>96.6 ± 14.0*</td>
</tr>
<tr>
<td>PVA (Fc 25 Hz) (( \mu V ))</td>
<td>1,057.7 ± 258.7</td>
<td>1,056.8 ± 293.9*</td>
<td>733.3 ± 239.6t</td>
</tr>
<tr>
<td>RMSA (Fc 25 Hz) (( \mu V ))</td>
<td>399.3 ± 98.5</td>
<td>379.6 ± 113.9*</td>
<td>276.3 ± 86.3t</td>
</tr>
<tr>
<td>PVA (Fc 70 Hz) (( \mu V ))</td>
<td>277.6 ± 80.2</td>
<td>282.1 ± 71.1*</td>
<td>203.0 ± 65.1t</td>
</tr>
<tr>
<td>RMSA (Fc 70 Hz) (( \mu V ))</td>
<td>96.6 ± 26.4</td>
<td>93.4 ± 27.1*</td>
<td>66.4 ± 19.7t</td>
</tr>
<tr>
<td>99% bandwidth (Hz)</td>
<td>156.7 ± 41.2</td>
<td>155.4 ± 45.9*</td>
<td>172.4 ± 43.1*</td>
</tr>
<tr>
<td>SA (total) (mV x Hz)</td>
<td>1,495 ± 1,036</td>
<td>1,337 ± 359*</td>
<td>958 ± 320t</td>
</tr>
<tr>
<td>SA (50-250 Hz) (mV x Hz)</td>
<td>16.7 ± 61</td>
<td>14.4 ± 46*</td>
<td>97 ± 43*</td>
</tr>
<tr>
<td>SA (70-110 Hz) (mV x Hz)</td>
<td>28 ± 25</td>
<td>37 ± 23*</td>
<td>30 ± 17*</td>
</tr>
</tbody>
</table>

\*p = NS; \( p < 0.001; \) \( p < 0.05 \). n = number of signal-averaged electrocardiograms (ECGs); \( \Sigma V \) = summed voltage computed from leads I, II, III, V_1 and V_6 of the standard ECG; other abbreviations as in Table 1.
Table 3. Changes in the Electrocardiographic Variables in the Absence of Rejection and at the Time of Rejection in Comparison With a Reference Tracing in 25 Patients

<table>
<thead>
<tr>
<th></th>
<th>Repeat Normal (%)</th>
<th>Mild Rejection (%)</th>
<th>Severe Rejection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 97)</td>
<td>(n = 14)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td>2V (I, II, III, V1, V6)</td>
<td>−0.84 ± 18.27</td>
<td>1.26 ± 22.61*</td>
<td>−3.34 ± 25.61*</td>
</tr>
<tr>
<td>PVA (unfilt)</td>
<td>0.21 ± 23.90</td>
<td>0.38 ± 16.43*</td>
<td>−23.50 ± 26.64†</td>
</tr>
<tr>
<td>RMSA (unfilt)</td>
<td>0.95 ± 25.81</td>
<td>−2.53 ± 16.65*</td>
<td>−21.48 ± 24.13‡</td>
</tr>
<tr>
<td>DUR (unfilt)</td>
<td>−0.97 ± 7.41</td>
<td>0.19 ± 3.26*</td>
<td>4.45 ± 7.48‡</td>
</tr>
<tr>
<td>PVA (Fc 25 Hz)</td>
<td>3.63 ± 27.89</td>
<td>6.31 ± 19.77*</td>
<td>−22.21 ± 23.42†</td>
</tr>
<tr>
<td>RMSA (Fc 25 Hz)</td>
<td>3.19 ± 26.81</td>
<td>0.39 ± 14.44*</td>
<td>−21.57 ± 21.47†</td>
</tr>
<tr>
<td>PVA (Fc 70 Hz)</td>
<td>5.98 ± 33.57</td>
<td>9.19 ± 17.38*</td>
<td>−20.17 ± 23.78†</td>
</tr>
<tr>
<td>RMSA (Fc 70 Hz)</td>
<td>5.30 ± 29.52</td>
<td>−0.56 ± 18.12*</td>
<td>−22.26 ± 21.15†</td>
</tr>
<tr>
<td>99% bandwidth</td>
<td>5.81 ± 28.35</td>
<td>−3.13 ± 12.65*</td>
<td>4.15 ± 25.38*</td>
</tr>
<tr>
<td>SA (total)</td>
<td>9.28 ± 87.40</td>
<td>−6.92 ± 28.14*</td>
<td>−23.27 ± 23.15†</td>
</tr>
<tr>
<td>SA (50–250 Hz)</td>
<td>17.41 ± 62.65</td>
<td>5.77 ± 22.98*</td>
<td>−13.83 ± 43.85*</td>
</tr>
<tr>
<td>SA (70–110 Hz)</td>
<td>36.01 ± 134.45</td>
<td>−2.11 ± 37.97*</td>
<td>6.75 ± 84.29*</td>
</tr>
</tbody>
</table>

*p = NS; †p < 0.01; ‡p < 0.05. Abbreviations are as in Tables 1 and 2.

encountered, even in the absence of rejection. At least two mechanisms accounting for these false positive results were observed. The first was massive pneumonia, which induced a >33% decrease in the peak and root-mean-square voltage amplitudes of the QRS complex. The second mechanism was an intermittent conduction defect occurring in a patient who never experienced rejection. This abnormality induced important fluctuations in time domain data (Fig. 4).

Value of time and frequency domain variables in detecting rejection (Tables 2 and 3). The accuracy of detecting severe rejection was evaluated only for the seven variables that were significantly altered for both absolute and relative measurements. Therefore, QRS duration, 99% bandwidth of the spectrum and spectral areas of the 50- to 250-Hz and 70- to 110-Hz ranges were not analyzed. The compromise between sensitivity and specificity was analyzed by means of receiver operating characteristic curves obtained for different rejection criteria. The curves for the most discriminative variables (all in the time domain) are shown in Figure 5. The most accurate rejection criterion was an 11% decrease in the root-mean-square voltage of the 70-Hz high pass filtered QRS complex between two consecutive ECGs. This criterion had 87.5% sensitivity with 78.4% specificity.

Detection of rejection in the early versus late postoperative period. Three of the 16 severe rejection episodes occurred in the 1st 6 weeks after transplantation. As assessed by our rejection criteria, all 3 of these episodes and 11 of the 13 episodes occurring later were correctly identified (p = NS). Moreover, the number of false positive results was similar between the early and late postoperative periods in the case of repeat biopsy with normal findings (24.6% [16 of 65] versus 21.8% [7 of 32], respectively; p = NS).

Discussion

Time domain analysis of the signal-averaged QRS complex provides a noninvasive indication of rejection after orthotopic cardiac grafting. The rejection process induces decreases in the peak and root-mean-square voltage amplitudes of the QRS complex, which are easier to identify after

![Figure 1. Example of rejection demonstrated by time domain analysis of the signal-averaged electrocardiogram (ECG). Top, Unfiltered data; bottom, 70-Hz high pass filtered data. Panels A and B show baseline data obtained 25 and 32 days after transplantation. Panel C corresponds to rejection, with a prominent decrease in the initial forces of the QRS complex occurring 46 days after transplantation. The numbers beside each ECG indicate the root-mean-square voltage of the entire QRS complex and the relative change (%) from the preceding recording. t = time.](image-url)
The magnitude of these alterations seems to depend on the histologic severity of the rejection crisis because mild rejection induces nonsignificant ECG changes. In concordance with published studies (1–3,9), our work confirms that the standard ECG, unlike the signal-averaged ECG, cannot detect rejection episodes.

**Frequency domain analysis.** The conclusions concerning the value of frequency domain analysis in our study conflict with those of Haberl et al. (10), who presented both averaged and nonaveraged high resolution data obtained from two distinct bipolar leads. Unfortunately, their methods were not clearly detailed with regard to the leads or acquisition technique. Respiratory changes in QRS and T wave axes may have affected the results obtained from only two nonorthogonal bipolar leads. Furthermore, normalization of the spectral plots by setting the predominant frequency equal to 1 rendered the approach difficult to evaluate. Thus, the rejection criterion advocated in the study of Haberl et al. (10) was a 20% increase in the 70- to 110-Hz frequency content of the QRS amplitude between two consecutive recording sessions. We found that this variable was poorly reproducible because the mean difference between two recordings on the same day was evaluated to be 26.8% in our control group. Moreover, changes occurring within this range at the time of rejection were found to be nonsignificant.

**Time domain analysis.** The time domain results are consistent with those obtained by other investigators. Keren et al. (9) reported that the total root-mean-square voltage of the averaged filtered QRS complex is a promising variable for the identification of rejection in the late postoperative period. These investigators (9) as well as Aleixo et al. (8) observed a rejection-induced decrease of about 15% to 20% in the QRS voltage amplitude and, similar to our findings, mild forms of rejection were not detected. The use of high pass filters may seem paradoxic because rejection causes overall spectral changes. However, according to our results, the higher the corner frequency, the greater the accuracy in detecting rejection. This observation suggests that the high frequency content of the QRS complex is probably less subject to spontaneous variations than are the low frequency components after cardiac transplantation.

The mechanism of the alterations occurring in the time domain remain speculative. We did not observe any increase in the high frequency content, suggesting the lack of fractionation of the QRS forces. Moreover, the rapid reversibility during treatment and the involvement of the total spectrum indicate edema as the possible origin of the voltage decrease. The predominance of this decrease in the initial forces of the QRS complex is consistent with previous studies (17) that emphasized the extent of histologic damage in the subendocardial regions.

With our method, we did not find the lower sensitivity in the early postoperative period reported by Keren et al. (9). This discrepancy could be due to the use of a different filtering technique, but the number of rejection episodes
during this period was too small to allow definitive conclusions.

Limitations. False positive and, in particular, false negative results are the major limitations of this technique. Such drawbacks also exist for the endomyocardial biopsy technique as evidenced by the occurrence of a fatal rejection crisis 24 h after a normal biopsy finding in our study. This normal biopsy finding was accompanied by overt ECG changes. False positive or false negative results related to intermittent conduction defects were not unexpected because incomplete bundle branch block unrelated to rejection has been reported (18), with a high prevalence after operation. An unsteady hemodynamic state, changes in the electrolyte balance or the effects of immunosuppressive drugs may also account for some of the discrepancies observed between ECG data and biopsy results.

Conclusions. Additional studies utilizing signal-averaged electrocardiography and methods such as echocardiography or nuclear magnetic resonance imaging are needed to improve the noninvasive monitoring of rejection and reduce the number of endomyocardial biopsy procedures.

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References