edge, this is the first continuous murmur associated with an acquired VSD. In fact, there has been only one instance of a continuous VSD murmur reported in a patient with a congenital VSD. We postulate that the murmur was continuous as a result of elevated left ventricular diastolic pressure secondary to the acute anterior myocardial infarction. A marked pressure disparity between the left and right ventricles during diastole could explain the continued left-to-right shunting during this latter period. Continued shunting during diastole was likely facilitated by the small diameter of the defect, which prevented the rapid equilibration of pressures between the two chambers.

The specially equipped stethoscope in conjunction with a handheld computer used in this study was valuable in the assessment and teaching of cardiac sounds. In this instance, the visual displays provided confirmation of the continuous nature of this murmur; moreover, this apparatus allowed for repeated playback at both full speed and half-speed, providing a means for additional auditory review.

**References**


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**Myocardial Apoptotic Index Based on *In Situ* DNA Nick End-Labeling of Endomyocardial Biopsies Does Not Predict Prognosis of Dilated Cardiomyopathy**

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**Background:** DNA breaks detected largely by terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate *in situ* nick end-labeling (TUNEL) are observed in the hearts of patients with diseases such as dilated cardiomyopathy (DCM).

**Study objectives:** To determine the prognostic value of TUNEL assays in cases of DCM.

**Design, setting, and participants:** DCM patients were selected from among patients who had undergone left ventricular (LV) biopsy during the period from 1994 to 2001 in our hospital. Of those, 46 (35 men and 11 women; mean [± SD] age, 58 ± 11 years) who were followed up for > 3 years after the undergoing the biopsy (mean follow-up period, 4.9 ± 2.0 years) or died during the follow-up period were entered into the present study. The myocardial apoptotic index was assessed in deparaffinized biopsy specimens that were stained using a conventional TUNEL assay. In addition, all surviving patients received a follow-up echocardiographic examination.

**Results:** Ten of the 46 biopsy specimens (22%) contained TUNEL-positive myocytes; their mean apoptotic index was 0.44 ± 0.05%. The apoptotic index showed no relation to cardiac functional parameters determined at the time of biopsy, however. Seven patients died during the follow-up period, and 19 of the surviving patients were readmitted to the hospital because of a worsening of their heart failure. There was no significant difference in the apoptotic indexes of biopsy specimens from the dead and surviving patients, or between the surviving patients who were readmitted to the hospital and those who died.
were not. There was also no significant correlation between the apoptotic index and changes in the LV ejection fraction, LV end-diastolic diameter, or LV posterior wall thickness during follow-up. Conclusion: The apoptotic index derived from TUNEL assays is not predictive of the prognosis of patients with DCM-induced heart failure.

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Key words: apoptosis; heart failure; dilated cardiomyopathy; prognosis

Abbreviations: CHF = congestive heart failure; DCM = dilated cardiomyopathy; LV = left ventricle, ventricular; LVEF = left ventricular ejection fraction; LVEDD = left ventricular end-diastolic diameter; LVPWT = left ventricular posterior wall thickness; TUNEL = terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick end-labeling

DNA breaks detected largely by terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate in situ nick end-labeling (TUNEL) have been observed in the hearts of patients with diseases such as dilated cardiomyopathy (DCM). This suggests that the loss of cardiac myocytes due to apoptosis may contribute to the progression of congestive heart failure (CHF), though so far the data are inconclusive. If correct, however, the level of apoptosis within the myocardium may influence the prognosis of CHF patients; in other words, the apoptotic index measured in specimens of myocardial tissue may have predictive value as to patient outcome. To test this hypothesis, we carried out a tracking study in a group of DCM patients who had undergone endomyocardial biopsy > 3 years earlier and were followed up thereafter.

Materials and Methods

After obtaining approval for this study from our local ethics committees, patients with DCM diagnosed according to the definition and classification proposed by the World Health Organization/International Society and Federation of Cardiology task force were selected from among those who had undergone left ventricular (LV) biopsy in our hospital during the period from 1994 to 2001. Of those, 46 patients who were followed up for > 3 years after the biopsy (mean [± SD] follow-up period, 4.9 ± 2.0 years) or died during the follow-up period were entered into the present study. These participants included 35 men and 11 women with a mean age of 58 years (age range, 23 to 75 years). The endomyocardial biopsy specimens were obtained from the posterior free wall of the LV, and the myocardial apoptotic index was determined in deparaffinized biopsy specimens that were stained using a conventional TUNEL assay. In each specimen, a mean of 276 ± 66 cardiomyocytes were evaluated within a mean area of 1.4 ± 0.5 mm². In addition, the sections were subjected to Taq polymerase-based in situ DNA ligation assays using a method previously described. We also examined the specimens under an electron microscope, observing at least five grids from each case.

Results and Discussion

The biopsy specimens from 10 of the 46 patients (22%) contained TUNEL-positive myocytes; the mean apoptotic index was 0.44 ± 1.05%. However, the apoptotic index was found to have no relation to any functional parameters or to the anatomic status of the hearts, which were assessed at biopsy by cardiac catheterization (ie, LV pressures, LV ejection fraction [LVEF], and LV end-diastolic volume index) and echocardiography (LVEF and LV dimensions, including LV end-diastolic diameter [LVEDD], and LV posterior wall thickness [LVPWT]) (Table 1). In addition, the results of the Taq polymerase-based in situ DNA ligation assays were negative in all biopsy specimens, although, as expected, positive reactions were noted in lining epithelial cells from mouse thymus tissue (ie, the positive control). Using the electron microscope, we failed to find even one myocyte showing the typical ultrastructural features of apoptosis in any of the specimens. These patients were treated with digitalis, diuretics, vasodilators, or some combination of the three, but there was no specific difference in the medication used between patients whose biopsy specimens were TUNEL-positive and those that were not.

We also tested whether the apoptotic index derived from the TUNEL assays could reflect the patients’ prognosis with respect to survival or progression of the CHF. Seven patients died during the follow-up period, and 19 of the surviving patients were readmitted to hospitals due to a worsening of their CHF. The biopsy specimen from one of the dead patients was TUNEL-positive, but the other six were not. Moreover, there was no significant difference between the apoptotic indexes of the biopsy specimens from dead and surviving patients (0.38 ± 0.06% vs 0.45 ± 0.10%, respectively; p = 0.87), or between the surviving patients who were readmitted to the hospital and those who were not (0.40 ± 0.95% vs 0.55 ± 1.21%, respectively; p = 0.55).

All surviving patients received a follow-up echocardiographic examination. Earlier echocardiography findings showed that at biopsy the mean LVEF was 40.2 ± 11.3%, the mean LVEDD was 60.6 ± 14.8 mm, and the mean LVPWT was 11.4 ± 4.0 mm. Over the course of the > 3-year follow-up period (mean follow-up period, 4.9 ± 2.0 years), changes in these functional parameters were small. LVEF increased by about 11.7% to 44.9 ± 14.0%, while LVEDD and LVPWT stayed about the same (60.5 ± 9.6 and 9.7 ± 1.4 mm, respectively).

Table 1—Comparison of Hemodynamic Parameters at the Time of Endomyocardial Biopsy Between the Patients With and Without a Positive Apoptotic Index*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apoptotic Index</th>
<th>Apoptotic Index</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (n = 36)</td>
<td>Positive (n = 10)</td>
<td></td>
</tr>
<tr>
<td>Cardiac catheterization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD, mm Hg</td>
<td>11.2 ± 6.9</td>
<td>14.7 ± 10.1</td>
<td>0.22</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>37.9 ± 12.6</td>
<td>40.6 ± 11.4</td>
<td>0.55</td>
</tr>
<tr>
<td>LV end-diastolic volume</td>
<td>126.3 ± 34.0</td>
<td>107.4 ± 34.0</td>
<td>0.13</td>
</tr>
<tr>
<td>index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>40.0 ± 10.6</td>
<td>46.1 ± 10.2</td>
<td>0.11</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>60.1 ± 6.6</td>
<td>57.1 ± 3.4</td>
<td>0.17</td>
</tr>
<tr>
<td>LVPWT, mm</td>
<td>9.7 ± 1.8</td>
<td>9.7 ± 0.9</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*Values given as mean ± SD, unless otherwise indicated.
There was no significant correlation between the apoptotic index and changes in these functional parameters (Fig 1).

The present data clearly do not support the hypothesis that conventional TUNEL assays of endomyocardial biopsy specimens from patients with DCM-induced CHF have prognostic value with respect to either survival or the functional status of the heart. But because LV endomyocardial biopsy specimens represent only a small part of the LV, they do not exclude the possibility that the presence or absence of apoptosis in other parts of the LV could reflect in vivo status. Having said that, however, the high proportion of TUNEL-positive cells reported in earlier studies that analyzed whole hearts might lead one to expect that the affected patients inevitably died in short order, but that does not fit with the clinical observations. More importantly, to our knowledge, there has never been a report identifying cardiomyocytes with the characteristic apoptotic ultrastructure in DCM patients, which we also failed to see in the present study. Still, TUNEL assays measure only a small portion of the total apoptotic cascade, and a biopsy study including electron microscopy records only a tiny segment of time during the course of a disease that stretches over decades, leaving open the possibility that apoptosis may not be rare in patients with DCM.

Bearing these findings in mind, along with the known limitations of the TUNEL assay (e.g., its lack of specificity), we suggest that TUNEL assays cannot be used as the sole test to identify apoptosis in failing hearts, as the derived apoptotic indexes are not predictive of the prognosis of DCM-induced CHF.

REFERENCES

Etanercept for Refractory Ocular Sarcoidosis*

Results of a Double-Blind Randomized Trial

Robert P. Baughman, MD, FCCP; Elyse E. Lower, MD; Deborah A. Bradley, MD; Lawrence A. Raymond, MD; and Adam Kaufman, MD

Purpose: Study a tumor necrosis factor receptor antagonist (etanercept) in the treatment of chronic ocular sarcoidosis.

Subjects and methods: Eighteen patients with ocular sarcoidosis and ongoing inflammation in the eyes. All patients had received at least 6 months of therapy with methotrexate and were currently receiving corticosteroids. Patients were randomized to receive either etanercept, 25 mg subcutaneously twice a week, or placebo in a double-blind randomized trial. Treatment for ocular inflammation with systemic and local corticosteroids at the beginning and end of 6 months of treatment was noted. All patients underwent an ophthalmic examination at the beginning and the end of the study by one ophthalmologist who was unaware of what treatment the patient was receiving.

Figure 1. Correlation between the apoptotic index derived from LV endomyocardial biopsies and the percentage change in the indicated cardiac functional and anatomical parameters during the follow-up period. △LVEF (top, A); △LVEDD (middle, B); and △LVPWT (bottom, C).