Patterns of Serological Markers for Cellular Immune Activation in Patients with Dilated Cardiomyopathy and Chronic Myocarditis

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We determined serum concentrations of neopterin and \( \beta_2 \)-microglobulin, soluble markers of cellular immune activation, in 27 patients with either dilated cardiomyopathy (DCM) or chronic myocarditis. Neopterin and \( \beta_2 \)-microglobulin concentrations were respectively increased in 2 and 5 of 11 patients with DCM and in 11 and 9 of 16 patients with chronic myocarditis. A higher cardiac functional class (according to the New York Heart Association) was associated with greater neopterin and \( \beta_2 \)-microglobulin concentrations. During follow-up of patients, both neopterin and \( \beta_2 \)-microglobulin concentrations in serum correlated with the course of disease. Additionally, correlations were significant between left ventricular functional tests (end-diastolic volume, end-systolic volume, and ejection fraction) and neopterin and \( \beta_2 \)-microglobulin concentrations. We conclude that measurement of neopterin and \( \beta_2 \)-microglobulin are useful to monitor disease development in patients with myocardial inflammation.

Additional Keyphrases: neopterin \( \cdot \) \( \beta_2 \)-microglobulin \( \cdot \) congestive heart failure \( \cdot \) inflammation

Dilated cardiomyopathy (DCM) is considered to be the end stage of chronic myocarditis.4 Diagnosis is based mainly on the result of endomyocardial biopsy, and various tests may support differential diagnosis between DCM and chronic myocarditis. However, reliable markers for monitoring disease progression in patients with congestive heart failure are still lacking.

Immunological abnormalities are frequent in patients with DCM (1), suggesting that viral infections or autoimmune phenomena may be involved in the pathogenesis of the disorder (2). Moreover, about half of the patients with acute myopericarditis present with enterovirus-specific IgM responses (3), and enterovirus-specific RNA sequences can be found in myocardial biopsies (4).

Neopterin and \( \beta_2 \)-microglobulin concentrations in serum have been shown to be increased in some patients with myocardial inflammation (5). These soluble markers of immune-cell activation can be readily detected in serum. Patients with acute viral infections and with autoimmune disorders have increased neopterin concentrations in body fluids. The frequency of abnormally increased neopterin concentrations, as well as the amount of the increase, are strongly correlated with the severity of disease (6–9). Neopterin and \( \beta_2 \)-microglobulin concentrations are predictors of disease progression in patients with human immunodeficiency virus infection (9–11).

Here we evaluated the pattern of neopterin and \( \beta_2 \)-microglobulin concentrations in serum during the follow-up of patients with myocardial inflammation.

Patients and Methods

We studied 11 men (mean age 41, SD 11, years) with DCM according to World Health Organization criteria (12) and 16 patients with chronic myocarditis (15 men, 1 woman, mean age 37, SD 12, years) referred to the All-Union Cardiology Research Center. Nineteen patients underwent coronary angiography, which demonstrated that none had significant coronary artery disease; endomyocardial biopsy was performed in 16 of the 19. In one patient diagnosis was confirmed at autopsy. Twelve patients had histologic evidence of chronic myocarditis. The mean (SD) New York Heart Association (NYHA) functional class was 2.25 (0.75) for patients with DCM and 2.50 (0.63) for those with chronic myocarditis. Clinically significant thromboembolic events were documented in nine patients. Only two patients had clinical and laboratory features of intercurrent infections (increased body temperature, mildly increased blood sedimentation rate) at the beginning of the study, and one at the end. These patients received specific antibacterial therapy. Other patients were treated conventionally with diuretics, glycosides, and vasodilators.

Echocardiographic measurements (M-mode and two-dimensional) were performed in all patients. Standard echocardiographic values of left and right ventricles were assessed. Left ventricle end-diastolic volume (EDV) and end-systolic volume (ESV) were calculated according to Teicholz et al. (13). Ejection fraction (EF) was calculated as follows:

\[
\text{EF(\%)} = \frac{(\text{EDV} - \text{ESV}) \times 100}{\text{EDV}}
\]

Neopterin and \( \beta_2 \)-microglobulin concentrations in serum were measured with radioimmunoassays (neopterin: Henning, Berlin, F.R.G.; \( \beta_2 \)-microglobulin: Pharmacia, Uppeala, Sweden). In the hospital, 23 of 24 healthy controls had serum neopterin concentrations <9.0 nmol/L (5), which agrees well with the normal range obtained earlier (6) for healthy controls in Aus-
tria (<8.7 nmol/L). The upper limit of the normal range for $\beta_2$-microglobulin was 2.4 mg/L.

We measured neopterin and $\beta_2$-microglobulin concentrations simultaneously in serum from each patient at the time of initial presentation and at various intervals at follow-up visits (after 3, 6, 12, 18, and 24 months).

Besides neopterin and $\beta_2$-microglobulin, we also determined serum creatinine and hemoglobin concentrations and the erythrocyte sedimentation rate, using routine techniques.

For statistical evaluation, we used analysis of variance (ANOVA) for group comparisons and computation of Spearman's rank correlation coefficients ($r_s$). ANOVA was done by the Brown--Forsythe method (14) because the variances in the subgroups were significantly different by Levene's test (15).

Results

At the start of the investigation, serum neopterin concentrations were increased in 2 of 11 patients with DCM and in 11 of 16 patients with chronic myocarditis. Serum $\beta_2$-microglobulin concentrations were increased in 5 of 11 patients with DCM and in 9 of 16 patients with chronic myocarditis. Higher NYHA class was associated with the greater neopterin and $\beta_2$-microglobulin concentrations (Table 1). Serum creatinine and hemoglobin concentrations and erythrocyte sedimentation rate did not differ in patients with distinct NYHA class differences (data not shown). The correlation between neopterin and $\beta_2$-microglobulin concentrations in patients with DCM and chronic myocarditis was significant ($r_s = 0.585, P < 0.001$).

Significant correlations between left-ventricular functional tests (end-diastolic volume, end-systolic volume, and ejection fraction) and neopterin and $\beta_2$-microglobulin concentrations were found in patients at first visits (Table 2). The strength of correlation was greater for neopterin concentrations. Similar observations were made during follow-up visits of patients. In general, the correlations were even better during follow-up visits, particularly for $\beta_2$-microglobulin concentrations (Table 2).

Both neopterin and $\beta_2$-microglobulin concentrations in serum correlated significantly with the course of disease; these concentrations either did not change or even decreased during follow-up of patients with a stable course of disease, i.e., those without increasing NYHA class and (or) without thromboembolic complications (Figure 1, lower panels). In contrast, in patients with poorly controlled heart failure despite therapy, or in those having thromboembolic complications, neopterin and $\beta_2$-microglobulin concentrations increased (Figure 1, upper panels). Six of 14 patients from this group died during the study period.

There was no association between the clinical course of disease and the other laboratory analytes in our study (including erythrocyte sedimentation rate and hemoglobin and creatinine concentrations; data not shown in detail).

Discussion

This study indicates that changes of neopterin and $\beta_2$-microglobulin concentrations are associated with the functional class of myocardial inflammation in patients with DCM and chronic myocarditis. In longitudinal examinations, increasing serum concentrations of neopterin and $\beta_2$-microglobulin were associated with disease progression. Patients whose clinical conditions

| Table 1. Dependence of Serum Neopterin and $\beta_2$-Microglobulin Concentrations on NYHA Class in Patients with Chronic Myocarditis and DCM |
|-----------------|-----------------|-----------------|-----------------|
| NYHA class      | I $^a$          | II $^b$         | III $^b$        |
| n               | 2               | 11              | 14              |
| Neopterin, nmol/L $^c$ | 7.2 (8.1)       | 7.6 (5.2--6.9)  | 11.5 (8.1--16.5) |
| $\beta_2$-Microglobulin, mg/L $^c$ | 1.9 (2.0)       | 2.4 (1.5--2.8)  | 2.8 (2.6--3.6)  |

$^a$ Individual measurements.

$^b$ Medians (and 25th--75th percentiles).

$^c$ Significantly related to NYHA class: $p < 0.01$ (ANOVA).

| Table 2. Correlation between Neopterin and $\beta_2$-Microglobulin Concentrations and Left-Ventricular Function Tests in Patients with DCM and Chronic Myocarditis |
|-----------------|-----------------|-----------------|-----------------|
| Function test $^a$ | First visit | Follow-up | First visit | Follow-up |
| EDV $^b$         | 0.487$^b$      | 0.812$^b$     | 0.359$^b$      | 0.843$^b$     |
| <0.01 $^b$       | <0.001 $^b$    | <0.05 $^b$    | <0.001 $^b$    | <0.001 $^b$   |
| ESV              | 0.569          | 0.653          | 0.385          | 0.683        |
| <0.005          | <0.001         | <0.05          | <0.001         | <0.01        |
| EF $^b$          | -0.582         | 0.516          | 0.354          | 0.478        |
| <0.005          | <0.001         | <0.05          | <0.01          |

$^a$ EDV, end-diastolic volume; SV, end-systolic volume; EF, ejection fraction.

$^b$ Spearman rank correlation coefficients are shown.
were stable or improving exhibited stable, low, or decreasing concentrations of both immune markers.

We demonstrated several correlations between functional tests of the left ventricles and the concentrations of neopterin and \( \beta_2 \)-microglobulin. Left-ventricular measures such as end-diastolic volume, end-systolic volume, and ejection fraction are significantly associated with outcome in patients with DCM (16). Thus, the correlations found between the functional tests and immune activation markers point to a potential predictive value of neopterin and \( \beta_2 \)-microglobulin in patients with congestive heart failure.

Neopterin and \( \beta_2 \)-microglobulin concentrations in serum can be influenced by kidney function. Chronic inflammation in patients with DCM or chronic myocarditis may cause renal impairment in some patients in later stages of the disease. However, the creatinine concentration in serum was practically normal in all of our patients at their initial examinations as well as in follow-up investigations. Therefore, the increases of neopterin and \( \beta_2 \)-microglobulin clearly did not result from renal impairment.

In agreement with in vitro data and with clinical observations from several other disease conditions, increased neopterin and \( \beta_2 \)-microglobulin appear to indicate the degree of immune activation in patients (6, 7, 10). High neopterin concentrations are known to be closely associated with endogenous formation of cytokines during immune stimulation. Interferon gamma is the most potent inducer of neopterin formation and release by human monocytes and macrophages (6, 7). Thus, activation of the immune system parallels the course of DCM and chronic myocarditis. Immune activation may contribute to the spectrum of symptoms in patients and to progression of the disease. From the neopterin and \( \beta_2 \)-microglobulin data we were not able, however, to distinguish whether viral infection or autoimmune disease or both were involved in the etiology of myocardial inflammation in patients with DCM or chronic myocarditis.

We conclude that measurement of neopterin and \( \beta_2 \)-microglobulin concentrations in serum may serve as a simple tool to monitor the clinical course of patients with myocardial inflammation. Further studies are required to determine whether these variables are also of value for predicting outcome in patients with DCM and chronic myocarditis.

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References