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Prognostic role of myocardial tumor necrosis factor-alpha and terminal complement complex expression in patients with dilated cardiomyopathy

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Abstract

Background: In patients with dilated cardiomyopathy (DCM), elevated plasma levels of tumor necrosis factor-α (TNF-α) are associated with poor prognosis. The terminal complement complex (C5b-9) stimulates myocardial TNF-α expression.
Aims: To investigate whether myocardial TNF-α and C5b-9 expression correlate with clinical outcome in DCM.
Methods and results: 71 patients with DCM underwent myocardial biopsy. Biopsies were analyzed for TNF-α, C5b-9, markers of inflammation and for viral genome. Patients were divided into three groups according to biopsy results: group A: no TNF-α and no C5b-9; group B: TNF-α or C5b-9; and group C: TNF-α and C5b-9. NYHA classification, ECG and echocardiography were documented. Patients received conventional treatment of heart failure and, in a few cases, additional treatment with interferon β_{1b} (virus positive) or prednisolone (inflammatory DCM).

There were 13 patients (18%) in group A, 19 patients (27%) in group B, and 39 patients (55%) in group C. All groups had a similar and significant improvement in NYHA classification and echocardiographic parameters. TNF-α and C5b-9 did not significantly correlate with the presence of viral genome or with markers of inflammation.
Conclusion: TNF-α and C5b-9 are widely distributed in the myocardium of DCM patients. Neither of the antigens correlates with clinical outcome. Myocardial TNF-α may not be a useful prognostic marker in DCM.

Keywords: Dilated cardiomyopathy; Biopsy; Heart failure; TNF-α; Terminal complement complex

1. Introduction

It has been suggested that TNF-α, a proinflammatory cytokine, is involved in the pathogenesis of atherosclerosis, myocardial infarction and chronic heart failure [1–6]. Indeed, the potential importance of plasma TNF-α in chronic heart failure has been demonstrated in animal models and clinical trials [1,2,4]. TNF-α may be directly involved in the progression of heart failure, because cardiomyocytes in the failing heart express TNF-α, and myocardial TNF-α may exert direct negative inotropic effects and trigger apoptosis in cardiomyocytes [5,6]. In animal experiments, myocardial TNF-α expression correlates with increased mortality rates [7]. We have recently shown that C5b-9, the terminal complement complex, induces TNF-α synthesis in cardiac myocytes in vitro, and that C5b-9 and TNF-α are associated in the myocardium of DCM patients [8].

Although it is well established that elevated *plasma* levels of TNF-α correlate with the severity of chronic heart failure, there is no reliable information about whether *myocardial* TNF-α expression in humans suffering from chronic heart failure provides information about clinical outcome. In this study, we investigated whether TNF-α and

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C5b-9 expression in myocardial biopsies correlate with clinical outcome in patients with DCM.

2. Methods

2.1. Study group

71 patients with DCM admitted to Ulm University Medical Centre between 2000 and 2002 were included in the study. All patients presented with clinical symptoms of cardiac failure. Diagnostic evaluation included NYHA classification, ECG, echocardiography and cardiac catheterization. Inclusion criteria have been described in detail elsewhere [9,10].

2.2. NYHA classification

NYHA functional classification was performed following the guidelines of the Criteria Committee of the New York Heart Association [11].

2.3. ECG

The 12-lead ECG was analyzed for rhythm, QR-axis, heart rate, bundle branch block, ST deviation and Sokolow index I. Pacemaker triggered conduction was not assessed.

2.4. Echocardiography

Echocardiography was carried out as described previously [9]. Left ventricular function (LVF) was assessed qualitatively in the apical view and classified as either normal, mildly, moderately or severely decreased. Fractional shortening (FS) was calculated by M-mode in a standard manner in order to assess left ventricular systolic function.

2.5. Myocardial biopsies

Analysis of myocardial biopsies was performed as described in detail previously [9,10]. In brief, six right ventricular (septal) endomyocardial biopsies were analyzed from each patient to reduce the sampling error. Histomorphological diagnosis of dilated cardiomyopathy was performed by examination of the following criteria: interstitial fibrosis, cellular infiltrates, cellular hypertrophy and myocardial cell degeneration.

Immunohistochemical analysis included staining for TNF-α and C5b-9 with mouse monoclonal antibodies. Five sections from a single biopsy were analysed. Peroxidase-conjugated horse anti-mouse antibody was used as a secondary antibody. TNF-α and C5b-9 were assessed as either positive or negative, independent from their localisation in the biopsy tissue or staining intensity. Immunohistochemical analysis also included staining for CD2. CD2 positive cells were counted under a light microscope (cells/mm^2). Biopsies were classified as borderline inflammation (3 to 10 CD2 positive cells/mm^2) or inflammatory cardiomyopathy (>10 CD2 positive cells/mm^2).

Virological analysis for enteroviral RNA and adenoviral DNA was performed by PCR using extracted nucleic acid. The GAPDH gene was used to demonstrate equal loading of intact DNA.

2.6. Treatment protocol

Drug therapy was administered as described in detail previously [9]. In summary, patients were treated with standard medication according to ACC/AHA guidelines [12]. In a few cases, additional treatment with interferon β_{1b} (virus positive) or prednisolone (inflammatory DCM) was administered.

2.7. Follow-up

For outcome evaluation, the following parameters were assessed [9]:

(A) NYHA-classification (see above)
(B) ECG-parameters, as follows:
  i. rhythm: For follow-up within groups, sinus rhythm was assigned. For comparative follow-up between groups, changes from sinus rhythm to arrhythmia, stable rhythm and changes from arrhythmia to sinus rhythm were documented.
  ii. QR-axis: For follow-up within groups, an angle from −30° to 60° was considered as normal QR-axis. For comparative follow-up between groups, changes and no changes of the QR-axis were documented.
  iii. heart rate: For heart rate, absolute values were considered.
  iv. bundle branch block: For follow-up within groups, presence of a bundle branch block was considered. For comparative follow-up between groups, changes from normal to bundle branch block, no change and changes from bundle branch block to normal were documented.
  v. ST deviation and/or Sokolow index I: For follow-up within groups, presence of ST-deviation and/or Sokolow index I was assessed. For comparative follow-up between groups, changes from normal to ST deviation and/or Sokolow index I, no changes and changes from ST deviation and/or Sokolow index I to normal were considered.
(C) Echocardiography: For qualitative assessment of LVF, a rank was applied to each echo (normal, mildly decreased, moderately decreased, and severely decreased). For FS, absolute values were considered.

2.8. Statistical analysis

Statistical analysis was performed using SigmaStat version 2.0 software. Tests included one-way analysis of variance (ANOVA), Kruskal–Wallis one-way analysis of variance on ranks, Wilcoxon Signed Rank Test, paired *t*-test, McNemar's Test, Mann–Whitney Rank Sum Test, and Chi-squared Test as indicated. Tests were calculated for two sides. Paired tests were performed for follow-up within groups, unpaired tests for comparative follow-up between groups. Overall, tests were performed first. If these tests revealed a $p<0.05$, pairwise tests were calculated. Statistical significance was assumed only in case of a $p<0.05$ for both, overall and pairwise analysis.

3. Results

3.1. Endomyocardial biopsy evaluation and clinical characteristics

The main demographic characteristics of the patient population are shown in Table 1. Patients were allocated to either group A, B, or C according to biopsy results: group A: no TNF-α and no C5b-9; group B: TNF-α or C5b-9; and group C: TNF-α and C5b-9. There were 13 (18%) patients in group A, 19 (27%) patients in group B and 39 (55%) patients in group C.

Age, male to female ratio, EF at the time of myocardial biopsy, and all other baseline parameters were not significantly different between the groups. TNF-α and C5b-9 did not significantly correlate with presence of viral genome or with inflammatory infiltrates.

One patient in group A and one patient in group B received additional treatment with prednisolone. One patient in group A and two patients in group C received additional treatment with interferon β_{1b}. No complications were reported for prednisolone or interferon β_{1b} treatment.

Table 1
Main clinical characteristics of patient population

	(A) Neither TNF-α nor C5b-9 ($n=13$)	(B) TNF-α or C5b-9 ($n=19$)	(C) TNF-α and C5b-9 ($n=39$)
Age (years)	49 (28–68)	59 (33–73)	55 (20–73)
Sex (M/F)	7/6	13/6	31/8
Duration of symptoms to biopsy (months)	2 (1–39)	5 (1–252)	3 (0–60)
Duration from diagnosis to biopsy (months)	1 (0–72)	1 (0–96)	0 (0–60)
Follow-up period (months)	10 (5–25)	6 (1–35)	12 (1–30)
EF at biopsy (%)	24 (15–53)	36.5 (22–46)	30 (7–48)
Inflammation	6	9	18
Detection of viral genome	2	2	4
Specific therapy			
Prednisolone	1	1	0
Interferon β_{1b}	1	0	2

Quantitative parameters: median (range). Qualitative parameters: absolute number of patients.

3.2. Follow-up results within groups

For NYHA classification and echocardiographic parameters all groups improved significantly. For ECG parameters, group B ($p\leq0.001$) and group C ($p\leq0.001$) showed a significant decrease in heart rate, presumably due to the use of beta receptor blockers. For group A ($p=0.031$) and group C ($p=0.042$) a significant reduction in ST deviation/Sokolow Index I was detected. This may indicate an improvement in cardiac function and correlate with improvement in EF and NYHA class.

3.3. Comparative follow-up between groups

No significant differences were detected for NYHA classification, most ECG parameters and echocardiography between the three groups. Group A showed a significantly better follow-up for ST deviation/Sokolow I than group B ($p=0.044$).

3.4. Cardiac events during follow-up

There were no differences between the groups for automatic implantable cardiac defibrillator implantation ($p=0.506$), pacemaker implantation ($p=0.110$), cardiac resynchronisation therapy (i.e. implantation of a biventricular pacemaker, $p=0.430$), cardiac decompensation ($p=0.229$), symptomatic arrhythmia ($p=0.818$) and death ($p=0.250$).

4. Discussion

To our knowledge this is the first study to investigate a possible prognostic role of *myocardial* TNF-α and C5b-9 expression for clinical outcome in patients with DCM. 71 patients were assessed. Three major observations were made:

(1) Neither myocardial TNF-α nor C5b-9 correlates with clinical outcome in DCM patients.
(2) Both antigens are widely distributed in the myocardium of DCM patients.
(3) TNF-α and C5b-9 did not significantly correlate with presence of viral genome or with inflammatory infiltrates.

Numerous reports have shown that increased *plasma* levels of TNF-α are associated with the evolution and progression of chronic heart failure [2,3,6]. Experimental data suggested that TNF-α may be pathobiologically involved in the progression of cardiac disease [1–8]. These observations led to the idea that administration of TNF-α antagonists like infliximab and etanercept may be beneficial in the treatment of heart failure. Although animal models

and pilot trials initially provided encouraging results, larger multicenter studies were ultimately not able to show any clinical improvement in patients with chronic heart failure [13,14].

In this study, we investigated whether *myocardial* TNF-α expression might provide prognostic information in DCM. As complement activation may play a causal role in myocarditis and DCM and as C5b-9 induces TNF-α expression in cardiomyocytes and is associated with TNF-α in biopsy specimens in DCM [8], we studied both antigens. However, neither *myocardial* TNF-α nor C5b-9 correlates with clinical outcome in DCM patients. Furthermore, TNF-α and C5b-9 do not significantly correlate with the presence of viral genome or with inflammatory infiltrates, and thus, they may not be considered as diagnostic parameters.

The fact that neither myocardial TNF-α nor C5b-9 correlate with outcome in our DCM patients, again indicates that the pathogenic role of TNF-α in chronic heart failure may have been overemphasized in the past. Our results may explain in part why large clinical trials targeting TNF-α in heart failure have not shown any beneficial effect.

A limitation of the study is the retrospective study design. Given the low incidence of DCM, however, the study population of 71 patients is relatively large. Another limitation is the lack of data on TNF-α and C5b-9 plasma levels at the time of myocardial biopsy. Furthermore, immunohistochemical staining only provides a qualitative analysis, quantitative measurement of myocardial TNF-α and C5b-9 is not possible. Finally, no statement concerning a possible chronology of presence and expression of TNF-α and C5b-9 can be made.

In summary, our data suggest that assessment of TNF-α and C5b-9 in myocardial biopsy specimens is not helpful for the evaluation of clinical outcome in DCM patients. In accordance with other experimental [15,16] and clinical data [13,14], our study provides evidence that the pathobiological role of TNF-α in chronic heart failure may have been overemphasized.

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