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Nicole de Leeuw
Dirk J. Ruiter
Aggie H. M. M. Balk
Nicolaas de Jonge
Willem J. G. Melchers
Jochem M. D. Galama

Histopathologic findings in explanted heart tissue from patients with end-stage idiopathic dilated cardiomyopathy

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N. de Leeuw
Department of Human Genetics,
University Medical Centre Nijmegen,
P.O. Box 9101, 6500 HB Nijmegen,
The Netherlands

D.J. Ruiter
Department of Pathology,
University Medical Centre Nijmegen,
P.O. Box 9101, 6500 HB Nijmegen,
The Netherlands

A.H.M.M. Balk
Department of Cardiology,
University Hospital Rotterdam-Dijkzigt,
P.O. Box 2040, 3000 CA Rotterdam,
The Netherlands

N. de Jonge
Department of Cardiology,
University Hospital Utrecht,
P.O. Box 85500, 3508 GA Utrecht,
The Netherlands

J.M.D. Galama
Department of Medical Microbiology,
University Medical Centre Nijmegen,
P.O. Box 9101, 6500 HB Nijmegen,
The Netherlands

W.J.G. Melchers (✉)
Department of Medical Microbiology,
University Medical Centre Nijmegen,
P.O. Box 9101, 6500 HB Nijmegen,
The Netherlands,
e-mail: W.Melchers@MMB.AZN.NL,
Fax: + 31-243540216

Abstract Explanted hearts were examined to determine whether specific histopathologic features are present in the myocardium of patients with end-stage idiopathic dilated cardiomyopathy (IDC). Extensive histopathologic examination by light microscopy, electron microscopy and immunohistochemistry revealed marked fibrosis in the hearts of 21 of 37 IDC patients and in 26 of 35 patients with heart diseases of known causes. Reactive (interstitial and perivascular) fibrosis predominated in the IDC hearts, whereas both reparative (replacement) fibrosis and reactive fibrosis were found in the comparison group. Endocardial fibroelastosis was found in nine patients with IDC and in 14 patients from the comparison group. Distinct patterns of fibrosis were the sole significant histopathologic difference between myocardial samples from patients with IDC and from those with heart diseases of known causes. The diffuse presence of reactive fibrosis in IDC patients suggests a more generalised dysfunction that affects the composition of the myocardial extracellular matrix.

Keywords Idiopathic dilated cardiomyopathy · Explanted hearts · Histopathology · Fibrosis · Endocardial fibroelastosis

Abbreviations *IDC* Idiopathic dilated cardiomyopathy · *EMB* Endomyocardial biopsy

Introduction

Cardiomyopathies are defined as diseases of the myocardium associated with cardiac dysfunction [18]. They are classified into four main categories: dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular dysplasia. Dilated cardiomyopathy is characterised by dilation of one ore more cardiac chambers, ventricular hypokinesis, and a depressed left ventricular ejection fraction ($< 40\%$). In the majority of patients with this disease, the aetiology is unknown and the disease is referred to as idiopathic dilated cardiomyopathy (IDC). The reported annual incidence of IDC ranges from 5–8 per 100,000 persons [3, 12]. If medication fails to improve the condition of a patient suffering from IDC, heart transplantation is the only therapeutical option. Next to ischemic heart disease, dilated cardiomyopathy is the second most common cause of heart failure as well as the second most com-

Table 1 Baseline characteristics of patients with end-stage heart disease

Diagnosis	*n*	Sex (male/female)	Average age (range) (in years)	Duration of symptoms:	
				Median in years	Range
Patient group	37	21/16	47 (14–63)	4	3 Months to > 10 years
Idiopathic dilated cardiomyopathy					
Comparison group	35	28/7	52 (32–65)	> 10 Years	3 Months to > 10 years
Ischemic heart disease	29				
Hypertrophic cardiomyopathy	3				
Valvular heart disease	2				
Congenital heart disease	1				

mon indication for heart transplantation. By definition, the cause of IDC is not known. Factors thought to be involved in the pathogenesis of this disease are genetic predisposition, abnormal modulation of the immune response, and metabolic, energetic, and contractile abnormalities. IDC is believed to be preceded by (viral) myocarditis in some patients [3, 12]. In a previous study [5], we analysed multiple myocardial tissue samples from 37 IDC patients for the presence of enteroviruses, because these viruses are the main cause of myocarditis [26]. We did not find evidence of persistent enteroviral RNA in any of the IDC patients or in 39 patients with end-stage heart disease of known cause who were included for comparison. In an attempt to identify immune abnormalities, both the explanted hearts, serum samples, and HLA profiles were examined from patients with IDC. No signs of an active autoimmune process could be demonstrated in patients from either the IDC group or the comparison group [6].

In the present study, we focus on the histopathologic aspects of myocardium from patients with IDC. One of these aspects concerns the myocardial collagen matrix, which lends the heart mechanical stability. Alterations in the composition and/or extent of this structural network may lead to weakening and dilatation of the ventricular wall. We histologically examined the explanted hearts from patients with IDC and from patients with heart diseases of known causes. We compared the histopathologic features of these explanted hearts to identify characteristics specific for IDC.

Patients and methods

Patients

Our study population comprised a total of 72 patients with end-stage heart disease. By exclusion of obstructive coronary artery disease, congenital- or valvular heart disease, hypertension, alcoholism, insulin-dependent diabetes mellitus, and systemic disease, 37 patients were included in the IDC group. The comparison group consisted of 35 patients who suffered from heart diseases of known causes. The baseline characteristics of all patients are summarised in table 1. We obtained control hearts from two male individuals (aged 43 and 47 years) who died of non-cardiac diseases. Our study was performed with approval of the Committees for Scientific Research with Humans of the University Hospitals of Rotterdam, Utrecht and Nijmegen. Informed consent was obtained from all patients or their parents or guardians.

Clinical specimens

At the time of heart transplantation, myocardial tissue samples were collected from each patient within 1 h after explantation of the heart. From both the left and the right heart ventricle, two transmural specimens were randomly excised near the apex. From every specimen (average size was 0.6 cm^3) a fragment of 1 mm^3 was cut and fixed in 4% glutaraldehyde for electron microscopic analysis. The remainder of each tissue sample was immediately fixed in a buffered solution of formaldehyde. These samples were subsequently further processed, embedded in paraffin and cut into serial 4 μm tissue sections for histological staining.

Histopathologic evaluation

From each patient, two left and two right heart ventricle tissue sections were stained with Haematoxylin Eosin and, likewise, four consecutive tissue sections were stained with Masson's Trichrome according to standard protocols. All heart tissue sections (average size of 80 mm^2) were examined in a blinded fashion by two observers. Various histopathologic features were evaluated, including hypertrophy, fibrosis, liposis (defined as myocardial accumulation of adipose tissue), inflammatory cells, lipofuscin, atrophy, necrosis, myofibrillolysis, and vacuolar dystrophy/cellular oedema. The observations were noted on scoring forms. For each feature, the grade was specified as 'none or marginal', 'mild', 'moderate' or 'marked', the distribution was indicated by 'local', 'focal', or 'diffuse' and the location by '(sub)epicardial', 'intramural' or '(sub)endocardial'. In addition, fibrosis, the disproportionate accumulation of fibrillar collagen, was further classified as 'reactive' (interstitial or perivascular) or 'reparative' (replacement) [23]. In interstitial fibrosis, fibrillar collagen appears in intermuscular spaces previously devoid of collagen. Perivascular fibrosis refers to accumulation of collagen within the adventitia of intramyocardial coronary arteries and arterioles. A reparative or replacement fibrosis represents microscopic scarring that follows myocyte necrosis. Endocardial fibroelastosis is defined as diffuse thickening of the endocardium with layering of collagen and elastic fibres.

Table 2 Histopathologic features in patients with end-stage heart disease

	IDC group ($n = 37$)	Comparison ($n = 35$)
Marked myocardial liposis	24 (65%)	27 (77%)
- left ventricle samples only	1 (4%)	1 (4%)
- right ventricle samples only	17 (71%)	11 (41%)
- left and right ventricle samples	6 (25%)	15 (56%)
LEFT versus RIGHT VENTRICLE	29 vs. 96% [a]	60 vs. 97% [a]
Marked epicardial liposis	18 (49%)	20 (57%)
- left ventricle samples only	3 (17%)	2 (10%)
- right ventricle samples only	5 (28%)	5 (25%)
- left and right ventricle samples	10 (55%)	13 (65%)
LEFT versus RIGHT VENTRICLE	72 vs. 83%	75 vs. 90%
Marked epicardial fibrosis	14 (38%)	20 (57%)
- left ventricle samples only	5 (36%)	1 (5%)
- right ventricle samples only	1 (7%)	5 (25%)
- left and right ventricle samples	8 (57%)	14 (70%)
LEFT versus RIGHT VENTRICLE	93 vs. 64% [a]	75 vs. 95%
Marked epicardial liposis with fibrosis	11 (30%)	16 (46%)
- left ventricle samples only	4 (36%)	2 (13%)
- right ventricle samples only	2 (18%)	5 (31%)
- left and right ventricle samples	5 (45%)	9 (56%)
LEFT versus RIGHT VENTRICLE	81 vs. 63%	69 vs. 87%
Marked fibrotic lesions	21 (57%)	26 (74%)
- left ventricle samples only	15 (71%)	14 (54%)
- right ventricle samples only	1 (5%)	2 (8%)
- left and right ventricle samples	5 (24%)	10 (38%)
LEFT versus RIGHT VENTRICLE	95 vs. 29% [a]	92 vs. 46% [a]
Reactive fibrosis[b]	16/21 (76%)	16/26 (62%)
- perivascular only	4 (25%)	3 (19%)
- perivascular and interstitial	12 (75%)	13 (81%)
LEFT versus RIGHT VENTRICLE	94 vs. 13% [a]	81 vs. 25% [a]
Reparative fibrosis	7/21 (33%)	16/26 (62%) [c]
- focal/local only	5 (71%)	6 (38%)
- diffuse only	2 (29%)	6 (38%)
- focal/local and diffuse	0	4 (25%)
LEFT versus RIGHT VENTRICLE	71 vs. 43% [a]	75 vs. 38% [a]
Reactive and reparative fibrosis	4/21 (19%)	6/26 (23%)
Endocardial fibroelastosis (≥ 100 µm)	9/21 (43%)	14/26 (54%)
- left ventricle samples only	7 (78%)	12 (86%)
- right ventricle samples only	0	1 (7%)
- left and right ventricle samples	2 (22%)	1 (7%)
LEFT versus RIGHT VENTRICLE	100 vs. 22% [a]	93 vs. 14% [a]

[a] $P < 0.05$ for prevalences in left versus right ventricle
[b] The pattern of reactive fibrosis was predominantly diffuse
[c] $P < 0.05$ for prevalence in IDC group versus comparison group

Immunohistochemistry

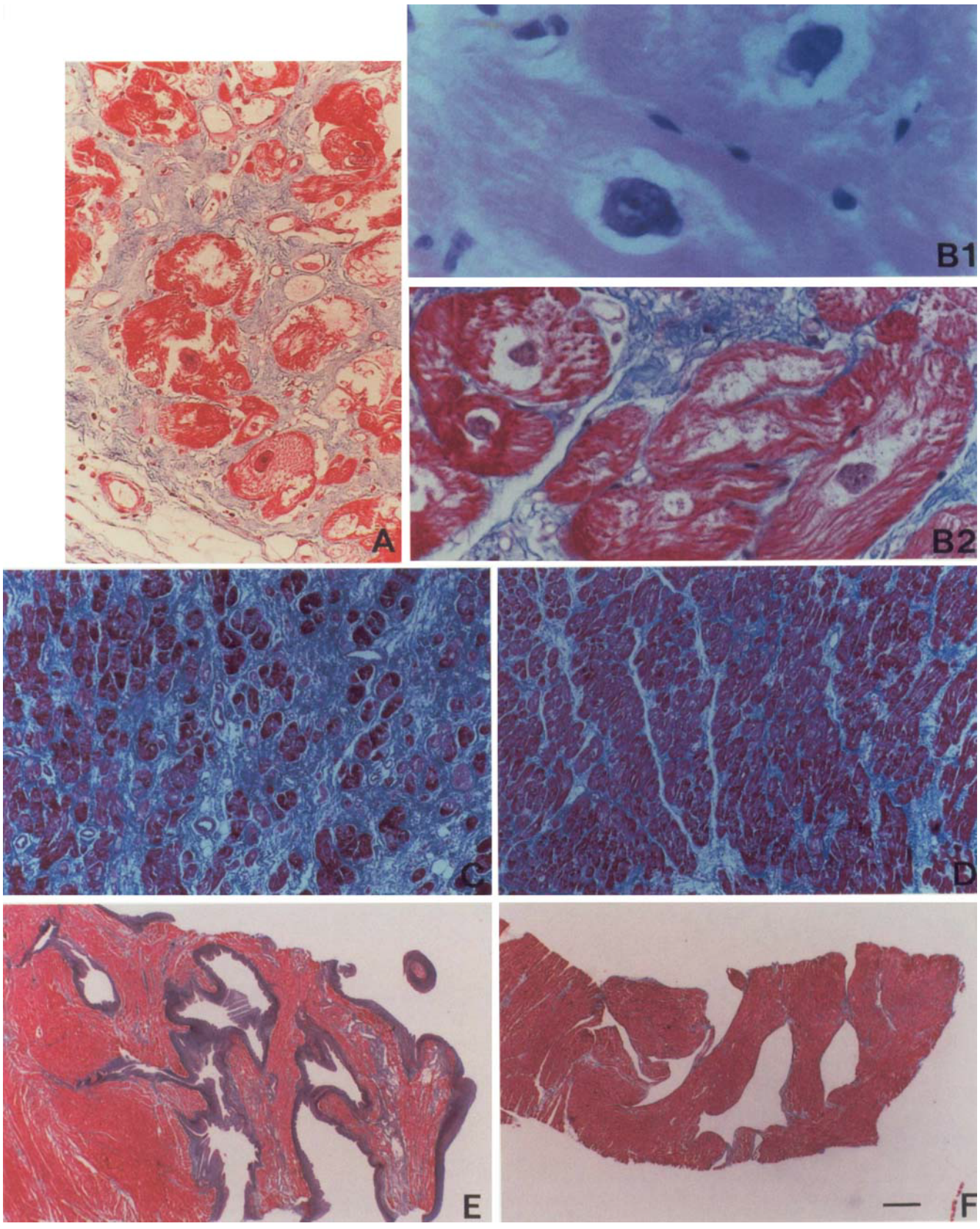
Myocardial tissue sections that contained inflammatory cell infiltrates were further evaluated by means of immunohistochemical analysis. Antibodies against CD3 (pan T cells) and CD8 (suppressor/cytotoxic T cells) were used as previously described [6] to identify T lymphocytes in the myocardium.

Electron microscopy

The tissue samples fixed in 4% glutaraldehyde were processed for evaluation by transmission electron microscopy. Only tissue samples in which abnormalities were observed that could not be readily identified by light microscopic examination were further analysed.

Statistical analysis

The differences between the data for histological scores from both groups were tested for significance by means of the two-tailed chi-square test at the 5% level.


A
B1
B2
C
D
E
F

Results

Histopathologic evaluation

Histopathologic evaluation of the myocardial tissue sections revealed variable hypertrophy of cardiomyocytes, vacuolar dystrophy, myofibrillolysis, lipofuscin, atrophy and necrosis (figure 1A). These features did not differ significantly between patients from the IDC group and those from the comparison group. Table 2 summarises the main histopathologic features of the myocardial samples from our study patients. To further characterise the fibrotic lesions, they were classified as reactive (interstitial and/or perivascular) fibrosis or reparative (replacement) fibrosis [23]. Reactive fibrosis significantly prevailed in patients with IDC ($P < 0.05$), whereas in the comparison group, reactive and reparative fibrosis were equally represented. In both groups, there was a gradient in frequency of marked fibrosis from the subepicardial layer (24–34%) to the subendocardial layer (37–51%; data not shown).

Inter- and intra-observer reproducibility were determined and qualified as highly satisfactory (> 95% agreement). Histopathologic examination of the two control hearts from patients who died of non-cardiac diseases revealed a mild degree of interstitial fibrosis and focal liposis. Otherwise, no abnormalities were observed.

Immunohistochemistry

Few, small inflammatory cell infiltrates (less than 100 mononuclear cells) were found in tissue sections from seven patients with IDC, in sections from ten patients with ischemic heart disease, and in sections from one patient with hypertrophic cardiomyopathy. The infiltrates consisted of lymphocytes, macrophages, and mast cells. Additional immunohistochemical analysis revealed that T cells were scarcely represented in these infiltrates. The mononuclear cellular infiltrates were predominantly located in the subepicardial layer and were found in absence of myocyte necrosis or degeneration.

◀ **Fig. 1** Paraffin sections of myocardial samples from patients with end-stage heart disease. All tissue sections were stained with Masson's Trichrome stain except for tissue section B1, which was stained with Alcian Blue. Histopathologic features found in both patient groups included myofibrillolysis, vacuolar dystrophy, and interstitial fibrosis **A**. Vacuolar inclusions in the nuclei of cardiomyocytes were seen in a patient with autosomal dominant hypertrophic cardiomyopathy **B1**, **B2**. Significant differences were observed between left and right ventricle samples with regard to interstitial fibrosis (**C** and **D**, respectively), and endocardial fibroelastosis (**E** and **F**, respectively). *Bar* represents 50 μm for **A**, 15 μm for **B1**, 25 μm for **B2**, 250 μm for **C** and **D**, and 500 μm for **E** and **F**

Electron microscopy

In a 34-year old female patient with autosomal dominant hypertrophic cardiomyopathy, vacuolar inclusions were seen in the nuclei of multiple myocytes in the absence of mononuclear cells (figure 1B). By electron microscopy, intranuclear tubular inclusions were found (figure 2), but we could not further identify or specify these structures. There was no evidence that this patient had recently had an infectious disease that may have clarified these structures.

Discussion

Although by definition the cause of IDC is unknown, it appears that various pathogenic pathways may lead to a common end-stage in IDC [3]. The affected myocardium in patients with IDC shows generalised and non-specific morphologic features. These aspects of myocardial morphology in IDC have most often been characterised in endomyocardial biopsy (EMB) specimens and/or autopsy hearts. However, EMB specimens have limited representative value in IDC and autolytic processes in autopsy hearts may influence the actual morphology. Identification of disease markers specific for IDC would be of great value in order to improve the diagnosis and to optimise the treatment and prognosis of patients suffering from IDC. In an attempt to find morphological clues to solve the problem of idiopathy, we extensively examined the histopathologic features in explanted IDC hearts and compared them to those found in hearts of patients with other cardiac diseases.

Our histopathologic observations are in agreement with the reported morphologic features of dilated cardiomyopathy [2, 19, 20]. In the majority of heart tissue samples, hypertrophic cardiomyocytes and interstitial myocardial fibrosis were seen in the absence of inflammatory cells and myocyte degeneration. Myocarditis, a possible precedent of dilated cardiomyopathy, has been diagnosed in patients with IDC [10, 17, 28]. In our study population, signs of myocarditis were not observed in any of the 72 patients. Although minor inflammatory cell infiltrates were found in some heart tissue sections from 7 of 37 IDC patients, the (immuno)histologic features did not meet the criteria for the diagnosis of myocarditis [1, 10]. In none of the patients was the mean number of T lymphocytes greater than 6.0 per square mm of myocardial tissue. The absence of myocarditis in our study population could be due to the fact that we included only patients with end-stage heart disease, whereas Zee-Cheng et al. [28], Parrillo et al. [17], and

Kühl et al. [10] examined endomyocardial biopsies of patients in an earlier phase of heart disease.

In our study, intranuclear tubular inclusions were observed in heart tissue sections from a patient with hypertrophic cardiomyopathy. Although the inclusions looked virus-like and were of a size compatible with that of virus particles (60 nm), they were most probably aspecific degenerate cell products, such as altered chromatin filaments [22, 25].

Marked liposis and fibrosis were more prevalent in the comparison group than in the IDC group. Although all study patients suffered from end-stage heart disease, the duration of symptoms after clinical onset was significantly longer in the comparison group. This could mean that disease progression in IDC proceeds more rapidly, but it could also mean that the asymptomatic phase in IDC is much longer. In IDC patients, marked fibrosis was predominantly of the reactive type, whereas patients from the comparison group suffered from both reactive and reparative fibrosis. In most patients from the comparison group, chronic ischemic heart disease was most probably the cause of diffuse, reactive fibrosis in combination with multiple small lesions of scar tissue (reparative fibrosis). The diffuse presence of an increased interstitial and perivascular fibrotic network in IDC may be one of the main causes of reduced heart contractility leading to severe cardiac dilation and dysfunction [8]. A variety of insults may cause damage to the heart and initiate myocardial degeneration and fibrosis. Progression of degenerative and fibrotic changes in the myocardium could eventually be responsible for fatal cardiac failure [2]. The diffuse pattern of reactive fibrosis, which predominates in IDC, suggests a generalised dysfunction that affects the composition of the extracellular matrix rather than reparative fibrosis following myocardial damage [8]. Both, enhanced collagen synthesis as well as degradation, may contribute to changes in matrix composition. This metabolic dysfunction could be on the genetic level or it could be due to continuous stimulation and activation of macrophages and fibroblasts by cellular debris [21]. Elevated levels of circulating fibrogenic factors such as effector hormones of the renin-angiotensin-aldosterone system may also be involved in the disproportionate accumulation of collagen [23]. Previous studies used immunohistochemical analysis to show the localisation of various collagen types in myocardium from patients with dilated cardiomyopathy [13, 16, 24, 27]. Increased amounts of collagen types I and III were observed in patients with dilated cardiomyopathy and in patients with ischemic heart disease. In addition, Schaper et al. [20, 21] demonstrated disproportionate increases of various cytoskeletal proteins in myocardial and interstitial cells of patients with end-stage dilated cardiomyopathy. Recently, dilated cardiomyopathy was found to be associated with deficiency of the cytoskeletal protein metavinculin

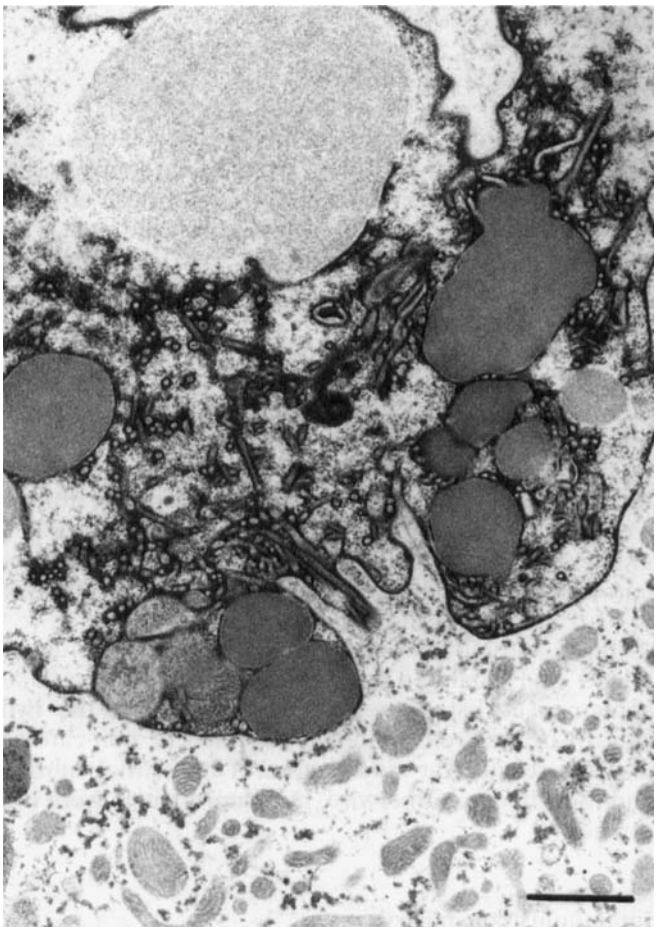


Fig. 2 Intranuclear tubular inclusions in the nuclei of cardiomyocytes from a patient with autosomal dominant hypertrophic cardiomyopathy. *Bar* represents 1 μm

[11]. Moreover, a number of defective genes that were found to be involved in dilated cardiomyopathy have been identified to code for cytoskeletal proteins [reviewed in 9].

Increased fibrosis in combination with alterations of the myocardial cytoskeleton appears to contribute considerably to the diminishing of cardiac contractile function. An important general observation in this study was that myocardial damage was not always of a comparable degree in the left and right ventricular samples (figure 1; table 2). Based on our data, a right EMB cannot be considered to be representative for the condition of the left heart ventricle. In 50–70%, examination of right EMBs could result in a false-negative diagnosis ('no fibrotic abnormalities'). In addition to the safety aspects [4] and the limited prognostic value [7], our findings suggest an even further diagnostic devaluation of right EMBs, in particular in patients with dilated cardiomyopathy. Because the majority of marked fibrotic lesions in our study population were either found suben-

docardially or transmurally, EMBs do seem to represent the transmural condition of the myocardium. For example, endocardial fibroelastosis, an important cause of reduced myocardial contractility, can be diagnosed well. Endocardial fibroelastosis was frequently observed in patients from the IDC group (24%) as well as in patients from the comparison group (40%). This suggests that endocardial fibroelastosis is a non-specific response to chronic myocardial dysfunction, as was previously stated by Lurie [14] and Newbould et al. [15].

In conclusion, the myocardial degenerative fibrotic changes in IDC are diffuse. They are also more prevalent in the left ventricle. We could not identify histopathologic features specific for IDC. Therefore, histologic evaluation of heart tissue samples is unlikely to provide a tool to differentiate between IDC and heart disease of known cause. However, detailed examination of the myocardial cytoskeleton and the extracellular fibrotic processes, hereby including study on the levels of circulating fibrogenic factors such as aldosterone and angiotensin II, may identify factors that contribute to the extensive remodelling of the heart ventricles in IDC. In addition, the role of gene defects in the aetiology of IDC is of growing importance, particularly since several of these are being elucidated. The application of molecular genetic technologies, such as mutation detection, DNA micro array studies, and gene expression profiling analyses, will most certainly yield crucial data to elucidate the pathogenesis of IDC and to enable the improvement of (presymptomatic) diagnostic and therapeutical strategies.

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