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Correlation of Vascular Reaction in Endomyocardial Biopsies with Micro- and Macrovascular Graft Vessel Disease after Heart Transplantation

Nicola E. Hiemann, Rudolf Meyer, Ernst Wellnhofer, and Roland Hetzer

Graft Vessel Disease (GVD) is the predominant long-term complication after heart transplantation. The study tests the correlation of vascular reaction with micro- and macrovascular evidence of GVD in cardiac transplants. The authors studied 41 heart transplant patients (9 women, 32 men, mean survival 58 months), 15 with and 26 without any angiographic signs of GVD. Paraffin-embedded right ventricular endomyocardial biopsies ($n = 272$) were graded for vascular reaction (H&E staining), that is, endothelial cell swelling and vascular wall thickening (both grade 0-2). Immunohistochemical investigations for vascular smooth muscle cells (SMCs) (α -actin, clone 1A4) were performed and evaluated by light microscopy. Patients with angiographic evidence of GVD had more microvascular endothelial cell swelling than patients without macrovascular GVD ($P < 0.05$). There was a positive correlation of α -actin-positive microvessels with early posttransplant endothelial cell swelling ($P < 0.05$). In contrast, there was a positive correlation of α -actin-positive microvessels early after heart transplantation, with endomyocardial vessel wall thickening in the later postoperative period ($P < 0.05$). Development of GVD in large and small coronary arteries may coincide. Proliferation of medial SMCs plays the dominant role in the pathogenesis of small vessel disease. The serial evaluation of microvascular status provides early evidence of pathologic alterations associated with GVD.

Introduction

Graft vessel disease (GVD) is the predominant, lethal long-term complication after heart transplantation.¹⁻⁷ It is characterized by diffuse stenosis of large and small blood vessels, due to an intimal thickening and a proliferation of smooth muscle cells (SMCs).^{5,7-20} Many immunological^{3,21-23} and nonimmunological pathogenetic factors^{19,24-27} may be involved. The roles of acute myocardial rejection episodes and of the severity of vascular reaction are unclear as of yet.^{4,28-32}

It is well accepted that endothelial cells (ECs) and vascular smooth muscle cells play a central role in the pathogenesis of this disease.^{1,7-16,32-36} The aim of

the study was to investigate whether severity of vascular reaction in endomyocardial biopsies is associated with vasculopathy of large coronary arteries and may predict small vessel disease after heart transplantation.

Materials and Methods

We studied 41 heart transplant patients (9 women and 32 men) who underwent transplantation because of dilated cardiomyopathy ($n = 26$), coronary artery disease ($n = 14$), and Eisenmenger's syndrome ($n = 1$). According to their angiographic results, patients were divided into 2 groups: 15 patients with angiographic signs of GVD (4 women,

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Table 1 | DEMOGRAPHICAL DATA OF PATIENTS WITH AND WITHOUT MACROVASCULAR GVD

Parameter	GVD Patients (Mean ± SD)	Controls (Mean ± SD)	P Value
Age at HTx/years	50 ± 4.9	47 ± 7.6	ns
Mean survival time/months	67 ± 22.4	60 ± 27.2	ns
Age at exitus letalis/years	55 ± 5.2	52 ± 7.9	ns
Ischemic time/min	159 ± 23.3	150 ± 66.0	ns
Reperfusion time/min	56 ± 20.6	51 ± 18.9	ns
Donor age/years	36 ± 11.7	37 ± 11.3	ns

SD = standard deviation, ns = not significant.

Table 2 | CLASSIFICATION OF VASCULAR REACTION, CARDIOVASCULAR PATHOLOGY STUDY GROUP, DEUTSCHES HERZZENTRUM BERLIN

Grade	Definition
0/0	Endothelial cells inconspicuous, no vessel wall thickening, no proliferation
0/1	Endothelial cells inconspicuous, mild vessel wall thickening by proliferation
0/2	Endothelial cells inconspicuous, intensive vessel wall thickening by proliferation
1/0	Endothelial cells prominent (not swollen), vessel wall inconspicuous
2/0	Intensive endothelial cell swelling, vessel wall inconspicuous
2/1	Intensive endothelial cell swelling, mild vessel wall thickening by proliferation
1/1	Mild endothelial cell swelling, mild vessel wall thickening by proliferation
1/2	Mild endothelial cell swelling, intensive vessel wall thickening by proliferation
2/2	Intensive endothelial cell swelling, intensive vessel wall thickening by proliferation

11 men, mean survival 57 months) and 26 patients without (5 women, 21 men, mean survival 59 months). All patients were treated with cyclosporine 2–4 mg/kg, prednisolone 0.1–0.5 mg/kg, and azathioprine 1–5 mg/kg. For further demographical data, see Table 1.

Two hundred seventy-two consecutive serial right ventricular endomyocardial biopsies, harvested during the first 14 months after heart transplantation, were subjected to retrospective investigations. All biopsies were classified for acute myocardial rejection according to the Working Formulation of the International Society for Heart and Lung Transplantation.³⁷ The evaluation of vascular reaction was done by light microscopy at × 200, according to the classification shown in Table 2.

The immunohistochemical preparation of the tissue sections was based on the avidin-streptavidin method.³⁸ We used as the immunohistochemical marker for SMCs α -actin (clone 1 A4, Dako Industries®).³⁹ Histomorphometric analysis of immunohistochemical sections was done by light microscopy at × 500, counting antibody-positive blood vessels in a test field of 100 compartments.

All data were analyzed using nonparametric tests; a *P* value of < 0.05 was considered to be significant.

Results

There were no significant differences between the 2 groups concerning their demographical data (Table 1). According to the results of acute myocardial rejection, there was a trend toward a higher number of mild acute rejection episodes in GVD patients.

There was a positive immunohistochemical reaction in all studied endomyocardial biopsies, and all biopsy specimens were subjected to histological, immunohistochemical, and histomorphometric evaluations. In all observed intervals, patients with angiographic evidence of GVD had significantly more α -actin-positive microvessels than patients without macrovascular disease. Microvascular endothelial cell swelling was more pronounced in GVD patients in the 3rd, 5th, and 11th months after heart transplantation than in patients without evidence of macrovascular GVD (Fig 1). Microvascular vessel wall thickening was more pronounced in GVD patients in the 5th month after

ETIOPATHOGENESIS:

The doctrine of the causes and development of diseases.

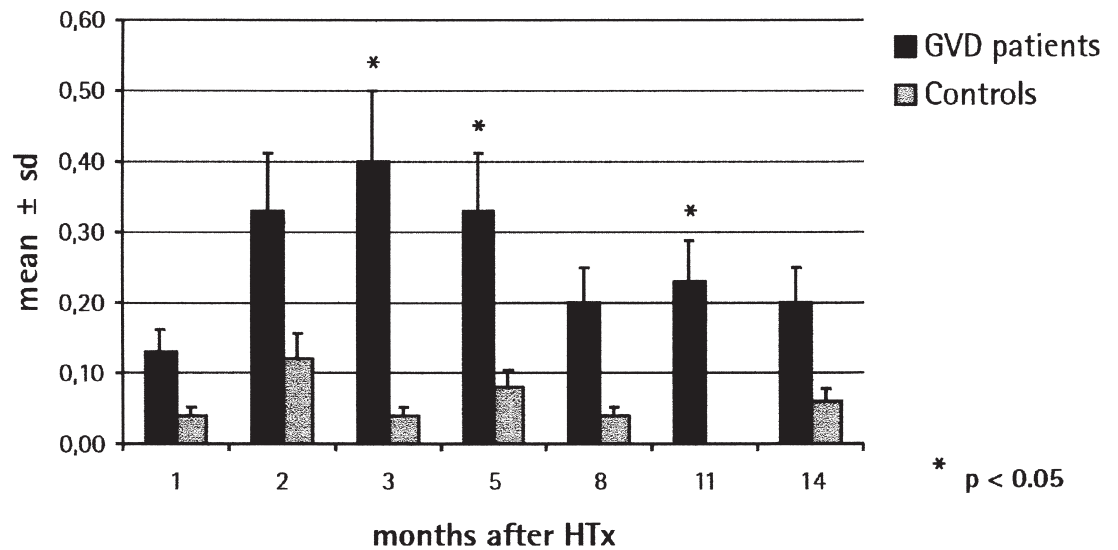


Figure 1. Endothelial cell swelling in patients with and without macrovascular graft vessel disease (GVD).

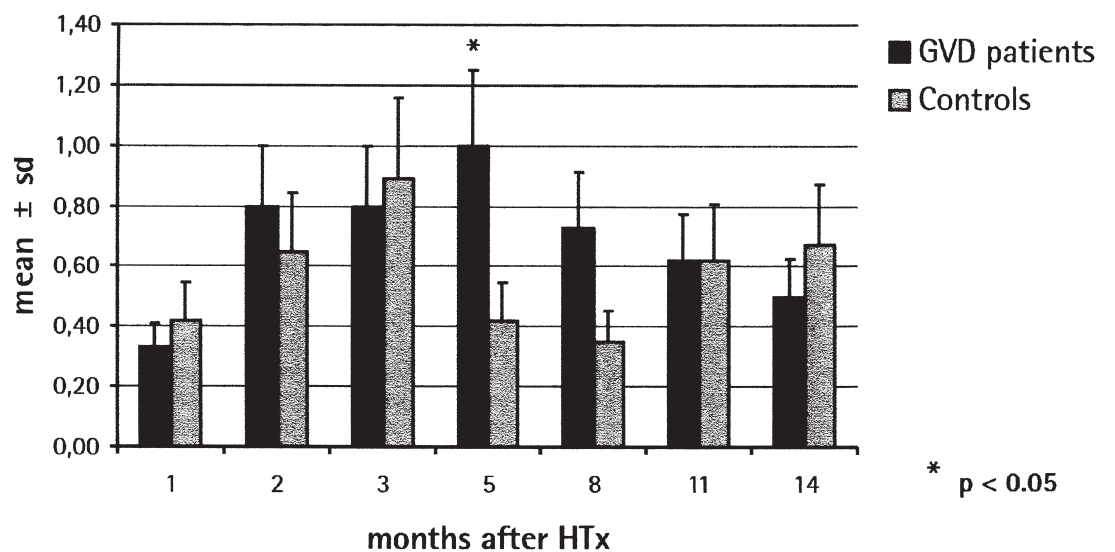


Figure 2. Vessel wall thickening in patients with and without macrovascular graft vessel disease (GVD).

heart transplantation than in patients without GVD of the large coronary arteries (Fig 2). There was a positive correlation of α -actin-positive microvessels in the later period after heart transplantation, with early posttransplant endothelial cell

swelling. In contrast, there was a positive correlation of α -actin-positive microvessels early after heart transplantation, with endomyocardial vessel wall thickening in the late postoperative period (see Table 3).

Table 3 | SPEARMAN'S CORRELATION—VASCULAR REACTION AND α -ACTIN-POSITIVE MICROVESSELS

Endothelial Cell Swelling				
	Months after HTx	1	2	11
α -actin-positive microvessels	8	ns	ns	0.333*
	14	0.378*	0.356*	ns
Vessel Wall Thickening				
	Months after HTx	5		
α -actin-positive microvessels	1	0.317*		
	5	0.329*		
	8	0.368*		
Endothelial Cell Swelling				
	Months after HTx	1	2	8
Vessel wall thickening	1	0.363*	ns	ns
	2	0.395*	0.533**	ns
	8	ns	ns	0.402**

ns = not significant, * $P < 0.05$, ** $P < 0.01$.

Discussion

GVD after heart transplantation affects large and small coronary arteries,^{1,8-16} based on morphological intimal and medial alterations.⁶⁻²⁰ The dominant feature of these changes is a proliferation of SMCs and a transformation of capillaries to blood vessels that display morphological characteristics of arterioles.^{8,9,11,15}

According to our results, there were no significant differences between the 2 groups with regard to their demographic data. This situation has also been described by others,^{1,13,40-42} whereas some investigations revealed an increased risk for the development of GVD in association with older donor age,^{43,44} increased ischemic time, and increased reperfusion time.^{26,27} In view of the association between the development of GVD with the number, duration, and grade of acute rejection episodes, the literature presents conflicting results,^{4,28-30} which may result from different immunosuppressive regimens.⁴⁵

The differentiation between cell-mediated responses to allograft parenchyma and those to allograft vasculature has been suggested by Hosenpud and others³¹ and may explain progressive vascular disease, despite the absence of acute cellular rejection. The importance of diagnosis and grading of vascular rejection has been published by Hammond and others.³² In this article, endothelial cell swelling and/or vasculitis on light microscopy pro-

vided clinical impact and prognostic value on outcome of cardiac recipients. We complemented the classification of morphological features of the terminal vascular system with evaluation of the vessel wall, preferring the term vascular reaction rather than rejection, because what kind of etiology these morphological changes have, and at what time they should be considered disease, is still unknown.

In our own, as in other studies,^{1,8-16,36} GVD occurred in large extramural and small intramural blood vessels, whereas some published data failed to find development of GVD within the terminal vascular system.^{14,46}

It is well accepted that ECs play a central role in the pathogenesis of GVD after heart transplantation,^{7,32-35} by secretion of several mediators that regulate vascular tone³⁰ and influence proliferation^{23,33,47} or phenotypic appearance⁴⁸ of SMCs. They also act as antigen-presenting cells³² and may sustain ongoing immune stimulation by the graft.⁴⁷ According to our results, endothelial cell swelling preceded evidence of SMC proliferation as indicated by immunohistochemical investigations and may reflect endothelial activation and/or endothelial damage.

In summary, these data underline the important role of EC-SMC interaction in the development of GVD after heart transplantation, and we suggest that serial investigations of endomyocardial biopsies of cardiac transplant recipients provide impor-

VASCULAR REACTION:

The morphological appearance of the small intramyocardial blood vessels, that is, the morphology of the endomyocardial microvascular endothelial cells (i.e. not swollen, prominent or swollen), and the morphology of the medial vessel wall (i.e. absence of thickening, mild thickening by proliferation or strong thickening by proliferation).

tant information with impact for the diagnosis of GVD in small coronary arteries.

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