

Hypoxia and Myocardial Remodeling in Human Cardiac Allografts: A Time-course Study

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- Background:** Cardiac allografts are known to develop myocardial fibrosis, which may be a cause of progressive cardiac dysfunction. Apart from the renin-angiotensin and transforming growth factor- β system, hypoxia has been proposed as an important player in the pathogenesis of fibrosis, but its significance remains unclear. This study examines the degree of myocardial fibrosis, cellular remodeling and hypoxic signaling over a time-course of 10 years after human cardiac allograft transplantation.
- Methods:** Serial right ventricular biopsies of 57 patients were collected in 6-month intervals after cardiac transplant surgery for a total of 10 years to allow a retrospective longitudinal analysis. Over this period, tissue remodeling, including interstitial fibrosis and cellular changes, were determined morphometrically. Immunohistochemistry (IHC) was used to analyze expression of the following hypoxia-related proteins: hypoxia-induced factor 1-alpha (HIF1 α); the oxygen sensor prolyl hydroxylase 3 (PHD3); and vascular endothelial growth factor (VEGF).
- Results:** Fibrosis increased significantly from $12.6 \pm 6.5\%$ at the point of transplantation throughout follow-up to $28.8 \pm 7.7\%$ at 10 years. The DNA content and number of nuclei changed over the period of follow-up, displaying signs of cellular hypertrophy and a loss of myocytes. Whereas HIF1 α expression revealed a U-shaped pattern with both early and late elevation during fibrogenesis, PHD3 and VEGF expression patterns showed a gradual increase with PHD3 decreasing again in later fibrogenesis.
- Conclusions:** In cardiac allografts, extensive and progressive tissue remodeling is present. Hypoxia may play a role in this process by up-regulating HIF1 α and leading to differential regulation of pro-angiogenic signals. *J Heart Lung Transplant* 2009;28:1119–26. Copyright © 2009 by the International Society for Heart and Lung Transplantation.

In the past 40 years, cardiac transplantation has become a routine procedure for patients with end-stage heart disease. Despite great advances in post-transplant therapy and significantly longer survival of heart transplant recipients, some long-term sequelae remain as significant problems. Among these are neoplastic diseases, cardiac allograft vasculopathy (CAV) and myocardial fibrosis. Although it has been established that some degree of myocardial fibrosis occurs after transplanta-

tion, specific causes for this remodeling, particularly with a long-term perspective, have not been identified. It has been suggested that cardiac allograft remodeling, CAV and myocardial fibrosis contribute to these functional changes, which include myocardial stiffness and diastolic and, to a certain degree, systolic dysfunction.¹

Several factors have been proposed as participants in allograft fibrogenesis: one may be cyclosporine A (CsA) toxicity. Although CsA has significantly improved outcomes after organ transplantation, its use may lead to interstitial fibrosis.² The renin-angiotensin and transforming growth factor-beta (TGF- β) system as well as hypoxic signaling may participate in CsA-induced fibrogenesis.^{3–5} Other factors in allograft fibrogenesis may be donor ischemic time⁶ and CAV.⁷ Both factors imply oxygen supply and blood flow as influencing tissue fibrosis. Thus, it seems likely that, apart from immune mechanisms, non-immune mechanisms could also contribute to allograft fibrogenesis. In particular, acute and/or chronic hypoxia may play a role in this process.

An insufficient oxygen supply is often a cause for myocardial dysfunction, cardiac remodeling and fibrogenesis, which may cause systolic and diastolic heart failure and atrial fibrillation.^{8,9} Cardiac remodeling and

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Submitted April 2, 2009; revised May 22, 2009; accepted May 24, 2009.

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fibrogenesis may also impair coronary microcirculation and, therefore, may impede myocardial tissue perfusion.¹⁰ Cardiac fibrosis may increase oxygen diffusion distances from capillaries to myocytes by accumulating myocyte-encircling collagen within the interstitium. This would further lead to local hypoxia and could therefore trigger cellular adaptation.

The hypoxia-inducible factor (HIF) pathway has been implicated in disease development. In the absence of oxygen, HIF binds with the hypoxia-response elements (HREs) and, thus, induces genes such as vascular endothelial growth factor (VEGF) and TGF- β , a pro-fibrotic cytokine.¹¹ In normoxic conditions, however, prolyl hydroxylases (PHDs) modify HIF for proteasomal degradation, making the PHDs oxygen sensors.¹²

The aim of this study was to further characterize cardiac allograft remodeling focusing on fibrosis formation over a 10-year follow-up period. Moreover, we assessed potential associations between ventricular fibrogenesis and the regulation of hypoxic signaling throughout this period.

METHODS

Study Population

All patients regularly followed-up at our institution after transplantation were screened before participation. Patients with relevant comorbidities, such as malignancies or chronic inflammatory diseases, were excluded. A total of 57 patients were included. All patients received their allografts between November 1993 and November 1997. There were 50 male and 7 female recipients. The pre-transplantation cardiac diagnoses were atherosclerotic heart disease ($n = 27$), non-ischemic dilated cardiomyopathy ($n = 29$) and rheumatic cardiomyopathy ($n = 1$). The right ventricular (RV) allograft biopsies used were taken at 6-month intervals after transplantation. All biopsies ($n = 1,119$) were fixated in formalin and paraffin-embedded for histologic study. Rejection reaction was judged according to the revised International Society for Heart and Lung Transplantation (ISHLT) classification (Grades 0 to 3R).¹³ CAV was judged by reviewing coronary angiograms ($n = 282$) according to a scoring system described by McGiffin et al.¹⁴ This score represents a measure for the severity of CAV. Generally, a score of >15 is associated with severe 3-vessel disease. In cases in which biopsies appeared to originate from a previous biopsy site, the biopsies were excluded from the study and a different biopsy from the same time-point was used (Table 1).

Morphometry

Deparaffined tissue sections were stained with Sirius red stain or according to Feulgen's protocol (Figure 1A and B). A minimum of two characteristic areas per biopsy were photographed and analyzed using OPENLAB

(version 2.2.5) software (Improvision, UK). To evaluate fibrosis, Sirius red-stained sections were examined. To judge DNA content and the number of nuclei as surrogate marker for myocytes, Feulgen-stained sections were analyzed. The size, intensity and number of nuclei of each section were calculated in a standardized fashion.

Immunohistochemistry for HIF1 α , PHD3 and VEGF

In dewaxed and rehydrated sections antigen recovery was performed in citrate buffer (0.1%, pH 6.0) using a microwave oven. Endogenous biotin and peroxidase activity were blocked using a biotin blocking system (X0590; Dako, Denmark) and peroxidase blocking reagent (S2001; Dako), respectively. As primary antibody (PAB), ESEE 122 (anti-HIF1 α), EG188e/E6 (anti-PHD3) and VG1 (anti-VEGF) were used in different dilutions (HIF1 α 1:30, PHD3 1:5, VEGF 1:2). The following procedures were carried out according to the EnVision System (Dako, Denmark). Diaminobenzidine D5905 (Sigma, Germany) was used as chromogene. Computerized quantification was performed using OPENLAB software, as detailed elsewhere.¹⁵ Sample stainings are shown in Figure 1C-E.

Statistical Analysis

Analysis of variance (ANOVA) was performed for pairwise comparison of longitudinal distributions. A multifactorial covariance analysis was used to study the effects of the variables shown in Table 1 on fibrosis (in percent). With ANOVA/unpaired *t*-tests (where appropriate), all factors were screened to filter the potentially relevant ones with an impact on fibrosis. Only factors with a $p < 0.2$ were considered to be potentially influential and included in the multifactorial covariance analysis. Results are presented as mean \pm SD. A global significance level of $\alpha = 5\%$ was used for all tests. Because analyses were conducted in an exploratory manner,¹⁶ $p \leq 0.05$ was considered statistically significant.

RESULTS

Donor Hearts and Ischemic Time

There were 45 male and 12 female heart donors, aged 28 ± 6 years. The mean donor body mass index (BMI) was 24.7 ± 3.5 years. Donor age and disparate donor and recipient body mass index were not found to consistently influence the amount of myocardial fibrosis or signs of cellular hypertrophy at $p < 0.05$. Graphical analysis did not detect a significant correlation confirming these results.

The average donor heart ischemic time was recorded at 153 ± 45 minutes. Explosive brain death was found to be present in 5 organ donors prior to heart explant-

Table 1. Patient Characteristics and Results

	Time of follow-up						<i>p</i> -value
	0 year	2 years	4 years	6 years	8 years	10 years	
Part I: Patient characteristics							
Age (years)	54.5 ± 8.76	56.5 ± 8.76	58.5 ± 8.76	60.5 ± 8.76	62.5 ± 8.76	64.5 ± 8.76	–
Gender (M/F)	50/7	–	–	–	–	–	–
Body mass index	26.9 ± 4.9	26.7 ± 4.6	27.3 ± 5.2	27.4 ± 5.1	27.8 ± 4.7	28.4 ± 4.3	NS
NYHA class (1–4)	2 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1	NS
TR (degree 0–3)	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	1 ± 1	NS
RVSP (mm Hg + CVP)	26.3 ± 5.4	26.5 ± 5.4	27.3 ± 9.6	27.7 ± 5.7	26.6 ± 7.4	26.8 ± 4.5	NS
LVEF (%)	57.0 ± 5.9	62.0 ± 8.4	60.0 ± 9.9	58.4 ± 7.3	59.1 ± 11.9	58.2 ± 8.8	NS
LA (mm)	39.3 ± 6.7	40.6 ± 6.9	41.0 ± 4.4	41.4 ± 5.6	41.1 ± 8.2	40.1 ± 7.3	NS
LV (mm)	44.6 ± 5.01	46.9 ± 6.59	46.2 ± 4.67	46.8 ± 5.89	47.6 ± 4.72	48.0 ± 5.66	NS
DD (Grade 1–4)	1.42 ± 0.51	1.56 ± 0.73	1.49 ± 0.68	1.62 ± 1.02	1.69 ± 0.64	1.64 ± 0.79	NS
Hypertension (<i>n</i> /%)	16/29	16/29	17/30	17/30	18/32	18/32	NS
IVS (mm)	12.2 ± 3.2	11.8 ± 1.6	12.3 ± 2.2	12.4 ± 1.9	12.1 ± 2.5	12.7 ± 2.3	NS
DM (<i>n</i> /%)	20/35	20/35	22/39	22/39	23/40	24/42	NS
Cyclosporine (mg/kg)	8.35 ± 2.29	5.01 ± 1.62	3.68 ± 1.87	3.49 ± 1.11	3.27 ± 0.97	2.87 ± 1.51	<0.05
Azathioprine (mg)	150 ± 53	99 ± 47	75 ± 34	50 ± 36	46 ± 27	38 ± 22	<0.05
Prednisone (mg)	19.2 ± 13.2	7.0 ± 4.6	6.5 ± 2.0	4.5 ± 2.1	4.2 ± 1.2	2.5 ± 1.9	<0.05
Spirinolactone (<i>n</i> /%)	10/18	12/21	9/16	9/16	8/14	8/14	NS
ACE-I (<i>n</i> /%)	28/49	30/53	31/54	30/53	35/61	34/60	NS
β-blocker (<i>n</i> /%)	40/70	42/74	44/77	41/72	45/79	44/77	NS
Statin (<i>n</i> /%)	47/82	49/86	51/89	47/82	48/84	50/88	NS
ISHLT AR (0–3)	1.02 ± 0.08	0.74 ± 0.04	0.31 ± 0.03	0.53 ± 0.03	0.56 ± 0.03	0.93 ± 0.09	NS
Treated AR (<i>n</i> /%)	8/14	3/5	6/11	3/5	4/7	5/9	NS
AR with HC (<i>n</i> /%)	4/7	1/2	2/4	1/2	2/4	1/2	NS
CAV (0–105)	0.81 ± 0.03	1.20 ± 0.12	2.27 ± 0.21	3.92 ± 0.52	6.76 ± 0.85	9.18 ± 1.02	<0.05
Part II: Results							
Fibrosis (%)	12.6 ± 6.5	18.2 ± 7.1	21.9 ± 3.9	25.2 ± 6.3	26.8 ± 2.9	28.8 ± 7.0	See Fig 2
DNA content (relative)	1.5 ± 0.7	2.2 ± 1.5	3.2 ± 2.1	3.9 ± 1.9	5.0 ± 2.6	6.1 ± 2.3	<0.05
Number of nuclei	91.1 ± 12.4	86.2 ± 9.4	81.9 ± 16.1	79.3 ± 7.4	77.2 ± 4.7	76.1 ± 10.1	<0.05
HIF1α (%)	21.6 ± 6.1	17.1 ± 3.4	11.6 ± 6.4	9.9 ± 3.6	9.2 ± 4.7	12.0 ± 4.0	See Fig 3
PHD3 (%)	2.3 ± 2.0	6.0 ± 4.5	8.7 ± 4.8	8.1 ± 4.0	9.0 ± 2.8	5.2 ± 2.0	See Fig 4
VEGF (%)	11.4 ± 3.2	19.4 ± 5.8	27.4 ± 7.3	28.7 ± 7.4	24.1 ± 8.9	17.1 ± 6.8	See Fig 5

NYHA, New York Heart Association; TR, tricuspid regurgitation (0 = none, 1 = mild, 2 = moderate, 3 = severe); RVSP, right ventricular systolic pressure; CVP, central venous pressure; LVEF, left ventricular ejection fraction; LA, left atrium; LV, left ventricle; DD, diastolic dysfunction determined by transmitral blood flow; IVS, intraventricular septum; DM, diabetes mellitus; ACE-I, angiotensin-converting enzyme inhibitor; ISHLT, International Society for Heart and Lung Transplantation; AR, acute rejection; HC, hemodynamic compromise; HIF, hypoxia-induced factor; PHD3, prolyl hydroxylase 3; VEGF, vascular endothelial growth factor; NS, not statistically significant. *p*-value: $\alpha = 5\%$ ($p < 0.05$).

tation. Analysis of donor heart ischemic time and explosive brain death did not reveal a significant correlation between ischemic time and the amount of myocardial fibrosis or myocyte hypertrophy.

Right Ventricular Allograft Remodeling

To examine myocardial fibrosis Sirius red stainings were analyzed (Figure 1A). At the time of transplantation, the allograft RV biopsies displayed fibrosis involving only minor areas between cardiomyocytes and a thickened interstitial matrix. Fibrotic area was calculated to be 12.6 ± 6.5%. Fibrosis increased gradually and started to form fibrotic bundles separating groups of cardiomyocytes. Finally, fibrosis covered large areas, with a total fibrotic area of 28.8 ± 7.0% (Table 1 and Figure 2).

With fibrogenesis, relative nuclear DNA content and the number of nuclei according to Feulgen's stainings also changed significantly (Figure 1B). Cardiomyocytic DNA content gradually increased from 1.5 ± 0.7 relative units (RU) shortly after transplantation to 6.1 ± 2.3 RU after 10 years ($p < 0.05$). In contrast, the number of nuclei per image (and thus, indirectly, cardiomyocytes) significantly decreased throughout follow-up from 91.1 ± 12.4 at the time of transplantation to 76.1 ± 10.1 after 10 years ($p < 0.05$).

Medications

Patients received different amounts of the standard immunosuppressants CsA, azathioprine and prednisone. Generally, the doses could be tapered over time. The daily mean

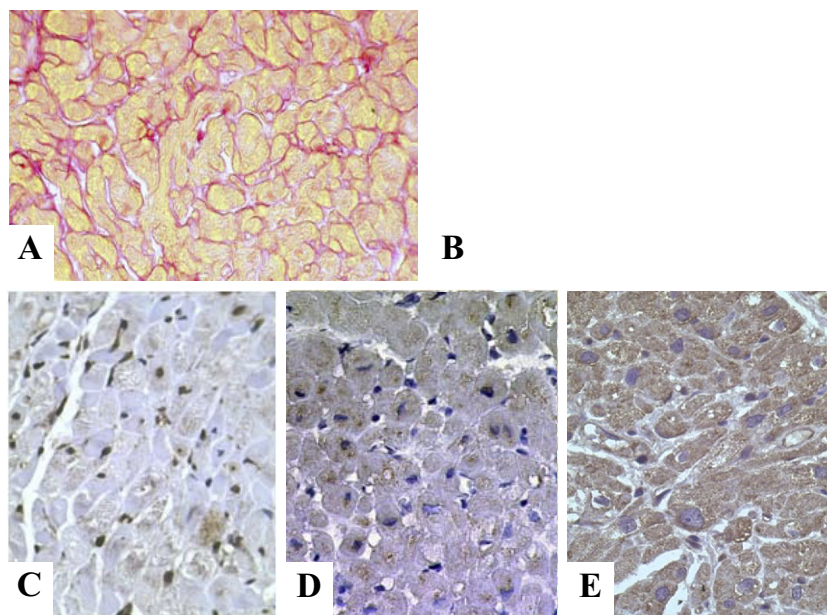


Figure 1. Representative sections (2 μ m) processed according to the Sirius red protocol to stain collagen (A) and Feulgen's protocol to stain DNA (B). Images (C) and (D) show representative immunohistochemistries using diaminobenzidine (DAB) as a chromogen for HIF1 α (C), PHD3 (D) and VEGF (E). The brown color identifies the respective antigens. Original magnification: $\times 40$.

dose of CsA decreased from an initial 8.35 ± 2.29 mg/kg to 2.87 ± 1.51 mg/kg at 10 years after transplantation. For azathioprine and prednisone the respective doses were 150 ± 53 mg decreasing to 38 ± 22 mg, and 19.2 ± 13.2 mg decreasing to 2.5 ± 1.9 mg.

During follow-up (depending on time-point) 29% to 32% of patients were treated for systemic hypertension ($\geq 140/90$ mm Hg; see Table 1). Various anti-hypertensive agents were used: 69% to 75% were on a diuretic ($31.3 \pm 11.4\%$ of maximum dose); 63% to 69% were on a calcium channel blocker ($67.6 \pm 5.9\%$ of maximum dose); 39% to 51% took an angiotensin-converting enzyme inhibitor ($27.6 \pm 7.1\%$ of maximum dose); and 70% to 79% took a beta-adrenergic blocker ($59.3 \pm 9.5\%$

of maximum dose). Statistical analysis did not detect a significant relationship between immunosuppressive agent (or dose), anti-hypertensive regimen (or dose) and RV fibrosis.

Post-transplant Clinical Conditions

Potentially influential factors on myocardial remodeling after transplantation were studied, including systemic hypertension ($\geq 140/90$ mm Hg), CAV, degree of ISHLT allograft rejection and the number rejection episodes with hemodynamic compromise, and/or need for treatment. Reviewing angiographic data, CAV revealed a significant increase over time (Table 1). Analysis of average ISHLT grades showed a slight, non-significant trend to increase during the follow-up period. All factors were evaluated for potential influence on myocardial fibrosis. A univariate analysis was performed followed by a multivariate analysis if the univariate analysis yielded $p < 0.2$. Apart from CAV, however, we did not detect a statistically significant relationship between the presence or absence of the respective factors and RV fibrosis.

Sensing Hypoxia

To determine possible hypoxia we performed immunostainings for HIF1 α (Figure 1C). After an initially elevated HIF1 α level of $21.6 \pm 6.1\%$ (shortly after transplantation) the expression of HIF1 α decreased to $9.0 \pm 4.3\%$ at 6.5 years after transplantation. Next, levels appeared not to change significantly until 8.5 years after surgery, then expression rose to $12.0 \pm 4.0\%$ at 10 years post-transplantation (Table 1 and Figure 3).

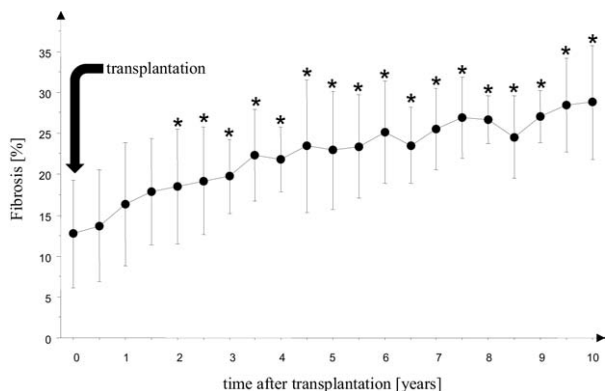


Figure 2. Right ventricular fibrosis: Results of morphometrically determined fibrosis using Sirius red stainings. Data displayed as mean \pm standard deviation. The black arrow indicates the point of allograft transplantation surgery. * $p < 0.05$ vs baseline at 0 year.

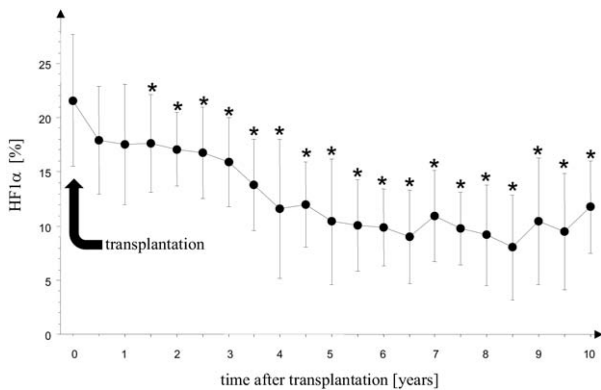


Figure 3. HIF1 α : Right ventricular biopsies were stained using immunohistochemistry with DAB as chromogen. Computer analysis identified the amount of HIF1 α staining. Results are displayed as mean \pm standard deviation. The black arrow indicates the point of allograft transplantation surgery. * $p < 0.05$ at baseline at 0 year.

Sensing Oxygen

Next, immunostainings for PHD3 were performed (Figure 1D). Here, a different picture was found, with initial levels being relatively low at $2.3 \pm 2.0\%$. Levels increased significantly and reached $10.2 \pm 4.9\%$ at 4.5 years after transplantation. Next, PHD3 expression decreased slightly. Nine years after transplantation the decrease appears to have accelerated to reach expression levels of $5.2 \pm 2.0\%$ after 10 years (Table 1 and Figure 4).

Pro-angiogenic Signaling

Finally, we performed immunohistochemistry on our biopsies to assess VEGF expression as a potential hypoxia-related target gene (Figure 1E). VEGF expression in these specimens started at $11.4 \pm 3.2\%$ and increased from there to reach a maximum expression level of $30.8 \pm 7.3\%$ at 5 years after transplantation. For the next 2.5 years, levels stayed relatively constant and then started to decrease again to reach $17.1 \pm 6.8\%$ at 10 years after transplantation (Table 1 and Figure 5).

In an effort to ascertain possible associations between myocardial fibrosis (i.e., Sirius red staining) and the factors listed in Table 1, a univariate analysis was carried out. Although age, CAV, HIF1 α , PHD3 and VEGF could be identified as potentially influencing factors (defined as $p < 0.2$), no other factors, including hypertension and myocardial hypertrophy, showed significant influence. However, when the effect of these parameters on RV fibrosis was analyzed in a multifactorial covariance analysis, only CAV, HIF1 α , PHD3 and VEGF emerged as powerful predictors of RV fibrosis.

DISCUSSION

The present study is first to investigate myocardial allograft remodeling over a 10-year follow-up period. A

novel and most important finding the significant fibrogenesis that may be associated with a differential regulation of hypoxia-related proteins.

Allograft Myocardial Remodeling

Serial RV biopsies revealed a significant progression of allograft fibrosis and a marked loss of myocytes with the remaining cells displaying signs of hypertrophy. Armstrong et al, in their study of allograft myocardial remodeling, also demonstrated progressive fibrosis and cellular hypertrophy.¹ General levels of fibrosis were similar. However, the kinetics of fibrogenesis appears to have differed from ours: They found that fibrosis increased mostly in the first year after transplantation, whereas we found increasing fibrosis throughout follow-up. Different follow-up periods (6 vs 10 years) and improved immunosuppressive therapy, possibly delaying allograft remodeling, may explain this finding. However, average fibrosis 6 years after transplantation was comparable in the studies (20% vs 25.2%).

Although we did not find a significant correlation between donor heart ischemic time and myocardial remodeling, donor ischemia may influence remodeling. Evidence concerning this matter has been conflicting.^{1,6,17} One study suggesting such an influence limited investigation of donor organ ischemia-related remodeling to one measurement 5 to 10 days after surgery.⁶ It appears that only early allograft fibrosis may correlate with ischemic time.

Explosive brain death in organ donors has been suggested to increase inflammatory cytokines and elevate levels of epinephrine with subsequent ischemic damage to the heart and poor functional recovery.^{18,19} An influence on the development of CAV has also been reported.²⁰ However, in the present study, no signifi-

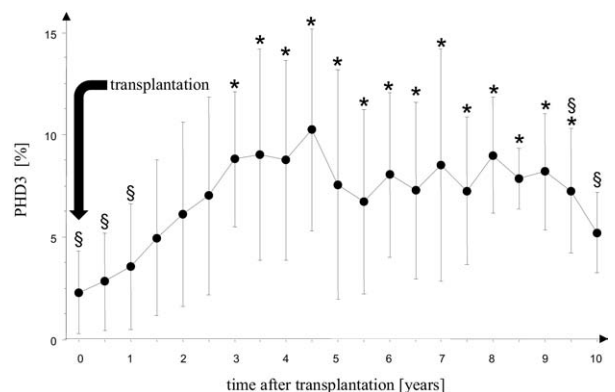


Figure 4. PHD3: Right ventricular biopsies were stained using immunohistochemistry with DAB as chromogen. Computer analysis identified the amount of PHD3 staining. Results are displayed as mean \pm standard deviation. The black arrow indicates the point of allograft transplantation surgery. § $p < 0.05$ vs peak at 4.5 years; * $p < 0.05$ vs baseline at 0 year.

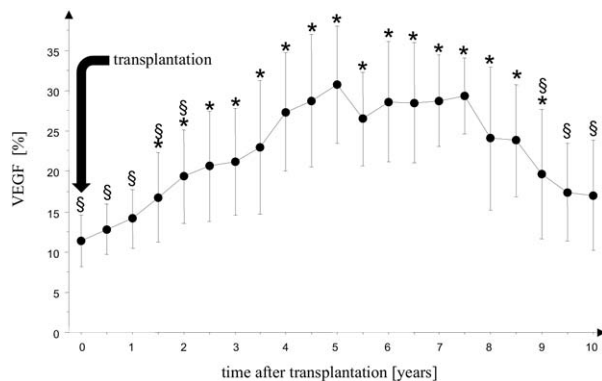


Figure 5. VEGF: Right ventricular biopsies were stained using immunohistochemistry with DAB as chromogen. Computer analysis identified the amount of VEGF staining. Results are displayed as mean \pm standard deviation. The black arrow indicates the point of allograft transplantation surgery. § $p < 0.05$ vs peak at 5 years; * $p < 0.05$ vs baseline at 0 year.

cant influence was found, which may be explained by the relatively small number of donors affected ($n = 5$).

Calcineurin inhibitors such as CsA have been suggested to cause fibrosis² by inducing TGF- β .²¹ The relevance of calcineurin inhibitors for allograft remodeling is unclear.¹⁷ Information regarding *cardiac* allografts is scarce. We did not detect a significant influence of immunosuppressants on fibrosis. One reason may be the low and decreasing CsA blood levels in our study. CsA is known to induce systemic hypertension.²² Hypertension is a possible stimulus for myocardial fibrosis.²³ TGF- β is an established pro-fibrotic cytokine in cardiac fibrosis²³⁻²⁵ and is closely related to the renin-angiotensin system.^{26,27} Inhibition of the renin-angiotensin system (and, thus, indirectly TGF- β) has been shown to prevent CsA-induced fibrosis.⁵ However, studying angiotensin-converting enzyme (ACE) inhibition and its influence on remodeling revealed no significant influence. One reason may be the moderate number of patients having received ACE inhibition (39% to 51%) and the low average percentage of the maximum dose ($27.6 \pm 7.6\%$).

A donor-recipient graft size mismatch may lead to allograft remodeling.^{28,29} Such an influence, however, was not detected in our study as the ratio between donor-recipient graft size was 0.93. Generally acceptable are ratios as low as 0.71,³⁰ putting our ratio well into the range.

Graft rejection may cause myocardial fibrosis. The underlying mechanism may be recurrent inflammatory episodes ultimately leading to tissue scarring with interstitial fibrosis.²³ CAV itself has been associated with ventricular echocardiographic remodeling.³¹ This remodeling has been suggested to be linked to cardiac fibrosis.³² In our study the average rejection among all patients was only mild to moderate. Throughout follow-

up, acute rejection episodes did not change significantly; however, a non-significant trend to more rejection episodes was found after Year 4 post-transplantation. Longer follow-up and/or more patients may be necessary for this trend to reach significance. Angiographically determined CAV increased significantly throughout follow-up. It thus seems possible that CAV may have contributed to the histologic changes observed.

Sensing Hypoxia

HIF1 is a heterodimer consisting of HIF1 α and HIF1 β . It is constitutively expressed and has an extremely short half-life of <5 minutes under normoxia.³³ Oxygen promotes the hydroxylation of HIF1 by HIF-specific PHDs, a required condition for von Hippel-Lindau protein (VHL) to associate with and inactivate HIF1.³⁴ A deficient oxygen supply (i.e., hypoxia) may be a cause for myocardial injury and dysfunction. The latter, in turn, commonly develops in cardiac allograft recipients.⁷ We found HIF1 α to be up-regulated early after transplantation and then to be significantly down-regulated over the following years. This finding points to early myocardial allograft hypoxia, which then may trigger adaptive mechanisms to reduce hypoxic stress. Although the early hypoxic response may have been influenced by mechanisms such as graft ischemia time, peri-transplant hemodynamic status of the donor and myocardial reperfusion damage, the chronic hypoxic response needs additional explanation. Allograft rejection, characterized by an inflammatory reaction, could serve as such an explanation. HIF1 may help counteract inflammation and remodeling because it has anti-inflammatory properties.³⁵ Apparently however, HIF1 is unable to stop the progression of fibrogenesis.

Sensing Oxygen

In normoxia, PHDs hydroxylate HIF1 α and thus target it for proteasomal degradation. In acute hypoxia, PHD expression is down-regulated, leading to HIF1 α stabilization. In chronic hypoxia, however, the oxygen-sensing PHDs may be up-regulated.³⁶ In the present study we observed early low levels of PHD3, which then increased in parallel with myocardial fibrosis. Finally, PHD3 reached a plateau and then decreased again. Our results show that the initial up-regulation of PHD3 may transpire despite chronic hypoxia. After years of chronic hypoxia and increasing fibrosis it then seems to be eventually down-regulated. From animal studies it is known that mice react to chronic hypoxia by overexpressing PHDs. This may help the organism in the prevention of chronic hypoxic HIF-triggered signaling.³⁶ Our data allow us to speculate that, in long-standing hypoxia, compensatory mechanisms may fail once myocardial fibrogenesis reaches a critical point.

Pro-angiogenic Signal

In the absence of oxygen, HIF1 α binds to the HRE, and thus induces genes such as VEGF.¹¹ VEGF then may interact with and activate its receptors, leading to pro-angiogenic signaling. We found VEGF expression to be differentially regulated in cardiac allograft recipients. In the first years after transplantation, VEGF expression increased significantly and then peaked at 5 years after surgery. In the following years, VEGF levels decreased again slightly. Because in our study VEGF expression did not track HIF1 α expression, there must be other, additional factors at work. For kidney allografts, a link between VEGF expression and interstitial fibrosis has been postulated,^{4,37} and these findings were linked to CsA toxicity. Furthermore, it has been shown that corticosteroid treatment may reduce VEGF expression.³⁸ In our study, initial high doses of corticosteroids may have contributed to lower VEGF expression. However, it appears that, despite a significant reduction in corticosteroid dose during the follow-up period, VEGF started to decline again 5 years after transplantation. This suggests other additional mechanisms at work. CAV and rejection episodes have been suggested to increase VEGF expression.³⁹ These factors may have influenced VEGF regulation in the present study.

In conclusion, in the cardiac allografts studied, extensive and progressive tissue remodeling was present. Myocardial fibrosis was central to this process. Hypoxia may have also played a role in this process, leading to differential regulation of pro-angiogenic signals: After early post-operative tissue hypoxia, as indicated by elevated HIF1 α levels, hypoxia seems to have improved, as confirmed by decreasing HIF1 α and rising PHD levels. This improvement in hypoxia may have been caused by an increase in pro-angiogenic signaling, such as in the elevation of VEGF levels. However, later after cardiac transplantation these processes appeared to have been reversed: Progressive fibrogenesis and CAV were observed and may have hampered tissue oxygen supply and thus helped to promote tissue hypoxia.

DISCLOSURE STATEMENT

None of the authors have conflicts of interest to disclose.

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