TRANSPLANT CORONARY ARTERY DISEASE

Coronaire atherosclerose na harttransplantatie

Cover:
Transverse section of an epicardial artery of a heart transplant recipient illustrating the hyperplasia of the intimal layer.

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Proefschrift

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Since the first successful orthotopic cardiac transplantation in 1967, the survival after transplantation has gradually been improved due to the introduction of cyclosporin-A, the monitoring of acute rejection by endomyocardial biopsies and optimized patient care[1]. In the last decade, clinical studies indicate that coronary artery disease in the graft is the most common cause of death in the first postoperative years[2-4]. The earliest change consist of concentric fibrosis and smooth muscle cell proliferation with collagen accumulation creating diffuse intimal thickening. This process involves not only the large epicardial vessels but also the intramyocardial branches[5]. The specific angiographic morphology of the lesions found after transplantation was described by the group of Stanford, distinguishing 3 categories: type A, discrete or short tubular stenosis in the proximal, middle or distal segments of major coronary arteries or their branches, type B; diffuse concentric luminal narrowing in the middle to distal segment branches; and type C, diffusely narrowed irregular distal branches that are squared of and end abruptly, the latter two groups both unique to the post-transplant patients[6]. Despite this clear categorization, pathological examinations showed that the process of accelerated coronary artery disease is underestimated by visual interpretation of coronary angiography[7]. Thus, visual interpretation of coronary angiography has limitations for both clinical and research purposes.

The aim of this thesis is to describe the changes in the coronary vasculature in the first six years after cardiac transplantation and to correlate these changes with previously identified risk factors. In addition to morphological epicardial coronary artery changes, assessed by quantitative coronary angiography, myocardial flow reserve and atrial pacing stress were used to assess the influence of accelerated coronary artery disease on myocardial flow and cardiac function. Chapters II-V describe the results of quantitative analysis of serial coronary angiograms at different postoperative stages. The influence of changes in coronary microvasculature on myocardial flow reserve is described in chapters VI and VII. Chapter VIII describes the functional correlates of diffuse coronary artery disease using atrial pacing stress at the time of routine angiography.

Quantitative coronary angiography

Coronary angiography has been used as a diagnostic tool for more than 30 years. In the last decade the introduction of computer assisted analysis provided a more objective and reproducible method to describe the changes in the coronary luminal diameter. This technique has evolved to be the optimal method to study coronary artery dimensions in trials concerning the progression and regression of coronary artery disease[8].

The computer-assisted Cardiovascular Angiography Analysis System (CAAS) has been in operation at the Thoraxcenter since 1982. The boundaries of a selected coronary segment are detected automatically from optically magnified regions of interest (512 x 512 pixels) of an end diastolic cineframe. The mean, minimal and maximal coronary width of each

segment are calculated in millimetres.

Two parameters are used in this thesis to describe the progression or regression of diffuse coronary artery disease. The patient global score, a description of the coronary status of an individual patient, is used to describe progression or regression of *diffuse* coronary artery disease. In addition, the minimal segment width is measured to assess development of *focal* disease[8].

Myocardial flow reserve

The concept of myocardial flow reserve has been developed as a means to describe the functional capacity of a coronary artery i.e. the relationship between the angiographic severity of coronary artery disease and the resulting limitation in maximal myocardial blood flow[9]. In theory, this is especially relevant in cardiac transplant recipients, where a diffuse arteriopathy involving the microvasculature, can reach a flow limiting significance, without changes in the angiographic appearance. Several techniques are currently under development to allow the assessment of myocardial flow reserve in humans. In general, these techniques are designed for application during a catheterization procedure, like venous blood flow measurements in the coronary sinus, or the assessment of phasic coronary blood flow velocities using ultrasonic Doppler catheters[9,10].

Early studies concerning the calculation of coronary blood flow by analysis of contrast passage in the coronary arteriogram were reported almost 25 years ago[11]. Analysis of these changes in contrast density, delineation of so-called time-density curves and subsequent calculation of time parameters from these curves, was referred to as videodensitometry. Shortly thereafter it was shown that visualisation of contrast agent through the myocardium could be enhanced by ECG-triggered subtraction imaging. If digital methods are used for this purpose, the term digital radiography is applied. Compared with the other invasive techniques to measure myocardial flow, this approach has several advantages. First, the technique is more easily applicable during routine catheterization, because no additional catheter or intracoronary device has to be used. Moreover, the analysis of multiple regions of interest provides flow information from various subsegments of the coronary artery tree, which is not possible or more time consuming with other invasive techniques.

Atrial pacing stress test

In patients with coronary artery disease a narrowing of the vasculature can lead to subnormal perfusion of specific regions of the myocardium, with decreased mechanical function. A reduction in global myocardial function may not become manifest except during physical activity or other types of stress. Atrial pacing as a stress test to investigate the dynamic response of the heart in these patients was introduced as attractive method[13]. Advantages of this method are the rapid reversibility, its precise control in both duration and cardiac frequency, and the removal of psychological factors, since the patient is unaware of

the precise level of stress. Atrial pacing increases myocardial oxygen consumption by means of an increase in heart rate and augmented wall stress. Hemodynamic measurements at peak pacing reveal differences in left ventricular end-diastolic pressure, peak positive rate of isovolumic left ventricular pressure rise and calculated maximal myocardial velocity of shortening in patients with coronary artery disease as compared to controls[13]. We therefore used the atrial pacing stress test to verify its application in early detection of the diffuse coronary artery disease as present in cardiac transplant recipients.

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CHAPTER 2

PROGRESSION OF FOCAL ARTERIOSCLEROSIS AND DIFFUSE CORONARY DISEASE AFTER CARDIAC TRANSPLANTATION. CASE REPORTS.

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INTRODUCTION

Coronary arteriosclerosis of the cardiac allograft is the leading cause of morbidity and mortality in late survivors (>1 year) after cardiac transplantation[1-3]. In order to detect the onset and progression of allograft coronary disease, most transplant centers perform coronary cine-angiography at regular intervals after transplantation. Angiographycally there is typically diffuse luminal narrowing with distal vessel obliteration. Accordingly this type of coronary disease is usually not amendable to balloon angioplasty or bypass graft surgery[4]. In addition to diffuse disease, however, some cardiac transplant recipients may develop more sharply demarcated lesions. In our series of 200 patients receiving an orthotopic cardiac transplant from June 1984 to January 1993 one year survival was 92%, confidence interval 88-96%[5]. Five patients underwent a balloon dilatation or atherectomy for localized coronary stenosis. The rate of progression of focal and diffuse disease in these patients was studied by quantitative analysis of sequential coronary angiograms using an automated contour detection technique[6].

CASE REPORTS

A 37-year-old man (patient A) underwent cardiac transplantation in January 1985 because of end stage heart failure, due to dilated cardiomyopathy. He received a donor heart from a 34-year-old woman (Table 1). Immunosuppressive therapy consisted of cyclosporine and low dose steroids. After transplantation he experienced several upper airway infections. The patient resumed smoking. One episode of acute rejection was treated with methylprednisolone. In the first postoperative years, the serum cholesterol level averaged

Table 1. Risk factors

Patient	A	B	C	D	E
Age recipient (years)	37	28	50	46	55
Age donor (years)	33	26	35	36	34
Gender mismatch	yes	yes	no	no	no
Number of HLA mismatches	3	4	3	4	6
Number of rejection episodes	1	6	2	2	3
Smoking	yes	no	yes	no	no
Serum Cholesterol (mmol/l)	5.9	6.0	8.4	7.6	9.0
Serum HDL-Cholesterol (mmol/l)	1.8	1.5	1.2	1.2	2.3
Serum Triglyceride (mmol/l)	1.4	1.8	3.7	3.3	1.9

5.9 mmol/l. Focal luminal narrowing in the left circumflex (LCX) coronary artery was observed on the third annual coronary angiogram. In the fifth postoperative year a 50-60% stenosis had developed (Figure 1). Since ^{99m}Tc methoxyisobutyl isonitrile (MIBI) perfusion scintigraphy showed reversible ischemia of the left ventricular posterior wall, a percutaneous coronary intervention was scheduled. Directional coronary atherectomy was performed, because of the eccentricity of the lesion. The angiograms before and after the intervention are shown in figure 1. At follow-up, 7 months later, no restenosis was visible. Quantitative analyses of three visually "normal" segments of this patient are shown in figure 2. Although there is considerable variation over the years, no significant diffuse luminal narrowing of these segments could be detected. At 6 year follow-up, the perfusion scintigraphy showed reversible ischemia of the anterior wall. Coronary angiography revealed diffuse wall irregularities and aneurysmal dilatation of the left anterior descending branch. One year later, seven and a half years after transplantation, at a meeting of cardiac transplant recipients, he collapsed because of a cardiac arrest. He underwent successful cardiopulmonary resuscitation. In the hospital no electrocardiographic signs of myocardial infarction or elevated plasma levels of creatine kinase were detected. His cardiac arrest was probably related to reversible ischemia of the myocardium resulting in a ventricular arrythmia. A few days later he was declared brain death and the ventilatory support was terminated.

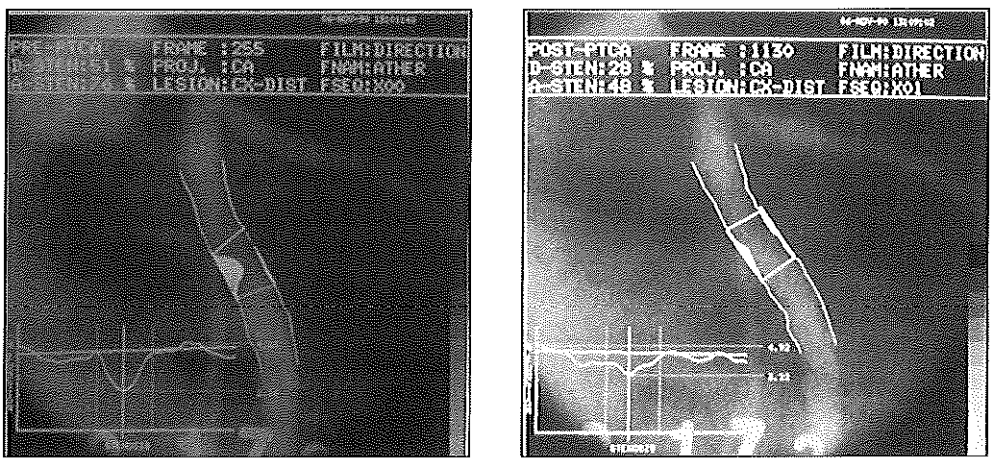

Figure 1. Quantitative analysis of the obstruction in the middle segment of left circumflex coronary artery of patient A, filmed both before and after atherectomy. The white area is a measure of the atherosclerotic plaque and is derived form the actual luminal contour and a interpolated reference contour. *Reprinted by permission of the American Heart Journal, Strikwerda S, Umans V, Van der Linden MMJM, Van Suylen RJ, Balk AHMM, De Feyter PJ, Serruys PW; volume 123: pp 1686-1690, 1992. Copyright 1992. All rights reserved.*

The second patient (patient B) was a 28-year-old woman, who developed postpartum cardiomyopathy after the birth of her second child in June 1985. In April 1986 she received the heart of a 26-year-old man. There were six acute rejection episodes, which were treated with pulsed doses of methylprednisolone, and polyclonal and monoclonal anti-T-cell antibodies. Because of ongoing rejection, azathioprine was added to her maintenance immunosuppressive therapy consisting of cyclosporine and prednisolone. Hypertension was present, which was treated with nifedipine. The mean serum cholesterol level after transplantation was 6.0 mmol/l, and mean high density lipoprotein cholesterol and triglyceride levels were 1.5 and 1.8 mmol/l, respectively. On the third annual angiogram, a 50% stenosis in the left anterior descending (LAD) coronary artery was detected just distal to a large diagonal side branch. One year later, this stenosis had progressed to approximately 80% diameter reduction. ^{99m}Tc MIBI perfusion scintigraphy showed evidence of reversible ischemia of the anteroseptal and anterior myocardium and a balloon dilatation was performed successfully. At 5 year follow-up angiography, no restenosis of the culprit lesion

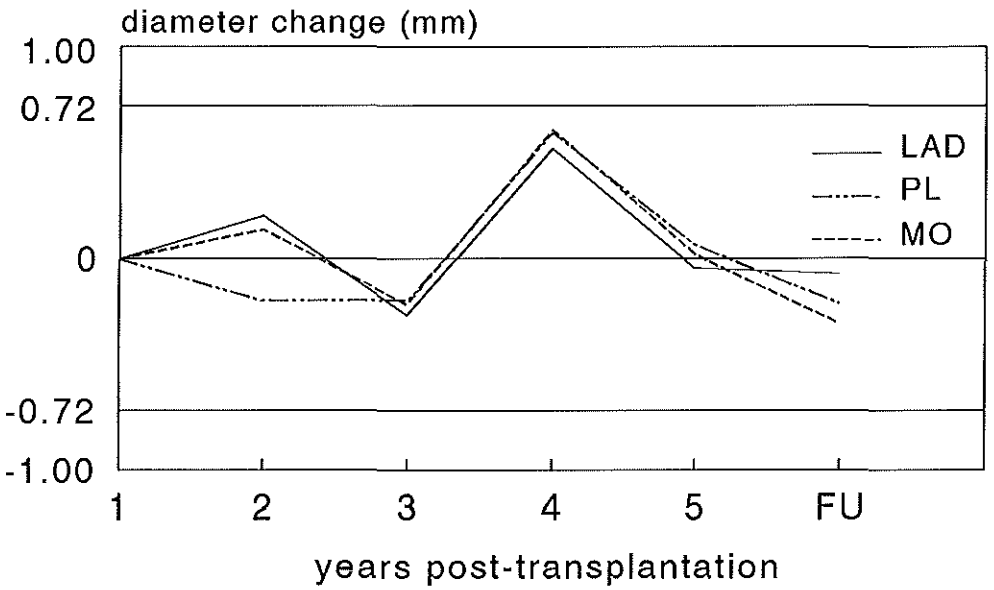

Figure 2. Change in mean diameter of the proximal obtuse marginal branch (MO), proximal left anterior descending (LAD) coronary artery, and posterior lateral branch (PL) after cardiac transplantation in patient A, averaged after quantitative analysis in multiple matched views, relative to the first year angiograms in millimetres. A change in diameter exceeding twice the long-term variability of our quantitative angiography system[8] (0.72 mm), represented by the two horizontal lines, was considered a significant change from baseline, taking into account that the first coronary angiograms were not acquired under standard conditions, i.e. no special care was taken to reduce the potential sources of variability (X-ray system settings and vasomotor tone).

was found. However, other segments of the coronary tree showed progression of both diffuse and focal coronary disease. She died five and a half years after transplantation from severe heart failure due to ischemia of the myocardium.

The third patient (patient C) was a 50-year-old man, who developed cardiomyopathy as a result of alcoholism. In 1989 he received the heart of a 35 year old man. This patient suffered from two episodes of acute rejection in the first year after transplantation. The mean serum cholesterol level averaged 8.4 mmol/l. In the first postoperative year he resumed smoking. Coronary angiography after one year revealed normal epicardial coronary arteries. Minor wall irregularities were seen of the smaller septal branches. After two years a focal stenosis was seen of the LAD (< 50% by visual assessment), which showed progression in the subsequent years and was treated by directional coronary atherectomy in 1993 because of angina and electrocardiographic signs of ischemia. The disease of the smaller branches did not increase in severity. In 1994 has was readmitted because of a small inferior myocardial infarction (peak plasma level of creatine kinase 363 U/l). Coronary angiography revealed a closure of the proximal right coronary artery. The treated lesion in the LAD only showed minor wall irregularities. In february 1996 ^{99m}Tc MIBI perfusion scintigraphy showed no evidence of reversible ischemia. Echocardiography revealed a decreased left ventricular function. In July 1996 coronary angiography showed no additional focal coronary lesions. In august 1996, almost 8 years after transplantation, he was admitted to the hospital with signs of heart failure and died a few weeks later.

The fourth patient (patient D) was a 46-year-old man, who underwent cardiac transplantation because of ischemic heart disease. In 1987 he received the heart of a 36 year old man. Within a few weeks after hospital discharge he was readmitted because of acute rejection. He suffered from another episode of acute rejection in the fourth postoperative year. Annual coronary angiography revealed diffuse coronary artery disease within two years after transplantation. After five years two focal lesions developed in the left anterior descending branch, which were treated by directional atherectomy. Recently, a coronary angiography was performed which showed a significant stenosis distal in the left anterior descending branch and diffuse wall irregularities of all coronary artery branches. Since a ^{99m}Tc MIBI perfusion scintigraphy did not show signs of ischemia, an intervention was not performed. At this moment, 9 years after transplantation, he is in excellent condition. He resumed working and does not have any complaints.

The fifth patient (patient E) was a 55-year-old woman. In 1988 she received the heart of a 34 year old woman. Annual coronary angiography revealed only minor wall irregularities of the small septal branches of the LAD, which did not increase in severity. She was treated for mild hypertension with nifedipine from the first postoperative year. The serum cholesterol level averaged 9.5 mmol/l and mean high density lipoprotein cholesterol and triglyceride levels were 1.89 and 2.30 mmol/l, respectively. In the fourth postoperative year

she was admitted to the hospital because of an acute anterior myocardial infarction. Coronary angiography revealed wall irregularities in all major epicardial vessels and all smaller branches of the coronary tree. Furthermore, the coronary angiogram showed a significant stenosis (>50% stenosis) of the LAD, which was treated by balloon angioplasty. In 1994, 6 years after transplantation, a ^{99m}Tc MIBI perfusion scintigraphy showed signs of ischemia, and a coronary angiography was repeated. Severe coronary atherosclerosis was seen with significant stenoses in the proximal and distal segments of the right coronary artery, and proximal and distal segments of both the circumflex and anterior descending branch of the left coronary artery. Before the results of this coronary angiogram could be discussed by the cardiologists of the transplant team and the surgeons, she died.

DISCUSSION

The pathogenesis of accelerated coronary artery disease in cardiac transplant recipients remains unclear. It is thought, however, that an immune-mediated insult to the vascular endothelium initiates the process, since it affects the coronary arterial system over its entire length. Diffuse intimal hyperplasia, due to smooth muscle cell proliferation and accumulation of collagen in a circumferential distribution, has been observed as early as 1 week after transplantation[8,9]. A progressive rise in the degree of luminal narrowing and a greater proportion of focal, eccentric plaques have been observed as a function of allograft survival time[8]. Once the initial injury to the coronary endothelium has occurred, other factors may influence the rate of development and the morphologic features of both diffuse and focal transplant coronary lesions. In particular, accelerated coronary artery disease has been reported to be associated with the occurrence of HLA incompatibilities[1], acute rejection episodes[2,3], higher donor age[1,10], elevated serum triglyceride levels[1,10], elevated total cholesterol and low density lipoprotein levels[3] and Cytomegalo virus infections[11]. Gao et al. compared concentrations of serum lipids in patients with the morphologic type of coronary artery disease on the coronary angiogram. Surprisingly, a greater association of higher triglyceride and total cholesterol levels was found with diffuse concentric distal obliterative lesions than with more focal discrete disease located in proximal and middle portions of vessels[9]. In our series we could not find any relation between either focal or diffuse disease and these risk factors[6,12].

As in these case reports, most patients show no lumen abnormalities on their first year angiogram at the site of subsequent obstruction. Progression of focal coronary artery disease appears to be as rapid and unpredictable as native coronary atherosclerosis[13]. It should be appreciated that coronary arteriography is relatively insensitive for detection of early vascular lesions[14]. In particular, focal lesions graded as having <25% diameter narrowing on microscopic examination and diffuse disease are often underdiagnozed by coronary angiography[14], even when (semi)quantitative techniques are used[15].

In our series of 293 cardiac transplant recipients 9 patients underwent a coronary

intervention (directional atherectomy or balloon angioplasty) because of significant stenoses of the coronary artery tree with evidence of reversible ischemia on ^{99m}Tc MIBI perfusion scintigraphy.

Histologic examination of the coronary atherosclerosis specimen retrieved by atherectomy in patient A revealed a very interesting finding. The tissue had a low-to-moderate cellularity, embedded in collagen bundles and cellular matrix, resembling ordinary atherosclerotic plaque[16]. Results from a polymerase chain reaction analysis of variable number of tandem repeat (VNTR) gene loci showed that the coronary arteriosclerotic tissue of patient A and donor myocardial biopsy performed immediately after transplantation shared the same alleles and that this pattern consistently differed from that of the recipients blood[17]. These findings support the concept that focal lesions in graft atherosclerosis may represent progression of preexisting though angiographically undetectable coronary disease of the donor heart. However, these observations do not exclude the possibility of accelerated development of a de novo "ordinary" atherosclerotic plaque after cardiac transplantation.

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CHAPTER 3

CORONARY LUMINAL CHANGES IN THE FIRST YEAR AFTER CARDIAC TRANSPLANTATION: QUANTITATIVE ANALYSIS OF CORONARY ANGIOGRAPHY.

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ABSTRACT

In order to evaluate the changes in coronary luminal morphology in cardiac transplant recipients, serial quantitative coronary angiography was performed 1 month and 1 year after transplantation in 30 patients. Between 6 and 8 segments of the coronary artery tree were analyzed in multiple matched projections using an automated edge detection technique. Twenty-five patients were treated with calcium-channel blockers.

The mean segment diameter of the 249 analyzed segments showed a small decrease from 3.40 ± 0.82 mm at 1 month to 3.36 ± 0.83 mm at 1 year (p=0.007). Twelve patients (40%) showed luminal narrowing, using the variability of the analysis system (0.11 mm) as a cut off level for significance. In 12 other patients (40%), coronary luminal diameter did not change, and in 6 patients (20%) an increase in diameter was found. Patients, transplanted because of severe coronary artery disease showed a larger reduction in lumen diameter than patients with other indications for transplantation. No other risk factors for reduction of coronary artery diameter were identified. This study using quantitative analysis of coronary angiograms confirms the minimal reduction in diameter of epicardial arteries in the first year after transplantation in patients using calcium-channel blockers.

INTRODUCTION

Accelerated coronary artery disease is one of the limiting factors for long term survival of cardiac transplant recipients[1]. Due to the lack of innervation of the cardiac allograft, angina pectoris is usually absent, and electrocardiographic signs of myocardial infarction, congestive heart failure or sudden death may be the first signs of graft coronary arteriosclerosis[2].

In order to detect the onset and progression of coronary disease in an early phase, coronary arteriography is performed yearly in many transplant centers. The reported incidence of visually detectable coronary artery disease varies considerably, ranging from 2 to 34 % at 1 year, and from 50 to 73 % at 6 years after transplantation[3-11]. This study was undertaken to provide a more accurate and objective evaluation of the development of coronary artery disease in the first postoperative year by quantitative analysis of large epicardial coronary branches filmed both 1 month and 1 year after transplantation. Also, the relation between such changes and potential risk factors for transplant atherosclerosis[12-22] was explored.

PATIENTS AND METHODS

Patients

From September 1989 until September 1990 thirty-one patients underwent cardiac transplantation at the Thoraxcenter. Early coronary angiography was performed in 30 patients to establish initial coronary status after transplantation. All 30 patients underwent follow-up angiography 1 year after transplantation. One other patient died 2.5 months after transplantation from multi-organ failure.

Early prophylactic immunosuppressive therapy consisted of 7 day courses of polyclonal (Horse anti-thymocyte globulin, antilymphocyte IgG2, Lymphoglobulin, Institute Merieux) or monoclonal anti-T cell antibodies (OKT3, Ortho Pharmaceutical, Raritan, N.J.) in 15 patients each[23]. Maintenance immunosuppression consisted of low dose steroids and cyclosporine. Additional immunosuppressive treatment was instituted in cases of moderate rejection with definite myocyte necrosis (Billingham grade 2)[24], and consisted of pulsed high dose steroids and poly- or monoclonal anti-T cell therapy. In 5 patients, azathioprine was added to the maintenance regimen because of ongoing or recurrent rejection.

Cytomegalo virus (CMV) seronegative recipients received anti-CMV hyperimmunoglobulin (Cytotect, Biotest Pharma GmbH, Dreiech, Germany) during the first 10 weeks after transplantation[25]. CMV infection was defined as any rise in serum IgM, isolation of CMV from urine, throat or buffycoat, or evidence of CMV immediate early antigen. CMV disease was defined as infection accompanied with fever >38°C for at least 2 days, and either leukocytopenia (< 2.5 x 10^9/l) or thrombocytopenia (<100 x 10^9/l), or symptoms of organ involvement[25].

All patients were managed with antiplatelet agents, consisting of either dipyridamole 75 mg tid or aspirin 80 mg daily. Hypertension was preferably treated with nifedipine.

Quantitative coronary angiography

All patients underwent left heart catheterization and selective coronary angiography, using the femoral approach. To reduce variations in dynamic vessel tone, 5 mg of isosorbide-dinitrate was administered sublingually before the first coronary contrast injection.

The coronary angiograms were analyzed by automated edge detection, using the computer based Cardiovascular Angiography Analysis System (CAAS), which has been described in detail previously[26,27]. An example of an analysis is shown in figure 1. The variability of the contour detection system, defined by the standard deviation of the differences of the computed results from repeated analyses, is 0.11 mm for the mean width in non-obstructed segments[27]. Progression of coronary artery narrowing was defined as a decrease of segment width by more than 0.11 mm[12]. At baseline and follow-up coronary angiography, nine proximal coronary segments were measured in multiple matched

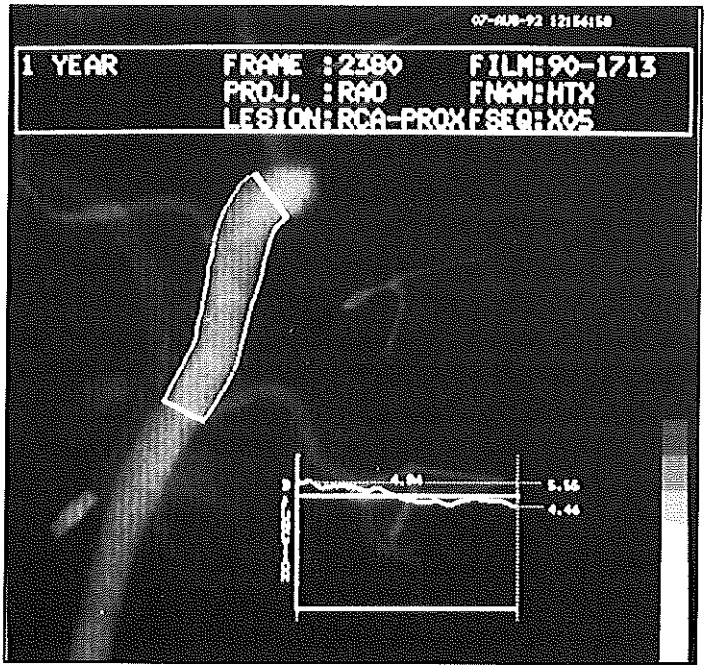

Figure 1. Quantitative analysis of the proximal right coronary artery of a 48 year old cardiac transplant recipient, one year after cardiac transplantation. A single frame of the angiogram is shown with superimposition of the contours, as traced by the automated contour detection system.

projections: segments 1-3, 5, 6-8, 11 and 13 or 14 according to the classification by the American Heart Association[28].

The results of present study are expressed in a patient global score and a segment global score as described earlier[29]. Patient global score was defined as the average of the mean widths of the measured segments per patient. The patient global score change was defined as the average change in the mean width of the segments. The segment global score was defined as the average of mean widths of all measured segments.

Statistical analysis

All data are presented as the mean ± 1 SD. Statistical analysis of the change in coronary diameter was performed using the Wilcoxon Matched-pairs Signed-ranks test. Logistic regression and chi square tests were used to identify risk factors for accelerated coronary artery disease. Statistical significance was defined as a p value of 0.05 or less.

Table 1. Clinical data of the cardiac transplant recipients

Recipient age (yrs)		45 ± 12
Donor age (yrs)		26 ± 8
Gender		2F, 28M
Gender mismatch (pts)		9
Heart disease	CMP	14
	IHD	14
	VHD	2
HLA-mismatch	A (no)	1.4 ± 0.6
	B	1.6 ± 0.5
	DR	1.3 ± 0.6
Cold ischemia time (min)		154 ± 30
Rejection episodes (no, median) *		1 (range 0 - 4)
No of patients with	0 AR *	12
	1 AR	14
	2 AR	4
Cholesterol (mmol/l) †		7.4 ± 1.7
Triglycerides (mmol/l) †		2.3 ± 0.8
HDL-cholesterol (mmol/l) †		1.5 ± 0.5
Smoking (yes / no) ‡		7 / 23
Hypertension (150/95 mmHg) (pts)		22
Blood pressure	Systolic (mmHg) §	141 ± 20
	Diastolic (mmHg)	90 ± 11
Diabetes Mellitus (pts)		2
CMV preop serostatus neg (pts)		13
CMV infection (pts)		12
CMV disease (pts)		7
Use of nifedipine (pts) ‡		25

F:female; M:male; CMP:cardiomyopathy; IHD:ischemic heart disease;
VHD:valvular heart disease; AR:acute rejection;
HDL=High Density Lipoproteins; CMV=Cytomegalo virus;
* between 1 month and 1 year follow-up; † mean value in the first year after transplantation; ‡ during the first postoperative year;
§ at follow-up catheterization

RESULTS

The clinical data of these 30 cardiac transplant recipients are described in table 1. In 29 patients early coronary angiography was performed at a median of 34 days after transplantation (range 19 - 109 days). In one patient, who suffered from recurrent rejection episodes, the first angiogram was made 6 months after transplantation. Follow-up coronary angiography was performed at a median of 12.2 months after transplantation (range 10.7 - 13.1 months).

In total 249 segments were available for paired quantitative measurement. In 2 patients 6 segments were analyzed, in 1 patient 7 segments, in 13 patients 8 segments and in 14 patients 9 segments. The length of the selected coronary segments averaged 21.2 ± 9.3 mm in the early angiogram and 21.1 ± 9.4 mm at follow-up (difference not statistically significant).

Patient global score

Patient global score was similar at 1 month and 1 year after transplantation, respectively 3.39 ± 0.34 and 3.35 ± 0.34 mm. If the variability of the CAAS system, 0.11 mm, is applied

Table 2. Change in patient global score

change (mm)	number of patients	mean ± SD (mm)
< -0.11	12	-0.23 ± 0.10
-0.11 - +0.11	12	-0.01 ± 0.07
> +0.11	6	0.25 ± 0.11
total	30	-0.05 ± 0.20

Table 3. Segment global score by vessel size

Segment mean width at baseline (mm)	number of segments	segment global score (mm)		change in segment global score		
		baseline	1 year	absolute (mm)	relative (%)	
> 3.77	83	4.29 ± 0.38	4.19 ± 0.48	-0.10 ± 0.38	-2.3 ± 9.0	p=0.01
3.07 - 3.77	83	3.43 ± 0.21	3.40 ± 0.39	-0.03 ± 0.33	-0.9 ± 9.4	NS
< 3.07	83	2.48 ± 0.43	2.48 ± 0.47	-0.00 ± 0.23	0.2 ± 9.1	NS
total	249	3.40 ± 0.82	3.36 ± 0.83	-0.05 ± 0.32	-1.0 ± 9.2	p=0.007

Table 4. Change in segment global score change

diameter change (mm)	number of segments	change in segment global score absolute (mm)	relative (%)
< -0.11	105	-0.32 ± 0.18	-9.0 ± 4.2
-0.11 - 0.11	75	-0.02 ± 0.07	-0.6 ± 2.3
> 0.11	68	0.34 ± 0.21	10.7 ± 6.1
total	248	-0.05 ± 0.32	-1.0 ± 9.2

as a cut off level for significance, 12 patients (40%) showed luminal narrowing. In 12 patients (40%) coronary luminal diameter did not change, and in 6 patients (20%) an increase was found (Table 2).

Segment global score

Comparison of segment global score 1 and 12 months after transplantation (Table 3), showed small decrease from 3.40 ± 0.82 to 3.36 ± 0.83 mm (p=0.007). The mean relative reduction of segment diameter was 1.0 %. This reduction in segment global score proved to be statistically significant only for vessel-segments larger than 3.77 mm (Table 3). The smaller segments did not show any reduction of segment diameter. Based on the variability of the CAAS system, 105 segments showed luminal narrowing, 75 segments did not change, and in 69 segments luminal diameter increased (Table 4). Minimal segment diameter of all 249 segments decreased from 2.97 ± 0.83 to 2.92 ± 0.84 mm (p=0.01), a reduction of 1.5 %.

Risk factors

Patients, with coronary artery disease prior to cardiac transplantation (N=14), showed a reduction of patient global score in contrast with patients with other indications for transplantation (-0.13 ± 0.17 mm versus 0.03 ± 0.20 mm, p<0.05). Using the 0.11 mm threshold as a cut of level for significance, this indication related risk could not be confirmed by chi square test. No other risk factors for reduction of coronary artery diameter (Table 1) were identified.

DISCUSSION

In the present study of 30 consecutive patients only minimal changes in coronary diameter were apparent. This is in contrast with a distinct reduction of coronary artery

diameter (-0.23 ± 0.19 mm) as reported in 25 patients during the first postoperative year at Stanford[12]. The present data are in agreement with subsequent data from Stanford[13] in patients treated with the calcium-channel blocker diltiazem.

Assessment of transplant coronary artery disease

Transplant coronary artery disease is a major problem affecting long term survival of cardiac transplant recipients. Publications from various transplant centers and the registry from the International Society for Heart and Lung Transplantation show that over 40% of all deaths after the first postoperative year are caused by this disease. Various methods and definitions to determine the onset or progression have been used[3-12,29-31]. It should be appreciated that customary visual interpretation of the coronary angiograms underestimates the degree and extent of this process[31]. Histologic evidence of minimal coronary artery disease is present in all patients who die 1 year or later after transplantation, independent of the cause of death and the interval after transplantation[30,31]. These pathological findings were confirmed by in vivo intracoronary ultrasound in cardiac transplant recipients[32,33]. Nevertheless angiographic evidence of accelerated coronary disease is reported in only 2 to 34% at one year[3-11].

Quantitative coronary angiography

Since visual reading of angiograms underestimates the extent of coronary disease, a more objective method for detecting and monitoring cardiac allograft coronary artery disease is needed. De Feyter et al[29] discussed the value of quantitative coronary angiography in clinical trials to describe the progression and regression of coronary artery disease. The parameter most suitable to assess the progression of *diffuse* coronary artery disease, is the mean segment width. Therefore, this measurement was used in this study to describe the changes in the coronary arteries of the cardiac transplant recipients. Changes within each patient were analyzed, based on a "patient global score" of all analyzable segments of the coronary tree. No significant differences of this patient global score were apparent in this group of transplant recipients (Tables 2). Similar, in a larger series of 119 patients who underwent a transplantation at our center, the prevalence of anatomical significant lesions (>50% stenosis in the epicardial branches by visual assessment) one year after transplantation was very low (1%). In this study the prevalence of minimal wall irregularities and other minor abnormalities of the coronary artery tree, was 34%[8].

Comparison with other studies using quantitative coronary angiography

The changes in the first year after transplantation were first described in 1990[12] in a series of 25 patients. The segment global score decreased by 9.2% in both proximal and distal segments. The patient global score also showed a significant reduction from 2.44 ± 0.26 mm

Table 5. Comparison of quantitative coronary angiography-studies

Institution	Thoraxcenter Rotterdam	Stanford University
Number of patients	30	25
Number of segments	249	353
Patient global score (mm)		
baseline	3.39 ± 0.34	2.44 ± 0.26
1 year	3.35 ± 0.34	2.21 ± 0.34
	NS	p<0.001
Segment global score		
baseline	3.40 ± 0.82	2.39 ± 0.93*
1 year	3.36 ± 0.83	2.17 ± 0.86*
change (mm)	-0.05 ± 0.32	-0.22
(%)	-1.0 ± 9.2	-9.2
Clinical parameters		
Rejection episodes †	1.2 ± 1.0	2.9 ± 1.5
Maintenance immunosuppression		
Cyclosporine and prednisone (pts)	25	0
Cyclosporine, prednisone and azathioprine (pts)	5	25

* measurements in a subgroup of patients (n=18)
† during the first postoperative year

to 2.21 ± 0.34 mm. In contrast, we observed a reduction of 2.3% only in segment global score of vessels with a diameter exceeding 3.77 mm. No difference was observed in smaller vessels (Table 3), while also the patient global score did not change. Furthermore, only 40% of the patients in this study showed coronary artery narrowing, compared to 84% in the patients in a comparable report[12].

Both studies are very similar with regard to the methods used. The measurements were performed using an automated edge detection technique and the cut off levels for significant diameter change were 0.11 mm and 0.10 mm respectively. In present study quantitative analysis was limited to larger epicardial segments (baseline segment global score 3.40 ± 0.82 mm versus 2.39 ± 0.93 mm), and fewer segments per patient were measured (mean 8.3 versus 20.6, table 5). This selection cannot explain the difference between both studies,

since we observed no changes in the smaller vessels.

Clinical and laboratory data of both studies are also comparable, except the number of rejection episodes in the first postoperative year, which was lower at the Thoraxcenter, and the immunosuppressive regimen, which seemed less extensive for maintenance therapy (Table 5). Neither study, however, could detect a relation between the number of rejection episodes or immunosuppressive therapy and the changes in coronary vessel diameter.

The small changes in lumen diameter in the present study may be a result of specific characteristics of the treatment strategy. In contrast to many other centers, 83% of the patients in present study received the calcium-channel blocker nifedipine for 8.1 ± 3.8 months. This may have reduced changes in coronary diameter since a recent randomized trial reported a similar favourable effect of another calcium-channel blocker: diltiazem[13]. Moreover, the patients in present study received at least one bloodtransfusion prior to transplantation[34]. CMV disease was infrequent, probably due to the prophylactic use of CMV hyperimmunoglobulin after transplantation in seronegative patients. Only 12 patients were infected with CMV of whom 7 patients developed CMV disease[35]. Still it remains speculation whether these factors have contributed to the more favourable coronary artery diameter in our series, since no specific risk factors for accelerated coronary artery disease were identified uptill now.

Conclusions

In comparison with other early post transplant studies[12] we observed only minimal changes in coronary luminal diameter, expressed in a patient global score and a segmental score in the first year after cardiac transplantation. Most of the patients used a calcium-channel blocker. No relation with potential risk factors, as described in the literature could be found. To overcome the differences in definition of accelerated coronary artery disease, serial quantitative coronary angiography, should be applied in large series to achieve better understanding of the pathophysiology of accelerated coronary artery disease, and to investigate the influence of different treatment protocols on this process.

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CHAPTER 4

SHORT- AND LONG-TERM QUANTITATIVE ANGIOGRAPHIC FOLLOW-UP AFTER CARDIAC TRANSPLANTATION.

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INTRODUCTION

Survival after cardiac transplantation has improved over the last years with a 1-year survival rate of greater than 80% in most transplant centers[1]. At present, one of the most limiting factors for medium and long-term survival is the process of graft vasculopathy, an accelerated and diffuse form of coronary atherosclerosis[2]. It accounts for approximately 60% of all retransplantation procedures[3].

The exact cause of this disease is thought to be immunologic, however direct proof is lacking. A major problem comparing the incidences at individual centers are the different definitions and methods to assess this diffuse vasculopathy. Therefore quantitative coronary angiography is thought to be a more sensitive and objective method for assessment, which offers potential for better understanding the pathophysiology and for investigating the influence of different treatment strategies on this process.

In the beginning of this century the first experimental work on cardiac transplantation was performed by Carrel and Guthrie. They transplanted the heart of one dog into the neck of another dog: the first heterotopic transplantation. The first successful clinical cardiac transplantation was performed by Barnard in 1967. Following this promising experience a large number of transplants were performed throughout the world. However, the immediate results after transplantation did not meet the expectations, because of acute allograft rejection and infection and consequently only a few centers continued with the development of this technique. During the 1970's indications and contraindications for cardiac transplantation were defined. Treatment of rejection was greatly enhanced by the use of rabbit anti-thymocyte-globulin. The detection and surveillance of rejection were facilitated by the introduction of transvenous right ventricular biopsy and the development of a grading system for the histologic findings[4-6].

One year survival increased from 22% in 1968 to 65% in 1978[7]. The greatest step forward however, has been made in the early 1980's by the introduction of cyclosporin A for immunosuppression[8].

As a result of the use of cyclosporin, cardiac transplantation has been developed to a generally accepted treatment for end-stage heart disease. According to the ninth report of the Registry of the International Society for Heart and Lung Transplantation, up to december 1991 over 19,000 heart transplantations have been performed and the one-year survival rate has increased to approximately 80%[1].

Accelerated coronary artery disease

With the improvement of short term survival, it became clear that the process of accelerated coronary artery disease is one of the important factors limiting the long term survival of cardiac transplant recipients[2]. Due to the lack of innervation of the cardiac allograft, angina pectoris is usually absent, and electrocardiographic signs of myocardial infarction, congestive heart failure or sudden death may be the first signs of graft coronary

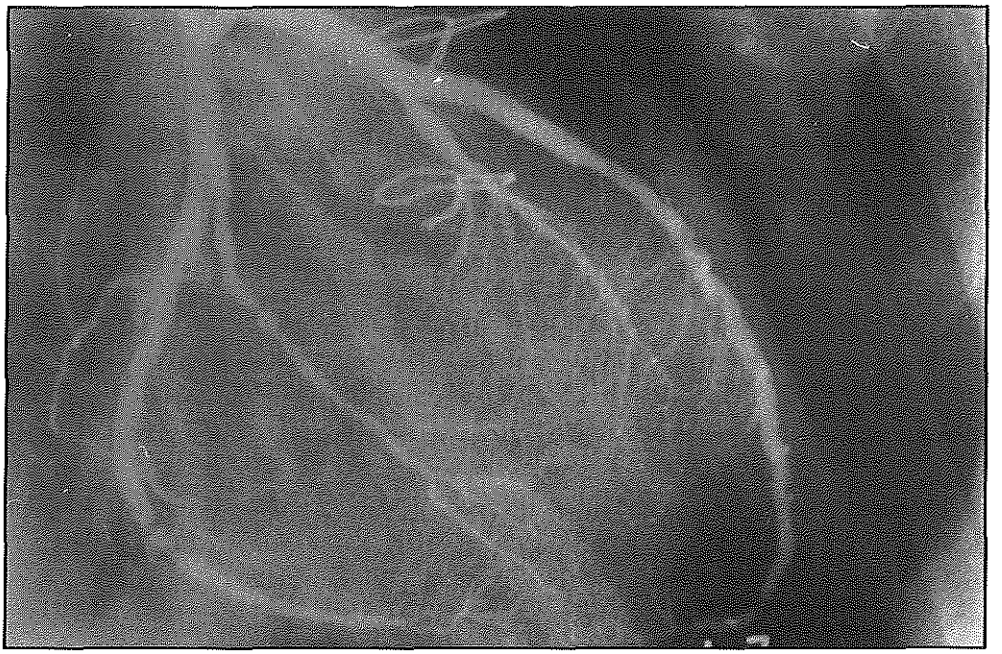

Figure 1. Example of a coronary angiogram in right anterior oblique view of a 60 year old male cardiac transplant recipient, 5 year after transplantation, showing the typical diffuse wall-irregularities and local stenoses.

arteriosclerosis[9].

The histologic findings of graft coronary arteries after human transplantation were first described by Bieber et al.[10] and confirmed by others[11-14]. The earliest change consists of concentric fibrosis and smooth muscle cell proliferation with collagen accumulation creating diffuse intimal thickening. This is seen as early as one week after transplantation. Subsequently, these lesions may progress to diffuse obliterative lesions creating longitudinal narrowing and distal pruning. The lesions are generally present in the large epicardial vessels as well as in the small intramyocardial branches. Therefore, because of the diffuse nature of the process, standard revascularization techniques such as bypass grafting and percutaneous transluminal coronary angioplasty are of limited value.

To detect the onset and progression of coronary disease in an early phase, coronary angiography is performed annually in most transplant centers. Gao et al.[15] first described the specific angiographic morphology of the lesions found after transplantation by dividing them into 3 categories: type A, discrete or short tubular stenosis in the proximal, middle or distal segments of major coronary arteries or their branches, type B; diffuse concentric luminal narrowing in the middle to distal segment branches; and type C, diffusely narrowed irregular distal branches that are squared of and end abruptly, the latter two groups both unique to the post-transplant patients (Figure 1). Despite this clear categorization, the

reported incidences of visually detectable coronary artery disease vary considerably, ranging from 2 to 34 % at 1 year, and ranging from 50 to 73 % at 6 years after transplantation[15-23]. Thus, visual interpretation of coronary angiography has limitations for both clinical and research purposes: The results of different transplant centers are not comparable, and the influence of different treatment strategies cannot be assessed.

Quantitative coronary angiography

Quantitative coronary angiography has the advantage of being more accurate and reproducible for the assessment of coronary artery disease. De Feyter et al.[24] discussed the value of quantitative coronary angiography in clinical trials concerning progression or regression of coronary artery disease. A specific tool to assess the progression of *diffuse* coronary artery disease is the mean segment width measured in millimeters. It is also the single measurement to detect both *diffuse* and *focal* atherosclerosis. Therefore mean segment width should be the appropriate measurement to be used in cardiac transplant recipients. The description of progression and regression of allograft coronary artery disease should include 2 parameters: Firstly, a description based on the coronary status of an individual patient: *the patient global score*, which is defined as the average of the mean width of all analyzable segments of the coronary tree (including those with lesions) per patient. The patient global score change is defined as the average change in the mean width of the segments (Table 1). Secondly, a description based on all the segments measured in a study: *the segment global score*, which is defined as the mean of the coronary segment widths of all these measured segments. Segment global score change is defined as the average change of all segments (Table 1).

O'Neill et al.[23] was the first to report a significant reduction of coronary luminal diameter, using quantitative coronary angiography in cardiac transplant recipients. Mean coronary diameter of the left main coronary artery decreased from 5.4 ± 0.9 mm at 1 year to 4.7 ± 0.8 mm at 3 years after transplantation in 20 patients having serial coronary angiography. The coronary luminal diameters of the proximal and mid left epicardial artery segments also showed a significant decrease. However, distal epicardial segments did not change significantly. Quantitative analysis was performed by two observers. In this study vessel borders were manually traced in end diastolic frames and measured using digital calipers. The patient global score was not presented. Furthermore no relation was found between these changes and potential risk factors for development of accelerated coronary artery disease.

Stanford University reported the use of quantitative coronary angiography by automated computerized edge detection in a study describing the changes in coronary luminal diameter in the first year after transplantation[26]. In a group of 25 patients mean coronary diameter decreased from 2.44 ± 0.26 mm at an average of 5.1 weeks after transplantation to 2.21 ± 0.34 mm at 1 year follow-up ($p < 0.001$). Although absolute changes were less in smaller arteries, there was no significant difference between large (> 2.9 mm), medium (2.0 - 2.9

Table 1. Example of the calculation of patient global score and patient global score change in a cardiac transplant recipient.

SEGMENT NUMBER *	BASELINE † (mm)	FOLLOW-UP ‡ (mm)	CHANGE (mm)
1	3.75	3.64	-0.11
2	3.65	3.28	-0.37
3	3.29	3.23	-0.06
5	4.00	3.47	-0.53
6	4.44	3.99	-0.45
7	3.19	3.23	0.04
8	2.40	2.28	-0.12
11	2.70	2.57	-0.13
13	2.24	2.13	-0.11
patient global score	3.30 ± 0.74	3.09 ± 0.63	-0.20 ± 0.20

* Segment number according to the American Heart Association Classification[25]

† Mean segment width at baseline coronary angiography

‡ Mean segment width at follow-up coronary angiography

mm) and small (< 2.0 mm) vessels with regard to percentage change (-9.4, -10.9 and -6.4%, respectively). In this study also no relation with potential risk factors for transplant coronary artery disease could be found. Mills et al.[27] recently reported coronary artery segment measurements in 18 patients from 1 to 3 years after cardiac transplantation, using cine-videodensitometry. All angiograms were visually interpreted as "normal" by an experienced investigator, using side-by-side projectors. No loss of distal branches was seen. Using quantitative analysis all segments except the proximal left anterior descending segment showed a significant decrease from the first to the third postoperative year (range -0.19 to -0.48 mm). They concluded that graft arteriopathy is ubiquitous in heart transplant recipients, however no new insights on the pathogenesis of graft arteriopathy were given.

THE THORAXCENTER EXPERIENCE

Visual analysis of the coronary angiograms of all patients who underwent a cardiac transplantation between June 1984 and May 1990 at the Thoraxcenter, made as part of their annual follow-up protocol, revealed a prevalence of abnormalities of the epicardial vessels in this patient group increasing from 34% at one year to 79% after 5 years. A very low threshold for assessment of visual coronary artery disease was used by two observers

experienced in the reading of post transplant coronary angiograms. However, the prevalence of anatomical significant lesions (>50% stenosis in the epicardial branches or abrupt ending/proximal occlusion of tertiary branches) was only 1% at one year and 11% after 5 years[20].

In order to provide a more accurate and objective evaluation of the development of coronary artery disease, a study was initiated at the Thoraxcenter to describe the changes in coronary luminal diameter of the epicardial branches using serial quantitative coronary angiography. Furthermore, these changes were correlated with potential risk factors, as described in the literature.

PATIENTS

All cardiac transplant recipients who underwent a coronary angiography, as part of their annual follow-up protocol, between September 1989 and September 1990 were included in this study. Five subgroups could be identified: The first group consisted of 30 patients undergoing early angiography within one month after transplantation. The second, third, fourth and fifth groups consisted of 28, 21, 23 and 9 patients having angiography 1, 2, 3, and 4 years after cardiac transplantation respectively (Tables 2 and 3). In the subsequent year all patients underwent follow-up coronary angiography, thus achieving serial one year follow-up coronary angiography.

Six patients were excluded from this study: 1 patient died before follow-up angiography, in 2 patients follow-up angiography was not performed because of severe kidney failure, and in 3 patients follow-up angiography was postponed because of either infective disease or rejection.

Early prophylactic immunosuppressive therapy consisted of either polyclonal anti-T cell antibodies (Horse anti-thymocyte globulin, anti-thymocyte IgG2, Lymphoglobulin, Institute Merieux) or monoclonal anti-T cell therapy (OKT3, Ortho Pharmaceutical, Raritan, N.J.)[28]. Maintenance immunosuppression consisted of low dose steroids and cyclosporin. Azathioprine was added to this regimen in 16 patients because of recurrent rejection, detected and monitored by endomyocardial biopsies. The histologic findings of these biopsies were graded according to Billinghams criteria[5] until December 1990, and by the guidelines of the International Society for Heart and Lung Transplantation[6] from January 1991. In cases of moderate rejection with definite myocyte necrosis (Billingham grade 2) or grade 3A according to latter criteria, additional treatment was instituted, consisting of pulsed high dose steroids and poly- or monoclonal anti-T cell therapy.

In Cytomegalo virus (CMV) seronegative recipients, anti-CMV hyperimmunoglobulin (Cytotect, Pharma GmbH, Dreiech, Germany) was administered during the first 10 weeks after transplantation[29]. CMV infection was defined as any rise in serum IgM, isolation of CMV from urine throat or blood, or evidence of CMV immediate early antigen. CMV disease was defined as infection accompanied with fever >38°C for at least 2 days, and either

Table 2. Clinical data

	group 0-1yr	group 1-2yr	group 2-3yr	group 3-4yr	group 4-5yr
Number of patients	30	28	21	23	9
Number of segments	249	227	173	186	75
Immunosuppressive regimen *					
- Cyclosporine and prednisone (pts)	25	25	17	20	8
- Triple therapy (pts)	5	3	4	3	1
Recipient age (yr) †	45 ± 12	45 ± 11	47 ± 7	44 ± 10	36 ± 13
Donor age (yr)	26 ± 8	25 ± 8	23 ± 7	24 ± 8	21 ± 8
Gender (F/M)	2 / 28	4 / 24	2 / 19	2 / 21	1 / 8
Gender mismatch (pts)	9	9	7	10	5
HLA-mismatch					
A	1.4 ± 0.6	1.3 ± 0.7	1.3 ± 0.6	1.3 ± 0.6	1.2 ± 0.7
B	1.6 ± 0.5	1.6 ± 0.6	1.5 ± 0.5	1.5 ± 0.7	1.7 ± 0.5
DR	1.5 ± 0.7	1.4 ± 0.7	1.5 ± 0.6	1.2 ± 0.5	1.3 ± 0.9
Rejection episodes between angiography median (range)	1 (0-5)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Cholesterol (mmol/l) ‡	7.4 ± 1.7	7.0 ± 1.6	7.9 ± 1.5	7.3 ± 1.5	7.6 ± 1.2
Triglyceride (mmol/l) ‡	2.3 ± 0.8	2.2 ± 0.8	2.4 ± 0.9	2.4 ± 1.3	2.5 ± 1.0
HDL-cholesterol (mmol/l) ‡	1.5 ± 0.5	1.3 ± 0.4	1.5 ± 0.5	1.3 ± 0.4	1.5 ± 0.3
Donor heart ischemia (min)	154 ± 31	169 ± 38	151 ± 38	159 ± 45	190 ± 44
Smoking (Yes/No) *	7 / 23	6 / 22	1 / 20	5 / 18	2 / 6
Diabetes (pts) *	2	1	1	1	2
CMV infection (pts) *	12	3	3	3	1
CMV disease (pts) *	7	0	0	0	0
Use of nifedipine (pts) §	20	18	14	16	5

F = female; M=male; CMV=cytomegalo virus; HDL=High Density Lipoprotein;
* = during the follow-up period; † = at the time of the transplantation;
‡ = mean during the follow-up period; § = at follow-up catheterization;

leukocytopenia (< 2.5 x 10^9/l) and thrombocytopenia (< 100 x 10^9/l), or symptoms of organ involvement[29].

In patients, who never received a blood transfusion before transplantation, a transfusion was administered pre-operatively[30]. All patients were treated with antiplatelet agents, consisting of either dipyridamole 75 mg tid or aspirin 80 mg daily. Hypertension was preferably treated with nifedipine.

Quantitative coronary angiography

All patients underwent left heart catheterization and selective coronary angiography by the femoral approach. Right heart catheterization with pressure measurements was performed and five endocardial biopsies were obtained, using the percutaneous transjugular approach.

To reduce the influence of dynamic vessel tone, isosorbide-dinitrate (5 mg) was given sublingually before contrast injection. At baseline coronary angiography, standard projections were used and replicated at follow-up.

Off-line quantitative analysis was performed using the computer-assisted Cardiovascular Angiography Analysis System (CAAS), which has been described in detail previously[31,32].

Nine epicardial coronary segments, identified by anatomic landmarks[25], were selected and analyzed in two orthogonal views avoiding foreshortening. Of the right coronary artery the proximal, mid and distal segment were analyzed; of the left coronary artery the main branch and segments 6, 7, 8, 11 and 13 were chosen for analysis.

The results were expressed in a patient global score and a segment score as described before.

Statistical analysis

All data are presented as the mean ± SD. Statistical analysis was performed using the Wilcoxon Matched-pairs Signed-ranks test. Unpaired *t* tests, one-way analysis of variance and logistic regression were used to compare differences in potential risk factors for accelerated coronary artery disease. Statistical significance was defined as a p value of 0.05 or less.

RESULTS

Clinical data

The clinical data of the cardiac transplant recipients in the different subgroups are described in Table 2. The number of rejections and Cytomegalo-virus infections was, as expected, higher in the first year after transplantation in comparison with the other periods.

Table 3. Patient global score

	number of patients	patient global score (mm) baseline	follow-up	patient global score change mm	%	
group 0-1yr	30	3.39 ± 0.34	3.35 ± 0.34	-0.05	-1.5	NS
group 1-2yr	28	3.18 ± 0.37	3.19 ± 0.39	0.01	0.3	NS
group 2-3yr	21	3.34 ± 0.40	3.26 ± 0.38	-0.08	-2.3	NS
group 3-4yr	23	3.17 ± 0.27	3.16 ± 0.41	-0.01	-0.3	NS
group 4-5yr	9	3.23 ± 0.51	3.23 ± 0.46	0.00	0.0	NS

Table 4. Segment global score

	number of segments	segment global score(mm) baseline	follow-up	segment global score change mm	
group 0-1yr	249	3.40 ± 0.82	3.36 ± 0.83	-0.05	p=0.007
group 1-2yr	227	3.17 ± 0.85	3.17 ± 0.82	0.00	NS
group 2-3yr	173	3.35 ± 0.87	3.28 ± 0.81	-0.07	p=0.005
group 3-4yr	186	3.18 ± 0.78	3.19 ± 0.83	0.00	NS
group 4-5yr	75	3.23 ± 0.90	3.24 ± 0.86	0.00	NS

There was no significant difference between groups for mean recipient- and donor age, gender mismatch, HLA-A+B or -DR mismatch, total serum cholesterol, triglyceride, high-density lipoprotein cholesterol levels, and the other described risk factors for graft atherosclerosis. The only risk factor we could identify was the presence of coronary artery disease prior to transplantation. In the first subgroup of patients, a significantly larger patient global score change was found in patients with this disease (N=14), than in patients with other indications for transplantation (-0.13 ± 0.17 mm versus 0.03 ± 0.20 mm, $p<0.05$). The changes in minimal luminal diameter were -0.15 ± 0.16 mm and 0.04 ± 0.23 mm ($p=0.01$), respectively.

Quantitative angiography

In the 5 different subgroups of patients 249, 227, 173, 186 and 75 segments were analyzed respectively. Only one patient in the last subgroup showed a significant narrowing (> 50%) of the left anterior descending artery.

Patient global score

The results are outlined in Table 3. The largest decrease in patient global score occurred in the first and third postoperative year. These changes didn't prove to be significant.

Segment global score

In Table 4 the results of segment global score calculations are described. It can be appreciated that, according to these calculations, the largest changes also occurred in the first and third year after transplantation. This decrease was 0.05 and 0.07 mm respectively, and proved to be statistically significant.

DISCUSSION

In view of the described studies, the changes in this group of patients were small, both for patient global score and segment global score, ranging from -0.08 to 0.01 mm in the different yearly postoperative periods.

The exact cause of accelerated coronary artery disease is not yet elucidated. However, it is widely believed that immune mediated phenomena play an important role in the pathogenesis of this disease, because of it's diffuse nature, involving the entire length of the coronary artery tree with sparing of the native vessels, and it's development in patients of all ages. The "response to injury mechanism", due to damage to the endothelium during graft rejection, is widely believed to be the most basic etiologic factor, although both in this study as in others this hypothesis could not be confirmed[19,20,26,33,34]. A number of potential additional risk factors such as the presence of Cytomegalo virus infection[35-37], the presence of B-cell antibodies[38] or anti-HLA antibodies[39], the immunosuppressive regimen[40], plasma triglycerides[41], diabetes mellitus[42], donor age[37,39,42] could also not be confirmed by other studies. Please note that most of these studies are based on visual, and thus subjective, interpretation of coronary angiography. Our study shows that quantitative coronary angiography offers potential for a better and more objective description of the changes in the coronary artery tree and for investigating the factors that influence the development of the disease.

One of the first studies comparing the effect of different treatment strategies using an objective edge detection system was described recently in a report of Stanford[43]. In a placebo controlled study in 106 patients, the beneficial effect of the calcium-antagonist diltiazem to inhibit early post-transplant coronary luminal narrowing was described. In the 54 patients, who received placebo, segment global score decreased significantly from 2.41 ± 0.27 mm at baseline (median, 19 days after transplantation) to 2.22 ± 0.26 mm at 2 year follow-up. In the same period, segment global score of the patients who received the calcium-antagonist, changed from 2.32 ± 0.22 mm to 2.36 ± 0.22 mm, a not statistically significant change. In

our study most transplant recipients also received a calcium-antagonist (nifedipine), but no relation was found between the use of nifedipine and the development of post transplant coronary artery disease.

An increase in coronary artery dimension was first reported by Von Scheidt et al.[44] in 5 out of a group of 68 patients after transplantation (7.3%). No causal relation with clinical data could be determined. However, this process was assessed by visual interpretation of coronary angiography and has not been confirmed by quantitative methods.

CONCLUSION

The coronary luminal diameter, expressed in a patient global score and a segmental score, decreased in the first and third year after cardiac transplantation. However, we observed only minimal changes in comparison with other post transplant studies[23,26,27]. Furthermore, these changes seemed to be clinically insignificant. Pre-transplant coronary artery disease of the recipient was identified as a risk factor for the development of transplant coronary atherosclerosis in the first postoperative year. No relation was found with other described potential risk factors.

To overcome the differences in definition of accelerated coronary artery disease, serial quantitative coronary angiography, if used in larger studies, can be an objective method to assess the incidence of this disease. Therefore, it offers potential for better understanding the pathophysiology of accelerated coronary artery disease, and for investigating the influence of different treatment protocols on this process.

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CHAPTER 5

EPICARDIAL LUMINAL CHANGES DURING SIX YEARS AFTER CARDIAC TRANSPLANTATION: SERIAL QUANTITATIVE CORONARY ANGIOGRAPHY.

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ABSTRACT

Reported incidences and risk factors of accelerated diffuse coronary disease after cardiac transplantation (HTX) vary considerably. To further elucidate the development of transplant coronary artery disease and the risk factors for such development, a 2 year follow-up study was performed in 104 patients, starting between 1 month and 4 years after transplantation.

Initial quantitative coronary angiography was performed in 30, 26, 20, 20 and 8 patients, respectively at 1 month and 1, 2, 3 and 4 years after the operation, and replicated in each patient 1 and 2 years later. Maintenance immunosuppression consisted of low dose steroids and cyclosporine in 90 patients; in 14 patients azathioprine was added. Hypertension was preferably treated with nifedipine. Cytomegalo virus (CMV) seronegative patients received anti-CMV hyperimmunoglobulin after transplantation.

Changes in the epicardial coronary arteries were evaluated by quantitative angiography using an automated edge detection system at different intervals after transplantation. The average change in mean lumen diameter in the first year after transplantation was -0.05 mm. In the following years the changes were -0.01, -0.03, +0.01, +0.05 and +0.12 mm respectively. None of these changes proved to be statistically significant on a patient based score. On a per patient basis, 33 patients showed progression of focal atherosclerosis, 39 patients showed regression and 32 had either a mixed response or no change during 2 year follow-up. Although the development of coronary disease is thought to be of immunologic origin, no relation with rejection episodes could be established. No other risk factors for development of coronary disease could be identified.

Conclusion: Serial quantitative coronary angiography demonstrated no significant reduction in mean diameter of epicardial coronary arteries during 6 years follow-up after heart transplantation, and no risk factors for changes in individual patients could be identified. Larger studies with longer follow-up are warranted in patients receiving different medical regimens after transplantation.

INTRODUCTION

Long term survival of cardiac transplant recipients is limited by the development of accelerated coronary artery disease. This diffuse and progressive type of coronary artery disease accounts for the majority of deaths after cardiac transplantation, and for 60% of the retransplantation procedures[1-3]. The exact cause of this disease has not yet been elucidated. Damage to the endothelium during graft rejection, is thought to be an etiologic factor (response to injury mechanism), although most studies could not confirm a relation between recognized rejection episodes and the development of transplant coronary artery disease[4-7]. Conflicting data have been presented concerning the association of additional risk factors and the presence of accelerated coronary artery disease. Incriminated factors include Cytomegalo virus infections[8-10], the presence of B-cell antibodies[11] or anti-HLA antibodies[12],

the employed immunosuppressive regimen[4,8,13,14], elevated plasma triglycerides[13,16], diabetes mellitus[10] and higher donor age[8,10,16].

Most studies were based on visual interpretation of the coronary angiograms. The assessment of the disease was thus subjective and not comparable between centers. Quantitative coronary angiography offers a more objective method to monitor luminal changes, and has been applied in a few studies in small patient groups, during a limited follow-up period[16-19]. Therefore, we performed quantitative analysis of serial coronary angiograms in a larger series of patients, to describe the changes in coronary luminal diameter at different postoperative stages up to 5 years after transplantation and to correlate these changes with previously identified risk factors. Data from patients at different intervals after transplantation were combined to obtain a complete pattern of angiographic changes during the first 6 years after transplantation.

PATIENTS AND METHODS

Patients

From October 1989 until October 1990 113 consecutive cardiac transplant recipients underwent coronary angiography at different postoperative intervals, as part of an annual follow-up protocol[20]. All patients had undergone orthotopic heart transplantation at the Thoraxcenter of the University Hospital Rotterdam, Dijkzigt. Five subgroups were identified as described in table 1. Thirty patients (group A) underwent coronary angiography within one month after transplantation. In the other groups the baseline coronary angiogram for this study was made at 1, 2, 3 and 4 years after transplantation respectively. All patients were asked to return for coronary angiography in the subsequent two years, thus achieving serial one and two year follow-up coronary angiography. These data were complete in 104

Table 1. Distribution of the patients in the different groups, according to the time of the angiography.

	Serial quantitative angiography						
Group	1 month - 1 year	1 - 2 years	2 - 3 years	3 - 4 years	4 - 5 years	5 - 6 years	Lost to 2 year follow-up
A	30	30					-
B		30	26				4
C			21	20			1
D				23	20		3
E					9	8	1

patients. Each patient served as its own control for the measurement of changes in coronary diameter. Thus five patient cohorts were studied with observations at different intervals after transplantation: 0-2, 1-3, 2-4, 3-5 and 4-6 years respectively (Table 1).

Immunosuppression and other prophylactic pre- and peri-operative measures

Induction immunosuppressive therapy consisted of either polyclonal anti-T cell antibodies (Horse anti-thymocyte globulin, Lymphoglobulin, Institute Merieux, Lyon, France) or monoclonal anti-T cell therapy (OKT3, Ortho Pharmaceutical, Raritan N.J., U.S.A.)[21]. Maintenance immunosuppression consisted of low dose steroids and cyclosporine. Azathioprine was added to this regimen in 14 patients with frequent rejection episodes. Endomyocardial biopsies were used to detect and monitor acute rejection. The histologic findings were graded according to Billinghams criteria[22] until December 1990, and by the guidelines of the International Society for Heart and Lung Transplantation[23] from January 1991. In cases of moderate rejection with definite myocyte necrosis[22] or grade 3A[23] additional treatment was instituted, consisting of pulsed high dose steroids or poly- or monoclonal anti-T cell therapy.

Cytomegalo virus (CMV) seronegative recipients received anti-CMV immunoglobulin (Cytotect, Biotest Pharma GmbH, Frankfurt, Germany) during the first 10 weeks after transplantation[24]. CMV infection was defined as any rise in serum immunoglobulin M, isolation of CMV from urine throat or blood, or evidence of CMV immediate early antigen. CMV disease was defined as infection accompanied with fever of more than 38°C for at least 2 days, and either leukocytopenia (less than 2.5 x 10^9/l), thrombocytopenia (less than 100 x 10^9/l), or symptoms of organ involvement[25].

In patients who never had received a blood transfusion before transplantation, a transfusion was administered during the screening period. Anti-leukocyte-antibodies were measured preoperatively to determine the necessity of preoperative cross matching with donor blood, which was performed if the percentage of antibodies, measured with a standard leucocyte panel, exceeded 5%[26].

All patients received antiplatelet therapy, consisting of either dipyridamole 75 mg three times daily or aspirin 80 mg/day. Hypertension was preferably treated with the calcium-channel blocker nifedipine in a slow release preparation.

Quantitative coronary angiography

All patients underwent left heart catheterization and selective coronary angiography by the femoral approach at three consecutive years. The projections used at baseline coronary angiography were documented and replicated at follow-up. To reduce the influence of dynamic vessel tone, isosorbide dinitrate (5 mg) was given sublingually before the first coronary contrast injection.

Off-line quantitative analysis was performed using the computer-assisted Cardiovascular

Angiography Analysis System (CAAS)[27,28]. After correction for pincushion distortion caused by the image intensifier, the mean and minimal coronary width of each segment were calculated in millimeters. The catheter tip or shaft, filmed without contrast, was used for calibration.

Nine epicardial coronary segments, identified by anatomic landmarks, as defined by the American Heart Association[29], were selected: the proximal, mid and distal segments of the right coronary artery, the main branch and the proximal, mid and distal segments of the anterior descending branch as well as the proximal and mid segment of the circumflex branch of the left coronary artery. All segments were analyzed in two views, preferably orthogonal or separated by at least 30°, avoiding foreshortening.

Two parameters were used in this study to describe the progression or regression of coronary artery disease[30]. The patient global score provided a description of *diffuse* coronary artery disease, and was defined as the per-patient average of the mean widths of the 9 measured segments. The threshold used to assess significant change in patient global score was 0.20 mm, which is two times the standard deviation of the measurement of mean width in non-obstructed segments[27]. In addition, the minimal luminal diameter per segment (MLD) was used to assess development of *focal* disease. A change of 0.40 mm was considered to be clinically significant[31]. Table 2 summarizes the definitions of progression or regression of focal coronary artery disease[32].

Statistical methods

All data are presented as the mean ± SD. Statistical analysis of the changes in patient global score was performed using the Wilcoxon matched-pairs signed-rank test. One-way analysis of variance (ANOVA) and linear regression analysis were used to assess the association of potential risk factors with accelerated coronary artery disease. A chi square test was performed to calculate the risk for development of transplant coronay artery disease for each potential risk factor. Continuous variables were dichotomized by cutpoints on the

Table 2. Definition of changes in focal coronary artery disease

Progression:	At least one segment progressed and none regressed
Mixed:	One segment regressed and at least one progressed
Stable:	No segments progressed or regressed
Regression:	At least one segment regressed and none progressed

basis of the median value. Multivariate analysis was performed using logistic regression to identify variables independently correlated with the occurrence of accelerated coronary artery disease. All statistical analyses were carried out with a commercial statistical package (BMDP statistical Software Package). P values (two tailed) were considered significant only when less than 0.05.

RESULTS

Clinical data

Complete data at baseline, one and two year follow-up, were obtained in 104 patients (Table 1). Nine patients were lost to 2 year follow-up: one patient died; in 2 patients the final angiogram was not made because of severe renal failure; one patient had a cerebro vascular accident during the second angiogram and therefore the third follow-up angiogram was cancelled, and in 5 patients the final angiogram was cancelled because of other medical and organisational reasons.

The baseline characteristics of the eligible patients in the different subgroups are shown in table 3. Of these 104 patients, 95 were male and 9 female. Recipient ages ranged from 13 to 61 years (mean 44 ± 7 year). As expected, the numbers of rejection episodes and cytomegalo-virus infections were higher in the first year after transplantation than in the other periods. There was no difference between the groups with respect to mean values of recipient- and donor age, gender mismatch, HLA-A+B or HLA-DR mismatch, total serum cholesterol, triglyceride, high-density lipoprotein cholesterol levels and the other described risk factors for graft atherosclerosis.

Angiography

At baseline angiography, no significant narrowing (more than 50% by visual assessment) was observed. By visual assessment 27% of the patients showed evidence of diffuse coronary artery disease in the large epicardial vessels. The smaller branches were affected in 54% of these patients. At 1 year follow-up, the angiogram of 1 patient in subgroup E showed a significant narrowing of the left anterior descending artery, which was treated by balloon angioplasty. At 2 year follow-up, 4 patients had developed a ≥ 50% stenosis in one segment of the coronary tree.

Quantitative coronary angiography

In the 5 different subgroups of patients 827 segments were analyzed. The mean segment diameter was 3.26 ± 0.82 mm at baseline angiography. In table 4 the changes in patient global score in the different postoperative stages are shown. These changes were small, and

Table 3. Baseline characteristics

	group A	group B	group C	group D	group E
Angiographic follow-up *	0 - 2	1 - 3	2 - 4	3 - 5	4 - 6
Number of patients	30	26	20	20	8
Recipient age (yr) †	45 ± 12	45 ± 11	47 ± 7	44 ± 10	36 ± 13
Donor age (yr)	26 ± 8	5 ± 8	23 ± 7	24 ± 8	21 ± 8
Gender (F/M)	2 / 28	3 / 23	1 / 19	2 / 18	1 / 7
Gender mismatch (pts)	9	7	6	9	4
HLA-mismatch					
A	1.4 ± 0.6	1.3 ± 0.7	1.3 ± 0.6	1.3 ± 0.6	1.2 ± 0.7
B	1.6 ± 0.5	1.6 ± 0.6	1.5 ± 0.5	1.5 ± 0.7	1.7 ± 0.5
DR	1.5 ± 0.7	1.4 ± 0.7	1.5 ± 0.6	1.2 ± 0.5	1.3 ± 0.9
Immunosuppressive regimen ‡					
- Cyclosporine and prednisone (pts)	25	24	16	18	7
- Triple therapy (pts)	5	2	4	2	1
Donor heart ischemia (min)	154 ± 31	168 ± 37	151 ± 38	159 ± 44	183 ± 42
Indication for HTX (ischemic/other)	14 / 16	12 / 14	11 / 9	11 / 9	3 / 5
Rejection episodes between initial and 1 year	1 (0-5)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
1 and 2 year, median (range)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-0)
Smoking ‡ (Yes / No)	7 / 23	6 / 20	1 / 19	5 / 15	2 / 6
CMV serology (pos/neg)	17 / 13	17 / 13	11 / 10	12 / 11	3 / 6
CMV infection (pts) ‡	12	3	3	3	1
CMV disease (pts) ‡	7	0	0	0	0
Cholesterol (mmol/l) $	7.4 ± 1.7	7.0 ± 1.6	8.0 ± 1.5	7.3 ± 1.5	7.6 ± 1.2
Triglyceride (mmol/l) $	2.3 ± 0.8	2.3 ± 0.8	2.4 ± 0.9	2.4 ± 1.3	2.5 ± 1.0
HDL-cholesterol (mmol/l) $	1.5 ± 0.5	1.4 ± 0.4	1.5 ± 0.5	1.3 ± 0.4	1.5 ± 0.3
Diabetes (pts) ‡	2	1	1	1	2
Use of calcium antagonist (pts)	26	22	15	16	6

F=female; M=male; CMV=Cytomegalo virus; HDL=High Density Lipoproteins; *=study period related to the post-transplant survival;
†=at transplantation; ‡=during the study period; $=mean during the follow-up period

Table 4. Changes in patient global score in the different post-transplant intervals (mm)

		Follow-up period					
Group	n	1 month - 1 year	1 - 2 years	2 - 3 years	3 - 4 years	4 - 5 years	5 - 6 years
A	30	-0.05 ± 0.20	-0.03 ± 0.21				
B	26		0.01 ± 0.15	0.00 ± 0.14			
C	20			-0.08 ± 0.18	0.02± 0.16		
D	20				-0.01± 0.21	0.08± 0.14	
E	8					0.00 ± 0.20	0.12 ± 0.27
Patient global score		-0.05 ± 0.20	-0.01 ± 0.18	-0.03 ± 0.16	0.01 ± 0.19	0.05 ± 0.16	0.12 ± 0.27

n=number of patients in each group with three complete angiograms (see table 1).

statistically not significant. There was a trend towards narrowing of mean segment diameter in the first 3 years, and widening in subsequent years (Figure 1). However, these changes were not statistically significant.

Changes in patient global score in 50 patients with and 54 without visually detected progression of transplant coronary artery disease, according to the criteria as described by the group of Stanford[15] were equally distributed (Figure 2).

Progression of focal coronary artery disease was found in 33 patients. Both progression in some and regression in other segments was observed in 19 patients, while no such changes were apparent in 13 patients. Thirty-nine patients showed regression of focal coronary artery disease.

Risk factors

By univariate analysis, the incidence of diffuse transplant coronary artery disease appeared lower in patients who continued smoking after transplantation (p=0.02) (Table 5). After correction for co-variates using multiple logistic regression analysis, not-smoking was retained in the model as an independent predictor of the occurence of *diffuse* accelerated coronary artery disease.

The described risk factors for diffuse coronary artery disease were also included in a logistic regression model to assess the likelihood of the occurrence of *focal* coronary artery disease. Both not-smoking and the occurence of rejection between the baseline and two year follow-up angiogram were retained in the model as an independent predictor of the occurrence of focal coronary artery disease (Odds ratio smoking 0.33; 95% CI 0.08-1.15 and rejection 3.0; 95% CI 1.10-8.19, respectively).

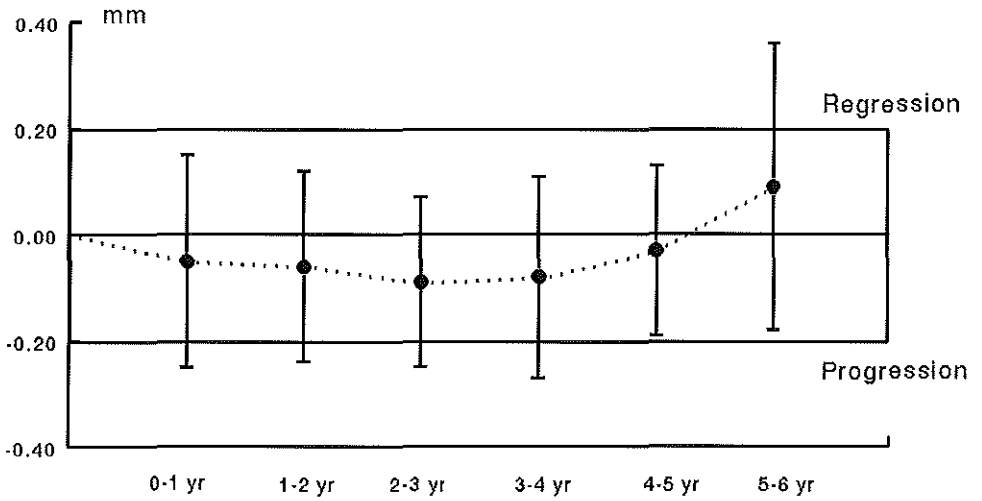

Figure 1. Changes in patient global score at different post-transplant intervals. Changes within each year have been added to changes in previous years to demonstrate the 6 year trend in patient global scores. Mean change and standard deviation are shown. The threshold used to assess significant change in patient global score (0.20 mm) is drawn in the figure.

DISCUSSION

In contrast with earlier reports[16-18], serial quantitative coronary angiography revealed no significant reduction of mean diameter of epicardial coronary arteries up to 6 years after transplantation. Nevertheless, progression of focal coronary artery disease occurred in 32% of the patients. The role of previously identified risk factors for the development of transplant coronary artery disease could not be confirmed.

In this study the severity of accelerated diffuse coronary artery disease was less extensive in comparison with other studies. In particular, other groups reported larger changes in coronary luminal diameter, both in the first year after transplantation (9.2% decrease of mean coronary diameter, corresponding to 0.22 mm)[16], and between the first and third year after transplantation (7% decrease of mean coronary diameter = 0.20 mm)[17]. Yet, our findings were in agreement with an earlier report from the Thoraxcenter[20] in which the incidence of abnormalities in primary and secondary epicardial branches, by visual interpretation of the coronary angiograms, increased from 15 to 57% in the first 5 postoperative years in 119 patients who received similar treatment as in the present study. This may have contributed to the excellent five year patient survival rate of 84% (CI 78-90%) at our center.

Table 5. Progression of coronary artery disease and risk factors *

Risk factor	Patient positive for the risk factor: CAD / total	Patient negative for the risk factor: CAD / total	Odds ratio (95% CI)
Age recipient (> 50.2 years)	10 / 52	11 / 52	0.89 (0.31 to 2.36)
Age donor (> 23 years)	12 / 52	9 / 52	1.43 (0.49 to 4.19)
Males	20 / 95	1 / 9	2.13 (0.24 to 48.12)
Gender mismatch	8 / 35	13 / 69	1.28 (0.42 to 3.82)
Number of HLA mismatches (> 4)	6 / 45	15 / 59	0.45 (0.14 to 1.41)
Ischemia of the donorheart (> 160 min)	7 / 44	14 / 60	0.62 (0.20 to 1.87)
Indication for HTX: IHD	8 / 51	13 / 53	0.57 (0.19 to 1.68)
Number of rejection episodes (> 0) †	7 / 28	14 / 76	1.48 (0.46 to 4.62)
Number of early rejection episodes (> 0) ‡	26 / 90	5 / 24	1.83 (0.56 to 6.33)
Smoking	1 / 25	20 / 79	0.12 (0.01 to 0.95)
CMV infections	5 / 27	16 / 77	0.87 (0.24 to 2.94)
CMV disease	1 / 7	20 / 97	0.64 (0.03 to 5.99)
Serum Cholesterol (> 7.55 mmol/l)	10 / 52	11 / 52	0.89 (0.31 to 2.36)
Serum triglyceride (> 2.26 mmol/l)	9 / 50	12 / 54	0.77 (0.26 to 2.22)
Serum HDL-Cholesterol (> 1.29 mmol/l)	13 / 52	8 / 52	1.83 (0.62 to 5.47)
Use of Nifedipine (< 22.8 months)	10 / 52	11 / 52	0.89 (0.31 to 2.36)
Hypertension	9 / 41	12 / 63	1.22 (0.42 to 3.55)

* all 104 patients with 2 year follow-up; †=during the study period; ‡=in the first postoperative year; IHD=Ischemic Heart Disease; CMV=Cytomegalo virus; CAD=Patients with diffuse transplant coronary artery disease, assessed by quantitative coronary angiography (threshold of 0.20 mm)

The small changes in patient global score in this study may be a result of specific characteristics of the postoperative treatment strategy. Most patients received the calcium-channel blocker nifedipine. Other groups already reported the beneficial effect of the use of other calcium-channel blockers in patients with coronary artery disease[33], especially in cardiac transplant recipients[19].

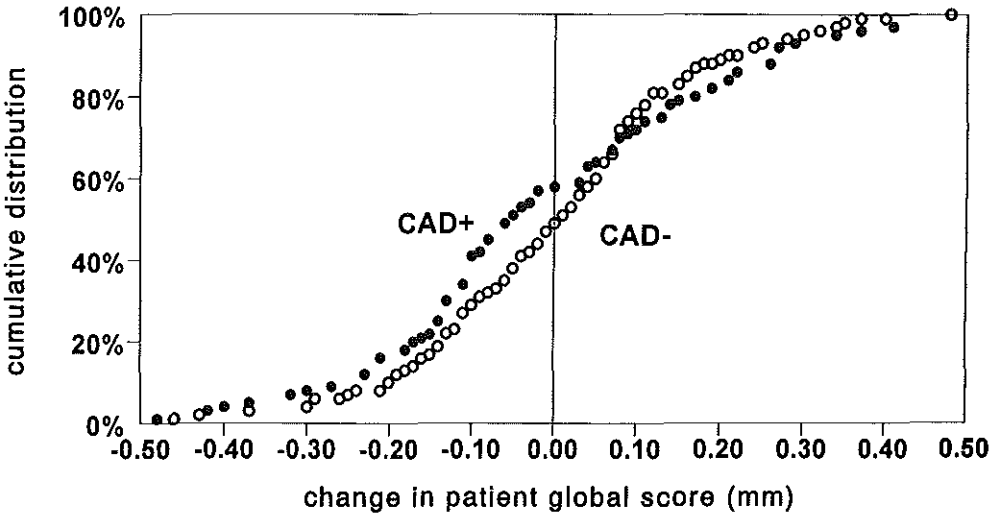

Figure 2. Cumulative curves of changes in patient global score in 50 patients with and 54 without visually assessed progression of diffuse coronary artery disease in epicardial coronary arteries (CAD).

Accelerated coronary artery disease involves the entire length of the coronary artery tree. Histologic findings include concentric fibrosis and smooth muscle cell proliferation with collagen accumulation creating diffuse intimal thickening. Subsequently, these lesions may progress to diffuse obliterative lesions and distal pruning[34-36]. The selective involvement of the coronary tree of the graft, its diffuse nature and its development in patients of all ages, suggest that immune-mediated phenomena may play an important role in the pathogenesis of accelerated coronary artery disease. Therefore, the number of HLA-mismatches and the number of rejections are thought to be risk factors for the development of coronary artery disease[5-7,37-39]. This study was underpowered to confirm this relation. Using the odds ratio's of table 5, sample size calculations show that a larger study in more than 560 patients will be necessary to demonstrate a relation between the number of rejection episodes and the occurrence of diffuse coronary artery disease.

The presence of Cytomegalo virus (CMV) infection as a risk factor was described by Cameron et al[8], and an association between viral infections and atherosclerosis was shown in animal experiments[40]. In the present study such association was not confirmed (Odds ratio 0.99; 95% CI 0.34 to 2.83). Again, the sample size may have been inadequate to detect such variation. It is however, also possible that the profylactic treatment of CMV negative patients with anti-CMV immunoglobulin may have prevented accelerated transplant coronary artery disease in part of our patients. Also the effect of known risk factors for non-transplant coronary artery disease as serum lipid levels and diabetes mellitus [41] could not be confirmed.

Surprisingly, the smoking habits of the patients appeared to influence the occurrence of both focal and diffuse coronary artery disease. This unexpected observation can not be easily explained and may be a chance finding.

Limitations

Quantitative analysis of coronary angiograms was applied to provide an objective description of the specific changes in cardiac transplant recipients. This method has been used in various progression and regression studies[33,42], however the number of publications applying this method in cardiac transplant recipients is limited.

It should be appreciated that the patient global score is an average of 9 measurements. Therefore, within one patient an increase in mean width in one segment can compensate a decrease in another segment resulting in small changes in patient global score. Furthermore, it is possible that the assessment of luminal narrowing by quantitative measurement does not reflect the extent of the intimal hyperplasia[35,36] whenever intimal thickening is balanced by compensatory enlargement or aneurysmal medial dilatation[43,44]. This is illustrated by the marked changes in measurements of focal disease in the present series, with little change in patient global scores.

Intravascular ultrasonography is a more suitable technique to assess the changes in the vessel wall in proximal segments, and can thus be a useful addition to the angiographic measurements. The application of this technique in cardiac transplant recipients has been described in a few recent studies[45-47]. Several studies have shown the prognostic importance of intimal thickening, measured by intravascular ultrasound, with regard to the risk for development of accelerated coronary artery disease[45,46]. Yet, these studies also show conflicting data about the association of risk factors and the presence of transplant coronary artery disease. Therefore further studies in larger patient groups remain necessary.

Conclusions

In this group of patients, the changes in coronary luminal diameter in the first 5 years after cardiac transplantation were small in comparison with other studies applying quantitative coronary angiography. The patient global score did not change significantly at different postoperative intervals. No relation with described risk factors could be found, although smoking seemed to have a beneficial effect. Therefore, no specific recommendations can be made concerning the prevention of accelerated coronary artery disease and further evaluation in a larger patient groups remains necessary to verify these findings.

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CHAPTER 6

ON-LINE VERSUS OFF-LINE ASSESSMENT OF CORONARY FLOW RESERVE.

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INTRODUCTION

Since its introduction, coronary arteriography has been of great importance for the diagnosis and management of patients with ischemic heart disease[1]. Although location and morphology of coronary artery stenoses can sufficiently be assessed by this technique, information about their functional significance cannot always be obtained from the arteriogram alone[2-4].

The concept of coronary flow reserve (CFR) has been developed to describe the relationship between the angiographic severity of coronary artery disease and the resulting reduction or limitation of maximal coronary blood flow in the myocardium[3-7]. Even in the absence of focal atherosclerosis or other flow limiting factors in major epicardial vessels, CFR measurements can be used to evaluate dysfunction of the microcirculation. This is especially relevant in cardiac transplant recipients, where a diffuse arteriopathy can reach a flow limiting significance, without changes in the angiographic appearance[8,9].

Different techniques of coronary flow reserve measurement have been described, and used in clinical practice. In general, these techniques are designed for application during the catheterization procedure, like venous blood flow measurements in the coronary sinus, or the assessment of phasic coronary blood flow velocities using ultrasonic Doppler catheters. The latter technique requires the insertion of hardware in the coronary artery tree, and is extremely "space dependent"[10]. Finally, the radiographic assessment of myocardial perfusion, using contrast media, combines the videodensitometric approach with digital subtraction angiography. Compared with the other invasive techniques to measure CFR, this approach has several advantages. First of all, the digital subtraction technique is more easily applicable during routine catheterization, because no additional catheter or intracoronary device has to be used, which makes this procedure safer, less time consuming and less expensive. Moreover, the analysis of multiple regions of interest (ROI) provides flow information from various subsegments of the coronary artery tree, which is not possible or more time consuming with other invasive techniques.

In the setting of pharmacological or mechanical interventions, where the results have to be estimated directly after the catheterization, these on-line assessment techniques of coronary flow reserve are very useful[5]. Off-line assessment of CFR, on the other hand, allows objective evaluation of multicenter trials in core laboratories, where the selection of ROI's can be carried out by an independent analyst, thereby non-biased by the investigator. However, only on-line assessments of CFR have been validated in animal experiments as well as in a clinical setting using flow calculations from simultaneously intravascular Doppler velocity measurements as a reference[11,12].

ON-LINE AND OFF-LINE ASSESSMENT OF CORONARY FLOW RESERVE

To assess coronary flow reserve by videodensitometry, a fixed amount of non-ionic contrast medium is injected at 37°C into the coronary artery using an ECG-triggered infusion pump. The injection rate of the contrast medium is judged to be adequate when back flow of contrast medium into the aorta occurs. The heart is atrially paced at a level approximately 10 beats/minute above spontaneous heart rate. The X-ray exposure per frame is kept constant by selecting the lock-in mode on the X-ray generator. After intracoronary administration of 2 mg isosorbide dinitrate, basal coronary angiography is performed. Thirty seconds after pharmacologically induced maximal hyperemia, using an intracoronary bolus injection of 12.5 mg papaverine, the angiogram is repeated.

Off-line

Coronary flow reserve measurement with digital subtraction cineangiography from 35 mm cinefilm has been implemented in the Thoraxcenter research version of the Cardiovascular Angiography Analysis System (CAAS)[7]. Thereby, five end-diastolic cineframes are selected from successive cardiac cycles. Logarithmic non-magnified mask-mode background subtraction is applied to the image subset to eliminate non-contrast medium densities, using the last end-diastolic frame prior to contrast administration as a mask. The principle of mask mode subtraction techniques allows the determination of myocardial time-density curves before and during coronary vasodilatation. In the CAAS system at the Thoraxcenter, the appearance-time-contrast density approach, according to Vogel et al.[13], is used. From the sequence of background subtracted images, a contrast arrival time image is automatically determined, using an empirically derived fixed density threshold[7]. Each pixel is labelled with the sequence number of the cardiac cycle in which the pixel intensity level exceeds the threshold, starting from the beginning of the ECG-triggered contrast injection. Arrival time numbers are displayed color coded in the CAAS. In addition to the contrast arrival time image, a density image is computed, with each pixel intensity value being representative for the maximal local contrast medium accumulation.

On corresponding basal and hyperemic end-diastolic frame sequences, identical regions of interest (ROI) are selected in such a way that the epicardial coronary arteries visible on the angiogram, the coronary sinus and the great cardiac vein are excluded from the analysis.

For the calculation of relative blood flow within these regions of interest two parameters are required: the relative regional vascular volume and the mean contrast appearance time. The relative regional vascular volume can be calculated from the maximal density image, the intensity value being proportional to the transradiated amount of contrast medium within the vessel. Therefore, the regional vascular volume for a user-defined ROI is proportional to the mean radiographic density within the ROI. The mean contrast appearance time is derived from the contrast arrival time image.

Regional flow values are quantitatively determined using the following videodensitometric principle: Q=V/T (Q=regional blood flow, V=regional volume and T=mean appearance time).

The coronary flow reserve for one ROI is then calculated as follows:

$$CFR = \frac{Q_h}{Q_b} = \frac{V_h/T_h}{V_b/T_b} = \frac{V_h \times T_b}{V_b \times T_h} = \frac{D_h \times T_b}{D_b \times T_h}$$

(D=mean maximum contrast density; h=hyperemic; b=baseline)

On-line

The on-line method as implemented in the Philips Digital Cardiac Imaging System (DCI) uses the same basic principle as the off-line method. After manual selection of the mask, the computer automatically determines the end-diastolic images. Interaction by the analyst is possible according to visual inspection and the ECG recording. After logarithmical transformation of the data, the mask image is subtracted from the subsequent images. Usually 5 to 8 of these images are necessary to perform the calculations. From these sequences 3 parametric images are constructed:

A contrast arrival time image (T_{arr}), where each pixel is related to the cardiac cycle in which the maximal change in density of contrast is achieved.

A contrast density image (D_{max}), where each pixel intensity value is representative for the maximal value for contrast density in the sequence of subtracted images.

Finally, a parametric flow image is constructed, in which the contrast density is divided by the arrival time.

When parametric images are obtained under baseline and hyperemic conditions, a fourth image, a CFR image, can be obtained by exactly superimposing both images, in which each pixel intensity value is representative for the calculated CFR. This is performed by the computer, but can also be corrected by the analyst, using anatomical landmarks. Gray scaling allows quick inspection of the CFR in different areas of the myocardium.

THE THORAXCENTER EXPERIENCE

At the Thoraxcenter a study has been initiated to compare, in a clinical setting, off-line assessment of CFR, using the cinefilm based analysis system, which is implemented in the Cardiovascular Angiography Analysis System (CAAS, digital matrix 512 x 512) with the corresponding on-line software, which is implemented in the Phillips Digital Cardiac Imaging System (DCI, pixel matrix 512 x 512).

Patients

Elective heart catheterization is performed in all cardiac transplant recipients as part of their annual follow-up protocol. This procedure consists of right ventricular biopsy, selective coronary angiography and assessment of CFR off-line. Since the DCI system has been installed at the Thoraxcenter, 18 patients (age 46 ± 14, mean ± SD) were included in this comparative study. The mean interval after transplantation was 3.2 ± 1.1 year. All patients were free of acute rejection at the time of the procedure. They were investigated without premedication and their anti-hypertensive medication was discontinued the evening before the catheterization.

Methods

On- and off-line measurements of CFR were performed as described. Simultaneous videocamera acquisition and cinefilm exposure was made possible by selecting the CINE-DCI mode, using a standard partially transmitting silver mirror. A print-out was made of the on-line CFR image with the selected regions of interest, to allow off-line assessment of CFR in the same areas, using anatomical landmarks (Figure 1).

To assess left ventricular function, left ventricular angiography was performed in 60° left anterior oblique and 30° right anterior oblique projection. Left ventricular ejection fraction (EF) was calculated by the Dodge technique[14], and regional wall motion was assessed using the "Centerline-method", as described by Sheehan, using fractional shortening in 100 chords, perpendicular to a centerline drawn between the end-diastolic and end systolic contours of a ventriculogram[15].

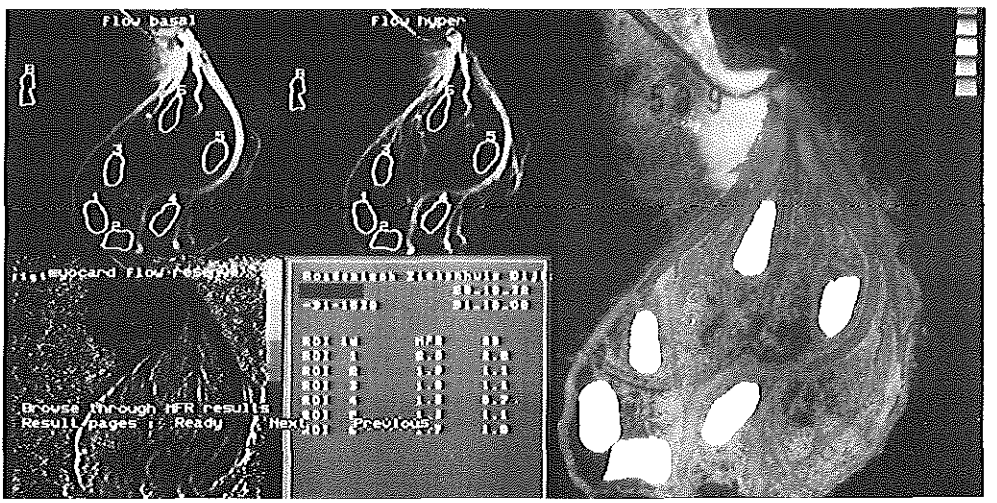

Figure 1. Illustration of the image acquisition in this study in a 58 year old male heart transplant recipient. Left: On-line assessment of CFR. Right: Off-line assessment of CFR.

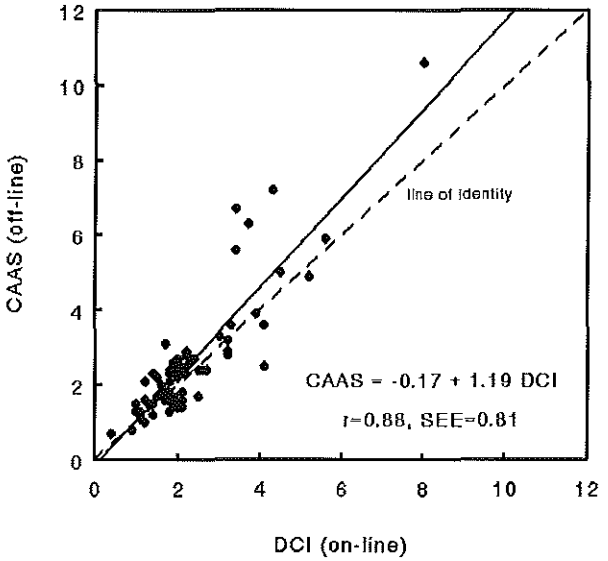

Figure 2. Results of comparison of digital and cinefilm measurements: The CFR results of the DCI are plotted against the results obtained by the CAAS system. The results of the linear regression analysis and the line of identity are included in the graph.

Statistical methods

Statistical analysis was performed using linear regression analysis and the Student's T test and Wilcoxon Matched-pairs Signed-ranks test for paired analysis. To assess the agreement between both measurements, the individual differences between CFR measured by CAAS and DCI were plotted against individual mean values, according to the statistical approach proposed by Altman and Bland[16]. Mean value and standard deviation of the signed differences in CFR between both methods were then calculated. Statistical significance was defined as a p value of 0.05 or less.

Results

In 18 cardiac transplant recipients a total of 68 ROI's (3.7 per patient, range 3-6) were analyzed with both techniques. All patients had a normal left ventricular function with normal regional wall motion. Ejection fraction could be assessed in 17 patients and gave a mean value of 69 ± 7%. Mean systolic blood pressure at the time of hyperaemia was 119 ± 15 mmHg and mean diastolic blood pressure was 84 ± 11 mmHg.

Among the patients included in this study, there was no angiographic evidence of

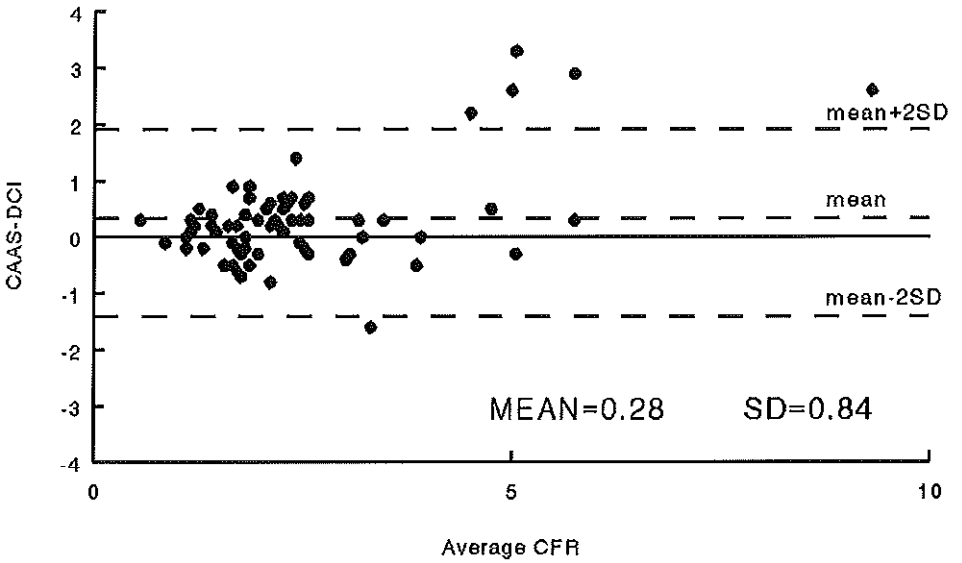

Figure 3. Comparison of digital and cinefilm measurements according to the method of Altman and Bland[16]: The differences between DCI and CAAS measurements are plotted against the mean values. The mean difference and 2-fold standard deviation are shown in the figure.

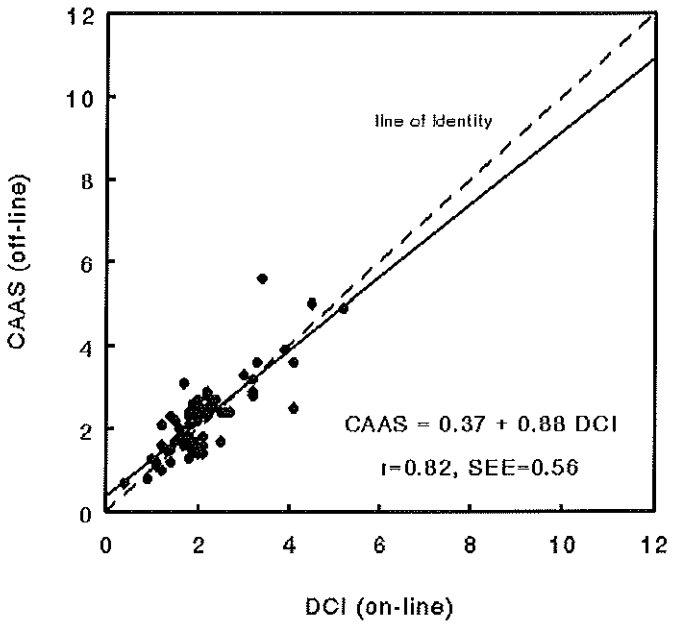

Figure 4. Results of comparison of digital and cinefilm measurements, excluding the 2 patients with high values for CFR as assessed by the off-line (CAAS) system.

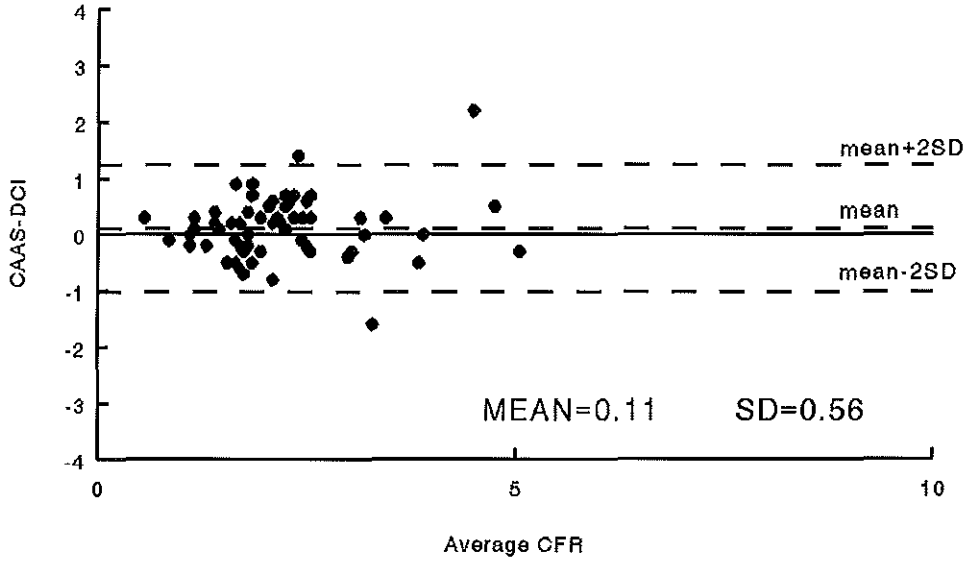

Figure 5. Comparison of digital and cinefilm measurements according to the method of Altman and Bland[16], excluding 2 patients with high values for CFR as assessed by the CAAS system.

collateral circulation or flow limiting stenosis (>50% diameter reduction) by either visual assessment or quantitative analysis, using automated edge detection.

The linear regression analysis, as shown in figure 2, revealed a reasonable correlation between CFR measurements using the DCI and CAAS system (r=0.88, y = -0.17 + 1.19 x, SEE = 0.81). However, the CAAS measurements (mean 2.62 ± 1.70) were significantly higher than the DCI measurements (mean 2.33 ± 1.25) (p = 0.01, Wilcoxon Matched-pairs Signed-ranks test). According to the approach of Altman and Bland, as shown in figure 3, the mean difference between both methods was 0.28 ± 0.84.

As illustrated by figure 2, the difference between the results of on- and off-line measurements is more pronounced for high values of CFR (> 5,0 by CAAS; 6 ROI data points). These datapoints were derived from 2 patients only. In one of these patients the level of inspiration during the basal and hyperemic coronary angiogram was not identical. Therefore the position of the diaphragm could have influenced the result of CFR measurements. In the second patient the injection of contrast medium was performed selectively in de circumflex artery.

After exclusion of these 2 patients from the analysis, the relationship between off-line and on-line assessment of CFR improved (r = 0.82, y = 0.37 + 0.88 x, SEE = 0.56), as shown in figure 4. There was no significant difference between the results of both measurements. The mean value for off-line measurements was 2.26 ± 0.97, for the DCI ystem 2.15 ± 0.91. The mean difference between both methods was 0.11 ± 0.56 (Figure 5).

DISCUSSION

During the last decade several studies have demonstrated that the functional significance of a coronary obstruction cannot always completely be evaluated by visual interpretation of stenosis morphology or quantitative measurement of it's geometric dimension[17,18]. As already stated in this book, additional assessment of myocardial blood flow provides better insight in the functional significance of a coronary stenosis.

Furthermore, assessment of CFR provides information concerning the specific characteristics of myocardial perfusion in patients with cardiomyopathy, syndrome X and diffuse coronary artery disease, as present in cardiac transplant recipients. The influence of different treatment strategies can be assessed by multicenter trials, where analyses are performed in an independent core laboratory, blinded for treatment and therapy.

The introduction of digitized facilities in the catheterization laboratories made it possible to perform on-line videodensitometric CFR measurements. However, since to date only 5% of the European catheterization laboratories (estimation by the industry) are equipped with digital angiographic facilities, there is a need for off-line analysis systems based on conventional cinefilm. Furthermore, the storage capacity for digital images, which is important for the transfer of the digital information to a core-laboratory, is still limited.

Animal experiments and in vivo validation studies of videodensitometric CFR measurements on myocardial regions of interest have shown excellent results[12,19]. The validation using intravascular Doppler assessment of blood flow velocity is eminently relevant because the methodological approach of both techniques is completely different. In a study of 21 patients undergoing elective PTCA for angina pectoris[20], the CFR measurements using off-line digital subtraction cineangiography (OLDSC) were compared with CFR measurements using intracoronary blood flow velocity assessed by a Doppler balloncatheter (DOP). There was a good relationship between the measurements, irrespective whether the flow was limited by the severity of the stenosis (OLDSC = 0.88 DOP + 0.12, r = 0.85, SEE = 0.38) only, or whether additional factors were present with potential influence on the outcome of CFR measurements like left ventricular hypertrophy or coronary artery dissection (OLDSC = 0.96 DOP + 0.01, r = 0.87, SEE = 0.34).

Animal experiments using microspheres show that despite the possible error sources, there is a good correlation between videodensitometric measurements of CFR and the application of microspheres (N = 86, r = 0.79, y = 0.58 + 0.81 x, SEE = 0.80)[12].

Since the analysis of the cineangiogram includes the selection of ROI's in the end-diastolic images, and the boundaries are drawn by the observer using a writing tablet, interfaced with the computer, this procedure can introduce some interobserver variability. However, as shown in table 1, both the inter- and intra-observer variabilities, as well as the short-, medium-, and long-term variabilities of CFR show a reasonable reproducibility of this technique[20]. Interobserver variability from 2 observers, measured in 12 regions of interest in 7 patients, was 0.08 ± 0.52 and intraobserver variability, measured in 11 regions of interest in 6 patients, was -0.01 ± 0.07. The short-term variability, based on the analysis

Table 1. Reproducibility of the digital sebstraction technique [21].

Intra-observer	-0.001 ± 0.007	NS
Inter-observer	0.08 ± 0.52	NS
Short-term	-0.02 ± 0.26	NS
Medium-term	-0.06 ± 0.52	NS
Long-term	0.11 ± 0.63	NS

of 2 coronary angiograms made 5 minutes apart and including 13 regions of interest, was -0.02 ± 0.26, the medium-term variability, based on repeated coronary cineangiograms within 1-3 hours, was found to be -0.06 ± 0.52 and the long-term variability from repeated coronary cineangiograms within 3 - 5 months, was 0.11 ± 0.63. In all these variability studies, no significant difference was found between both measurements.

To assess the relation between CFR, measured by digital subtraction technique, and the severity of coronary artery disease, assessed by quantitative coronary angiography, a precision study from the Thoraxcenter on 17 patients with single vessel coronary artery disease, and 12 patients with normal coronary artery dimensions, showed a good relation between CFR and the minimal luminal cross-sectional area (r = 0.92, SEE = 0.73) as well as between CFR and the percent area stenosis (r = 0.92, SEE = 0.74)[7]. In visually normal coronary arteries a CFR of 5.0 ± 0.8 was calculated, which differed significantly from CFR of the coronary arteries with obstructive disease providing values between 0.5 and 3.9. In both this study as in later studies[19,21] a normal CFR was defined as greater or equal to 3.4 (2 SD below the mean CFR of angiographically normal coronary arteries).

In this study the mean value of the measured CFR is 2.33 ± 1.25 by DCI, and 2.62 ± 1.70 by CAAS. The distribution of the ROI over the myocardium, using the coronary tree as a reference, is shown in figure 6. As can be appreciated from this scheme, only in a small percentage of ROI's a normal CFR is measured, which is not an unexpected finding in such a group of patients[21].

The results of this study show that where estimated CFR was ≤5.0, a good correlation was found between the CFR measurements using both systems. However, where estimated CFR was >5.0 by CAAS, the DCI yielded lower CFR values. There are a few possible reasons for discrepancy between the methods. First of all, the relation between the local amount of contrast and the resulting video brightness, has a different sign for both systems, i.e. negative for DCI and positive for CAAS. Despite the applied logarithmic subtraction technique, non-linear terms may remain in the transfer function between contrast and videosignal level because of non-linear amplification stages in the DCI-chain and because of the non-linear relation between light exposure of the cine-film and its resulting optical transparency.

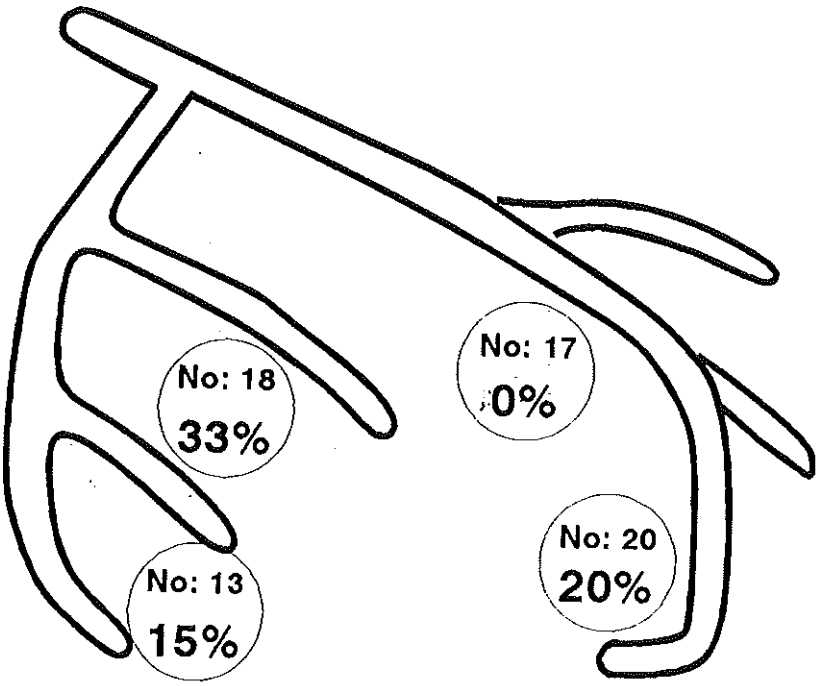

Figure 6. Distribution of the 68 ROI's over the myocardium with reference to the coronary artery tree. In bold the percentage of ROI's having a normal CFR is given.

Moreover, the fixed density threshold, used in the contrast arrival time image to calculate contrast arrival time, is different for both systems. For the off-line system this threshold, expressed in percentage of the brightness scale, was empirically derived by analyzing the relationship between the baseline and the hyperemic myocardial contrast appearance times as well as the resulting CFR in 12 patients with visually normal coronary arteries [7]. With a low threshold of 4% above video black level and to a lesser extent with a threshold of 8%, background density was not eliminated, resulting in very short contrast medium appearance times. Therefore, a threshold of 12% was defined for the CAAS system to completely exclude the influence of background noise on the calculation of contrast medium appearance times. The DCI system uses a threshold of 50% of maximal pixel intensity for the calculations of contrast arrival time and maximal contrast density in a ROI.

Limitations

Comparing these methods, one has to realize the limitations of this technique. The videodensitometric method requires the use of contrast media, which have substantial vascular effects, although non-ionic media, like Iopamiro used in this study, may disturb

blood flow less than ionic agents[15,22]. Furthermore, because longterm variability is 0.11 ± 0.63, this approach is only suitable to detect rather large changes in flow reserve (>1.37, mean + 2 SD) and should therefore be used in specific patients, in whom large changes of myocardial flow are expected.

For all techniques, the CFR is based on the ratio between maximal coronary blood flow and resting flow. The latter is mainly determined by the aortic pressure and heart rate, and therefore slight changes in these 2 parameters can influence CFR measurements. Flow during maximal hyperemia is linearly related to the perfusion pressure. This can result in a scatter of CFR data in a single patient. The recently described hyperemic versus perfusion pressure relationship[23] theoretically overcomes this problem, but is difficult to assess with angiography.

Conclusion

The digital subtraction technique to measure CFR is a reliable method, which can be assessed on-line or off-line. This method is easily applicable, and less time consuming than other methods to assess CFR. In view of the good correlation between both the on- and off-line system it is reasonable to propose the use of the off-line technique to assess CFR in large multicenter trials where cinefilm is used. The design of the "REGRESS" study already included these measurements.

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CHAPTER 7

MYOCARDIAL FLOW RESERVE MEASUREMENTS IN CARDIAC TRANSPLANT RECIPIENTS DURING LONG TERM FOLLOW-UP.

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(Submitted)

ABSTRACT

Accelerated transplant coronary artery disease is a major impediment of long-term survival after cardiac transplantation. In order to evaluate this diffuse arteriopathy of the coronary artery tree in cardiac transplant recipients, myocardial flow reserve measurements (MFR) were performed in 99 cardiac transplant recipients at different postoperative intervals.

Initial measurements were performed in 29, 17, 21 and 32 patients respectively at one month and 1, 2 and 3 years after transplantation.

Serial follow-up measurements of both quantitative coronary angiography and MFR was obtained during 77 procedures. The mean MFR in the first postoperative year 2.34 ± 0.82. In the following years the mean MFR were 2.16 ± 0.84, 2.63 ± 0.84, 2.43 ± 0.79, 2.22 ± 0.83 and 1.78 ± 0.97 respectively. Only 14 % of the measurements exceeded the normal value of MFR (3.4).

No relation was found with visually detected transplant coronary artery disease and with changes in luminal diameter of the large epicardial coronary arteries as measured by quantitative coronary angiography. No immunologic and non-immunologic risk factors for accelerated coronary artery disease could be identified.

Conclusion: MFR reserve measurements in this group of patients were impaired. No significant decrease in the consecutive postoperative years was found. Risk factors for changes in individual patients could not be identified.

INTRODUCTION

Transplant coronary artery disease is one of the limiting factors for long term survival after cardiac transplantation[1]. The earliest change consists of concentric fibrosis and smooth muscle cell proliferation with collagen accumulation creating diffuse intimal thickening. This is seen as early as one week after transplantation[2,3]. Subsequently, these lesions may progress to diffuse obliterative lesions creating longitudinal narrowing and distal pruning. The lesions are generally present in the large epicardial vessels as well as in the small intramyocardial branches. To detect the onset and progression of coronary disease in an early phase, coronary angiography is performed in most transplant centers. Three categories describing the specific angiographic morphology of the lesions found after transplantation are distinguished[4]: type A, discrete or short tubular stenosis in the proximal, middle or distal segments of major coronary arteries or their branches, type B; diffuse concentric luminal narrowing in the middle to distal segment branches; and type C, diffusely narrowed irregular distal branches that are squared of and end abruptly, the latter two groups both unique to the post-transplant patients.

It has been demonstrated that coronary angiography is relative insensitive for the detection of early vascular lesions[3]. Both not significant focal lesions (< 25% stenosis) and

diffuse disease are often underdiagnozed by coronary angiography, even when quantitative techniques are used[3-5].

Myocardial Flow Reserve (MFR) measurements describe the relation between the angiographic severity of coronary artery disease and the resulting reduction or limitation in maximal blood flow in the myocardium[6-10]. Even in the absence of focal atherosclerosis or other flow limiting factors in major epicardial vessels, MFR measurements can be used to evaluate dysfunction of the microcirculation. This is especially relevant in cardiac transplant recipients, where a diffuse arteriopathy can reach a flow limiting significance, without changes in angiographic appearance[11,12].

The purposes of present study were 1) to describe the changes in MFR at different postoperative stages up to five years after transplantation, 2) to correlate these changes with changes in luminal diameter as assessed by quantitative and visual analysis of the coronary angiograms, and 3) to correlate these changes with previously identified risk factors[13-20].

PATIENTS AND METHODS

Patients

The study population consisted of 99 adult heart transplant recipients, who underwent coronary angiography at different postoperative stages, as part of an annual follow-up protocol[21]. This procedure consisted of right ventricular biopsy, selective coronary angiography and assessment of MFR. All patients had undergone orthoptic heart transplantation at the Thoraxcenter of the University Hospital Rotterdam, Dijkzigt. In 59 patients MFR measurements were repeated 1 and 2 years after the baseline coronary angiography thus achieving serial one year and two year follow-up. In 34 patients only one year follow-up measurements were obtained. Six patients were lost to follow-up.

All patients were treated with standard immunosuppressive regimens, including prophylactic anti-lymphocyte antibody therapy during the early postoperative period. Maintenance immunosuppressive therapy consisted of low dose steroids and cyclosporine. In 12 patients azathioprine was added because of recurrent rejection.

Cytomegalo virus (CMV) seronegative recipients received anti-CMV immunoglobulin (Cytotect, Biotest Pharma GmbH, Frankfurt, Germany) during the first 10 weeks after transplantation.

All patients were investigated without premedication and their anti-hypertensive medication was discontinued the evening before the catheterization.

Myocardial flow reserve measurements

The technique to assess myocardial flow reserve by digital subtraction angiography has been extensively described[22-28]. Coronary angiography was carried out by femoral approach using Judkins technique. An 8 French guiding catheter (Type Judkins, Cordis, Miami, Florida, USA) was advanced to the aortic root. To assess myocardial flow reserve, non-ionic contrast medium (Iopamiro, Bracco, Italy; 370 mg iodine/ml) was injected at 37°C into the left coronary artery using an ECG-triggered infusion pump (Medrad IV, Medrad, U.S.A.). The heart was atrially paced at a level approximately 10 beats/minute above spontaneous the heart rate, ranging from 90 to 120 beats/min (mean 107 ± 11). Filming speed was set at 50 images per second to obtain equal images in successive cardiac cycles. The X-ray exposure per frame was kept constant by selecting the lock-in mode on the X-ray generator. Basal coronary angiography was performed in either 90° left anterior oblique or 30° right anterior oblique projection, after intracoronary administration of 2 mg isosorbide-dinitrate. Thirty seconds after pharmacologically induced maximal hyperaemia, using an intracoronary bolus injection of 12.5 mg papaverine, the angiogram was repeated.

Myocardial flow reserve measurement with digital subtraction cineangiography from 35 mm cinefilm has been implemented in the Thoraxcenter Research version of the Cardiovascular Angiography Analysis System (CAAS)[29]. Thereby, five end-diastolic cineframes are selected from successive cardiac cycles. Logarithmic non-magnified mask-mode background subtraction is applied to the image subset to eliminate non-contrast medium densities, using the last end-diastolic frame prior to contrast administration as a mask. The principle of mask mode subtraction techniques allows the determination of myocardial time-density curves before and during coronary vasodilatation. In the CAAS system at the Thoraxcenter, the appearance-time-contrast density approach, according to Vogel et al.[26], is used. From the sequence of background subtracted images, a contrast arrival time image and a density image are automatically determined, using an empirically derived fixed density threshold[10].

On corresponding basal and hyperaemic end-diastolic frame sequences, identical regions of interest (ROI's) are selected in such a way that the epicardial coronary arteries visible on the angiogram, the coronary sinus and the great cardiac vein are excluded from the analysis. For the calculation of relative blood flow within these regions of interest two parameters are required: the relative regional vascular volume and the mean contrast appearance time. The relative regional vascular volume can be calculated from the maximal density image, the intensity value being proportional to the transradiated amount of contrast medium within the vessel. Therefore, the regional vascular volume for a user-defined ROI is proportional to the mean radiographic density within the ROI. The mean contrast appearance time is derived from the contrast arrival time image.

Regional relative flow values are quantitatively determined using the following videodensitometric principle: $Q=V/T$ (Q=regional blood flow, V=regional volume and T=mean appearance time).

The MFR for one ROI is then calculated as follows:

$$MFR = \frac{Q_h}{Q_b} = \frac{V_h/T_h}{V_b/T_b} = \frac{V_h x\ T_b}{V_b x\ T_h} = \frac{D_h x\ T_b}{D_b x\ T_h}$$

(D=mean maximum contrast density; h=hyperaemic; b=baseline)

The normal value of MFR is defined as greater or equal to 3.4 (2 SD below the mean MFR of angiographically normal coronary arteries (5.0 ± 0.8, mean ± SD)[30]. The threshold used to assess significant change in MFR was 0.8, which is the standard deviation of the measurement as described above.

Quantitative coronary angiography

Off-line quantitative analysis was performed using the computer-assisted Cardiovascular Angiography Analysis System (CAAS)[31,32]. After correction for pincushion distortion caused by the image intensifier, the mean coronary width of each segment was calculated in millimetres. The catheter tip or shaft, filmed without contrast, was used for calibration.

The main branch and segments 6, 7, 8, 11 and 13 of the left coronary artery, identified by anatomic landmarks, as defined by the American Heart Association[33] were selected for analysis. All segments were analyzed in two views, preferably orthogonal or separated by at least 30°, avoiding foreshortening.

The "patient global score" provided a description of the coronary status of an individual patient, and was defined as the average of the mean widths of the measured segments per patient.

Statistical methods

Statistical analysis was performed using linear regression analysis. The Student's T test and Wilcoxon Matched-pairs signed ranks test were used for paired analysis. All data are presented as the mean ± SD. Multivariate analysis was performed using logistic regression to identify variables independently correlated with the changes in MFR. All statistical analyses were carried out with a commercial statistical package (BMDP statistical Software Package). P values (two tailed) were considered significant only when less than 0.05.

RESULTS

Clinical data

The baseline characteristics of the patients are shown in table 1. There was no difference between patients studied at different intervals with respect to mean values of recipient- and

Table 1. Baseline characteristics

	group A	group B	group C	group D
Baseline coronary angiography (timing after HTX)	1 month	1 year	2 years	3 years
Number of patients	29	17	21	32
Recipient age (yr) †	44 ± 12	47 ± 9	43 ± 10	45 ± 10
Donor age (yr)	26 ± 8	24 ± 6	25 ± 9	23 ± 7
Gender (F/M)	2 / 27	5 / 12	4 / 17	2 / 32
Gender mismatch	8	6	8	12
HLA-mismatch				
A	1.4 ± 0.6	1.3 ± 0.7	1.3 ± 0.7	1.3 ± 0.6
B	1.6 ± 0.5	1.5 ± 0.6	1.7 ± 0.5	1.6 ± 0.6
DR	1.3 ± 0.6	1.6 ± 0.5	1.4 ± 0.7	1.4 ± 0.6
Immunosuppressive regimen				
- Cyclosporine and prednisone (pts)	28	15	17	27
- Triple therapy (pts)	1	2	4	5
Donor heart ischemia (min)	154 ± 31	163 ± 34	162 ± 44	156 ± 40
Indication for HTX (ischemic/other)	13 / 16	9 / 8	9 / 12	19 / 13
CMV serology (pos/neg)	16 / 13	10 / 7	10 / 11	17 / 15
Cholesterol (mmol/l)	5.1 ± 1.8	7.2 ± 1.2	7.4 ± 1.9	7.5 ± 1.5
Triglyceride (mmol/l)	1.3 ± 0.7	2.3 ± 0.7	2.5 ± 0.8	2.3 ± 1.0
HDL-cholesterol (mmol/l)	1.2 ± 1.2	1.4 ± 0.4	1.4 ± 0.5	1.4 ± 0.4
Use of calcium antagonist (pts)	18	15	16	24
Smoking (pts)	3	0	4	3
Diabetes Mellitus (pts)	1	2	1	1

F=female; M=male; CMV=Cytomegalo virus; HDL=High Density Lipoproteins

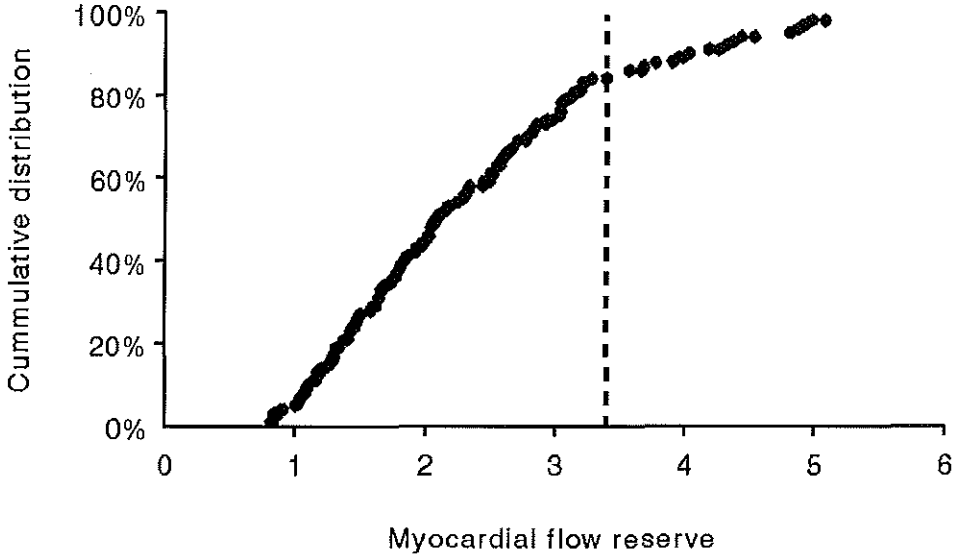

Figure 1. Cummulative curve of the myocardial flow reserve measurements in this group of patients (n=158). The normal value of myocardial flow reserve (3.4) is drawn in the figure.

donor age, gender mismatch, HLA-A+B or HLA-DR mismatch, total serum cholesterol, triglyceride, high-density lipoprotein cholesterol levels and the other described risk factors for graft atherosclerosis.

Myocardial flow reserve

In this study 243 coronary angiograms were analyzed in 99 patients at different post-operative intervals. MFR measurements could not be performed in 28 follow-up catheterizations because of both clinical and mechanical reasons (arrythmias, total amount of contrast already given to the patient and equipment failure). Eighty-five angiograms could not be analyzed because of low contrast density after mask mode-subtraction, arrythmia's or not identical background subtracted images .

Table 2 shows the distribution of the results of the MFR measurements in the consecutive posttransplant years. Only 14 % of the measurements exceeded the normal value of MFR (Figure 1).

Serial follow-up measurements of both quantitative coronary angiography and MFR was obtained during 77 procedures. No correlation was found between the changes in patient global score and MFR with linear regression analysis ($r = -1.19$, $y = 0.02 - 1.56\ x$, SEE = - 0.18). Subgroup analyses for ROI's in areas of the myocardium perfused by either the left anterior descending branch or the circumflex branch of the left coronary artery, did not

Table 2. Myocardial flow reserve measurements at different postoperative intervals.

Postoperative interval	Myocardial flow reserve	Number of measurements
1 month	2.34 ± 1.18	23
1 year	2.16 ± 1.14	29
2 years	2.63 ± 1.45	35
3 years	2.43 ± 1.12	38
4 years	2.22 ± 0.88	25
5 years	1.78 ± 0.69	8

Table 3. Decrease in myocardial flow reserve and risk factors *

Risk factor	Patient positive for the risk factor: decrease / total	Patient negative for the risk factor: decrease / total	Odds ratio (95% CI)
Age recipient (> 50.2 years)	8 / 36	12 / 41	0.64 (0.20 to 2.04)
Age donor (> 23 years)	10 / 37	10 / 40	1.11 (0.36 to 3.46)
Males	13 / 68	5 / 9	0.19 (0.24 to 0.97)
Gender mismatch	10 / 27	10 / 50	2.35 (0.73 to 7.61)
Number of HLA mismatches (> 4)	7 / 33	13 / 44	0.67 (0.20 to 2.07)
Ischemia of the donorheart (> 160 min)	9 / 36	11 / 41	0.91 (0.29 to 2.84)
Indication for HTX: IHD	6 / 36	14 / 41	0.39 (0.11 to 1.28)
Number of rejection episodes (> 0) †	6 / 17	14 / 60	1.79 (0.48 to 6.58)
Smoking	7 / 22	13 / 55	1.51 (0.44 to 5.10)
CMV infections	4 / 18	16 / 59	0.77 (0.18 to 3.05)
CMV disease	2 / 7	18 / 70	1.16 (0.52 to 5.49)
Serum Cholesterol (> 7.55 mmol/l)	13 / 43	7 / 34	1.67 (0.52 to 5.49)
Serum Triglyceride (> 2.26 mmol/l)	9 / 39	11 / 38	0.74 (0.23 to 2.30)
Serum HDL-Cholesterol (> 1.29 mmol/l)	9 / 35	11 / 42	0.98 (0.31 to 3.05)
Use of nifedipine	15 / 56	5 / 21	1.17 (0.32 to 4.42)
Hypertension	5 / 20	15 / 57	0.93 (0.25 to 3.42)

* all 77 patients with 1 or 2 year follow-up using 0.8 as a threshold to identify a significant decrease in myocardial flow reserve, IHD=Ischemic Heart Disease; CMV=Cytomegalo virus transplant; †=during the study period

improve this relation. There was no significant difference between the myocardial flow reserve measurements performed at consecutive postoperative intervals (Table 2).

Statistical analysis using a students t-test could not reveal a correlation between the presence of visually detected coronary artery disease of both the epicardial and intramyocardial branches of the coronary tree and changes in MFR (p=0.48).

Risk factors

In contrast with other studies no relation was found between the occurrence of rejection during the catheterization and MFR[11].

As is shown in Table 3 no risk factors for accelerated coronary artery disease could be identified both using univariate analysis and logistic regression model. A decrease in MFR of more than 0.8 was used as a threshold to define the development of diffuse coronary artery disease.

DISCUSSION

This study was designed to investigate the process of accelerated transplant coronary artery disease by measurement of myocardial flow reserve.

The number of reports concerning the use of myocardial flow reserve to monitor transplant coronary artery disease and identify specific immunologic and non-immunologic risk factors for progression of accelerated coronary artery disease, is limited[11,12].

These studies demonstrated that in the absence of allograft rejection, acquired left ventricular hypertrophy and regional wall motion abnormalities, myocardial flow reserve was normal after cardiac transplantation. During heart rejection episodes the myocardial flow reserve was impaired[11]. In contrast, in a more recent study, the impairment of myocardial flow reserve during cardiac rejection was not demonstrated[36]. In this study mild diffuse coronary occlusive disease was associated with a decrease of peak flow response to papaverine and thus a decrease of myocardial flow reserve.

All these studies were performed using intracoronary Doppler. In this study, we used the videodensitometric technique instead of coronary Doppler. Since the videodensitometric technique can be performed without the insertion of hardware in the coronary tree, it is more applicable in large patient groups. Furthermore, the analysis of the cineangiogram is performed off-line, and thus offers an objective method to assess myocardial flow reserve[37]. Validation studies of off-line videodensitometric myocardial flow reserve measurements on myocardial regions of interest have shown excellent results compared to intracoronary blood flow velocity measurements assessed by a Doppler catheter[38]. Both the inter- and intra-observer variabilities, as well as the short-, medium-, and long-term variabilities of myocardial flow reserve show a reasonable reproducibility of this technique[39].

The present data show that the myocardial flow reserve in this group of patients is rather low. This is somewhat surprising since the incidence of diffuse coronary artery disease is limited in this patient group, when assessed by visual or quantitative epicardial coronary artery changes[21,40]. However, as was already stated in the introduction, coronary angiography is relatively insensitive for the detection of early vascular lesions[41] and dysfunction of the small intramyocardial arteries. Therefore, the reduction of myocardial flow reserve in this group of patients can be a result of functional damage to the microvasculature. This is in agreement with the histologic findings of Johnson and coworkers[2,3] which show smooth muscle cell proliferation and accumulation of collagen and ground substance as early as one week after transplantation both in large epicardial vessels as in the small myocardial branches.

The exact mechanisms leading to the development of accelerated coronary artery disease and the resulting impairment of coronary vascular function are not elucidated. The number of HLA-mismatches and the number of rejection episodes are thought to be risk factors for the development of accelerated coronary artery disease[14-16]. After the initial injury, hyperlipidaemia, Cytomegalo virus infections and other risk factors may be a cofactor[4,5,13,17-20]. Unfortunately, in this study no relation was found with previously described risk factors for accelerated coronary artery disease (Table 3). Excluding the patients with an acute rejection during the catheterization, as assessed by right ventricular myocardial biopsies, did not alter these results. A larger number of serial measurements will be necessary to reveal the influence of potential risk factors on the function of the microvasculature.

During allograft rejection, the impairment of myocardial flow reserve is thought to be linked to abnormalities of the coronary vasculature. The reversible alteration of myocardial flow reserve in these patients can be a result of change in responsiveness of the vascular wall to papaverine, compression of intramyocardial vessels as a result of interstitial edema, or the release of vasoactive mediators as leukotrienes due to perivascular or intimal lymphocyte and monocyte infiltration[11].

Limitations

Several potential methodological limitations must be considered when interpreting these data. Because myocardial flow reserve depends on the level of basal coronary blood flow, the small differences between patients in the different groups could be a result of a higher basal myocardial flow due to the high pacing stimulus (mean 107 beats/min) in this study. Secondly, myocardial flow reserve measurements may be influenced by pharmacological alterations in resting or maximal hyperaemic blood flow, despite the fact that intracoronary nitroglycerine was administered to obtain maximal epicardial coronary vasodilatation and all vasoactive medication was discontinued the evening before the actual catheterization. Finally, the number of sequential measurements of myocardial flow reserve in this study is low (n=77).

Conclusions

In this group of patients following cardiac transplantation the myocardial flow reserve is impaired, probably as a result of structural damage to the microvasculature. The myocardial flow reserve measurements do not change significantly in the subsequent posttransplant years. Therefore, the present data support the earlier findings in patients transplanted at the Thoraxcenter in which no overall progression of accelerated coronary artery disease was found during long-term follow-up[40]. No relation was found between the myocardial flow reserve and described risk factors. Visual and quantitative assessment of coronary artery disease in epicardial coronary arteries were not related with changes in myocardial flow reserve measurements. Further studies will be necessary to define the exact role of these technologies in the assessment of transplant coronary artery disease. More attention should be given to vessels of the second and third order. The intracoronary volume measurements by videodensitometry offer a new and reliable approach to the assessment of progression and regression of coronary artery disease in these vessels[42]. Therefore, this technique could provide a reliable technique to assess diffuse coronary artery disease in cardiac transplant recipients.

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CHAPTER 8

EVALUATION OF THE HEMODYNAMIC RESPONSE TO ATRIAL PACING STRESS TESTING FOLLOWING CARDIAC TRANSPLANTATION: COMPARISON TO CHANGES IN EPICARDIAL CORONARY ARTERY SIZE OVER A 1 YEAR PERIOD.

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ABSTRACT

Previous investigations have used quantitative coronary angiography (QCA) of major epicardial vessels to monitor progression of transplant coronary disease, a vasculopathy potentially involving the coronary microvasculature. The physiologic significance of these changes, however, is uncertain. Therefore, 91 patients without focal angiographic stenosis >50% underwent initial evaluation 1 month to 5 years after cardiac transplant with 1 year follow-up. QCA and rapid atrial pacing to matched maximum heart rate were performed with measurement of left ventricular pressure and pressure-derived parameters (+dP/dt, Vmax, Tau) by tip manometry. At follow-up study, mean coronary segment diameter (mn ± SE) decreased 30 ± 11 microns (733 segments, $p < 0.05$). No changes in left ventricular systolic or diastolic parameters at rest or with maximum pacing occurred over the 1 year follow-up. Similarly, in two subsets of patients with greater decreases in mean coronary segment width (a: 1 month initial study group, QCA decrease 58 ± 25 microns, $p < 0.05$; b: greater than 220 micron one year QCA decrease in patient mean segment diameter group), LVEDP, Tau, +dP/dt, and Vmax remained similar to initial study.

Therefore, over a one year time interval, left ventricular function remained unchanged at rest and with rapid atrial pacing. Detectable changes in epicardial vessel size were not associated with changes in the coronary microvasculature sufficient to result in a decrease in left ventricular functional reserve as assessed by atrial pacing stress testing. QCA and rapid atrial pacing may provide complementary anatomic and functional assessments of the coronary vasculature following cardiac transplant.

INTRODUCTION

Transplant coronary disease is the leading cause of mortality in patients surviving greater than 1 year following cardiac transplant[1]. Angiographic[2] and pathologic[3] studies have identified that transplant coronary disease can be manifest as a diffuse arteriopathy, potentially involving small epicardial or intramyocardial arteries. Even in the absence of high grade focal stenoses by coronary angiography, allograft arteriopathy may be functionally significant and pathologically characterized by diffuse involvement of epicardial and intramyocardial arteries[4,5].

Although visual analysis of coronary angiograms may be adequate for the detection of high grade focal coronary artery lesions, quantitative coronary angiography (QCA) of epicardial arteries has been proposed as a method to assess patients for the development of diffuse arteriopathy following cardiac transplant[6,7]. However, the functional significance of decreases in mean coronary segment diameter are uncertain.

Rapid atrial pacing is a well characterized method to assess the functional significance of coronary artery disease. In previous studies, hemodynamic measurements at peak pacing revealed highly significant differences in left ventricular end-diastolic pressure, peak

positive rate of isovolumic left ventricular pressure rise (+dP/dt), and calculated maximal myocardial velocity of shortening (Vmax) in patients with coronary artery disease as compared to controls[8]. Thus, atrial pacing may be useful for functional assessment and possible early detection of diffuse arteriopathy involving the entire vasculature of the myocardium. Therefore, in patients 1 month to 5 years following cardiac transplant without high grade focal angiographic coronary obstructions, we assessed the effects of QCA-detected changes in average epicardial arterial lumen diameter on global left ventricular function using the atrial pacing stress test over a one year time interval.

PATIENTS AND METHODS

Patients

Ninety seven patients 1 month to 5 years following cardiac transplant underwent initial functional assessment. Resting and pacing data from a previous report of 57 patients without identifiable coronary disease at the time of catheterization were used as controls[8]. Transplant patients with high grade focal coronary obstructions were excluded from this study since they were expected to have myocardial ischemia with atrial pacing on this basis alone without warning symptoms of angina pectoris. Five patients had significant coronary artery disease (focal stenosis > 50%) at the time of initial study and one patient had normal

Table 1. Hemodynamics of cardiac transplant and normal patients at rest.

Hemodynamic Maeasurement	Normal pts.	Transplant pts.	
		Baseline	1 Year
Heart Rate (bpm)	68 - 80	93 ± 12	92 ± 12
LV peak systolic pressure (mmHg)	114 - 170	129 ± 17	133 ± 20
LV end-diastolic pressure (mmHg)	7 - 14	11 ± 5	12 ± 5
+ dP/dt (mmHg/sec)	1465 - 2255	1791 ± 375	1786 ± 353
Vmax (s^{-1})	49 - 67	61 ± 11	60 ± 12
Tau (msec)	N.A.	42 ± 10	43 ± 9

Normal patient data is presented as the 67% range of normal control patients described previously[8]. Transplant patient data is presented as mean ± standard error. Bpm = beats per minute, LV = left ventricle, N.A. = not available, Tau = time constant of isovolumic relaxation, Vmax = peak velocity of contractile element, + dP/dt = peak rate rise of left ventricular pressure.

coronary arteries at initial 1 month study but developed severe graft atherosclerosis over the next year requiring retransplantation. Results of endomyocardial biopsy were not used to exclude patients. The remaining 91 transplant patients were reassessed at one year follow-up and had the following characteristics: the mean age of recipients was 45 ± 12 years and the mean age of their donors was 25 ± 8 years. An average of 4.3 ± 1.1 HLA mismatches were present. Patients had an average cholesterol of 285 ± 62 mg/dl. Immunosuppressive protocols and other patient population features have been previously described[9].

Methods

In the cardiac catheterization laboratory, a left ventricular micromanometer tipped pigtail catheter was advanced into the left ventricle from the right femoral artery. A temporary pacing catheter was advanced into the right atrium from the right internal jugular vein. The following measurements were made with use of a previously described digital analysis system[8,10]: heart rate; left ventricular peak systolic and end-diastolic pressures; the maximum first time-derivative of left ventricular pressure (+dP/dt); the relation between left ventricular (dP/dt)/pressure and pressure linearly extrapolated to pressure = 0 (Vmax, the theoretical maximal velocity of the myocardial contractile element); and tau, the simple exponential time constant of isovolumic pressure decay. Tau measurements were not obtained in the control group.

Baseline resting left ventricular measurements were made followed by the initiation of atrial pacing at a rate slightly above baseline heart rate. Atrial pacing was performed with 20 bpm increments every 3 minutes to AV block or a maximum rate of 180 bpm with repeat left ventricular measurements made at each rate.

Coronary angiography was then performed in the transplant population with nonionic contrast (Iopamiro, Bracco, Italy; 370 mg iodine/ml) in multiple orthogonal views preceeded by 5 mg sublingual isosorbide dinitrate. Coronary angiogram cine films were analyzed off-line by automated edge detection using the computer-based Cardiovascular Angiography Analysis System (CAAS) which has been previously described in detail[11,12]. Nine epicardial coronary segments were selected for initial and follow-up measurements: American Heart Association classification segments 1-3, 5, 6-8, 11 and 13 or 14[12]. All segments were analyzed in two orthogonal views and the results were averaged per segment. Segments showing foreshortening, overlap or inappropriate filling were excluded from the analysis. The standard deviation of the CAAS QCA system repeated measurement of identical coronary segments was 110 microns. Results are reported as a segment based mean coronary segment width ± standard error of the mean as described by de Feyter and coworkers[13].

In the transplant patients, the atrial pacing protocol and angiographic projections were replicated 1 year after initial study. Identical coronary segments were compared to obtain changes in mean segment diameters.

Statistical methods

Hemodynamic data are presented as the mean ± SD. Rest and matched maximum heart rate hemodynamic variables were compared at initial and at 1 year follow-up using paired Student's t-test.

RESULTS

Comparison of hemodynamic measurements following transplant to normal controls (Table 1 and 2).

In the transplanted patients, the hemodynamic response to pacing was heterogeneous with no identifiable trends for any patient subgroup. Transplant patients had a significantly higher resting heart rate of 93 ± 12 bpm as compared to controls ($p < 0.05$). Other than a higher resting heart rate, hemodynamics in this group of transplant patients were similar to controls and remained stable over 1 year.

Changes in epicardial artery size over 1 year (Figure 1).

Mean coronary segment width of all patients decreased slightly but with statistical significance over the 1 year period of follow-up ($p < 0.01$). In the subgroup of patients with

Table 2. Hemodynamics of cardiac transplant and normal patients during maximal atrial pacing.

Hemodynamic Maeasurement	Normal pts.	Transplant pts.	
		Baseline	1 Year
Heart Rate (bpm)	130 - 170	144 ± 21	144 ± 21
LV peak systolic pressure (mmHg)	107 - 136	123 ± 18	128 ± 17
LV end-diastolic pressure (mmHg)	1 - 8	6 ± 4	6 ± 5
+ dP/dt (mmHg/sec)	1800 - 3200	2225 ± 522	2352 ± 512
Vmax (s^{-1})	63 - 96	70 ± 16	74 ± 14
Tau (msec)	N.A.	38 ± 8	38 ± 8

Normal patient data is presented as the 67% range of normal control patients described previously[8]. Transplant patient data is presented as mean ± standard error. Bpm = beats per minute, LV = left ventricle, N.A. = not available, Tau = time constant of isovolumic relaxation, Vmax = peak velocity of contractile element, + dP/dt = peak rate rise of left ventricular pressure.

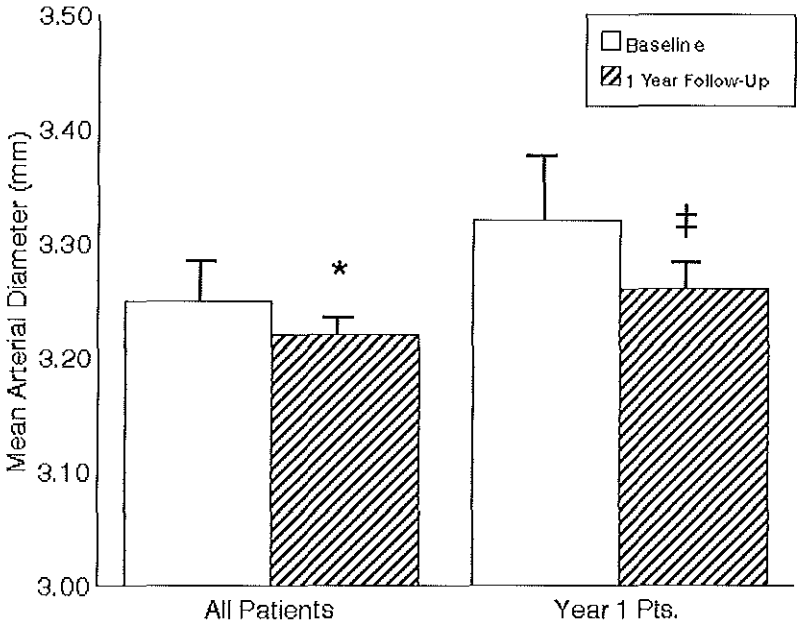

Figure 1. Comparison of QCA-detected regression of mean segment diameter measurements from baseline to 1 year follow-up in all patients (n = 91) and those studied initially 1 month post-cardiac transplant (n = 22). Error bars represent the standard error of the mean. QCA technique was based on mean segment analysis by de Feyter et al.[13]. * $p < 0.01$, † $p < 0.02$.

initial studies 1 month following transplant, changes in mean artery segment size were approximately twice that of the entire transplant group (Figure 1: $p < 0.02$).

Left ventricular hemodynamics with atrial pacing to maximum matched heart rate (Table 1 and 2).

By design, heart rate was increased to a matched 144 ± 21 bpm with pacing at initial and 1 year follow-up evaluation. With pacing, left ventricular (LV) systolic pressure fell slightly as LV end-diastolic pressure decreased by approximately 50% at both times of evaluation. Indices of contractility, +dP/dt and Vmax, were similar at rest and maximum pacing over the 1 year interval. Tau, the rate of isovolumic relaxation, was also similar over this time period.

Despite greater changes in mean coronary segment diameter over the first year of transplant, no significant changes in resting or rapid pacing left ventricular function were seen, although changes were heterogenous for the entire population (Table 3). In addition, in 14 patients with >220 micron change in mean coronary segment diameter at 1 year (2x standard deviation of QCA-system repeated measurement), no changes in baseline or rapid pacing measurements were detected (Table 4).

DISCUSSION

Other than a higher resting heart rate in this group of transplant patients, hemodynamics at rest and with rapid atrial pacing were similar to non-transplant controls and remained stable over 1 year including subgroups with greater changes in epicardial artery lumen diameter. Neither indices of systolic (+dP/dt, Vmax) or diastolic (LVEDP, Tau) function were affected over this time period. Thus, in the absence of significant focal stenoses, detectable decreases in epicardial vessel lumen size may not imply changes in the coronary vasculature sufficient to result in a decrease in left ventricular function as assessed by atrial pacing stress testing.

Johnson and coworkers described allograft vasculopathy and other coronary artery pathologic changes in transplant patients dying of a variety of causes[14]. In patients surviving at least 12 months, 15 of 25 showed coronary lesions confined to the proximal epicardial arteries without involvement of the coronary microvasculature; 10 of 25 exhibited a diffuse fibrous intimal thickening with or without atheromatous plaques possibly as a sequela of a previous arteritis involving arteries possessing discrete medial layers. The latter subgroup was hypothesized to have been subjected to greater myocardial immunologic rejection with associated inflammation directed toward the coronary vasculature.

Quantitative coronary angiography (QCA) has been considered a potentially useful method to characterize allograft arteriopathy since pathologic[3,4] and intravascular

Table 3. Hemodynamics of patients 1 month post-transplant at rest and during maximal atrial pacing.

Hemodynamic Maeasurement	Baseline		1 Year follow-up	
	Rest	Maximal pacing	Rest	Maximal pacing
Heart Rate (bpm)	91 ± 12	151 ± 17	94 ± 13	150 ± 17
LV peak systolic pressure (mmHg)	130 ± 19	114 ± 19	135 ± 28	125 ± 19
LV end-diastolic pressure (mmHg)	13 ± 5	6 ± 4	12 ± 5	5 ± 5
+ dP/dt (mmHg/sec)	1827 ± 355	2177 ± 485	1734 ± 251	2300 ± 377
Vmax (s^{-1})	63 ± 11	72 ± 17	58 ± 10	74 ± 12
Tau (msec)	45 ± 13	42 ± 10	43 ± 8	38 ± 9

N = 22; Patient data is presented as mean ± standard error. Bpm = beats per minute, LV = left ventricle, Tau = time constant of isovolumic relaxation, Vmax = peak velocity of contractile element, + dP/dt = peak rate rise of left ventricular pressure.

Table 4. Hemodynamics at rest and during maximal atrial pacing for >200 µm diameter reduction group.

Hemodynamic Maeasurement	Baseline		1 Year follow-up	
	Rest	Maximal pacing	Rest	Maximal pacing
Heart Rate (bpm)	93 ± 13	154 ± 15	91 ± 11	154 ± 20
LV peak systolic pressure (mmHg)	123 ± 14	121 ± 14	125 ± 21	126 ± 17
LV end-diastolic pressure (mmHg)	12 ± 5	6 ± 4	12 ± 6	6 ± 5
+ dP/dt (mmHg/sec)	1764 ± 347	2173 ± 424	1725 ± 270	2345 ± 378
Vmax (s^{-1})	60 ± 11	65 ± 18	59 ± 8	74 ± 8
Tau (msec)	43 ± 10	37 ± 9	41 ± 6	35 ± 6

N = 14; Patient data is presented as mean ± standard error. Bpm = beats per minute, LV = left ventricle, Tau = time constant of isovolumic relaxation, Vmax = peak velocity of contractile element, + dP/dt = peak rate rise of left ventricular pressure.

ultrasound[5,15] studies have shown significant diffuse, arterial intimal thickening without visually detected focal stenoses by angiography. Furthermore, in evaluating changes in epicardial artery diameter, coronary vascular pathology may be underestimated if intimal thickening is offset by compensatory or aneurysmal medial dilatation, the so-called "Glagov phenomenon"[16]. Compensatory enlargement, however, may be less within intramyocardial arteries, presumably due to physical myocardial constraints[5]. Thus, if mean segment diameter changes detected within epicardial vessels were manifest diffusely throughout the coronary vasculature, increasing oxygen demand could be associated with progressive myocardial ischemia affecting left ventricular functional reserve.

Atrial pacing stress testing has been applied extensively in patients with coronary artery disease to measure left ventricular functional reserve[8,17,18], but not previously in patients following heart transplantation. With provoked myocardial ischemia, changes in diastolic function may precede changes in systolic function[19]. In this study, as in previous studies[17,19], changes were assessed at peak pacing with heart rates matched at initial and 1 year follow-up. Although pacing may induce changes secondary to ischemia, conditions including left ventricular hypertrophy and cardiomyopathy could also be associated with changes in systolic or diastolic parameters at rest or at peak pacing[20].

The heterogenous results of this study are consistent with the pathologic findings of Johnson, et al.[14] in that transplant coronary disease may be manifest by a spectrum of

anatomic involvement and functional changes. Small decreases in proximal epicardial artery size may be accompanied by a functional decrease in atrial pacing stress test reserve only when associated with significant small vessel coronary disease. Whether rapid atrial pacing alone can identify these patients, however, remains unclear. Complementary measurements of epicardial arterial size, intimal thickness, and reserve to functional stress testing may be necessary to fully characterize the manifestations of graft arteriopathy within a given patient or group of patients.

Our angiographic findings are similar to those of Gao and coworkers from Stanford[6] in that first year changes in coronary artery size were greater than in subsequent years although the magnitude of first year change was less in our study. Patients with known angiographically significant focal stenoses were excluded for safety reasons from follow-up atrial pacing stress testing in this group of patients with denervated hearts. This may in part account for the relatively small changes in mean coronary artery segment size observed in this 1 year follow-up study. Even in subgroups with more marked change in epicardial artery size, however, no change in myocardial reserve to atrial pacing stress testing could be detected.

Limitations

Atrial pacing, although used for many years, is not a maximal stimulus for myocardial ischemia compared to other stress tests including exercise and dobutamine infusion. However, the relative reproducibility and ease of application in the catheterization laboratory was ideal for testing the functional status of these transplant patients. Thus, pacing may be relatively insensitive to small changes in epicardial lumen diameter, but could be utilized for functional assessment of patients with focal stenoses or suspected vasculopathy involving the entire coronary vasculature.

Current methods to diagnose allograft vasculopathy.

Table 5 lists potential advantages and limitations of the major methods that have been applied to the assessment of coronary artery disease in the patient following heart transplant. Intravascular ultrasound is the most promising of newer methods that can precisely define pathologic changes within the vascular wall[5,15], but may not yet represent a mature technology for obtaining consistent, reproducible circumferential images of the intima-media interface throughout the epicardial coronary vasculature. Doppler derived coronary flow reserve[21] should correlate with changes following tachycardic or pharmacologic stress but also may be limited due to nonspecific increases in baseline flow associated with systemic hypertension or left ventricular hypertrophy[22,23]. Angioscopy may discriminate qualitatively between predominantly fibrous versus fatty plaque as well as thrombus, but may not yield quantitative data for comparative studies[24].

Table 5. Measurements available to assess transplant coronary artery disease.

Technique	Advantages	Limitations
Visual Angiographic Stenosis Sizing % or minimal lumen diameter by angiography.	* Hemodynamic significance can be assessed by conventional criteria. * Defines lesions amendable to intervention.	* Does nog detect diffuse changes.
Quantitative Coronary Angiography Mean width per vessel segment.	* Can detect diffuse changes. * Operator independent with good reproducibility.	* Cannot use as surrogate for entire coronary vasculature. * "Glagov phenomenon" may affect interpretation.
Atrial Pacing Stress Testing	* Assesses potential for ischemia throughout coronary vasculature.	* May be affected by non-coronary cardiac conditions. * Provides only a moderate stress of coronary flow reserve.
Intravascular Ultrasound Lumen diameter and intimal thickness	* Detects biologic process within epicardial arteries.	* Imaging still inconsistent in visualization of intimal-medial interface throughout coronary arterial tree.
Coronary Flow Reserve By Doppler	* Assesses potential for ischemia throughout coronary vasculature.	* May be affected by non-coronary cardiac conditions. * Sensitivity unknown.
Angioscopy	* Qualitatively characterizes endovascular pathology.	* Not suitable to quantitate arterial changes.

Conclusions

In patients following orthotopic cardiac transplantation, hemodynamics at rest and with rapid atrial pacing remain within normal limits over a 1 year time duration despite suspected development of mild coronary allograft vasculopathy. Quantitative coronary angiography

and rapid atrial pacing stress testing assess complementary aspects of allograft vasculopathy that may not necessarily be associated. From this study, the changes in QCA appear sooner and may be a more sensitive predictor of epicardial arteriopathy than atrial pacing stress testing, although it's role in defining intimal hyperplasia within the intramyocardial arteries remains undefined. Further studies will be necessary to define the relative merit of these as well as emerging technologies in the complete assessment of the patient with possible transplant coronary disease.

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CHAPTER 9

DISCUSSION

DISCUSSION

In most centers, transplant coronary artery disease has become the leading cause of death beyond the first 5 years after cardiac transplantation[1] and up to 79% of the patients have evidence of coronary disease on coronary angiograms made after 3 to 5 years[2-5]. World wide, about 40% (33% and 68% in early reports[6,7]) of the causes of death beyond the first year after cardiac transplantation are related to accelerated coronary artery disease[8]. In our experience of 293 transplanted patients, actuarial survival is higher compared to the overall survival at different transplant centers collected by the International society for Heart and Lung Transplantation[8] (Table 1). Only 24% of the deaths beyond the first year after transplantation were related to coronary artery disease. The most frequent causes of death were rapidly progressive malignant lymphoproliferative disease and other forms of cancer (39%, figure 1). The low incidence of coronary death after transplantation at our center and the relatively higher overall risk of malignancy may be related to the more intensive immunosuppression used at our center in case of an acute rejection. A reduction of the immunosuppressive regimen might result in a decrease of the incidence of neoplasia, possibly at the cost of a higher incidence of transplant coronary artery disease.

Figure 2 shows the incidence of coronary interventions, myocardial infarction and death related to accelerated coronary artery disease at our center. Retransplantation because of severe coronary disease has been performed in several centers[8,10]. We have considered this procedure since it is the only available therapy available to prolong survival in transplant recipients with severe coronary artery disease. However, we have refrained from such intervention because of the discouraging survival rates after a second cardiac transplantation[8] and the long waiting time before transplantation because of the increasing waiting list.

Progression of transplant coronary artery disease

Visual analysis of the coronary angiograms has confirmed progression of coronary

Table 1. Survival of the first 267 patients transplanted at the Thoraxcenter (June 1984-May 1996)[9], compared to the registry of the International Society for Heart and Lung Transplantation[8].

	Thoraxcenter Rotterdam	Registry
1 year	90%	79%
3 years	85%	70%
5 years	81%	64%
8 years	66%	50%

lesions in patients transplanted at our center. The prevalence of abnormalities in all coronary branches increased from 34% after 1 year to 79% after 5 years[5]. At 5 year follow-up coronary angiography 57% of the patients showed abnormal primary and secondary epicardial branches; in 21% of the patients abnormal tertiary branches were seen. Only 11% of the patients showed presumed anatomical significant lesions (more than 50% lesions in the epicardial branches or abrupt ending / proximal occlusion of tertiary branches[11]), some of which might be amendable to PTCA or CABG. In our series PTCA was performed in 9 patients. Yet, 5 of these patients died within 3 years after PTCA because of progression of coronary artery disease, as is illustrated in chapter 2.

In this thesis morphological epicardial coronary artery changes were studied by quantitative serial coronary angiography. Progression of focal coronary disease was found in 50% of the patients of whom in 18% a mixed form of progression in some and regression in other segments was shown. Regression of focal coronary artery disease was seen in 38% of the patients. No such changes were apparent in 13% of the patients (chapter 5). The "patient global score" was used to quantitatively characterize the progression of diffuse coronary artery disease. The measured changes were small in the first 6 years after cardiac transplantation (-0.05 - 0.12 mm, N=104 patients) and did not reach statistical significance (chapters 3,4 and 5). These changes were smaller in comparison with other studies (chapters 3-5). Apparently, the changes in patient global score in these studies underestimate the severity of the disease, since it is a mean value of 9 segments, particularly if intimal hyperplasia is offset by compensatory medial dilatation[12,13]. On an individual base, 20% of the patients showed progression of diffuse disease (chapter 5, table 5). In contrast, 50% of the patients showed progression of focal disease by quantitative coronary angiography, while in 48% of the patients progression of visually detectable accelerated coronary artery disease was found.

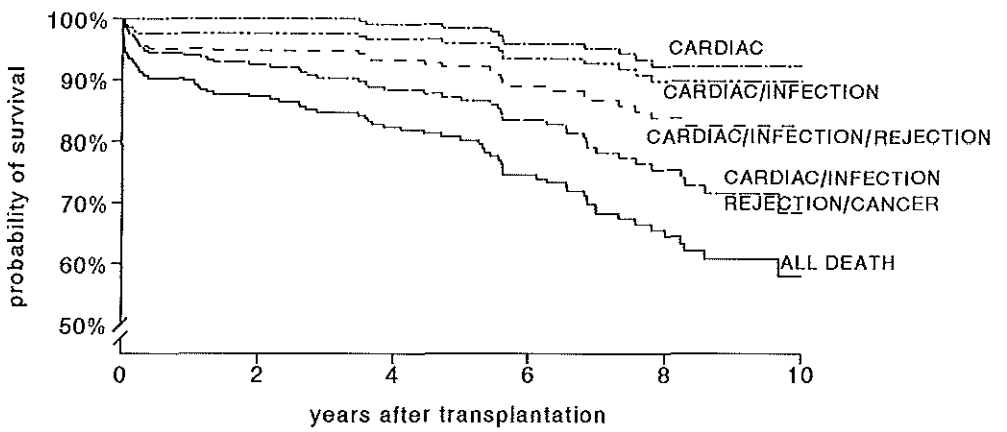

Figure 1. *Kaplan-Meier curve showing the incidence of deaths related to transplant coronary artery disease (cardiac), combined with infection, acute rejection, cancer and other causes of death at our center.*

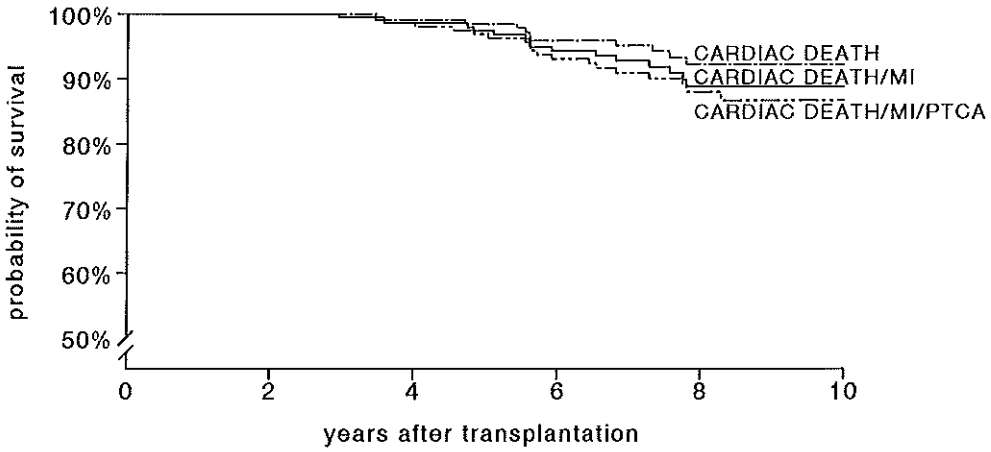

Figure 2. *Kaplan-Meier curve showing the incidence of death related to transplant coronary artery disease (cardiac death), combined with myocardial infarction (MI) and coronary interventions (PTCA) at our center.*

In addition to the description of morphological epicardial coronary artery changes, functional measurements were performed using myocardial flow reserve and atrial pacing stress testing to quantify the influence of accelerated coronary artery disease on myocardial flow and cardiac function. The measured myocardial flow reserve (MFR) was rather low. Only 14% of the measurements exceeded the normal value for MFR (Figure 1, chapter 7). Rapid atrial pacing stress tests revealed that hemodynamic characteristics of the transplanted patients at rest and during maximal atrial pacing were similar to controls and remained stable over one year (chapter 8, tables 1 and 2). Both techniques did not reveal overall progression of transplant coronary artery disease (chapters 7 and 8).

In spite of the clear progression of focal and visually detected accelerated coronary artery disease, no significant changes were seen in the overall patient global score and myocardial flow reserve measurements. Histologic studies already have shown that accelerated coronary artery disease is present in all cardiac transplant recipients as a heterogeneous phenomenon with variable distribution, morphologic features and severity[14,15]. Since the risk of coronary incidents seems to be related to the progression of focal disease (chapter 2), the patient global score and myocardial flow reserve measurements are probably not suitable to identify the patients at risk for coronary death.

Comparison with progression/regression trials

In this thesis only small changes in the mean luminal diameter were found (chapter 5, table 4), similar to the results of trials concerning the angiographic progression and regression of both focal and diffuse atherosclerosis in patients with coronary artery disease,

who were not transplanted[16-18]. For example, in the MAAS trial the influence of the lipid-modifying drug simvastatine on coronary artery disease was studied[18]. In the reference group a decrease in mean luminal diameter of 0.02 mm/year was found. In the treatment group this decrease was smaller (0.005 mm/year). In a more recent report in which the results of a number of these trials were combined, the decrease in mean luminal diameter was 0.034 mm/year in the overall reference group[19]. This thesis showed a change in mean luminal diameter from the first to the sixth postoperative year of -0.05, -0.01, -0.03, +0.01, +0.05 and +0.12 mm respectively (chapter 5, table 4). These changes are of the same order of magnitude compared to the reference group of large angiographic progression and regression trials. Therefore, we could not confirm the accelerated nature of diffuse transplant coronary artery disease in comparison with coronary artery disease in not transplanted patients. The increase in patient global score in the fifth and sixth postoperative year should be interpreted with great caution, since it is an average measurement in only 29 and 8 patients respectively.

Risk factors

In this thesis we tried to identify immunologic and non-immunologic risk factors for transplant coronary artery disease. The progression of coronary artery disease was expressed by either progression of visually detected coronary artery disease, decrease in patient global score or progression of focal coronary artery disease as measured by quantitative analysis of coronary angiograms, or decrease in myocardial flow reserve. No specific risk factors could be identified (chapters 3-5 and 7). The "beneficial effect" of smoking is probably a chance finding (chapter 5).

Different risk factors as Cytomegalo virus infections, the presence of B-cell antibodies or anti-HLA antibodies, the employed immunosuppressive regimen, elevated plasma triglycerides and higher donor age, have been suggested in several publications but could not be confirmed by others (chapter 2-5). Since the incidences of accelerated transplant coronary artery disease at the different transplant centers are based on various detection techniques and concern smaller patient groups, larger multicenter studies or registries should be performed to identify specific risk factors and investigate the influence of different treatment protocols on the process of accelerated coronary artery disease.

Prevention of accelerated coronary artery disease

One report clearly showed favourable effects of the use of the calcium-channel blocker diltiazem on the progression of coronary luminal narrowing in the first postoperative year. In the patient group which received no calcium-channel blocker (n=54), a decrease in luminal diameter of 0.22 mm was found, whereas luminal diameter did not change in the patient group receiving diltiazem (n=52)[20]. We used another calcium-channel blocker, nifedipine, for the treatment of hypertension in most patients in our series. It is uncertain

whether diltiazem and nifedipine have similar effects in patients with coronary artery disease[21-23]. Different and specific receptor sites for verapamil, dihydropyridines (nifedipine and nicardipine) and diltiazem have been identified on the cell surface, selectively binding these drugs. Verapamil enhances the receptor dependent uptake and metabolism of LDL cholesterol in arterial smooth muscle cells in vitro[24]. Nifedipine raises intracellular cholesterol hydrolysis and thus reduces cholesterol in lipid-laden cultured smooth muscle cells[25]. Furthermore, antiproliferative effects and a reduction in cell migration have been described[26]. A lowering of total and LDL cholesterol has been described using diltiazem[27]. Further studies are necessary to verify whether these findings are mutual characteristics of all calcium-channel blockers or specific properties of individual drugs.

Recently, the use of calcium-channel blockers has been the issue of much controversy, since the analysis of different trials and meta-analyses have shown contradictory results with regard to safety and efficacy of these drugs[28-30]. Review of data of clinical trials has raised concern as to whether some or all of the calcium-channel blockers could increase the risk of infarction and mortality. Due to the great variation in the design of these studies with regard to patient selection, duration of treatment and follow-up, and end-points, it remains unclear whether the positive effect of nifedipine on coronary artery disease influences the cardiovascular mortality, or whether the use of nifedipine is associated with an increase of all-cause mortality[31]. For example, in the INTACT trial a 28% reduction of new coronary lesions (0.59 lesions per patient in the treatment group versus 0.82 lesions in the placebo group) was found with an excess of 6 cardiovascular deaths among 214 patients treated with nifedipine (2.8%). Although we did not find a relation between the incidence of transplant coronary artery disease and the use of the calcium-channel blocker nifedipine in our patient group (chapters 3 and 5), the supposed anti-atherogenic effects of calcium-channel blockers can be useful to inhibit the progression of transplant coronary artery disease.

Since it has been suggested that immune-mediated phenomena may play an important role in the pathogenesis of accelerated coronary artery disease, the exact pathophysiology of the effect of calcium-channel blockers especially in cardiac transplant recipients remains unclear. Larger prospective randomized trials are necessary to determine the specific differences between the calcium-channel blockers with regard to their effect on accelerated coronary artery disease.

The lipid levels after cardiac transplantation are often elevated[32]. In our series more than 70% of the patients have a serum cholesterol above 6.5 mmol/l (chapter 5, table 2). Since angiographic progression/regression trials have shown beneficial effects of lipid lowering strategies in patients with coronary artery disease[16-19], the influence of a HMG-CoA reductase inhibitor, pravastatine, on the progression of coronary disease has been studied in cardiac transplant recipients (n=97)[33]. The pravastatine group showed less progression of maximal intimal thickness in the first postoperative year (-0.11 ± 0.09 mm v. 0.23 ± 0.16 mm, p=0.002). This effect may be a result of a decrease of the level of serum low-density lipoproteins (LDL) and therefore a reduction of LDL-bound cyclosporin, leaving a higher level of free cyclosporin[34]. Yet, in our studies we could not identify elevated lipid levels as

a risk factor for accelerated coronary artery disease. In addition to the effects of pravastatine on plasma LDL concentrations, other mechanisms may result in a beneficial effect of this drug[35]. Intravascular ultrasound was used to assess the progression of coronary artery disease in the cardiac transplant recipients versus angiography in patients with coronary artery disease. Since intravascular ultrasound studies vessel wall morphology and pathology beneath the endothelial surface whereas angiography studies the effect of atherosclerosis on the vascular lumen, a direct comparison between the study in cardiac transplant recipients and the angiographic progression and regression trials in patients with coronary artery disease is not possible. Yet, lipid-modifying strategies offer potential to reduce the progression of accelerated coronary artery disease.

Detection of transplant coronary artery disease, which technique to use?

In view of the above, a reliable assessment technique able to detect transplant coronary artery disease in an early phase, and progression over time, is needed to evaluate both the origin of this process and the effects of different treatment strategies.

The application of quantitative coronary angiography for the assessment of accelerated transplant coronary artery disease in epicardial coronary arteries is a reliable and easily applicable technique[36]. It is hampered by the fact that it's role in defining arterio-sclerosis in the small intramyocardial vessels is limited. Furthermore, this technique only assesses the intimal hyperplasia in the epicardial vessels by it's effect on luminal diameter.

Myocardial flow reserve can describe the physiological significance of transplant coronary artery disease. The number of reports describing the use of this technique in cardiac transplant recipients is limited[37,38]. Myocardial flow reserve measurements can be performed both on-line and off-line (chapter 6). Several potential methodological limitations must be considered. First of all, the small differences between patients in the different groups in our studies can be a result of a higher basal myocardial flow due to the high pacing stimulus (in our studies: mean 107 beats/min). Secondly, the MFR measurements can be influenced by the presence of visually not detected coronary fistula as a result of the large number of ventricular biopsies, performed to monitor rejection. Finally, MFR measurements may be influenced by pharmacological alterations in resting or maximal hyperaemic blood flow, despite the fact that intracoronary nitroglycerine is administered to obtain maximal epicardial coronary vasodilatation and all vasoactive medication was discontinued before the actual catheterization. The development of smaller Doppler devices and combined flow velocity and pressure gradient measurements offer potential to improve this technique[39,40].

Rapid atrial pacing is a well characterized method to assess the functional significance of coronary artery disease[41,42] and may be useful for functional assessment and possible early detection of diffuse arteriopathy after cardiac transplantation. The application of this technique in cardiac transplant recipients has not been described earlier. The changes in epicardial coronary artery size may be accompanied by a functional decrease in atrial pacing

stress test reserve, only when associated with significant changes in the small intramyocardial branches. Despite the low MFR measured in our group of patients, indicating significant disease of the intramyocardial vessels, the hemodynamics at rest and with rapid atrial pacing were similar to non-transplant controls and remained stable over 1 year, including subgroups with greater changes in epicardial luminal diameter. Therefore, the value of the atrial pacing stress test to detect the onset or progression of transplant coronary artery disease remains unclear.

Recent publications have shown the ability of intravascular ultrasound to image the coronary artery in cross section, delineate vessel wall morphology and quantitate intimal thickness in cardiac transplant recipients[43-46]. The early studies demonstrated a good reproducibility and feasibility of this technique[32]. The miniaturization of the ultrasound catheters have permitted application of these techniques during diagnostic procedures in smaller vessels. Since the remodelling capacity of the vessel wall can make coronary artery disease angiographically undetectable in the early phases of the development of focal lesions[47], intravascular ultrasound offers potential for the assessment of accelerated coronary artery disease in an early phase.

Several studies have confirmed the prognostic importance of the measurement of intimal thickening in cardiac transplant recipients[45,46]. For example, mean intimal thickness of >0.3 mm was associated with a significantly worse 4 year actuarial and cardiac survival[45]. This specific study was performed in 145 heart transplant recipients during routine coronary angiography 1 to 10 years after transplantation. However, conflicting data exist about the association of immunologic and non-immunologic risk factors and the severity of intimal thickening[45,48]. Although intravascular ultrasound offers distinct advantages over routine coronary angiography with regard to characterize changes in wall thickness, larger studies in a higher number of patients are necessary.

At our center we use the visual analysis of the coronary angiograms after the first postoperative year for clinical practice. Since there is evidence that the development of transplant coronary artery disease is related to events occurring in the first year after transplantation[20,49], we now repeat coronary angiography after one or two years in patients with visual evidence of accelerated coronary artery disease on their first year coronary angiogram. In patients with normal epicardial branches 1 year after transplantation, follow-up angiography is postponed until the fourth post-operative year. ^{99m}Tc methoxyisobutyl isonitrile (MIBI) perfusion scintigraphy is applied in patients with anatomical significant abnormalities to evaluate reversible ischemia of myocardium and thus the necessity of a coronary intervention.

Because the development of transplant coronary artery disease is related to the changes in the first postoperative year, a trial assessing the extend of coronary artery disease in the first postoperative year and at later postoperative intervals by intravascular ultrasound and coronary angiography would be useful to elucidate the development of this disease.

CONCLUSIONS

The detection and progression of transplant coronary artery disease has been the focus of this thesis. A prospective 2 year follow-up study in cardiac transplant recipients was designed to obtain a complete pattern of morphological and functional changes of the coronary artery tree during the first 6 years after cardiac transplantation. Long-term yearly follow-up angiographic studies in a large number of patients are hampered by the development of severe impairment of renal function due to the prolonged use of cyclosporin in many patients[50]. Therefore, our results represent a unique data set, covering 6 years after transplantation.

Quantitative coronary angiography measurements did not confirm an overall progression of accelerated coronary artery disease during long term follow-up. We observed a variable pattern of changes of both focal and diffuse coronary artery disease within patients as measured by quantitative coronary angiography. Therefore, global measurements in large patient groups are not useful to describe the clinical severity of accelerated coronary artery disease. The progression of focal disease appeared more useful to identify patients at risk for coronary death after cardiac transplantation. Progression was not shown in decrease of myocardial flow and function. The additional value of Myocardial Flow Reserve and Atrial Pacing Stress measurements to identify the progression of accelerated coronary artery disease in the small intramyocardial branches and patients at risk for coronary incidents was not confirmed.

No specific immunologic and non-immunologic risk factors for progression of accelerated coronary artery disease could be identified, probably as a result of the slow progression of transplant coronary artery disease in the studied group of patients. Therefore, no specific recommendations can be made with regard to the peri- and postoperative treatment regimen. As described in the literature, the use of calcium-channel blockers, lipid lowering drugs, the prophylactic use of CMV hyperimmunoglobin and the administration of a blood transfusion prior to transplantation, may have contributed to the more favourable results in our series.

In comparison with other transplant centers the number of deaths related to accelerated coronary artery disease is low in our patient group. This is consistent with the small changes in coronary diameter found in our studies.

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SUMMARY

Chapter 1

The history of cardiac transplantation is presented as well as the use of different diagnostic tools to assess the progression of accelerated coronary artery disease. In particular, the application of quantitative coronary angiography, myocardial flow reserve measurements and atrial pacing stress test are described.

Chapter 2

Progression of both diffuse and focal coronary artery disease is described in 5 patients. Progression of focal coronary artery disease in cardiac transplant recipients appears to be as rapid and unpredictable as native coronary atherosclerosis. It has an unfavourable outcome, which is illustrated in this chapter. Four of the described patients died within 3 years after a coronary intervention.

In our series of 293 patients, 9 transplant recipients underwent coronary intervention because of a significant stenosis of the coronary artery tree. Polymerase chain reaction analysis was performed in a coronary atherosclerosis specimen received by atherectomy in one of the patients. The coronary arteriosclerotic tissue of this patient showed the same alleles as the tissue of the donor. Therefore, focal lesions in cardiac transplant recipients may represent progression of preexisting donor heart lesions.

Chapter 3

Early changes in coronary luminal diameter may predict the development of accelerated coronary artery disease. Therefore the changes in coronary luminal diameter were studied in the first year after cardiac transplantation by quantitative coronary angiography in 30 patients.

The mean segment diameter of the 249 analyzed segments showed a small but significant decrease from 3.40 ± 0.82 mm at one month after transplantation to 3.36 ± 0.83 mm at one year. On a patient based patient global score 40% of the patients showed luminal narrowing, in 40% coronary luminal diameter did not change, and in 20% an increase in luminal diameter was found. These changes were small compared to those in a similar report concerning 25 patients transplanted in Stanford. In this group of patients a distinct reduction of 0.23 ± 0.19 mm was found.

No risk factors for accelerated coronary artery disease could be identified in our series.

Chapter 4

Serial one year follow-up quantitative coronary angiography was performed in 111 patients at different postoperative intervals, ranging from 1 month to 5 years after cardiac transplantation. The number of rejections and Cytomegalo-virus infections were higher in the first postoperative year. There was no significant difference between the groups for other immunologic and non-immunologic risk factors for accelerated coronary atherosclerosis.

In the 5 subgroups with different follow-up intervals 249, 227, 173, 186 and 75 segments were analyzed respectively. The largest change occurred in the first and third postoperative year (-0.05 and -0.07 mm respectively) and proved to be statistically significant. Patient global score did not show significant changes.

Since serial quantitative coronary angiography can be an objective method to assess the incidence of transplant coronary artery disease, larger studies are proposed to investigate the influence of different treatment protocols on the process of accelerated coronary artery disease.

Chapter 5

In 104 patients the changes in coronary luminal diameter at different postoperative intervals were described in a 2 year follow-up study. The progression of both diffuse and focal coronary artery disease was evaluated using the patient global score and the minimal luminal diameter per segment respectively.

There was a trend towards narrowing of mean diameter in the first 3 years, and widening in subsequent years. These changes were not statistically significant. The changes in patient global score were equally distributed in patients with and without visually detected progression of accelerated coronary artery disease. Progression of focal disease was found in 33 patients. Thirty-nine patients showed regression of focal disease. A mixed form was found in 19 patients and in 13 patients no changes were seen.

Not-smoking was identified as an independent predictor of both diffuse and focal coronary artery disease by multivariate analysis using multiple logistic regression. This may be a chance finding.

Chapter 6

The concept of coronary flow reserve (CFR) has been developed to evaluate the relationship between the angiographic severity of coronary artery disease and the resulting reduction of maximal coronary blood flow in the myocardium. In the absence of focal arteriosclerosis these measurements can be used to evaluate dysfunction of the myocardium. Since the introduction of digitized facilities in catheterization laboratories CFR can be

performed both on- and off-line.

In 18 cardiac transplant recipients both systems were compared. A total of 68 regions of interest (ROI's) were analyzed using both techniques. The linear regression analysis revealed a good correlation between both systems ($y = 0.37 + 0.88x$, $r = 0.82$, SEE = 0.56). According to the approach of Altman and Bland, the mean difference between the measurements performed by both methods was 0.28 ± 0.84.

The off-line technique can be used to assess CFR in large (multicenter) trials, where selection of ROI's can be carried out by an independent analyst.

Chapter 7

Myocardial flow reserve measurements (MFR) were performed in 99 patients at different postoperative intervals to monitor the presence and progression of accelerated coronary artery disease. Serial follow-up measurements of both quantitative coronary angiography and MFR were obtained during 77 procedures.

The MFR in this studied group of patients was low, only 14% of the measurements exceeded the normal value of MFR (3.4). No significant decrease was found during long-term follow-up. No correlation was found between the changes in patient global score and MFR using linear regression analysis ($y = 0.02 - 1.44\ x$, $r = -1.75$, SEE = -0.18). Subgroup analysis for ROI's in areas of the myocardium perfused by either the left anterior descending branch or the circumflex branch of the left coronary artery, did not improve this relation.

No relation was found between the presence of visually detectable coronary artery disease and changes in MFR ($p=0.48$). Risk factors for accelerated coronary artery disease were not identified in a logistic regression model using a decrease in MFR of more than 0.8 as a threshold to define the development of diffuse coronary artery disease.

Chapter 8

Atrial pacing stress tests were studied in 97 patients following cardiac transplantation. The hemodynamic measurements at rest and at maximal pacing were heterogeneous with no identifiable trends. Only the resting heart rate was higher as compared to controls (93 ± 12 beats per minute versus 68-80 beats per minute respectively).

Neither in patients studied over the first postoperative year nor in patients with >0.22 mm change in patient global score, changes in baseline or rapid pacing hemodynamic measurements were detected.

In our experience only 24% of the deaths beyond the first year after transplantation are related to transplant coronary artery disease. Visual analysis of coronary angiography has confirmed progression of coronary lesions at our center. The use of quantitative coronary angiography, myocardial flow reserve measurements and atrial pacing stress tests in cardiac transplant recipients is discussed.

Progression of focal coronary disease was found both by quantitative and visual analysis of the coronary angiograms. The patient global score was used to describe the progression of diffuse disease. The changes in patient global score were small and of the same order of magnitude compared to the reference groups in large angiographic progression and regression trials. The additional value of MFR measurements and atrial pacing stress tests was not shown. Since the risk of coronary events seems to be related to the progression of focal disease, the patient global score and MFR measurements are probably not suitable to identify the patients at risk for coronary events. Intravascular ultrasound studies offer potential to quantitate intimal thickness in cardiac transplant recipients and thus describe the progression or regression of accelerated coronary artery disease in more detail in future studies.

No immunologic or non-immunologic risk factors for accelerated coronary artery disease could be identified using the described techniques. The use of calcium-channel blockers, lipid lowering drugs, the prophylactic use of CMV hyperimmunoglobin and the administration of a blood transfusion prior to transplantation, may all have contributed to the more favourable results in our series.

In view of our results no specific recommendations could be made concerning the prevention of accelerated coronary artery disease. Further evaluation in larger patient groups remains necessary to verify these findings.

SAMENVATTING

Hoofdstuk 1

Versnelde coronaire atherosclerose is een van de problemen die optreden na een orthotope harttransplantatie. In dit hoofdstuk worden verschillende methoden (kwantitatieve coronaire angiografie, coronaire flow reserve en atrium-pacing-stress-test) beschreven die gebruikt kunnen worden om progressie van deze ziekte vast te stellen.

Hoofdstuk 2

Progressie van zowel diffuse als focale atherosclerose wordt beschreven bij vijf patiënten. Progressie van focale atherosclerose bij harttransplantatie patiënten lijkt even snel en onvoorspelbaar op te treden als atherosclerose bij niet-getransplanteerde patiënten. De ernst van de ziekte wordt geïllustreerd door de beschreven patienten. Vier van hen overleden binnen 3 jaar na een coronaire interventie.

In Rotterdam zijn inmiddels 293 patiënten getransplanteerd, waarvan 9 patiënten een coronaire interventie ondergingen wegens een significante stenose van één of meer kransslagaderen. Bij één van de patiënten werd de herkomst van de gladde spiercellen in een primaire coronaire vernauwing onderzocht. De studies werden verricht op DNA, dat geïsoleerd werd uit een atherectomie fragment. De resultaten van DNA fingerprinting bevestigden dat de atherosclerose voornamelijk bestond uit gladde spiercellen afkomstig van de donor. Focale vernauwingen na een harttransplantatie kunnen derhalve ontstaan door progressie van reeds bestaande donor atherosclerose.

Hoofdstuk 3

De veranderingen van de coronaire diameter in het eerste post-operatieve jaar werden bestudeerd bij 30 patiënten, omdat beschreven is dat veranderingen in coronaire diameter in het eerste jaar na een harttransplantatie de ontwikkeling van versnelde atherosclerose zouden kunnen voorspellen.

De gemiddelde diameter van de 249 segmenten was 3.40 ± 0.82 mm één maand na transplantatie en toonde een geringe doch significante vermindering resulterend in een gemiddelde diameter van 3.36 ± 0.83 mm één jaar na transplantatie. Op patiënt basis (patient global score) toonde 40% van de patiënten een afname van gemiddelde coronaire diameter. Bij 40% van de patiënten werd geen verandering gevonden. Twintig procent van de patiënten toonde een toename van de coronaire diameter.

In vergelijking met een publikatie uit Stanford waren deze veranderingen klein. Bij 25 patiënten vond deze groep een vermindering van de gemiddelde coronaire diameter van 0.23 ± 0.19 mm in het eerste postoperatieve jaar.

Er werden geen risicofactoren voor versnelde coronaire atherosclerose gevonden.

Hoofdstuk 4

Bij 111 patiënten werd seriële 1-jaars follow-up kwantitatieve coronair angiografie verricht op verschillende tijdstippen na de transplantatie. De groepen verschilden onderling niet voor wat betreft immunologische en niet-immunologische risicofactoren voor versnelde coronaire atherosclerose. Het aantal afstotingen en Cytomegalo-virus infecties was, zoals verwacht, hoger in het eerste post-operatieve jaar.

In de 5 verschillende subgroepen werden respectievelijk 249, 227, 173, 186 en 75 segmenten geanalyseerd. De grootste vermindering van coronaire diameter werd in het eerste en derde post-operatieve jaar gevonden (respectievelijk -0.05 en -0.07 mm). Deze veranderingen bleken statistisch significant. Op patiënt basis werden tijdens de studieperiode geen veranderingen in coronaire diameter gevonden.

Geconcludeerd werd dat grotere studies noodzakelijk zijn om met behulp van kwantitatieve coronair angiografie de invloed van verschillende behandelingsprotocollen op het proces van versnelde coronaire atherosclerose vast te stellen.

Hoofdstuk 5

Een twee-jarige follow-up studie, waarin de verandering in coronaire diameter op verschillende post-operatieve tijdsintervallen werd beschreven, werd uitgevoerd bij 104 patiënten. De progressie van diffuse coronaire atherosclerose werd onderzocht door middel van de "patient global score". Progressie van focale coronaire atherosclerose werd onderzocht door gebruik te maken van de minimale coronaire segment diameter.

Deze studie toonde een trent waarbij de gemiddelde diameter in de eerste drie jaren na transplantatie een afname vertoonde. In de daarop volgende jaren werd een niet-significante toename gevonden. De individuele veranderingen in coronaire diameter waren gelijkelijk verdeeld over patiënten met en zonder visuele toename van coronaire atherosclerose. Progressie van focale ziekte werd gevonden bij 33 patiënten. Regressie werd gevonden bij 39 patiënten. Negentien patienten toonden enerzijds progressie en anderzijds regressie in verschillende segmenten. Bij 13 patienten werden geen veranderingen gevonden.

De toename van zowel focale als diffuse coronaire atherosclerose bleek gerelateerd te zijn aan het rookgedrag van de individuele patiënt. Dit is waarschijnlijk een toevalsbevinding.

Hoofdstuk 6

Bepaling van de coronaire doorstromingsreserve is een techniek die ontwikkeld is om de relatie tussen de angiografische ernst van de vernauwing van de kransslagaderen en de als gevolg daarvan optredende vermindering van bloeddoorstroming van het myocard te beschrijven. Indien geen focale atherosclerose aanwezig is, kan deze techniek worden gebruikt om dysfunctie van de capillaire doorstroming van het myocard te evalueren. Deze techniek kan zowel tijdens als na de angiografie worden verricht (on-line en off-line).

Een vergelijkende studie tussen beide systemen werd verricht bij 18 getransplanteerde patiënten. In totaal 68 regio's van het myocard werden geanalyseerd. Lineaire regressie analyse toonde een goede relatie tussen beide technieken (y=0.37 + 0.88x, r=0.82, SEE=0.56). Met behulp van de statistische analyse techniek, zoals beschreven door Altman en Bland, werd een gemiddeld verschil in de resultaten van beide technieken gevonden van 0.28 ± 0.84.

Geconcludeerd werd dat de off-line techniek een goede mogelijkheid bied om coronaire doorstromingsreserve te bestuderen in grote studies.

Hoofdstuk 7

Het proces van versnelde coronaire atherosclerose werd geëvalueerd bij 99 patiënten door middel van coronaire doorstromingsmetingen op verschillende tijdsintervallen na een harttransplantatie. Volledige gegevens van zowel seriële kwantitatieve metingen van coronaire diameter en coronaire doorstromingsreserve waren beschikbaar bij 77 procedures.

De gemiddelde coronaire doorstromingsreserve in de studie groep was laag en er werden geen significante veranderingen gevonden gedurende lange termijn follow up. Er werd geen relatie gevonden tussen de veranderingen in individuele diameter en coronaire doorstromingsreserve met behulp van lineaire regressie analyse (y = 0.02 - 1.44x, r= -1.75, SEE = -0.18). Specifieke analyses van de coronaire doorstromingsreserve in zowel het doorbloedingsgebied van de LAD als RCX gaven geen verbetering van deze resultaten.

Er bleek geen relatie te bestaan tussen de visueel aanwezige versnelde coronaire atherosclerose en de resultaten van veranderingen in coronaire doorstromingsreserve. Er werden geen risicofactoren voor het ontstaan van versnelde coronaire atherosclerose geïdentificeerd.

Hoofdstuk 8

Bij 97 patiënten werden atrium-pacing-stress testen verricht. De haemodynamische metingen toonden een heterogene verdeling, zowel in rust als bij maximale pacing. In vergelijking met een controlegroep van niet-getransplanteerde patiënten werd een hogere basis hartfrequentie gezien (respectievelijk 68 - 80 slagen per minuut en 93 ± 12 slagen per

minuut).

Zowel bij patiënten waarbij de metingen werden verricht in het eerste post-operatieve jaar als bij patiënten met een significante vermindering in individuele coronaire diameter werden geen veranderingen in haemodynamische metingen vastgesteld.

Hoofdstuk 9

Van de mensen die getransplanteerd zijn op het Thoraxcentrum en overlijden langer dan 1 jaar na de transplantatie, overlijdt slechts 24% aan de gevolgen van versnelde coronaire atherosclerose. Visuele beoordeling van de coronair angiogrammen heeft de toename van lesies bevestigd. Een vergelijking wordt gemaakt tussen het gebruik van kwantitatieve coronair angiografie, coronaire flow reserve en atrium-pacing-stress test.

Met behulp van visuele analyse en quantitatieve analyse werd een toename van focale lesies gevonden. De veranderingen in "patient global score", als maat voor diffuse atherosclerose waren van dezelfde orde van grootte als de veranderingen die gevonden worden in progressie/regressie studies. Coronaire doorbloedingsmetingen en atriale-pacing-stress testen gaven geen additionele informatie.

Risicofactoren voor versnelde coronaire atherosclerose konden met behulp van ons onderzoek niet worden vastgesteld. Het gebruik van calcium-antagonisten, CMV antilichamen en pre-operatieve bloedtransfusies kunnen hebben bijgedragen tot de gunstige resultaten in onze patiëntengroep.

In het licht van onze resultaten kunnen geen specifieke aanbevelingen worden verricht voor voorkoming van versnelde coronaire atherosclerose. Nadere evaluatie in grotere groepen patiënten is aan te bevelen om deze bevindingen te verifiëren. Intravasculaire echografie is mogelijk een nieuwe techniek die de toename van de intima-dikte van de coronair arteriën beter kan beschrijven.

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CURRICULUM VITAE

Marc van der Linden werd geboren op 18 augustus 1964 te Bergen op Zoom. In 1982 behaalde hij het Gymnasium diploma aan het Juvenaat Heilig Hart te Bergen op Zoom. In hetzelfde jaar werd begonnen aan de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. In 1987 werd het doctoraal examen afgelegd en in 1989 het artsexamen. Zijn afstudeeronderzoek, onder leiding van dr. A.H.M.M. Balk, betrof de factoren die de inspanningstolerantie na harttransplantatie bepalen.

Na zijn militaire dienst werkte hij in het Thoraxcentrum van het Academisch Ziekenhuis Rotterdam-Dijkzigt aan het onderzoek "Ontwikkeling van coronair afwijkingen na harttransplantatie", gesubsidieerd door de Nederlandse Hartstichting, welke te grondslag ligt aan dit proefschrift (promotor: Prof. Dr. M.L. Simoons). Sinds januari 1994 is hij in opleiding tot cardioloog (opleider: Prof. Dr. J.R.T.C. Roelandt) en volgde daartoe de 2 jarige stage Inwendige Geneeskunde in het Merwede Ziekenhuis te Dordrecht (opleider: Dr. J. van der Meulen). Sinds 1996 wordt de opleiding vervolgd aan het Thoraxcentrum te Rotterdam.

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