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A QUANTITATIVE ANGIOGRAPHIC STUDY OF CORONARY ARTERIAL DISEASE IN THE TRANSPLANTED HUMAN HEART.

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ABSTRACT

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A QUANTITATIVE ANGIOGRAPHIC STUDY OF CORONARY ARTERIAL DISEASE IN THE TRANSPLANTED HUMAN HEART.

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This study examined the early phase transplant coronary artery disease (TxCAD), as measured by the quantitative coronary angiography (QCA) mean lumen diameter loss (MLDL). A comparison was made between the large primary versus the smaller branch vessels. The relationship of different non-immunological and immunological factors to the development of TxCAD was examined, and we correlated the QCA data to that obtained from intracoronary ultrasound (ICUS). 121 patients who were transplanted between September 1994 and June 1999 were studied and followed up for a period of one to five years. 103 patients were males (85%) and the mean age was 48.5±10 years.

We found that TxCAD was predominantly a disease of the large vessels, as evidenced by greater MLDL in the first years after transplant. The MLDL increased with time in both the large and small vessels and the greatest loss occurred in the first year. The first year MLDL was a predictor of long-term MLDL. The relative changes in lumen diameter measured by QCA and ICUS were similar. A low early left ventricular echocardiographic ejection fraction was related to greater MLDL in the large vessels as was donor male sex. Domino and non-domino hearts did not differ in the long-term MLDL. Total ischaemic time, RATG induction of immunosuppression, baseline vessel tone, acute rejection, CMV infection, total number of HLA mismatches and the first year mean antivimentin antibodies level, were not related to MLDL, nor was the baseline right and left heart catheter haemodynamic data.

In conclusion, TxCAD as measured by QCA-MLDL was predominantly a disease of the large epicardial vessels and was predicted by a low early echocardiographic ejection fraction. Both QCA and ICUS could be used to assess TxCAD and their measurements were well correlated.

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STATEMENT OF PERSONAL CONTRIBUTION.

Quantitative coronary angiography started to be performed in heart transplant patients at Harefield Hospital by Dr Nicholas Banner, in the late 1994. The main aim was to eventually create a database and perform a follow-up study to evaluate the changes in the coronary vasculature with time.

When I joined Harefield Hospital in 1998, I was inspired and motivated by Dr. Banner's work. I carried on performing quantitative coronary angiography on the transplant patients and performed the follow up. I performed the quantitative angiographic analysis and analysed the haemodynamic data. I performed the data collection and collected and analysed the intracoronary ultrasounds that were performed on some of these patients. Immunological tests were gratefully performed by the immunologists at Harefield Hospital.

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Finally, immense appreciation goes to my wife Linda, and my parents, for their understanding and support in tolerating my absence from many domestic activities during the compilation of this thesis.

ABBREVIATIONS

AECA:	Antiendothelial cells antibodies.
AMI:	Acute myocardial infarction.
ANOVA:	Analysis of variance.
BP:	Blood pressure.
CF:	Calibration factor.
CMV:	Cytomegalovirus.
CO:	Cardiac output.
CT:	Computed tomography.
Cx:	Circumflex artery.
D1:	First diagonal artery.
D2:	Second diagonal artery.
DSE:	Dobutamine stress echo
EF:	Ejection fraction.
FS:	Fractional shortening.
HDL:	HDL cholesterol.
HLA:	Human leucocyte antigen.
IA:	Intimal area.
ICUS:	Intracoronary ultrasound.
II:	Intimal index.
ISHLT:	International society for heart and lung transplantation.
LAD:	Left anterior descending artery.
LCSA:	Lumen cross-sectional area.
LDL:	LDL cholesterol.

LV: Large vessels.

LVEDP: Left ventricular end diastolic pressure.

LVEF: Left ventricular ejection fraction.

MHC: Histocompatibility gene complex.

MIT: Maximal intimal thickening.

MLD: Mean lumen diameter.

MLDL: Mean lumen diameter loss.

MM: Mismatch / mismatches.

NS: Not significant.

OM1: First obtuse marginal artery.

OM2: Second obtuse marginal artery.

PAMP: Pulmonary artery mean pressure.

PAWP: Pulmonary artery wedge pressure.

PBS: Phosphate buffer saline.

PDA: Posterior descending artery.

PET: Positron emission tomography.

PRA: Panel reactive antibodies.

QCA: Quantitative coronary angiography.

QCU: Quantitative coronary ultrasound.

RAP: Right atrial pressure.

RATG: Rabbit antithymocyte globulin.

RCA: Right coronary artery.

RI: Remodelling index.

RVBr: Right ventricular branch of right coronary artery.

SD: Standard deviation.

SIP:	Scion image programme.
SV:	Small vessels.
TA:	Total area.
TC:	Total cholesterol.
TG:	Triglycerides.
TIT:	Total ischaemic time
TxCAD:	Transplant coronary artery disease.

CHAPTER 1

INTRODUCTION

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1.1 Background.

Heart transplantation has become established as the treatment of choice for selected patients with advanced heart failure, as the survival rates achieved with transplantation[1] greatly exceeds that achieved with optimum medical therapy[2, 3]. Nevertheless, the long-term results of heart transplantation are far from perfect. The Registry of the International Society of Heart and Lung Transplantation (ISHLT)[1] indicates that the overall patient half-life (time to 50% survival) is only 9.3 years and the conditional half-life (time to 50% survival for patients who survive $\geq$ 1 year) is only 11.8 years. After the first year, there is a constant mortality rate of 4% a year. The leading causes of death more than one year after transplantation are coronary artery disease, infection and malignancy[1, 4], with transplant coronary artery disease being the most common cause of death and re-transplantation[5, 6].

Transplant coronary artery disease (TxCAD) was first reported in animal heart transplant models. After their successful orthotopic transplant in a dog in 1961[7] , Lower et al[8] in 1968 drew attention to the proliferative and obliterative intimal changes of chronic vascular rejection in the epicardial coronary arteries of long surviving canine transplants.

Following the first human heart allograft by Christiaan Barnard[9] in December 1967, Thomson[10] in 1968 gave the first description of the pathologic findings in a transplanted human heart, that of Barnard's patient, who had survived the operation for 18 days. The first description of a human heart with the obliterative vascular changes that characterize chronic rejection appeared in 1969, when Thomson described the donor heart of a transplanted patient who had survived for 19 ½ months in the first

long-term survivor reported from South Africa[11]. That patient died with extensive coronary artery disease after transplantation for ischaemic cardiomyopathy. Thereafter, detailed reports appeared on the cardiac findings and complications in a series of orthotopic transplants in the early 1970s[12-18].

A number of important questions about transplants, including protocols for immunosuppression[19], correlation of the surface electrocardiogram with allograft rejection[20], and reversal of these changes with augmented immunosuppression, were subjects of the early laboratory study. The first heart transplant initiated a great amount of interest in other centres around the world. However, the one-year survival was only 15 percent, and as a result, the enthusiasm for heart transplantation had waned by the end of 1971. Through the decade of the 1970s, investigators refined recipient selection criteria[21], developed transvenous endomyocardial biopsy for diagnosing rejection[22], developed rabbit antithymocyte globulin as an effective treatment of acute rejection[23], and defined many of the late post operative complications and management principles[24]. Application of heart transplantation depended on the development of better immunosuppressive therapy; this was achieved with the discovery that ciclosporin A (formerly Cyclosporin A), a peptide of fungal origin, which selectively blocks the early phase of T-cell activation and the expression of interleukin 2 [25-28].

With improved outcomes in terms of both quality of life and patient survival, cardiac transplantation became the accepted therapy for many patients with end stage heart failure. Survival of 79% after transplantation at one year[1] compares favourably with survival from heart failure at one year of 57% in men and 64% in women[3]. Nevertheless, heart transplant long-term results are still far from perfect.

Transplant coronary artery disease assumes greater importance as the numbers of long-term survivors after transplantation increases. This chapter provides an overview of this clinical problem as a framework for the clinical, pathological and diagnostic aspects of TxCAD.

1.2 Incidence.

The reported incidence of TxCAD varies with the sensitivity of the method used for diagnosis. Using conventional qualitative coronary angiography, the incidence was found to be 11 % at one year and 40% at 5 years in the Canadian Study of Cardiac Transplantation Atherosclerosis[29]. In a multiinstitutional study using qualitative angiography, Constanzo et al[30] found the incidence to be 42% at 5 years.

Using more sensitive technique of intracoronary ultrasound (ICUS), the incidence was generally found to be higher than that of conventional qualitative angiography. Yeung et al[31] found in a multicentre trial the incidence to be 40% within the first 4 weeks post transplantation, and 75% at one and 2 years. Julius et al[32] found the incidence to be 43, 64 and 58% at 3 months, 1 and 2 years respectively.

Examination of the coronary arteries at autopsy shows that TxCAD is much higher than that detected by either of these methods. In one series, Johnson et al[33] found coronary lesions in 100% of patients who died more than 1 year after transplantation, even when death was not directly related to a cardiac event. In another series[34], he found the incidence to be 89% in a study of 61 cardiac allografts examined 1 day to 11.9 years after transplantation.

1.3 Pathology.

The pathologic findings in the coronary arteries of the human cardiac allografts show a wide spectrum of disease features which are not typical of conventional coronary atherosclerosis [34, 35]. On the basis of both light microscopic appearance and distribution of the coronary lesions in 61 human cardiac allografts of short and long-term survival, Johnson et al[34] showed that TxCAD is a heterogeneous phenomenon with variable distribution, morphologic features and severity. Allografts were divided into two broad groups: those with fibrous or atherosclerotic lesions confined to the proximal region of epicardial arteries and those with diffuse necrotizing vasculitis or atherosclerosis of the entire coronary arterial system. Within the two groups, coronary artery morphologic features varied in a time-dependent fashion. Disease in the proximal region began as concentric fibrous intimal thickening, with atheromatous lesions observed after 1 year of transplantation. The earliest form of diffuse disease was a necrotizing vasculitis, which was invariably associated with acute myocardial rejection. Long-term survivors with diffuse disease showed severe fibrous or fibrofatty intimal lesions of the large and small epicardial and intramyocardial arteries. In some, diffuse disease may have resulted from healing of necrotizing vasculitis.

Arbustini et al[36] investigated at autopsy or at re-transplantation the frequency of coronary thrombosis in 76 cardiac allografts: 37 in place $\leq$ 2 months (early) and 39 in place 2 to 99 months (late). They found that coronary thrombosis was common in epicardial coronary arteries > 2 months after cardiac transplantation, as it was found only in one allograft in the early group and 24 (62%) of the late allografts (32±30 months), all of the late allografts had TxCAD.

1.4 Angiography.

The angiographic features of coronary disease in the transplanted heart are variable and include features that are unusual in the arterial disease within the native heart. Gao et al[37] in an angiographic study in 81 transplant patients exhibiting coronary vascular disease, had classified the lesions into three categories: type A, discrete or tubular stenoses; type B, diffuse concentric narrowing; and type C, narrowed irregular vessels with occluded branches. The 81 arteriograms showing transplant coronary vascular disease were contrasted with 32 from non transplant patients with coronary artery disease analysed in a similar fashion. The results are shown in Table 1.1 below.

Table 1.1: Angiographic features of non-transplant vs. transplant coronary artery disease.

Parameter	Non-TxCAD (32 arteriograms, 178 lesions)	TxCAD (81 arteriograms, 461 lesions)	p-value
Lesion type	A- 100%	A- 76% B- 24%	
Vessels involved	Primary-75% Secondary-25%	**Type A:** Primary- 57% Secondary- 42% Tertiary- 1.4% **Type B:** Primary- 25% Secondary- 44% Tertiary- 31%	0.05
Total occluded vessel segment	Proximal & Middle- 96% Distally 4%	Proximal & Middle- 51% Distally- 49%	<0.002
Poor collaterals in presence of total vessel occlusion	7%	92%	<0.002

Quantitatively, TxCAD presents as progressive coronary luminal narrowing after cardiac transplantation. Gao et al[38] assessed 25 patients after a mean of 5.1 weeks post-

transplantation and annually thereafter. Five hundred and fifteen coronary segments in the 25 patients having 1-year follow-up and 353 segments in 18 patients reaching 2-years follow-up were compared with baseline angiograms. Significant change was defined as ± 0.10 mm, equal to 3.8% change in mean lumen diameter (MLD) based on three standard deviations obtained from estimation of measurement error. Coronary MLD fell from 2.44 ± 0.26 mm at baseline to 2.21 ± 0.34 mm ($p<0.001$) at 1-year follow-up. This rate of mean lumen diameter loss (MLDL) was 20 fold more rapid during the initial post-transplantation year than the rate of change of visually normal segments in non-transplant patients with coronary atherosclerosis elsewhere. There was no significant MLDL between the first and second year in those patients who had second year studies. Absolute MLDL for vessels greater than 2.9 mm significantly exceeded that for smaller vessels but did not differ when considered as a ratio of vessel diameter. In 21 of the 25 patients MLDL exceeded the three standard deviation threshold at their last angiogram, but only two of these patients had visually detectable TxCAD.

1.5 Clinical Features.

The clinical presentation of TxCAD is different from coronary disease in the general population. It is often clinically silent because the allograft is denervated and transplant patients, lacking afferent pain fibres, do not appear able to experience angina pectoris. However, reinnervation starts to occur within the first year, and increases with time[39], and some patients experience chest pain characteristic of angina pectoris[40].

The presentation of acute myocardial infarction (AMI) is also different from that in the general population. Gao et al[41] examined the clinical presentation of 25 episodes of AMI in 22 transplant patients. AMI occurred at a mean of 3.86 years post transplant. The commonest symptom was fatigue and weakness (64%), followed by dyspnoea (44%), palpitations (32%), dizziness (28%), diaphoresis (24%), nausea and vomiting (20%), syncope (16%), chest or arm pain (12%) and paresthesia (8%). The infarct was clinically silent in three episodes (12%). The commonest sign was a new S3 (32%), followed by cyanosis, confusion and pulmonary oedema (each accounting for 12%), liver enlargement (8%) and new S4 (4%). ECG changes typical of Q wave AMI was present in 7 of the 18 patients hospitalised for symptoms (39%). Five patients had non-specific ST segment changes (28%) and 2 had no documented changes (11%). Two had old Q waves (11%).

Myocardial damage and fibrosis lead to deteriorating cardiac function and present non-specifically as effort intolerance, exertional dyspnoea and fatigue. Overt congestive cardiac failure may occur due to systolic or diastolic ventricular dysfunction[42, 43]. Arrhythmia, bradycardia and sudden death can occur from damage and fibrosis to the conducting system and / or the myocardium[44, 45].

Coronary artery spasm is known to occur in transplanted hearts. It commonly occurs during catheterisation of the coronaries[46-48]. It has also been reported to occur spontaneously[46, 49]and during exercise testing[46]. Clinically, coronary spasm can present with syncope[50 46], hypotension or chest pain. The latter might not occur due to cardiac denervation.

1.6 Prognosis.

The development of epicardial coronary disease in the transplanted heart conveys a poor prognosis[42, 51, 52]. The relative risk of cardiac events is 3 times higher and the relative risk of cardiac death is 4.6 times higher in recipients with angiographic evidence of TxCAD than in those without[42]. Keogh et al[51]found that the actuarial survival 5 years after the detection of moderate or severe angiographic disease was only 17%. Disease that develops early after transplantation appears to be more aggressive and to be linked to a worse prognosis[53, 54].

1.7 Diagnosis of Transplant Coronary Artery Disease.

As early TxCAD is asymptomatic due to denervation, and as the late presentation is usually by a catastrophic event such as myocardial infarction or sudden death[41], it is essential to screen patients regularly for TxCAD. The ultimate clinical goal of any test is to detect TxCAD and to provide prognostic information to identify those patients who might benefit from any available intervention. Specifically, the test should be able to identify both the anatomic, and more importantly, the physiologic consequences of the disease process in both the large epicardial arteries and the microcirculation. For convenience, these tests can be divided into non-invasive and invasive tests, and each has its merits and limitations.

1.7.1 Non-invasive tests.

Non-invasive tests are attractive because of their low morbidity and potential ability to supply physiologic information. Many of the non-invasive techniques to assess atherosclerotic coronary artery disease in the non-transplant patient have been applied

to the cardiac transplant population, including exercise ECG, stress echocardiography, stress myocardial scintigraphy, positron emission tomography and ultrafast computed tomography.

1.7.1.1 Exercise electrocardiography.

Interpretation of exercise electrocardiographs in transplant patients is difficult. This is because the baseline ECGs are abnormal in the majority of transplant patients and that make the interpretation of stress-induced changes less sensitive and specific[55]; in a study of non-invasive means to diagnose TxCAD, Mairesse et al[56] found that only 59% of the exercise electrocardiograms are interpretable for ischaemia. Absence of angina in the great majority of transplant patients further reduces the sensitivity, as does the inability to achieve the desired target heart rates required for adequate exercise stress testing[57, 58]. However, development of hypotension during stress testing was found to be quite specific for focal grafts coronary artery disease and predicts future cardiac events[59, 60].

1.7.1.2 Radionuclide scintigraphy.

Thallium-201 and technetium-99 are well established in the diagnosis of coronary artery disease in non-transplant patients[55, 61]. This allows imaging of the regional distribution of myocardial perfusion, as these radiopharmaceuticals accumulate proportional to regional myocardial blood flow. Single photon emission computed tomography[62] (SPECT) has improved the diagnostic accuracy. These perfusion tracers have also been coupled to vasodilatory (i.e. dipyridamole) and inotropic (i.e. dobutamine) pharmacologic stress agents.

Smart et al[63] and Redonnet et al[64] studied the role of dipyridamole scintigraphy, which is not dependent on heart rate response for effect, in cardiac transplant recipients. This had moderate specificity (64-84%) for TxCAD when both fixed and reversible defects were considered. However, it lacked the sensitivity (21-58%) when rather more conservative angiographic criteria were used as the gold standard (i.e. epicardial coronary narrowing >50%). Exercise protocols, and the inclusion of angiographically diffuse distal stenosis into the definition of TxCAD, appears to improve both the sensitivity (67-77%) and specificity (100%) of myocardial perfusion imaging[65, 66]. However, inadequate heart rate response in these patients limit the sensitivity[58]. Thus, myocardial scintigraphy has good specificity but low sensitivity in non invasive detection of TxCAD.

1.7.1.3 Stress echocardiography.

Stress echocardiography has been shown to have good sensitivity and specificity in diagnosing TxCAD[55, 67-69], especially when dobutamine is used as the stressing agent. Dobutamine has been a particularly useful stressing agent because it increases contractility, heart rate and wall stress in a graded dose-dependant fashion and likely reflects the cumulative effects of both epicardial and small vessel TxCAD in a physiological manner. In addition, dobutamine improves the poor sensitivity of exercise protocols because the heart rate response is augmented and the transplant heart is more sensitive to catecholamine stimulation than the non-transplanted hearts[70].

Compared to the sensitivity of scintigraphy, that of dobutamine echo of 79-95% is better[68, 71], even when the definition of TxCAD is more liberal and expanded to include angiographic stenosis of <50%, diffuse distal tapering or intimal thickening by

intracoronary ultrasound. However, there appears to be some loss of specificity (55-91%) with this increase in sensitivity in studies using qualitative angiography as the reference method[67, 68]. This loss may be due to the insensitivity of conventional (qualitative) angiography in the detection of coronary disease[55]. If significant ultrasound intimal thickening (Stanford grade III or IV; see table 1.2) is used as the standard, dobutamine stress echocardiography is quite specific[71]. Angina when it occurs during this test is also quite specific for TxCAD and probably reflects re-innervation of the transplanted heart[72].

Dobutamine echocardiography also has prognostic value in this population. Akosah et al[73] found that a positive test (new or worsening regional wall motion abnormality or failure of augmentation) was associated with 33% chance of a cardiac event (myocardial infarction, angina or heart failure) over a 2 year period. The most severe abnormalities were associated with a risk ratio of six (confidence interval 2.3 to 14.4) for a cardiac event.

In summary, because stress echocardiography, especially with dobutamine, has good sensitivity and specificity, it can be used as both a screening test and as an adjunct to angiography in the management of TxCAD.

1.7.1.4 Positron emission tomography.

Positron emission tomography (PET) represents an advanced form of nuclear imaging technology[74]. It has become established as the most accurate non-invasive means for the diagnosis of coronary artery disease in non-transplant patients using myocardial perfusion radiotracers, which include rubidium-82, N-13-ammonia, and O-15-water.

Studies in cardiac transplant recipients have demonstrated that resting myocardial blood flow does indeed decrease with time and this decrease is more profound when angiographic coronary artery disease is present[75]. However, studies with oxygen-15 water as the perfusion tracer suggest that maximal hyperaemic blood flow may remain normal and the loss in coronary blood flow reserve (defined as the hyperaemic to baseline blood flow) may actually be due to elevated baseline coronary blood flow rather than microvascular coronary disease[76]. Allen-Auerbach et al[77] in a study of 19 patients found that the degree of abnormalities in endothelial independent myocardial flow as detected by PET one to two years after transplantation is associated with morphological indices of disease progression by ICUS.

Unfortunately, the limited published experience with this technology in transplanted patients has generally excluded patients with significant TxCAD. In addition, the high cost and limited availability of PET limits its use. More research is needed in this field before the sensitivity and specificity of PET in transplant patients can be accurately ascertained.

1.7.1.5 Ultrafast computed tomography scanning.

Ultrafast computed tomography (CT) scanning has been shown to be useful to detect or rule out high-grade coronary-artery stenosis and occlusions in the non-transplant population[78]. The use of ultrafast CT scanning in detecting TxCAD has been studied by Babir et al[79-81]. In a study of 102 cardiac transplant recipients, they found that ultrafast CT scanning had a sensitivity of 83% and a specificity of 80% in detection of coronary calcification, and hence TxCAD, when an angiographic stenosis of >24% was taken as

significant. The positive predictive value was 74% and the negative predictive value was 88%. Raising the threshold for the diagnosis of coronary artery disease to >49% stenosis increased the negative predictive value to 97%.

Detection of coronary calcification by ultrafast CT scanning has a prognostic significance. Lazem et al[81] has demonstrated that absence of coronary calcification by ultrafast CT scanning is a significant predictor of event-free survival.

However, ultrafast CT scanning is not widely available. In addition, the sensitivity and specificity is lower than that of stress echocardiography.

1.7.1.6 Limitations of non-invasive testing.

Although the use of non-invasive testing for TxCAD is appealing, their routine clinical use is limited for several reasons:

1- Most of the studies of non-invasive testing have used the presence or absence of at least one angiographically focal epicardial coronary stenosis greater than 50% to define the sensitivity and specificity. This is a poor gold standard and is unreliable, as it has been documented before, that qualitative angiography is insensitive means to detect TxCAD[82].

2- Most studies have not correlated ischaemic territories by non-invasive testing with angiographic anatomy.

3- Few studies, with limited numbers of patients, have studied the sensitivity and specificity of these tests. In addition, frequency of clinical events has generally been low

4- Few studies have directly compared the sensitivity and specificity of various non-invasive testing methods.

5- Cardiac transplant recipients cannot achieve heart rates that are adequate for a meaningful interpretation of exercise stress testing.

6- When the test is dependant on an assessment of the relative coronary flow reserve between myocardial vascular territories, the interpretation of ischaemia may be difficult if there is diffuse arteriosclerotic involvement of the microcirculation and a concomitant reduction in coronary blood flow.

7- Although the potential prognostic use of thallium scintigraphy, dobutamine echocardiography and ultrafast CT scanning is encouraging, only small single centre experiences with limited numbers of patients over short periods of time have demonstrated this use.

1.7.2 Invasive tests.

The invasive tests used to assess atherosclerotic coronary artery disease in non-transplanted patients are also used for the cardiac transplant population. These include qualitative coronary angiography, quantitative coronary angiography (QCA) and intracoronary ultrasound (ICUS). In addition, myocardial biopsies have also been used.

1.7.2.1 Qualitative coronary angiography.

Although coronary angiography was one of the most important cardiological advances in the 20th century, it has too often been placed inappropriately on a pedestal of infallibility. The role of conventional coronary angiography in diagnosing TxCAD is subject to considerable misunderstanding and the limitations are many.

TxCAD is a diffuse disease that involves both the epicardial vessels and the microcirculation[83]. There is usually no "reference" unaffected segment, and thus although there might be a considerable loss of lumen diameter, the angiograms may appear normal, unless there is a focal narrowing. This insensitivity has been demonstrated by Mills et al[84] and O'Neill et al[82], when they compared qualitative coronary angiography with the quantified loss of lumen diameter demonstrated by QCA.

Luminal irregularities are often present which give a clue to the presence of disease. In an attempt to compensate for the insensitivity of qualitative angiography, most cardiologists report the presence of even minor irregularities. Despite this approach, considerable amount of disease is missed. In addition, there is always a risk of over reporting disease in the small tortuous vessels.

The angle of view is also a potential source of misinterpretation of qualitative angiography. The angiograms provides a two-dimensional silhouette of a three dimensional structure. In the presence of focal stenosis, many different luminal sizes and shapes can yield the same silhouette on angiography[85]. Moreover, the angle of the angiographic view can misrepresent the degree of stenosis.

The spatial resolution of coronary angiography is limited[38, 85, 86]. Most coronary arteries are between 2 and 3 mm in diameter, but angiography can resolve only approximately 0.1 mm[85-87]. This degree of resolution is not sufficient to allow for the precise diagnosis of all lesions. Angiography cannot detect structures smaller than 0.1 mm, and so can miss small but important lesions.

Another reason why angiography can fail to identify coronary artery disease is that a vessel can actually adapt its structure to accommodate, and thereby conceal atherosclerosis within the vessel wall. The mechanism of the vessel remodelling was first described in 1987 by Glagov et al[88], who found that when an atheroma first develops on the wall of a normal artery, the adventitia sometimes responds by remodelling outward, while the lumen maintains its original size. Because angiography shows only the lumen, a remodelled vessel will appear normal when, in fact, it is harbouring an atheroma. This can occur in patients whose level of coronary disease is severe. Angiography cannot identify the diseased lesion until the adventitia reaches a point of maximum expansion and the lumen is finally reduced in size. Of interest, there is also a "reverse Glagov phenomenon". Just as atherosclerotic material will induce the adventitia to remodel outwards, regression of a lesion will cause the same adventitia to remodel back inwards again, while the size of the lumen remains constant. Because angiography cannot detect the reverse Glagov phenomenon, it cannot demonstrate whether drug therapy is proving successful in promoting lesion regression.

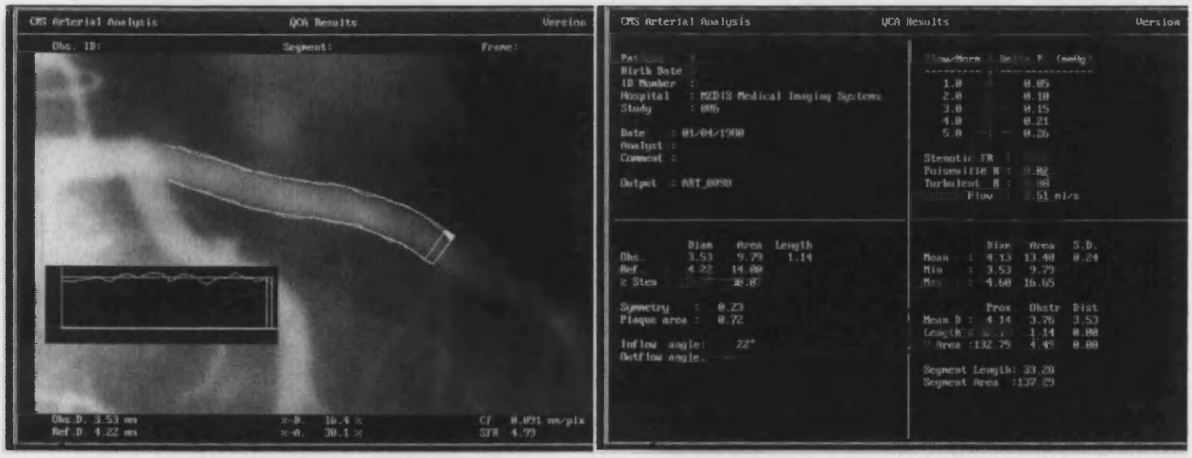
Atheromas have a predilection for arterial bifurcations. However, plaques at a vessel bifurcation are difficult to detect by angiography because the conjoining of two vessels often shields the lesion from view. All bifurcations have some degree of overlap between the "parent" and "child" vessel and this overlap prevents the angiograms from accurately depicting the lumen. As a result, lesions in the sites that are most likely to become diseased are also the most difficult to detect on angiography.

In summary, conventional qualitative angiography is insensitive in diagnosing TxCAD. In addition to the conventional limitations of angiography in diagnosing coronary disease in non-transplanted hearts, such as angle misinterpretation, remodelling and bifurcation disease, it cannot diagnose loss of lumen diameter as there is no "normal" reference segment for comparison.

1.7.2.2 Quantitative coronary angiography.

In quantitative coronary angiography, computer assisted quantitative image analysis is applied to a series of coronary angiograms to measure change in lumen diameter[89, 90]. Edge-detection algorithms remove the subjective element from the measurement. High quality angiographic images are required and the radiographic techniques used are standardized to provide comparable images for measurement from each study. Intracoronary nitrates are given to eliminate any variation due to changing vascular tone. Figure 1.1 shows an example of such measurement.

Figure 1.1: QCA analysis of the proximal LAD.



QCA does not provide an absolute measurement of lumen diameter. This is because each image is calibrated against the diameter of the angiography catheter used for the study. The catheter does not lie in the same plane as the artery and a magnification error will occur. Nevertheless, provided the technique is standardised the magnification ratio will remain constant between examinations, allowing valid comparisons to be made. QCA is much more sensitive than qualitative angiography for detecting vascular narrowing after transplantation[82, 84, 90]. The method has been used to evaluate therapy aimed at preventing TxCAD[91].

Apart from quantification of lumen diameter size, QCA suffers from the other limitations of qualitative angiography. Unlike intracoronary ultrasound (ICUS), early disease and disease in remodelling vessels[92] that does not affect the lumen size can be missed.

However, QCA has got important advantages over ICUS. It is less invasive, and requires only minor modifications to the conventional angiographic technique, making it more acceptable to patients and ideal for incorporation into a routine follow up protocol. Patients do not need systemic anticoagulation, which is required for ICUS, and there is no concern about performing a cardiac biopsy during the same procedure. The cost is much less than ICUS, as the ultrasound probe is relatively expensive. Most importantly, the distal branch vessels, which are often involved in transplant disease can be easily examined angiographically; these are inaccessible to the currently available ICUS probes.

In summary, QCA is much more sensitive than conventional qualitative coronary angiography in diagnosing TxCAD. It is less sensitive to ICUS; however, it is less invasive, less expensive and unlike ICUS, can be used to assess the coronary branch vessels.

1.7.2.3 Intracoronary ultrasound.

The images produced by introduction of an ICUS probe into the coronary arteries have been compared to those produced by histology[93]. ICUS has several inherent characteristics, which are of value in the precise quantification of TxCAD. The tomographic orientation of ultrasound enables visualization of the full 360 degrees circumference of the vessel wall, rather than a 2-dimensional projection of the lumen. Accordingly, measurement of the lumen area by direct planimetry is performed on a cross sectional image, which unlike angiography, is not dependant on an angle of projection. Unlike QCA in which vessel sizing depends on careful calibration of the analysis system, ultrasound devices rely on an electronic distance scale which is internally generated and overlaid on the image. Because the velocity of the sound within soft tissues is nearly constant, ultrasound measurements are inherently accurate and require no special calibration method[94].

The tomographic perspective of ultrasound enables characterization of the extent of atherosclerotic disease in vessels that are typically difficult to assess by conventional angiographic techniques. These include the diffusely diseased transplant coronary arteries, bifurcation lesions, ostial stenoses and eccentric plaques[94]. In each of these circumstances, the need for an unaffected reference segment, overlapping structures or

foreshortening can preclude accurate angiographic imaging. However, ICUS is unaffected by these factors, enabling accurate imaging.

As the transducer is placed in close proximity to the vessel wall, high ultrasound frequencies are used for intravascular imaging, typically 20 to 50 MHz[94]. The use of high frequencies provides excellent theoretical resolution because the ultrasound wavelength, which determines the maximum resolution, is inversely proportional to the frequency. At 30 MHz, the wavelength is approximately 50μm, which permits axial resolution of approximately 100μm. Determinants of lateral resolution are more complicated and dependent on imaging depth and beam shape. Typically, lateral resolution for a 30 MHz device averages approximately 250μm at typical distances most prevalent in coronary imaging[94].

Currently available ultrasound catheters for intracoronary application have an outer diameter between 2.9 and 3.5 F (diameter of 0.96 10 1.17 mm). These smaller diameter probes are suitable for examination of more distal locations within the coronary vessels[94]. However, examination of the most distal and branch vessels is not possible because of the size factor. Putting an ICUS probe into too small a vessel may cause coronary spasm.

In a normal healthy young adult, the thickness of the intima is below the level of ultrasound resolution and the artery wall has a uniformed single layered appearance. In older adults, and in disease, thickening of the intima allows it to be imaged as a separate structure from the media and adventia, giving the artery wall a characteristic 3 layered appearance. The intima area can be planimetered to measure the plaque burden

as a proportion of the vessel area or area inside the external elastic lamina[95]. The degree of intimal thickening has been quantitatively divided into four groups according to severity in the Stanford Clssification[96], as shown below in table 1.2.

Table 1.2: Stanford ultrasound classification of coronary artery disease in cardiac transplant recipients.

	Class			
	I	II	III	IV
Severity	Minimal	Mild	Moderate	Severe
Intimal thickness	<0.3mm <180°	<0.3mm >180°	0.3-0.5mm or >0.5mm, <180°	>1mm or >0.5 mm, >180°

Using ICUS, Tuzcu et al[97] in a study of 132 patients 1 to 9 years after transplantation, demonstrated that TxCAD is detected in more than 80% of patients, with proximal segments most frequently focally affected. Diffuse and circumferential atherosclerosis was more common in mid and distal segments. They also demonstrated in a separate study unequivocal atherosclerosis in 56% of patients studied within 1 month after transplantation, indicating pre-existing abnormality or donor related disease[98]. These studies suggest that TxCAD has a dual aetiology with many donor transmitted, early, focal, non circumferential plaques in proximal segments and more diffuse, concentric pattern in distal segments. Botas et al[99] noted that pre-existent donor coronary disease does not accelerate the progression of TxCAD within the first few years. However, Yeung et al[31] found that the progression of intimal thickening occurs during the first year, followed by slow but inexorable progression over time.

Prognostically, Rickenbacher et al[100] demonstrated that the finding of moderate to severe intimal thickening by ICUS predicts the development of angiographically

apparent TxCAD. Moreover, Mehra et al[101] demonstrated that recipients with severe intimal thickening were ten times more likely to suffer cardiac events than those without severe hyperplasia.

The safety of ICUS has been questioned. Pinto et al[102] had demonstrated that the use of ICUS in transplant recipients is not associated with serious immediate complications, increased mortality, myocardial infarction or acceleration of atherosclerosis in the imaged segment.

In summary, ICUS is more sensitive than QCA in detecting TxCAD, and provides information about the vessel wall in addition to the diameter and area. However, it is highly invasive, requires anticoagulation, and prolongs the cardiac catheterisation procedure and radiation exposure. It cannot be applied to the smaller branch vessels of the coronary tree. The cost of the single use ultrasound catheters is relatively high and adds appreciably to the cost of cardiac catheterisation.

1.7.3 Molecular analysis of myocardial biopsy specimens.

The standard morphological features of endomyocardial biopsy specimens do not correlate well with graft vascular disease[103]. However, immunohistochemical analysis provides a potentially valuable tool for the evaluation of TxCAD. About 80% of biopsy procedures locate vessels suitable for analysis[103, 104]. Endomyocardial biopsy specimens taken in the first 3 months after transplantation reveal arteriolar / arterial endothelial changes (i.e. the expression of HLA-DR or ICAM-1 and the depletion of tissue plasminogen activator) that are strongly associated with the development of TxCAD and outcome[105-108]. Weis at al[109] detected an association between early enhanced

myocardial endothelin mRNA expression (but not endothelial nitric oxide synthase mRNA expression) and the development of new intimal thickening and epicardial endothelial dysfunction during follow up. Thus, microvascular markers of increased immunologic activity that are present soon after transplantation may predict later development of TxCAD in larger vessels. Whether the presence of early microvascular endothelial activation is merely a warning signal for TxCAD or is fundamentally involved in its pathogenesis remains unknown.

1.7.4 Problems of a gold standard for the diagnosis of transplant coronary artery disease.

The evaluation of any medical test is based on its ability to identify the presence or absence of disease accurately. For this accuracy to be established, a gold standard test is usually used to evaluate all other tests. Unfortunately, due to the diffuse nature of TxCAD and the involvement of both the epicardial vessels and the microcirculation, this does not exist.

Qualitative coronary angiography is very insensitive to TxCAD, as for interpretation, it needs an unaffected segment for comparison. Sensitivity can be improved by the use of QCA. However, QCA is a pure measure of the lumen diameter, and does not give information about TxCAD until the lumen diameter starts to change. ICUS has proved to be a more suitable gold standard in detecting early TxCAD, and it gives information about the lumen diameter and arterial wall. However, due to the time and cost involved, it is usually performed only as part of research studies in one coronary artery and is rarely a part of a routine catheterisation.

Neither QCA nor ICUS give us information about the microcirculation. The microcirculation can be assessed indirectly by coronary blood flow studies. However such studies are not easily performed and are rarely available for comparison with non-invasive techniques.

In summary, information from both QCA and ICUS is needed to evaluate epicardial TxCAD. There is no direct test as yet to evaluate the microcirculation; the development of such a test is needed for the better understanding of TxCAD at the microvascular level.

1.8 Non-immunological factors associated with transplant coronary artery disease.

1.8.1 Cytomegalovirus infection.

1.8.1.1 Definition.

Cytomegalovirus (CMV) is a member of the beta herpesvirus group and has double-stranded DNA, a protein capsid, and a lipoprotein envelope. Like other members of the herpesvirus group, CMV demonstrates icosahedral symmetry, replicates in the cell nucleus, and can cause either a lytic and productive or a latent infection. Viral replication is associated with the production of large intranuclear inclusions and smaller cytoplasmic inclusions.

CMV which was initially isolated from patients with congenital cytomegalic inclusion disease, is now recognized as an important pathogen in all age groups[110]. In addition to inducing severe birth defects, CMV causes a wide spectrum of disorders in older

children and adults, ranging from an asymptomatic, sub-clinical infection to a mononucleosis syndrome in healthy individuals to disseminated disease in immuno-compromised patients[111].

1.8.1.2 Epidemiology.

CMV has a worldwide distribution. Approximately 1 percent of newborns are infected with CMV, and the percentage is higher in many less developed countries[110, 111]. Perinatal and early childhood infections are common. Virus may be present in milk, saliva, faeces, and urine. The virus is not readily spread by casual contact but requires repeated or prolonged intimate exposure for transmission[112]. In late adolescence and young adulthood, CMV is often transmitted sexually, and asymptomatic viral carriage in semen or cervical secretions is common. Transfusion of whole blood or certain blood products containing viable leukocytes also may transmit CMV.

Once infected, an individual probably carries the virus for life. The infection usually remains latent[110]. However, CMV reactivation syndromes develop frequently when T lymphocyte-mediated immunity is compromised, for example, after organ transplantation[112]. Most primary CMV infections in organ transplant recipients result from transmission of the virus in the graft itself.

1.8.1.3 Pathogensis.

Once acquired, CMV persists indefinitely in tissues of the host[112, 113]. The sites of persistent or latent infection are unclear but probably include multiple cell types and various organs. Autopsy studies suggest that salivary glands and bowel also may be areas of latent infection.

When the host's T cell responses become compromised by immunosuppression, latent virus can be reactivated to cause a variety of syndromes[111]. Chronic antigenic stimulation in the presence of immunosuppression, following tissue transplantation, appears to be an ideal setting for CMV activation and CMV induced disease. Certain particularly potent suppressants of T cell immunity, such as antithymocyte globulin, are associated with a high rate of clinical CMV syndromes, which may follow either primary or reactivation infection. CMV may itself contribute to further T lymphocyte hypo-responsiveness, which often precedes super-infection with other opportunistic pathogens, such as Pneumocystis carinii.

The pathology of CMV associated vasculitis ranges from local involvement of the virus to widespread CMV disease[114]. Although the pathogenesis is not entirely clear, it probably concerns active infection of the virus in endothelial cells, as shown by Persoons et al[115]. An interesting observation in this field is the detection of CMV infected endothelial cells in the blood of acutely infected patients[116, 117].

1.8.1.4 Clinical Features.

In organ transplant recipients, CMV induces a variety of syndromes, including fever and leucopenia, myocarditis, hepatitis, pneumonitis, oesophagitis, gastritis, colitis, and retinitis[111]. The period of maximal risk is between 1 and 4 months after transplantation Clinical disease is related to various factors, such as the degree of immunosuppression; patients receiving certain immunosuppressive agents, such as antithymocyte globulin, appear to be more likely to have severe infections than those receiving other agents, such as ciclosporin.

1.8.1.5 Clinical studies correlating Cytomegalovirus and transplant coronary artery disease.

The association between CMV and TxCAD has been suggested in several clinical studies[118]. This was initially suggested by Grattan et al[119] in 1989, who in a study of 301 patients, found that 28% of CMV infected patients developed severe coronary obstructive lesions, whereas only in 10% of patients not infected, did the same degree of TxCAD develop. Koskinen et al[120] reported a cohort of 53 heart transplantation recipients and the correlation of coronary angiograms and capillary and arteriolar changes in endomyocardial biopsy specimens and their CMV status. They documented that biopsy specimens showed significant change after cytomegalovirus infection that was diagnosed on the basis of specific immunoglobulin M, a positive viral culture from blood, urine or bronchial alveolar lavage fluid together with a four fold immunoglobulin G rise or positive CMV antigenaemia test. The significant changes in coronary angiograms followed in two years after transplantation. In a different study, Wu et al[121] demonstrated that allograft explantations with TxCAD had a much higher incidence of positive in situ hybridization for CMV nucleic acid in the vascular intima compared with explantations without TxCAD. A similar positive association between CMV and TxCAD has also been by others[122, 123].

However, not all investigators agree with the above mentioned positive association. Balk et al[124] failed to find such a relationship in their study of 100 patients who survived at least 1 year after transplantation. There was no significant difference in the incidence of TxCAD between CMV seropositive and CMV seronegative patients, between patients with and without CMV infection (culture positivity), or between patients with and those without clinical CMV syndrome. Weimar et al[125] studied the

clinical course of 87 cardiac transplants and found no association between acute or chronic rejection and patient pre-transplant CMV serostatus or post-transplant seroconversion. Stovin et al[126] in a more limited study of 43 heart transplant recipients (22 of whom seroconverted), found no evidence of CMV associated inflammation in endomyocardial biopsies taken up to the time of CMV seroconversion (approximately 57 days post-transplant), and no difference in rejection frequency between these patients and those who remained CMV-seronegative. In a retrospective analysis of 210 cardiac transplant recipients, Radovancevic et al[127] found no correlation between TxCAD with CMV infection.

Thus, the role of CMV in the pathogensis of TxCAD is not clear, and studies have shown contradicting results. The issue is complicated by the fact that the diagnosis of TxCAD and CMV have been made by various methods, which have different sensitivities and specificities for the diagnosis of TxCAD. Therefore it is difficult to draw a clear conclusion from these studies due to the variety of methods used to determine CMV infection and detect TxCAD.

1.8.2 Hyperlipidaemia.

Hyperlipidaemia is a potentially important risk factor for heart graft recipients. Many individuals awaiting cardiac transplantation have pre-morbid hyperlipidaemia[128]. This hyperlipidaemia tends to normalize by the time end stage heart failure has developed.

Hyperlipidaemia with hypercholesterolaemia, hypertriglyceridaemia, and increased low density lipoproteins often develop in the months following transplantation. Farmer et al[129] studied the pattern of change of lipid profile in 41 cardiac allograft recipients,

before and after transplant. Post-transplant, patients received triple immunosuppression in the form of prednisolone, azathioprine and ciclosporin. Comparing the baseline levels to that obtained 3 months post-transplantation, the mean (± SEM) values increased for total plasma cholesterol (from 4.68±0.21 to 5.93±0.21 mmol/L, $p \leq 0.001$), triglycerides (from 1.39±0.12 to 2.28±0.15 mmol/L, $p \leq 0.001$), HDL cholesterol (from 1.01±0.05 to 1.27±0.08 mmol/L, $p \leq 0.002$) and LDL cholesterol (from 3.09±0.18 to 3.59±0.18 mmol/L, $p < 0.02$). Apolipoprotein A-1 and B-100 also increased, but lipoprotein(a) decreased from 11.7±1.7 to 6.8±1.1 mg/dl; $p \leq 0.0001$) after transplantation. Although total cholesterol, triglycerides, LDL cholesterol, apolipoprotein A-1 and B-100 increased dramatically after cardiac transplantation, so did HDL cholesterol, thereby keeping the LDL/HDL cholesterol ratio constant. The decrease in lipoprotein(a) after cardiac transplantation suggests that metabolism of lipoprotein(a) is independent of LDL cholesterol and that immunosuppressive drugs either decreased the synthesis or increased catabolism of lipoprotein (a).

The cause of these lipid abnormalities is unclear, although immunosuppressive agents, particularly steroids, are probably important. Renlund et al[130] have demonstrated that the total cholesterol level was significantly higher in recipients receiving chronic corticosteroid treatment compared to those who were not. Insulin resistance induced by corticosteroids is also an important pathway leading to hyperlipidaemia[131]. This will enhance VLDL secretion and impede the removal of triglycerides from VLDL in the circulation. This will result in hypertriglyceridaemia. The expanded VLDL pool increases the transfer of cholesterol out of HDL and probably out of LDL to VLDL. This in turn leads to low levels of HDL cholesterol and the formation of small cholesterol depleted LDL. Barbir et al[132] also demonstrated that patients who received

immunosuppression by means of prednisolone and azathioprine developed coronary arterial disease compared to patients who were treated with ciclosporin and azathioprine without routine oral prednisolone. Ciclosporin may also be responsible because it inhibits prednisolone clearance by the liver[133]. Its interaction with cytochrome P 450 system may also increase steroid induced effects. It also increases hepatic lipase activity, with impairment of very low density and low density lipoprotein clearance. This may lead to hypertriglyceridaemia and increased endothelial contact with small low density lipoprotein particles.

Rickenbacher et al[134] studied 116 adult heart transplant recipients with a mean age of 44.7±2.0 years (89 men and 27 women) undergoing annual coronary angiography and ICUS 3.4±2.7 (range, 1.0-14.6) years after transplantation. Prevalence of any transplant coronary artery disease (TxCAD) was 85% by ICUS and 15% by angiography. By multiple regression analysis, average fasting plasma triglyceride level ($P < 0.006$) was significantly correlated with severity of intimal thickening ($R = 0.54$, $P < 0.0001$). Average fasting plasma triglyceride level ($P < 0.009$) was significantly correlated with stenosis by angiography. Furthermore, hypertriglyceridaemia predicted intimal thickening as early as 1 year.

Hypercholesterolaemia has also been implicated in the pathogenesis of TxCAD. Eich et al[135] reviewed 38 heart transplant recipients who survived more than 3 years after surgery and looked at the development of hypercholesterolemia for a possible correlative or predictive value to the development of early coronary artery disease after heart transplantation. Eleven patients were identified as having coronary disease by the third year after transplantation. They found that high cholesterol value at 6 months after

transplantation was a strong predictor for development of accelerated coronary artery disease and early graft failure. Barbir et al[132] investigated the relationship of levels of serum lipids to the subsequent development of coronary arterial disease in 95 patients with angiographically normal coronary arteries one year after cardiac transplantation. The cumulative probability of coronary arterial disease in those with total cholesterol greater than 5.8 mmol/l was at 2, 3 and 4 years was significantly higher than those with a total cholesterol less than 5.8 mmol/l. Similar results were also found by Sharples et al[136]. However, other investigators found no association between lipid levels and TxCAD[137, 138].

Lowering cholesterol and triglycerides by lipid lowering agents has been associated with a reduction in TxCAD. Wenke et al[139] in a 4-year prospective randomized study with heart transplant recipients, the efficacy of primary anti-hypercholesterolaemic therapy with simvastatin was compared with that of general dietary therapy. The aim of the treatment was to maintain post-transplantation LDL-cholesterol levels at <3.12 mmol/L. Seventy-two heart transplant recipients receiving standard triple immunosuppression were randomly assigned to an active treatment group (low-cholesterol diet and simvastatin, n=35) or a control group (general dietary measures, n=37). In the course of 4 years after transplantation, the simvastatin group had significantly lower LDL-cholesterol concentrations than the control group (mean±SD, 2.99±0.36 versus 4.06±0.44 mmol/L, p=0.002), a significantly lower incidence of TxCAD in the coronary angiographic findings (16.6% versus 42.3%, p=0.045). In a separate study, Stapleton et al[140] used gemfibrozil to lower the triglycerides level in a prospective study of 56 of 137 heart transplant recipients with ≥ 1 year post-transplant survival, and assessed the morbidity and mortality associated with the development of

TxCAD following transplantation. At baseline, patients on the treatment arm had higher total cholesterol (6.97±0.94 vs. 5.8±1.33 mmol/L), LDL cholesterol (4.34±1 vs. 3.54±1.1 mmol/L), and triglycerides (3±0.36 vs. 1.96±1.18 mmol/L) compared to the untreated patients. Levels of HDL cholesterol were statistically similar in both groups (1.1±0.29 vs. 1.17±0.1 mmol/L). In the treated group, there was a 3% decrease in total cholesterol (6.73±0.83 mmol/L) and an 8% decrease in LDL cholesterol (3.98±0.88 mmol/L) following intervention with gemfibrozil (p=NS). In addition, an 11% increase in HDL cholesterol (1.22±0.31 mg/dl) and a 19% decrease in serum triglycerides (2.41±1.19 mg/dl) were observed (both $p<0.01$). The death rate was 5.4 % in the treated group compared to 14.8 % in the untreated group, in a mean cohort survival time of 4.3±2.1 years. There was a significant difference in mortality (p=0.01) between those patients with high lipids who were treated (5.4%) compared to those patients with high lipids who were untreated (27.3%).

1.8.3 Hypertension.

According to the registry of the ISHLT[1], hypertension develops in 72% of heart transplant recipients by 1 year and 95% by 5 years. The use of ciclosporin has been shown to be associated with its development[141, 142]. Several mechanisms, including endothelin mediated systemic vasoconstriction[143], impaired vasodilation secondary to reduction in nitric oxide[144], and altered cytosolic calcium translocation[145], have been proposed to underlie ciclosporin induced hypertension. In addition, other studies have shown activation of the sympathetic nervous system[146] and the renin angiotensin system[147], as well as abnormalities in prostaglandin metabolism, as culpable mechanisms. Haemodynamic features of ciclosporin induced hypertension consist of

elevated peripheral vascular resistance[148], ventricular vascular uncoupling contributing to left ventricular hypertrophy[149], [146]and abnormalities in the diastolic function of the allograft.

Studies investigating the role of recipient blood pressure in the incidence of TxCAD have generally been negative. Hauptman et al[150] in a study of 204 patients using ICUS, found no significant difference in the progression of intimal thickening and the presence of hypertension. Rickenbacher et al[134] found no significance in post-transplant blood pressure in the incidence of TxCAD as measured by conventional angiography or ICUS. Interestingly, Costanzo et al[30] in a multiinstitutional study of donor and recipient risk factors, found that donor hypertension is a risk factor for TxCAD as measured by angiography (p=0.07).

The pulse pressure has recently come to light as an important predictor of cardiovascular mortality in both the hypertensive and normotensive non-transplant populations. Fang et al[151] initially showed in a retrospective study of a population of 5730 participants in a follow-up of 5.43 years of follow-up that a large pulse pressure difference was the most powerful measure available of initial blood pressure to identify, in advance, those hypertensive patients at greatest risk for a subsequent myocardial infarction. The same group[152] subsequently showed in a retrospective study of 7346 normotensive participants, aged 25-74 years, in a follow-up period of 17.4 years, that that among young subjects with a very low risk of cardiovascular disease, a wide pulse pressure was associated with increased cardiovascular mortality. Similar results relating pulse pressure to increased incidence of cardiovascular mortality and myocardial infarction has been shown by other investigators[153, 154].

Although the effect of systolic and diastolic blood pressure post-transplant on the incidence of TxCAD has generally been negative in previous studies, the effect of the pulse pressure has not been investigated before. Thus our study provided an opportunity to do so.

1.8.4 Diabetes.

Induction of diabetes in rat models is associated with increase incidence of TxCAD in the allografts compared to non-diabetic allografts[155]. In addition, treatment of diabetes in heterotopic heart transplant rat models is associated with decreased diabetes-induced TxCAD[156].

The effect of diabetes on TxCAD in humans has also been investigated. Ladowski et al [157] investigated whether non-insulin and insulin diabetic patients who received a heart transplant had higher incidence of TxCAD, as diagnosed be qualitative angiography, compared to non-diabetics 4 years after transplant. They found that they did not have a higher incidence of graft atherosclerosis. Valentine et al[158] investigated insulin resistance in 66 patients without overt diabetes, in the development of TxCAD, 2 to 4 years after transplant using ICUS and measuring the plasma glucose and insulin after oral glucose. Coronary artery intimal thickness (IT) and subsequent outcome were compared in patients stratified as having "high" versus "low" plasma glucose (>8.9 mmol/L) and insulin (>760 pmol/L) 2 hours after glucose challenge. Patients with high glucose or insulin concentrations had greater IT: 0.38±0.05 versus 0.22±0.02 mm, $P \leq 0.05$, and 0.39±0.05 versus 0.20±0.02 mm, $P \leq 0.01$, respectively. Thus insulin resistance plays a role in TxCAD as diagnosed by ICUS. In our study, we investigated serum glucose level to the incidence of TxCAD, as diagnosed by QCA.

1.8.5 Smoking.

The literature is deficient in studies relating post-transplant smoking and the development of TxCAD. Radovancevic et al[127] found that smoking after transplantation was related significantly to the occurrence of TxCAD as diagnosed by coronary angiography, postmortem examination or examination of the transplanted heart at the time of re-transplantation. Kapadia et al[159] found that conventional atherosclerosis risk factors, including smoking, do not predict development of TxCAD, as diagnosed by ICUS. In our study, none of the transplant recipients smoked after transplantation.

1.8.6 Donor risk factors.

Donor risk factors had been implicated in the pathogenesis of TxCAD[30, 134, 150]. These risk factors include brain death, donor age and sex. Total ischaemic time, although not found in previous studies to affect survival and cardiac function[160-162], might have an effect on the development of TxCAD.

1.8.6.1 Brain death.

Organs for heart transplantation are usually obtained from brain dead patients[163, 164] who are on ventilators, after appropriate consent has been verified. Less commonly, hearts from the heart-lung blocks obtained from patients undergoing heart-lung transplantation is used (domino hearts)[165]. Whether the hearts from brain dead patients are more likely to develop TxCAD compared to domino hearts remains to be investigated adequately.

Brain death has been suggested to have an impact, although not clearly defined, on the quality of peripheral organs[166]. The influence of brain death on outcome of kidney transplantation has been suggested by clinical observation that the behaviour of grafts from living donors is consistently superior from those from cadavers[167]. These intriguing results suggest that events surrounding brain death may alter the donor organ so that it is more prone to recipient alloimmunity.

Several animal experiments had been performed to assess the effects of brain death on the heart[168, 169]. Bittner et al[169] designed a study to establish a validated canine brain death model. Ten consecutive dogs were studied to investigate the effects of brain death on hemodynamic, metabolic, and hormonal function. Brain death was induced by inflation of a subdurally placed balloon and was validated neuropathologically. Functional data and blood samples were collected before and 15, 45, 90, 240, 360, and 420 minutes after the induction of brain death. No inotropic or vasoactive support was given. The results were expressed as mean ± standard error of the mean. The Cushing reflex occurred in all animals and lasted 13.3±1.5 minutes. Raised catecholamine levels were documented at 15 minutes, whereas the pituitary gland hormones vasopressin and adrenocorticotrophic hormone decreased significantly after 15 and 45 minutes, respectively. Triiodothyronine, thyroxine, and glucagon decreased significantly from 0.58±0.05 ng/ml, 2.20±0.15 μg/dl, and 49.7±9.1 pg/ml to 0.34±0.03 ng/ml ($p < 0.05$), 1.14±1.14 μg/dl ($p < 0.05$), and 6.9±1.4 pg/ml ($p < 0.05$) respectively. Insulin and lactate dehydrogenase showed a moderate increase after brain death. Diabetes insipidus occurred after 45 minutes in nine animals (urine output 13.5±1.8 ml/kg/hour). Left and right ventricular end-diastolic pressure increased significantly toward the end of all

experiments. Cardiac output increased and systemic and pulmonary vascular resistance decreased, but heart rate remained unchanged.

Smith et al[165] studied the outcome of 72 cardiac transplants using domino hearts, as regards to outcome and the development of TxCAD. There were four deaths (5.6%) at less than 30 days (2 from multiple organ failure, 1 from primary allograft failure and 1 from acute rejection). Actuarial survival estimates at 1 and 5-year were 77±5.2% and 69±6.3%, respectively. This compared favourably with survival data obtained in 234 non-domino cardiac recipients. There was no difference in the incidence of freedom from graft atherosclerosis (74±3% versus 70±3% at 5-years) as diagnosed by conventional angiography between the domino and non-domino groups.

The incidence of TxCAD in domino heart transplants hasn't been investigated in detail. Our study thus provided an excellent opportunity to study this relationship, as TxCAD was measured by QCA rather than qualitative angiography. In addition, the incidence of TxCAD in the primary versus the small coronary arteries will be assessed.

1.8.6.2 Donor age.

The relationship between donor age and the incidence of TxCAD has been investigated before; the results have been contradictory.

One of the large studies was conducted by Costanzo et al[30]. In this multi-institutional study, 2609 patients one to five years post cardiac transplant were studied by coronary angiography to diagnose coronary artery disease. They found that angiographic TxCAD was very common after heart transplantation, occurring in approximately 42% of the

patients by 5 years. By multivariate analysis, older donor age was found to be a risk factor for development of TxCAD ($p < 0.0001$). Recipient's age was not found to be a significant risk factor.

In a multicentre ICUS study, Hauptman et al[150] studied 204 post-transplant patients. ICUS was performed at baseline and 1 year post transplant in 70 patients, at year-1 and year-2 in 46 patients, year-2 and year-3 post transplant in 49 patients and 39 patients had ICUS at different times. The maximal intimal thickening (MIT) and Intimal Index (II) were measured. For patients studied at baseline and year-1, both MIT and II correlated significantly with donor age ($p=0.013$ and $p=0.025$ respectively). However, with the use of cut off point of 500 μm for critical MIT at baseline, there was no significant difference in donor age. There was also no significant relation to recipient's age.

In a study using both qualitative angiography and ICUS to detect TxCAD, Rickenbacher et al[134] studied 116 heart transplant recipients. The patients were 1 to 14.6 years (mean 3.4±2.7) after transplantation. Prevalence of any TxCAD was 85% by ICUS and 15% by angiography. Donor age ($P < 0.006$) was significantly correlated with stenosis by angiography but using ICUS, donor age was not significantly correlated with intimal thickening ($p < 0.8$).

The above studies showed that by using qualitative angiography a relationship can be shown to exist between increasing donor age and TxCAD, while by using ICUS, this relationship doesn't exist. Whether this relationship exists when TxCAD is diagnosed by using QCA is not known.

1.8.6.3 Donor gender.

The role of donor gender in the development of TxCAD has also been questioned, and again the results are inconclusive. Costanzo et al[30] in their multi-institutional study using coronary angiography to diagnose TxCAD, found that donor male sex predict the development of TxCAD (p=0.006). In addition, they found that recipient male sex was also a risk factor in TxCAD (p=0.02).

However, using conventional angiography and ICUS, Rickenbacher et al[134] found no relationship between donor gender and TxCAD ($p<0.2$ and $p<1$ respectively). In addition, Hauptman et al[150] in the multi-institutional study using ICUS, found no significant difference in intimal thickening, as measured by MIT and II, as regards donor or recipient gender in the incidence of TxCAD. The role of donor gender in the pathogenesis of TxCAD thus needs further evaluation.

1.8.6.4 Total ischaemic time.

Physiologic and metabolic changes develop in organs lacking circulation[128]. Whether these changes have any effect on the subsequent development of TxCAD when the heart is transplanted has been questioned.

Rickenbacher et al[134] investigated the development of TxCAD and its relation to total ischaemic time (TIT) in cardiac transplant patients using conventional coronary angiography and ICUS. They found that increased TIT has no significance in the development of TxCAD ($p = 0.3$ by both methods). Similar results were found by Costanzo et al[30] using conventional angiography, and by Hauptman et al[150] using ICUS.

The relationship between TIT and TxCAD hasn't been studied using QCA. Our research thus provides an opportunity for that.

1.8.7 Coronary artery tone.

The endothelium plays a critical role in the control of vasomotor tone by the release of vasoactive substances[170]. It releases a number of mediators that regulate tone and growth in response to changes in shear stress and other haemodynamic and metabolic factors[171]. An important product is nitric oxide, which is formed from L-arginine by the enzyme nitric oxide synthase. Damage to the endothelium may result in reduced production of nitric oxide and/or other vasoactive substances such as prostacyclin[172]. Functional endothelial disturbances are clinically detectable as abnormal vasomotor responses to endothelium dependent vasodilators such as acetylcholine or substance P. Reduced coronary vasodilation or vasoconstriction in response to these agents has been observed in a substantial proportion of cardiac transplant recipients[173-177]. In contrast, endothelium independent vasodilators such as nitroglycerine and adenosine usually elicit normal vasodilator responses[173, 178, 179].

The predictive value of endothelial dysfunction in the later development of TxCAD is not clear. Different studies have shown different results. Angiographic studies have shown that acetylcholine induced coronary vasodilation 2 months after transplantation may be impaired to the same extent in patients with and without angiographic evidence of TxCAD at 1 year follow up[180]. However, in an ICUS study, Davis et al[181] showed that coronary segments with endothelial dysfunction, compared to normally dilating segments, early after transplant has a greater increase in intimal thickness at 1 year.

This suggests that endothelial dysfunction has a predictive value for the subsequent development of TxCAD.

As the endothelium plays a critical role in regulating the coronary tone, and as endothelial dysfunction has been shown by ICUS studies to predict later development of TxCAD, the question arises whether early change in vascular tone is a predictor of later development TxCAD. On review of the literature, such study hasn't been undertaken as yet. We have thus investigated this question in our current study.

1.8.8 Change in cardiac haemodynamics secondary to transplant coronary artery disease.

Review of literature shows that very little information is available on the impact of TxCAD on cardiac function. Grocott-Mason et al[182] studied such relationship in the decade following cardiac transplantation. They performed a retrospective analysis on 137 patients who survived at least 1 year and returned for annual assessment. Any irregularity on conventional angiography was interpreted as indicative of TxCAD. The incidence of TxCAD was 8.8% (12 of 137) at one year. Only 34 of 137 patients (25%) were alive and free from TxCAD at 10 years. The mean time post-transplant to diagnose TxCAD was 4.4±2.4 years for single vessel disease and 5.8±2.6 years for triple vessel disease. Cardiac function was impaired if TxCAD was present, with higher filling pressures and lower left ventricular ejection fraction (LVEF); five years post-transplant, the left ventricular end diastolic pressure (LVEDP) was 11.5±3.6 vs. 13.8±5.7 mmHg, mean right atrial pressure (RAP) was 4.9±2.3 vs. 6.9±3.2 mmHg, LVEF was 66.2±11.3% vs. 59.4±13.9%.

They also analysed the one-year haemodynamic data of all recipients with normal coronary arteries. These were grouped into 2 groups. Group 1, those documented not to have developed TxCAD during the first decade (n=34); Group 2, those who were documented to have developed TxCAD between 1 to 10 years (n=81). Those who died before10 years were excluded, unless they were shown to have developed TxCAD, as it was unknown whether they would have developed TxCAD if they had survived 10 years. Patients in Group 1 (who never developed TxCAD) had significantly better cardiac function than those in Group 2 (i.e. prior to the diagnosis of TxCAD): LVEF of 70.3±0.4% vs. 63.3±13.4%, mean RAP of 3.7±1.8 vs. 6.1±4 mmHg, respectively, both $P < 0.05$. Similar differences in cardiac function were found comparing 1 and 5 years haemodynamic data for patients with or without TxCAD at 5 or 10 years, respectively.

The data above confirms that even with normal coronary arteries on conventional angiography, differences in cardiac function are detectable in those who develop TxCAD at a later date. Whether these data can be reproduced when TxCAD is measured by the more sensitive QCA is not known. Our study thus provided an opportunity to do that.

1.9 Immunological factors associated with transplant coronary artery disease.

Although the pathogensis of TxCAD is thought to be multifactorial, immunological factors probably play a major role in its development, as the vascular endothelium is known to be immunologically active, and as it is highly responsive to cytokines and express numerous molecules which interact with ligands on lymphocytes[183]. This section will deal with the different immunological aspects that are thought to be implicated in the pathogenesis of TxCAD.

1.9.1 Acute rejection.

The immune system mounts a response to the allograft which may lead to hyperacute, acute or chronic rejection. Hyperacute rejection occurs within hours after transplantation, and is due to preformed circulating antibodies from prior transfusion, pregnancy or ABO incompatibility. In the immunosuppressed patient, acute rejection might occur from the second week onwards after transplantation, and is characterized by deterioration in cardiac function and cellular infiltration of the myocardium by mononuclear cells. Chronic rejection is the most important cause of death after the first year, due to the accelerated coronary vascular disease[184]. The process is a fibrointimal hyperplasia that can go undetected by conventional coronary arteriography as has been discussed previously; this then progress to diffuse atherosclerotic changes.

The "gold standard" for the diagnosis of acute cardiac allograft rejection is by histologic examination of an endomyocardial biopsy (EMB)[185, 186]. Noninvasive methods can be used in conjunction with histology to aid diagnosis[187]. These include the use of electrophysiology (QRS summation voltage, fast-Fourier-transformed electrocardiography and intramyocardial electrocardiography[188]), echocardiography[189] (intramyocardial wall thickness, myocardial mass, fractional shortening), immunologic methods[190, 191] (cytoimmunological monitoring, transferring receptors, and interleukin 2 receptors), radioisotopic techniques[192, 193] (anti-myosin-monoclonal antibodies, thallium, technetium, and gallium scintigraphy and indium labelled cells), as well as resonance imaging[194].

At the instigation of the International Society of Heart Transplantation (now known as the International Society for Heart and Lung Transplantation [ISHLT]), a standardized

grading system for heart transplant biopsy specimens was published in the Journal of Heart Transplantation in November/December 1990[195, 196]. The purpose of this grading is to provide a system into which other previously used systems could be translated for the purpose of multicentre clinical trials and for publications so that results from different centres could be compared effectively. The ISHLT has classified acute cardiac rejection into the following grades:

0- No rejection.

1- This is subdivided into:

    1A- Focal (perivascular or interstitial) infiltrate without necrosis.

    1B- Diffuse but sparse infiltrate without necrosis.

2- One focus only with aggressive infiltration and / or focal myocyte damage.

3- This is subdivided into:

    3A- multifocal aggressive infiltrates and / or myocyte damage.

    3B- diffuse inflammatory process with necrosis.

4- Diffuse aggressive polymorphous infiltrate ± oedema ± haemorrhage ± vasculitis, with necrosis.

Early acute rejection has been considered the critical risk factor for chronic rejection (TxCAD)[128]. Subclinical host alloreactivity may persist despite putatively satisfactory maintenance immunosuppression; indirect presentation of donor MHC antigens may become increasingly important over time. In addition, noncompliance by the recipient may allow continuing low-grade host alloresponsiveness. As a result, several studies have been undertaken to investigate the relationship between acute rejection and subsequent development of TxCAD.

One of the initial studies was by Uretsky et al[137]. They found that occurrence of two or more major rejection episodes were associated with the development of TxCAD ($p < 0.005$), as diagnosed by qualitative angiography. In a separate study, Radovancevic et al[127] subsequently showed that the total number of rejection episodes correlated significantly with the occurrence of TxCAD ($P < 0.05$), showing that patients who experienced two or more rejection episodes had an incidence of TxCAD of 40%, as opposed to a 23% incidence in patients who experienced no rejection. A composite rejection score derived from multivariate regression analysis of the severity, frequency, and timing of acute cardiac rejection episodes was found to correlate with the development of CAD ($P < 0.05$). Narrod et al[197] in a study of 173 heart transplant recipients found a significant relationship between acute rejection episodes after the first year of transplant and the subsequent development of TxCAD. Zerbe et al[198] in a review of 146 allografts established a positive association between moderate rejection in the first 3 months after transplantation and development of TxCAD.

However, not all investigators agree about this relationship. Gao et al[199] analysed 126 consecutive heart transplant recipients and devised an arbitrary method to incorporate the number, duration, and severity of myocardial rejection episodes during the first postoperative year, resulting in a rejection score for each patient. They then correlated the later incidence (mean follow-up, 4 years) of angiographic accelerated graft coronary artery disease with this rejection score. They found no significant difference between patients with and without TxCAD in their rejection parameters. In a separate study, Stovin et al[126] studied the grades of rejection seen at all biopsies performed in the first 6 months after heart transplantation in 108

patients who survived more than 6 months. The development of TxCAD was assessed at routine follow-up coronary angiography in 101 patients and at necropsy in seven patients. No significant association was found between either moderate rejection or any level of rejection and the later development of TxCAD, nor did the absence of any rejection protect against its development.

All the above studies used qualitative angiography to assess the development of TxCAD. This is a subjective and insensitive method of diagnosing TxCAD; QCA or ICUS would be more appropriate methods. In a multicentre study using ICUS, Kobashigawa et al[200] studied 68 heart transplant recipients from seven heart transplant programmes. All patients underwent baseline ICUS and again at 1 year after transplantation. Rejection episodes were recorded for each patient during the first year after transplantation, and was categorized into 3 groups; the Treated Endomyocardial Biopsy group (ISHLT $\geq$3A requiring augmented immunosuppression), Treated Rejection group (any rejection, confirmed clinically or by biopsy, requiring treatment) and Average Biopsy Score (when a biopsy score was obtained by assigning a numerical score to each ISHLT grade). There was no significant difference in the development of TxCAD between patients in the Treated Endomyocardial Biopsy and the Treated Rejection groups and those who did not reject. However, there was a significant correlation between increased average biopsy score at 0 to 3 months after transplantation and increased intimal thickening. This suggests that recurrent mild rejection episodes may have an important role in the development of transplant coronary artery disease.

In an autopsy study, Winters et al[201]compared the severity of rejection to the degree of luminal narrowing, as measured by means of digitization on a video image analysis system, in the coronaries of 15 allografts. Rejection episodes, considering all degrees of rejection, were strongly related to percent luminal narrowing ($p = 0.01$).

In summary, the role of acute rejection in the development of TxCAD seems to vary depending on the method used to diagnose it. We thus investigated the role of acute rejection in the development of TxCAD in the main and secondary vessels when QCA is used.

1.9.2 Mismatches at the HLA locus.

1.9.2.1 Introduction.

Antigenic differences between members of a species are called *alloantigens*, and when these play a determining role in the rejection of allogeneic tissue grafts, they are called *histocompatibility antigens*[202]. Evolution has conserved a single closely linked region of histocompatibility genes, the products of which are prominently displayed on cell surfaces and provide a strong barrier to allotransplantation. The terms *major histocompatibility antigens* and *major histocompatibility gene complex* (MHC) refer to the gene products and genes of this chromosomal region. The response to a given antigenic determinant is now known to require the binding of the appropriate peptide fragment to an MHC molecule. In humans, the MHC is called HLA (Human Leucocyte Antigen)[202] and is located on the short arm of chromosome 6. The individual letters of HLA have various unofficial meanings, and by international agreement HLA is the logo for the human MHC.

Three classes of gene products are encoded within the 4000-kilobase region of HLA[202]. Class I molecules, expressed on virtually all cell surfaces, consist of one heavy and one light polypeptide chain and are the products of three reduplicated loci: HLA-A, HLA-B, and HLA-C. Class II molecules are restricted in expression to B lymphocytes, dendritic cells, monocytes, antigen-activated T lymphocytes, and to epithelial and endothelial cells that have been activated by interferon. They consist of two noncovalently linked polypeptide chains (α and β) of unequal length. They are the products of several closely linked genes, collectively termed the HLA-DR (D related). The class II heterodimers form a structure similar to that of class I.

1.9.2.2 Clinical studies.

Clinical studies show that HLA histocompatibility between donor and recipient is an important determinant of allografts outcome in kidney transplantation[203]. In combination with pharmacological modification of the host immune response using immunosuppressive agents, HLA matching is effective at weakening the potency of host T cells to destroy donor tissue, reducing both the frequency and severity of rejection and graft loss[204-206].

In contrast, in human heart transplantation, published clinical studies have produced conflicting results between histocompatibility and survival[207, 208]. In addition, only a few studies have examined the effect of HLA mismatch on the occurrence of TxCAD. Numerous complex reasons account for these inconsistencies.

1- Donor-recipient HLA matching is not done prospectively in heart transplantation, and the number of fortuitously well matched recipients is very small.

2- Tissue typing has been performed with serologic rather than with DNA genotyping techniques. Although serologic methods for the recognition of distinct HLA types have improved, at present by no means are all MHC antigens distinguishable. Often when one antigen is reported for a given locus, it is assumed that the patient is homozygous when in fact the second allele is an as yet untypeable antigen.

3- Data on the relationship between HLA mismatch and heart allografts rejection may be affected by inter-institutional variability in the diagnosis, grading, and threshold for treatment of rejection.

4- Lack of sensitivity of invasive (coronary angiography) and non-invasive methods for detecting TxCAD makes it difficult to assess the impact of donor-recipient tissue matching on the occurrence and progression of this disease.

Cocanougher et al[209] investigated the relationship between HLA matching and allografts failure and recipients death as a result of TxCAD. An HLA score was obtained by assigning two points for each completely mismatched A, B, and DR antigen, one point for tolerated cross reactive antigens, and no points for completely matched antigens. Total and B-DR HLA scores were higher in patients who died because of TxCAD than in long term survivors with angiographic evidence of TxCAD (10.2±1.4 vs. 8.8±2.2, $p = 0.02$ respectively). This intriguing finding suggests that patients with greater degree of HLA mismatch have greater mortality when TxCAD develops.

Some investigators found no relationship between HLA mismatch and the development of TxCAD. Hornick et al[210]studied 550 heart transplant recipients with postmortem data

or yearly angiograms, donor: recipient serological HLA typing, and biopsy data. They did not find any significant association between the mean number of mismatches for Class I or Class II antigens that exerted a protective or deleterious effect. In a different study, Zerbe et al[198] studied 146 allografts obtained either at re-transplantation or autopsy. Epicardial coronary vessels were measured by planimetry of photographs of coronary artery sections with use of a digitizing tablet. Only rejection rate in the first 3 post-operative months, but not the degree of HLA mismatch, were associated with severity of coronary artery luminal narrowing.

Interestingly, an inverse relationship between HLA mismatch and development of and outcome from TxCAD has been suggested. Kerman et al[211] in a review of 448 ciclosporin treated heart transplant recipients examined the relationship of donor-recipient HLA compatibility to survival, rejection and death from TxCAD. An inverse relationship was found between HLA A and B mismatch, but not HLA DR mismatch, and death from coronary artery disease in that 17% (19/111) of well matched ($\leq$2 A and B mismatches) vs. 9% (32/327) of poorly matched patients (>2 A and B mismatches) died from TxCAD ($p = <0.01$). Similar results had been found by Radovancevic et al[212] in a separate study of 167 patients who underwent cardiac transplantation. They found that at the HLA-A locus, there was significantly higher incidence of TxCAD ($p<0.01$) in the group of patients with zero mismatches (10 of 17, 58%). At the HLA-B locus, there were no statistically significant differences between the number of mismatches and the development of TxCAD. At the HLA-DR locus, there was no correlation at all. Generally, the group of patients with four to six mismatches had a significantly lower incidence of TxCAD ($p <0.05$) than the patients with three or fewer mismatches. One can only speculate that that these well matched patients experienced fewer acute

rejection episodes and consequently received less immunosuppression which allowed a greater degree of alloreactivity to proceed undisturbed leading to TxCAD.

1.9.2.3 Animal studies.

Evidence from animal models militates against the hypothesis that antigens of the MHC are the sole targets of the recipient's immune responses, which eventually lead to the occurrence and progression of TxCAD. Adams et al[213] have developed a heterotopic heart transplant model in which Lewis rats serve as heart donors and Fisher 344 rats serve as recipients. These rat strains differed only at minor histocompatibility loci. Despite the absence of MHC antigenic differences, allografts blood vessels developed intimal proliferative lesions, which by light microscopy, were very similar to the histopathologic changes typical of human TxCAD. Grusby et al[214] generated mice lacking major histocompatibility complex (MHC) antigens and demonstrated that they reject allogeneic skin grafts with little delay. Thus allografts rejection might be mediated by alternative immune responses.

1.9.2.4 Conclusion.

The role of MHC donor/ recipient differences in the pathogenesis of TxCAD has not yet been completely elucidated. Clinical studies have been inconclusive in defining the relationship, together with the associated morbidity and mortality. Animal studies do not support the role of MHC alloimmune mechanisms in the development of TxCAD.

1.9.3 Anti-HLA antibodies and the relationship of the lymphocytic cross-match and panel reactive antibodies in the development of transplant coronary artery disease.

The presence of preformed lymphocytotoxic antibodies reactive against donor lymphocytes in recipient serum, detected in a routine crossmatch, is considered a contraindication to solid organ transplantation because of the high incidence of humoral allografts rejection, early graft failure and poorer patient survival[215-217]. In addition, the development of TxCAD in transplant recipients has been linked with the development of anti-HLA antibodies after transplantation[218-220]. These antibodies are directed primarily against donor major histocompatability complex (MHC) class I HLA antigens constitutively expressed by the allografts endothelium, since non-activated endothelium does not express MHC class II HLA antigens[215].

Lymphocytotoxic crossmatch is usually performed by mixing donor spleen lymphocytes with recipients serum, which has been pre-treated with Dithiothreitol (DTT) to digest IgM autoantibodies. Rabbit compliment is then added. The donor lymphocytes, both T and B, are isolated from donor spleen lymphocytes by antibody coated magnetic beads. Positive crossmatch is usually defined as a greater than 10% killing of donor lymphocytes above background levels[216]. Lymphocytotoxic antibodies can also be tested against lymphocytes from a donor panel representative of all established HLA specificities of class I and II MHC, collectively referred to as measurements of panel reactive antibodies (PRA)[215]. Recipient sera is incubated with the lymphocytes, in addition to rabbit complement (complement dependent cytotoxicity-CDC). The PRA test is usually considered positive if the panel reactivity was greater than 5% and the strength of the reaction was greater than 10% above background levels[216].

A positive cross match does not always lead to hyperacute or accelerated graft rejection[221]. Investigators thus started to characterise the antibodies responsible for positive crossmatches. Experience in renal transplant patients suggested that a positive crossmatch due to IgM antibodies may be irrelevant and may not affect primary allografts survival[222]. These IgM antibodies may be either non-HLA lymphocytotoxic autoantibodies or non deleterious anti-HLA class I antibodies[223]. Scheinin et al[221] in a retrospective study of 125 heart transplant patients, demonstrated that an IgM positive crossmatch did not have a deleterious effect on survival. There was no statistically significant difference in the development of TxCAD in those with a positive crossmatch.

Rose et al[219] studied serial serum specimens from 118 heart transplant recipients and tested them against a reference panel of 70 cells for anti-HLA lymphocytotoxic antibodies. Patients with positive sera on at least three separate samplings at minimal intervals of 1 week were considered to be antibody producers (Ab+), and those with less than three positive sera samplings were considered non-producers (Ab-). Graft atherosclerosis confirmed by coronary angiography or autopsy developed in 12 Ab+ patients (16%), compared with 1 of 42 Ab- patients (2.3%) ($p < 0.05$).

Reed et al[224] have investigated the role of various demographic and immunologic parameters as prognostic indicators of TxCAD in a population of 274 heart allograft recipients. Using HLA-A2 as a marker for the release of soluble HLA antigens from the donor, they established that recipients displaying circulating donor alloantigens for more than 26 weeks following transplantation are at increased risk of developing

TxCAD (P=0.008). This association suggests that the release of alloantigens from the allograft is indicative of chronic injury and/or that it stimulates chronic rejection via the indirect allorecognition pathway.

The role of a positive lymphocytotoxic crossmatch or PRA before transplantation on subsequent development of TxCAD as diagnosed by QCA has not been studied before. We have thus investigated their role in relationship to the development of large and small vessel TxCAD.

1.9.4 Non HLA antiendothelial cell antibodies.

Non HLA, antiendothelial cell antibodies (AECA) have been detected initially in kidney transplant recipients[225, 226]. This was followed by a similar detection of these antibodies in heart transplant recipients[227-229]. Western blotting studies[230, 231] have shown that the patients can make antibodies against many different endothelial peptides; two-dimensional gel electrophoresis has shown in excess of 40 different protein spots which react with IgM in sera from patients with TxCAD[183, 232]. Many of these were identified as normal cytosolic proteins / enzymes including triose phosphate isomerase and 75 Kd glucose regulated protein which belongs to the head shock 70 family of proteins[183]. The most frequent response was against a doublet of peptides at 56/58 kDa shown by N-terminal analysis and absorption studies to be the intermediate filament protein vimentin.

Vimentin is the intermediate filament protein characteristic to endothelial cells and fibroblasts. However, it is not restricted to these cells only. Proliferating and migrating smooth muscle cells also express vimentin, in addition to the desmin that they also

express at rest. Staining normal heart sections with antibodies to vimentin provides a picture similar to that obtained with antibody against CD31 (a marker of endothelial cells). Vimentin is diffusely expressed in the intima and media of normal coronary arteries and is upregulated in diseased coronary arteries[183]. Antibodies against vimentin reflect disease activity in the coronary arteries, but to date an effector function hasn't been ascribed. It is not known how vimentin, a cytosolic protein, is exposed to the immune system. It may be that nonomeric peptides of vimentin are normally present on the cell surface, presented within endothelial MHC molecules. Alternatively, damage to endothelial cells leads to novel or further exposure or release of vimentin peptides.

Dunn et al[230] in our institution investigated the frequency of AECA against human umbilical vein endothelial cells by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis and western blotting. Peptide-specific AECA were found in 15/21 heart transplant recipients with accelerated coronary artery disease, and 1/20 transplant patients who had not developed the disease. Jurcevic et al[233] in retrospective study of 109 up to 5 years post-transplant patients, using 880 samples, found that the mean titres of antivimentin antibodies, calculated up to 1, 2, and 5 years, were significantly higher in patients who developed TxCAD than those who remained disease free ($P<0.0001$, $P<0.0038$, and $P<0.0001$, respectively). A predictive test based on the first-year mean vimentin titre alone (> or = 120) produced a test with 63% sensitivity and 76% specificity.

However, Hosenpud et al[234] found no relationship between AECA and TxCAD. Recipient serum obtained between 6 and 8 weeks (early) and 1 year (late) after transplantation were reacted with recombinant human interferon (rhIFN)-gamma pre-

treated donor-specific human aortic endothelial cells (HAECs) in 10 recipients with and 10 recipients without TxCAD at 1 year after transplantation. HAEC IgM binding was assessed by flow cytometry and complement fixation and HAEC lysis was measured using standard chromium release assays. Seven of 10 and 5 of 10 patients with TxCAD had IgM detected by flow cytometry at early and late time points, respectively (14±2 and 16±5 mean channel shift), whereas 5 of 10 and 6 of 10 patients without TxCAD had IgM detected by flow at early and late time points, respectively (15±4 and 14±3 mean channel shift). This finding was not different between groups. Despite between 50% and 70% of all patients having detectable IgM binding to endothelial cells, no patient's serum was cytotoxic to its donor-specific HAECs. They concluded that IgM antibody to endothelial cells is common (at low titers) after transplantation and that it is not cytotoxic and in their study provided no discrimination between those with and without chronic rejection.

It is thus obvious that the opinion is divided about the relationship of AECA and TxCAD. No study has been performed to date to relate the presence of these antibodies to the development of TxCAD in a large population of patients over time, using QCA to diagnose TxCAD. Our study thus provided an excellent opportunity to explore this.

1.9.5 Induction of immunosuppression with rabbit antithymocyte globulin.

Immediately after transplantation, immunosuppression has to be instituted in order to prevent rejection of the transplanted heart. This will have to be continued throughout the patient's life. Numerous protocols are being used by different transplant centres for maintenance of immunosuppression, and they are continually changing. Currently, the most common protocol involves triple drug therapy of ciclosporin, azathioprine and

prednisolone. These are usually given in higher doses in the early post-transplant period, with weaning to lower doses and less toxic levels for chronic administration.

Rabbit antithymocyte globulin (RATG), a "custom-made" polyclonal antibody produced in rabbits, is the immunosuppressive medication of choice for severe rejection, not usually responsive to conventional therapy; it is also associated with a lower incidence of subsequent acute rejection[235]. It is also used initially after transplantation when the renal function is poor, in place of ciclosporin. Some centres have adopted giving RATG to induce immunosuppression in the first 3 days post transplant. The effect of this on the subsequent development of TxCAD has been looked into before.

Carrier et al[235] conducted a retrospective study to evaluate the cardiac transplant outcome, in their 10 year experience of induction of immunosuppression with RATG. 163 patients who had a 3-day course of intravenous RATG immediately following heart transplantation (Group 1), were compared to 48 patients who had intravenous and oral ciclosporin immediately following heart transplantation (Group 2). One, 5- and 10-year actuarial survival rate averaged 85±3%, 77±4% and 67±5% in Group 1 compared with 88±5%, 81±6% and 76± 6%in Group 2 ($p = 0.5$). At 1, 5 and 10 years, the freedom rate from TxCAD as diagnosed by conventional coronary angiography averaged 93±2%, 68±5 %and 50±7% in Group 1 compared with 93±4, % 58±8% and 30±8 %in Group 2 ($p = 0.1$). Thus there was a trend towards a lower incidence of coronary atherosclerosis 5 and 10 years after transplantation in the RATG induction group, but this did not reach statistical significance. At 1 year, the freedom rate from an episode of acute rejection averaged 43±4% in Group 1 and 30±7% in Group 2 ($p = 0.03$). Using Minnesota

Antilymphocyte Globulin (ALG), Dresdale et al[236] found that ALG might reduce the occurrence of TxCAD as diagnosed by conventional coronary angiography in their follow up of cardiac transplant recipients up to a median duration of 34 months.

Thus the relationship of RATG induction of immunosuppression and subsequent development of TxCAD is not clear. Our study thus provided an opportunity to further investigate this issue.

CHAPTER 2

METHODS

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2.1 Study objectives.

We investigated coronary arterial disease in the transplanted heart using quantitative coronary angiography (QCA) up to 5 years after transplantation. Our aim was to determine the relationship of disease in the larger vessels (LV) compared to that in the smaller vessels (SV). QCA unlike ICUS, has the advantage of examining the small vessels (SV). We also investigated the pathogenic risk factors associated with TxCAD, and the effect of disease on the haemodynamics of the transplanted heart.

2.1.1 Specific research questions.

The study was designed to determine:

1- The reliability and validity of our QCA data.

2- The rate of change in coronary lumen diameter segments from immediately after transplant (baseline), to years 1, 3, and 5 years.

3- The changes in the large and the smaller epicardial vessels. The large vessels (LV) category included the proximal and mid left anterior descending artery, proximal circumflex artery, proximal and mid right coronary artery. The smaller vessels (SV) category included the distal segments of the three main coronary arteries, the first and second diagonal arteries, first and second obtuse marginal arteries, right ventricular branch of the right coronary artery and the posterior descending artery. Table 2.1 shows the abbreviations of these segments that will be used throughout this study.

4- The effects of the LV and SV TxCAD on the haemodynamic parameters of the heart.

5- The relationship of different immunological and non-immunological factors to the development of SV and LV TxCAD. These factors are shown in the table 2.2.

Table 2.1: Abbreviations of the main coronary arteries and their measured segments.

Artery/ Arterial Segment	Abbreviation
Left Anterior Descending Artery	LAD
Cirumflex Artery	Cx
Right Coronary Artery	RCA
Proximal	Prox.
Middle	Mid.
Distal	Dist.
First Diagonal	D1
Second Diagonal	D2
First Obtuse Marginal	OM1
Second Obtuse Marginal	OM2
Right Ventricular Branch	RVBr.
Posterior Descending Artery	PDA

Table 2.2: Factors investigated for their role in the development of TxCAD.

Pattern of early change in vessel diameter.
Donor and recipient age and sex.
Vessel tone
Domino and non-domino transplants.
Cause of donor death.
Total ischaemic time.
RATG induction of immunosuppression.
Prednisolone treatment during the first year.
Conventional cardiovascular risk factors.
Immunological factors, including acute rejection. and antivimentin antibodies.
Pre-transplant CMV serology and post-transplant CMV count.

2.1.2 Main outcome measure.

The main outcome measure was mean lumen diameter loss (MLDL) in the large and small sized vessels as determined by QCA.

2.2 Study population.

The study population consisted of patients who have undergone heart transplantation at Harefield Hospital between September 1994 and June 1999, and were consented for the angiographic follow-up. In total, 121 patients were enrolled in this study. These patients were followed up for a period ranging from one to five years. The study was approved by the Hillingdon Ethics Committee, which at the time of the start of the study was responsible for ethical approval of our research studies.

One hundred and three patients were males (85%) and 18 were females (15%). The mean age was 48.5±10 years. Out of 121 patients, 114 (94%) were Caucasians, 5 (4%) were Asians, 1 (1%) was Afro-Caribbean and 1 (1%) was Middle Eastern. The cause of the heart failure, which necessitated transplantation, was ischaemic in 74 (61%) patients, idiopathic dilated cardiomyopathy in 29 (24%), congenital in 6 (5%) and other causes in 12 (10%) patients. The mean organ ischaemia time was 155±57 minutes.

2.3 Angiographic method.

Quantitative coronary angiography (QCA) was performed initially within the first month after transplant, and subsequently at 1-year, 3-years and 5-years. The angiograms were performed through the femoral approach by the standard

percutaneous Judkins technique[237, 238]. To allow for the quantitative analysis of the angiograms, specific modifications of the angiographic technique were made:

1- 8-Fr Cordis catheters[239] were used in all the angiographic procedures, in order to standardise QCA calibration for analysis.

2- 200 μg of intracoronary glycerine trinitrate (GTN)[240] was injected into the LCA after an initial angiographic run in the shallow RAO projection, and a repeat run taken 30 seconds after the GTN injection in the same projection, to assess the vessel tone. Subsequently, different runs were taken of the LCA after contrast injection. All runs for the RCA were taken 30 seconds after 200μg of GTN was injected into it.

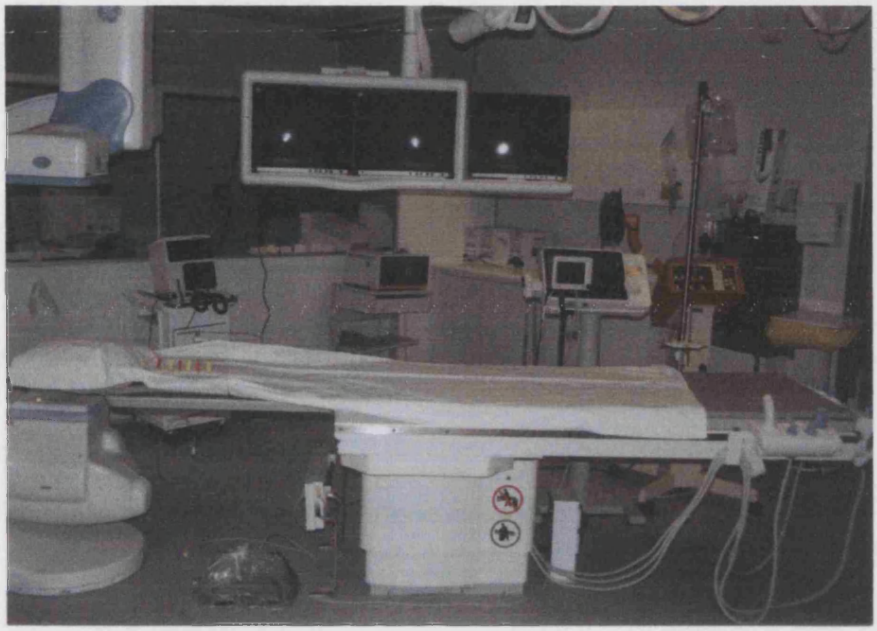
3- Standardised projections were taken for the left and right coronary arteries following the 200μg GTN injection into these vessels. The X-ray tube and the image intensifier were moved to different positions, to acquire cinefilm runs of these vessels in different projections when contrast was injected. These positions were documented to establish the exact angle, table height, image intensifier and magnification used, so that the exact positions and projections were used in the following years for each particular patient. In some cases a repeat of the angiogram obtained in the first projection was obtained following the other angiographic projections to help assess the reproducibility of the method.

4- The same catheterisation laboratory was used for all the angiographic procedures (figure 2.1). All angiograms were recorded on cine films.

Right heart catheterisation was performed through the femoral vein using the standardised Seldinger approach[237]. A 7 Fr multipurpose catheter was used to document

the right-sided cardiac pressures[241], and the cardiac output calculated using the Fick principle[242].

Figure 2.1: The cardiac catheter laboratory.



2.4 QCA analysis.

2.4.1 Instruments.

The Cardiovascular Measurement System (QCA-CMS) workstation (figure 2.2) distributed by MEDIS Medical Imaging Systems BV, Nuenen-the Netherlands, was used for analysis of the cine films. This has been shown to provide more precise measurements in quantitative analysis of coronary lumen diameters than other systems[243]. The basic hardware configuration consisted of:

1- A Pentium Processor.

2- High quality frame grabber for digitisation of images.

3- High-resolution monitor.

4- A laser printer.

5- A mouse.

This system was used in conjunction with a high quality cine-projector (CAP-35E) with built-in optical zoom lens.

Figure 2.2: QCA "Cardiovascular Measurement System" workstation.

QCA analysis software, V 2.3S, was used. This system provided an automated calibration procedure based on the angiographic catheter and computer assisted edge detection to allow measurement of the lumen diameter of individual coronary segments.

2.4.2 Analysis method.

2.4.2.1 Calibration.

Each film was put onto the cine-projector. An initial reference frame was marked and the counter zeroed. The cine was then forwarded to the desired view for analysis and the system calibrated choosing a frame that showed the angiographic catheter filled

with contrast. The frame had to show a non-tapering segment of the catheter in a stable position. If there was any doubt about the accuracy of the detected catheter contour, the procedure was repeated. The zoom and brightness were adjusted such as the calibration factor (CF) was around 0.09 mm/pixel prior to making the measurements in adherence with the operational procedure recommended by the manufacturer. For a particular view, when comparing different cines of the same patient, the variability of the CF was always kept to be less than 0.005[244].

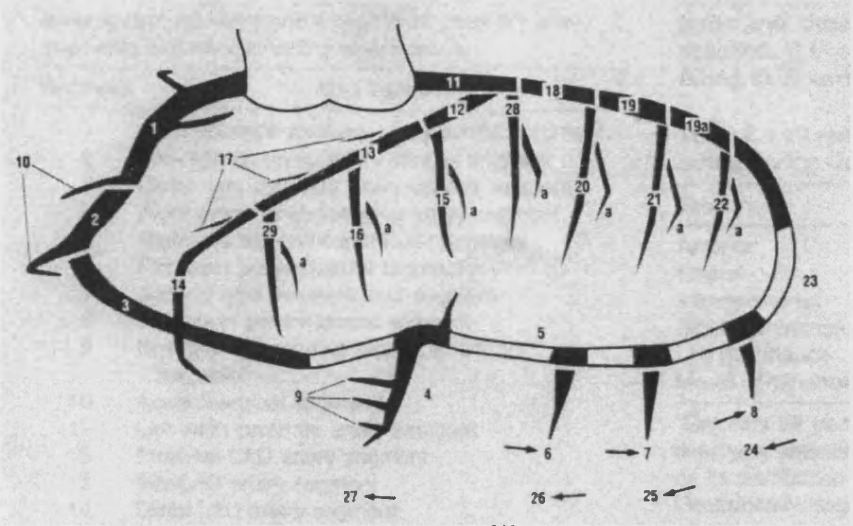
2.4.2.2 Measurement of coronary lumen.

A frame that showed clearly the segment to be studied with its start and end points (bifurcation points) was selected for the segment analysis. The frame number, CF, brightness and zoom were documented. The second or third cardiac cycle following contrast injection was usually chosen, making sure that the segment of interest was fully opacified[244]. The frame had to be at end-diastole or in the diastasis period so that the effect of motion blur was minimal.

The analysis of each coronary segment was done in a standardized manner according to the guidelines of the Bypass Angioplasty Revascularization Investigation (BARI) investigators. These guidelines were approved by the American College of Cardiology and American Heart Association for analysis of coronary angiograms[245]. BARI investigators used angiographic definitions used in formats of prior revascularisation trials, particularly the Coronary Artery Surgery Study (CASS). These guidelines endorse the system developed by the BARI investigators and published by Alderman and Stadius in *Coronary Artery Disease*[246]. This system provided for nomenclature of

the most frequently encountered coronary arterial segments as shown in figure 2.3. Table 2.1 shows the abbreviations of the main arteries and their measured segments.

Figure 2.3: The coronary artery map used by the BARI investigators.



Reproduced with permission from Alderman et al[246].

The arterial segments measured in our analysis are as follows:

1- LAD segments; proximal (segment 12), middle (segment 13 between segments 15 and 16) and distal (segment 14).

2- LAD branch vessels; first (segment 15) and second (segment 16) diagonal.

3- Circumflex; proximal (segment 18) and distal (segment 23 / 19a).

4- Circumflex branch vessels; first (segment 20) and second (segment 21) obtuse marginal vessels.

5- RCA segments; proximal (segment 1), middle (segment 2) and distal (segment 3) segments.

6- RCA branch vessels; right ventricular branch (segment 10) and posterior descending artery (segment 4).

These segments were measured in all the cases, except when there was an anatomical variation, overlap of vessel segment in all the views or poor opacification of the segment by contrast. The bifurcation points were manually set inside the corresponding bifurcations. When these points were approved, the automated edge detection algorithm of the CMS outlined the luminal borders of that segment. Whenever possible, no manual corrections were made; the exception to that was when a branch occurred within the measured segment, in which case the branch point was edited by visual inspection. When the outlined segment was approved, the computer automatically calculated the mean lumen diameter (MLD) of the segment, which was documented for our study.

When the view was changed to assess a different vessel, or a different segment of the same vessel, the calibration process was repeated. In analysing yearly consecutive cines of the same patient, exactly the same projection for each segment was used on each occasion, with the same magnification, view and skew, together with the same table and image intensifier height. Figures 2.4 and 2.5 show the images from the same patient at baseline and at Year-1.

Figure 2.4: An example of QCA analysis of the proximal LAD at baseline.

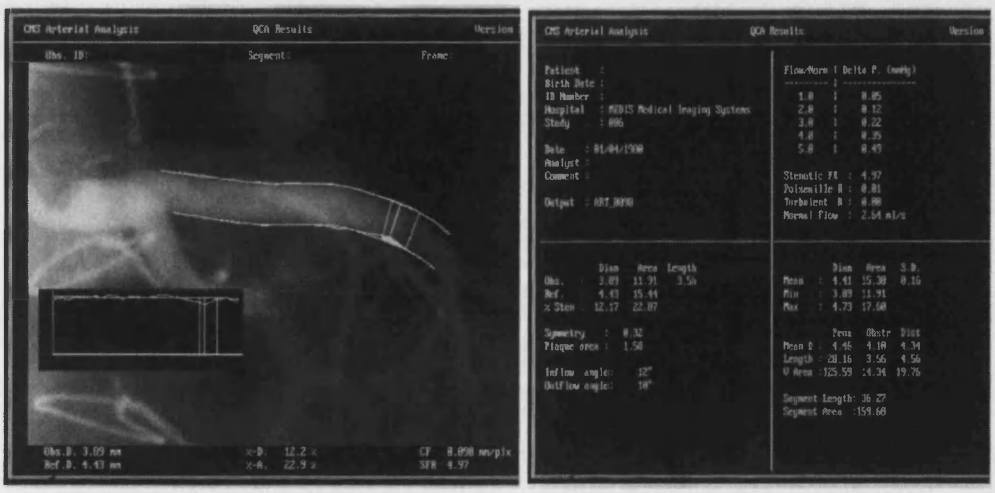
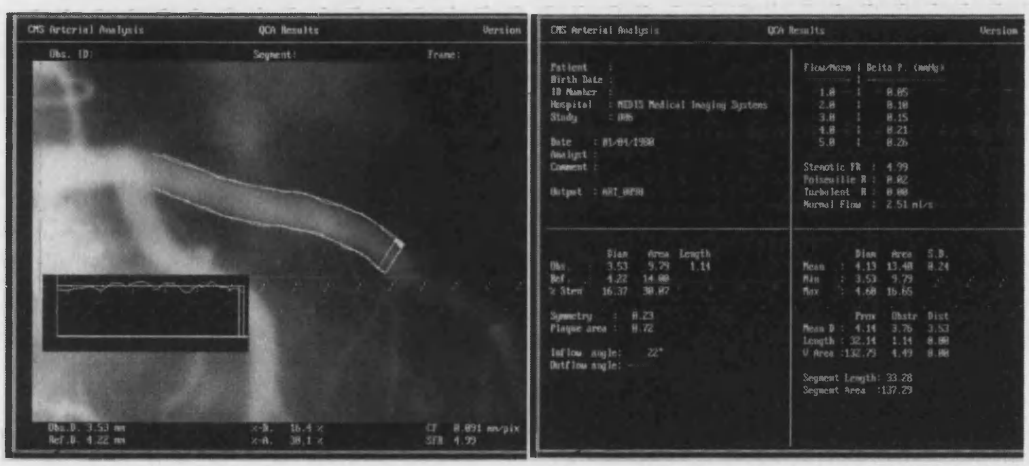


Figure 2.5: QCA analysis of the proximal LAD at Year-1 in the same patient analysed in figure 2.4.



2.5 Intracoronary ultrasound.

2.5.1 Ultrasound catheters.

The equipment used to perform intracoronary ultrasound (ICUS) consisted of 2 major components, a catheter incorporating a miniaturized ultrasound transducer and a console containing the electronics necessary to reconstruct the image[94]. Transducers with ultrasound frequencies of 30MHz were used; these had a wavelength of 50μm, and permitted an axial resolution of approximately 100μm, and lateral resolution of 250μm[94]. The transducers used were of the mechanical type, which used a drive cable running the length of the catheter that rotates a single piezoelectric transducer element mounted near the distal catheter tip.

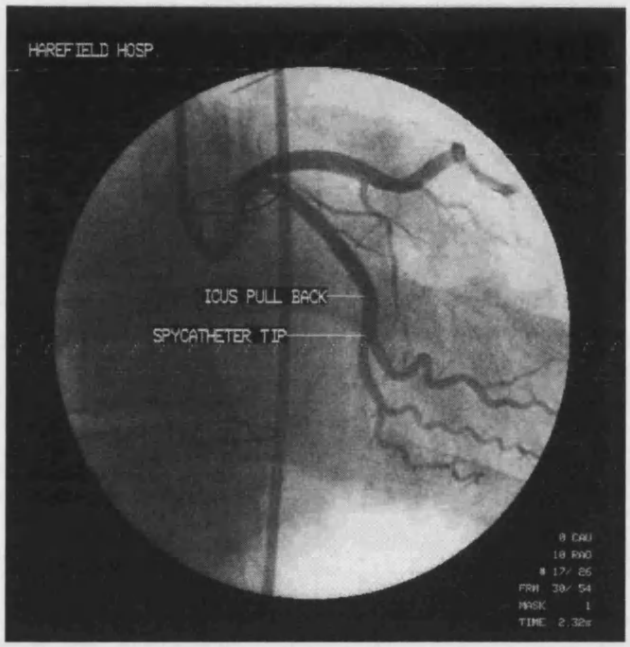
The outer diameters of the catheters used varied from 3Fr to 4Fr. Catheters with two different delivery devices had been used. In the first (Sonicath catheter, Boston

Scientific Cooperation, Watertown MA, USA), the distal catheter contained a short monorail segment (5 to 10mm in length). With the transducer positioned just proximal to the monorail portion of the catheter, the vessel was selectively cannulated with the guide-wire, and the device was then advanced and retracted to interrogate the vessel. The second type (Spycath, Boston Scientific Cooperation), a sheath was advanced to a distal location in the artery. The transducer was then advanced within the sheath to image the vessel. Both devices with or without the sheath, worked well, although with the catheter without a sheath, some difficulty was experienced tracking tortuous vessels.

2.5.2 Examination technique.

Standard interventional techniques for intracoronary catheter delivery were used for intraluminal coronary ultrasound examination. After the QCA had been performed, intravenous heparin (to maintain activated clotting time between 200 to 250 seconds) was administered before imaging. An 8 Fr guiding catheter was used to subselectively cannulate the vessel of interest to be studied using a steerable 0.014-in angioplasty guidewire. A stable guiding catheter position with good support was essential, because the ultrasound catheters have less trackability and larger profile than modern balloon angioplasty catheters. The imaging catheter was carefully advanced and positioned at an obvious landmark on the vessel, usually a branching point as shown in figure 2.6. The imaging catheter was then withdrawn slowly using a motorized pullback device at a constant speed (between 0.25 and 1 mm/s). The images were recorded on a super VHS tape for subsequent quantitative analysis.

Figure 2.6: Tip of the ICUS catheter positioned at a landmark, the OM1 on this patient



2.5.3 Intracoronary ultrasound data analysis.

The recorded super VHS images were digitised using a Pinnacle Systems Studio DC10 Plus capture card. This allowed image capture at a resolution of 720 x 540 Pixel in 8 bits matrix at a rate of 25 frames per second. The images were stored in a computer hard disc.

There are two general methods of using ICUS to quantitate TxCAD[247]. In the first, measurements can be taken at several selected sites in a coronary artery and followed up serially over time. The site may be chosen because of certain TxCAD characteristics, such as the presence of heavy calcium or severe narrowing; alternatively, it can be any site that is easily known and reproduced in subsequent studies (usually close to branch points). In the second method, the average TxCAD severity can be determined and followed up serially. The average severity of intimal

thickening, lumen diameter, and intimal volume density can be accurately determined with the use of the principles of morphometry. With this method, the sites are chosen randomly without regard to severity of the disease or proximity to vessel branches. As TxCAD is a diffuse disease morphometric analysis was used. Morphometry is a mathematical science in which three-dimensional information can be obtained from data collected in one or two dimensions. This principle was demonstrated in 1847 by the French geologist Delesse, and states that the ratio of two cross sectional surface areas are equal to the ratio of the two corresponding volumes[248]. A similar relationship is true for surface densities (the ratio of surface area to volume). In the case of coronary arteries, the volume density of the intima (volume of intima/volume of intima + lumen) is equal to the intimal index (cross-sectional area of intima/cross-sectional area of intima + lumen).

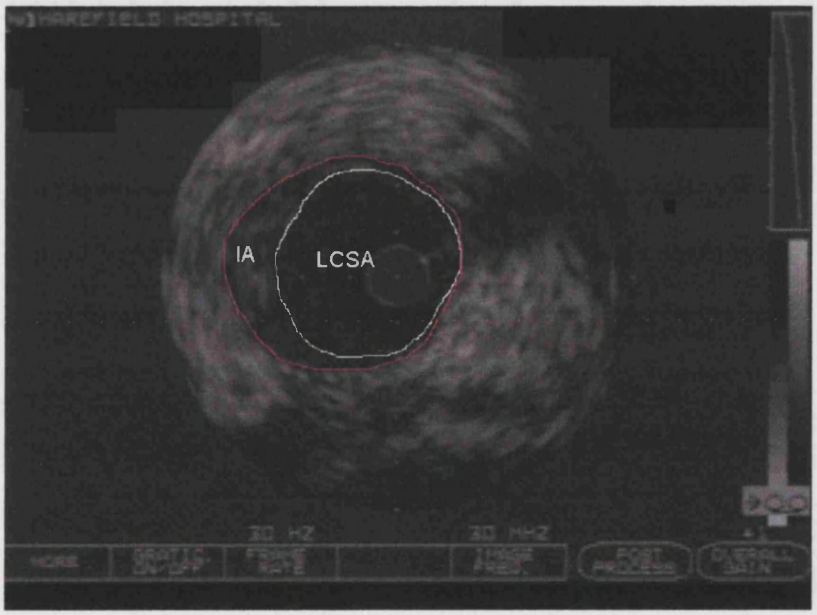
In the artery studied, during pullback, 10 frames at equal intervals of time, were obtained for morphometric analysis. Johnson et al[249, 250] have shown that no more than 10 random sites on the coronary segments studied are necessary to precisely determine the mean severity of TxCAD. The frames were taken at end-diastole from the digitised cardiac cycles, at a time of maximal dilatation of the vessel. The frames were then imported for analysis by the Scion Image Programme (SIP) version 4.02, the PC version of NIH Image, distributed by Scion Corporation. The cross hairs recorded with the ICUS images were used for calibration. The following parameters of quantitative coronary ultrasound (QCU) were derived[94], and their abbreviations are shown in table 2.3:

1- **Lumen cross-sectional area** *(LCSA)*: the lumen-vessel wall interface was traced by planimetry, and the area was automatically calculated by the SIP.

When a three-layer appearance of the vessel was present, but the intima consisted only of a thin line (<100μm), this line was considered the lumen boundary; this is shown as the inner line in figure 2.7. In the absence of a three layer appearance, the innermost interface (presumably the adventitia) was considered the border.

2- **Total Area** *(TA)*, also known as the external elastic membrane cross sectional area (EEM-CSA): the leading edge of the adventitia was traced by planimetry, when the three-layer appearance was present. This is shown as the area within the outer red boundry in figure 2.7. When the three-layer appearance was absent, the TA was considered to be equal to the LCSA.

Figure 2.7: LCSA (area within inner white boundary) and TA (area within outer red boundary) in a vessel with the three layer appearance, with the IA being the area between the two boundaries.



3- **Intimal area** *(IA)*, also referred to as the intimal cross-sectional area (IT-CSA): this was used to describe the severity of TxCAD when the three-layer

appearance was present, and was calculated from the difference between TA (EEM-CSA) and the LCSA. This method includes the media in the measurement of the intima (or plaque) [94] as the media is not always distinguished as a clear layer because its thickness is slightly greater than the axial resolution of the imaging systems. When the three-layer appearance was absent, the IA was considered to be zero.

4- **Intimal Index** *(II)*: this was calculated by dividing the IA by TA. It has been used by some investigators to describe the severity of the lesion. It is a more sensitive measurement of disease severity than IA, which has a disadvantage that it is directly proportional to the TA.

5- **Remodelling Index** *(RI)*: this was calculated by dividing the change in total area by the change in intimal area (RI=ΔTA/ΔIA) [251]. The coronary segments were divided into the following categories:

i- RI $\geq$1, the ΔTA$\geq$ΔIA, *reflecting adequate compensation or overcompensation.*

ii- RI >0 and <1, ΔTA < ΔIA, *reflecting partial compensation.*

iii- RI$\leq$0, *no increase in TA, representing no compensation or even a decrease in TA, reflecting shrinkage of the vessel.*

Table 2.3: Table of abbreviations of the Quantitative Coronary Ultrasound parameters and their meaning.

Abbreviation	Meaning
LCSA	Lumen cross sectional area.
TA	Total area
EEM-CSA	External elastic membrane cross sectional area
IA	Intimal area
IT-CSA	Intimal cross sectional area
II	Intimal index
RI	Remodelling index

2.6 Haemodynamic measurements.

Right and left heart pressure data were obtained during angiography using fluid filled catheters. These were recorded on hard copies and interpreted in conjunction with the QCA data. The right atrial, pulmonary artery, pulmonary wedge and left ventricular end diastolic pressures data were documented, and the data obtained by the standard methods[252].

2.7 CMV antigen measurement.

Over the past few years, the direct detection of pp65 antigen, which is a matrix protein between the viral capsid and envelope, in blood leucocytes has been successfully applied to permit early diagnosis of CMV infection[253-255]. The method allows early diagnosis, usually before the onset of clinical symptoms, and is used by our immunology laboratory. The pp65 antigen is detected in cytospin preparations made from blood leucocytes using immunological methods. It is found primarily in the cell nucleus of granulocytes. Sensitivity and specificity is approximately 90%. The assay is usually completed 6hrs from receipt of sample of 7ml of heparinised blood or blood in EDTA. The results were reported as the number of positive cells per 200,000 white cells.

2.8 Analysis of the immunological parameters.

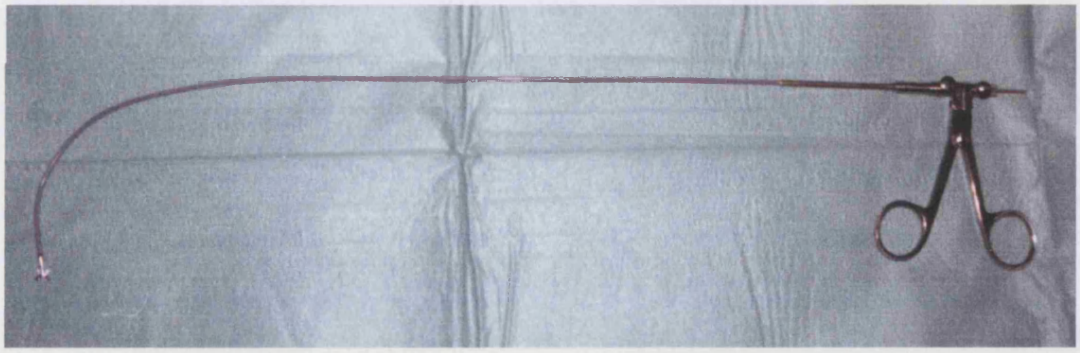
2.8.1 Cardiac biopsies.

Right ventricular (RV) cardiac biopsies were taken for histological evidence of rejection starting from the first week post-transplant. They were done at regular time schedule during the first year, according to the Harefield Hospital protocol. This protocol requests that regular biopsies are to be taken weekly for the first six weeks

post transplant, then fortnightly for the subsequent 6 weeks, then monthly for the subsequent 3 months, then every 2 months for the subsequent 6 months. Additional biopsies were done during the first year, regardless of the protocol, if there was a clinical indication or to monitor the response of a rejection episode to immunosuppressive treatment. After the first year, cardiac biopsies were only done if there was a clinical indication.

The procedure was normally performed through the right internal jugular (RIJ) vein using the percutaneous transvenous approach[256] using Yacoub's bioptome (figure 2.8). At least four good myocardial biopsies were taken from each patient in each biopsy session and the specimens put into a formalin filled pot for subsequent histological analysis.

Figure 2.8: Yacoub's bioptome.



Endomyocardial biopsies were then fixed in 10% formol saline and processed routinely. Histopathological changes of rejection are graded according to the International Society for Heart and Lung Transplantation Working Formulation for Heart Rejection[195].

2.8.2 HLA typing.

HLA typing was performed by PCR-SSP (Sequence Specific Primers) developed by Olerup et al[257] using 20 ml of blood anticoagulated with sodium citrate which was collected from recipients and/ or donors. The kits used were purchased from Protrans (Heidelberg, Germany) and incorporated primers for the typing of HLA-A, -B, -Cw, -DR and –DQ alleles. This method incorporated the polymerase chain reaction and sequence specific primers (PCR-SSP) for the rapid determination of HLA alleles and was used for HLA typing of both the recipient and donor. The oligonucleotide primers were designed to obtain amplification of specific alleles. The discrimination between alleles took place during the in vitro amplification. This reduced the post-amplification processing to a minimum[257].

2.8.3 HLA mismatching.

An HLA mismatch is defined as the presence of a particular HLA A, B or DR specificity on the donor HLA phenotype that is not present on the recipient HLA type. The minimum HLA mismatch for a given locus is 0 with a maximum mismatch of 2 disparate HLA specificities.

2.8.4 Panel reactive antibody screening.

Panel reactive lymphocytotoxic antibody status was determined using an HLA class I (A, B, C) and class II (DR and DQ) typed panel of lymphocytes from 40 healthy volunteers and 10 patients with chronic lymphocytic leukaemia in a modified NIH microcytotoxicity technique[258]. The panel was selected to include the majority of class I and class II specificities. All sera were screened by a modified microcytotoxicity technique. Blood samples were collected from patients into plain tubes.

2.8.5 Cross-matching.

Donor spleen samples approximately 1.5 cm cubed in size were used for cross-matching. These were pushed through a metal sieve into RPMI medium to form a suspension. This suspension was then treated with an equal volume of Dextran 110 solution (6% in 0.9% NaCl) and a small amount of carbonyl iron (Sigma, Poole, UK) for 30 minutes at 37°C with occasional mixing. T and B lymphocytes were then purified and were then incubated with recipient sera for 60 mins at 22°C followed by a further 60 minutes after the addition of 5µl rabbit complement (Cedarlane, Ontario, Canada). Cell viability was assessed after staining with a mixture of ethidium bromide and acridine orange using a fluorescent microscope (Carl Zeiss, Welwyn Garden City, UK) with a X20 objective lens. A positive reaction was defined as greater than 10% killing of donor cells above background levels of cell death seen in the wells of donor spleen cells incubated with a negative control serum (Pooled Human Male AB serum).

2.8.6 Antivimentin antibodies ELISA.

Recombinant human Vimentin (Cymbus, Chandlers Ford, UK) was diluted to 10µg/ml in PBS and 100µl coated overnight at 4°C onto 96 well polystyrene plates to give a concentration of 1 µg/well. After washing the wells of the plate five times with 200µl of PBS-Tween, 100 µl of serum at 1/50 was added and left for 1 hour at room temperature, and subsequently washed. 100µl of a monoclonal rabbit anti-human IgM-HRP (horse radish peroxidase) conjugated antibody was added for a further 45 minutes. After a further 5 washes with PBS-Tween, the presence of antivimentin IgM antibody was determined colorimetrically using tetramethyl benzidine (TMB) as a substrate. The colour reaction was allowed to develop for 4 minutes before the addition of 1M sulphuric acid to stop the reaction. The absorbance was measured at 450 nm with a

reference at 620 nm in the Labsystems Multiskan plate reader. Using a standard curve generated from the dilutions of a well characterised positive serum which was tested in each assay and expressed in titre units, the relative amount of antivimentin antibodies present in the test sera was determined[259].

2.9 Statistical analysis.

Standard statistical methods for medical research were adopted for data analysis. The data were originally stored on Microsoft Excel spreadsheets and transferred onto the Minitab software package (Release 12; Minitab Inc., State College, Pennsylvania) for statistical analysis. Continuous variables were expressed as mean ± standard deviation and categorical variables as proportions. The Normality of continuous variables was checked using a Normal probability plot and assessed using the Anderson-Darling test. Non-parametric tests or transformations were used when variables did not appear to be normally distributed. Table 2.4 (below) shows the continuous and categorical clinical variables that were analysed.

Table 2.4: The continuous and categorical clinical variables that were analysed.

Continuous Variables	Categorical Variables
Mean lumen diameter	Gender (males versus females)
Age	Race (Caucasians versus non-Caucasians)
Blood pressure variables	Donor and recipient CMV immunological status (positive versus negative)
Lipid profiles	Pre-transplant Lymphocytic crossmatch (positive versus negative)
Total ischaemic time	Pre-transplant PRA (positive versus negative).
Haemodynamic variables	Acute rejection score
ECG summation voltage	Number of mismatches
CMV count	
Antivimentin antibodies titre	
ICUS variables	

Initially, univariate analysis was performed on all our data for the LV and SV groups. Subsequently, multivariate analysis was performed to derive the multivariate models for the respective groups.

2.9.1 Univariate analysis.

2.9.1.1 Demographics.

The age was expressed as mean ± SD, and gender and race as percentages.

2.9.1.2 Change in segment diameter using QCA.

The decrease in individual segment diameter from baseline was calculated both in terms of absolute and proportional (percentage) mean lumen diameter loss (MLDL). The percentage decrease from baseline to, for example, Year-1 was calculated as 100 × ("Year-0" value – "Year-1" value)/ "Year-0" value.

Subsequently, the vessels were grouped into LV and SV and the MLDL for each group was calculated for each patient. The significance of the difference in MLDL between the LV and SV was evaluated using a paired t-test, pairing by patient

When more than two group means were compared, analysis of variance (ANOVA) was used.

2.9.1.3 Increase in vessel segment diameter after GTN.

The paired t-test was used to compare the increase in mean lumen diameter (MLD) between the LV and SV, after intracoronary GTN was given.

2.9.1.4 Change in ICUS measurements.

Again, paired t-test was used to assess the significance of change in intimal area, lumen cross-sectional area, total area and intimal index between successive measurements.

2.9.1.5 Change in vessel diameter related to clinical parameters.

The correlations of donor and recipient ages and subsequent MLDL were evaluated and their significance evaluated using a t-test. The two-sample t-test was used to assess whether MLDL differed between the sexes. ANOVA was used to assess whether MLDL differed between the races. To test for the significance of the correlation between the blood pressure and lipid profile parameters and MLDL, the t-test was used. ANOVA was used to assess whether donor cause of death was associated with different mean lumen diameter loss. To test for a difference in MLDL between domino and non-domino donors, the two-sample t-test was used and the same test was used for the RATG induction therapy and first year prednisolone treatment. The significance of the relationship between ischaemic time, glucose and creatinine levels and the MLDL were investigated using the t-test.

2.9.1.6 Endomyocardial biopsies.

A biopsy score was initially derived for each patient. The relation of the biopsy score and MLDL was tested using the t-test. Using the same test, the relation of the MLDL and summation ECG voltage and the echocardiographic fractional shortening/ ejection fraction were tested.

2.9.1.7 Cytomegalovirus count.

The CMV count was not normally distributed as tested by the Anderson-Darling test. The Mann-Whitney test was used to test for any significant relation between the count and pre-transplant CMV serological status. In order to obtain a more "normal" distribution, the transformation ln(1 + CMV count) was used; the relation of this transformed variable and MLDL was tested using a t-test.

2.9.1.8 Immunological factors.

Patients were grouped by their total number of mismatches; the MLDL for the different groups were compared using the ANOVA test. The significance of the pre-transplant lymphocytic crossmatch, Pre-transplant PRA and antivimentin antibody titre (positive or negative) to MLDL was tested using the two-sample t-test.

2.9.1.9 Haemodynamic data.

The correlation of the different haemodynamic data and MLDL was tested using the t-test.

2.9.2 Multivariate analysis.

After performiong univariate analysis on the relationship between the different potential predictor variables and MLDL, all variables with p-value < 0.2 and more than 80% of values present (i.e. not missing) were used. The multivariate model was obtained using standard linear regression with MLDL as the response variable, starting with the candidate regressor variables and eliminating non-significant variables using the backward elimination procedure, using a nominal 5% significance level.

CHAPTER 3

QCA RELIABILITY & VALIDATION.

To determine the reliability and validity of the QCA measurements of coronary lumen diameter obtained in our laboratory, we carried out a preliminary study comparing QCA with ICUS and also examined the variation between repeated QCA measurements within the same subject.

3.1 Terminology and previous studies.

Four important terms used in mensuration[260, 261] are defined in table 3.1. Validation refers to the process of establishing that a technique provides a suitable method for measuring the quantity of interest, whilst accuracy is the determination of how far the measured value, derived from repeated observations, deviates from the 'true value' obtained from a reference method i.e. the size of any 'systematic error' in the measurement. The reliability of a measurement determines how far a single observation may be expected to deviate from the true value and this is often expressed as the precision of the measurement or the 95% confidence interval for repeated measurements; these are derived from repeated observations of the same object and are a measure of the size of the 'random error'. The effect of a random measurement error may be reduced by making repeated observations (or by increasing the sample size from a study population) where systematic errors cannot be eliminated in this way.

In practice, accuracy is either determined from repeated measurements made with the same apparatus under different conditions or by comparison with another (reference) measurement system. In the first case, it is an indicator of how robust the results of the measurement process are against changes in the conditions under which the observations were made; in the second case, accuracy is an assessment of the validity of the measurement in relation to the reference method.

Table 3.1: Terminology.

Term	Definition
Validation	Determining whether the instrument is actually measuring what it purports to be measuring[260].
Accuracy	The mean, signed, difference between the true and measured values or between the values from measurements repeated under different conditions[261].
Reliability	The test that demonstrates whether the instrument will produce the same result when administered repeatedly to an individual[260].
Precision	The standard deviation of the differences between the true and measured values or between the values from repeated measurements[261].

Reiber et al[262] assessed the *accuracy* and *precision* of measurements of coronary lumen diameter derived from computer-assisted quantitation of 35 mm coronary cineangiograms. A computer-assisted technique was developed to assess absolute coronary arterial dimensions to reduce the subjective aspect of vessel edge detection. The boundaries of optically magnified and video-digitised coronary segments and the intracardiac catheter were defined by automated edge-detection techniques. The *accuracy* and *precision* of the edge detection procedure as assessed from cinefilms of contrast-filled acrylate (Perspex) models (phantoms) were -30 μm and 90 μm respectively. Short-, medium-, and long-term variability measurements were assessed from repeated coronary angiographic examinations performed 5 min (with unchanged geometry of X-ray system and patient position), 1 hr (the X-ray system and geometry and patient position were changed between the acquisitions), and 90 days apart, respectively. For all studies the mean differences in absolute diameters were less than 0.13 mm. The variability in obstruction-diameter ranged from 0.22 mm for the best controlled study (medium-term) to 0.36 mm for the least-controlled study (long-term); the variability in reference-diameter ranged from 0.15 to 0.66 mm, respectively.

Lowry et al[263] compared lumen diameter measurements obtained from transplanted hearts by QCA to that of ICUS. Twenty-five patients underwent both procedures

following cardiac transplantation (20 < 1 year, 5 > 1 year). Lumen diameter and area measurements of proximal coronary artery segments were compared using both techniques. Overall, lumen diameter and area measurements correlated closely between the two procedures, both for the early and late follow-up patients.

3.2 Method.

In order to assess the variation of our QCA results, a repeat angiogram of the first angiographic projection in the RAO projection was taken at the end of the left coronary artery catheterisation, and the proximal segments of the LAD and Cx measured quantitatively. This was done as follows. One minute after intracoronary GTN (200μg) was administered as described in chapter 2, a cineangiographic aquisition was obtained. The X-Ray settings (angle, skew, table height and image intensifier height) were all documented. The X-ray system was then adjusted to acquire further angiograms of the left coronary artery. At the end of the sequence of left coronary views, the X-ray system was positioned in the same settings of the initial angiogram and a repeat angiogram was then obtained. Subsequently, the proximal segments of the LAD and Cx in these repeat angiograms were analysed quantitatively to determine the variation in measurements obtained under these conditions.

3.3 Reliability.

The diameter of 99 proximal LAD segments (LAD1) with their corresponding proximal segments from the repeat angiogram (LAD2) was measured quantitatively. The corresponding proximal segments of the Cx artery in the initial (Cx1) and repeat (Cx2) angiograms were also measured; however due to technical factors such as poor opacification of the vessel and wire overlap, 5 proximal Cx segments could not be

measured and we were able to measure 94 pairs of segments. Table 3.2 shows the mean lumen diameter (MLD) of the segments and their standard deviation, and Table 3.3 shows the differences between the first and repeat measurements together with the means for the total LAD and Cx segments. Figure 3.1 and Figure 3.2 show the scatter plot of the measured LAD and Cx segments respectively. The repeat measurements were very closely related to the initial ones and the accuracy (mean difference) between the initial and repeat measurements was 19μm ($p<0.004$) for the LAD and 10μm($p=0.24$) for the circumflex artery.

Table 3.2: Mean diameter of the segments and their standard deviation.

Segment	Number of segments	Mean lumen diameter (mm)	Standard deviation (mm)
LAD1	99	4.441	0.687
LAD2	99	4.461	0.688
Cx1	94	3.735	0.882
Cx2	94	3.745	0.879

Table 3.3: Difference between initial and repeat measurements of the mean lumen diameters of the LAD and Cx proximal coronary segments.

Variable	Number of segments	Mean lumen diameter in mm	Standard deviation in mm
LAD Difference	99	-0.019 (*accuracy*)	0.065 (*precision*)
LAD Mean	99	4.451	0.687
Cx Difference	94	-0.010 (*accuracy*)	0.082 (*precision*)
Cx Mean	94	3.740	0.880

Figure 3.1: Scatter plot of LAD replicate 1 against replicate 2.

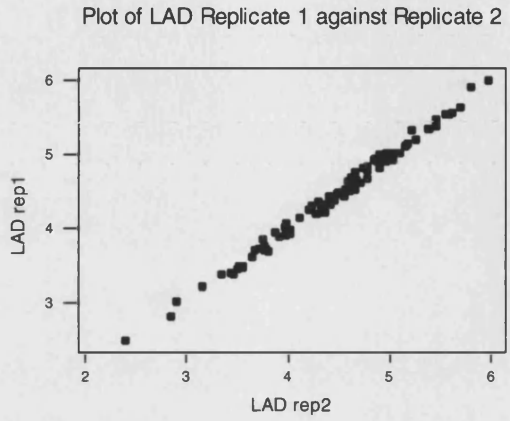
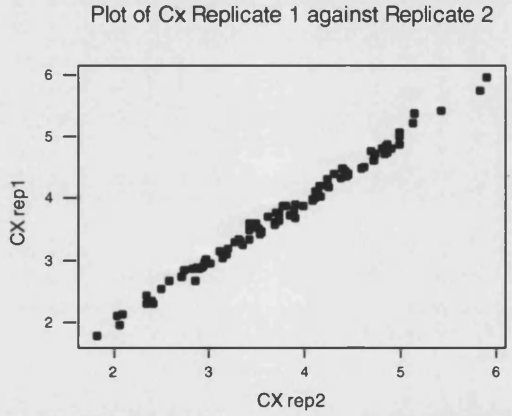


Figure 3.2: Scatter plot of Cx replicate 1 against replicate 2.



We used the probability plot to assess whether the difference between the initial and repeat measurements of the LAD and Cx arteries follow a normal distribution. The probability plot is a graphic technique for assessing whether or not a data set follows a given distribution such as the normal distribution[264]. The data were plotted against a theoretical normal distribution in such a way that the points should form or approximate a straight line. Deviation from the line indicates deviation from

normality[264]. Figures 3.3 and 3.4 show that the LAD and Cx difference between the initial and repeat measurements were normally distributed.

Figure 3.3: Difference between LAD replicates.

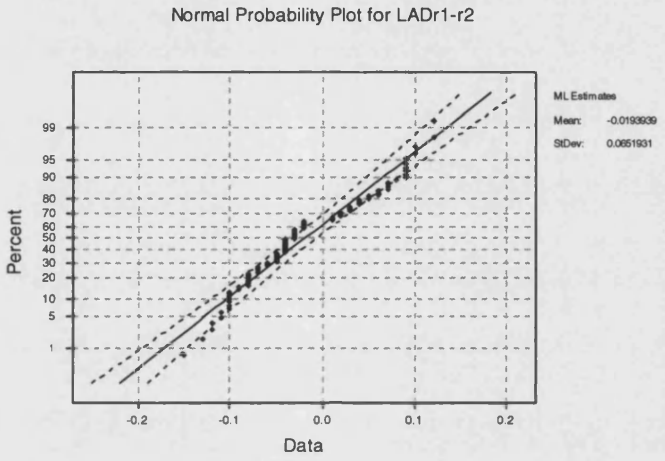
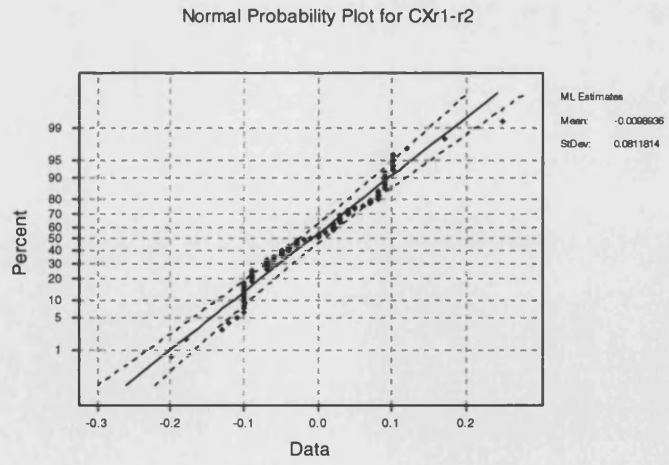


Figure 3.4: Difference between Cx replicates.



We used the repeated measurements to assess the degree of variation that might be expected to occur between a pair of QCA measurements of the same vessel segment obtained from a pair of cineangiograms using the same projection in the same patient. If the variance of the measurement is σ^2, the variance of the difference between a pair

of replicate measurements will be $2\sigma^2$ and hence an estimate of σ^2 is given by half the sample variance of the differences[265]. So, for the LAD, our estimate of σ^2 was $0.065^2/2$ (see table 3.3) and the estimate of the standard deviation was $0.065/\sqrt{2}$ i.e. 0.046. For a "normal distribution", there is approximately a 95% chance of the second of a pair of measurements falling within 2 standard deviations of first. So, there was 95% probability range for a repeated LAD measurement lying within approximately ± 0.10mm of the first measurement.

Similarly, for the circumflex, the sample variance of the 94 differences was 0.082^2 and standard deviation = $0.082/\sqrt{2}$ i.e. 0.058. So, there was 95% probability range for a repeated Cx measurement lying within approximately ±0.12mm of the first measurement.

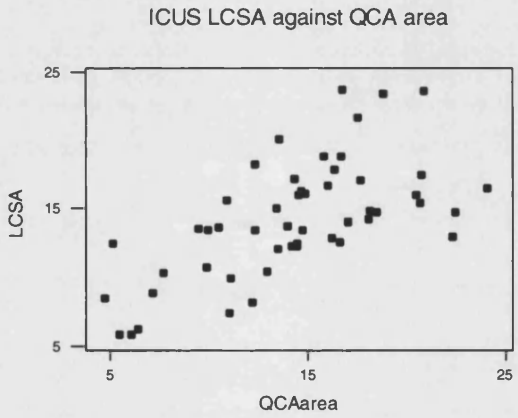
3.4 Validation of QCA data using intracoronary ultrasound.

We assessed the validity of our QCA data by comparing it to data obtained from ICUS in 18 transplant patients. Direct comparisons between the 2 techniques are limited by several factors. First, QCA measures the lumen diameter from a single angiographic projection of the vessel segment. Lumen area can only be calculated by assuming the vessel cross sectional to be approximately circular so the area is approximately $\Pi d^2/4$. This assumption appears justified in transplant patients without gross coronary disease. Second QCA cannot measure an absolute vessel diameter because of the magnification error created because the catheter located in the aortic root which is used to calibrate the image does not lie in the same plane as the vessel segment to be measured. This, systematic, magnification error will however be constant when further measurements of

the same vessel segment are made in the same angiographic projection. We have therefore not only compared absolute QCA and ICUS measurements but also compared the relative change in vessel diameter over time measured by the two techniques.

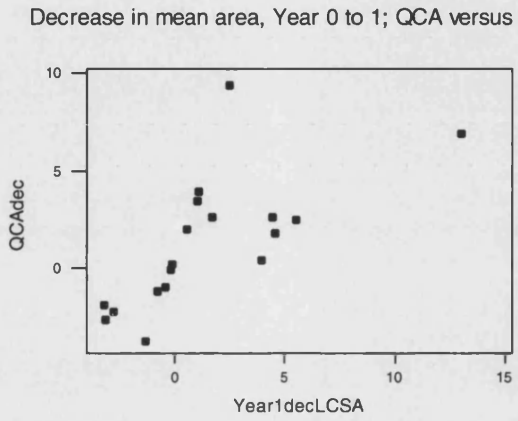
Figure 3.5 shows the plot graph of the correlation between the ICUS lumen cross sectional area and QCA mean area in all the vessels. There was a strong evidence of a correlation between the two, correlation factor being 0.641 ($p<0.001$).

Figure 3.5: Plot of ICUS lumen cross-sectional area against QCA mean lumen area in all vessels in μm^2.



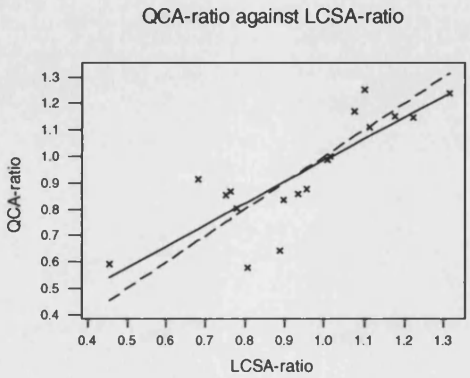
We also tested the hypothesis that the decrease in ICUS lumen cross-sectional area from baseline to Year-1 was similar to the decrease in QCA mean area over the same period. The plot graph in figure 3.6 shows the result that the measured losses were correlated, but can sometimes vary from each other by a large margin. The sign was nearly always the same for both measurements i.e. when ICUS indicated a decrease, then so did QCA and vice versa.

Figure 3.6: Plot of the loss in ICUS luminal cross-sectional area against loss in QCA mean area in μm^2.



We then assessed whether the relative (percent) diameter change from baseline to Year-1 as measured by one modality is reflected by the other. Figure 3.7 shows the scatter plot of QCA ratio (QCA at Year-1/QCA at Year-0) to that of the ICUS ratio. The dashed line represents the line of identity (QCA-ratio = LCSA-ratio) and the solid line represents the regression line obtained from our data. The two ratios were very well correlated (R^2= 0.682). The F-test[266], which tests whether the standard deviations of two populations are equal, showed the p-value to be 0.42 implying no significant evidence of a poor fit.

Figure 3.7: plot of QCA-ratio versus LCSA-ratio.



3.5 Discussion.

We have assessed both the reliability and the validity of QCA measurements in our laboratory. The 95% confidence intervals for a single repeat measurement of the LAD or Cx were ±0.10mm and ±0.12mm respectively. This was similar to that found by Gao et al[38] in their QCA study (± 0.1mm). Under ideal circumstances, modern radiographic imaging techniques provide spatial resolution of 5 line pairs per millimetre[87] (5 black lines alternating with 5 white lines at intervals of 100μm). Thus this 100μm error reflects the inherent error of the radiographic technique and X-Ray image rather than an error in QCA system.

Although only the proximal LAD and Cx coronary artery segments have been studied here, we have no reason to suspect that the right coronary artery (RCA) will behave differently. The systematic error identified in the repeat LAD measurement is unexplained. It is unlikely to reflect a general error in the method since there was no significant change in the circumflex measurements. It may reflect some change in LAD vessel tone between the first and second measurements. However, its magnitude is small in comparison to both the long term changes observed in the rest of this project and to the 'random' errors observed in the reliability study.

Direct comparison between QCA and ICUS data is flawed because of the magnification error inherent in the QCA method. Nevertheless, our QCA data correlated with the ICUS data. Changes in vessel lumen diameter with time measured by QCA were very closely related to those observed by ICUS. These findings were in agreement with those of Lowry et al[263].

These observations support the use of QCA in the rest of this study. Although QCA has some limitations including the failure to image the vessel wall, we have demonstrated that it can provide valid and reliable measurements of vessel lumen diameter. In addition QCA has many advantages over ICUS including its less invasive nature, the ability to avoid anticoagulation, the ability to study smaller branch vessels, its lower cost and the ease with which it can be incorporated into a routine angiographic follow up protocol.

CHAPTER 4

RESULTS I

CHANGE IN VESSEL MEAN LUMEN DIAMETER AND TONE WITH TIME.

4.1 Mean lumen segment diameter at each year.

4.2 Loss in the individual segment mean lumen diameter from baseline.

4.3 Mean lumen diameter of the "large" and "small" vessel segments.

4.4 Change in mean lumen diameter from baseline & between successive years in the "large" and "small" vessels.

4.5 Relationship of change in the vessel diameter in the first year to long-term changes.

4.6 Increase in vessel segment diameter after GTN with time: basic statistics.

4.7 Correlation between increase in segment mean lumen diameter after GTN at baseline and subsequent loss of diameter.

4.8 Change in intracoronary ultrasound parameters.

- 4.8.1 Intimal area, lumen cross-sectional area and the total area as determined by ICUS.
- 4.8.2 Intimal and remodelling indices.

4.9 Discussion.

- 4.9.1 QCA results.
- 4.9.2 ICUS results.
- 4.9.3 Conclusion.

This chapter describes the changes that were observed in vessel diameter and tone with time. The methods used were described in chapter 2. We also compared changes in the larger coronary vessels (LV) with those in the smaller vessels (SV) which, in life, can only be studied by QCA because they are usually too small to admit an ICUS probe. Although histological studies[34, 37, 184] have emphasised the importance of these SV in coronary disease within the transplanted heart, these vessels have not been analysed in detail in previous clinical studies. Our study population consisted of 121 patients.

We noticed that the vessels tone, as detected by the difference in vessel diameter before and after GTN, was different at the baseline angiogram compared to that at year-1. As the endothelium plays a critical role in regulating the coronary tone[267, 268] we also investigated whether early change in vascular tone was a predictor of later mean lumen diameter loss (MLDL).

We also performed ICUS in a subgroup of 22 patients who also underwent QCA examination and investigated the changes that occur in the vessel segment areas and indices over the first three years after transplant. The implications of the ICUS findings for the QCA study will be discussed.

4.1 Mean lumen segment diameter at each year.

The diameter of individual coronary segments at Year-0 (baseline), 1, 3 and 5 are shown in table 4.1A and 4.1B for the LV and SV respectively. The segments measured at baseline were slightly less than the number of patients in the study as a few segments could not be measured due for technical reasons (e.g. inadequate opacification of the segment or problems caused by overlapping structures). With time, there was a decrease in the number of patients analysed because of those who had not reached their

3 or 5 year follow up before the end of the study. There was a continuous loss in the diameter of vessel segments with time in both the LV and SV; the small increase in mean diameter seen at Year-5 in some segments was probably due to the small sample size.

Table 4.1A: Mean lumen diameter of the "large" vessel segments.

Vessel	Year	*n*	Mean (mm)	Standard deviation	Median
Proximal LAD	0	116	4.50	0.72	4.51
	1	117	4.21	0.72	4.28
	3	48	4.11	0.67	4.01
	5	8	3.81	0.55	3.98
Mid LAD	0	115	3.12	0.74	3.00
	1	110	2.92	0.69	2.80
	3	44	2.76	0.67	2.78
	5	6	2.73	0.6	2.65
Proximal Cx	0	118	3.86	0.82	3.84
	1	115	3.65	0.76	3.71
	3	47	3.68	0.80	3.70
	5	8	3.19	1.09	3.06
Proximal RCA	0	118	4.40	0.70	4.41
	1	115	4.14	0.67	4.14
	3	46	4.05	0.70	4.13
	5	7	4.00	1.08	4.52
Mid RCA	0	116	3.84	0.75	3.90
	1	112	3.67	0.69	3.75
	3	46	3.50	0.70	3.54
	5	7	3.85	0.54	3.99

For meaning of abbreviations, please see table 2.1

Table 4.1B: Mean Lumen diameter of the "small" vessel segments.

Vessel	Year	*n*	Mean (mm)	Standard deviation	Median
Distal LAD	0	112	1.77	0.44	1.65
	1	106	1.71	0.41	1.59
	3	46	1.62	0.37	1.54
	5	7	1.62	0.30	1.56
D1	0	113	1.89	0.50	1.89
	1	110	1.83	0.46	1.83
	3	43	1.84	0.42	1.84
	5	6	1.59	0.49	1.59
D2	0	89	1.46	0.40	1.34
	1	87	1.45	0.39	1.41
	3	36	1.43	0.39	1.47
	5	3	1.51	0.22	1.44
Distal Cx	0	113	1.97	0.75	1.79
	1	107	1.91	0.68	1.65
	3	45	1.92	0.78	1.76
	5	8	1.82	0.39	1.77
OM1	0	109	1.90	0.55	1.82
	1	109	1.83	0.49	1.74
	3	46	1.77	0.47	1.70
	5	6	1.70	0.29	1.76
OM2	0	84	1.43	0.38	1.36
	1	82	1.40	0.36	1.35
	3	40	1.45	0.30	1.37
	5	5	1.58	0.21	1.61
Distal RCA	0	117	3.56	0.87	3.57
	1	113	3.33	0.86	3.44
	3	46	3.22	0.86	3.42
	5	8	3.02	1.07	3.41
RVBr.	0	113	1.70	0.35	1.76
	1	110	1.67	0.35	1.69
	3	47	1.57	0.32	1.61
	5	8	1.49	0.18	1.42
PDA	0	109	1.78	0.41	1.74
	1	105	1.72	0.39	1.70
	3	42	1.71	0.35	1.70
	5	6	1.83	0.56	1.72

For meaning of abbreviations, please see table 2.1

4.2 Loss in the individual segment mean lumen diameter from baseline.

We then calculated the loss in segment mean lumen diameter from baseline to Year-1, 3 and 5, to assess its magnitude and time course. This was expressed both in terms of absolute and proportional (percentage) change. The proportional decrease to a

particular year, e.g. Year-1, was calculated as 100 × ("Year-0" value – "Year-1" value)/ "Year-0" value. Tables A4.1A &B and tables A4.2A&B in the appendix detail the decrease in segment diameter from baseline in absolute and proportional terms. There was gradual MLDL with time in both the "large" and "small" vessels.

4.3 Mean lumen diameter of the "large" and "small" vessel segments.

Vessel segments were grouped into large (proximal and mid LAD, proximal Cx, and proximal and mid RCA) and small vessel (distal LAD, Cx and RCA, D1 and D2, OM1 and OM2, RV branches and PDA) groups as described in chapter 2. A composite mean lumen diameter (MLD) for each category of vessels was then calculated as shown in tables 4.2 A&B. There was MLDL with time; the slight increase in diameter at Year-5 in the small vessels is probably due to the small sample size.

Table 4.2 A: Mean lumen diameter of all the "large" vessel segments.

Year	*n*	Mean	Standard deviation	Median
0	120	3.9559	0.4938	3.9430
1	118	3.7307	0.4553	3.7540
3	48	3.6302	0.4645	3.5690
5	9	3.470	0.501	3.710

Table 4.2 B: Mean lumen diameter of all the "small" vessel segments.

Year	*n*	Mean	Standard deviation	Median
0	120	2.0076	0.3493	1.9428
1	118	1.9116	0.2697	1.8622
3	48	1.8448	0.2291	1.8344
5	9	1.8612	0.1820	1.8529

4.4 Change in mean lumen diameter from baseline & between successive years in the "large" and "small" vessels.

We calculated the absolute MLDL from baseline, in both the LV and SV separately (table 4.3) together with the proportional change (table 4.4). The absolute and proportional MLDL progressed with time.

Table 4.3: Absolute mean lumen diameter loss from baseline in the "large" and "small" vessels.

Year	*n*	Large Vessels		Small Vessels	
		Mean (mm)	Standard deviation	Mean (mm)	Standard deviation
1	117	0.23	0.27	0.0812	0.1557
3	47	0.42	0.27	0.1615	0.2070
5	8	0.52	0.29	0.271	0.379

Table 4.4: Proportional mean lumen diameter loss from baseline in the "large" and "small" vessels.

Year	*n*	Large Vessels		Small Vessels	
		Mean (%)	Standard deviation	Mean (%)	Standard deviation
1	117	5.314	7.040	2.633	7.739
3	47	10.135	6.461	5.96	7.55
5	8	13.05	8.03	8.62	10.61

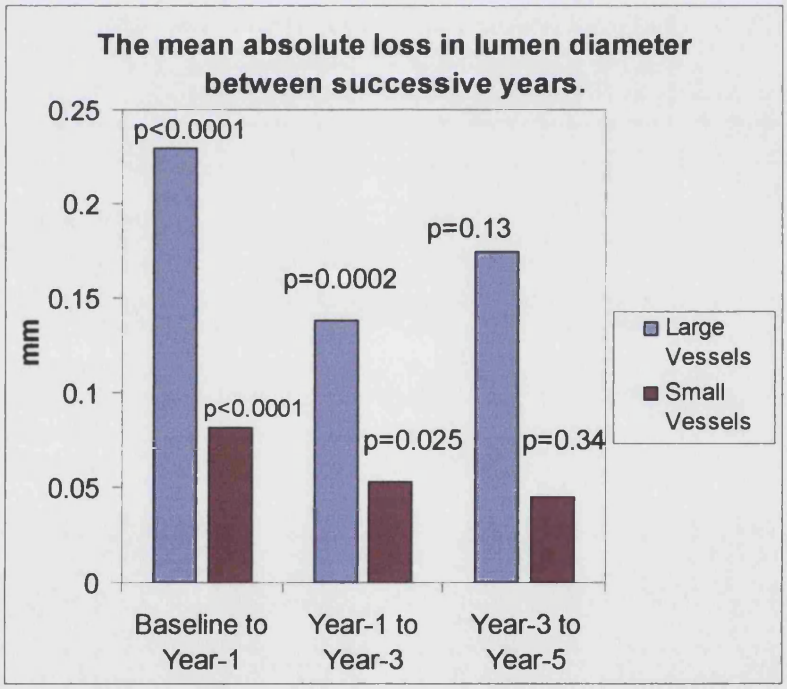
The difference between the MLDL in the LV and SV was then calculated, both in absolute and percentage values (table 4.5). Using the t-test, the p-value examined the hypothesis that the absolute (or percentage) LV-MLDL equals the absolute (or percentage) SV-MLDL. The absolute and proportional MLDL was significantly greater in the LV up to Year-3.

Table 4.5: Absolute and proportional difference between the "large" and "small" vessel mean lumen diameter loss.

Year	*n*	Absolute difference			Proportional difference		
		Mean (mm)	Standard Deviation	p value	Mean (%)	Standard Deviation	p value
1	117	0.1475	0.2011	<0.0001	2.681	6.389	<0.0001
3	47	0.2619	0.2665	<0.0001	4.18	7.36	0.0003
5	8	0.245	0.388	0.12	4.42	11.90	0.33

We also calculated the LV and SV-MLDL *between successive years.* The results are shown in figure 4.1 and displayed in table A4.3; using the t-test, the p-value signifies whether the mean loss equals zero. There was significant MLDL from baseline to Year-1 and from Year-1 to Year-3, in both the LV and SV.

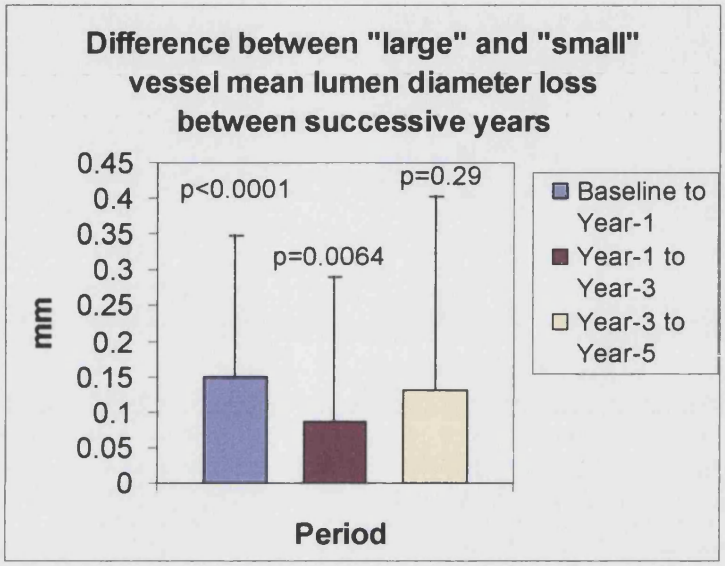
Figure 4.1



Subsequently, we calculated *the difference* between LV-MLDL and SV-MLDL *between successive years.* The results are shown in figure 4.2, and displayed in table

A4.4. There was evidence that the LV had significantly more MLDL than the SV from baseline to Year-1 and from Year-1 to Year-3.

Figure 4.2



We investigated whether or not MLDL from baseline to Year-1 was similar to MLDL from Year-1 to Year-3 using the matched paired t-test. There was significant evidence that the first year MLDL was higher than MLDL from Year-1 to Year-3 for both LV and SV ($p < 0.0001$ for both cases).

4.5 Relationship of change in the vessel diameter in the first year to long-term changes.

We investigated whether diameter change in the first year was predictive of subsequent loss of diameter. Table A4.5 A and A4.5 B show the summary statistics and a p-value for each group, investigating whether the mean long-term decrease was the same for the different groups. The long-term MLDL increased with group with greater loss during

the first year. Long-term reflects the maximum reductions in diameter from baseline to Year-3 or to Year-5. Using ANOVA (which is used to compare the means of more than two independent groups) there was significant difference in the long-term (from baseline up-to Year-5) MLDL between the groups ($p<0.001$).

Figure 4.3 shows the scatter plot of the long-term percentage MLDL against percent MLDL from baseline (Year-0) to Year-1 in the LV. Initial MLDL was a good predictor of long-term MLDL. A linear regression of long-term percent decrease gave the following formula:

$$\text{long-term \% MLDL} = 6.92 + 0.614 \times \text{initial \% MLDL} \quad (p<0.001)$$

For example, if there is no change in the first year, the long-term percent MLDL is predicted to be 6.9%; if there is a 10% MLDL in the first year, the long-term mean percent MLDL is predicted to be 13%.

Figure 4.3: Long-term "large" vessel percentage mean lumen diameter loss against percentage loss from baseline to Year-1

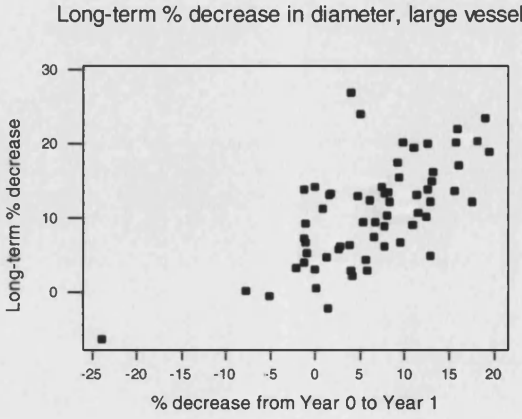
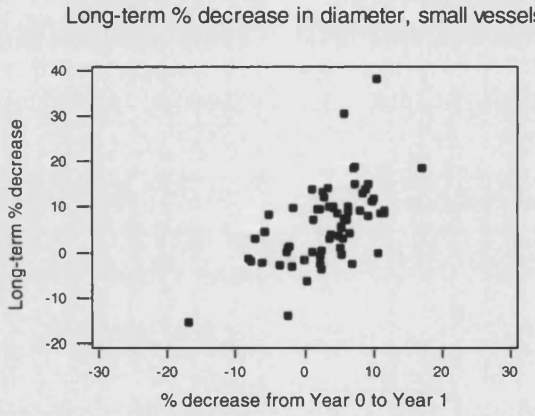


Figure 4.4 shows the scatter plot of the long-term percent MLDL in the SV against percent MLDL from baseline to year-1. Although the relationship was not as strong as for the LV, initial MLDL was still a good predictor of long-term MLDL. A linear regression of long-term percent MLDL gave the following formula:

$$\text{long-term \% MLDL} = 3.51 + 0.874 \times \text{initial \% MLDL} \quad (p<0.001)$$

Figure 4.4: Long-term "small" vessel percentage mean lumen diameter loss against percentage loss from baseline to year-1



4.6 Increase in vessel segment diameter after GTN with time: basic statistics.

We measured the MLD of the proximal LAD and Cx and the OM 1, before and after glycerine trinitrate (GTN) was given to assess whether a change in vessel diameter (a reflection of vessel tone) was related to long-term TxCAD, as measured by MLDL. Figure 4.5A and 4.5B shows QCA of the proximal Cx before and after GTN respectively. Tables 4.6 to 4.8 show the basic statistics for the increase in diameter (in mm), from before GTN to after GTN, for each year. The p-value tests the hypothesis of zero mean increase in diameter, using a paired t-test. There was a significant increase in diameter in all segments after GTN administration for all three vessels; however the

least mean increase occurred at baseline (Year-0), indicating that the resting vessel tone was least at that time.

Figure 4.5A: QCA of the proximal Cx before GTN.

Figure 4.5B: QCA of the proximal Cx after GTN.

Table 4.6: Proximal LAD absolute mean lumen diameter increase after GTN.

Year	*n*	Mean lumen diameter increase (mm)	Standard deviation (mm)	p-value
0	55	0.2882	0.2109	<0.0001
1	69	0.4191	0.4653	<0.0001
3	36	0.3625	0.2960	<0.0001
5	5	0.3700	0.0474	0.0001

Table 4.7: Proximal Cx absolute mean lumen diameter increase after GTN.

Year	*n*	Mean lumen diameter increase (mm)	Standard deviation (mm)	p-value
0	59	0.3415	0.2326	<0.0001
1	69	0.5291	0.2232	<0.0001
3	35	0.4666	0.3133	<0.0001
5	6	0.3850	0.2224	0.0082

Table 4.8: OM1 absolute mean lumen diameter increase after GTN.

Year	*n*	Mean lumen diameter increase (mm)	Standard deviation	p-value
0	58	0.1771	0.1745	<0.0001
1	68	0.2649	0.1412	<0.0001
3	32	0.2447	0.1488	<0.0001
5	4	0.2500	0.1140	0.022

4.7 Correlation between increase in segment mean lumen diameter after GTN at baseline and subsequent loss of lumen diameter.

Having demonstrated that the least diameter increase after GTN administration was at baseline, we then investigated whether there was a correlation between the extent of this increase in diameter after GTN at the baseline angiogram, and the subsequent MLDL compared to baseline, thus reflecting TxCAD on the long-term. The correlations could not be computed for MLDL between Year-0 to Year-5, due to small data size at Year-5. Table 4.9 shows the results of these correlations and their p values (for testing zero correlation). The negative correlations indicate that a larger increase in diameter after GTN is associated with a smaller subsequent MLDL. For the proximal LAD, there was marginal evidence that a higher diameter increase after GTN was associated with a smaller MLDL between Year-0 and Year-1; there was little evidence of a longer-term correlation. For the Proximal Cx, there was marginal evidence that a higher increase after GTN was associated with a smaller MLDL between Year-0 and Year-3; there was little evidence of other correlations. For the OM1, and contrary to

what was found in the proximal LAD, there was evidence that a higher increase after GTN was associated with a higher MLDL between Year-0 and Year-1; there was no evidence of another correlation. Thus overall, there was no consistent evidence that response to GTN at baseline angiogram predicted subsequent change in mean lumen diameter.

Table 4.9: Correlation between baseline increase in proximal LAD, proximal Cx, and OM1 segment diameter after GTN and the long-term MLDL.

Period of diameter loss	Proximal LAD segments		Proximal Cx. segments		OM1 segments	
	Correlation	p-value	Correlation	p-value	Correlation	p-value
Year-0 to Year-1	-0.263	0.052	0.027	0.84	0.323	0.013
Year-0 to Year-3	-0.245	0.50	-0.672	0.047	0.020	0.96

4.8 Change in intracoronary ultrasound parameters.

4.8.1 Intimal area, lumen cross-sectional area and the total area as determined by ICUS.

Table 4.10 shows the summary statistics of the intimal area (IA), lumen cross-sectional area (LCSA) and the total area (TA) of all the vessels as analysed using morphometric analysis (see sections 2.5.2 and 2.5.3). Twenty-two patients were studied in total, and the follow up ranged from 1 to 3 years. Note that only 18 patients had follow up at Year-1. The LAD was examined in 10 patients, the Cx in 6 and the RCA in another 6 patients. Changes in these areas are shown in figures 4.6, 4.7 and 4.8 respectively from baseline to Year-1. Based on a matched paired t-test, the p-value evaluates the hypothesis of zero mean change. There was significant evidence in most cases that mean IA increased. The LCSA decreased, but the evidence was not significant, probably because of the small sample size. There was little evidence that mean TA changed.

Table 4.10: Summary statistics for all the vessels intimal area, lumen cross-sectional area and the total area.

Year	*n*	Intimal Area			Lumen Cross-Sectional Area			Total Area		
		Mean (mm^2)	Standard deviation (mm^2)	Median (mm^2)	Mean (mm^2)	Standard deviation (mm^2)	Median (mm^2)	Mean (mm^2)	Standard deviation (mm^2)	Median (mm^2)
0	22	0.50	1.18	0.00	14.68	4.46	14.53	15.18	4.34	15.75
1	18	1.43	1.73	0.96	13.15	3.65	13.66	14.58	3.58	15.25
2	8	1.78	1.87	1.61	14.62	3.28	13.46	16.41	3.89	14.95
3	4	4.33	3.94	2.69	14.43	3.68	14.53	18.77	5.99	19.14

Figure 4.6

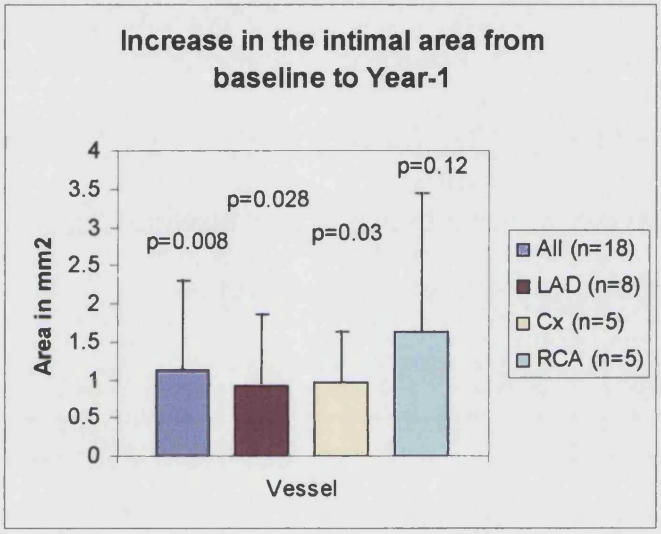


Figure 4.7

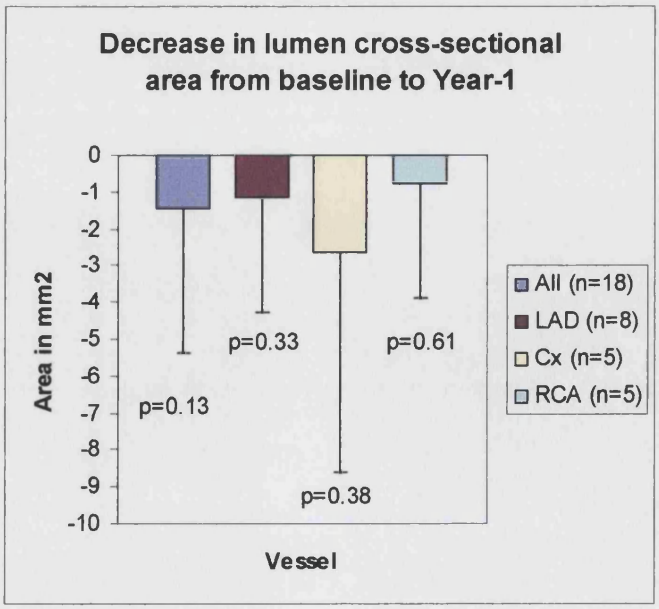
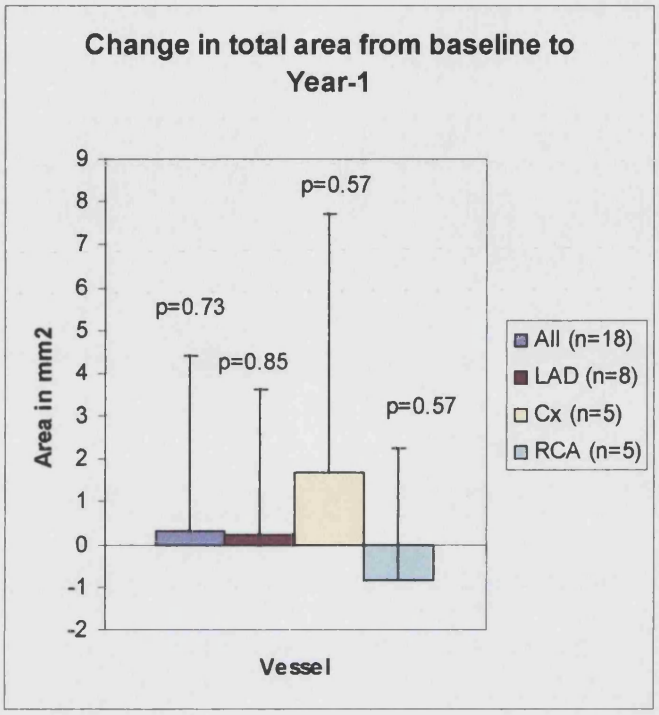


Figure 4.8



4.8.2 Intimal and remodeling indices.

Table 4.11 shows the summary statistics of the intimal index (II) and remodelling index (RI); section 2.5.3 shows the definitions of II and RI. Change in II is shown in figure 4.9. The II significantly increased when all the vessels were considered together. However, the increase was not always significant when the vessels were considered separately, probably because of the small sample size.

Figure 4.10 shows the frequencies of the different remodelling patterns over the first year and over the three years period. Remodelling indices were classified into the 3 groups shown in section 2.5.3. During the first year, "overcompensation" and "absence of compensation and vessels shrinkage" occurred with similar frequencies of 39% and

"partial compensation" occurred in 22% of vessels. Overall during the 3 years of the study, "absence of compensation and vessel shrinkage" was the commonest, accounting for 47% of the RI, followed by "overcompensation" accounting for 33%, followed by "partial compensation" accounting for 20%.

Table 4.11: Summary statistics of the intimal and remodelling indices.

Year	*n*	Intimal Index			Remodelling Index		
		Mean	Standard deviation	Median	Mean	Standard deviation	Median
0	22	0.0305	0.0698	0.0000	-	-	-
1	18	0.0947	0.1093	0.0550	-1.29	8.64	0.39
2	8	0.0985	0.0917	0.0829	-3.89	13.72	0.11
3	4	0.2038	0.1308	0.1410	-0.265	0.933	-0.496

Figure 4.9

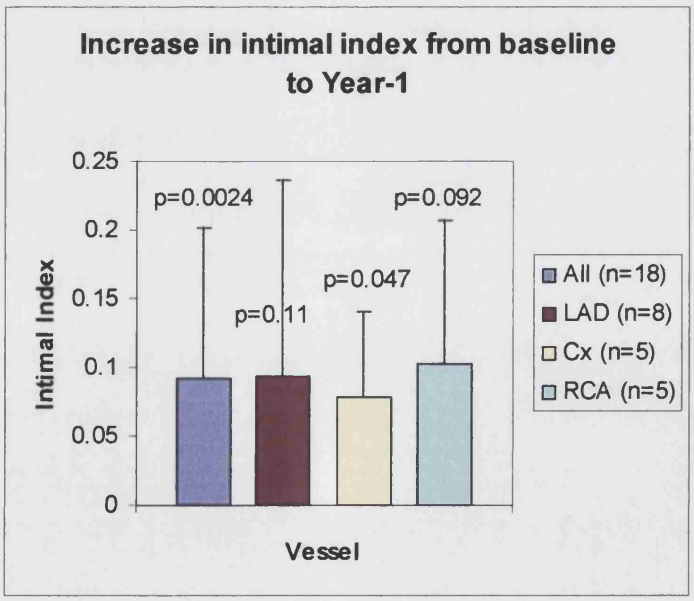
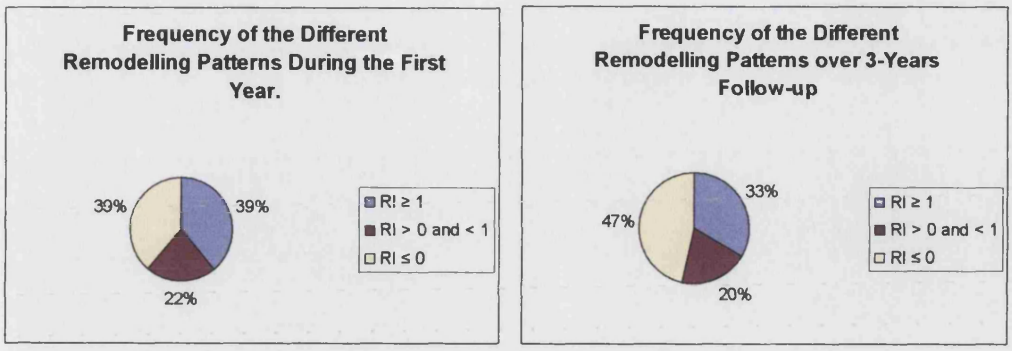


Figure 4.10



4.9 Discussion.

4.9.1 QCA results.

We have shown that there was a progressive MLDL in the LV and SV with time, the greatest loss of diameter occurred in the first year. The absolute and proportional MLDL was significantly more in the LV compared with SV in the first 3 years after transplant. The degree of initial MLDL in the first year was quite a good predictor of long-term MLDL.

Our finding that a progressive MLDL occurred during the first 3 years after transplantation contrasts with the results reported by Gao et al[38]; although they found that there was significant loss in vessel diameter in the first year post-transplant, they did not demonstrate significant loss in mean diameter between the first and second year. In addition, they found that the loss in absolute diameter for vessels greater than 2.9 mm (large vessels according to their classification) significantly exceeded diameter loss for smaller vessels. However they found that the proportional (percentage) loss did not differ for the LV and SV. Their classification of LV and SV was according to

absolute vessel size, rather that ours of proximal and mid primary coronary arteries versus distal primary and secondary coronary vessels, might have contributed to this difference. In addition, their study contained only 25 patients which would have reduced their ability to detect changes in the coronary lumen diameter.

Our finding that an initial MLDL in the first year was a predictor of long-term MLDL is in keeping with another study by Gao et al[53]. In their study of 139 consecutive patients who underwent transplantation, 45 patients progressed from a normal qualitative angiogram to the presence of 50% or greater stenosis in one or more major vessels within 1 year (fulminant group); 94 did not (indolent group). At 1, 3, and 5 years after initial detection of TxCAD, 50%, 33%, and 16% of patients in the fulminant group and 89%, 70%, and 60% of patients in the indolent group were free of ischemic events ($p < 0.0001$).

An important implication of our finding that an initial MLDL was a predictor of long-term MLDL is the need for tighter control of cardiovascular risk factors and immunosuppression, if a large initial MLDL was detected. In addition, the extent of initial MLDL will help determine the frequency of follow-up angiograms.

However, detecting early loss in vessel diameter can only be done by QCA. Most clinicians base their findings on qualitative angiography, which is an insensitive means of detecting change. Our findings can be applied in routine clinical practice if computer assisted techniques are used.

Vessel tone was low immediately after transplant in both the LV and SV, but it tended to increase later. Review of literature showed that a similar study relating endothelium independent vasodilation to TxCAD was not undertaken before. Our study showed that in general endothelium independent vasodilation by GTN at baseline was not a predictor of MLDL on the long term. An exception to this was our finding that higher tone at baseline in the OM1 was associated with greater MLDL between baseline and Year-1; however, that association disappeared on longer-term follow-up and hence its significance is questionable.

Other studies have investigated whether endothelium dependant vasodilation is related to subsequent TxCAD. Aptecar et al[180] compared the responses of epicardial coronary arteries to stepwise intracoronary infusion of acetylcholine (10^{-8}M to 10^{-5}M) in 18 patients who had undergone transplants within 2 months after surgery, to that in 7 control subjects. Follow-up at 1 year showed angiographically normal coronary arteries in 12 patients and coronary atherosclerosis in 6 patients. In patients who had undergone transplants, acetylcholine-induced endothelium-dependent coronary artery dilation was similarly impaired early after surgery (within 2 months) in patients with and without coronary atherosclerosis at 1-year follow-up. Thus, the response to acetylcholine was not a predictor of secondary atherosclerosis. Nitenberg et al[174] showed that the acetylcholine response is persistently abnormal in transplant recipients compared with that in normal control subjects and that this abnormality may not be related simply to the presence of atherosclerosis.

However, Davis et al[181] studied the endothelium-dependent vasomotion early post-transplant in 20 patients by serial intracoronary acetylcholine infusion, and the percent

change in diameter was measured by quantitative angiography. The development of arteriosclerosis was studied by use of ICUS in the same 20 patients by quantifying the changes in II and maximal intimal thickness of 46 matched coronary segments between initial and 1-year follow-up studies. They found that coronary segments with endothelial dysfunction demonstrated a significantly greater increase in mean II and maximal intimal thickness by 1-year post-transplant compared with segments with normal endothelial function.

4.9.2 ICUS results.

In our study, using ICUS, there was significant increase in both the mean IA and the II from baseline to Year-1. There was a decrease in the LCSA from baseline to Year-1; however the decrease was not significant in our study, possibly due to the small sample size. There was no change in the TA. During the 3 years of our study, "absence of compensation and vessel shrinkage" was the commonest remodelling pattern, followed by "overcompensation", followed by "partial compensation".

Our finding that that the mean IA and II increased from baseline to Year-1 is in keeping with the finding of Yeung et al[31], who in their multicentre ICUS study found that in 92 patients evaluated at 1 year, there was an increase the IA. This is further confirmed by the findings of Rickenbacher et al[269] who found that the mean intimal thickness and II were significantly higher at Year-1 after transplantation.

We found that the commonest remodelling patterns early after transplantation (Year-1) are "overcompensation" and "absence of compensation and vessel shrinkage" (39% each), and the latter was the commonest pattern occurring later in transplant (47% at

Year-3). This seems to be in keeping with the findings by Lim et al[251] who studied 75 heart transplant recipients with 151 matched coronary segments for the presence of intimal disease progression as detected by serial ICUS examinations 1 to 3 years apart. They found that early after cardiac transplantation, a large proportion of the coronary segments with progression of intimal thickening had "overcompensation" of the vessel wall; however, a substantial number of coronary segments (22%) showed "absence of compensation and vessels shrinkage".

An important study correlating the different ICUS parameters in transplanted hearts was undertaken by Tsutsui et al[270]. In 38 transplant recipients, serial ICUS examinations were performed 3.7±2.2 weeks after transplantation and annually thereafter for 5 years. They found that most of the intimal thickening occurred during the first year after heart transplantation. Changes in the TA showed a biphasic response, consisting of early expansion and late constriction. Thus, different mechanisms of lumen loss were observed during 2 phases after transplantation: early lumen loss primarily caused by intimal thickening and late lumen loss caused by TA area constriction. Again, our study seems to be in keeping with these findings, although changes in our parameters were not always significant. This is probably due to the fewer number of patients in our study, and to the shorter follow-up.

Comparison of our QCA data to the ICUS data was made as mentioned in section 3.4. Assuming that the vessel is circular, ICUS lumen cross sectional area and QCA mean area were strongly correlated, correlation factor being 0.641 ($p<0.001$). Also the QCA ratio (QCA at Year-1/QCA at Year-0) was well correlated to the ICUS ratio (R^2= 0.682). Our ICUS findings, coupled with those of others indicate that progressive loss

of MLD observed by QCA may be the result of two processes- intimal thickening or vessel shrinkage. It is not possible to differentiate these by QCA.

4.9.3 Conclusion.

In conclusion, TxCAD is predominantly a disease of the large epicardial vessels, rather than the small distal and secondary branch vessels in the first year after transplantation. This finding contrasts the histological concept that TxCAD is mainly a disease of the SV[34, 37, 184]; although disease in the SV is readily recognizable on histological examination, it is the LV that tend to lose their luminal diameter more when assessed by QCA. Useful information about TxCAD progression can be gained by assessing the change in vessel diameter during the first year. Vessel tone cannot be used solely to predict the development of TxCAD. Many different factors affect endothelial function[271, 272], and thus the endothelial response to different agents is not a pure reflection of atherosclerosis.

The ICUS study provided us with valuable information. Although intimal thickening, which is the first manifestation of TxCAD, can reduce lumen diameter, compensatory vessel expansion can mask the early phase of the disease. Thus, absence of lumen narrowing by angiography does not exclude intimal thickening.

As QCA, unlike ICUS, can be used to measure both LV and SV, and due to the good correlation between the QCA and ICUS results, QCA was used to study the factors underlying the early development of TxCAD in patients treated in our centre.

CHAPTER 5

RESULTS II

RELATIONSHIP OF NON-IMMUNOLOGICAL FACTORS TO MEAN LUMEN DIAMETER LOSS.

5.1 Donor and recipient age and sex.

5.2 Recipient race.

5.3 Recipient aetiology of heart disease.

5.4 Recipient post-transplant blood pressure.

5.5 Recipient post-transplant plasma lipids.

5.6 Donor Factors.

- 5.6.1 Cause of donor death.
- 5.6.2 Non-domino versus domino hearts.
- 5.6.3 Total ischaemic time.

5.7 Creatinine and glucose.

5.8 Echocardiographic ejection fraction and fractional shortening.

5.9 Cytomegalovirus.

- 5.9.1 Basic statistics.
- 5.9.2 Correlation of the CMV count to mean lumen diameter loss.
- 5.9.3 Relationship of the pre-transplant CMV serological status to mean lumen diameter loss.

5.10 Haemodynamic Data.

- 5.10.1 Cardiac output.
- 5.10.2 Angiographic ejection fraction.
- 5.10.3 Filling pressures.

5.11 Discussion

This chapter examines the significance of the different non-immunological factors in the development of MLDL in the LV and SV, from baseline to Year-5. Both recipient and donor factors are considered. Only univariate analysis is considered in this chapter, with multivariate analysis discussed in chapter 7.

5.1 Donor and recipient age and sex.

The mean donor age was 34±12 years. Table A5.1 shows the correlation of donor age to the development of LV and SV disease, as measured by the yearly absolute MLDL compared to baseline diameters. There was no evidence of any correlation between change in vessel size and donor age.

Of 119 donors whose sex was known to us, 47 were women and 72 men (i.e. 39.5% and 60.5% respectively). Table 5.1 shows the result of the comparisons between female and male patients and the MLDL from baseline to Year-1, 3 and 5, in both the LV and SV groups. The p-values test the hypothesis of equal MLDL for men and women (two-sample t-test). In the LV group, there was evidence of greater MLDL for hearts from male donors between Year-0 and 1. In the SV group, there was significant evidence of a greater MLDL for men during the same time period, but otherwise the evidence was less compelling than for the LV.

Table 5.1: Comparison between donor sex and the mean lumen diameter loss in "large" and "small" vessel groups.

Year	Sex	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	Female	46	0.129	0.253	0.0008	0.042	0.156	0.020
	Male	69	0.297	0.258		0.111	0.151	
0 to 3	Female	19	0.335	0.229	0.063	0.176	0.274	0.74
	Male	27	0.481	0.290		0.153	0.153	
0 to 5	Female	3	0.508	0.162	0.95	0.548	0.539	0.30
	Male	5	0.520	0.364		0.104	0.119	

Table A5.2 shows the result of the correlation of the recipient age to the development of LV and SV disease, as measured by the yearly absolute MLDL compared to baseline diameters. Positive correlations indicate larger percentage diameter loss with increasing age. In the LV group, there was no evidence of a correlation between change in vessel diameter and recipients age at transplant. In the SV group, there was significant evidence of greater MLDL between Year-0 and Year-3 with increasing recipient age. However, the result is not sustained on follow-up.

Comparison was made between female and male recipients and the MLDL from baseline to Year-1, 3 and 5, in both the LV and SV groups as shown in table A5.3. There was no evidence of a difference in MLDL between the sex in both groups.

5.2 Recipient race.

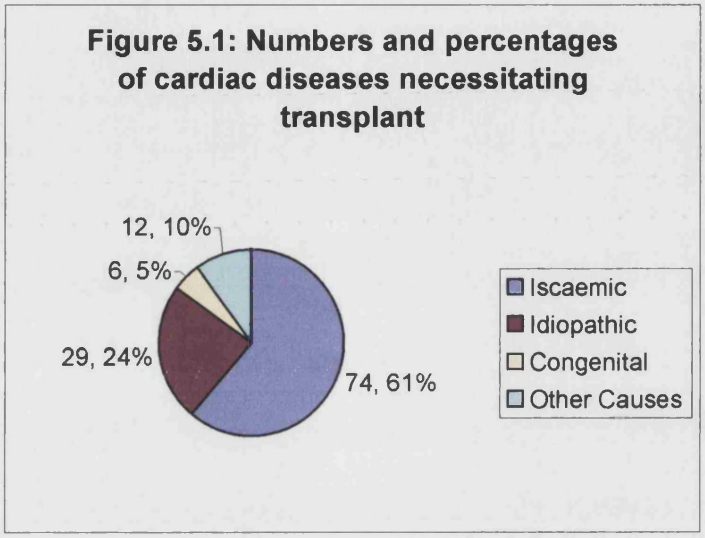
We examined the MLDL from baseline (in mm) in the LV and SV in the Caucasians and non-Caucasians, and tested the significance of this diameter loss. Table 5.2 shows the result. Overall, there are indications of a smaller loss in mean lumen diameter of the SV for Caucasians, but with such a small sample of non-Caucasians, the results remain uncertain.

Table 5.2: Comparison of the loss in mean lumen diameter from baseline (in mm) for "large" and "small" vessels for Caucasians and non-Caucasians.

Year	Race	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value for the	Mean loss	Standard deviation	p-value for the
0 to 1	Non-Caucasian	7	0.384	0.254	0.11	0.2685	0.2216	0.001
	Caucasian	110	0.219	0.266		0.0693	0.1439	
0 to 3	Non-Caucasian	3	0.452	0.335	0.85	0.1411	0.0835	0.86
	Caucasian	44	0.422	0.271		0.1629	0.2132	

5.3 Recipient aetiology of heart disease.

Figure 5.1 shows the aetiology of the underlying heart disease necessitating transplant.



In order to obtain reasonable sample sizes, we formed three groups; ischaemic, idiopathic and others. The number of men and women in the three groups are shown in Figure 5.2.

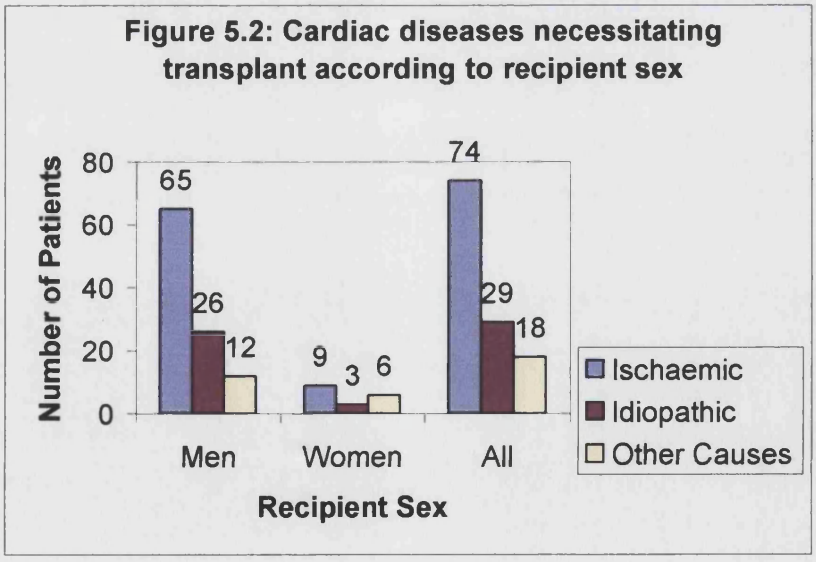


Table 5.3 summaries the mean age for the three groups. There was strong evidence that the mean age differs between the groups, with patients in the ischaemic group tending to be older. Table A5.4 compares the absolute MLDL from baseline in the LV and SV for the three aetiology groups. There was little evidence that MLDL differed between the aetiology groups, for either the LV or SV.

Table 5.3: Mean age for the different aetiological groups.

Group	*n*	Mean age	Standard deviation	p-value
Ischaemic	74	52.2	6.2	<0.001
Idiopathic	29	46.2	11.5	
Other	18	37.1	10.6	

5.4 Recipient post-transplant blood pressure.

Table A5.5A, A5.5B, A5.5C and A5.5D show the pattern of post-transplant systolic, diastolic, mean and pulse blood pressure (BP) respectively over the years. For all four measurements, the pattern was for quite a large increase in BP from pre-transplant to 1-month post-transplant, a smaller increase from 1 to 6 months post-transplant and then a steady level. Figure 5.3 shows a plot of mean BP against time. We have therefore taken three possible predictors of TxCAD: (i) increase in BP from pre- to 1 month post-transplant; (ii) increase in BP from 1 month to 6 months post-transplant; (iii) value at 6-months post-transplant. We only analysed the mean BP.

Table 5.4 shows the basic statistics of the different mean BP parameters used in our analysis, and table 5.5 shows the correlation between these parameters and MLDL between year-0 and subsequent years, and p-values for testing zero correlation. Negative correlations indicate that a larger increase in BP was associated with a smaller

subsequent loss in diameter. There was no significant correlation between the parameters used and development of significant MLDL in either LV or SV. An exception to that was the significant negative correlation between increase in BP from baseline to one month, and SV-MLDL between baseline and 3 years. The significance of this isolated correlation is not clear.

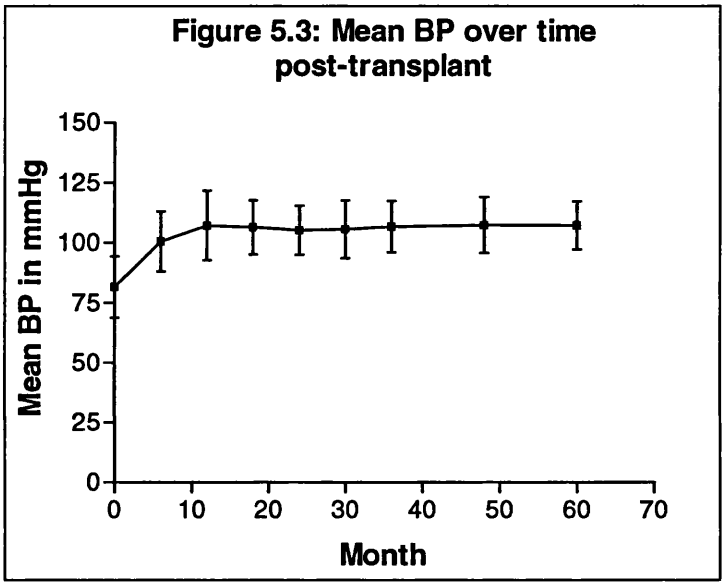


Table 5.4: Basic statistics of the different mean blood pressure parameters used for our analysis.

Blood Pressure	Number of values, *n*	Mean (mmHg)	SD	Median (mmHg)
Pre to 1-month	115	19.15	17.71	18.0
1-month to 6-months	120	6.56	16.98	5.33
At 6-months	120	107.24	14.44	107.00

Table 5.5: Correlation between the different mean blood pressure parameters and the "large" and "small" vessels mean lumen diameter loss from baseline to subsequent years.

Blood Pressure	Period in years	Large Vessels		Small Vessels	
		Correlation	p -value	Correlation	p-value
Pre to 1-month	Year-0 to Year-1	0.157	0.10	-0.022	0.82
	Year-0 to Year-3	0.032	0.84	-0.329	0.026
1-month to 6-months	Year-0 to Year-1	-0.028	0.77	0.088	0.35
	Year-0 to Year-3	0.140	0.35	0.136	0.36
At 6-months	Year-0 to Year-1	0.048	0.61	0.065	0.49
	Year-0 to Year-3	0.170	0.25	0.118	0.43

5.5 Recipient post-transplant plasma lipids.

The patients were divided into those on statins (89, or 73.6%) and those not on statins (32, or 26.4%). Table A5.6 compares the two groups with respect to MLDL from baseline to Year-1, and the different lipid fractions at Year-1. Although patients on statins had significantly lower total cholesterol, total triglycerides and LDL cholesterol, there was no difference in MLDL in the LV or SV between those on and not-on statins.

Table A5.7 correlates MLDL from Year-0 to Year-1 for the LV and SV, and the various lipid fractions. There was no evidence of any correlation with the level of the different lipid fractions.

5.6 Donor Factors.

This section examines the correlation of three donor risk factors to the later MLDL. The three donor factors are the cause of donor death, brain death (domino and non-domino hearts) and the total ischaemic time.

5.6.1 Cause of donor death.

Of the 121 donors, the transplanted hearts were obtained from living donors as part of domino transplants in 24 patients (20%), and the rest were obtained from brain dead donors. Table 5.6 shows the causes of donor death and the number of female donors and table A5.8 shows the underlying disease in the donor patients in domino transplants. We grouped the causes of donor death into 5 groups as shown in table A5.9, to allow statistical analysis. Note that the numbers used for the causes of death correspond to that used in Table 5.6. Table 5.7 shows that there was significant

evidence that donors who died from intra-cranial haemorrhage had less MLDL in the first year for both the LV and SV.

Table 5.6: Causes of donor death and the number of female donors.

Cause	Number	% of total	Number of women	% women
1= Intra-cranial haemorrhage	56	46.28	30	54.55
2= Head injury	24	19.83	4	17.39
3= Meningiococcal meningitis	2	1.65	0	0
4= Hypoxic arrest	4	3.31	3	75.00
5= Paracetmol overdose	1	0.83	1	100.00
6= Brain abscess	1	0.83	1	100.00
7= Brain tumour	5	4.13	1	20.00
8= Carbon monoxide poisoning	2	1.65	1	50.00
9= Hanging	2	1.65	1	50.00
10= Domino =XXXX	24	19.83	5	20.83
Total	121	100.00	47	39.50

Table 5.7: Comparison of mean lumen diameter loss in the five donor groups.

Year	Cause of death	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	Intra-cranial haemorrhage	55	0.1541	0.2980	0.041	0.0401	0.1512	0.022
	Head injury	23	0.2503	0.2235		0.0767	0.1421	
	A = 3, 4, 6, 7.	12	0.3643	0.2804		0.1718	0.2228	
	B = 5, 8, 9	4	0.2435	0.1376		0.0684	0.1593	
	Domino	23	0.3120	0.1858		0.1391	0.1047	
0 to 3	Intra-cranial haemorrhage	19	0.4220	0.2841	0.49	0.1248	0.1326	0.077
	Head injury	7	0.3374	0.3954		0.1495	0.2073	
	A = 3, 4, 6, 7.	6	0.3079	0.2559		0.2029	0.2719	
	B = 5, 8, 9	2	0.5733	0.0896		0.5547	0.7499	
	Domino	13	0.5021	0.1827		0.1421	0.0970	

5.6.2 Non-domino versus domino hearts.

Table 5.8 shows the result of the comparison of MLDL in the dominos and non-dominos in the LV and SV respectively. Between Year-0 and Year-1, the MLDL seemed to be greater for the domino patients, for both LV and SV. After that, though, the evidence ceased to be significant, the domino patients had slightly smaller SV-MLDL.

Table 5.8: Comparison of the absolute mean lumen diameter loss from baseline to Year-1 in "large" and "small" vessels for non-domino and domino donors.

Year	Domino status	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	Non-domino	94	0.208	0.280	0.037	0.067	0.163	0.012
	Domino	23	0.312	0.186		0.139	0.105	
0 to 3	Non-domino	34	0.393	0.295	0.14	0.169	0.237	0.58
	Domino	13	0.502	0.183		0.1421	0.0970	

5.6.3 Total ischaemic time.

The correlation between the total ischaemic time and vessel lumen diameter has also been investigated. Table A5.10 shows the summary statistics for the total ischaemic time in minutes. Table A5.11 shows the correlations of the ischaemic time and MLDL from Year-0 to subsequent years. There was no evidence that longer ischaemic time was associated with change in mean diameter for LV and SV.

5.7 Creatinine and glucose.

We correlated the recipients' serum creatinine and glucose levels to MLDL. We used the immediate pre-transplant level, maximum level during the first year and the Year-1 level for the correlation. Table A5.12 shows that there was marginal evidence of a non-zero correlation of 0.231 between the maximum creatinine level during Year-1 and the SV-MLDL between Year-0 and Year-1. Although there was some evidence that the correlation was non-zero, it was not a very strong correlation between the two variables. Table A5.13 shows the glucose correlation. There was no evidence of any significant correlation.

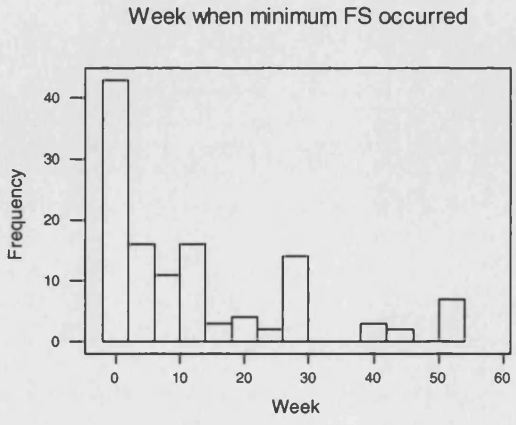
5.8 Echocardiographic ejection fraction and fractional shortening.

Echocardiographic ejection fraction (EF) was very highly correlated to the fractional shortening (FS) (correlation = 0.963, $p < 0.0001$), and thus their correlation with other variables yielded similar results. This was expected as echocardiographic EF and FS are mathematically coupled. Table 5.9 shows the correlations of minimum EF in first year and MLDL between baseline and subsequent years. There was significant evidence of negative correlations for the LV. The correlations were also negative for the SV, though there was no longer significant evidence of non-zero correlation. The minimum ejection fraction / fractional shortening tend to occur early after transplant as shown in figure 5.4.

Table 5.9: Correlation of the minimum echocardiogram ejection fraction in the first year and the mean lumen diameter loss between baseline and subsequent years for the "large" and "small" vessels.

Period of lumen diameter loss	Large vessels		Small vessels	
	Correlation	p- value	Correlation	p-value
Year-0 to Year-1	-0.267	0.004	-0.120	0.20
Year-0 to Year-3	-0.322	0.027	-0.135	0.37

Figure 5.4: Time of occurrence of minimum fractional shortening.



The mean±SD of the first recorded FS after transplant was 35±6.5% and the median was 35%. Table 5.10 show the first fractional shortening above and below the median, and its relationship to MLDL for the LV and SV. There was strong evidence of smaller LV-MLDL in the early stages for patients with higher initial fractional shortening.

Table 5.10: The relationship of the first fractional shortening measured after transplant, to mean lumen diameter loss in the "large" and "small" vessels.

Year	Initial FS	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	≤35%	60	0.290	0.262	0.0098	0.106	0.143	0.076
	>35%	57	0.164	0.259		0.055	0.165	
0 to 3	≤35%	28	0.428	0.272	0.89	0.147	0.151	0.61
	>35%	19	0.416	0.277		0.183	0.273	

5.9 Cytomegalovirus.

5.9.1 Basic statistics.

Of the 121 patients, the pre-transplant CMV serological status was positive in 70 (57.8%) and negative in 51 (42.2%). The serological CMV status was known for only 91 donors; of these 39 (42.9%) were positive and 52 (57.1%) negative. Table 5.11 shows the two-way frequencies and table 5.12 shows the basic statistics of the post transplant CMV count.

Table 5.11: Pre-transplant CMV status of the donors and recipients.

Recipient	Donor Negative	Donor Positive	All
Negative	24	14	38
Positive	28	25	53
All	52	39	91

Table 5.12: Basic statistics of the CMV count.

Variable	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value
CMV count	1337	9.21	67.73	0	0	1475

5.9.2 Correlation of the CMV count to mean lumen diameter loss.

CMV count was extremely skewed to the right. In order to try to analyse it, we found that it was helpful to work with ln(1 + CMV count) (where "ln" stands for natural logarithm). Table A5.14 shows the correlation of the mean ln(CMV count + 1) in the first year to MLDL from baseline to subsequent years, for the LV and SV respectively. There was no significant evidence of any correlation.

We also compared the MLDL for patients with zero CMV count throughout the first year and patients with at least one non-zero count in the first year. Table A5.15 shows the result. Again, there was no significant evidence that MLDL was linked to positive CMV count.

5.9.3 Relationship of the pre-transplant CMV serological status to mean lumen diameter loss.

Table 5.13 shows the relationship of the pre-transplant CMV serological status to the development of LV and SV disease. The p-value was based on an ANOVA test. For both LV and SV there was significant evidence that hearts from CMV positive donors tend to have less MLDL over the first year.

Table 5.13: Relationship of the pre-transplant serological status to the development of "large" and "small" vessel mean lumen diameter loss.

Year	CMV status	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	Donor -ve, recipient -ve	24	0.2791	0.2319	0.022	0.0923	0.1019	0.037
	Donor -ve, recipient +ve	27	0.3218	0.2343		0.1530	0.1745	
	Donor +ve, recipient -ve	13	0.1482	0.2563		0.0298	0.0918	
	Donor +ve, recipient +ve	23	0.1108	0.3202		0.0295	0.2218	
0 to 3	Donor -ve, recipient -ve	8	0.5177	0.3116	0.59	0.1725	0.1805	0.84
	Donor -ve, recipient +ve	12	0.4174	0.3457		0.1957	0.3187	
	Donor +ve, recipient -ve	6	0.4715	0.2623		0.1217	0.1572	
	Donor +ve, recipient +ve	4	0.2583	0.2668		0.0828	0.0987	

We also examined serological CMV negative donors, and assessed LV and SV-MLDL in those who became CMV viraemia positive compared to those who remained CMV viraemia negative. Fifty-one patients had donor negative hearts; of these 37 had a zero CMV count throughout the first year, so that the median equals zero. The MLDL from baseline (in mm) for patients with zero and non-zero CMV counts in the first year is shown in table 5.14; the p-value was determined using a 2-sample t-test. There was no significant evidence of a difference in MLDL between the groups.

Table 5.14: Relationship of serologically negative CMV donor hearts to mean lumen diameter loss in those who developed CMV antigenaemia compared to those who did not develop CMV antigenaemia.

Year	Any positive Year-1 CMV count?	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	No	37	0.296	0.222	0.78	0.124	0.134	1.0
	Yes	14	0.318	0.263		0.125	0.183	
0 to 3	No	15	0.416	0.335	0.33	0.217	0.291	0.27
	Yes	5	0.583	0.303		0.095	0.166	

5.10 Haemodynamic data.

5.10.1 Cardiac output.

The basic statistics of the cardiac output (CO), as measured by right heart catheterization is shown in Table A5.16. This is illustrated in figure 5.5. Mean CO increased from baseline to Year-1 and then stayed fairly constant. Table 5.15 assessed the significance of the increase in CO from baseline to Year-1, which turned out to be significant.

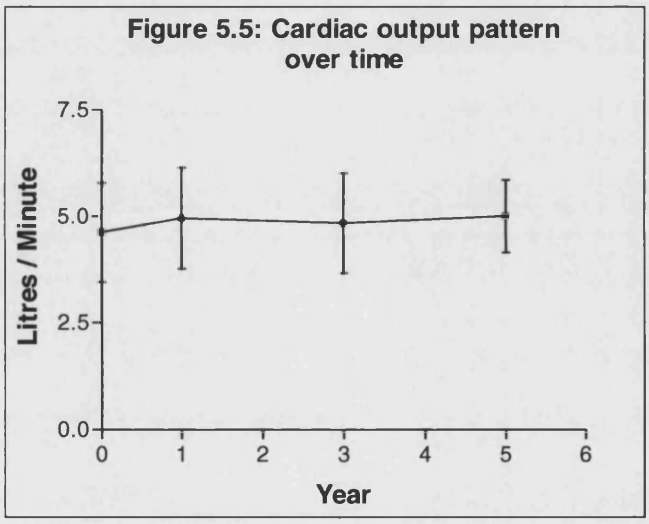


Table 5.15: Increase in cardiac output from baseline to Year-1.

n	Mean increase	Standard deviation	Median	Minimum value	Maximum value	p-value
107	0.362	1.299	0.27	-3.6	2.7	0.0048

In investigating any association between the cardiac output and MLDL, we used the cardiac output value at baseline and the increase in cardiac output from baseline to Year-1 as possible prognostic variables. Tables A5.17 and A5.18 show the correlation between cardiac output at baseline and the increase from baseline to Year-1, to subsequent MLDL. There was little evidence of an association with MLDL.

5.10.2 Angiographic ejection fraction.

Figure 5.6 shows the angiographic ejection fraction (EF) pattern over time, and table A5.19 shows the basic statistics. Mean EF decreased from baseline to Year-1 and then stayed fairly constant. Table 5.16 shows the relationship of the decrease in EF from baseline to Year-1, which was significant. The correlation between the baseline angiographic EF and decrease in EF from baseline to Year-1, to MLDL was investigated, as shown in Tables A5.20 and Table A5.21 respectively. Although the correlation with the baseline EF was negative, the evidence of an association was not significant. There was no significant correlation between the decrease in EF from baseline to Year-1, to the MLDL.

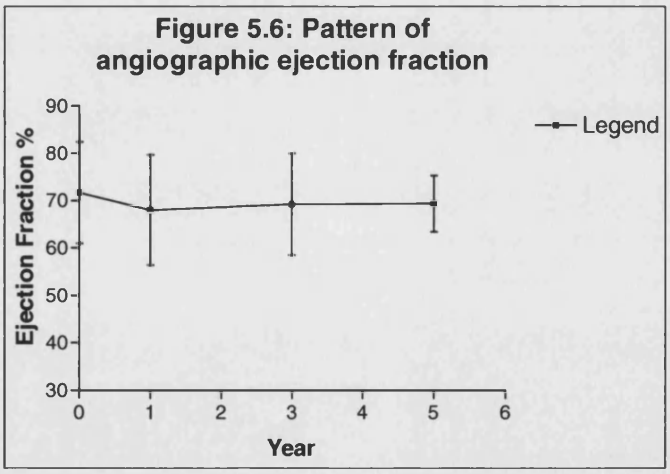
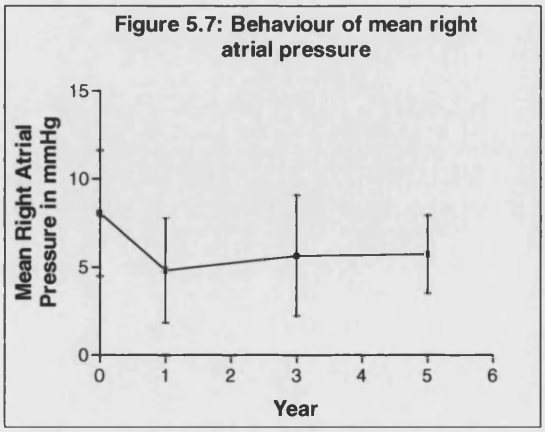


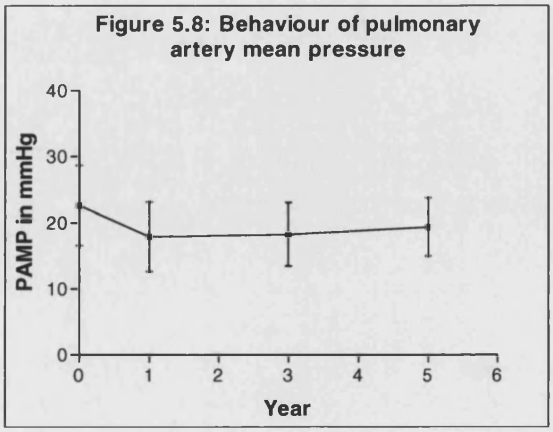
Table 5.16: Basic statistics of the decrease in angiographic ejection fraction from baseline to Year-1.

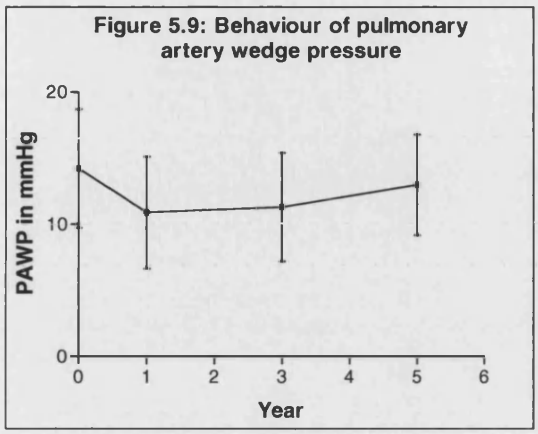
n	Mean decrease	Standard deviation	Median	Minimum value	Maximum value	p-value
101	3.12	12.54	4.0	-34	29	0.014

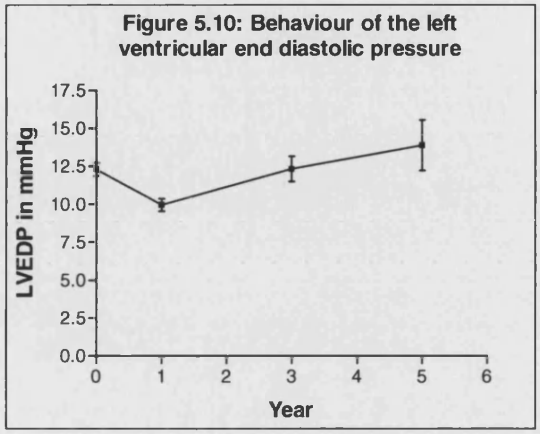
5.10.3 Filling pressures.

The basic statistics of the mean right atrial pressure (RAP), mean pulmonary artery mean pressure (PAMP), mean pulmonary artery wedge pressure (PAWP) and the mean left ventricular end diastolic pressure (LVEDP) over time are shown in tablesA5.22, A5.23, A5.24 and A5.25 respectively. Figures 5.7, 5.8, 5.9 and 5.10 show the respective plot of the values. The mean RAP, PAMP and PAWP appeared to decrease from baseline to Year-1 and then stayed fairly constant; the values at Year-5 are likely not representative due to the small sample size. The LVEDP decreased from baseline to Year-1 and then started to rise again. The decrease in mean RAP, PAMP and LVEDP values from baseline to Year-1 was statistically significant as shown in tables A5.26, 5.27 and A5.28; the values of the PAWP were closely related to that of the PAMP, and thus will not be discussed further. In investigating the correlation between the mean RAP, PAMP and LVEDP to MLDL, we used the values at baseline and the decrease from baseline to Year-1 as prognostic variables, and correlated them to the MLDL as shown in tables A5.29 to A5.34 respectively. There was no significant correlation of any of these parameters to MLDL.









5.11 Discussion.

Donor and recipient age and sex.

In this univariate analysis, donor age was not a predictor of LV or SV-MLDL. Donor male sex was associated with greater MLDL from baseline to Year-1 in the LV and SV, although the evidence for the SV was not as compelling as for the LV. Increasing recipient age was associated with greater MLDL in the SV between baseline and Year-3; however, the result was not sustained on follow-up and thus its significance is questionable. Recipient sex was not related with MLDL.

Costanzo et al[30] in their multicentre qualitative angiographic study found that donor age was a risk factor, while Hauptman et al[150] in their multicentre ICUS study found no relationship between the two. Rickenbacher et al[134] found that donor age was related to the development of TxCAD when qualitative angiography was used, but it was not related when ICUS was used. Thus it seems that when the insensitive qualitative angiography was used, donor age was found to be related with the development of TxCAD, and when the sensitivity of the diagnosis of TxCAD was increased by using

ICUS, no relation was found. Our QCA results are in agreement with the ICUS studies that there is no relation between donor age and TxCAD as assessed by MLDL.

In a study based on qualitative angiography, Costanzo et al[30] in a large multicentre study found that donor sex was related to the development of TxCAD. Hauptman et al[150] and Rickenbacher et al[134] in smaller studies using ICUS could not demonstrate such a relation. Thus it seems clear that the relation with TxCAD depends on the method used to diagnose TxCAD.

Recipient age was not a risk factor for MLDL in our study. This is consistent with the findings of Costanzo et al[30] in their multicentre qualitative angiographic study, and of Hauptman et al[150] in their multicentre ICUS study. We also found that recipient's sex was not a risk factor for MLDL, and this was shown previously by Hauptman et al[150]; however, Costanzo et al[30] found that recipient's male sex was a risk factor for TxCAD.

Recipient Race.

Caucasians had a smaller initial SV-MLDL in our study. However with such a small sample of non-Caucasians (6%), and lack of this relationship on long-term follow up, the significance of this finding is questionable. Costanzo et al[30] in their large multicentre qualitative angiographic study, found that recipient black race was a risk factor to the development of TxCAD. Differences in population size and means used to diagnose TxCAD prevents accurate comparison of our study with that of Costanzo et al[30].

Recipient aetiology of heart disease.

Recipient native heart disease was not related to MLDL in either the LV and SV. This is in agreement with the results of the multicentre study of Costanzo et al[30], although they used qualitative angiography to diagnose TxCAD.

Recipient post-transplant blood pressure.

Increase in mean BP from pre-transplant to 1-month post-transplant, from1-month to 6 months post-transplant and the mean value at 6 months post-transplant were not related to MLDL. This is in keeping with the findings of Rickenbacher et al[134] and Hauptman et al[150]. A reasonable explanation for this is that the majority of heart transplant recipients (72%) develop hypertension by one year[1]. In addition, hypertension is always treated and the antihypertensive medications may have other beneficial effects on atherosclerosis, other than by lowering the BP[273, 274].

Recipient post-transplant plasma lipid level.

We found that post-transplant plasma lipid level was not associated with MLDL in either the LV and SV. Although patients on statins had significantly lower total cholesterol, LDL cholesterol and low triglycerides, there was no difference in MLDL. Rickenbacher et al[134] found that increased triglycerides were related to the development of TxCAD, when the latter was diagnosed with either ICUS or conventional angiography. Eich et al[135] found that high cholesterol level at 6 months was a good predictor for the development of TxCAD on the long term. The follow up in the first study was 3.4±2.7 years (range 1-14.6) and in the latter all the patients had at least 3 years follow up. This might have contributed to the difference in their results compared

to ours. In addition, other investigators found no relationship between lipid levels and TxCAD[137, 138].

Donor Factors.

Aetiology of donor death

Although the aetiology of donor death relation to graft survival has been studied before[275], the literature is quite deficient in research relating the aetiology of donor death to the development of TxCAD. We found that donors who died from intracranial haemorrhage had less MLDL in the first year in both the LV and SV. The reason for this is unclear.

Non-domino versus domino hearts.

We found an increase in MLDL in domino transplants in the first year after transplant. There are no previous studies with QCA or ICUS in the domino population. Smith et al[165] showed that there was no difference in the incidence of freedom from graft atherosclerosis (74±3% versus 70±3% at 5 years) between the domino and non-domino groups, as diagnosed by conventional angiography. Similarly, Anyanwu et al[276] in a study of 131 domino transplants found that the 1, 5 and 10-year graft survival to be good, and that late death caused by coronary disease was uncommon. Other work has been done mainly on kidney transplants. Terasaki et al[167] found that the behaviour of kidney grafts from living donors was consistently superior from those from cadavers. The reason why non-domino hearts and hearts from donors who sustained intracranial haemorrhage in our study had less MLDL in the first year is not clear.

Total ischaemic time.

We found that total ischaemic time was not correlated to MLDL. Hauptman et al[150] using ICUS, Costanzo et al[30] using conventional angiography and Rickenbacher et al[134] using ICUS and conventional angiography found that there was no relationship between total ischaemic time and the development of TxCAD. Thus, our study is in agreement with these studies.

Echocardiographic ejection fraction and fractional shortening.

The "fractional shortening" was strongly correlated with the "ejection fraction" and so the results using any of the two parameters were similar. This strong correlation was expected as both parameters are mathematically coupled. The minimum ejection fraction/ fractional shortening in the first year was strongly correlated to the long-term MLDL in the LV, and tended to occur early after transplant. Low FS obtained in the first week after transplant was related to significant MLDL from baseline to Year-1 in the LV and SV. Review of literature found that such relationship has not been investigated before.

There is nothing in the heart transplant literature relating injury at time of transplant and subsequent development of TxCAD. However, experience from kidney transplant shows that ischaemia-reperfusion injury is related to subsequent development of chronic graft rejection[277-280]. Myocardial preservation of the cardiac allograft is related to endothelial damage[281], in addition to subsequent cardiac function[282-284]. It is thus possible that the effect organ preservation which leads to ventricular dysfunction after transplant might also have an effect on the development of TxCAD. Thus, patients who are found to have a low echocardiographic ejection fraction early after transplantation

need to be monitored closely for the development of TxCAD, and the cardiovascular risk factors well controlled.

Cytomegalovirus

CMV count was not related to MLDL. Hearts from serologically positive CMV donors tended to have less MLDL over the first year in both the LV and SV. There was no significant evidence of a difference in MLDL between serologically negative CMV donors who later develop CMV antigenaemia compared to those who don't.

Our finding of the lack of association between CMV count and MLDL is in agreement with that of Balk et al[124] who in their study of 100 patients who survived at least 1 year after transplantation, found no significant difference in the incidence of TxCAD between patients with and without CMV infection (culture positivity). Radovancevic et al[127] in a retrospective analysis of 210 cardiac transplant recipients, found no relation between TxCAD with CMV infection. However, other investigators found a positive relationship. Grattan et al[119] in a study of 301 patients, found that 28% of CMV infected patients developed severe coronary obstructive lesions, whereas only in 10% of patients not infected, did the same degree of TxCAD develop. CMV infection was diagnosed on the basis of a positive culture, fourfold increase in CMV serological titre, or demonstration of CMV inclusion bodies in the tissues. Koskinen et al[120] in a cohort of 53 patients demonstrated that CMV infection, as diagnosed by positive culture or fourfold rise in specific IgG or specific IgM or CMV antigenaemia, developed angiographically detectable TxCAD two years after the diagnosis of the CMV infection. Conventional angiography was used. An obvious cause for the discrepancies

between the different results is the means by which CMV infection and TxCAD were diagnosed.

An interesting finding in our study was that hearts from serologically positive CMV donors tend to have less MLDL over the first year for both the LV and SV. Thereafter this effect tended to disappear. Weimar et al[125] assessed the donor/recipient serostatus combination in 81 transplant patients and correlated this to the development of TxCAD as diagnosed by conventional angiography. They found no difference in the incidence of TxCAD between the groups. Conventional angiography, rather than QCA was used.

Haemodynamic data.

Cardiac output, as measured by right heart catheterisation, increased from baseline to Year-1 and then stayed fairly constant. There was no association between the CO at baseline, or the increase from baseline to Year-1, and the MLDL in either the LV or SV. The angiographic EF decreased from baseline to Year-1 and then stayed fairly constant. There was no significant relationship between the baseline angiographic EF, or the decrease in EF from baseline to Year-1, to the MLDL in either the LV or SV. The mean RAP, PAP and PAWP decreased from baseline to Year-1, and then remained fairly constant. There was no association between these variables at baseline or the decrease to Year-1, with the MLDL in either the LV or SV. The LVEDP decrease from baseline to Year-1 and then started to rise again. There was no association between the LVEDP at baseline or the decrease from baseline to Year-1, to the MLDL in either the LV or SV.

Review of literature showed that very little research has been done to investigate the haemodynamic aspects of TxCAD. Grocott-Mason et al[182] studied the angiographic EF at Year-1 in 111 patients and related it to the development of TxCAD in the first 10 years post transplant (34 patients). Conventional angiography was used to diagnose TxCAD. They found that those who never developed TxCAD in the first 10 years after transplant had significantly better cardiac function at Year-1 than those who did (LVEF of 70.3 ± 10.4% vs. 63.3 ± 13.4%). Our finding of a negative correlation between the baseline angiographic EF and the MLDL in both the LV and SV is in keeping with the findings of Grocott-Mason et al[182], although our relationship was not statistically significant. One reason that might have accounted for this is the shorter follow up period in our study (3 years) compared to theirs (10 years). Although baseline angiographic EF and echocariographic EF should basically be similar, the difference in results that we found in relation to MLDL could well be related to the timing when these tests were done.

Grocott-Mason et al[182] found a negative relationship between the right atrial pressure and the development of TxCAD in the 10 years following transplant. Again, as for the angiographic EF, the follow up period and the use of different means to diagnose TxCAD might have accounted for the difference in results.

Thus in conclusion, we found that donor male sex was associated with greater MLDL in the LV and SV between baseline and Year-1, although the evidence for the SV was not as compelling as for the LV. A new finding was that the low initial echocardiographic FS during the first week post transplant and minimum fractional shortening during the first year were strongly correlated to MLDL in the LV. This

could be a marker of graft injury and surrogate for the effectiveness of organ preservation. The sensitivity of the method used to diagnose TxCAD, in addition to the size of the study populations and period of follow up, could account for the difference in results between the different investigators.

CHAPTER 6

Results III

RELATIONSHIP OF IMMUNOLOGICAL FACTORS TO MEAN LUMEN DIAMETER LOSS.

This chapter outlines the results of the role of the immunological factors that are thought to play a major role in the development of MLDL in the LV and SV. These immunological factors were discussed in Chapter 1, section 1.9.

6.1 RATG induction therapy.

We investigated the relationship between RATG induction of immunosuppression and MLDL. Patients were divided according to whether or not they had RATG induction or RATG rescue (as treatment for severe rejection or temporarily in place of ciclosporin because of renal impairment). Table A6.1 shows the results, and table A6.2 shows the acute rejection episodes in those who had or did not have RATG in the first 3 and 12 months. Although the use of induction therapy was associated with a lower incidence of acute rejection from baseline to 3-months and 1-year after transplant (p=0.019 and 0.026 respectively), there was no evidence that its use was associated with a reduction in MLDL up to 3 years after heart transplantation (p=0.31 for the LV and 0.27 for the SV).

6.2 Prednisolone treatment.

We investigated whether the MLDL was related to those who had prednisolone treatment during the whole of the first year post-transplant compared to those who had had prednisolone discontinued before the end of the first year. Table A6.3 shows the results. There was no evidence of any difference between the two groups in MLDL to Year-1 (p=0.14 for the LV, and p=0.17 for the SV) or to Year-3 (p=0.99 for the LV, and p=0.1 for the SV).

6.3 Acute rejection.

As rejection occurs more commonly in the first 3 months after transplant, we investigated the rejection data according to whether the rejection occurred in the first 13 weeks, or in the first year. The following parameters were considered:

1- Average rejection score, each rejection being graded from 1 to 4 with A and B subgroups being ignored (ISHLT rejection 1A and 1B=1, 2=2, 3A and 3B=3, 4=4).

2- Average rejection score according to their grade from 1 to 6 with A and B subgroups being considered (ISHLT rejection grade 1A=1, 1B=2, 2=3, 3A=4, 3B=5, 4=6).

3- The number of the rejections, irrespective of whether treated or not.

Table 6.1 shows the basic statistics of the rejection episodes, according to the 3 groups mentioned above, and according to whether these episodes occurred in the first 3 months or in the first year.

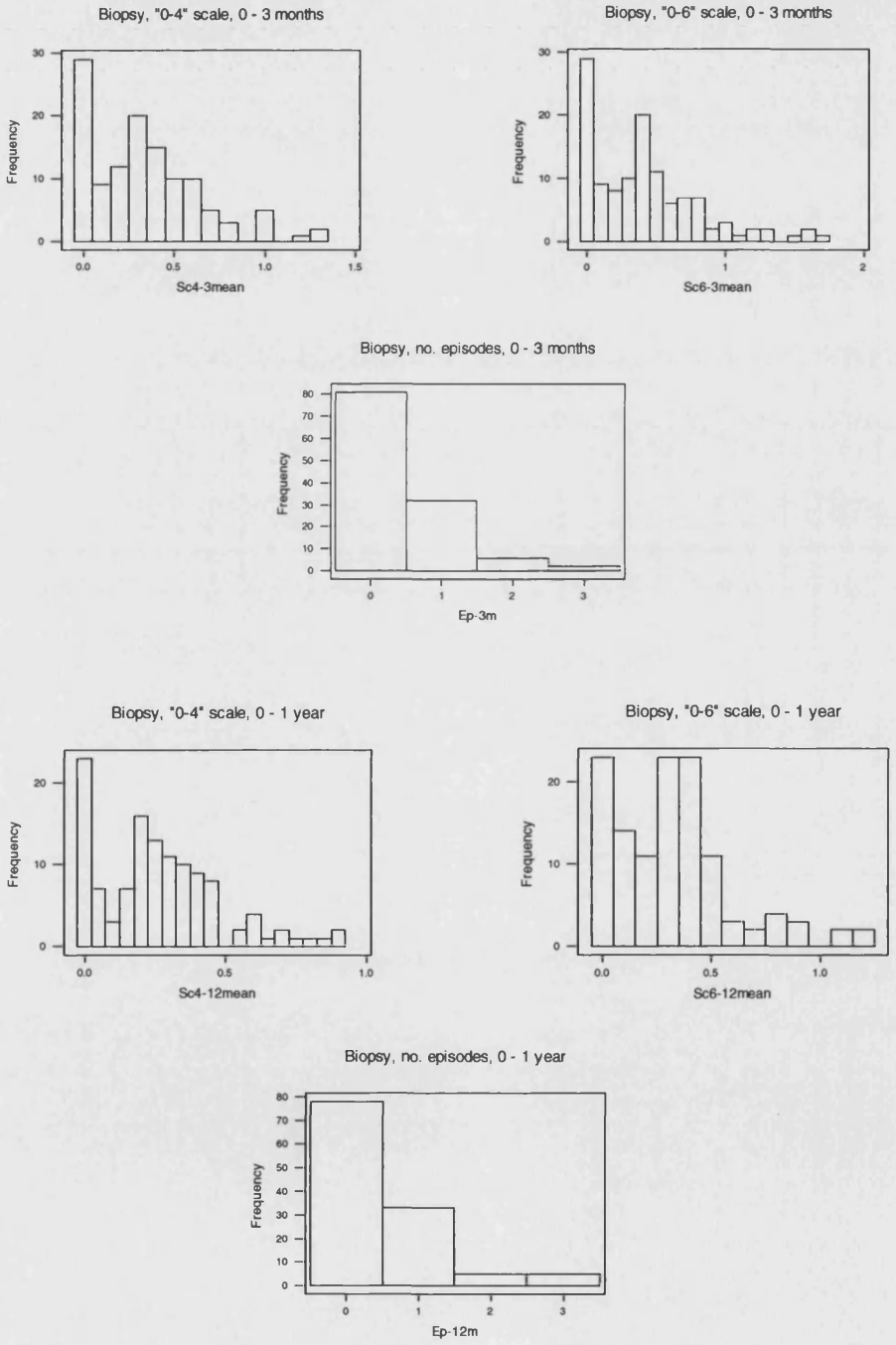
Table 6.1: Basic statistics of the rejection episodes.

Period	Variable	*n*	Mean	Standard deviation	Median
Baseline to 3-months	"0-4"score	121	0.3430	0.3051	0.3333
	"0-6"score	121	0.4201	0.3931	0.4000
	Number of rejection episodes	121	0.4132	0.6667	0.0000
Baseline to Year-1	"0-4"score	121	0.2619	0.2135	0.2353
	"0-6"score	121	0.3182	0.2738	0.2667
	Number of rejection episodes	121	0.4793	0.7648	0.0000

The frequency of occurrence of the rejection episodes is shown in the histograms and bar charts in figure 6.1. There was little difference between the 0-4 and 0-6 average scoring systems; therefore the average 0-6 score was used. In addition, there was little

difference when rejection was considered in the first 3 months or in the first 12 months; therefore the 12 months data was used in our analysis.

Figure 6.1: Histograms and bar charts of the rejection variables.



Sc = score; Sc4 = score 1-4; Sc6 = score 1-6; Ep = episodes.

6.4 Mean lumen diameter loss correlated to biopsy score.

Table 6.2 shows the result of the correlation of the 1-6 biopsy score over the first year to the MLDL in the LV and SV over the same period. Positive correlations indicate that more rejection was associated with greater subsequent loss in diameter. There was no significant evidence of any non-zero correlation.

Table 6.2: Correlation of 1-6 biopsy score to the "large" and "small" vessel mean lumen diameter loss.

Period of decrease	Large Vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	-0.023	0.81	0.003	0.98
Year-0 to Year-3	-0.241	0.10	0.015	0.92

6.5 Mean lumen diameter loss correlated to summation ECG voltage.

Table A6.4 shows the basic statistics of the rejection grade correlated to the summation ECG voltage (summation of leads 1, 2, 3, V1 and V6), and Table A6.5 shows the estimated difference from no rejection of each grade of rejection, allowing for patient-to-patient differences. The mean ECG voltage tended to decrease with increasing grade of rejection.

We looked at (i) average summation ECG voltage over the first year; (ii) standard deviation of the summation ECG voltage over the first year; (iii) minimum value of summation ECG voltage over the first year. The latter was correlated with average rejection score (on "0-6" scale) - smaller minimum values are associated with larger rejection scores ($p = 0.053$).

Table 6.3 correlates the minimum summation ECG voltage in first year and MLDL from Year-0 to subsequent years for the LV and SV. There was just one significant

result, for a negative correlation for the small vessels. Some of the other estimated correlations were positive, however, so no clear picture emerged. Maximum summated ECG voltage drop during the first year produced similar results.

Table 6.3: Correlation of the minimum ECG volatage in first year and mean lumen diameter loss from baseline to subsequent years in the "large" and "small" vessels.

Period of decrease	Large vessels		Small vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	0.166	0.074	0.173	0.064
Year-0 to Year-3	-0.106	0.48	-0.339	0.021

6.6 HLA Mismatches.

6.6.1 Total number of mismatches.

The total number of mismatches (MM) is the sum of the A MM, B MM and DR MM. Of the 119 patients for whom information was available, 1 had no mismatches, 2 had one mismatch, 8 had two mismatches, 27 had three mismatches, 32 had four mismatches, 31 had five mismatches and 18 had six mismatches. Details of the MLDL are shown in table A6.6 for the LV and SV. The p-values are based on ANOVA tests. There was no significant evidence of any association between the number of mismatches and MLDL, nor even any significant evidence of any trends.

Correlations of total number of mismatches and MLDL are shown in table A6.7 for the LV and SV. Positive correlations would suggest greater diameter loss with greater number of mismatches. There was no strong evidence of any significant correlation here.

6.6.2 Number of "A" mismatches.

Of the 120 patients for whom information was available, 14 had no A MM, 60 had one MM and 46 had two MM. Details of the MLDL and the A MM are shown in table A6.8 for the LV and SV respectively. There was no significant evidence of any association between the number of MM and MLDL; if anything, for the LV, there seemed to be a smaller loss in lumen diameter when there were more MM.

Correlations of the total number of MM at A and diameter loss are shown in table A6.9 for the LV and SV. As indicated by the analysis, there was no strong evidence of any correlation here.

6.6.3 Number of "B" mismatches.

Of the 119 patients for whom information was available, 8 had no MM, 35 had one MM and 76 had two MM. Details of the MLDL are shown in table A6.10 for the LV and SV. There was no significant evidence of any association between the number of MM and MLDL.

Correlation of the total number of MM at B and MLDL are summarised in table A6.11 for the LV and SV. There was no strong evidence of any correlation here.

6.6.4 Number of "DR" mismatches.

Of the 120 patients for whom information was available, 11 had no MM, 64 had one MM and 45 had two MM. Details of the MLDL are shown in table A6.12 for the LV and SV. There was no significant evidence of any association between the number of

MM and MLDL; however, there was a trend towards a greater diameter loss with increasing number of MM, especially if the small sample size rows are discounted.

Correlations of total number of MM at DR and MLDL are shown in table A6.13 for the LV and SV. There was no strong evidence of any correlation here.

6.7 Pre-transplant lymphocytic crossmatch & PRA.

Of the 116 patients for whom information was available, 114 were B cell negative, 2 were B cell positive. Since there were so few positives, no meaningful comparison could be made. Details of the MLDL are shown in Table A6.14 for the LV and SV.

Of the 121 patients studied, 118 were negative for pre-transplant PRA, 2 were positive 11-50%, and 1 was positive 51-100%. Because of the small numbers, we have pooled the last two groups. Details of the MLDL are shown in table A6.15 for the LV and SV. Due to the number of positives, no meaningful comparison could be made.

6.8 Antivimentin Antibodies.

Table A6.16 shows the basic statistics of our antivimentin antibodies data, and table 6.4 shows the summary of the data. We used a mean level of $\geq$120 units in the first year to investigate the relationship of antivimentin antibodies to the development of significant MLDL. This level was based on research conducted by Jurcevic et al[233], who found out that a first-year mean titre of $\geq$120 units produced a test with a sensitivity of 63% and a specificity of 76% to diagnose TxCAD. Table 6.5 shows the MLDL from baseline (in mm) for patients whose 1-year mean antivimentin titre was <120 and $\geq$ 120 units.

There was no significant evidence of a difference in MLDL between the groups, although in the LV there was more narrowing in the positive group.

Table 6.4: Summary statistics of the antivimentin antibodies data.

Statistic	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value	Quartiles 1st	Quartiles 3rd
1-yr mean	59	94.90	57.42	71.00	50.00	275.0	50.00	118.00
3-yr mean	107	118.5	103.9	85.9	50.0	743.	56.3	144.5
5-yr mean	118	117.29	83.28	89.25	50.00	540.0	64.67	139.25
1-yr max	59	109.47	69.69	83.00	50.00	300.0	50.00	150.00
3-yr max	107	144.0	127.1	106.0	50.0	890.	65.0	193.0

Table 6.5: First year mean antivimentin antibodies level related to the mean lumen diameter loss in the "large" and "small" vessels.

Year	First year mean titre ≥ 120 unit	*n*	Large Vessels			Small Vessels		
			Mean decrease	Standard deviation	p-value	Mean decrease	Standard deviation	p-value
0 to 1	No	44	0.191	0.281	0.34	0.073	0.192	0.92
	Yes	14	0.269	0.252		0.069	0.105	
0 to 3	No	5	0.473	0.243	-	0.1625	0.1703	-
	Yes	1	0.70000			0.28889		

6.9 Discussion.

The main findings were that RATG induction therapy did not affect the MLDL in either LV or SV, although it was associated with reduction in acute rejection. Prednisolone treatment during the whole of the first year was not associated with a reduction in the MLDL compared to those who did not have it for the whole year. A high endomyocardial biopsy score or rejection episodes were not associated with a higher incidence of MLDL in either the LV or SV. The mean summation ECG voltage tended to decrease with increasing grade of rejection and the minimum value of ECG voltage was associated with a larger 0-6 rejection score. However, the association of the minimum ECG voltage and TxCAD is not clear. Considering the HLA MM separately

or together, there was no association between them and the MLDL in either the LV or SV.

We also found no association between the pre-transplant lymphocytic crossmatch, PRA or the mean 1-year antivimentin antibodies level of ≥120 units to MLDL, although patients with ≥120 units level had more MLDL in the LV.

Our study found that RATG induction had no effect on MLDL, and hence TxCAD, 1 to 3-years after transplantation. Using conventional angiography, Carrier et al[235] and Dersdale et al[236] showed that RATG induction was associated with reduced incidence of TxCAD. Direct comparison between our study and these mentioned studies is not possible due to the different sensitivities of the methods used to diagnose TxCAD.

Several investigators studied the association between early prednisolone withdrawal from standard triple immunosuppression and the incidence of TxCAD[285-288] and found that there was no difference in TxCAD incidence between the groups on prednisolone and those not on it. All these studies used conventional angiography to diagnose TxCAD. Our QCA study is in keeping with results of these investigators, although we did not look into other factors which might affect the development of TxCAD, like lipid levels and the use of statins, in those who were on steroids compared to those who were not.

Our finding of lack of correlation between a high endomyocardial biopsy score to increased MLDL, and hence TxCAD, is in agreement with that of Gao et al[199]; they found that the rejection score and other seven parameters of rejection in the first year

post-transplant were not predictive of TxCAD in a 4 year mean follow-up. Qualitative angiography was used to diagnose TxCAD. Stovin et al[126] also found no relationship between acute rejection and TxCAD; conventional angiography and necropsy were used to diagnose TxCAD. Kobashigawa et al[200] in a multicentre ICUS study found no significant difference in the development of TxCAD between patients in the treated endomyocardial biopsy and the treated rejection groups and those who did not reject; however, there was a significant correlation between increased average biopsy score at 0 to 3-months after transplantation and increased intimal thickening, suggesting that recurrent mild rejection episodes might have an important role in the development of TxCAD. Using conventional angiography, Uretsky et al[137], Radovancevic et al[127], and Narrod et al[197] found a correlation between acute rejection and the subsequent development of TxCAD. Thus it seems that even by using the same means to diagnose TxCAD, researchers are divided in the role acute rejection plays in the development of TxCAD, although differences in the factors that might affect TxCAD such as immunosuppression protocols, lipid levels and the use of statins might have played a role.

It is well recognised that the summation ECG voltage decreases with acute rejection[289]. Our finding that it was not related to the development of TxCAD is not surprising. Golshayan et al[290] studied different aspects of the post-transplant ECG, such as RBBB and SVT, and found no correlation with the development of TxCAD. The fact that the ECG voltage is influenced by lots of factors, such as myocardial and pulmonary oedema and pericardial effusion, makes it an unreliable tool to correlate with disease aspects without worrying that the results might have been contaminated by these factors. Intramyocardial ECG amplitudes, which involves direct implantation of

epicardial leads and hence eliminates lots of the factors which influence the ECG amplitude, have been successfully used previously to diagnose acute rejection and monitor rejection treatment[291-294].

Our finding of no correlation between the total number of MM and the MLDL for either the LV and SV agrees with the work of Hornick et al[210] who studied 550 post transplant patients and found no association between the mean number of MM for Class I or Class II antigens and TxCAD as diagnosed by qualitative angiography, and of Zerbe et al[198] who studied of 146 allografts obtained either at re-transplantation or autopsy and found no association between the degree of HLA MM and the severity of coronary artery luminal narrowing as measured by planimetry of photographs of coronary artery sections with use of a digitising tablet. On the other hand, Radovancevic et al[212] found that patients with four to six MM had a significantly lower incidence of TxCAD ($p < 0.05$) than the patients with three or fewer MM.

Radovancevic et al[212] in their study of 167 patients who had cardiac transplantation found that at the HLA-A locus, there was significantly higher incidence of TxCAD ($p<0.01$) in the group of patients with zero MM (10 of 17, 58%). This interesting finding was also reflected in our study; although there was no evidence of any association between the number of A MM and MLDL, there seemed to be a smaller reduction in the LV-MLDL when there were more MM. This inverse relationship was also reported by Kerman et al[211].

Our finding of absence of association between B MM and MLDL is in keeping with the findings of Radovancevic et al[212]; however, Kerman et al[211] found an inverse

relationship. Considering the DR MM, our finding of absence of any association between them and MLDL is in keeping with the findings of Kerman et al[211] and Radovancevic et al[212].

On review of the literature, we found that there is lack of research in the association between the pre-transplant lymphocytic crossmatch and PRA and the MLDL. Our ability to investigate this relationship was limited by the very small number of patients who were PRA positive or who had a positive crossmatch.

Jurcevic et al[233] found that a 1-year antivimentin mean titre of ≥120 units predicts the development of TxCAD up to 5 years post-transplant with a sensitivity of 63% and specificity of 76%. This contradicts our finding of no association. Although the use of immunosuppression and the numbers of the two populations of patients were comparable (109 patients in their study), we had half the number of antivimentin samples in our study (440 vs. 880 samples). In addition, their use of conventional qualitative angiography to diagnose TxCAD, and the differences in the periods of follow up might have played a role. Hosenpud et al[234] found no relationship between the IgM antiendothelial cells antibodies obtained 6-8 weeks(early) and Year-1 (late) from recipients and donor specific human aortic endothelial cells of those with and without TxCAD at 1 year after transplantation. Thus the role of antiendothelial endothelial cells antibodies as a predictor of TxCAD needs further investigation.

Thus in conclusion, although the pathogensis of TxCAD is thought to be mainly immunological, our research and that of others show that the association between the different immunological factors and TxCAD pathogensis in patients receiving

pharmacological immunosuppression including ciclosporin, azathioprine and corticosteroids with or without induction is far from clear. Acute rejection is diagnosed and treated early, which might have masked its effect on TxCAD. Further research with standardisation of the methods used to detect TxCAD and investigate the different immunological factors is needed.

CHAPTER 7

MULTIVARIATE ANALYSIS.

In order to learn more about the relationship between the several independent or predictor variables and MLDL (dependent or criterion variable), we performed *multivariate analysis* using standard multiple linear regression. The multivariate model was obtained using stepwise regression by the backward removal method in which we eliminated non-significant variables one-by-one, using a nominal 5% significance level.

To obtain a regression equation (model), the general computational problem that needs to be solved in multiple regression analysis, is to fit a straight line (regression line) to a set of data (variables)[295]. A line in a two dimensional or two-variable space is defined by the equation $Y=a+b*X$; on other words, the Y variable can be expressed in terms of a constant (or intercept - a) and a slope (regression or B coefficient - b) times the X variable. In general, multiple regression procedures will estimate a linear equation of the form:

$$Y = a + b_1*X_1 + b_2*X_2 + ... + b_p*X_p$$

The regression line expresses the best prediction (model) of the dependent variable (Y), given the independent variables (X). However, usually there is variation of the observed points around the fitted regression line. The deviation of a particular point from the regression line (its predicted value) is called the residual value. The smaller the magnitude of the residual values around the regression line relative to the overall variability, the better the model fits the observed data. If there is no relationship between the X and Y variables, then the ratio of the residual variability of the Y variable to the original variance is equal to 1.0. If X and Y are perfectly related then there is no residual variance and the ratio of variance would be 0. In all cases, the ratio must fall somewhere between these 0 and 1.0. 1.0 minus this ratio is referred to as *R-square* or

the *coefficient of determination.* The *R-square* value is an indicator of how well the model fits the data (e.g., an *R-square* close to 1.0 indicates that we have accounted for almost all of the variability with the variables specified in the model).

However, multivariate analysis has its limitations[295]. Only associations between variables can be ascertained, and one can never be sure about the underlying casual mechanism. In linear regression analysis, it is assumed that the relationship between variables is linear, although in practice this assumption usually can not be confirmed. Fortunately, multiple regression procedures are not greatly affected by minor deviations from this assumption. The choice of the number of variables is essential. One would be capitalizing on chance when simply including as many variables as can be thought of as predictors of a variable of interest, as usually at least a few of them will appear to be significant by chance. This problem is compounded when, in addition, the number of observations is relatively low. Most authors recommend that one should have at least 10 to 20 times as many observations (cases, respondents) as one has variables, otherwise the estimates of the regression line are probably very unstable and unlikely to replicate if one were to do the study over again with another set of data. In addition, instability can occur when a model is constructed using independent variables that are closely related or highly correlated (e.g. using both FS and EF to assess left ventricular function). This problem is described as *multicollinearity*, and must be avoided by a careful selection of predictor variables.

7.1 Variables considered for multivariate analysis.

All the variables that were found to have a significance value of $p \leq 0.2$ in the univariate analysis, and which had completed data of $\geq 80\%$ were used as candidate variables for

the multivariate analysis. The candidate variables considered, together with their codes, are shown on table 7.1.

Table 7.1: Variables considered in the multivariable analysis, and their codes.

Variable	Code
Donor Sex	0= female; 1=male
Race	0=non-caucasians, 1=caucasians
Intracranial Haemorrhage	0=not intracranial haemorrhage, 1=intracranial haemorrhage
Initial FS (>36 or ≤36%)	0= >36%, 1=≤36%
Domino Transplant	0=not domino, 1=domino
ECG voltage (drop)	Maximum fall in ECG summation voltage in Year-1
Prednisolone in first year	0=no, 1=yes
Maximum creatinine in first year	Maximum serum creatinine in the first year.

7.2 Multivariate analysis of the "large" vessel variables.

Table A7.1 shows a provisional model when all variables were fitted in the analysis of the LV- MLDL. Table 7.2 shows the final multivariate model which was obtained after dropping non-significant variables one-by-one. The R-square value of the model was 22.6%. Donor sex, initial fractional shortening ≤36% and prednisolone treatment during the first year were positively correlated with MLDL, whereas intracranial haemorrhage was negatively correlated with it. In other words, the use of male donor or a donor who died from a cause other than intracranial haemorrhage, an initial echocardiographic fractional shortening ≤36% or prednisolone treatment continuing to 1-year after transplantation all predicted greater MLDL in the LV.

Table 7.2: "Large" vessel mean lumen diameter loss multivariate model.

Predictor	Coeffecient	SD	p-value
Constant	-0.1561	0.1091	0.155
Donor Male Sex	0.15068	0.04925	0.003
Intracranial Haemorrhage	-0.09779	0.04753	0.042
Initial FS (>36 or ≤36%)	0.12849	0.04610	0.006
Prednisolone in first year	0.15234	0.05948	0.012

7.3 Multivariate analysis of the "small" vessel variables.

A provisional model when all variables were fitted in the analysis of the SV- MLDL is shown in Table A7.2. Table 7.3 shows the SV multivariate model, after dropping the non-significant variables one-by-one. The R-square value of the model was 22.5%. Domino transplants, maximum drop in ECG voltage in Year-1, and maximum creatinine in Year-1 were significantly positively correlated to the MLDL, whereas being a Caucasian was significantly negatively correlated.

Table 7.3: "Small" vessel mean lumen diameter loss multivariate model.

Predictor	Coeffecient	SD	p-value
Constant	0.00445	0.08386	0.958
Caucasians	-0.15978	0.06187	0.011
Domino Transplant	0.08406	0.03777	0.029
ECG voltage drop	0.020768	0.008682	0.019
Maximum creatinine in first year	0.0004178	0.0001527	0.008

7.4 Comparison of the factors affecting the "large" and "small" vessels.

We then investigated whether the variables affected the two types of vessels differently, by regressing the difference in MLDL between the LV and SV on the 8 variables earlier found to be significant. The overall significance for whether the variables were non-zero was marginal at $p = 0.035$. On dropping non-significant terms one by one, we ended up with the model shown in table 7.4.

Only the variable "donor male" seemed to significantly affect the LV and SV MLDL differently with all other variables seemed to have an equal influence on MLDL of the LV and SV. When the donor was male, the MLDL in the LV tended to be relatively high ($p = 0.01$).

Table 7.4: Multivariate model obtained by the regression analysis of the difference between the "large" and "small" vessels mean lumen diameter loss on the eight key variables.

Predictor	Coeffecient	SD	p-value
Constant	0.08744	0.02903	0.003
Male Donor	0.09860	0.03748	0.010

7.5 Discussion.

We found out that apart from male donor sex, the factors that caused MLDL in the LV were similar to those that caused MLDL in the SV, although they only reached statistical significance in only one or the other type of vessels. Male donor sex caused significant LV MLDL. Thus, it appears that TxCAD is a homogenous disease affecting both the LV and SV to similar extent.

Considering the LV, male donor sex was the strongest predictor for large vessel MLDL (p=0.003). In addition, it was the only factor that only affected MLDL in the LV, rather than the SV (p=0.01). Our findings are in agreement with that of Costanzo et al[30] in their multi-institutional study using qualitative angiography and with that of Tuzcu et al[98] using ICUS. However, Rickenbacher et al[296] did not find such a relationship using either qualitative angiography or ICUS.

The fact that hearts from patients who sustained intracranial haemorrhage had less LV MLDL is perhaps surprising. Although this has not been studied before, one would have expected that, this group would do worse, due to the profound physiological derangements[128, 297]such as labile blood pressure, elevated catecholamines[298], and activation of surface molecules in peripheral organs by massive release of macrophages and T-cell associated cytokines and adhesion molecules from the injured brain into the

circulation[299]. However using qualitative angiography, Luckraz et al[300] found that the 2-year freedom from TxCAD in brain-dead and non-brain-dead donors is the same.

Although dobutamine stress echocardiography (DSE) early after heart transplantation was found to predict development of TxCAD[301], the role of simple transthoracic echocardiography has not been studied before. We found that an initial echocardiographic fractional shortening of <36% was a predictor of LV-MLDL. Thus, this very early parameter can guide us to intensify our efforts to control risk factors that might lead to TxCAD. It is quite conceivable that organ preservation methods immediately after harvesting the heart, and which affect the immediate post-transplant cardiac function, are implicated in the development of TxCAD. DSE has also been found to predict the progression[302] of TxCAD and the development of cardiovascular events[301]. Further studies are needed to evaluate the role of early left ventricular function as a risk factor for subsequent TxCAD.

Using conventional angiography, several investigators[285-288] found that there was no difference in the incidence of TxCAD between patients on prednisolone compared to those not on it. We have now shown that using QCA steroid therapy during the first year cause more LV-MLDL. Our findings are in keeping with those of Ratkovec et al[287]. Using qualitative angiography up to 3 years in a study of 102 patients, they found that TxCAD was not increased in patients withdrawn from maintenance corticosteroids when compared with their corticosteroid-requiring counterparts; in fact, with each 1 gm increment in cumulative corticosteroid use, there was a slightly increased risk of TxCAD was noted.

Domino transplant, high ECG voltage drop during Year-1, and high maximum creatinine in Year-1 were associated with larger MLDL in the SV, whereas Caucasian recipients had smaller SV-MLDL. It is not possible for us to compare our findings with those of others as the factors that affect SV disease have not been studied by other investigators.

Thus TxCAD is a homogenous disease with factors that affect both LV and SV similarly, but to different extent. As regards the LV, our findings were compatible with those of previous studies. However the association between left ventricular systolic function early after transplantation and the subsequent development of TxCAD has not been reported previously.

Chapter 8

SUMMARY & CONCLUSIONS

8.1 Change in vessel diameter and tone with time.

8.2 Factors causing "large" vessel mean lumen diameter loss.

8.3 Factors causing "small" vessel mean lumen diameter loss.

8.4 Multivariate analysis.

8.5 Clinical implications and importance of the study.

8.6 Limitations of the study.

8.7 Future directions.

In this study, we found that QCA could be reliably used to assess TxCAD and to investigate the pattern of MLDL in the LV and SV in the first few years after transplant. We then used QCA to investigate the relationship of the MLDL to the different potential aetiological factors. In this chapter, I will briefly review our findings.

8.1 Change in vessel diameter and tone with time.

Although there was MLDL in both the LV and SV, the loss was greater in the LV rather than the SV. Thus the earliest changes of TxCAD occur predominantly in the larger epicardial vessels. We also found that subsequent progression of the disease was related to the MLDL in the first year. Vessel tone immediately after transplant was not a predictor of development of TxCAD at Year-1or Year-3

8.2 Factors causing "large" vessel mean lumen diameter loss.

An important finding of our study that was not reported or investigated before was that a low initial echocardiographic fractional shortening during the first week post-transplant and a low minimum fractional shortening during the first year were strongly related to MLDL in the LV. Donor male sex and hearts from domino transplants were associated with greater MLDL from baseline to Year-1. Donors who died from intracranial haemorrhage and donors who were serologically CMV positive, had less lumen diameter loss in the first year.

8.3 Factors causing "small" vessel mean lumen diameter loss.

Significant SV-MLDL was related to male donors, increase in mean blood pressure from baseline to one month, domino hearts and those who had high maximum creatinine level in the first year. Caucasian recipients, hearts from donors who had

intra-cranial haemorrhage and donors who were serologically CMV positive had less MLDL. Minimum summation ECG voltage in the first year was negatively related to SV-MLDL from baseline to Year-3.

8.4 Multivariate analysis.

Donor male sex, echocardiographic fractional shortening ≤36% and prednisolone treatment during the first year were significantly positively related to LV-MLDL, whereas intracranial haemorrhage was significantly negatively related. The R^2 value was 22.6%. Domino transplants, maximum drop in ECG voltage in Year-1, and maximum creatinine in Year-1 were significantly positively related to SV-MLDL, whereas Caucasians recipients were significantly negatively related. The R^2 value was 22.5%.

Apart from donor male sex which caused greater LV-MLDL, the significant variables affected both the LV and SV equally.

8.5 Clinical implications and importance of the study.

We discovered that early graft function as assessed by the echocardiographic fractional shortening/ ejection fraction was a predictor of MLDL in the coronary arteries of the transplanted heart. This suggests that factors that affect the quality of a donor heart such as the process of brain death, medical management of the organ donor and the techniques used to protect the heart during transport back to the transplant centre may influence the subsequent development of coronary arterial disease in the transplanted heart. This hypothesis could be tested in randomized studies comparing different types of donor management or of myocardial protection. Early graft function could be used as a surrogate measure in pilot studies. A promising strategy could now be tested in a

larger scale randomized study using QCA and ICUS to determine the impact on TxCAD.

Closer monitoring and control of cardiovascular risk factors for the development of TxCAD will be needed in patients who have factors that have been associated with TxCAD. This could include more frequent QCA to follow up progress of the disease, and identify cases where further treatment is required.

8.6 Limitations of the study.

Our study has the following limitations. It was observational, and treatments were determined by physician preference and were not randomly allocated. Thus, for example, it is impossible to determine whether patients treated with corticosteroids up to 1 year had a greater MLDL because of this drug treatment or whether another unmeasured factor led both to the need for more prolonged corticosteroid therapy and to the MLDL. The sample size decreased with time. This was due to the length of follow up available when the study was concluded. Thus effectively, our study was limited to 3 years, and thus our conclusions relate only to the early phase of TxCAD. The number of patients was small in some subgroups, e.g. non-Caucasians, which limited our ability to detect some important relationships. The range of values present in the study population was sometimes large and represented the clinical practice during the study period (e.g. ischaemia time in which the mean±SD was 155±57 minutes).

The R-square values of the multivariate model indicate that the majority of the variance in MLDL has not been explained by these models. The results of such models should

be viewed as provisional until they have been validated in another group of patients. However, such models play an important role in generating hypotheses, which can be tested by further research.

8.7 Future directions.

Most transplant centres follow-up heart transplant recipients by conventional qualitative angiography. However, further information could be obtained by the routine use of QCA; simple modification of the angiographic technique can allow quantitative analysis.

ICUS remains the only method that can directly visualize disease in the arterial wall. However, its routine use is limited by its cost and highly invasive nature. ICUS will remain the "gold standard" for studies of therapeutic intervention in transplant coronary disease. However, since ICUS involves concomitant angiography, QCA techniques can be used to provide complementary information about the more distal vessels, and the two techniques should be used to assess specific therapeutic interventions in randomised clinical trials.

APPENDIX

Table A4.1A: Absolute "large" vessel mean lumen diameter loss from baseline.

Vessel	Year	*n*	Mean (mm)	Standard deviation	Median
Prox LAD	1	114	0.2956	0.3867	0.245
	3	47	0.5147	0.3909	0.450
	5	6	0.720	0.559	0.61
Mid LAD	1	107	0.1950	0.3222	0.150
	3	42	0.4586	0.3530	0.460
	5	4	0.410	0.291	0.34
Prox Cx	1	113	0.2253	0.3592	0.170
	3	45	0.3822	0.4211	0.380
	5	7	0.476	0.377	0.41
Prox RCA	1	113	0.2624	0.3493	0.240
	3	45	0.4149	0.3553	0.380
	5	5	0.416	0.577	0.38
Mid RCA	1	111	0.1779	0.2811	0.200
	3	45	0.3409	0.3404	0.370
	5	6	0.4817	0.2303	0.475

Table A4.1B: Absolute "small" vessel mean lumen diameter loss from baseline.

Vessel	Year	*n*	Mean (mm)	Standard deviation	Median
Distal LAD	1	103	0.072	0.24	0.05
	3	45	0.20	0.33	0.16
	5	6	0.46	0.73	0.14
D1	1	107	0.06	0.22	0.03
	3	41	0.10	0.28	0.07
	5	4	0.11	0.14	0.11
D2	1	86	0.02	0.28	-0.01
	3	35	0.05	0.27	-0.01
	5	2	0.11	0.20	0.11
Distal Cx	1	105	0.09	0.21	0.06
	3	43	0.26	0.75	0.14
	5	7	0.09	0.29	0.02
OM1	1	106	0.06	0.22	0.03
	3	42	0.06	0.22	0.04
	5	5	0.05	0.19	0.02
OM2	1	80	0.04	0.15	0.03
	3	38	0.05	0.20	0.04
	5	4	-0.04	0.18	-0.02
Distal RCA	1	111	0.23	0.34	0.20
	3	45	0.29	0.33	0.32
	5	6	0.44	0.27	0.49
RVBr	1	107	0.05	0.18	0.06
	3	45	0.12	0.18	0.12
	5	5	0.22	0.23	0.27
PDA	1	103	0.07	0.22	0.05
	3	40	0.11	0.20	0.12
	5	4	0.26	0.08	0.24

Table A4.2A:Proportional "large" vessel mean lumen diameter loss from baseline.

Vessel	Year	*n*	Mean (%)	Standard deviation	Median
Prox LAD	1	114	6.365	8.672	5.423
	3	47	10.74	8.05	9.43
	5	6	14.81	9.51	14.32
Mid LAD	1	107	5.65	10.79	4.86
	3	42	13.42	9.60	12.96
	5	4	11.80	4.95	10.48
Prox Cx	1	113	5.396	9.305	4.457
	3	45	9.18	9.50	10.04
	5	7	14.61	13.20	13.09
Prox RCA	1	113	5.633	7.697	5.974
	3	45	9.28	7.87	8.96
	5	5	9.38	14.21	7.47
Mid RCA	1	111	4.098	7.400	5.063
	3	45	8.26	8.59	9.44
	5	6	11.57	6.17	10.74

Table A4.2B:Proportional "small" vessel mean lumen diameter loss from baseline.

Vessel	Year	*n*	Mean (%)	Standard deviation	Median
Distal LAD	1	103	2.90	13.99	3.01
	3	45	9.07	14.37	9.09
	5	6	15.24	20.18	8.89
D1	1	107	1.95	11.82	1.57
	3	41	3.97	11.93	4.27
	5	4	7.16	8.16	6.84
D2	1	86	-0.73	22.12	-0.38
	3	35	2.15	20.60	-0.83
	5	2	6.24	12.09	6.24
Distal Cx	1	105	3.093	9.941	3.243
	3	43	9.07	22.85	6.45
	5	7	0.70	15.95	1.04
OM1	1	106	2.085	9.562	1.993
	3	42	1.68	11.97	1.77
	5	5	2.31	10.65	1.08
OM2	1	80	1.82	10.59	2.11
	3	38	1.38	13.49	2.08
	5	4	-4.54	13.49	-2.33
Distal RCA	1	111	5.915	10.077	6.531
	3	45	7.96	8.67	8.90
	5	6	12.53	7.74	12.77
RVBr	1	107	2.24	11.23	3.73
	3	45	6.34	10.21	7.78
	5	5	10.61	14.55	15.88
PDA	1	103	3.09	11.76	3.51
	3	40	4.99	11.13	5.72
	5	4	12.95	4.53	13.50

Table A4.3: Absolute mean lumen diameter loss *between successive years* in the "large" and "small" vessels.

Year	*n*	Large Vessels			Small Vessels		
		Mean	Standard deviation	p-value	Mean	Standard deviation	p-value
0-1	117	0.229	0.267	<0.0001	0.0812	0.1557	<0.0001
1-3	47	0.1385	0.2337	0.0002	0.0532	0.1572	0.025
3-5	6	0.175	0.2335	0.13	0.045	0.1056	0.34

Table A4.4: Difference between "large" and "small" vessel mean lumen diameter loss between successive years.

Year	*n*	Mean (mm)	Standard Deviation	p value
0-1	117	0.148	0.201	<0.0001
1-3	47	0.0853	0.2048	0.0064
3-5	6	0.13	0.272	0.29

Table A4.5 A: Relationship of change in the "large" vessels diameter in the first year to long-term change.

Change from Year-0 to Year-1	*n*	Max, Years 3 and 5	Standard deviation	p-value
Increase, >5%	3	-2.241	3.534	<0.001
Increase, <5%	9	7.449	4.221	
Decrease, <5%	14	8.369	7.406	
Decrease, >5%	18	11.572	5.456	
Decrease, >10%	19	15.391	4.945	

Table A4.5 B: Relationship of change in the "small" vessels diameter in the first year to long-term change.

Change from Year-0 to Year-1	*n*	Max, Years 3, and 5	Standard deviation	p-value
Increase, >5%	7	-0.614	7.529	<0.001
Increase, <5%	8	-0.988	6.569	
Decrease, <5%	20	5.879	6.301	
Decrease, >5%	21	9.775	7.495	
Decrease, >10%	7	13.592	12.279	

Table A5.1: Correlation between "large" and "small" vessel mean lumen diameter loss and donor age.

Period of diameter loss	Large Vessels		Small Vessels	
	Correlation	p-value	Correlation	p-value
Year-0 to 1	-0.161	0.083	-0.129	0.17
Year-0 to 3	0.115	0.44	0.190	0.20
Year-0 to 5	-0.573	0.14	-0.246	0.56

Table A5.2: Correlation between "large" and "small" vessel mean lumen diameter loss to recipient age.

Period of diameter loss	Large Vessels		Small Vessels	
	Correlation	p-value	Correlation	p-value
Year-0 to 1	0.008	0.93	0.069	0.46
Year-0 to 3	0.180	0.23	0.384	0.008
Year-0 to 5	0.059	0.89	0.553	0.6

Table A5.3: Comparison between the mean lumen diameter loss in "large" and "small" vessel groups and recipient sex.

Year	Sex	*n*	Large Vessels			Small Vessels		
			Mean decrease	Standard deviation	p-value	Mean decrease	Standard deviation	P value
0 to 1	Female	18	0.240	0.222	0.83	0.119	0.123	0.19
	Male	99	0.227	0.275		0.074	0.160	
0 to 3	Female	7	0.275	0.359	0.26	0.192	0.419	0.83
	Male	40	0.449	0.250		0.156	0.153	
0 to 5	Female	3	0.530	0.402	0.94	0.485	0.614	0.44
	Male	5	0.507	0.255		0.142	0.091	

Table A5.4: Comparison of the mean lumen diameter loss from baseline in the "large" and "small" vessels for the three aetiological groups.

Year	Aetiology group	*n*	Large Vessels			Small Vessels		
			Mean loss (mm)	Standard deviation	p-value	Mean loss (mm)	Standard deviation	p-value
0 to 1	Ischaemic	71	0.2475	0.2917	0.57	0.0868	0.1653	0.88
	Idiopathic	28	0.1843	0.2482		0.0759	0.1620	
	Other	18	0.2233	0.1837		0.0675	0.1040	
0 to 3	Ischaemic	31	0.4560	0.2908	0.50	0.2000	0.2203	0.18
	Idiopathic	10	0.3793	0.2491		0.1090	0.1777	
	Other	6	0.3287	0.1912		0.0503	0.1283	

A5.5A: Pattern of systolic blood pressure over the years.

Time	Number of values, *n*	Mean (mmHg)	Standard deviation	Median (mmHg)
Pre	116	109.24	17.40	109.0
1 month	120	133.49	17.18	130.0
6 months	120	141.24	20.40	140.0
1 year	119	139.21	15.80	140.0
18 months	113	137.81	14.53	140.0
2 years	100	137.24	17.76	135.5
30 months	79	139.59	16.71	140.0
3 years	66	142.21	17.27	140.0
4 years	31	141.65	16.81	140.0
5 years	10	132.20	10.81	130.0

A5.5B: Pattern of diastolic blood pressure over the years.

Time	Number of values, *n*	Mean (mmHg)	Standard deviation	Median (mmHg)
Pre	116	67.83	12.08	70
1 month	120	84.28	11.50	84
6 months	120	90.24	12.52	90
1 year	119	90.210	10.678	90
18 months	113	89.097	9.894	90
2 years	100	89.83	10.62	90
30 months	79	90.46	9.51	90
3 years	66	90.09	12.85	90
4 years	31	90.19	8.64	90
5 years	10	91.40	6.33	90

A5.5C: Pattern of mean blood pressure over the years.

Time	Number of values, *n*	Mean (mmHg)	Standard deviation	Median (mmHg)
Pre	116	81.63	12.84	80.00
1 month	120	100.69	12.48	100.00
6 months	120	107.24	14.44	107.00
1 year	119	106.54	11.33	106.67
18 months	113	105.34	10.23	104.00
2 years	100	105.63	12.09	103.67
30 months	79	106.84	10.64	106.67
3 years	66	107.46	11.61	106.67
4 years	31	107.34	10.02	106.67
5 years	10	105.00	7.09	103.33

A5.5D: Pattern of pulse pressure over the years.

Time	Number of values, *n*	Mean (mmHg)	Standard deviation	Median (mmHg)
Pre	116	41.41	12.24	40
1 month	120	49.21	11.75	50
6 months	120	51.00	12.50	50
1 year	119	49.00	11.78	50
18 months	113	48.72	11.83	48
2 years	100	47.41	12.38	48
30 months	79	49.14	13.43	50
3 years	66	52.12	18.34	50
4 years	31	51.45	14.01	50
5 years	10	40.80	8.34	40

Table A5.6: Comparison of patients who are not on statins post-transplant to those who are on statins.

Variable	Group	*n*	Mean	Standard deviation	p-value
Diameter loss in mm, Year-0 to 1, large vessels	No statins	31	0.220	0.264	0.84
	Statins	86	0.232	0.270	
Diameter loss in mm, Year-0 to 1, small vessels	No statins	31	0.066	0.136	0.49
	Statins	86	0.087	0.163	
TC at Year-1(mmol/L)	No statins	30	5.013	0.928	<0.0001
	Statins	85	6.08	1.30	
TG at Year-1(mmol/L)	No statins	30	1.637	0.892	0.0054
	Statins	83	2.25	1.27	
LDL at Year-1(mmol/L)	No statins	30	3.017	0.831	<0.0001
	Statins	73	3.89	1.08	
HDL at Year-1(mmol/L)	No statins	30	1.258	0.336	0.97
	Statins	80	1.255	0.479	

Table A5.7: Diameter loss in the "large" and "small" vessels from baseline to Year-1, and the correlation with the lipid profiles.

Lipid Fraction	Vessels	Correlation	p-value
Total Cholesterol	Large	0.008	0.94
	Small	-0.001	0.99
Triglycerides	Large	-0.068	0.48
	Small	0.077	0.42
LDL Cholesterol	Large	0.042	0.68
	Small	-0.027	0.79
HDL Cholesterol	Large	0.050	0.61
	Small	-0.022	0.82

Table A5.8: Underlying disease in the domino donor patients.

Underlying Donor Disease	Number of Patients	% of Patients
Cystic fibrosis	20	84
Eisenmenger PDA	2	8
Bronchiectasis	1	4
Primary Pulmonary Hypertension	1	4
Total	24	100

Table A5.9: Causes of donor death grouped into 5 groups.

Cause	Number	% of total	Number of women	% women
1= intra-cranial haemorrhage	56	46	30	53
2= head injury	24	20	4	17
A = 3, 4, 6, 7.	12	10	5	42
B = 5, 8, 9	5	4	3	6
Domino = XXXX	24	20	5	83
Total	121	100.00	47	39

NB- Cause of death numbers correspond to those mentioned in table 5.6.

Table A5.10: Summary statistics of the total ischaemic time in minutes.

n	Mean In minutes	Standard deviation	Minimum value	Maximum value	95% conf. int. From	To
120	155.06	56.59	17	279	144.83	165.29

Table A5.11: Correlation of the ischaemic time to the "large" and "small" vessel mean lumen diameter loss from baseline to subsequent years.

Period	Large Vessels		Small Vessels	
	Correlation	p-value	Correlation	p-value
Year-0 to Year-1	-0.043	0.65	-0.035	0.70
Year-0 to Year-3	-0.090	0.55	-0.050	0.59

Table A5.12: Correlation of recipient creatinine level and mean lumen diameter loss.

Creatinine variable	Vessel Type	Decrease to Year-1 or Year-3?	Correlation	p-value
Pre-transplant	Large	1	0.189	0.13
Pre-transplant	Small	1	0.125	0.31
Pre-transplant	Large	3	0.087	0.67
Pre-transplant	Small	3	0.072	0.73
Max value in Year-1	Large	1	0.001	0.99
Max value in Year-1	Small	1	0.231	0.024
Max value in Year-1	Large	3	-0.053	0.76
Max value in Year-1	Small	3	0.333	0.044
Value at Year-1	Large	1	-0.136	0.19
Value at Year-1	Small	1	-0.057	0.58
Value at Year-1	Large	3	-0.092	0.59
Value at Year-1	Small	3	0.041	0.81

Table A5.13: Correlation of recipient glucose level and mean lumen diameter loss.

Glucose variable	Vessel Type	Year of Lumen Diameter Loss	Correlation	p-value
Pre-transplant	Large	1	0.105	0.51
Pre-transplant	Small	1	0.110	0.49
Pre-transplant	Large	3	-0.105	0.71
Pre-transplant	Small	3	0.506	0.054
Max value in Year-1	Large	1	0.020	0.85
Max value in Year-1	Small	1	-0.008	0.94
Max value in Year-1	Large	3	-0.056	0.74
Max value in Year-1	Small	3	0.144	0.39
Value at Year-1	Large	1	-0.059	0.59
Value at Year-1	Small	1	-0.110	0.30
Value at Year-1	Large	3	-0.221	0.22
Value at Year-1	Small	3	0.182	0.32

Table A5.14: Correlation of the mean ln(CMV count + 1) in the first year to mean lumen diameter loss from Year-0 to subsequent years in the "large" and "small" vessels.

Period of decrease	Large vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	-0.079	0.40	-0.071	0.45
Year-0 to Year-3	-0.007	0.97	-0.099	0.51

Table A5.15: Comparison of the mean lumen diameter loss in the "large" and "small" vessels for patients with zero CMV count throughout the first year and patients with at least one non-zero count in the first year.

Year	CMV count greater than zero?	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	No	65	0.230	0.246	0.97	0.084	0.149	0.86
	Yes	52	0.228	0.294		0.078	0.165	
0 to 3	No	26	0.378	0.290	0.20	0.170	0.231	0.76
	Yes	21	0.479	0.241		0.151	0.177	

Table A5.16: Basic statistics of the cardiac output.

Year	*n*	Mean	Standard deviation	Median
0	117	4.626	1.166	4.500
1	111	4.960	1.186	4.880
3	38	4.839	1.175	4.755
5	5	5.010	0.858	4.600

Table A5.17: Correlation of cardiac output at baseline to subsequent mean lumen diameter loss.

Period of MLDL	Large Vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	-0.081	0.39	-0.113	0.23
Year-0 to Year-3	0.123	0.41	-0.113	0.41

Table A5.18: Correlation of increase in cardiac output from baseline to Year-1, to subsequent mean lumen diameter loss in the "large" and "small" vessels.

Period of diameter loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	0.039	0.69	0.078	0.42
Year-0 to Year-3	0.005	0.97	0.218	0.17

Table A5.19: Basic statistics of angiographic ejection fraction.

Year	*n*	Mean	Standard deviation	Median
0	114	71.70	10.73	75.0
1	108	68.03	11.65	70.0
3	40	69.28	10.74	69.0
5	8	69.37	5.93	68.5

Table A5.20: Correlation of angiographic ejection fraction at baseline to subsequent mean lumen diameter loss.

Period of diameter loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	-0.140	0.14	-0.154	0.11
Year-0 to Year-3	-0.280	0.062	-0.008	0.96

Table A5.21: Correlation between decrease in angiographic ejection fraction from baseline to Year-1 and the subsequent mean lumen diameter loss.

Period of decrease	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	-0.064	0.53	0.090	0.37
Year-0 to Year-3	0.084	0.60	-0.030	0.85

Table A5.22: Basic statistics of the mean right atrial pressure over time.

Year	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value
0	117	8.068	3.562	8.	0.	17.
1	113	4.823	2.977	4.	0.	14.
3	38	5.658	3.419	5.	1.	16.
5	4	5.75	2.22	6	3	8

Table A5.23: Basic statistics of the pulmonary artery mean pressure over time.

Year	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value
0	119	22.630	6.055	22.	11.	45.
1	114	17.921	5.241	17.	8.	46.
3	38	18.263	4.791	17.	11.	31.
5	5	19.40	4.39	18	16	27

Table A5.24: Basic statistics of the mean pulmonary artery wedge Pressure over time.

Year	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value
0	119	14.218	4.484	14.0	3.	30.
1	114	10.886	4.238	10.0	1.	28.
3	38	11.316	4.114	11.5	3.	20.
5	5	13.00	3.81	12.	9	19

Table A5.25: Basic statistics of the left ventricular end diastolic pressure over time.

Year	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value
0	118	12.297	4.854	12.00	2.	28
1	115	9.974	4.518	10.00	2.	25
3	48	12.333	5.774	11.50	4.	33
5	10	13.90	5.28	14.5	6	26

Table A5.26: Decrease in right atrial pressure from baseline to Year-1.

n	Mean decrease	Standard deviation	Median	Minimum value	Maximum value	p-value
109	3.257	4.334	3	-12	17	<0.0001

Table A5.27: Decrease in pulmonary artery mean pressure from baseline to Year-1.

n	Mean decrease	Standard deviation	Median	Minimum value	Maximum value	p-value
112	4.75	6.871	5	-22	24	<0.0001

Table A5.28: Decrease in left ventricular end diastolic pressure from baseline to Year-1.

n	Mean increase	Standard deviation	Median	Minimum value	Maximum value	p-value
112	-2.366	6.214	-3	-17	16	0.0001

Table A5.29: Correlation of the mean right atrial pressure at baseline to subsequent mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	0.051	0.59	0.047	0.62
Year-0 to Year-3	0.092	0.54	0.036	0.81

Table A5.30: Correlation of the decrease in mean right atrial pressure from baseline to Year-1, to subsequent mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	p value
Year-0 to Year-1	0.081	0.40	-0.055	0.57
Year-0 to Year-3	-0.025	0.87	-0.001	0.996

Table A5.31: Correlation of the pulmonary artery mean pressure at Baseline, to subsequent mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	0.079	0.40	0.029	0.76
Year-0 to Year-3	0.234	0.11	-0.022	0.89

Table A5.32: Correlation of decrease in pulmonary artery mean pressure from baseline to Year-1, to subsequent mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	0.102	0.28	-0.014	0.89
Year-0 to Year-3	0.043	0.78	-0.064	0.67

Table A5.33: Correlation of the left ventricular end diastolic pressure at baseline, to subsequent mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	-0.135	0.15	-0.114	0.22
Year-0 to Year-3	-0.050	0.74	-0.085	0.57

Table A5.34: Correlation of the decrease in left ventricular end diastolic pressure from baseline to Year-1, to subsequent mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	p value	Correlation	p value
Year-0 to Year-1	0.078	0.42	0.102	0.29
Year-0 to Year-3	0.224	0.14	0.016	0.92

Table A6.1: Relationship between the use of RATG induction therapy and mean lumen diameter loss.

Year	RATG	*n*	Large Vessels			Small Vessels		
			Mean loss (mm)	Standard error	p-value	Mean loss (mm)	Standard error	p-value
0 to 1	No	35	0.208	0.045	0.80	0.0532	0.019	0.49
	Induction	68	0.244	0.034		0.0869	0.021	
	Rescue	7	0.208	0.089		0.1101	0.046	
0 to 3	No	22	0.468	0.050	0.31	0.206	0.053	0.27
	Induction	14	0.476	0.068		0.114	0.038	
	Rescue	6	0.286	0.168		0.072	0.079	

Table A6.2: Acute rejection episodes in those who had and did not have RATG therapy.

Period	RATG treatment	*n*	Mean	Standard error	p-value
Baseline to 3 month	No	42	1.19	0.16	0.019
	Yes	68	0.82	0.14	
Baseline to 1 year	No	42	1.45	0.19	0.026
	Yes	68	1.04	0.17	

Table A6.3: Mean lumen diameter loss in those who were and were not on prednisolone treatment during the first year.

Year	Prednislone?	*n*	Large Vessels			Small Vessels		
			Mean diameter loss	Standard deviation	p-value	Mean diameter loss	Standard deviation	p-value
0 to 1	No	21	0.141	0.288	0.14	0.042	0.130	0.17
	Yes	93	0.245	0.263		0.088	0.161	
0 to 3	No	12	0.435	0.214	0.99	0.106	0.108	0.10
	Yes	32	0.434	0.265		0.191	0.229	

Table A6.4: Basic statistics of the rejection grades and summation ECG voltage.

Rejection grade	*n*	Mean	Standard deviation	Median
NR	602	5.3212	1.5694	5.1000
1A	303	5.2650	1.6721	5.1000
1B	53	4.455	1.528	4.100
2	4	3.687	1.149	4.000
3A	62	4.672	1.510	4.250
3B	2	5.875	0.460	5.875
4	1	3.2000	*	3.2000

NR = no rejection.

Table A6.5: Estimated difference in summation ECG voltage from no rejection of each grade of rejection.

Rejection grade	Mean difference from no rejection	Standard error of difference
1A	-0.014	0.05865
1B	-0.349	0.11703
2	-0.230	0.40232
3A	-0.522	0.10806
3B	-0.529	0.56447
4	-1.364	0.79679

Table A6.6: Total number of mismatches related to "large"and "small" vessel mean lumen diameter loss.

Year	Total number of mismatches	*n*	Large Vessels			Small Vessels		
			Mean	Standard deviation	p-value	Mean	Standard deviation	p-value
0 to 1	0	1	0.0460	0.0000	0.98	-0.0186	0.0000	0.90
	1	2	0.3040	0.3055		0.1258	0.0200	
	2	8	0.2352	0.2348		0.1120	0.1212	
	3	25	0.2412	0.2804		0.0592	0.1344	
	4	31	0.1967	0.2968		0.0798	0.1989	
	5	30	0.2397	0.2497		0.1057	0.1175	
	6	18	0.2398	0.2807		0.0682	0.1854	
0 to 3	0	0			0.38			0.11
	1	2	0.5140	0.1131		0.1169	0.0302	
	2	3	0.3420	0.2612		0.3574	0.2330	
	3	10	0.5262	0.2420		0.1558	0.1251	
	4	12	0.3027	0.2186		0.0797	0.1039	
	5	12	0.3903	0.3339		0.1107	0.1628	
	6	7	0.5264	0.2962		0.3099	0.3900	

Table A6.7: Correlation of total number of mismatches to "large"and "small vessel mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	0.015	0.87	0.019	0.84
Year-0 to Year-3	-0.002	0.99	0.050	0.74

Table A6.8: Number of A mismatches related to "large"and "small" vessel mean lumen diameter loss.

Year	Number of mismatches at A locus	*n*	Large Vessels			Small Vessels		
			Mean	Standard deviation	p-value	Mean	Standard deviation	p-value
0 to 1	0	14	0.2580	0.2828	0.85	0.0309	0.1417	0.35
	1	57	0.2312	0.2795		0.0973	0.1709	
	2	45	0.2124	0.2525		0.0750	0.1397	
0 to 3	0	7	0.4465	0.2746	0.79	0.1676	0.1232	0.81
	1	19	0.4444	0.2333		0.1352	0.1713	
	2	20	0.3874	0.3153		0.1789	0.2635	

Table A6.9: Correlation of total number of A mismatches to "large"and "small" vessel mean lumen diameter loss.

Period of decrease	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	-0.054	0.57	0.037	0.69
Year-0 to Year-3	-0.094	0.53	0.050	0.74

Table A6.10: Number of B mismatches related to "large"and "small" vessel mean lumen diameter loss.

Year	Number of mismatches at B locus	*n*	Large Vessels			Small Vessels		
			Mean	Standard deviation	p-value	Mean	Standard deviation	p-value
0 to 1	0	8	0.3628	0.2103	0.31	0.1468	0.1130	0.47
	1	32	0.2010	0.2457		0.0727	0.1322	
	2	75	0.2245	0.2821		0.0797	0.1683	
0 to 3	0	2	0.5300	0.1358	0.72	0.1744	0.1116	0.94
	1	14	0.3795	0.2605		0.1430	0.1795	
	2	30	0.4315	0.2881		0.1656	0.2290	

Table A6.11: Correlation of total number of B mismatches to "large"and "small" vessel mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	-0.069	0.46	-0.065	0.49
Year-0 to Year-3	0.018	0.91	0.030	0.84

Table A6.12: Number of DR mismatches related to "large"and "small" vessel mean lumen diameter loss.

Year	Number of mismatches at DR locus	*n*	Large Vessels			Small Vessels		
			Mean	Standard deviation	p-value	Mean	Standard deviation	p-value
0 to 1	0	11	0.1413	0.2915	0.26	0.0540	0.1196	0.81
	1	61	0.2091	0.2559		0.0801	0.1504	
	2	44	0.2737	0.2757		0.0880	0.1736	
0 to 3	0	6	0.4170	0.1844	0.74	0.2262	0.1981	0.41
	1	25	0.3939	0.2927		0.1218	0.1303	
	2	15	0.4645	0.2784		0.1945	0.3016	

Table A6.13: Correlation of total number of DR mismatches to "large"and "small" vessel mean lumen diameter loss.

Period of loss	Large Vessels		Small Vessels	
	Correlation	P value	Correlation	P value
Year-0 to Year-1	0.154	0.10	0.054	0.56
Year-0 to Year-3	0.085	0.57	0.021	0.89

Table A6.14: Pre-transplant cross-match related to "large"and "small" vessel mean lumen diameter loss.

Year	Crossmatch positive?	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	No	110	0.215	0.025	0.95	0.076	0.156	0.61
	Yes	2	0.230	0.19		0.217	0.279	
0 to 3	No	44	0.4075	0.2729		0.1535	0.2086	-
	Yes	0						

Table A6.15: Pre-transplant PRA correlated to "large"and "small" vessel mean lumen diameter loss.

Year	Pre-transplant PRA positive?	*n*	Large Vessels			Small Vessels		
			Mean loss	Standard deviation	p-value	Mean loss	Standard deviation	p-value
0 to 1	No	114	0.225	0.265	0.56	0.077	0.152	0.37
	Yes	3	0.377	0.382		0.247	0.252	
0 to 3	No	47	0.4234	0.2713		0.1615	0.207	-
	Yes	0						

Table A6.16: Basic statistics of the antivimentin antibodies data.

Month/ Year	*n*	Mean	Standard deviation	Median	Minimum value	Maximum value	Quartiles 1st	Quartiles 3rd
PreTx	121	113.09	94.41	86.00	49.00	648.	56.50	130.00
1month	10	63.00	21.08	50.00	50.00	112	50.00	77.7
3months	25	95.6	59.4	68.0	50.0	237	50.0	117.5
6months	23	85.6	58.2	59.0	50.0	289	50.0	92.0
1 year	51	95.31	66.21	68.00	50.00	300	50.00	106.0
3 years	66	135.7	147.5	92.0	50.0	890	50.0	154.0
5 years	23	104.0	68.3	95.0	50.0	353	52.0	127.0

Table A7.1: "Large" vessel mean lumen diameter loss provisional model.

Predictor	Coeffecient	SD	p-value
Constant	0.0120	0.1789	0.947
Donor Sex	0.11786	0.06654	0.080
Caucasians	-0.0875	0.1158	0.452
Intracranial Haemorrhage	-0.11392	0.06521	0.084
Initial FS (>36 or ≤36%)	0.11900	0.06067	0.053
Domino Transplant	-0.00746	0.08316	0.929
ECG voltage (drop)	0.00559	0.01764	0.752
Prednisolone in first year	0.12438	0.07165	0.086
Maximum creatinine in first year	-0.0001118	0.0002729	0.683

Table A7.2: "Small" vessel mean lumen diameter loss provisional model.

Predictor	Coeffecient	SD	p-value
Constant	-0.0065	0.1039	0.951
Donor Sex	0.02989	0.03864	0.441
Caucasians	-0.03298	0.03787	0.386
Intracranial Haemorrhage	0.01681	0.03523	0.634
Initial FS (>36 or ≤36%)	0.02299	0.04161	0.582
Domino Transplant	-0.16155	0.06727	0.019
ECG voltage (drop)	0.05208	0.04829	0.284
Prednisolone in first year	0.01534	0.01024	0.138
Maximum creatinine in first year	0.0003711	0.0001585	0.022

PRESENTATION IN INTERNATIONAL MEETINGS

The following abstracts of material from this thesis were presented in the following international meetings:

1. RATG induction does not attenuate cardiac allograft vasculopathy. IA Bolad, D Robinson, NR Banner. International Conference of Immunosuppression annual scientific meeting, California, USA. December 2001. Selected for the Young Investigators Excellence Award presentation.

2. Left ventricular systolic function early after heart transplantation predicts cardiac allograft vasculopathy. IA Bolad, D Robinson, NR Banner. American College of Cardiology 51st annual scientific meeting in Atlanta, USA. March 2002.

3. Loss of coronary lumen diameter early after Heart Transplantation is greatest in the larger epicardial vessels. IA Bolad, D Robinson, NR Banner. International Society of Heart and Lung Transplantation annual scientific meeting. Washington DC, USA. April 2002.

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