UNIVERSITY OF SOUTHAMPTON

Cardiovascular Risk after Liver Transplantation

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ABSTRACT

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CARDIOVASCULAR RISK AFTER LIVER TRANSPLANTATION

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Cardiovascular risk factors including hypertension and hypercholesterolaemia are common after liver transplantation. It has been reported that cardiovascular disease is an increasingly common cause of patient mortality after liver transplant.

Using data from patient records, I assessed the prevalence of risk factors for cardiovascular disease after liver transplant and compared these to a non-transplant population. The data were used to examine the effect of switching immunosuppression from cyclosporin to tacrolimus upon cardiovascular risk. Clinical trials involving liver transplant recipients have examined the role of endothelin, renin-aldosterone and arterial stiffness in the development of hypertension, the efficacy of different antihypertensive drugs and the value of brain natriuretic peptide (BNP) as a potential screening tool for left ventricular impairment in hypertensive patients.

The predicted 10 – year probability of coronary heart disease (CHD) increased after liver transplant and was higher than a matched non-transplant population. Hypertension and hypercholesterolaemia were the most common risk factors for CHD. Tacrolimus was associated with a reduced prevalence of cardiovascular risk factors compared with cyclosporin and switching to tacrolimus can reduce blood pressure, weight and serum cholesterol. Increases in arterial stiffness and plasma endothelin-1 were implicated in the development of hypertension during the first 6 months. Amlodipine was optimum first line treatment of hypertension, with lisinopril being superior to bisoprolol as second-line treatment. BNP levels were raised in transplant recipients, particularly those with hypertension. Hyperuricaemia is common after liver transplantation and is associated with an increased predicted risk of CHD.

CHD risk rises after liver transplantation. It is likely that this will lead to an increase in post-transplant morbidity and mortality form cardiovascular disease, but this is not apparent by 5 years. Management of hypertension, hypercholesterolaemia and attention to weight gain after transplant are important to reduce the burden of post-transplant cardiovascular disease.

Published and Peer Reviewed Papers from work submitted in this thesis

Neal DAJ, Gimson AES, Gibbs P, Alexander GJM. Beneficial effects of converting liver transplant recipients from cyclosporine to tacrolimus on blood pressure, serum lipids and weight. Liver Transpl 2001;7:533-539

Neal DAJ, Tom BDM, Gimson AES, Gibbs P, Alexander GJM. Hyperuricaemia, gout, and renal function after liver transplantation. Transplantation 2001;72:1689-1691

Neal DAJ, Tom BDM, Luan J, Wareham NJ, Gimson AES, Delriviere LD, Byrne CD, Alexander GJM. Is there disparity between risk and incidence of cardiovascular disease after liver transplant? Transplantation 2004;77:93-99

Neal DAJ, Brown MJ, Wilkinson IB, Byrne CD, Alexander GJM. Haemodynamic efects of amlodipine, bisoprolol and lisinopril in hypertensive patients after liver transplantation. Transplantation 2004;77:748-750

Neal DAJ, Brown MJ, Wilkinson IB, Alexander GJM. Mechanisms of hypertension after liver transplantation. Transplantation (in press).

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List of Abbreviations

ACE:	angiotensin-converting enzyme
AI:	augmentation index
ALT:	alanine transaminase
ANOVA:	analysis of variance
ANP:	atrial natriuretic peptide
ATP III:	Adult Treatment Panel III
ATP:	adenosine triphosphate
BMI:	body mass index
BNP:	brain natriuretic peptide
BP:	blood pressure
CAD:	coronary artery disease
CDBP:	central diastolic blood pressure
CHD:	coronary heart disease
CI:	confidence intervals
CPP:	central pulse pressure
CSBP:	central systolic blood pressure
CVS:	cardiovascular
CyA:	cyclosporin
ET-1:	endothelin-1
FK506:	tacrolimus
H20:	water
HCl:	hydrochloric acid
H_2S0_4	sulphuric acid
HDL:	high density lipoprotein
HR:	heart rate
IL-2:	interleukin-2
kg:	kilogramme
LDL:	low density lipoprotein

LV:	left ventricular
LVH:	left ventricular hypertrophy
MAP:	mean arterial pressure
mg/dl:	milligrammes per decilitre
mg:	milligrammes
MI:	myocardial infarction
mmHg:	millimetres of mercury
mmol/l:	millimoles per litre
mU/l:	milli units per litre
NS:	not significant
PDBP:	peripheral diastolic blood pressure
pg/ml:	picogrammes per millilitre
pmol/l:	picomoles per litre
PPP:	peripheral pulse pressure
PSBP:	peripheral systolic blood pressure
PT:	prothrombin time
PWA:	pulse wave analysis
rapa:	rapamycin
SBP:	systolic blood pressure
SVRI:	systemic vascular resistance index
TFA:	trifluoroacetic acid
µg/ml:	microgrammes per millilitre
µmol/l:	micromoles per litre

Introduction

Liver Transplantation

The first liver transplant in man was performed in 1963. Following the recommendations of the National Institutes of Health Consensus Development Conference in 1983 when it was accepted that liver transplantation was an effective treatment for liver disease rather than an experimental procedure, considerable progress in the field has been made [1]. Advances have been made in surgical technique, better organ preservation, improved recipient selection criteria and development of potent immunosuppressive drugs. It is true to say that liver transplantation has revolutionised the management of patients with end-stage liver disease.

Today liver transplant is indicated for acute or chronic liver failure from any cause. It is a well-accepted treatment for patients with primary biliary cirrhosis, primary sclerosing cholangitis, cirrhosis due to hepatitis B and C viruses, alcoholic liver disease complicated by cirrhosis, acute paracetamol toxicity as well as childhood liver diseases and a host of other adult hepatic disease. The commonest indications in the United Kingdom are autoimmune liver disease, cirrhosis secondary to alcoholic liver disease and hepatitis C virus cirrhosis [2]. With improvements in surgical expertise, management of infections and post-operative complications, and safer immunosuppressive regimens patient survival is steadily improving.

Current 1–year survival rates in the main exceed 90 % [3] whilst 5-year patient survival is 75 % [4]. With increasing expertise in liver transplantation, older patients and patients with additional medical problems are accepted into liver transplant programmes. Thus patients with diabetes mellitus and stable coronary artery disease are now considered for transplant. These factors combined with longer patient survival after transplant make it likely that cardiovascular disease after transplant will be an increasingly important field with an impact upon long-term patient survival.

For many years it was believed that the prevalence of coronary artery disease (CAD) was lower in patients with cirrhosis than in the general population, based largely on studies showing less evidence of atherosclerosis and myocardial infarct in patients with cirrhosis [5, 6]. In support of a lower prevalence of CAD is the fact that patients with end stage liver disease often have lower serum cholesterol levels and in view of peripheral vasodilatation blood pressure is often low too [7]. However, recent studies have re-evaluated the prevalence of CAD in patients with liver disease. Coronary angiographic studies in patients with liver disease and being considered for transplant demonstrate that the prevalence of CAD in liver disease varies from 2.5 % to 27 % [8-10]. Such differences are in part explained by different population ages; the study of Carey et al with the highest prevalence of CAD examined only patients over the age of 50 [8]. Furthermore different definitions of significant CAD have been employed and it is probable that the prevalence is somewhere between 5 and 10 % [10]. By comparison, angiographic abnormalities in asymptomatic men with electrocardiographic abnormalities was 2.5 % [11]. It would appear then that the prevalence of CAD in transplant recipients is higher than had previously been considered and is at least as high as figures from the general population.

It is therefore not surprising that the issues surrounding cardiovascular disease after liver transplant should be commanding attention. Not only do patients have a similar or higher prevalence of coronary heart disease at the time of transplant as the general population, but patients are exposed to drugs in the form of anti-rejection medication that carry with them the burden of numerous cardiovascular side-effects. Superimposed on this is the fact that patients are now less likely to succumb to early deaths from infection, graft dysfunction or post-operative complications and accordingly survive longer. Patients are therefore increasingly exposed to a number of risk factors for development of cardiovascular disease after liver transplant and for a greater length of time than ever before.

Cardiovascular risk factors after liver transplantation

Cardiovascular disease after liver transplant

Cardiovascular disease developing post-transplant and the development of risk factors for cardiovascular disease has been an area of increasing importance over the last 5 years. This is borne out by a small but growing number of studies which have reported that coronary heart disease (CHD) is a common cause of death after liver transplant [12-15]. Most of these have examined deaths occurring beyond the first post-transplant year because peri-operative deaths and deaths due to complications of surgery are excluded. This enables the number of deaths due to cardiovascular disease to be more readily put into context alongside other long-term complications. It has been reported that up to 21 % of deaths after the first year are due to cardiovascular causes [13] and 60-75 % of cardiovascular deaths are due to myocardial infarction [13, 14]. These studies, which are all retrospective, provide at first glance at least some evidence that cardiovascular disease is an important area after liver transplant and has an impact upon patient survival. The increasing age of the transplant population, as a result of older patients being transplanted and increasing patient survival, is undoubtedly one of the contributory factors to the development of cardiovascular disease. It is also emerging that the prevalence of risk factors for heart disease such as hypertension, dyslipidaemia, obesity and to a lesser extent diabetes mellitus is high after transplant. These risk factors will be considered in turn below and hypertension and hypercholesterolaemia will also be discussed further in subsequent sections of the introduction.

Hypertension

Hypertension is the commonest and most important risk factor for development of cardiovascular disease after liver transplantation. The prevalence of hypertension after liver transplant varies from 36 to 82 % [16-25]; the variation is due in part to different definitions of hypertension. Earlier studies used a threshold of 160/95

mmHg to define hypertension whereas it is now accepted that a blood pressure above 140/90 mmHg is indicative of hypertension [26].

There are a number of different reasons why hypertension could develop after transplant although the underlying mechanisms have not been fully explained. The principle mechanism is widespread systemic vasoconstriction under the influence of immunosuppressant drugs such as calcineurin inhibitors [27]. Both cyclosporin and tacrolimus cause systemic and renal vasoconstriction which contributes to the development and maintenance of hypertension [28]. Other possible causes of hypertension are abnormalities of endothelial function, elevation of serum endothelin-1, use of corticosteroid drugs, alteration in the stiffness of the vasculature after transplant and stimulation of the renin-aldosterone axis [29]. The role of endothelin-1 and the renin-aldosterone system are the subject of Chapter 3. Finally, factors such as post-transplant diabetes mellitus and weight gain may also play a part in evolving hypertension.

Dyslipidaemia

The primary area of concern is hypercholesterolaemia given its important contribution to development of cardiovascular disease. Elevated serum cholesterol is an important risk factor for coronary and cerebrovascular disease in the general population and lipid abnormalities are common after liver transplant. Serum cholesterol increases after transplant [20, 30-34] and hypercholesterolaemia develops in as many as 66 % of patients [19]. Increases in serum triglyceride are also seen after liver transplantation [19, 24, 35], with reported prevalence rates up to 59 % [36]. Immunosuppressant drugs, including the calcineurin inhibitors and corticosteroids, are implicated in the development of hypercholesterolaemia [31, 37, 38]. Serum lipid levels are also influenced by post-transplant weight gain, diabetes mellitus, diet and renal dysfunction [24].

Obesity

Weight gain and obesity are frequently encountered problems in liver transplant recipients. Body mass index (BMI), defined as weight (kg) divided by the square of the height (m^2), increases by up to 14 % in the first year after transplant [20, 22, 39]. The major period of weight gain is in the first two years after transplant [19, 39, 40]. The reason for the increase in weight are not clear but corticosteroid use is a risk factor for and cyclosporin is also associated with weight gain after transplant [41].

Diabetes mellitus

Several authors have observed that liver transplant recipients have an increased prevalence of diabetes mellitus compared to the general population [21, 23, 42]. Immunosuppression and corticosteroids are both linked to development of diabetes mellitus. Posttransplant diabetes mellitus is also associated with hepatitis C virus allograft hepatitis [43]. The incidence of diabetes after transplant in the USA ranges from 12 to 18 % [15, 22, 23]. However, there is a trend now towards using lower doses of immunosuppressants and of early withdrawal of corticosteroids after transplant and both these approaches may have an impact upon the current published incidence of diabetes mellitus [38, 44, 45]. Indeed, a recent publication from Birmingham, UK, recorded an incidence of diabetes mellitus after transplant of 3 % [12].

Cardiovascular disease after transplant: limitations of knowledge

There are however, limitations to the current published literature regarding the development of cardiovascular disease. Studies to date examining the development of cardiac or cerebrovascular disease are limited by having been restricted to examining mortality rather than incorporating morbidity. Whilst it is

clear that important risk factors for cardiovascular disease such as hypertension and hypercholesterolaemia are seen frequently after transplant it cannot be assumed from current available evidence that these and other risk factors translate into development of cardiovascular disease in transplant recipients. Do transplant patients suffer a greater amount of cardiovascular disease that befits the prevalence of the risk factors? In order to address this question, the transplant population under study must be compared with an appropriate matched non-transplant population. Failure to do this renders data on the incidence of cardiovascular disease after transplant of limited value. Only two studies, one each from the United Kingdom and USA, have compared transplant and non-transplant populations with differing conclusions on the relative frequency of cardiovascular disease in transplant recipients [12, 22]. From current evidence therefore it is unclear whether liver transplant is associated with a higher incidence of cardiovascular disease.

Study of the prevalence of cardiovascular risk factors and incidence of cardiovascular disease after liver transplant

The first part of my thesis has been to assemble data on the development of hypertension, hypercholesterolaemia, obesity, diabetes mellitus and the incidence of cardiac and cerebrovascular disease in patients following liver transplant in Cambridge. In order to try and assess the impact of liver transplant upon future risk of cardiovascular disease, data that I gathered has been used to calculate the predicted 10-year risk of developing CHD by using the coronary risk equations as set out in the Framingham study [46]. Furthermore, the incidence of coronary and cerebrovascular disease observed in the liver transplant recipients I studied can then be compared with expected incidence rates in a non-transplant United Kingdom population matched for age and sex. This should then enable me to comment upon the risk, both calculated and actually realised, of developing cardiovascular disease after liver transplantation.

Hypertension

Hypertension is a common development after liver transplantation. This contrasts with the situation in patients with cirrhosis awaiting transplant. Cirrhosis is known to be associated with a hyperdynamic circulation [47], manifest primarily as increased cardiac output and decreased systemic vascular resistance. Consequently, prior to transplant patients often have low or low-normal blood pressure as a result of the circulatory changes and only 6 % have a prior history of hypertension [22]. For the majority of patients therefore hypertension is a complication related to the liver transplant. There are two key issues relating to hypertension after liver transplant. Firstly, what causes hypertension to occur and secondly, how is it best treated?

Mechanisms of hypertension

During the first few weeks after liver transplant there is a restoration of the hyperdynamic circulation typical of cirrhosis to a normal circulation [29]. Elevated cardiac outputs gradually decrease over the first few weeks or months after transplant and systemic vascular resistance increases during the first month [29]. As a result, blood pressure commonly increases soon after liver transplant. Superimposed on these circulatory changes is the vasoconstriction that is a direct consequence of the use of calcineurin inhibitors [48]. As a result of haemodynamic changes, the principal one being widespread arterial vasoconstriction, development of hypertension post transplant occurs during the first 4 months in as many as 50 % of patients [20].

It is important to understand the underlying mechanisms contributing to vasoconstriction and increased vascular resistance in patients receiving calcineurin inhibitors as this would facilitate appropriate management of hypertension. A number of mechanisms have been proposed. These include disturbances in sympathetic neural activity, alteration in local mechanisms of vascular regulation, stimulation of the renin-angiotensin system and increased production of the

peptide endothelin-1. Of these the latter two mechanisms will be considered in more detail below.

Renin-Angiotensin System

Physiology

Within the kidney the juxtaglomerular apparatus is made up of specialised arteriolar smooth muscle cells situated on the afferent glomerular arteriole as it enters the glomerulus. These cells secrete renin. Renin release allows the conversion of angiotensinogen into inactive angiotensin-I and angiotensin-converting enzyme then converts angiotensin-I into active angiotensin-II. The latter is a potent vasoconstrictor acting directly on smooth muscle cells via angiotensin-II receptors (Type 1). The renin-angiotensin system provides short-term regulation of the cardiovascular system that becomes activated in acute conditions such as hypotension, hypovolaemia and severe heart failure. Once blood pressure is restored further renin release is suppressed [49]. In addition, angiotensin-II interacts with the sympathetic nervous system to increase vascular tone. It causes volume expansion through sodium retention, via aldosterone release and renal vasoconstriction, and fluid retention via antidiuretic hormone [49].

Involvement in liver transplantation

In view of the vasoconstriction and volume expansion accompanying release of angiotensin-II and aldosterone, activation of the renin-angiotensin system after liver transplant has been postulated to be a contributory mechanism in the causation of hypertension. In vitro cyclosporin induced a three-fold increase in renin secretion by a direct effect on juxtaglomerular cells [50]. Early work in animals pointed to a direct relationship between cyclosporine administration and stimulation of plasma renin activity [51, 52]. Spontaneously hypertensive rats respond to cyclosporin by a rise in hypertension associated with increases in plasma renin activity [53]. Recently cyclosporin has been shown to up-regulate

angiotensin II receptors in cultured human vascular smooth muscle cells rendering them more sensitive to the effects of angiotensin II [54]. Up-regulation of angiotensin-II receptors in vivo could be a factor leading to vasoconstriction and be another potential cause of hypertension after transplant.

There have been few studies in humans of changes in the renin-aldosterone axis after liver transplantation. A number of studies of cyclosporin treated patients have shown that circulating renin levels are in fact low during the first 4 months after transplant, at a time when blood pressure generally increases [28, 55, 56]. Serial measurements after the first year however, indicate that levels of plasma renin activity increase [57]. Furthermore, in a study of 12 liver transplant recipients with hypertension developing at a median of 8 months, plasma renin levels were found to be elevated compared to normal controls when measured at 13 months, the delay being due to establishing a diagnosis of sustained hypertension [58]. One can only speculate whether plasma renin was elevated prior to development of hypertension.

The studies of Julien [58] and Textor [57] provide evidence that plasma renin levels are raised after the first year. Neither study is able to address the issue of whether increased stimulation of the renin-aldosterone system is causally linked to hypertension. Many patients who develop hypertension after transplant do so during the first 6 months [20]. A study of changes in the renin-aldosterone axis over this time period comparing normotensive patients with those who develop hypertension is desirable to ascertain whether development of early hypertension can be linked to elevations in serum renin. I have investigated changes in serum renin and aldosterone levels after liver transplant by measuring them prior to transplant and serially during the 6 months after transplant. If levels of these hormones are raised during the first few months and I can demonstrate a link to hypertension, this could have useful clinical ramifications regarding treatment of early hypertension after liver transplant.

Endothelin-1

The endothelins are potent vasoconstrictor peptides synthesised by the vascular endothelium [59]. Endothelin-1 (ET-1) is the principle isoform present in human endothelium. When ET-1 is infused into animals or humans it elicits a strong sustained vasoconstriction and hypertensive response [60, 61]. In the kidney it causes renal vasoconstriction, decline in renal plasma flow, glomerular filtration rate and sodium excretion [59, 62]. ET-1 is produced and released from endothelial cells by various chemical and physical stimuli and may contribute to vasospasm in pathophysiological conditions where levels of ET-1 are significantly elevated such as in atherosclerotic vessels [63].

Endothelin-1 and liver transplant

There is mounting evidence that calcineurin inhibitors affect ET-1 levels. Animal studies have suggested that the vasoconstrictor properties of cyclosporin might be mediated through endothelin [64]. Experiments on cultured endothelial cells and in humans treated with cyclosporin and tacrolimus have indicated that calcineurin inhibitors are associated with an increase in ET-1 production [65-69]. Furthermore endothelin levels have been shown to increase at day 7 after liver transplant in patients with moderate to severe acute cellular rejection [70].

It can be speculated that calcineurin inhibitors mediate some of their vasoconstrictor properties through ET-1. Elevation in ET-1 after transplant is an attractive hypothesis as a mechanism of early hypertension. Plasma ET-1 has been shown to increase in the first week after liver transplantation and is associated with a rise in mean arterial blood pressure from 82 ± 4 to 103 ± 2 mmHg [71]. In another study 44 cyclosporin and 31 tacrolimus treated patients were evaluated before and after transplant [72]. Circulating levels of ET-1 were slightly elevated for 2 years after transplant, albeit not differing significantly from levels pre-transplant. Urinary endothelin levels rose after transplant and also remained

elevated for 2 years. In the same study 73 % of cyclosporin treated patients and 54 % of patients treated with tacrolimus were hypertensive at 2 years. Endothelin in this case may not on the face of it be implicated in the development of hypertension. However, studies indicate that the vasoactive effects of ET-1 are normally countered by vasodilatory mechanisms, such as release of prostacyclin [73]. Urinary prostacyclin levels are low after liver transplant [72] so the vasoconstrictor properties of ET-1 could be relatively unopposed. Thus it is possible that the levels of circulating ET-1 observed in Textor's study could contribute to vasoconstriction and therefore hypertension. Against a role of ET-1 in early transplant hypertension is the recently reported finding that plasma ET-1 did not increase during the first 6 weeks after transplant despite development of hypertension in all 15 patients studied [74].

It is fair to conclude that it has not yet been determined whether increases in circulating ET-1 during the first few months after transplant can be linked to development of hypertension. Furthermore very little data relates to immunosuppression with tacrolimus. An important implication for the involvement of ET-1 in transplant hypertension is the recent introduction of endothelin antagonist drugs. Animal studies showed that endothelin receptor antagonists could prevent the rise in blood pressure and the vasoconstriction that are associated with administration of cyclosporin [75, 76]. Endothelin antagonists have recently entered the clinical arena and an initial study demonstrated efficacy of one such drug, darusentan, for treatment of hypertension [77]. These agents have not as yet been utilised in the setting of transplant hypertension but could play a role if endothelin is causative in hypertension after liver transplant.

Arterial Stiffness

Another potential explanation for development of hypertension after transplant is increasing arterial stiffness. Arterial stiffening with age is acknowledged as the cause of isolated systolic hypertension [78] in the non-transplant population.

Arterial stiffness relates to medium and large arteries as opposed to the smaller arterioles and resistance vessels. Arterial stiffness is an independent predictor of cardiovascular mortality [79]. Stiffness may be determined in part by structural elements within the arterial wall but is also influenced by the balance of vasoactive mediators such as nitric oxide and endothelin-1 acting on the endothelium [80]. Arterial stiffness can be assessed in a number of ways. Peripheral pulse pressure is a marker of arterial stiffness and is a predictor of cardiovascular risk [81]. Its use is limited by the fact that although diastolic and mean arterial pressure are relatively constant throughout the arterial tree, systolic pressure and hence pulse pressure varies considerably. Peripheral pulse pressure therefore does not always provide a reliable estimate of central pulse pressure and arterial stiffness [82].

Pulse Wave Analysis

The arterial pressure waveform contains valuable information concerning both aortic and systemic arterial stiffness. Over 100 years ago Mahomed [83] showed that it was possible to record the peripheral pressure waveform. More recently non-invasive assessment of the central arterial waveform has become possible [84]. Central pulse wave analysis (PWA) utilises applanation tonometry to record pressure waves from either the carotid or the radial artery. Applanation tonometry is based on the same principle used to record intraocular pressures, i.e., that when two curved surfaces are flattened, circumferential pressures are equalised. A probe with a micromanometer at its tip is used to flatten the radial artery at the wrist. In this way tonometry gives an excellent representation of the intra-arterial peripheral pressure wave [84]. A generalised and validated transfer factor based upon data established from invasive recordings [85] is then used to generate the corresponding central arterial waveform. From this, arterial stiffness can be assessed in a non-invasive and reproducible manner [80] by calculating the augmentation index and the timing of the reflected pressure wave.

Arterial Waveform and Augmentation Index

The arterial pressure waveform and systolic pressure in particular varies throughout the arterial tree [86]. This is due to differences in vessel compliance and wave reflection [87]. Arteries are normally compliant and buffer the pressure changes caused by the intermittent ejection of blood from the left ventricle. Outgoing pressure waves are reflected back from the periphery, principally from the aortic bifurcation. The arterial waveform at any time is thus made up of the forward moving and backward going reflected waves. As a consequence, aortic systolic pressure can differ from brachial artery pressure by more than 20 mm Hg [88].

Normally the reflected wave arrives back at the aortic root in diastole, thereby helping to maintain coronary perfusion. However, with increasing age or under conditions that cause stiffening of the arterial tree, the amplitude and velocity of the reflected wave increase [89, 90]. Accordingly, a larger reflected wave returns to the aorta earlier and adds to or augments the systolic pressure. The augmentation index is a measure of the contribution of the reflected pressure wave to the ascending pressure waveform and is expressed as a percentage of the pulse pressure [91]. The amplitude and speed of the reflected wave are dependent upon the arterial stiffness and hence augmentation index provides a measure of systemic arterial stiffness. An advantage to measuring augmentation index is that it reflects the manner in which the arterial tree interacts as a whole rather than the technique of pulse wave velocity that measures stiffness in a single short arterial segment.

Arterial stiffness and hypertension after transplant

An increase in arterial stiffness, indicated by a higher augmentation index, is a cause of systolic hypertension. In addition arterial stiffness is itself increased by hypertension [92]. Increasing arterial stiffness, as detected by measuring augmentation index, after liver transplant could be an underlying cause of early

transplant hypertension. In order to assess this it is necessary to determine augmentation index at intervals after transplant but before hypertension is established, for this will tend to increase the augmentation index. This can be assessed accurately using the technique described above of measuring pulse wave analysis. This has not been investigated in liver transplant recipients and could provide additional clues as to the mechanisms underlying hypertension.

Arterial stiffness and cardiovascular risk

As arterial stiffness increases the central blood pressure rises. The central aortic pressure determines left ventricular workload [93]. It has been shown that left ventricular mass correlates well with the shape of the central waveform [94, 95]. An increase in central pressure therefore favours left ventricular hypertrophy and potentially increases cardiovascular mortality [80]. Therefore, comparison of arterial stiffness before and after transplant may provide an additional means of assessing changes in cardiovascular risk with liver transplant.

Arterial stiffness and endothelin-1

There may be a link between arterial stiffness and production by the endothelium of vasoactive mediators such as nitric oxide and ET-1 [80]. Endothelial dysfunction, a risk factor for cardiovascular disease, is associated with a shift in the balance of production of vasoactive mediators away from nitric oxide and towards increased synthesis of the vasoconstrictor ET-1. Circulating serum ET-1 levels appear to correlate with arterial stiffness [96] and endogenous ET-1 production directly regulates pulse wave velocity and hence large artery stiffness [97]. Analysis of arterial stiffness and serum ET-1 levels after liver transplant will enable me to examine their role in the development of hypertension after liver transplant.

Arterial stiffness, wave reflections and antihypertensive agents

Pulse wave velocity, wave reflections and arterial stiffness are increased in essential hypertension [98-100]. Arterial waveform studies in the non-transplant population demonstrate that different antihypertensive drugs have differing effects on arterial haemodynamics and these effects can be useful additional guides as to appropriate choice of drug. Measurements of wave reflection taken during cardiac catheterisation revealed that nifedipine reduced the magnitude of the reflected pressure wave [99, 100] and the ACE-inhibitor captopril also reduced the size of wave reflection in hypertensive patients [101]. Administration of the beta-blocker propranolol has been shown to increase wave reflections and augmentation index in hypertensive patients despite adequate peripheral blood pressure lowering [98]. This would serve to maintain central aortic pressure which in the long run is undesirable.

These findings are based upon changes in wave dynamics after administration of single doses of drugs. In a study exploring the effects of longer term drug usage, applanation tonometry was used to measure carotid pressure waveforms non-invasively in 79 patients with mild hypertension who were treated for 8 weeks with the ACE-inhibitor fosinopril or the beta-blocker atenolol [102]. Both drugs reduced peripheral blood pressure to a similar extent. It was found that whilst both drugs reduced wave reflections, as estimated by the augmentation index, fosinopril reduced them to a greater extent than did atenolol. This suggests a more profound reduction in central systolic blood pressure with fosinopril. Similar effects if observed after liver transplant would provide important information that may influence the choice of long term antihypertensive drug.

Hypertension: management

Calcium channel antagonists

There is surprisingly little data on the management of transplant hypertension which is an area that has received very little attention in the form of clinical trials. Historically there is a vogue for using calcium channel antagonists and in particular the dihydropyridine class as first line treatment [27, 103]. Such drugs include nifedipine, isradipine, felodipine, nicardipine and amlodipine. A good case can be made on mechanistic grounds for using such drugs because as vasodilators they act upon vascular smooth muscle to reduce systemic vascular resistance and they are potentially able to counteract the systemic vasoconstriction that occurs after transplant as a result of calcineurin inhibitor immunosuppression [29]. Of the dihydropyridine class, nicardipine interferes with calcineurin inhibitor pharmacokinetics resulting in increased plasma levels of cyclosporin and is therefore less favoured [104]. Other calcium channel antagonists such as diltiazem and verapamil are not widely used as studies in renal transplant recipients showed that each drug inhibits cyclosporin metabolism leading to elevated blood levels of cyclosporin [105, 106]. Furthermore the cardiac side effects are greater [103].

Only three clinical trials have demonstrated efficacy of calcium channel antagonists, namely isradipine [107], nicardipine [104] and nifedipine [108]. Isradipine, a dihydropyridine calcium channel blocker, was given to 15 hypertensive patients within the first three months of transplant [107]. It was effective in lowering blood pressure but the drug was given at a time when corticosteroid doses were also being reduced and this could account for some of the observed drop in blood pressure. Another confounding factor is that the loop diuretic frusemide was given to control peripheral oedema. The second study demonstrating blood pressure lowering efficacy explored the use of nicardipine for immediate post-operative hypertension in 34 patients, 27 of whom continued the drug long-term [104]. 70 % of patients were normotensive on nicardipine. The absence of documented pre-treatment blood pressures minimises the usefulness of this data however. The third study which was published in abstract form only

showed that 16 patients on an unspecified dose of nifedipine reduced their systolic blood pressure from 161 ± 2 to 141 ± 5 mmHg [108]. No further details of the medication are given. A Pubmed search inputting hypertension, liver transplant and cardiovascular risk has not shown any other trials of antihypertensive agents after liver transplant.

Other antihypertensive drugs

Three studies from the United Kingdom report experiences with antihypertensive agents. In a retrospective analysis of 116 patients surviving more than 5 years after liver transplant, 29 % were receiving antihypertensive drugs [109]. Hypertension was reportedly controlled with nifedipine and in some cases this was combined with atenolol .The second study was primarily concerned with cyclosporine toxicity but in which 64 % of patients were hypertensive at 4 years. Initial management of hypertension was with the beta-blockers metoprolol or atenolol and the alpha-blocker prazosin was added in as necessary [110]. Finally, a series from King's College and Addenbrooke's Hospitals reports the use of alpha or beta blockade and vasodilators for the 17 % of cyclosporine treated patients who were hypertensive at a median of 40 months [111]. In none of these studies is any further information given on the choice of drug or dose used or indeed relative efficacy. The report from the Mayo Clinic, USA published in abstract form and mentioned above with reference to nifedipine, also showed that labetalol reduced blood pressure in 9 patients. Because the pre-treatment blood pressure quoted is the mean of the patients from both nifedipine and labetalol groups, the true drop in blood pressure for each drug is not clear. As with nifedipine the dose of labetalol used was not specified [108].

Because some studies have shown that serum renin levels are low in the first few months after transplant it has been suggested that angiotensin converting enzyme (ACE) inhibitors are of limited value when used alone in the first year post transplant [29]. According to such studies, ACE inhibitors should be more effective when used after the first transplant year [57]. These arguments remain theoretical as

there has not been any published work exploring the efficacy of ACE inhibitors after liver transplant. Evidence for beneficial effects of ACE-inhibitors does however, exist in animal studies where it has been shown that enalapril can prevent cyclosporin-induced hypertension [112].

To summarise, the liver transplant literature contains anecdotal evidence regarding the use and effectiveness of different classes of antihypertensive drugs but no clinical trials have examined and compared the relative blood pressure lowering abilities of these drugs.

Trial of Antihypertensive Drugs

In order to gain a better understanding of the efficacy of antihypertensive drugs and the management of hypertension after liver transplant I have conducted a clinical trial comparing treatment with three different drugs. In patients on no treatment I have examined the efficacy and tolerability of the calcium channel blocker amlodipine. In patients already on this drug or in those who have proved intolerant to it I have compared the ACE inhibitor lisinopril with the beta blocker bisoprolol in a cross-over study. Pulse wave analysis was undertaken to determine the effects of the drugs upon wave reflections, arterial stiffness and central aortic pressure. The study is presented in Chapter 3.

Conversion from cyclosporin to tacrolimus

Since its introduction in the early 1980's cyclosporin, in combination with azathioprine and corticosteroids, has been the mainstay of post-transplant immunosuppression [113]. In 1989 tacrolimus, a macrolide compound isolated from Streptomyces tsukubaensi, was introduced and has been increasingly used in liver transplantation following it's approval by the Food and Drug Administration in 1994. Although not chemically related to cyclosporin, tacrolimus has a similar

mode of action and both drugs inhibit interleukin-2 (IL-2) synthesis and expression of IL-2 receptors. Since the mid-1990s several studies have reported that there are differences in the side-effect profile of these drugs. Trials in liver transplantation have demonstrated small but clear differences between cyclosporin and tacrolimus with respect to the frequency of acute cellular rejection, refractory rejection and chronic rejection [16, 17, 114]. It has also been suggested that grafts with chronic rejection can be 'rescued' by switching from cyclosporin to tacrolimus [115], although good data in this regard are lacking.

Cardiovascular differences between cyclosporin and tacrolimus

As has been discussed calcineurin inhibitors are implicated in the development of such cardiovascular risk factors after liver transplant. Recently it has become apparent that patients treated with tacrolimus have a more favourable cardiovascular risk factor profile than those whose immunosuppression is with cyclosporin. Hypertension has been reported to occur significantly less frequently in patients whose immunosuppression is with tacrolimus rather than cyclosporin [16, 18-21, 32, 114]. Similarly, hypercholesterolaemia occurs in fewer patients after transplant where tacrolimus is used compared to when cyclosporin is used [19, 20, 32, 34]. The development of moderate or severe obesity after transplant has been described in over 34 % of patients with a normal body mass index (BMI) before surgery [22]. A trend towards reduced weight gain after transplantation with tacrolimus instead of cyclosporin has been described [19, 20, 116], although statistical significance was not reached in these particular studies. Early reports indicated that tacrolimus was associated with a higher prevalence of diabetes mellitus after transplant [16] although this has not been born out in recent studies that have used lower doses of tacrolimus [18, 20, 22].

Effect of switching from cyclosporin to tacrolimus

In general patients treated with tacrolimus develop less hypertension, less hypercholesterolaemia and to a lesser extent experience less weight gain after transplant. These differences may have an impact upon subsequent development of cardiovascular disease and possibly long term patient survival. One question that arises from the differences in side-effect profiles of the drugs is whether changing a patient's immunosuppression from cyclosporin to tacrolimus could result in a reduction in the observed blood pressure, serum lipids and weight.

This has been explored indirectly in a handful of small studies often in patients being converted from cyclosporin to tacrolimus because of liver graft rejection or cyclosporin related nephrotoxicity. Thus in one such study of 20 liver transplant recipients a reduced requirement for antihypertensive medication was noted after tacrolimus was substituted for cyclosporine [117]. An intriguing issue is that of the possible cardiovascular benefits, if any, of changing patients with normal graft function on cyclosporin over to tacrolimus. Evidence from two studies suggest an improvement in serum cholesterol upon conversion to tacrolimus but differing outcomes with respect to blood pressure in that only in one study did blood pressure improve [118, 119]. Weight gain can be dramatic in the first 2 years after liver transplant and has potential impact upon cardiovascular health as well as patients' emotional wellbeing. A literature search reveals that the effect of changing from cyclosporin to tacrolimus upon weight has not been assessed previously. No study has assessed whether a switch in immunosuppression to tacrolimus affects the predicted CHD as calculated using the Framingham risk prediction equations.

Study of switching immunosuppression with stable graft function

I have reviewed the case records of patients with normal graft function who have been converted from cyclosporin to tacrolimus. The effects upon blood pressure, serum cholesterol, blood glucose, weight, renal function and graft function are

discussed in Chapter 5. This study assesses whether changing immunosuppression after liver transplant can alter the risk of developing cardiovascular disease.

Brain Natriuretic Peptide

Left ventricular hypertrophy

Left ventricular hypertrophy is an independent risk factor for cardiovascular complications in hypertensive patients [120]. In a study of hypertensive patients, cardiovascular events occurred in a significantly higher proportion of patients with increased left ventricular mass identified by echocardiography than in those without (26 % versus 12 %) over a 10-year period [121]. Patients with increased ventricular mass were at greater risk for cardiovascular death and all-cause mortality. Risk stratification in hypertensive patients can be further refined with information on left ventricular mass and geometry. Patients with concentric hypertrophy as opposed to eccentric hypertrophy are at the highest risk for adverse outcome [121]. Hypertension is the most common antecedent of left ventricular hypertrophy in the general population [121]. The early identification of left ventricular hypertrophy is very important in the management of the hypertensive patient.

Electrocardiography is routinely performed in the assessment of hypertensive patients but is poor at detecting left ventricular hypertrophy (LVH) [122]. Echocardiography is more sensitive than electrocardiography in detecting LVH and provides useful information on the function of the left ventricle, but is more time consuming and the results are more difficult to interpret in obese patients or in those with pulmonary disease [122]. Thus there are limitations to both these established means of evaluating patients with hypertension, particularly with regard to echocardiography as a screening tool for LVH. In recent years determination of plasma levels of brain natriuretic peptide has emerged as a

potentially readily available and cost-effective diagnostic test of left ventricular hypertrophy.

Brian natriuretic peptide: introduction

Brain natriuretic peptide (BNP) is a hormone consisting of 32 amino acids and was first isolated from pig brain in 1988 [123]. Subsequently BNP was identified in human cardiac atria [124] and in human plasma [125]. The BNP gene and the atrial natriuretic peptide (ANP) gene are situated adjacent to each other but there are differences in the stimuli causing release of each peptide. ANP secretion is stimulated by atrial distension and is produced primarily by myocardial cells in the atria. BNP is secreted primarily by the ventricular myocardium [126]. Plasma levels of ANP reflect the severity of heart failure [127]. ANP levels also reflect the degree of elevation of blood pressure rather than the left ventricular mass. Production of BNP is increased in the presence of cardiac overload, such as occurs in congestive heart failure [126] and in acute myocardial infarction [128].

BNP has several actions. In the kidney it increases glomerular filtration and inhibits sodium reabsorption, causing natriuresis and diuresis. BNP relaxes vascular smooth muscle, causing arterial and venous dilatation leading to reduced blood pressure and ventricular preload. It also blocks cardiac sympathetic nervous system activity and has inhibitory effects on the renin-aldosterone axis [129].

Patients with essential hypertension with left ventricular hypertrophy also develop raised levels of plasma BNP [130, 131]. Elevated BNP levels are related to the severity of left ventricular hypertrophy rather than blood pressure level [130]. It has been shown that elevated plasma BNP is a more powerful marker of left ventricular systolic dysfunction and left ventricular hypertrophy than plasma ANP [132, 133]. Furthermore raised concentrations of BNP are independently associated with sudden cardiac death in patients with heart failure and after myocardial

infarction [134, 135], and BNP is more closely related to mortality than left-ventricular ejection fraction [129].

Brain natriuretic peptide and hypertension

The usefulness of measuring plasma BNP has been demonstrated in an outpatient setting with its ability to detect early abnormalities of left ventricular function and thus to serve as a potential screening tool [136, 137]. In Hirata's study [136], plasma BNP concentrations were measured in 415 patients with heart disease and/or hypertension, and in 65 control subjects. In those patients with both heart disease and hypertension, plasma BNP levels were higher in those who had abnormal echocardiograms and ECG's compared to those without such abnormalities. There was a significant correlation between plasma BNP levels and left ventricular wall thickness and left ventricular mass.

Yamamoto and colleagues measured BNP in 94 patients undergoing cardiac catheterisation and also 15 healthy controls [132]. An elevated value of BNP was defined as greater than the mean value in normal subjects plus 3 standard deviations. Thus a BNP value above 14.7 pmol/l (equivalent to 50.9 pg/ml) was a more powerful predictor of left ventricular systolic dysfunction, defined as an ejection fraction less than 45 % on echocardiography, than ANP with a sensitivity and specificity of 83 % and 77 % respectively.

In McDonagh's study, 1252 patients with ages ranging from 25 to 74 were randomly selected from general practitioner's lists in Glasgow and underwent echocardiography and electrocardiography [133]. A left-ventricular ejection fraction of 30 % or less was used to define left ventricular systolic dysfunction. Levels of plasma BNP were significantly higher in those with left ventricular dysfunction than in those without (24pg/ml vs. 7.7 pg/ml), and this applied to symptomatic and asymptomatic patients. A BNP level above 17.9 pg/ml had a sensitivity and specificity of 77 % and 87 % respectively for the identification of LV systolic dysfunction, findings similar to Yamamoto et al although it must be borne in mind

that the echocardiographic definition of systolic dysfunction was different in that study. McDonagh's work is important therefore in that it illustrates the ability of an elevated BNP to identify patients with asymptomatic left ventricular systolic dysfunction.

Left ventricular hypertrophy increases the risk of cardiovascular events in hypertensive patients, and plasma BNP has shown promise in being able to identify patients with hypertension who are likely to develop or who have early stages of left ventricular hypertrophy. Suzuki et al measured a single plasma BNP level in untreated hypertensive patients and an elevated plasma level of BNP was defined as 41 pg/ml (mean plus 2 standard deviations of the control population). They found that those with high levels of BNP at baseline (25 % of hypertensive patients) had significant increases in left ventricular wall thickness at 9 months follow-up compared with those with normal baseline BNP [138]. No such echocardiographic changes occurred in the patients with initially normal BNP levels. There was no difference in blood pressure between the two groups.

Nishikimi and co-workers looked at the ability of BNP in ninety patients to distinguish between hypertensive patients with concentric LV hypertrophy, with its associated worse outcome, and other types of LV hypertrophy such as eccentric hypertrophy. Plasma BNP levels tended to be higher in hypertensive patients than in controls. Levels were markedly increased however, in patients with concentric hypertrophy although there was no difference in blood pressure between these and other hypertensive patients [122]. In that study the sensitivity and specificity of a BNP value above 18 pg/ml (twice the upper limit of normal) for predicting concentric hypertrophy in hypertensive patients were 75 % and 74 % respectively.

Finally, a multicentre study of 1586 patients with dyspnoea revealed highest BNP concentrations in patients with decompensated heart failure but levels were also elevated in patients with left ventricular dysfunction [139]. A BNP level below 50 pg/ml was especially useful for ruling out heart failure, with a negative predictive value of 96 %.

The increased left ventricular wall tension seen with LVH activates the release of BNP (and to a lesser extent ANP). BNP has natriuretic, diuretic and vasodilatory properties and its secretion can be viewed as counter-regulatory to the actions of the renin-angiotensin-aldosterone axis. It has been suggested that BNP production may occur before the appearance of LV structural changes in hypertensive patients [138]. BNP release would appear to have two useful outcomes: firstly it represents a means of counteracting development of heart failure and secondly it serves as a marker of early LV dysfunction. Measurement of plasma BNP is feasible in outpatients and can be expected to identify patients with hypertension who are at increased risk of left ventricular hypertrophy.

Brain natriuretic peptide and liver transplantation

It is not known what happens to BNP in the setting of liver transplant hypertension. There are no data on levels of BNP in liver transplant patients, whether hypertensive or not. The prevalence of hypertension after liver transplant is high and if the same applies as in non-transplant hypertension, a single outpatient plasma sample analysed for BNP could provide valuable prognostic information in hypertensive transplant patients with regard to the presence or absence of left ventricular hypertrophy. This would enable patients at particular risk of cardiovascular disease consequent upon LV hypertrophy to be diagnosed early and to be targeted for cardioprotective treatment.

Chapter 4 comprises a study examining BNP levels in liver transplant recipients, both normotensive and hypertensive, and compares these with a non-transplant control population.

Uric Acid

Elevated serum uric acid was first linked to increased risk of cardiovascular disease in 1959 [140]. As efforts to identify treatable risk factors for cardiovascular disease intensify, there has in recent years been renewed interest in the association between hyperuricaemia and cardiovascular disease [141]. Several studies have now been published citing uric acid as a cardiovascular risk factor but there has been debate as to the nature of this association, particularly whether hyperuricaemia is an independent risk factor.

Uric acid synthesis

Dietary and endogenous nucleic acids are degraded ultimately to uric acid through the action of the enzyme xanthine oxidase. Uric acid itself is a weak acid present throughout the extracellular fluid as sodium urate. It is excreted renally and about 90 % of filtered uric acid is reabsorbed from the proximal renal tubule [142]. Active secretion into the distal tubule by an ATPase-dependent mechanism contributes to the overall clearance [143]. Serum uric acid levels are determined by the rate of purine metabolism, influenced by dietary and genetic factors, and the efficiency of renal clearance. Uric acid is sparingly soluble in aqueous solutions and exposure to high levels can over time predispose to deposition of urate crystals in soft tissues [142]. The classical manifestation of this is the attack of painful gout caused by deposition of urate crystals in the joints of the great toe.

Uric acid and calcineurin inhibition

It has long been recognised that hyperuricaemia occurs as a complication of cyclosporin therapy. Studies report that 30 to 84 % of patients on cyclosporine following heart or kidney transplants develop hyperuricaemia [144-151]. Hyperuricaemia has been shown to be associated with renal impairment and exacerbated by diuretic use [146, 150, 152, 153]. Mechanisms for hyperuricaemia

have been proposed. It has been shown that hyperuricaemia occurs as a consequence of reduced urate clearance rather than increased production of uric acid [150] and reduction in the glomerular filtration rate secondary to cyclosporin has been suggested as a mechanism of hyperuricaemia [150, 153]. A study by Marcen *et al* provides evidence that decreased tubular secretion of uric acid could also play a role in urate retention in renal transplant recipients [152].

Uric acid and liver transplantation

Uric acid levels after liver transplant have received little attention and there are only a handful of studies addressing the issue. Transient hyperuricaemia occurring during the first year after transplant has been described in 12 % of patients [154] and increases in serum urate during the first 3 weeks after transplant have been reported [155]. The current knowledge of uric acid levels after liver transplant is limited by small numbers of patients studied and the lack of any studies looking beyond one year after transplant.

Uric acid and cardiovascular disease

Observational studies show that serum uric acid concentrations are higher in patients with established coronary heart disease compared with healthy controls [156]. In a study of 7978 patients with mild to moderate hypertension followed up for a mean of 6.6 years high serum urate at baseline and during treatment was associated with an increased risk of developing cardiovascular disease [157]. Hyperuricaemia persisted despite control of blood pressure. Further evidence of an association between uric acid and cardiovascular disease came from the United States National Health and Nutrition Survey III showing that age-adjusted rates of myocardial infarction and stroke were higher across increasing serum uric acid quartiles among male and female hypertensive patients [158].

Uric acid and hypertension

There is evidence that hyperuricaemia and hypertension are linked. About one quarter of hypertensive patients have hyperuricaemia [159] and asymptomatic hyperuricaemia has been shown to predict subsequent development of hypertension, irrespective of renal function [160]. In the Olivetti Heart Study [161], baseline uric acid was the strongest predictor of new-onset hypertension among 547 middle-aged men, with a 1mg/dl increment in serum uric acid being associated with a 23 % increase in the risk of developing hypertension during a twelve year follow-up period. It is possible that hyperuricaemia contributes to the development of hypertension and thereby increases the risk of cardiovascular disease.

Confounding factors associated with hyperuricaemia

There has been however, controversy regarding the role of uric acid as an independent cardiovascular risk factor. Hyperuricaemia is associated with a number of confounding factors. These include elevated serum triglyceride and cholesterol, elevated or reduced blood glucose, fasting and post carbohydrate plasma insulin concentrations, increased or decreased body mass index and waist-hip ratio [162]. In addition, uric acid levels increase as renal function declines [163]. Distinguishing the effects of an elevated serum uric acid upon cardiovascular risk from the confounding effects of its association with other recognised risk factors has created difficulties in ascertaining the true impact of hyperuricaemia as a risk factor for cardiovascular disease.

Evidence for uric acid as an independent cardiovascular risk factor

Several studies have suggested that uric acid is an independent risk factor for cardiovascular disease [161, 164-166]. A significant association between raised serum uric acid and cardiovascular mortality independent of body mass index,

serum cholesterol concentrations, blood pressure, smoking status, age and diuretic use has been shown in three studies with more than 1000 patients in each study [164-166]. In the MONICA cohort of 1044 male patients followed up for 7 years [166] and Bickels's recent study of 1017 patients with coronary artery disease [165], those with the highest serum urate concentrations had higher age-adjusted risk of myocardial infarction and death from cardiovascular disease. In Alderman's study [157], after adjustment for the variables listed above together with serum creatinine, a difference of 1 standard deviation (0.03 mmol/l) in serum uric acid level was associated with a 22 % difference in total cardiovascular events (stroke, myocardial infarction, fatal and non-fatal events). This effect was greater than that associated with a 1.08 mmol/l difference in serum cholesterol or a 21mmHg difference in systolic blood pressure.

Evidence against uric acid as an independent cardiovascular risk factor

There are several studies that suggest the link between elevated uric acid and cardiovascular disease can be explained by confounding factors. Thus although an increased serum uric acid was shown to be associated with development of fatal coronary heart disease and increased risk of angina in large trials such as the Honolulu Heart Program of 7705 patients [167], this association disappeared once hypertensive patients on thiazide diuretics at baseline were excluded. In the Framingham Heart Study of 6763 patients [168] with median follow-up of 23 years, age-adjusted risk for coronary heart disease, cardiovascular death and all-cause mortality increased with increasing uric acid levels in women but not men. This effect was lost completely after adjustment for blood pressure, total cholesterol, smoking, diabetes mellitus and especially diuretic therapy.

A significant positive association between serum uric acid and risk of coronary heart disease was seen in the British Regional Heart Study of 7688 patients [169], but this relationship lost significance upon adjustment for serum cholesterol and blood pressure. Nevertheless, in the Honolulu study, elevated serum uric acid was associated with a 40 % increase in coronary risk independent of all other

confounding factors in a subgroup of alcohol abstainers. Furthermore, in the Framingham study men with gout, who had the highest uric acid levels, had a 60 % greater incidence of coronary heart disease independent of other risk factors [170]. These latter two observations suggest that even in the studies that have not demonstrated overall an independent effect of serum uric acid upon cardiovascular risk, there are important subgroups for whom elevated uric acid would appear to be implicated as an independent risk factor.

Determination of Hyperuricaemia after Liver Transplant

As there is evidence that hyperuricaemia is linked to increased cardiovascular risk in the general population, it is interesting to speculate whether it also predicts a greater risk of cardiovascular disease after liver transplant. It is first necessary to gain a better understanding of the prevalence of hyperuricaemia after liver transplant. I have undertaken a retrospective analysis of the case notes of patients at Addenbrooke's Hospital. This has enabled me to assess the prevalence of hyperuricaemia and to compare cardiovascular risk in hyperuricaemic patients versus non-hyperuricaemic patients. These findings are discussed in Chapter 6.

Chapter 1. Cardiovascular risk factors after liver transplantation

Introduction

With improved immunosuppressive regimens and better treatment of bacterial and viral infections after liver transplantation, long-term survival has increased and five-year survival rates now exceed 70 % [4, 22]. In recent years older patients and patients with co-morbidity, such as coronary heart disease, hypertension and diabetes mellitus, have been accepted for liver transplantation in greater numbers. Accordingly, transplant physicians have paid increasing attention to the importance of cardiovascular risk factors after transplantation. It is emerging that hypertension, hypercholesterolaemia and weight gain occur commonly [18-22, 24]. Most studies of cardiovascular complications after liver transplant [12, 17-19, 22, 23, 25] record that in the region of 50 % or more of patients are hypertensive after transplant and hypercholesterolaemia develops in as many as 66 % of patients [19]. Increases in body mass index of 3 kg/m^2 during the first two years after transplant are observed, representing an increase of 13 %. Development of cardiovascular risk factors from baseline may be an important factor in long-term survival although few studies have addressed this. I have examined retrospectively the development of hypertension, hyperlipidaemia, weight gain and diabetes mellitus in a cohort of liver transplant recipients followed up for a median of 52 months. Using the Framingham coronary risk scoring equations [46] I calculated the 10-year risk of developing CHD in our patients and determined by how much this risk changes as a consequence of liver transplantation. I have in addition compared the cardiovascular risk scores with a local age and sex matched population. Finally the incidence of myocardial infarction and stroke in this series was compared with expected rates in cohorts from the United Kingdom general population. My aim is that this information will provide a greater understanding of the prevalence of risk factors for CHD and the incidence of cardiovascular disease after liver transplant.

Patients and Methods

The casenotes of all patients aged eighteen years and over who underwent first liver transplantation for both acute or chronic liver disease between 01/01/1994

and 01/01/1999 at Addenbrooke's Hospital and who survived a minimum of six months were reviewed. Patients whose principal place of follow-up was elsewhere, notably Italian patients who were followed up in Italy, were excluded. I recorded the indication for transplant, age at transplant and baseline cardiovascular parameters including blood pressure, weight and body mass index (BMI), serum lipid levels and smoking status.

The three highest outpatient values at any time after transplant for blood pressure, cholesterol, triglyceride and weight were recorded and the average of these three was used in statistical analysis. Hypertension was defined as a blood pressure of 140/90 mmHg or greater on three separate occasions. An automated chemistry analyser (Dade Behring, Deerfield, Illinois, USA) was used to determine serum lipid levels. Hypercholesterolaemia was defined as a serum cholesterol greater than 5.2 mmol/l (200 mg/dl). A serum triglyceride greater than 2.0 mmol/l (176 mg/dl) was indicative of hypertriglyceridaemia. The prevalence of diabetes mellitus was determined by the number of patients requiring treatment with insulin, oral hypoglycaemic agents or diet. Rather than use the pre-transplant weight as the baseline measurement, which is often influenced by the presence of ascites, I chose the weight at the first outpatient visit after transplant with which to compare subsequent post-transplant weight. Body mass index (BMI) was divided into four categories according to World Health Organisation criteria [171]. Thus, underweight was defined as a BMI less than 20 kg/m^2, normal was defined as a BMI between 20-25 kg/m^2, a BMI between 25 and 30 kg/m^2 categorised overweight whilst a BMI above 30 kg/m^2 was considered obese. Cause of death and the incidence of cardiovascular and cerebrovascular events occurring beyond the first post-transplant year were also documented.

Coronary heart disease risk scores

The Framingham coronary risk equations provide an estimate of the 10-year risk of developing CHD [46]. This is based upon knowledge of patients' total cholesterol:high density lipoprotein-cholesterol (HDL) ratio, systolic blood pressure, smoking status, presence or absence of diabetes mellitus, age and

gender. For interpretation and for treatment purposes 10-year risk can be expressed as being < 15 %, 15-30 % and > 30 %. I used the Framingham equations to estimate coronary risk in 137 patients for whom *complete* data were available before and after transplant. The data needed to calculate post-transplant coronary risk were collected at a mean of 12 months (range 11-15 months) after transplant from a single clinic visit. In the remaining 44 patients there was incomplete documentation of pre-transplant fasting serum cholesterol and HDL-cholesterol and this prevented these patients from having their coronary risk scores calculated accurately.

A database of 1226 patients has been established for the population of Ely, a rural town in East Anglia close to Addenbrooke's Hospital. The Ely cohort was selected by chance and the response rate was 74 % [172]. This population provides an excellent comparison with our liver transplant recipients, the majority of whom are from East Anglia and surrounding areas. Data from the Ely cohort were used to predict coronary risk scores so as to compare with data from the liver transplant recipients.

Cardiovascular events

The incidence of myocardial infarct (MI) and stroke occurring after the first transplant year were compared with expected age and sex matched incidence rates in the general UK population. Events that occurred during the first year have been documented but not included in this analysis. The reason for excluding these is that there are several factors unrelated to cardiovascular disease in the first months after transplant that can contribute to cardiovascular events. What I have attempted to do is assess the impact of post-transplant hypertension and hypercholesterolaemia upon subsequent occurrence of MI and stroke. One year after transplant the majority of cases of hypertension and hypercholesterolaemia were evident and this therefore serves as a suitable baseline time point.

Comparison populations

I used data on the annual incidence of MI in a population of 568, 800 residents of Oxfordshire [173] to compare with the incidence in the transplant recipients. The Oxfordshire study was conducted in 1994/5 using prospective and retrospective case records. This is henceforward referred to as the Oxford cohort. Matching for age and sex, I calculated the number of MI's that would be expected in the general population given the same length of follow-up as the transplant patients. The same approach was used to compare the incidence of stroke after transplant with that of the non-transplant population. Data from community based stroke registers in London which had previously been used to ascertain the incidence of stroke between 1995 and 1997 [174] formed the basis of the comparative general population, which is referred to as the London cohort.

Finally the prevalence of hypertension and a cholesterol:HDL ratio ≥ 5 at one year after transplant were compared with age and sex matched figures for a general United Kingdom population. The Health Survey for England 1994 provided prevalence data on hypertension and cholesterol:HDL ratio in a sociodemographically representative sample of the English population [175, 176]. This is subsequently referred to as the English cohort. Hypertension in that study was defined as a blood pressure of 160/95 mmHg or greater and therefore I adjusted the blood pressure threshold for liver transplant patients accordingly to compare the two populations.

Statistical Analysis

The SPSS software statistical package was used. Student's t-test, Fisher's exact test, Mann-Whitney U-test and Chi-square tests were used as appropriate. Data are shown as mean ± standard error of the mean unless otherwise stated.

Results

Patient characteristics

A total of 181 patients met the criteria for analysis. The commonest indications for transplantation were primary biliary cirrhosis, alcohol related liver disease and chronic viral hepatitis B or C with cirrhosis (Table 1.1). Mean age at transplant was 53 years (Range 18 –68 years). The median length of follow-up was 54 months (Range 6-90 months). 92 patients were male and 89 female. 162 patients had one transplant, 13 had two transplants and 6 had three transplants.

Table 1.1 Patient Demographics

	All Patients n = 181	Cyclosporin n = 116	Tacrolimus n= 59	Rapamycin n = 6
Median Age (range) in years	53 (18-68)	52 (18-68)	54 (18-67)	57.5 (38-67)
Sex (M:F)	92:89	57:59	33:26	2:4
Indication for Transplant (%):				
Primary biliary cirrhosis	37 (20)	27 (23)	9 (15)	1 (17)
Alcoholic liver disease	32 (18)	21 (18)	11 (19)	1 (17)
Hepatitis B or C cirrhosis	30 (17)	16 (14)	12 (20)	2 (33)
Acute liver failure	21 (12)	14 (12)	7 (12)	*
Primary sclerosing cholangitis	18 (10)	12 (10)	6 (10)	*
Autoimmune liver disease	10 (6)	7 (6)	2 (3)	1 (17)
Others	33 (18)	19 (16)	12 (20)	1 (17)

Triple immunosuppression with a calcineurin inhibitor, prednisolone and azathioprine was used in the majority of patients. 116 (64 %) patients received cyclosporin, tacrolimus was used in 59 (33 %) patients whilst rapamycin was used in the remaining 6 (3 %). 44 patients switched immunosuppression during the course of the study period. Whichever immunosuppressant the patient was taking for the longest period was used to denote whether a patients' immunosuppression was with cyclosporin or tacrolimus. The cardiovascular data was then collected only for the period of time during which the patient was taking that agent. 40 patients switched from cyclosporin to tacrolimus, 2 switched from tacrolimus to cyclosporin and 2 from cyclosporin to rapamycin. 15 of the 40 patients converted to tacrolimus were classified as being on tacrolimus as main immunosuppression: the median time to conversion was 4 months. The remaining 25 patients were classified as being on cyclosporin and the median time to conversion was 33 months.

169 (93 %) patients received prednisolone. Patients treated with rapamycin and a further 6 who received Campath, a monoclonal antibody, did not get corticosteroids as primary immunosuppression. 44 out of the 174 patients surviving greater than one year were taking prednisolone or hydrocortisone for greater than 12 months. Of these, 10 had a primary diagnosis of autoimmune liver disease and 24 were on a dose of 5mg per day or less. Azathioprine was withdrawn at one year in the majority of patients. Acute cellular rejection episodes were treated with high-dose intravenous steroids. Mycophenolate mofetil was used in some cases of chronic, recurrent acute or unresolved acute rejection.

Cardiovascular Risk Factors

Cardiovascular risk factors were analysed for the whole population as well as by type of immunosuppression. Too few patients were treated with rapamycin for meaningful comparisons to be made with other immunosuppression. The peak values for the cardiovascular parameters occurred at any time from 2 months after transplant to the limits of the period of follow-up. The peak values for all cardiovascular parameters were scattered throughout the range of follow-up with

no discernible pattern. The exception was weight gain which tended to be maximal by 2 years.

Hypertension

Prior to transplantation 10 patients (5.5 %) were hypertensive or had a history of treated hypertension. These ten continued to require antihypertensive medication after transplant. Following transplantation 130 additional patients (71.8 %) developed hypertension ($P<0.001$). Hypertension developed in significantly more patients on cyclosporin compared to those treated with tacrolimus (Table 1.2). The commonest drugs administered were, in descending order of frequency, beta-blockers, calcium channel blockers, alpha-blockers, angiotensin converting enzyme inhibitors and diuretics. 30 % required more than one drug to control blood pressure.

Using a definition of hypertension of 160/95 mmHg or greater, to match that used in the English cohort, the prevalence of hypertension at a mean of 12 months after transplant was 54 %. The English cohort would be expected to have a prevalence of hypertension of 21.7 %. Younger transplant patients in particular had far higher rates of hypertension than their age matched counterparts in the English cohort: prevalence of hypertension in the under 45 age group was 37 % and 3 % for the transplant and English cohort patients respectively.

Table 1.2 Cardiovascular Risk Factors Before and After Liver Transplant

	Pre-transplant			Post-transplant		
	CyA	FK506	Rapa	CyA	FK506	Rapa
Hypertension (%) (n=181)	4.3	8.5	0	82.6 ^⊥	67.2 ^	83.3
Hypercholesterolaemia (%) (n=137)	15.9	16.1	20	65.7 ^	51.2 ^	60
Hypertriglyceridaemia (%) (n=137)	7.5	14	0	31.3 ^	48.8 ^	80
BMI > 25 kg/m^2 (n=181)	32.8	23.7	16.7	60.3 ^	52.5 ^	50
BMI > 30 kg/m^2 (n=181)	3.4	1.7	0	29.3	15.3	16.7
Diabetes Mellitus (%) (n=181)	7.8	10.2	16.7	12.2	15.3	16.7

CyA = cyclosporin. FK506 = tacrolimus. Rapa = rapamycin

^ denotes a significant difference between pre-and post-transplantation (P<0.05)

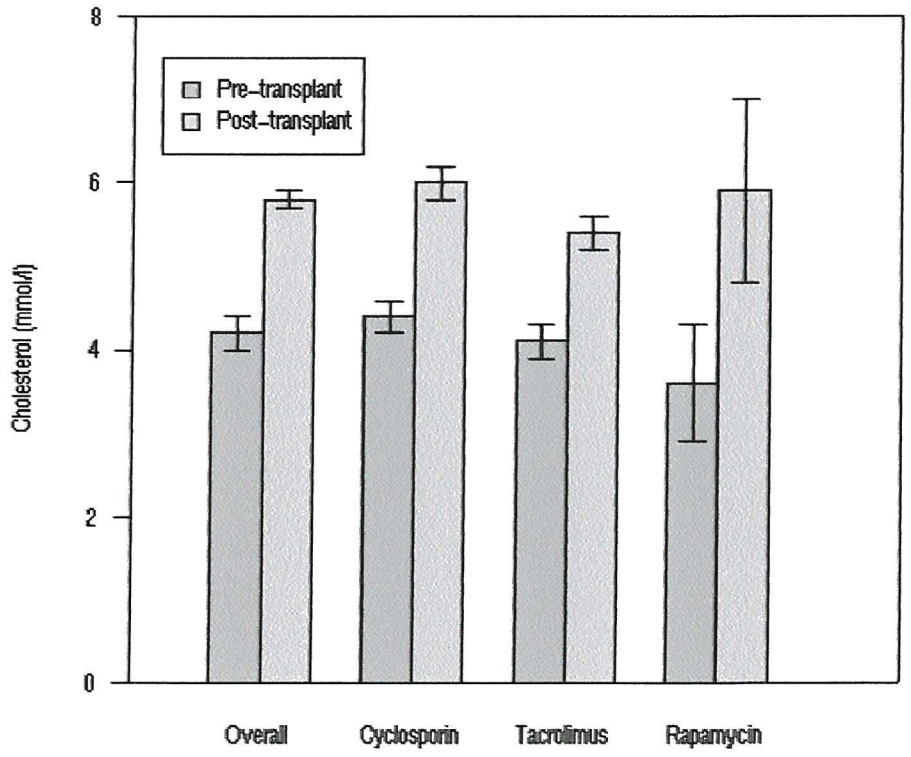
⊥ denotes a significant difference between cyclosporin and tacrolimus

Serum Cholesterol

Follow-up data for serum cholesterol were available in all bar one patient. 112 patients (62.2 %) had elevated serum cholesterol after transplant. Pre and post transplant serum cholesterol levels were available in 137 patients: 22 (16.1 %) had hypercholesterolaemia before transplant compared with 82 (59.9 %) after transplant (P<0.001). Two-thirds of patients with hypercholesterolaemia before transplant had cholestatic liver disease. Patients treated with cyclosporin were no more likely to have hypercholesterolaemia after transplant than those on tacrolimus (65.7 % versus 51.2 %) (Table 1.2).

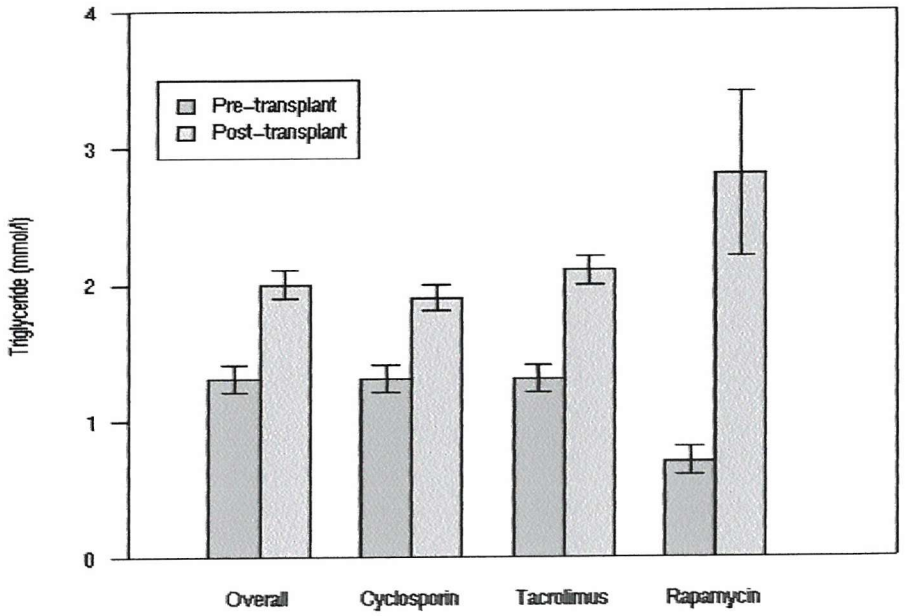
Serum cholesterol levels pre and post transplant are shown in Figure 1. Mean serum cholesterol increased after transplant from 4.2 ± 0.2 to 5.8 ± 0.1 mmol/l ($P<0.001$). Serum cholesterol showed a significant increase after transplant when cyclosporin, tacrolimus and rapamycin treated patients were analysed separately (Figure 1). There was no difference in the increase in serum cholesterol observed for cyclosporin compared with that for tacrolimus. The prevalence at one year after transplant of a cholesterol: HDL ratio >5 was 40 % after liver transplant. This compares with a figure of 28.9 % that would be expected from the English cohort.

Figure 1. Mean serum cholesterol pre and post transplant



All changes pre and post transplant are significant ($P<0.05$)

Figure 2. Mean serum triglyceride pre and post transplant



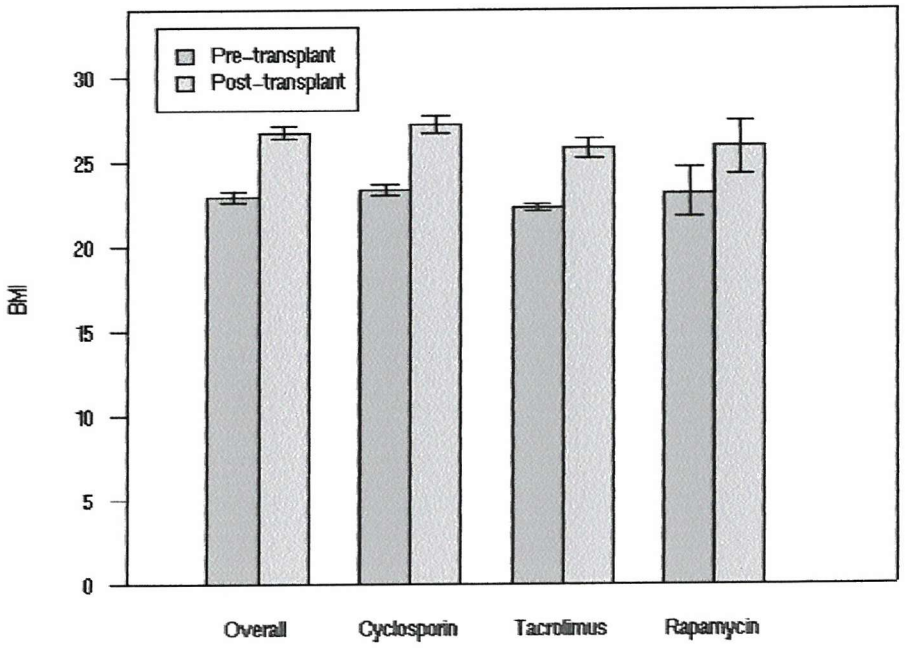


Figure 3. Mean BMI pre and post transplant

All changes pre and post transplant are significant ($P<0.05$)

Serum Triglyceride

Serum triglyceride levels were available in 180 patients. 74 (41.1 %) patients had high levels of serum triglyceride after transplant. Pre and post-transplant data were available in 137 patients: 13 (9.5 %) patients had hypertriglyceridaemia before transplant compared with 55 (40.1 %) after transplant (P<0.001). Tacrolimus treated patients were no more likely to have hypertriglyceridaemia after transplant than those on cyclosporin (Table 1.2). Mean serum triglyceride increased after transplant from 1.3 ± 0.1 to 2.0 ± 0.1 mmol/l (P<0.001). The effect of differing immunosuppression upon serum triglyceride is shown in Figure 2.

Body mass index

BMI increased after transplant from 23.0 kg/m^2 to 26.8 kg/m^2 (P<0.001) (Figure 3). At the first outpatient visit, 29.3% of patients had a BMI > 25 kg/m^2 compared with 57.5% after transplant. Thus the prevalence of being overweight rose by 28.2 %. The increase observed in BMI was significant for all types of immunosuppression (Figure 3). There was no difference in the change in BMI between cyclosporin and tacrolimus. A higher proportion of patients developed a BMI >30 kg/m^2 with cyclosporin compared to tacrolimus, although statistical significance was not reached (Table 1.2). Only 8 patients lost weight during follow-up. All of these 8 had low or normal BMI throughout.

Diabetes mellitus

16 patients had a pre-transplant diagnosis of diabetes mellitus. Following transplant a further 8 patients developed diabetes mellitus, 4 of whom became insulin dependent and 4 required oral hypoglycaemic agents. Two of these patients were able to discontinue treatment within one year. Four out of seven non-insulin dependent diabetics required insulin long term after transplant. The mean time on steroids for those who developed diabetes mellitus after transplantation was 12.1 ± 4.7 months compared with 12.5 ± 1.3 months for those without diabetes mellitus (P=0.91).

Smoking

At transplantation 18 % of patients were smokers, 23 % were ex-smokers and 59 % had never smoked. Of the current smokers, 50 % gave up within 1 year.

Deaths

3 patients died between 6 and 12 months after transplant. Causes of death for all patients are shown in Table 1.3. There were 16 deaths during the follow-up period. 7 deaths were due to a recurrence of the original liver disease and 3 were due to malignancy. One 62 year old male patient with a pre-transplant history of hypertension died from a subarachnoid haemorrhage 3.7 years after transplant. Compared to the Ely cohort those in the liver transplant group have a relative risk for death of 5.4 (95% CI, 2.9-10.2). Adjusting for age, sex, blood pressure, total and HDL- cholesterol, triglyceride, BMI and smoking, the relative risk for the transplant group was 3.8 (95% CI 1.6-9.1).

Table 1.3 Causes of death in patients surviving greater than 6 months

Cause of Death	Number of Patients
Recurrent cholangiocarcinoma	2
Recurrent hepatitis C cirrhosis	3
Recurrent primary sclerosing cholangitis	1
Recurrent hepatocellular carcinoma	1
Carcinoma of duodenum	1
Carcinoma of bronchus	1
Carcinoma of prostate	1
Non-Hodgkin's lymphoma	1
Sub-arachnoid haemorrhage	1
Septicaemia	1
Liver failure	1
Multi-organ failure	1
Bronchopneumonia	1

Cardiovascular events

Cardiac events are summarised in Table 1.4. Seven patients each had one event. One patient sustained a non-fatal MI 42 months after transplant. This patient had a history of hypertension prior to transplant and had also developed high serum cholesterol after transplant. This compares to an expected number of events of 1.82 in the matched Oxford cohort with an incidence ratio of 0.55 (95% CI: 0.01-3.06).

2 patients sustained their first stroke after the first transplant year. Each had a cerebellar infarct. Both had developed hypercholesterolaemia and one hypertension by the time of their strokes. The age and sex matched London cohort would be expected to have experienced 1.38 first strokes with an incidence ratio for first stroke of 1.45 (95% CI: 0.18-5.22). 3 additional patients had strokes during the first year; 1 after one month, 1 at three months and 1 at eight months after transplant. These occurred prior to development of any identifiable cardiovascular risk factors. No patient has any residual neurological disability.

Table 1.4 Non-fatal cardiovascular events beyond 1 year after transplant

Number of Patients	Cardiovascular Event	Interval to onset (months)
1	Myocardial infarct	42
1	New-onset angina	38
1	Atrial fibrillation	19
2	Heart failure	20
2	Stroke	50 (range 16-84)

Coronary Heart Disease risk

Cardiovascular parameters of the Ely cohort and the transplant recipients at one year post transplant are listed in Table 1.5. The mean 10-year predicted probability or risk of developing CHD in 1027 patients of the Ely cohort for whom complete data were available together with predicted probabilities pre- and post-transplant are shown in Table 1.6. The mean 10-year predicted risk of CHD, expressed as a percentage, increases after transplant, being 11.5 % compared with 6.9 % pre-transplant ($P<0.001$). It is also greater after transplant than that of the Ely cohort ($P<0.0001$). Subdivision of patients into the three different risk score groups is shown in Table 1.7. There is a markedly significant shift of patients into higher risk groups following transplant. There was no difference in the estimated CHD risk when comparing cyclosporin and tacrolimus. The estimated 10-year risk for cyclosporin treated patients, based upon data one year after transplant, is 12.1 % whilst that for tacrolimus treated patients is 10.7 % (P=NS).

Table 1.5 Comparison of Ely cohort and Liver transplant recipients at one year post-transplant

		Ely	Liver Transplant
		Mean	Mean
Men	Age (years)	59.6 ± 0.5	58.8 ± 1.0
	BMI (kg/m^2)	26.2 ± 0.2	28.5 ± 0.6
	Cholesterol (mmol/l)	6.4 ± 0.1	5.5 ± 0.2
	Systolic BP (mmHg)	131 ± 0.7	155.1 ± 2.2
	Diastolic BP (mmHg)	80.5 ± 0.5	91 ± 1.5
Women	Age (years)	59.5 ± 0.4	60.2 ± 1.0
	BMI (kg/m^2)	25.9 ± 0.2	27.2 ± 0.8
	Cholesterol (mmol/l)	6.4 ± 0.1	5.8 ± 0.2
	Systolic BP (mmHg)	126.7 ± 0.6	157.5 ± 2.8
	Diastolic BP (mmHg)	76.7 ± 0.4	91.9 ± 1.5

Table 1.6 Comparison of 10-year predicted probability of CHD in different cohorts

Cohort	n	Mean risk (%)	95 % CI lower (%)	95 % CI upper (%)
Pre-transplant	137	6.9	6.5	7.4
Post-transplant	137	11.5	10.1	13.0
Ely cohort	1027	7.0	6.7	7.4

95 % CI: 95 % confidence intervals

Table 1.7 Comparison of the percentage of patients in different CHD risk bands in the different cohorts

Cohort	10-year predicted probability for CHD			
	< 15 %	15 - ≤ 30 %	> 30 %	Total
Ely	77.8 %	20.0 %	2.2 %	100 %
Pre Liver Transplant	85.3%	14.7%	0	100 %
Post Liver Transplant	59.9 %	34.3 %	5.8 %	100 %

Pearson χ^2 test: $p < 0.001$ between pre and post transplant

Pearson χ^2 test: $p < 0.0001$ between Ely and post transplant

High Triglceride and low HDL-Cholesterol

There is evidence that a high serum triglyceride in combination with a low HDL-cholesterol is a predictor of ischaemic heart disease [177]. A high triglyceride was defined as above 1.69 mmol/l whilst a low HDL-cholesterol was below 1.03 mmol/l for men and below 1.29 mmol/l for women, according to the Adult Treatment Panel III (ATP III) criteria in the diagnosis of the metabolic syndrome

(Table 1.8). Using these definitions, 36 % of the total transplant patients had both a high triglyceride and low HDL-cholesterol. 32 % of patients with hypertension had this lipid abnormality.

Table 1.8. ATP III criteria for identification of the metabolic syndrome [178]

Abdominal obesity (waist circumference)	
Men	> 102 cm (40 in)
Women	> 88 cm (35 in)
Triglycerides	≥ 150 mg/dl
HDL cholesterol	
Men	< 40 mg/dl
Women	< 50 mg/dl
Blood pressure	≥ 130/ ≥ 85 mmHg
Fasting glucose	≥ 110 mg/dl

Diagnosis of the metabolic syndrome is made when 3 or more of the risk determinants shown are present.

Discussion

This study has shown that development of risk factors for cardiovascular disease, including hypertension and hypercholesterolaemia, is common following liver transplant. Utilising these data I have calculated that the predicted 10-year risk of developing CHD is much higher after transplant than before and exceeds that in age/sex matched general populations. However, the observed burden of cardiovascular disease, measured by cardiovascular mortality and the incidence of myocardial infarction and stroke, during 4.5 years of follow-up is low.

The development of cardiovascular risk factors and their contribution to accelerated cardiovascular disease after liver transplant is an important issue in the long-term management of transplant patients. On current available evidence however, it is questionable whether the development of hypertension and hypercholesterolaemia is accompanied by an equivalent increased incidence of cardiovascular disease. Many studies attest to the high frequency of cardiovascular risk factors but little has been reported on the development of cardiovascular *disease* after transplant and few studies have compared the incidence of MI and stroke with expected incidence rates from a comparable non-transplant population.

In common with other reported series hypertension, hypercholesterolaemia and weight gain occurred frequently after transplant. Hypertension was the commonest risk factor observed, affecting 77 % of patients at some point after transplant. This figure compares with prevalence rates from 36 to 82 % that have been reported in other studies [12, 16-23, 25, 33]. Hypertension was more common in transplant recipients compared to an age and sex matched general population and was observed in both young and older transplant recipients. Serum cholesterol increased significantly after transplant. Hypercholesterolaemia is well documented after liver transplantation [12, 20, 23, 30, 32-34] and develops in as many as 66.2 % of patients [19].

Obesity is a problem frequently encountered in the long term care of liver transplant recipients and in some patients weight gain is dramatic. Using the first outpatient BMI as a baseline, which typically is two to three weeks after transplant, I showed that BMI increased significantly after transplantation. The increase in BMI I observed is similar to that reported in other studies [20, 22, 39]. 39 patients (21.5 %) became obese (BMI > 30 kg/m^2) during follow-up whilst only 9 patients lost weight, all of whom had normal BMI throughout. Evidence suggests that the majority of weight gain occurs in the first post-transplant year, with only a slight increase during the second year and very little thereafter [19, 39, 40]. In this series weight gain persisted beyond 2 years in 96 %.

Several authors have observed that liver transplant recipients have an increased prevalence of diabetes mellitus [22, 23, 42]. I found that just 4.4 % of patients *became* diabetic with diabetes persisting beyond the first post-transplant year in 3 %. The incidence of diabetes after transplant in the USA ranges from 12 to 18 % [15, 22, 23]. The lower incidence of diabetes in our patients may reflect our policy of early withdrawal of corticosteroids. Corticosteroid withdrawal has been shown to improve diabetic control and may allow discontinuation of treatment [38]. A similar prevalence of diabetes mellitus to our own was reported from Birmingham, UK, [12] where prednisolone is typically withdrawn at three months after transplant.

Immunosuppressant drugs, including calcineurin inhibitors and corticosteroids, are implicated in the causation of hypertension, dyslipidaemia and weight gain after liver transplant [22, 29, 33, 107]. Serum lipid levels are also influenced by post-transplant weight gain, diabetes mellitus, diet and renal dysfunction [24]. There is evidence that transplant recipients receiving tacrolimus develop less cardiovascular risk factors than those receiving cyclosporin. In common with other studies I found a higher prevalence of hypertension in patients on cyclosporin compared to tacrolimus [16-20]. Patients on cyclosporin had a trend to higher serum cholesterol and greater weight gain echoing the findings of previous studies [12, 20, 30, 32, 34, 116, 179]. There was no difference between immunosuppression with respect to development of diabetes mellitus. Recent studies have also shown that diabetes mellitus develops equally with cyclosporin and tacrolimus [17, 20, 35, 179].

Major risk factors for cardiovascular disease such as hypertension and hypercholesterolaemia are undoubtedly common after liver transplant but it is not so clear whether transplant recipients are, as a result of development of such risk factors, at greater risk for development of coronary or cerebrovascular disease. The use of cardiovascular risk scores has recently been applied to liver transplant recipients [12]. I have used the same scoring system to compare the change in estimated risk of CHD before and after transplant and to relate the risk after

transplant with the risk in a matched local non-transplant population. This study is the first to demonstrate the marked increase in 10-year risk of developing CHD that occurs after liver transplant, with the estimated risk increasing from 6.9 % to 11.5 %. This is significantly higher than the estimated risk score for the Ely cohort of non-transplant patients. The majority of patients with elevated serum cholesterol pre-transplant had cholestatic liver disease and these patients are not thought to be at increased cardiac risk from their dyslipidaemia. The inclusion of these patients probably overestimates the overall cardiovascular risk pre-transplant such that in reality the change in CHD risk after transplant is perhaps greater than I have presented here.

The risk score of 11.5 % is also higher than that published from Birmingham, UK [12]. One possible explanation for the higher risk in our patients is that I looked at patients at one year after transplant when the majority have already developed hypertension and hypercholesterolaemia, the two principle factors determining the increase in coronary risk. The Birmingham cohort included patients from 0.1 years after transplant and this would probably underestimate the true coronary risk. To illustrate this the prevalence of systolic hypertension (Systolic blood pressure $\geq$ 140 mmHg) at 1 month in our patients was 35 % compared to 76 % at 12 months. Mean systolic blood pressure was 138 mmHg at 1 month and 157 mmHg at 12 months ($P<0.001$).

If we assume that patients require treatment aimed at reducing coronary risk when the risk is 15 % or greater, 40.1 % of patients after transplant should benefit from intervention aimed at primary prevention. This is a much higher percentage than the local non-transplant population. Regular blood pressure monitoring is important and efforts should be made to reduce systolic blood pressure to below 140 mmHg. Patients with an elevated serum cholesterol who have an estimated CHD risk score of 15 % or greater should be treated with a statin for primary prevention. A move to greater use of tacrolimus over cyclosporin may be beneficial in view of the lower prevalence of hypertension with tacrolimus although it is interesting that there was no difference in the estimated 10-year risk score between cyclosporin and tacrolimus treated patients. Shorter duration of

corticosteroid use after transplant may also reduce risk. Other risk factors for cardiovascular disease such as hyperuricaemia and hyperhomocysteinaemia may have a role to play as possible markers of cardiovascular risk in liver transplant recipients. Elevated serum uric acid occurs in 47 % of patients after liver transplant [180] (discussed in detail in Chapter 6) and increased serum homocysteine levels have also been documented [181], although their relationship to development of CHD in transplant recipients has not been shown.

Weight loss may become an increasingly important area to tackle in reducing cardiovascular risk. Liver transplant patients as a whole fulfil many of the criteria for the metabolic syndrome which is closely linked to insulin resistance [178] (Table 9). Insulin resistance is associated with heightened cardiovascular risk [182]. The majority of insulin resistant/hyperinsulinaemic patients have a fasting glucose concentration < 110 mg/dl and the combination of hypertension with a high serum triglyceride and low HDL-cholesterol is strongly suggestive of insulin resistance [183, 184]. Hypertensive patients with the highest ratio of triglyceride to HDL-cholesterol have the greatest CHD risk [185].

One-third of the liver transplant patients probably have insulin resistance, on the basis of hypertension and dyslipidaemia, although measurements of fasting insulin to support this assumption have not been performed. Interestingly hyperuricaemia which is present in almost half the patients after liver transplant [180] is associated with insulin resistance. It has been suggested that a substantial part of the CHD risk associated with hypertension in insulin resistant individuals is caused by other features of insulin resistance and treatment directed solely at blood pressure lowering may not reduce the CHD risk as much as is hoped [186, 187]. For the 32 % of liver transplant recipients who are insulin resistant, weight loss and increased physical activity could have as important a role to play as specific therapies targeting hypertension and hypercholesterolaemia. This is an area for further research.

Few studies have addressed the issue of long-term cardiovascular complications after liver transplant. Most have looked at deaths due to cardiovascular and in some cases cerebrovascular disease but data on non-fatal myocardial infarction or cerebrovascular accident are lacking. In a study of patients surviving at least one year after transplant, 13.8 % of subsequent deaths were from cardiovascular disease, of which 60 % were due to MI [15]. The mean time to death from cardiovascular disease was 55.6 months. Stroke accounted for 3.8 % of deaths. Asfar et al [188] report that 14.3 % of deaths beyond the first transplant year were from cardiovascular disease, 5.7 % from MI, and mean time to cardiac death was 3.9 years. Rabkin et al [14] report that 7.5 % of deaths in 40 patients who died after the first post-transplant year were from MI. A study of patients surviving more than three years after transplant reported that 15.8 % of deaths were due to MI with 21 % of deaths overall due to cardiovascular disease [13]. The majority of these patients had pre-existing coronary artery disease or risk factors before transplant. In all these studies the mortality from cardiovascular disease amounts to between 1.5 and 3 % of the total number of patients alive at one year [13-15]. It is not clear from the above studies whether these death rates are different from those expected in the general population.

The recent paper from Johnstone et al [12] compared cardiovascular deaths in transplant recipients with an age matched general population, albeit not local to the transplant centre. They showed a relative risk of death from cardiovascular disease of 2.56 compared to an age-matched non-transplant population. This was based on data acquired from 1312 transplant recipients over 4962 person-years of observation. Deaths due to ischaemic heart disease occurred at a median of 27 months post-transplant. Furthermore the relative risk of ischaemic cardiac events was 3.07 and the median times to MI and stroke were 32 and 34 months respectively. Sheiner et al [22], in a study of 96 patients surviving 5 or more years after transplant, also found a significantly higher prevalence of hypertension in transplant recipients compared to United States population figures. 6.1 % of deaths in that study were due to cardiac disease and overall 2.2 % died from cardiovascular disease. Interestingly the prevalence of heart disease including MI

after transplant was no different from that of an age and sex matched general population.

Most studies so far published suggest that cardiovascular disease is an important determinant of long term survival after transplant. In the current study however, not one patient died from MI or stroke. This is in contrast to the cardiovascular mortality evident in other studies [12-15, 188] and in stark contrast to renal transplantation in which cardiovascular disease accounts for up to 50 % of deaths, although pre-transplant cardiovascular disease is much more common in these patients [189]. It is noteworthy that the relative risk of dying in the Ely cohort only fell from 5.4 to 3.8 after adjustment for cardiovascular risk factors.

When assessing the long term outlook for transplant recipients with regard to potential cardiovascular mortality it is important to be able to relate the incidence of cardiovascular events in transplant recipients with that of the general population. I have compared the incidence of MI and stroke after transplant with that from general population studies in Oxford and London, United Kingdom. Whilst this is not ideal, as it would be preferable to have incidence rates of cardiovascular events from the local Ely population, the studies I chose to compare incidence rates were nevertheless from areas that a number of the transplant recipients come from. However, because of the small number of observed cardiovascular events and the associated wide confidence intervals it is difficult to draw statistically meaningful conclusions on the incidence of MI and stroke after transplant compared to non-transplant populations. Nevertheless, I feel it is an important clinical observation that in spite of a high prevalence of risk factors for CHD and stroke, only a small number of patients to date have had a cardiovascular event.

Why should cardiovascular mortality after transplant be lower in this study than others? One possible explanation is that the prevalence of diabetes mellitus is lower in these patients compared to other series. Secondly, the presence of CHD or risk factors for CHD prior to transplant has been implicated in the aetiology of post-transplant CHD [13] and to our knowledge very few of the patients studied

here had pre-existing CHD. Finally, tacrolimus is associated with fewer cardiovascular events and lower mortality from CHD after transplant than cyclosporin [190]. It is possible that proportionately more patients in our study were treated with tacrolimus than in earlier studies that reported a greater incidence of CHD [12, 15, 188].

Two further points deserve consideration. Firstly, the vast majority of patients having a liver transplant are pre-selected to be at low risk of cardiac disease at the time of transplant. It should not be surprising that it takes several years for those patients who subsequently develop risk factors such as hypertension to suffer a cardiovascular event as a result of de novo development of risk factors. Secondly, there is evidence that calcineurin, via a calcium-calmodulin-calcineurin-nuclear factor of activated T-cells (NFAT) signalling pathway, has a pivotal role in development of cardiac hypertrophy [191]. It is intriguing to speculate that calcineurin inhibitors could limit the cardiac hypertrophy that would be expected to develop in the face of post-transplant hypertension. In support of this are animal studies which have shown that following aortic constriction tacrolimus and cyclosporin partially prevent the expected consequent left ventricular hypertrophy [192, 193]. Against the idea of such a protective role are further animal studies showing no difference in development of left ventricular hypertrophy with cyclosporin and tacrolimus versus untreated animals [194, 195] and the recently reported autopsy evidence of asymmetric cardiac hypertrophy in both cyclosporin and tacrolimus treated liver transplant recipients [196]. A drawback of post-mortem studies is that they may select patients who are predisposed to cardiac disease that could be unrelated to the immunosuppression. For the present, it is an interesting notion that calcineurin inhibitors could offer some cardioprotective element in the face of the systemic vasoconstriction that accompanies their use.

Studies comparing transplant patients with matched non-transplant populations with longer periods of follow-up, ideally beyond 10 years, are required if the true extent of the burden of cardiovascular disease after transplant is to be established. This will need to be offset against the natural increase in cardiac morbidity and mortality with advancing age.

Chapter 2. Investigations into the mechanisms of hypertension

Introduction

Hypertension is the commonest cardiovascular complication that occurs after liver transplantation with several studies reporting a prevalence approaching 50 % or greater [18-20, 22, 23]. Hypertension is manifest frequently within 6 months of transplant [18]. Systemic vasoconstriction probably underlies post-transplant hypertension but the specific mechanisms contributing to this remain unresolved. Activation of the renin-angiotensin system may be implicated but the relationship between increases in plasma renin and development of hypertension is unclear. Increased plasma renin has been shown in transplant recipients with established hypertension 13 months after transplant [58] but low levels of renin have been reported during the first four months after transplant even in those who develop hypertension subsequently [27, 28, 55]. Endothelin-1 is a potent vasoconstrictor. Increases in plasma ET-1 levels have been observed in the first few days after liver transplant in association with increasing mean arterial blood pressure [71]. Urinary endothelin levels increase within a few months of transplant. A rise in plasma endothelin during the first weeks or months after transplant could have an impact upon the early increases in blood pressure that are frequently observed after transplant. Finally arterial stiffness is causally linked with hypertension and is acknowledged as the cause of isolated systolic hypertension [78, 79]. An increase in arterial stiffness after liver transplant could therefore contribute to development of hypertension. Interestingly it has recently been shown that an increase in ET-1 leads to a rise in pulse wave velocity and hence arterial stiffness [97].

The purpose of this study was to determine plasma levels of renin, aldosterone and endothelin-1 and to use pulse wave analysis to determine arterial stiffness [84] before and after liver transplant, in order to assess the contribution of these to the development of hypertension during the first 6 months after liver transplant.

Methods

Thirty-two consecutive patients on the waiting list for liver transplant were recruited into the study. Patients were enrolled into the study within one week of going on the waiting list. Patients with diabetes mellitus, past or current hypertension or clinical evidence of cardiovascular disease were excluded, as were patients taking beta-adrenoceptor antagonists for portal hypertension. Patients having a re-transplant or multiorgan transplant were also excluded. Diabetic patients were excluded because arterial stiffness is enhanced in diabetes mellitus [197] and because of the probability that ACE-inhibitors would be prescribed. Approval for the study was obtained from the local research ethics committee and informed written consent was given by each patient.

Renin and aldosterone

Blood samples were taken for renin and aldosterone estimation. After a 30 minute period of supine rest, a 5 ml sample of blood was collected into a lithium-heparin tube and centrifuged at 2000 rpm for 5 minutes within one hour of sampling. Plasma was then stored at minus 70 ^{o} Celsius until analysis. Shortly before analysis, the samples were thawed rapidly and maintained at room temperature. Renin was measured in 200 microlitre plasma with a commercially available immunoradiometric assay kit (Nichols Institute, CA, USA) following methods described previously [198]. Aldosterone was determined using a commercial radioimmunoassay kit (Diagnostic Products corporation, CA, USA) following the principles proposed by Kubasik et al [199]. The biochemistry department of Addenbrooke's Hospital performed the assays for me.

Endothelin-1

Samples for endothelin-1 were collected after patients had been lying down for 30 minutes. 5 ml samples were collected and placed immediately into lithium heparin tubes on ice. Within one hour the samples were centrifuged for 5 minutes at 2000 rpm and the plasma stored at minus 70 ^{o} Celsius until analysis.

Assay of endothelin in plasma:

Amprep C2 minicolumns (Amersham Biosciences, Little Chalfont, UK) were pre-conditioned with 2 ml methanol followed by 2 ml H20. Plasma samples (1.8 ml) were acidified by addition of 0.25 ml 2M HCl, centrifuged, and the supernatant applied to the pre-conditioned columns. The columns were then washed twice with 2.5 ml 0.1 % trifluoroacetic acid (TFA, Sigma, Gillingham, UK). Endothelins were eluted with 2 ml of 80 % methanol/0.1 % TFA/19.9 % H_2O and the eluate evaporated in an evacuated centrifuge (SpeedVac, Labsystems, Basingstoke, UK).

The eluate was reconstituted in 250 µl assay buffer and 100 µl were assayed in duplicate using a double-antibody enzyme-linked immunosorbent assay (ELISA) for endothelin (Endothelin-1 Biotrak ELISA System, Amersham Biosciences, Little Chalfont, UK) according to the manufacturers' instructions. Briefly, standard and unknown samples were added to 96 well plates coated with an antibody directed to the C-terminal hexapeptide of ET-1. After overnight incubation at 4 ^{0}C the plates were washed and an antibody directed to the N-terminal region of ET-1 conjugated to horseradish peroxidase was added. After 2 hours incubation at room temperature, the plates were washed and tetramethyl benzidine was added as a substrate. The colour reaction was stopped by addition of 1M H_2SO_4 and the resultant yellow colour read at 450 nm. Unknowns were compared to a standard curve of authentic endothelin-1 and the results expressed as pmol/L, corrected for the volume of plasma extracted.

Pulse wave analysis

Augmentation index (AI) and ascending aortic pressure were determined by pulse wave analysis (PWA) using the SphygmoCor apparatus (SCOR; PWV Medical, Sydney, Australia). A high fidelity micromanometer (SPC-301; Millar Instruments, Texas, USA) was used to flatten the radial artery at the wrist in the non-dominant hand using gentle pressure. Data were collected directly into a portable computer. After 20 sequential waveforms had been acquired, the

integrated software was used to generate an averaged peripheral waveform and corresponding central pressure waveform. The peripheral waveform was transformed into the central waveform using a generalised and validated transfer factor [85]. Recordings were excluded if the systolic or diastolic variability of the waveforms exceeded 5 %, or the amplitude of the waveform, a measure of the quality of the tracing, was < 100 mV.

AI and ascending aortic pressure were derived from the central pressure waveform using the computer software. AI was defined as the difference between the first and second systolic peaks of the central arterial waveform, expressed as a percentage of the central pulse pressure [84]. AI is a measure of systemic arterial stiffness and wave reflection.

Study Protocol

32 patients were recruited into the study. Males : Females 66 : 34, median age 51 years (26 – 67 years). The commonest indications for transplant were chronic hepatitis C virus cirrhosis, alcohol related cirrhosis and primary sclerosing cholangitis. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg [26].

Patients were seen at the time of listing for transplant and again at intervals of 1, 3 and 6 months after liver transplant. Measurements of renin, aldosterone, endothelin, pulse wave analysis and peripheral blood pressure were performed at the first three visits. For the final 6 month visit, all the measurements except PWA were performed. Pulse wave analysis was not measured at 6 months because for this study it was necessary to determine AI before development of hypertension. Blood pressure was measured in duplicate with the same mercury Sphygmomanometer after a 5 minute period of rest and pulse wave analysis was performed immediately after. Readings were collected in duplicate and the mean used for statistical analysis.

Where possible treatment of hypertension was avoided during the first 3 months. This is because during the first 3 months corticosteroids may contribute to hypertension. After 3 months however, the majority of patients were weaning off corticosteroids, as is our policy, and in some cases this is sufficient to lower blood pressure.

Immunosuppression Protocol

All patients received triple immunosuppression with tacrolimus, prednisolone and azathioprine initially. Tacrolimus was given in a twice daily dose adjusted to maintain the plasma concentration between 5 and 15 mcg/l for the first 3 months and between 5 and 10 mcg/l thereafter. Prednisolone was given in a dose of 20 mg daily for the first month and the dose reduced over the first three months. Prednisolone was continued beyond three months in those patients transplanted for autoimmune liver disease and sclerosing cholangitis associated with inflammatory bowel disease. Acute cellular rejection of moderate or severe grade 200 was treated with methylprednisolone 1g daily intravenously for three days in all cases.

Statistics

The data after transplant were compared using Student's t-test. Pearson's correlation coefficient was used where appropriate. The SPSS software was used.

Results

Hypertension

3 patients (9 %) developed hypertension within one month of transplant. 15 patients (47 %) were hypertensive by 3 months. At 6 months 16 (50 %) were hypertensive. The changes in haemodynamic parameters for the total patient population are shown in Table 2.1. Peripheral systolic blood pressure increased at

1 month in 22 patients (69 %) and decreased in 10 patients (31 %) compared with pre-transplant measurements, whilst at 3 months, only 3 patients (9 %) still had a blood pressure below that of the pre-transplant value. There was no overall change in heart rate.

Table 2.1. Haemodynamic parameters before and at 1 and 3 months after transplant

	Pre-transplant	1 month post-transplant	3 months post-transplant	P-value comparing pre and 1m	P-value comparing pre and 3m	P-value comparing 1m and 3m
PSBP	119 ± 3	123 ± 4	133 ± 3	0.348	<0.001	0.004
PDBP	75 ± 2	78 ± 3	84 ± 3	0.175	<0.001	0.003
CSBP	105 ± 2	109 ± 3	120 ± 3	0.156	<0.001	0.002
CDBP	76 ± 2	78 ± 2	85 ± 3	0.147	<0.001	0.002
MAP	88 ± 2	92 ± 2	100 ± 3	0.079	<0.001	0.001
HR	79 ± 3	84 ± 3	80 ± 2	0.058	0.223	0.113
AI	9.9 ± 2.6	14.7 ± 3.4	16.2 ± 1.9	0.264	0.035	0.07

PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; AI, augmentation index

Augmentation index

The augmentation index had increased significantly by 3 months after transplant (Table 2.1). The rise in AI could be inferred to be due to increasing arterial stiffness or it could be linked directly to the rise in blood pressure which itself causes augmentation index to increase. Logistic regression analysis was used to ascertain whether the increase in observed AI could be accounted for by changes

in heart rate and mean arterial pressure. Using a multiple regression model containing the peripheral systolic and diastolic blood pressure measurements, mean arterial pressure and heart rate, the adjusted $R^2 = 0.57$. Thus after adjusting for the variables listed above, it is inferred that 57 % of the change in AI could be accounted for by changes in HR and blood pressure. There was no correlation between AI at 3 months and plasma ET-1 ($r = 0.23$, $P = 0.42$).

Subgroup analysis

Patients were analysed according to whether or not they had developed hypertension at 3 months. This was so that changes in the haemodynamic parameters, in particular the AI, could be compared between the two groups *prior to* development of hypertension. Patient details of the two groups are shown in Table 2.2. The haemodynamic parameters between the groups were compared prior to transplant (Table 2.3), at one month after transplant (Table 2.4) and at 3 months after transplant (Table 2.5).

Table 2.2. Comparison of normotensive and hypertensive patients

	Age (range)	Sex	Pred 1m (mg)	Pred 3m (mg)	Plasma FK506 1m (mcg/l)	Plasma FK506 3m (mcg/l)	ACR
Normotensive :n=17	50 (22-64)	M 65%	14 ± 1	5 ± 1	8.8 ± 0.8	10.0 ± 0.7	N=8
Hypertensive: n=15	53 (41-66)	M 67%	14 ± 1	5 ± 1	8.2 ± 0.6	11.0 ± 0.9	N=8

Pred: median prednisolone dose; FK506: tacrolimus; ACR: acute cellular rejection
None of the differences between the two patient groups are significant at the 5 % level

Table 2.3. Pre-transplant haemodynamic parameters in patients who became hypertensive at 3 months compared to those who remained normotensive

	Hypertensive group N= 15	Normotensive group N= 17	P-Value
PSBP	123 ± 3	112 ± 4	0.036
PDBP	78 ± 2	70 ± 3	0.038
CSBP	110 ± 3	98 ± 3	0.012
CDBP	78 ± 2	71 ± 3	0.067
MAP	91 ± 2	82 ± 3	0.032
HR	72 ± 3	84 ± 4	0.042
AI	14.5 ± 4.1	7.1 ± 3.8	0.18

PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; AI, augmentation index

Table 2.4. Haemodynamic parameters at 1 month in patients who subsequently became hypertensive at 3 months compared to those who remained normotensive

	Hypertensive group N= 12	Normotensive group N= 17	P-Value
PSBP	132 ± 4	115 ± 3	0.0013
PDBP	84 ± 3	72 ± 2	0.0043
CSBP	118 ± 4	102 ± 3	0.0013
CDBP	85 ± 3	73 ± 2	0.0055
MAP	99 ± 3	86 ± 2	0.0025
HR	79 ± 3	87 ± 4	0.15
AI	19.3 ± 2.4	10.8 ± 3.5	0.046

N.B. These data exclude 3 patients who were already hypertensive at 1 month
PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; AI, augmentation index

Prior to transplant the patients who developed hypertension at 3 months had higher peripheral and central blood pressure than the normotensive patients. By 1 month after transplant 3 patients had developed hypertension. Because of the effects of a rise in blood pressure upon augmentation index these 3 were excluded from the analysis as I wanted to assess the change in augmentation index prior to developing hypertension to establish whether arterial stiffness is implicated in evolving hypertension. At 1 month peripheral systolic blood pressure was unchanged in the normotensive group (P=0.62) but had risen from 123 to 132 mmHg in the hypertensive group (P=0.04). Augmentation index was higher in the hypertension group (Table 2.4).

Data at 3 months after transplant are shown in Table 2.5. By 3 months 47 % were hypertensive and all these patients were included in the analysis of the hypertension group. Peripheral, central and mean arterial pressures and AI were higher in the hypertensive group.

Table 2.5. Haemodynamic parameters at 3 months in patients hypertensive at 3 months compared to those who remained normotensive

	Hypertensive group N=15	Normotensive group N=17	P-Value
PSBP	149 ± 2	118 ± 4	<0.0001
PDBP	93 ± 4	77 ± 2	0.001
CSBP	135 ± 3	107 ± 3	<0.0001
CDBP	94 ± 4	78 ± 2	0.0013
MAP	111 ± 3	91 ± 3	<0.0001
HR	78 ± 3	85 ± 4	0.18
AI	21.2 ± 2.7	11.9 ± 3	0.031

PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; AI, augmentation index

There was a rise in peripheral systolic blood pressure and mean arterial pressure at 3 months compared to pre-transplant in both the hypertensive and normotensive groups ($P < 0.001$ and $P=0.046$ respectively for systolic blood pressure and $P<0.0001$ and $P=0.012$ respectively for MAP). There was a trend towards an increase in AI by 3 months in both groups ($P=0.095$ and 0.124 for the hypertensive and normotensive groups respectively), the statistical significance having been lost due to the smaller patient numbers in each subgroup.

Renin and Aldosterone

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Changes in plasma renin and aldosterone are shown in Tables 2.6-2.8. The values are shown for the study group as a whole (Table 2.6) and subdivided into those patients who were normotensive and those who were hypertensive at the end of the study, i.e. at 6 months (Tables 2.7, 2.8). A plasma renin below 10 mU/l following a 30 minute period of supine rest is considered normal. The normal laboratory range for aldosterone is 100-800 pmol/l.

Table 2.6. Plasma renin and aldosterone before and during 6 months after transplant

	Plasma renin (mU/l)	% with high renin	Plasma aldosterone (pmol/l)	% with high aldosterone
Pre-transplant	440 ± 165	87.5	1244 ± 205	43.8
1 month	39.4 ± 8.4	84.4	311 ± 42	9.4
3 months	18.2 ± 2.9	71.9	324 ± 57	6.3
6 months	27.3 ± 5.6	65.6	425 ± 64	18.8

Levels of plasma renin and aldosterone were elevated pre-transplant. Plasma aldosterone fell to normal after transplant. Plasma renin also fell but levels remained slightly elevated.

Table 2.7. Comparison of plasma renin (mU/l) in patients hypertensive at 6 months versus those normotensive at 6 months

	Hypertensive (n=16)	Normotensive (n=16)	P-value
Pre-transplant	449 ± 327	433 ± 154	0.97
1 month	32.5 ± 13.0	45.0 ± 11.0	0.47
3 months	20.2 ± 5.0	15.7 ± 2.1	0.42
6 months	23.8 ± 5.9	29.2 ± 8.0	0.60

Table 2.8. Comparison of plasma aldosterone (pmol/l) in patients hypertensive at 6 months versus those normotensive at 6 months

	Hypertensive (n=16)	Normotensive (n=16)	P-value
Pre-transplant	1235 ± 344	1251 ± 255	0.97
1 month	307 ± 58	314 ± 62	0.94
3 months	407 ± 88	218 ± 57	0.087
6 months	459 ± 107	407 ± 82	0.70

These results illustrate that there was no difference between hypertensive and normotensive patients with respect to levels of renin and aldosterone at any timepoint.

Endothelin-1

Results of plasma ET-1 are shown in Tables 2.9 and 2.10. Table 2.9 shows the plasma ET-1 before and after transplant for all 32 patients whilst Table 2.10 compares the hypertensive and normotensive groups. An ET-1 level above 20 pmol/l was considered elevated.

Table 2.9. Plasma endothelin pre and post transplant for the whole population (n = 32)

	ET-1 (pmol/l)	% with high ET-1
Pre-transplant	19.4 ± 6.3	21.9
1 month	6.8 ± 1.5	15.6
3 months	10.6 ± 2.5	21.9
6 months	17.1 ± 4.2	31.3

The only significant difference pre- and post-transplant was between the pre-transplant and 1 month post-transplant samples (P=0.048).

Table 2.10. Comparison of plasma endothelin (pmol/l) in hypertensive and normotensive groups

	Hypertensive group (n=16)	% with high ET-1	Normotensive group (n=16)	% with high ET-1	P – value
Pre-transplant	27.0 ± 11.0	31.3	11.1 ± 5.4	12.5	0.021
1 month	10.8 ± 2.5	25	2.7 ± 1	6.3	0.008
3 months	14.4 ± 4.5	31.3	7.0 ± 2.1	12.5	0.16
6 months	28 ± 7	50	7.5 ± 3.6	12.5	0.019

The P - value refers to the difference in plasma ET-1 between the hypertensive and normotensive groups

For both groups plasma endothelin levels fell early after transplant but were similar to the pre-transplant levels by 6 months after transplant. Plasma ET-1 was higher in the hypertensive group throughout, with the exception of the 3 month level, and by 6 months hypertensive patients had a significantly greater plasma ET-1 than the patients without hypertension and the levels were elevated above the normal limit of 20 pmol/l. At 6 months, ET-1 was elevated in 8 of 16 patients

(50 %) with hypertension compared with 2 out of 16 patients (12.5 %) with normal blood pressure.

Abnormalities of plasma renin, ET-1 and pre-transplant state

11 of 32 patients (34.4 %) had diuretic resistant ascites at the time of transplant. Of these 91 % had plasma renin levels above 100 mU/l. Just one patient had a plasma renin level above 100 mU/l who did not have ascites. Of the 7 patients with elevated ET-1 prior to transplant, just 2 had ascites. Mean serum creatinine pre-transplant was 73 ± 3 μmol/l and at 6 months after transplant was 98 ± 4 μmol/l ($P < 0.001$). There was no correlation between serum creatinine and serum ET-1 either before or at 6 months after transplant ($r = 0.15$, $P = 0.43$ and $r = 0.03$, $P = 0.88$ respectively). There was no correlation between plasma ET-1 and plasma tacrolimus levels at 1, 3 and 6 months post-transplant ($r = -0.04$, $P = 0.86$, $r = 0.08$, $P = 0.71$, $r = -0.09$, $P = 0.67$ respectively).

Discussion

This study concerns the development of hypertension during the first 6 months after liver transplant. The involvement of the renin-aldosterone system and plasma endothelin -1 to development of early hypertension has been studied, in addition to changes in augmentation index after liver transplant.

Considering the total study population, blood pressure was unchanged at 1 month but had increased by 3 months after transplant. Both peripheral and central aortic blood pressures increased. 9 % were hypertensive at 1 month, 47 % were hypertensive at 3 months and 50 % at 6 months had developed hypertension. Thus the majority developed hypertension between the first and third months. All patients hypertensive at three months remained so at 6 months. Just one patient was treated for hypertension, with amlodipine, during the study period. The overall data were unaltered when this patient was excluded (not shown).

Since the study was completed, further outcome data has emerged regarding hypertension. Median follow-up is currently 27 months (Range 19 to 36 months). One hypertensive patient died from a myocardial infarct at 30 months. 94 % of the hypertensive patients have continued to require pharmacological treatment. 2 of the 16 patients (13 %) normotensive at 6 months have developed hypertension, one at 2 years and one at 2.5 years. This data supports the idea that most patients develop hypertension during the first 3 months with just a small percentage becoming hypertensive subsequently. Two patients have proved difficult to treat, both remaining hypertensive despite triple antihypertensive therapy with a calcium channel antagonist, an ACE-inhibitor and a beta-adrenoceptor antagonist. One of these had elevated ET-1 levels, the other did not.

Levels of plasma renin and aldosterone were elevated prior to transplant. This has been reported previously [28, 55] and is consistent with the vasodilatory circulatory system in many patients with cirrhosis awaiting transplant. Not all patients had deranged renin and aldosterone pre-transplant and this probably reflects differing degrees of circulatory disturbance associated with varying severity of liver disease. The presence of ascites, and in particular diuretic resistant ascites, reflects more marked circulatory dysfunction and this group of patients had the highest levels of plasma renin. The mean plasma ET-1 was elevated pre-transplant, as documented by some [74, 201] but not all [71]. Levels of ET-1 were not greater in those patients with more severe decompensation, as judged according to ascites and serum creatinine. Other studies have shown both increased plasma ET-1 [202] and unchanged plasma ET-1 levels [201, 203] in cirrhotic patients with ascites.

After transplant levels of renin fell but remained slightly above the normal level. There was no change in levels during the 6 month study period. There was no difference in plasma renin between hypertensive and normotensive patients. Similarly, plasma aldosterone fell to the low-normal range by 1 month after transplant and remained normal at 6 months. Again there was no difference between hypertensive and normotensive patients.

What of other studies examining the renin-aldosterone axis? The rise in arterial pressure that occurs following transplant that is perceived by the juxtaglomerular apparatus may contribute to reduction in renin synthesis with a consequent fall in aldosterone synthesis. Falls in plasma renin and aldosterone during the first 4 months after transplant have been reported in a small number of studies involving cyclosporin treated patients [28, 55, 57]. Furthermore, in 2 of these studies in which patients were followed up for 4 weeks [28] and 2 months [55] after transplant, the fall in renin and aldosterone occurred at a time when there was an increase in blood pressure. In the current study, the fact that there was no difference between the hypertensive and normotensive patients, at a time when blood pressure increased steadily, suggests that the mild stimulation of the renin-angiotensin axis does not contribute greatly to development of hypertension during the first 6 months after liver transplant.

What then could be the role for the renin-angiotensin system in early transplant hypertension? Levels of plasma renin activity are known to increase from 12 months after liver transplant [57] and it is possible that at this time point after transplant the renin-angiotensin axis may contribute to hypertension. Julien et al found increases in active and total renin in 16 hypertensive liver transplant recipients, samples being taken 13 months after transplant [58]. Aldosterone levels in the upright position were higher than in normal controls in the same study. In neither study was it possible to correlate the observed increases in renin with changes in blood pressure but a role for stimulation of the renin-angiotensin axis can be surmised. As I have observed however, in the first 6 months after transplant, which is the time when a large number of patients develop hypertension, it is likely that other mechanisms besides the renin-angiotensin axis are implicated.

The augmentation index is a marker of arterial stiffness. Mean AI increased at one month and increased further at 3 months. An increase in AI implies an increase in arterial stiffness. The most valuable time to determine AI in this study was prior to development of hypertension to see if a rise in AI preceded development of hypertension. This is important because established hypertension is itself

associated with a rise in AI [84] and it then becomes impossible to determine whether increasing AI due to increased arterial stiffness contributed to hypertension or whether the hypertension itself caused the increase in AI.

In order to investigate the possible influence of increasing arterial stiffness upon evolving hypertension I compared AI in patients who were hypertensive at 3 months but who were normotensive at 1 month with those who remained normotensive throughout. There was no difference between these groups with respect to tacrolimus levels, prednisolone dosage and number of patients receiving intravenous corticosteroids for acute cellular rejection.

The AI was higher in the group with evolving hypertension at one month even though they were normotensive at that time. Could this imply that increased arterial stiffness was a contributory factor to hypertension? Those in the group who developed hypertension at 3 months had higher mean blood pressure pre-transplant in addition to higher blood pressure at 1 month. It is likely that the increased blood pressure that persisted between the two groups itself contributed to the difference in observed AI rather than increasing arterial stiffness being wholly responsible. This is borne out by the regression analysis that demonstrated that 57 % of the difference in AI was associated with changes in blood pressure and heart rate. It is nevertheless plausible that increasing arterial stiffness was directly responsible for some of the increase in AI and hence can be implicated in the development of hypertension.

An interesting observation is that the blood pressure pre-transplant in the patients who became hypertensive post transplant was higher than in the group who remained normotensive after transplant. The study numbers are too small to examine whether there is a threshold blood pressure pre-transplant that serves as a marker of likely progression to hypertension, although I think it unlikely that such a threshold exists given the multiple factors implicated in post-transplant hypertension.

There is emerging evidence that circulating endothelin levels have a significant role in liver transplant recipients. A link with increased blood pressure has been postulated. Lerman et al report elevations in plasma endothelin throughout the first week after transplant [71] and blood pressure increased significantly during this time period. Textor et al [204] also documented increases in endothelin and blood pressure in the first week after transplant, although plasma endothelin levels fell towards pre-transplant values during weeks 2 to 4. Textor et al have documented increased levels of circulating and urinary endothelin for up to 2 years after liver transplant, with both cyclosporin and tacrolimus based immunosuppression, and 65 % of patients were hypertensive at 2 years [204]. Experimental studies have shown that administration of calcineurin inhibitors is associated with an increase in circulating levels of ET-1 [65, 66].

This study is the first to directly compare changes in circulating ET-1 pre and post-transplant in patients who developed hypertension with those who did not. In those patients who developed hypertension, plasma ET-1 was elevated by 6 months and was significantly higher than those with normal blood pressure. This could suggest a role for rising levels of ET-1 in the development of hypertension. Plasma tacrolimus levels were not higher in hypertensive patients and there was no correlation between plasma levels of tacrolimus and serum ET-1 levels. There may be limitations however, to the interpretation of ET-1 levels. For example, it has been suggested that measurements of circulating ET-1 are relatively imprecise markers of the release of endothelin, for endothelin is directed primarily toward the vascular smooth muscle cells rather than into the vessel lumen [72].

An important implication for the involvement of ET-1 in transplant hypertension is the recent introduction of endothelin antagonist drugs. Animal studies in rats showed that endothelin receptor antagonists could prevent the cyclosporin mediated rise in blood pressure, and also block vasoconstriction in afferent renal arterioles [75, 76]. They can also lower blood pressure in established cyclosporin-induced hypertension [205]. Endothelin antagonists have recently entered clinical trials in humans and an initial study demonstrates efficacy of one such drug, darusentan, in the treatment of hypertension, with significant reductions in systolic

blood pressure observed over a 6-week period [77]. These agents have not as yet been utilised in hypertension after liver transplant.

It is possible that the role of ET-1 in the development of hypertension after liver transplantation may not be known until trials of endothelin antagonists are underway, and their efficacy in transplant hypertension can be assessed. My data show that circulating ET-1 is elevated in hypertensive patients. This, together with the promise seen in early trials of endothelin antagonists in the non-transplant arena, suggests that clinical trials in transplant hypertension should be undertaken.

Chapter 3. Comparison of the efficacy of amlodipine, lisinopril and bisoprolol in the management of post-transplant hypertension

Introduction

Hypertension is common after liver transplant. Several studies report a prevalence approaching 50 % or greater [19, 20, 22, 23] [18]. Despite the fact that post-transplant hypertension is so common it is perhaps surprising that there are so very few clinical trials of antihypertensive drugs in transplant recipients. Traditionally calcium channel antagonists have been used as first line treatment of post-transplant hypertension and there is a theoretical basis for using them in that such drugs work by counteracting the intense vasoconstriction seen with calcineurin inhibitor immunosuppression. There are only 3 clinical studies that demonstrate efficacy of this class of drug but in no study were calcium channel antagonists compared directly with other agents [104, 107, 108]. Published series quote the use of a variety of other antihypertensives after liver transplant, including beta adrenoceptor antagonists, alpha adrenoceptor antagonists, angiotensin converting enzyme (ACE) inhibitors and diuretics, either used alone or in combination. However, there are no trials of such drugs as second line treatment of hypertension, i.e. after calcium channel blockers, and there is no available data that compares the use of commonly used antihypertensives such as ACE inhibitors and beta blockers. Accordingly it is not possible at this stage to formulate an evidence based management strategy for hypertension occurring after liver transplant.

The arterial pressure waveform contains useful information concerning aortic and systemic arterial stiffness. The waveform at any time is made up of a forward moving and backward going reflected wave. With increasing age or under conditions that cause stiffening of the arterial tree the amplitude and velocity of the reflected wave increase. A larger reflected wave returns to the aorta earlier and adds to or augments the systolic pressure. The augmentation index (AI) is a measure of the contribution of the reflected pressure wave to the ascending pressure waveform and is expressed as a percentage of the pulse pressure. AI provides a measure of systemic arterial stiffness and wave reflection.

AI can be measured non-invasively using the technique of pulse wave analysis which allows accurate recording of radial artery pressure waveforms and generation of the corresponding central aortic waveform. From this the central aortic pressure and augmentation index (AI) are derived. Central blood pressure and arterial stiffness are better predictors of cardiovascular risk and mortality than peripheral blood pressure [79, 81]. Monitoring the effects of antihypertensive agents on AI may provide information that complements peripheral blood pressure recordings and that could help in determining which drugs are preferred to treat post-transplant hypertension.

To address these issues, I have conducted a trial of antihypertensive therapy in liver transplant recipients. There are two arms to the study. The first arm assesses the calcium channel blocker amlodipine as initial antihypertensive treatment. The second arm examines in a randomised cross-over study the beta adrenoceptor antagonist bisoprolol and the ACE inhibitor lisinopril as second line drugs in patients intolerant of or unresponsive to amlodipine. Peripheral and central blood pressure and AI were measured before and after treatment with each drug.

Methods

Patients with hypertension were recruited from the liver transplant clinic. Hypertension was defined as an outpatient systolic blood pressure, as measured with a mercury sphygmomanometer after a 5 minute seated period of rest, of 140 mm Hg or greater on at least three separate clinic attendances. Systolic, as opposed to diastolic, blood pressure was studied in order to simplify the study in terms of assessing drug efficacy and deciding upon drug dosage increases, and also because it is more closely linked to CHD risk than diastolic blood pressure [206, 207]. Patients with diabetes mellitus were excluded, as were patients on diuretics and those already on antihypertensive drugs other than amlodipine. Approval for the study was obtained from the Local Research Ethical Committee and written informed consent was obtained from each participant.

The study design was in two parts.

Part 1. Using amlodipine as first-line treatment for hypertension

24 patients were recruited. These patients, who were not taking any antihypertensive medication, were commenced on the dihydropyridine calcium channel blocker amlodipine. This part of the study was open-labelled and uncontrolled. The starting dose was 5 mg once daily. If after one month of treatment systolic blood pressure was still above 140 mmHg the dose was increased to 10 mg once daily. Patients were reviewed 4-weekly for a total period of three months.

Part 2. Randomised cross-over study comparing bisoprolol with lisinopril

Patients were recruited into this arm of the study if they were intolerant of amlodipine or if peripheral systolic blood pressure was not controlled despite maximum tolerated dose of amlodipine. 13 patients entered this arm of the study, 11 of whom were recruited from the amlodipine study arm. 3 of the 13 patients were on amlodipine 5 mg or 10 mg once daily but were still hypertensive. The remainder were not taking any antihypertensive agents.

The study design was a randomised cross-over study comparing treatment with the beta-adrenoceptor antagonist bisoprolol with the ACE inhibitor lisinopril. The starting dose of each drug was 5 mg once daily. Patients were reviewed at 4-weekly intervals and if the systolic blood pressure remained above 140 mmHg, the dose of each drug was increased to 10 mg and if necessary 20 mg once daily. After a 12 week period of treatment the first drug was stopped. Following a 4 week washout period, the second agent was commenced. The study concluded after a further 12 weeks.

Peripheral blood pressure

Blood pressure was measured using the same mercury sphygmomanometer on each patient visit. Measurements were made in duplicate, after 5 minutes of seated rest, in the brachial artery of the dominant arm.

Pulse wave analysis

Peripheral pressure waveforms were recorded from the radial artery of the dominant hand at the wrist using a high-fidelity micromanometer (Millar Instruments, Texas, USA) and the SphygmoCor apparatus (SCOR; PWV Medical, Sydney, Australia). This technique has been described in more detail in the Introduction. 20 sequential waveforms were acquired and the integrated software was used to generate an averaged peripheral waveform and corresponding central pressure waveform. Recordings were excluded if the systolic or diastolic variability of the waveforms exceeded 5 %, or the amplitude of the waveform, a measure of the quality of the tracing, was < 100 mV. AI and ascending aortic pressure were derived from the central pressure waveform. AI, defined as the difference between the second and first systolic peaks of the central arterial waveform, was expressed as a percentage of the central pulse pressure.

Pulse wave analysis measurements were made in duplicate and the means of the two sets of measurements were used in analysis. Recordings were made prior to commencing each study drug and during the final treatment visit with each drug. Measurements were made after a 5 minute period of rest in a chair.

Plasma Renin and Aldosterone

Blood samples for serum renin and aldosterone were collected before initiating treatment with amlodipine, bisoprolol and lisinopril and again at the end of each treatment period. Blood sampling was done after a 20 minute period of supine rest. Samples were collected into lithium-heparin tubes. Samples were then spun at 2000 rpm for 5 minutes within one hour of sampling, and the plasma stored at – 70 Celsius until analysis.

The actual analysis was performed by the Department of Biochemistry at Addenbrooke's Hospital. Shortly before analysis, the samples were thawed rapidly and maintained at room temperature. Renin was measured in 200 microlitre plasma with a commercially available immunoradiometric assay kit

(Nichols Institute, CA, USA) following methods described previously [198]. Aldosterone was determined using a commercial radioimmunoassay kit (Diagnostic Products corporation, CA, USA) following the principles proposed by Kubasik et al [199].

Data analysis

Data were compared before and after treatment with amlodipine, bisoprolol or lisinopril using Student's paired t-test. To compare the responses to bisoprolol and lisinopril in the cross-over study I used repeated measures of analysis of variance (ANOVA). Values are reported as means ± standard error of the mean (unless otherwise stated). A p-value < 0.05 was considered significant.

Results

Baseline patient characteristics for each study arm are shown in Table 3.1. Two patients in the amlodipine study had a history of hypertension prior to transplant, as did one patient in the cross-over study. The commonest indications for liver transplant were cirrhosis due to chronic viral hepatitis or alcoholic liver disease, and primary biliary cirrhosis.

Table 3.1. Baseline characteristics

	Amlodipine study	Cross-over study
Male : Female (%)	58 : 42	46 : 54
Age (yrs) : mean (range)	59.8 (36-74)	55 (36-67)
Years since transplant	4.1 (0.5-14)	2.7 (0.75-14)
Immunosuppression	FK n = 20: CyA n = 4	FK 100 %

FK denotes immunosuppression with tacrolimus and CyA cyclosporin.

Drug tolerability: Amlodipine study arm

2 of the 24 patients discontinued the drug within 2 weeks. In one patient this was because of peripheral oedema and in the other because of palpitations. No outcome data were available for either patient, so both have been excluded from statistical analysis. Ten (42 %) of the total group of 24 patients developed side effects attributable to amlodipine. Peripheral oedema developed in 9 patients whilst tachycardia occurred in 1 patient. In all, 8 (33 %) of the total group had to stop taking the drug because of side effects. Of those who developed oedema with amlodipine, 78 % reported this on the 5 mg dose and 22 % on 10 mg daily.

Drug tolerability: Cross-over study

Throughout the duration of the cross-over study three patients were also taking amlodipine 5 mg daily. Both bisoprolol and lisinopril were well tolerated. One patient discontinued lisinopril after two doses because of probable hypotension. This patient has been excluded from statistical analysis. Two patients developed dry cough with lisinopril. One patient discontinued bisoprolol after 8 weeks because of headache with the 5 mg dose. This patient achieved a systolic blood pressure below 140 mmHg and sufficient data was available to allow inclusion in analysis. Intermittent claudication and cold peripheries were also reported with bisoprolol (1 patient each).

Haemodynamic changes

The peripheral and central haemodynamic changes for each drug are shown in Tables 3.2a, 3.2b and 3.2c. The data for bisoprolol and lisinopril are inclusive of the three patients who were also taking amlodipine. None of the haemodynamic changes were affected if these three patients were excluded from analysis (data not shown).

Table 3.2a. Haemodynamic changes with amlodipine

	HR beats min^{-1}	PSBP mmHg	PDBP mmHg	MAP mmHg	PPP mmHg	CSBP mmHg	CDBP mmHg	CPP mmHg	AI
Pre-amlodipine	75 ± 2	154 ± 2	89 ± 2	112 ± 2	65 ± 2	141 ± 2	91 ± 2	51 ± 2	27.3 ± 2
On amlodipine	78 ± 2	130 ± 2	80 ± 2	97 ± 2	50 ± 2	117 ± 2	81 ± 2	36 ± 2	19.3 ± 2
P-value	0.14	<0.001	<0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

HR, heart rate; PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; MAP, mean arterial pressure; PPP, peripheral pulse pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; CPP, central pulse pressure; AI, augmentation index

Table 3.2b. Haemodynamic changes with bisoprolol

	HR beats min^{-1}	PSBP mmHg	PDBP mmHg	MAP mmHg	PPP mmHg	CSBP mmHg	CDBP mmHg	CPP mmHg	AI
Pre-bisoprolol	76 ± 3	154 ± 2	92 ± 3	114 ± 3	62 ± 4	140 ± 3	94 ± 3	45 ± 3	21.7 ± 3
On bisoprolol	57 ± 3	142 ± 4	85 ± 3	106 ± 3	57 ± 4	135 ± 4	87 ± 3	49 ± 4	33.2 ± 3
P-value	< 0.001	< 0.001	0.004	0.001	0.042	0.07	0.003	0.07	<0.001

HR, heart rate; PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; MAP, mean arterial pressure; PPP, peripheral pulse pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; CPP, central pulse pressure; AI, augmentation index

Table 3.2c. Haemodynamic changes with lisinopril

	HR beats min^{-1}	PSBP mmHg	PDBP mmHg	MAP mmHg	PPP mmHg	CSBP mmHg	CDBP mmHg	CPP mmHg	AI
Pre-lisinopril	72 ± 3	154 ± 2	92 ± 3	113 ± 3	62 ± 2	140 ± 2	93 ± 4	47 ± 2	24.9 ± 1.9
On-lisinopril	71 ± 3	130 ± 5	81 ± 3	95 ± 4	50 ± 3	116 ± 5	81 ± 3	34 ± 2	14.7 ± 3.7
P-value	0.4	< 0.001	0.001	< 0.001	0.001	< 0.001	0.001	< 0.001	< 0.001

HR, heart rate; PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; MAP, mean arterial pressure; PPP, peripheral pulse pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; CPP, central pulse pressure; AI, augmentation index

Peripheral systolic blood pressure fell below 140 mmHg with 5 mg once daily of amlodipine in 17 of the 22 patients. However, 5 of these patients with a satisfactory response were unable to continue taking amlodipine because of side effects.

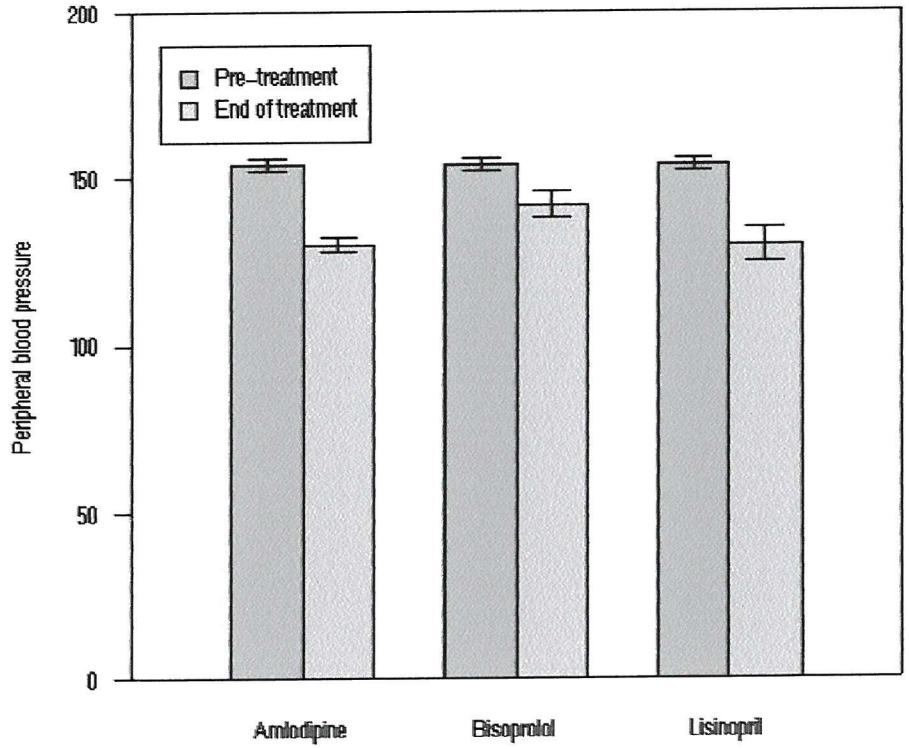
With bisoprolol, peripheral systolic blood pressure was reduced to below 140 mmHg in 7 patients with 5 mg, a further 2 patients with 20 mg but 3 remained hypertensive on 20 mg.

In the lisinopril arm, peripheral systolic blood pressure was lowered below 140 mmHg in 9 patients on 5 mg once daily, 2 further patients on 20 mg but one remained hypertensive on 20 mg daily.

All three antihypertensive agents reduced peripheral blood pressure significantly (Tables 3.2a, 3.2b, 3.2c and Figure 4). However, there were clear differences between the drugs and of particular interest between lisinopril and bisoprolol. Comparing bisoprolol with lisinopril using repeated measures of ANOVA,

lisinopril was associated with a greater fall in systolic blood pressure (F=7.04, P=0.022) and the change in AI (falling with lisinopril and rising with bisoprolol) was also significant (F=6.38, P=0.039). There was no order effect in terms of the blood pressure response in the cross-over study.

Figure 4. Peripheral blood pressure before and after treatment with antihypertensive drugs



The differing effects of the drugs upon peripheral and central pulse pressure can be illustrated by subtracting the central from the peripheral pulse pressure and comparing the differences pre- and post-treatment (Table 3.3). These data include the three patients who were also on amlodipine 5 mg throughout.

Table 3.3. The effects of antihypertensive drugs upon peripheral and central pulse pressure

	Pre-amlodipine	Post amlodipine	Pre-lisinopril	Post-lisinopril	Pre-bisoprolol	Post bisoprolol
PPP – CPP (mmHg)	14 ± 1	14± 1	15 ±1	16 ± 2	16 ± 1	8 ± 1
P-value	0.955		0.336		<0.001	

PPP = peripheral pulse pressure: CPP = central pulse pressure

There was no change in the difference between PPP and CPP with amlodipine and lisinopril reflecting the fact that both peripheral and central pressures were reduced by corresponding amounts with each drug. In contrast bisoprolol was associated with a fall in the difference between PPP and CPP, from 16 mmHg to 8 mmHg ($P < 0.001$). This illustrates that with bisoprolol, the central aortic pressure has not fallen as much as the peripheral pressure on account of the changes in wave reflection.

Plasma Renin and Aldosterone

The results of the plasma levels of renin and aldosterone before and at optimum dose of amlodipine, bisoprolol and lisinopril are shown in Table 3.4.

Table 3.4. Plasma renin and aldosterone during antihypertensive treatment

	Serum renin (mU/l)	Serum aldosterone (pmol/l)
Pre-amlodipine	19.5 ± 5.0	222.4 ± 37.1
On amlodipine	46.3 ± 10.2 **	368 ± 45.6 *
Pre-bisoprolol	41.8 ± 18.7	382.2 ± 24.3
On bisoprolol	18.9 ± 6.9	347.2 ± 42.5
Pre-lisinopril	13 ± 3.0	303.4 ± 26.2
On lisinopril	140.3 ± 29.2 **	190.8 ± 27.3 **

Normal lab range for renin: <10 mU/l

Normal lab range for aldosterone: 100-800 pmol/l

* denotes $P < 0.05$ comparing pre-treatment and during treatment

** denotes $P < 0.01$ comparing pre-treatment and during treatment

Baseline levels were similar with each drug. Treatment with amlodipine was associated with significant increases in both plasma renin and aldosterone, whereas lisinopril reduced plasma aldosterone. In contrast neither plasma renin nor aldosterone was influenced by bisoprolol. There was no difference in the data for bisoprolol and lisinopril when the 3 patients who were also taking amlodipine were excluded (data not shown).

Non-cardiovascular parameters

Table 3.5 shows the changes in non-cardiovascular parameters during the amlodipine study and the cross-over study. None of the changes in the various parameters were significant with the exception of the change in prothrombin time with p-values of 0.012 for the amlodipine limb and 0.003 for the cross-over limb. This was a clinically insignificant change, however. Neither liver nor renal function was affected during the study, nor was there any change in serum levels

of immunosuppression nor in prednisolone dosage. For those patients treated with lisinopril, there was no change in 24 hour creatinine clearance. Pre –lisinopril this was 53.7 ± 7.9 ml/minute and on maximum dose of lisinopril it was 51.1 ± 6.5 ml/minute.

Table 3.5. Changes in non-cardiovascular parameters with drug treatment

	Amlodipine study		Cross-over study	
	start	finish	start	Finish
FK 506 (μg/l)	8.0 ± 0.8	8.6 ± 0.9	8.9 ± 8	7.3 ± 0.9
CyA (μg/l)	140 ± 13	134 ± 15	***	***
Pred (mg)	1.8 ± 0.6	1.6 ± 0.6	1.2 ± 0	1.2 ± 0
Weight (kg)	81.6 ± 4.6	80.5 ± 4.5	90.7 ± 6.9	91.3 ± 6.5
Creatinine (μg/l)	118 ± 11	114 ± 12	140 ± 20	136 ± 19
ALT (U/L)	70 ± 24	67 ± 16	51 ± 8	69 ± 21
Albumin (g/L)	39 ± 1	38 ± 1	37 ± 1.6	38 ± 1.6
Bilirubin(μmol/l)	15.2 ± 3.3	15 ± 3.3	14.5 ± 4.4	11.3 ± 2.7
PT (secs)	13.8 ± 0.3	12.5 ± 0.3	14.5 ± 0.5	12.2 ± 2

FK506, tacrolimus level; CyA, cyclosporin level; Pred, mean prednisolone dose; Creatinine, serum creatinine; ALT, alanine aminotransferase; PT, prothrombin time

Discussion

I chose to study the effects of three commonly used classes of antihypertensive drug that are used to treat liver transplant recipients [29, 110, 208]. Following the principles that apply to treatment of hypertension in a non-transplant setting I utilised low doses of drugs initially and increased the dose at monthly intervals

until either the maximum dose was reached, therapeutic target blood pressure had been achieved or side-effects warranting cessation of treatment developed. The patients in this study all had established hypertension and with two exceptions were beyond one year after transplant. I have confirmed that amlodipine is an effective drug in liver transplant recipients. My data also show that lisinopril is more effective than bisoprolol and has an equal blood pressure lowering effect to that of amlodipine.

Calcium channel blockers have been traditionally used as first line treatment of transplant hypertension, on the basis that they are able to counter the vasoconstriction associated with calcineurin inhibition [27, 29, 103]. Studies to date have been few and not without limitations. Isradipine was found to be effective when given early after transplant and mean blood pressure decreased from 151 ± 3/91 ± 2 to 130 ± 3/81 ± 2 mmHg [107]. The blood pressure lowering effects may have been confounded however, by dosage adjustment of corticosteroids and cyclosporine, and co-administration of frusemide. Nicardipine has proven efficacy in transplant hypertension with 70 % of patients rendered normotensive, but the drug doses used are not clear and the pre-treatment blood pressures are not known [104]. 26 % of patients developed side effects, 15 % having to discontinue treatment. Finally, nifedipine was shown to reduce blood pressure in 16 patients at a mean of 15 months after transplant [108]. In this published abstract, the beta-adrenoceptor antagonist labetalol also reduced blood pressure in 9 additional patients. The study was not designed to compare the 2 drugs however, and it is noteworthy that the heart rate was identical in both groups and suggests the labetalol patients were not adequately beta-blocked.

After liver transplant, there is loss of the nocturnal decline in blood pressure as seen with other solid organ transplants [209] using cyclosporine immunosuppression. Loss of circadian blood pressure variation is associated with more rapid development of hypertensive end-organ damage [210, 211]. Interestingly nocturnal blood pressure decreased with isradipine in Taler's study. It is not known how this would impact upon subsequent long-term development of hypertensive complications.

In that study 40 % of patients complained of symptoms including tachycardia, oedema and headaches necessitating withdrawal of isradipine. This is remarkably similar to the findings in this study in which 42 % of patients developed side effects with amlodipine, principally peripheral oedema. Interestingly, Taler et al have recently shown that, for hypertension occurring in the first few months after transplant, the presence of resting tachycardia and a low peripheral vascular resistance measured non-invasively can identify which patients are likely to experience symptomatic intolerance to isradipine. Such measurements however may not be practical in everyday clinical practice. Patients developing symptoms of oedema or tachycardia in the study presented here generally did so within the first week of treatment with the 5 mg dose. 77 % responded to a 5 mg once daily dose whilst increasing the dose to 10 mg improved blood pressure in 2 patients. The remaining three either did not respond to or did not tolerate the increased dose. The fall in blood pressure with amlodipine is similar to that observed with isradipine [107]. The fall in peripheral blood pressure we observed was mirrored by a similar fall in the derived central aortic pressure (and mean arterial pressure).

Whilst amlodipine is clearly an effective antihypertensive drug in liver transplant recipients, it's use is potentially limited by the high percentage of patients developing side-effects. One option to limit these would be to keep the dose as low as possible, possibly using as little as 2.5 mg daily. Alternatively different calcium channel blockers could be tried. There are limitations to this however, for nicardipine has been shown to interfere with cyclosporin pharmacokinetics and causes increased levels [104] whilst diltiazem and verapamil can both lead to elevated levels of calcineurin inhibitor [103]. Therefore trying a different dihydropyridine calcium channel antagonist is favoured, and lercanidipine is a suitable alternatives to amlodipine.

Given that 40 % of patients develop side-effects with amlodipine there is a requirement for alternative agents. There are no published trials comparing antihypertensive drugs after liver transplant other than calcium channel blockers. The second part of my study was designed to address the question of what is appropriate second-line treatment. I compared once daily dosing of lisinopril and

bisoprolol in a cross-over study. The patients on bisoprolol were adequately beta-blocked as evidence by the resting heart rate of 57 beats /minute. Both drugs reduced peripheral blood pressure but the fall in peripheral systolic blood pressure was greater for lisinopril. Both drugs were equally well tolerated. There was no change in serum creatinine or in creatinine clearance during administration of lisinopril.

The elevation in serum renin observed in the patients prior to treatment is consistent with mild stimulation of the renin-angiotensin axis. After the first transplant year levels of plasma renin activity increase [57] and elevated plasma renin levels have been reported in hypertensive transplant patients at 13 months after transplant [58]. All bar one of our patients was beyond one year after transplant. The blood pressure response to lisinopril suggests that activation of the renin-angiotensin axis is one factor implicated in the causation of hypertension in these patients. The fall in aldosterone and increase in serum renin with lisinopril is consistent with the mechanisms of action of the drug, with negative feedback resulting in further release of renin. Because of studies suggesting suppression of the renin-angiotensin axis in the first year after transplant [55, 56] it has been felt that ACE-inhibitors have limited efficacy during this period [29, 103]. I have not been able to address this in the current study but lisinopril alone is certainly an effective agent for hypertension after one year. Amlodipine, by causing vasodilatation, stimulates both renin and therefore aldosterone release, and this is reflected in my observations. There was a trend towards a reduction in plasma renin with bisoprolol. Beta-blockers are known to suppress plasma renin, possibly through effects on the sympathetic nervous system [212].

Aside from the effects upon peripheral blood pressure, there were marked differences in the augmentation index and central aortic pressures which provide additional supportive information in favour of choosing lisinopril over bisoprolol. These can be explained by considering the dynamics of arterial pressure waves. Forward moving arterial pressure waves are reflected back from the periphery so that the waveform at any time is made up of the forward moving and backward going reflected waves. Normally the reflected wave arrives back at the aortic root

in diastole, thereby helping to maintain coronary perfusion. If wave reflection is increased, the reflected wave returns to the aorta earlier and augments the central systolic pressure. AI is a measure of the contribution of this reflected pressure wave to the ascending aortic pressure waveform.

AI increased with bisoprolol. Bisoprolol increases the AI because the length of systole is prolonged, allowing the reflected wave to return during systole. It has been shown that a fall in heart rate of 10 beats/minute is associated with a rise in AI of 4 % [213]. Thus the drop in heart rate observed with bisoprolol would account for around 8 % of the 11.5 % increase in AI. As the reflected wave returns to the heart it helps to maintain the central aortic pressure and hence the fall in central aortic pressure was smaller with bisoprolol than with lisinopril. The fall in central aortic pressure is therefore less than might be expected from measurements of the brachial artery pressure alone. Lisinopril on the other hand reduced both peripheral and central aortic systolic pressures equally. Amlodipine also reduced AI and central aortic pressure to a similar degree as lisinopril. The fall in AI observed with amlodipine, which acts as a vasodilator, may be explained by a reduction in arterial stiffness.

The different effects of these drugs on arterial wave dynamics mirror those encountered in the general population. Thus, nifedipine, captopril and fosinopril all reduce wave reflections [100-102] whilst propranolol increases reflections [98]. Using identical Sphygmacor apparatus to that which was used in my study, Deary et al showed similar findings to the current study: in patients with essential hypertension bisoprolol resulted in an increase in AI whereas lisinopril reduced AI [214].

The effect on central pressure may be clinically important because it is the central aortic pressure and not the brachial pressure that determines left ventricular workload [93]. Indeed left ventricular mass correlates well with the shape of the central waveform in normotensive and hypertensive patients [94, 95]. Because of its ability to lower central blood pressure in addition to a better peripheral pressure reduction, I suggest that lisinopril is preferred to bisoprolol. Whether the effects

upon central aortic pressure reduction that I observed with lisinopril are sustained and in the long term result in less left ventricular dysfunction are areas that have not been addressed as yet. In Chen's study [102] left ventricular mass was not altered after 8 weeks of treatment with fosinopril, possibly a reflection of the mild hypertension and too short a follow-up time to detect a change in ventricular mass.

Aside from pharmacological intervention using antihypertensive drugs there are other options to consider as means of reducing blood pressure after transplant. For patients who develop hypertension in the first few months after transplant corticosteroid withdrawal as part of the standard immunosuppressive regime may be sufficient to reduce blood pressure. Similarly if patients have continued on corticosteroids and have established hypertension steroid withdrawal may be appropriate. This has been shown to reduce blood pressure whilst not endangering the graft [44]. In a trial of 100 patients randomised to steroid withdrawal after 3 months, hypertension occurred less commonly in the steroid withdrawal group [215]. There was no difference in the incidence of acute or chronic rejection between the groups. An uncontrolled study looking at withdrawing corticosteroids at one year demonstrated a fall in the requirements for antihypertensive medications [216]. Finally reducing the dose of prednisolone from 10 mg to 5 mg daily was associated with discontinuation of antihypertensive medication in 9 % [23]. My work has also shown that switching immunosuppression from cyclosporin to tacrolimus can bring about a fall in blood pressure without adverse effects on graft function. This is discussed in detail in Chapter 5.

In summary, my findings support the general view that calcium channel blockers are the agents of choice for established hypertension. Among these the dihydropyridine class, which includes amlodipine, isradipine, felodipine and lercanidipine, are preferred. They have the most favourable side-effect profile of the three classes of drug studied with fewer contraindications to their use. I have shown that when amlodipine is not tolerated or is ineffective, lisinopril achieves greater peripheral blood pressure reduction than bisoprolol. Lisinopril's effects on reducing central aortic blood pressure and AI are further evidence that lisinopril should be preferred to bisoprolol. The finding that cyclosporin upregulates

angiotensin II receptors [54] suggests potential benefit of angiotensin II antagonists in transplant hypertension. This is an area for further study.

Chapter 4: Brain natriuretic peptide after liver transplantation

Introduction

Measurement of plasma BNP is performed easily in the outpatient clinic. It is known that plasma BNP rises in the setting of left ventricular hypertrophy in hypertensive patients. A level of BNP that is greater than the mean of the control population plus 2 standard deviations is generally defined as representing an elevated BNP. It is not known what happens to BNP in the setting of liver transplant hypertension. Based upon evidence from non-transplant hypertensive patients, a blood sample analysed for BNP should provide valuable prognostic information in hypertensive transplant patients in that patients with hypertension who have left ventricular hypertrophy and systolic dysfunction could be identified [132, 133]. This would enable patients at particular risk of cardiovascular disease, consequent upon LV hypertrophy, to be diagnosed and treated early.

There are no data on levels of BNP in liver transplant patients, whether hypertensive or not. The prevalence of hypertension after liver transplant is high. It is an attractive idea that BNP could serve as a useful screening tool for LVH in hypertensive patients, allowing resources to be targeted to those patients most likely to benefit from further investigations and intervention. BNP could in theory be utilised in liver transplant patients to provide additional information on cardiovascular risk.

Methods

A total of 104 people were recruited from the liver transplant clinics, hypertension clinic and normal healthy volunteers. Each participant had his or her blood pressure measured by me using the same mercury Sphygmomanometer (mean of two readings). Hypertension was defined as a blood pressure of 140 / 90 mmHg or greater, either on or off antihypertensive drugs. Patients taking beta-adrenoceptor antagonists were excluded as these drugs elevate BNP levels [214]. A single blood sample was taken from each participant for determination of serum BNP. The four subject groups are discussed below.

Liver transplant – non-hypertensive

25 patients were recruited from the liver transplant clinic. The case notes were used to identify patients who had been consistently normotensive following transplantation. On two consecutive clinic visits prior to being included in the study I had checked their blood pressure in duplicate. No patients had a pre-transplant history of hypertension and no patients were taking antihypertensive drugs.

Liver transplant – hypertensive

54 patients with hypertension following liver transplant, and who had no clinical signs of heart failure, were recruited from the liver transplant clinic. Patients were identified from their case notes as having been consistently hypertensive for a minimum of 12 months. I then measured their blood pressure on two consecutive clinic visits to confirm the presence of established hypertension. Only two patients had a history of hypertension prior to transplant.

Non-transplant – non-hypertensive

13 volunteers with normal blood pressure were recruited from staff at Addenbrooke's Hospital. Blood pressure was measured on two separate occasions over a one month period before inclusion in the study. No patients had a prior history of hypertension and no-one was taking any antihypertensive drugs.

Non-transplant – hypertensive

12 patients attending hypertension outpatient clinics at Addenbrooke's Hospital were recruited. These patients all had systolic blood pressures of 140 mmHg or above and 11 of the 12 were taking antihypertensive drugs (other than beta-blockers).

BNP analysis

A single 5 ml blood sample was taken and placed immediately into cooled EDTA tubes. These were centrifuged at 1800 rpm for 20 minutes at 4 ^{0}C. The separated plasma was put into tubes containing 50 µl trasylol and stored at - 70 ^{0}C until analysis. BNP was measured on the Advia Centaur immunoassay analyser. The assay was a 2-site sandwich immunoassay using direct chemiluminescence. The first antibody recognised the ring structure of BNP-32 and carried the acridinium label. Antibody 2 recognised the C-terminal portion of BNP-32 and is bound to the solid phase (magnetic particles). Antibody 1 was added before antibody 2 and the BNP pulled down by a magnet while the unreacted serum is washed away. The acridinium chemiluminescent label was activated and the signal measured. Blood samples were taken and stored by me. The analysis was performed by the Department of Biochemistry at West Suffolk Hospital, Bury St. Edmunds.

All patients gave written informed consent. The study was approved by the Local Research Ethics Committee.

Results

Table 4.1. Patient characteristics

	Transplant hypertension (n=54)	Transplant normal BP (n=25)	Non-transplant hypertension (n=12)	Non-transplant normal BP (n=13)
Sex M:F	56 : 44	44 : 56	58 : 42	54 : 46
Age (years)	61 (39 – 74)	54 (40 – 67)	61 (51 – 74)	38 (18 – 55)
Systolic BP	153 ± 2	125 ± 3	151 ± 3	119 ± 2
Diastolic BP	88 ± 2	79 ± 3	90 ± 3	75 ± 3

Liver Transplant Hypertension Group

Hypertension developed at a mean of 12 months (range 0.5 – 80 months) after liver transplant. The mean time since transplant was 49 months. This was identical to the time since transplant for the non-hypertensive transplant group. The samples for BNP were taken at a mean of 38 months following onset of hypertension (range 15 – 117 months). 30 of the 54 patients were taking antihypertensive medication. Of these, 24 were on one drug and 6 patients were taking two drugs. Calcium channel antagonists were the commonest antihypertensive agents used (25 patients), followed by angiotensin converting-enzyme inhibitors (9 patients) and alpha-adrenoceptor antagonists (2 patients). All patients were receiving tacrolimus.

Non-Transplant Hypertension Group

The patients in this group had been hypertensive for a mean of 12 years. All bar one patient were taking antihypertensive drugs. 4 were taking one drug, 5 were taking two drugs and 3 were taking three drugs. The commonest antihypertensive drugs used were, in descending order of frequency, calcium channel antagonists (7 patients), angiotensin-converting enzyme inhibitors (5 patients), alpha-adrenergic antagonists and diuretics (3 patients each).

Table 4.2 shows the serum levels of BNP in pg/ml.

Table 4.2. Serum levels of BNP in different patient groups

Group	Transplant hypertension	Transplant normal BP	Non-transplant hypertension	Non-transplant normal BP
BNP (pg/ml)	69.8 ± 11	48.4 ± 7.7	25.6 ± 7.2	14.9 ± 4.3
P-value		0.12		0.22

The P-values denote the significance between (i) the two transplant groups and (ii) the non-transplant groups.

Using the non-hypertensive non-transplant group as the control group, the cut-off plasma level of BNP was set at the mean plus 2 standard deviations giving a value of 44.6 pg/ml (14.92 + 2x14.85 pg/ml). 50 % of the hypertensive transplant group had BNP levels above 44.6 pg/ml compared with 17 % of the non-transplant hypertensive group. Serum levels were significantly higher in the transplant hypertensive group than the non-transplant hypertension group (P = 0.002). The non-hypertensive transplant group had higher values of BNP than the hypertensive non-transplant group (P = 0.039) and 48 % of this transplant group had BNP levels above 44.6 pg/ml. Finally, the control group had the lowest levels of BNP and just 8 % of the control group had BNP levels above 44.6 pg/ml.

There was no correlation between plasma BNP and systolic blood pressure (Pearson correlation coefficient 0.153, P = 0.3). Likewise there was no correlation between BNP and serum tacrolimus levels (Pearson correlation coefficient – 0.15, P = 0.38) nor between BNP and cyclosporin levels (Pearson correlation coefficient – 0.292, P = 0.383).

Table 4.3 shows the effect of sex upon the plasma levels of BNP in the two liver transplant groups.

Table 4.3. Effect of gender on BNP levels in liver transplant recipients

	Hypertensive group		Non-hypertensive group	
	Male (n = 30)	Female (n=24)	Male (n = 11)	Female (n=14)
Plasma level of BNP (pg/ml)	62.9 ± 13	74.2 ± 19	37.3 ± 10	54.1 ± 11
P-value	0.63		0.29	

In both groups, BNP levels tended to be higher in women although statistical significance was not reached.

Discussion

Plasma levels of BNP are elevated after liver transplant, both in hypertensive and non-hypertensive patients with the former group having higher values. The mean plasma levels of each transplant group were greater than the non-transplant hypertensive group. The cut-off level of BNP, derived from the control group, was similar to that reported in other studies [132, 138]. Approximately 50 % of liver transplant recipients had elevated levels of BNP. It is interesting that an equal proportion of non-hypertensive transplant patients have elevated levels of BNP, albeit not to the same magnitude as the hypertensive patients. One would be surprised if this many patients had LVH and caution should be used when interpreting the significance of an elevated BNP in the transplant patients. There is also a striking difference between the percentage of hypertensive non-transplant patients with elevated BNP and the two transplant groups.

What inferences can be drawn from the plasma levels of BNP observed after transplantation? The significant increases in BNP seen in transplant patients, both hypertensive and normotensive, could be related to the vasoconstriction that occurs with calcineurin inhibition. An early increase in BNP could be the left ventricle's protective response to an increase in arterial stiffness consequent upon calcineurin inhibitors. Stiff arteries are associated with increased pulse pressure and it is interesting that pulse pressure has been positively correlated with plasma levels of BNP [138]. It is possible that the transplant patients with elevated BNP represent those most at risk from development of cardiovascular disease. Indeed, as has been discussed earlier, BNP has been correlated with incident cardiovascular events in hypertensive patients [217].

The difference in BNP levels between the two transplant groups could represent the fact that the hypertensive patients have developed or are in the early stages of

left ventricular systolic dysfunction, which is known to be associated with elevated levels of BNP [132, 133]. Studies in non-transplant patients would suggest that the difference in blood pressure itself does not account for the increase in BNP in the hypertensive group. However, additional information, possibly from echocardiography, is required in order to clarify the relationship between plasma BNP and systolic dysfunction after liver transplantation.

It must be borne in mind that there are a number of confounding factors that should be considered in the interpretation of the levels of BNP. Female liver transplant recipients had a trend towards higher levels of BNP than males, in both hypertensive and normotensive patients. Patient numbers in the non-transplant groups were too small for meaningful gender comparisons to be made. Recent evidence points to there being a sex difference in plasma levels of BNP with women having higher levels than men [214, 218]. The largest study of BNP is Wang's analysis [218] of 911 patients from the Framingham Heart Study [219] who were healthy patients without hypertension, heart disease or heart failure: the strongest predictors of higher BNP levels were female sex and older age. Mean BNP levels were 8.0 ± 12.8 pg/ml and in women 13.9 ± 18.9, giving a combined mean BNP value of 11.7 pg/ml which is similar to our results.

The reasons for the difference are unknown, although an effect of female sex hormones upon BNP gene expression has been proposed [218]. Gender differences in BNP levels has important implications for the use of BNP in clinical practice, including transplant patients, but this has not been taken into account in the studies linking BNP to left ventricular dysfunction. Regarding the influence of age, in Wang's study [218] BNP levels were relatively constant up to the age of 70 but levels then rose considerably in the elderly, perhaps representing subclinical cardiac disease or reduced renal clearance of BNP [220]. Applying this to liver transplant recipients who are generally under the age of 70, it is probably reasonable to assume that age does not need to be factored in to the assessment of plasma BNP levels.

The final confounding factor to consider is that of co-existent antihypertensive medication. Antihypertensive drugs affect plasma levels of BNP in different ways. Beta-blockers are associated with an elevation of BNP levels, and in one study bisoprolol elevated BNP by more than 3-fold compared to placebo [214]. In clinical terms the increase in BNP could be regarded as a beneficial response to the effects of beta-blockade, particularly the resultant vasodilatation and natriuresis that are required to compensate for a reduction in cardiac output and renal perfusion pressure [214, 221]. Such an effect upon BNP levels have obvious implications for the usefulness of BNP as a screening tool for heart failure and for this reason, patients on beta-blockers were excluded from this study.

What of the effects of other drugs? In the same study, Deary et al looked at the effects of several antihypertensive agents on BNP secretion in hypertensive patients. Amlodipine and the thiazide diuretic bendrofluazide caused a significant decrease in BNP levels whereas plasma BNP following treatment with the ACE-inhibitor lisinopril or the alpha-blocker doxazosin was similar to placebo levels. It has also been shown that in patients treated with ACE-inhibitors whose left ventricular mass index falls, plasma BNP also falls. This effect however was related to regression of LVH rather than a direct effect of ACE-inhibition on BNP levels [222]. The liver transplant patients included in this study were treated with a variety of antihypertensive agents. When the plasma BNP levels were compared between those hypertensive patients on no treatment and those on antihypertensive medication, there was no difference (BNP levels of 67.8 ±14 pg/ml versus 71.7 ±17 pg/ml respectively, $P = 0.86$). Thus by excluding patients on beta-blockers, the effect of antihypertensive drugs on the BNP levels in the transplant patients appears to be of no significance.

This study is the first to examine plasma BNP levels in patients following liver transplantation. Half the patients have elevated levels, compared to a local control population. It would be adventurous to claim that elevated levels of BNP in transplant patients imply a risk of heart failure or systolic dysfunction. A rise in BNP may be the cardioprotective response to vasoconstriction due to immunosuppression after transplant and may not be indicative of left ventricular

impairment. Accordingly the high BNP values may not carry the same prognostic significance as in non-transplant hypertension. Certainly it seems unlikely that 48 % of normotensive transplant recipients have LVH, which their elevated BNP levels might suggest. Based on the data from this study, it is doubtful that plasma BNP levels will be useful in screening for or identifying LVH in transplant recipients.

What is now needed is further assessment by echocardiography, particularly of those transplant recipients with elevated BNP levels who were normotensive. If these patients have no LVH then it can be surmised that measurement of BNP will not be helpful, and the elevated levels observed represent the left ventricle's response to tacrolimus induced vasoconstriction. It would be interesting to obtain BNP levels in rapamycin treated patients for comparison. McDonagh et al showed that the negative predictive value in detecting left-ventricular systolic dysfunction of a BNP level below 17.9 pg/ml was 97.5 % [133]. It would be reasonable to surmise that after liver transplant a normal BNP would make the presence of LVH highly unlikely, and this could prove to be the most useful information derived from determining levels of BNP in transplant recipients.

Chapter 5. The effects of conversion from Cyclosporin to Tacrolimus upon cardiovascular risk factors

Introduction

Trials in orthotopic liver transplantation have demonstrated small but clear differences between cyclosporin and tacrolimus with respect to the frequency of acute cellular rejection, refractory rejection and chronic rejection [16, 17, 114]. It has also been suggested that grafts with chronic rejection can be 'rescued' by switching from cyclosporin to tacrolimus [115]. As 5-year survival rates continue to improve there is growing interest in factors that may affect long-term survival after liver transplantation, including the presence or absence of markers of cardiovascular disease. Hypertension, hyperlipidaemia and obesity are encountered frequently in the transplant recipient and may contribute to overall cardiovascular risk. Several studies suggest that cardiovascular risk profiles are more favourable in patients taking tacrolimus rather than cyclosporin. Thus the reported prevalence of hypertension [16, 18-21, 32, 114] and hypercholesterolaemia [19, 20, 32, 34] after transplant are lower with tacrolimus. The development of moderate or severe obesity after transplant has been described in over 34 % of patients with a normal body mass index (BMI) before surgery [22]. A trend towards reduced weight gain after transplantation with tacrolimus instead of cyclosporin has been described [19, 20, 116].

It is sometimes necessary to change immunosuppression from cyclosporin to tacrolimus. This could be because of graft dysfunction with cyclosporin or because a patient has side effects related to cyclosporin. Despite the reported differences between cyclosporin and tacrolimus with respect to development of cardiovascular risk factors there are few data on the effects of conversion from cyclosporin to tacrolimus upon blood pressure, serum lipids and weight. One study of 20 cyclosporin treated liver transplant recipients demonstrated a reduced requirement for antihypertensive medication after tacrolimus was substituted [117]. In another study of 31 patients converted to tacrolimus serum lipid levels fell significantly after three months [118]. To my knowledge, the effect of changing from cyclosporin to tacrolimus upon weight has not been assessed. There is therefore little information on what effect conversion to tacrolimus has upon

cardiovascular risk. I have reviewed the effect of converting 26 patients with and without cardiovascular risk factors from cyclosporin to tacrolimus upon blood pressure, serum lipids, blood glucose and weight. Using the Framingham coronary risk prediction equations I have compared the CHD risk before and after conversion to tacrolimus.

Methods

Patients

The outpatient case records of all 29 liver transplant recipients who had been converted from cyclosporin to tacrolimus over a 24-month period from 1997 to 1999 were evaluated. Three patients who were converted to tacrolimus because of chronic allograft rejection were excluded from the study on the basis that any resulting changes in cardiovascular parameters upon conversion to tacrolimus could be attributed to improvement in graft function rather than the drug alone.

This left 26 patients who were converted from cyclosporin to tacrolimus who had stable graft function during the months preceding conversion. The reasons for switching to tacrolimus are listed in Table 4.1. Six patients who were commenced on tacrolimus with the onset of late acute cellular rejection were included because graft function during the months prior to the episode of rejection had been stable. These patients all responded to 3 days treatment with intravenous methylprednisolone.

Table 5.1. Indication for Conversion to Tacrolimus

	Number Of Patients
Weight gain	8
Late acute cellular rejection	6
Pancytopaenia	1
Neurological symptoms	2
Lethargy	2
Nephrotoxicity	1
Itching	1
Hypertension	2
Hirsutism	1
Gum hypertrophy	1

Cardiovascular parameters

Blood pressure, total serum cholesterol and triglyceride, weight, random blood glucose, liver graft and renal function are collected routinely at each outpatient attendance. Seated blood pressure was measured after a period of rest in the outpatient clinic. Serum lipid and blood glucose levels were determined by an automated chemistry analyser (Dimension RXL, Dade Behring, USA). These parameters were evaluated on 3 outpatient attendances prior to changing immunosuppression. After conversion to tacrolimus and once patients had been established on this for two months, these same measurements were evaluated for the next three outpatient attendances. The time span during which the three sets of measurements were collected varied between patients according to the frequency of outpatient attendance, itself a reflection of graft function and time from transplant. This time varied from a mean of 7 ± 3 months whilst patients were

taking cyclosporin to a mean of 8 ± 3 months once patients were converted to tacrolimus.

The coronary risk prediction equations as used in the Framingham models, and which have been discussed in previous chapters, were used to predict 10-year coronary heart disease risk in the patients before and after conversion to tacrolimus using the data on blood pressure and serum cholesterol that was used in the above analysis together with age, gender, smoking status and presence of diabetes mellitus.

Immunosuppression protocol

All patients were taking cyclosporin to maintain their whole blood trough level between 80-150 μg/L. The day after cessation of cyclosporin, tacrolimus was started at a dose of 0.1mg/kg in two divided doses. The dose was subsequently adjusted to maintain trough plasma levels between 5 and 15 μg/L. Three patients also received azathioprine 75 mg daily. Two patients were on maintenance hydrocortisone for adrenal dysfunction, one of whom was also on azathioprine. Two were taking prednisolone 10 mg daily before immunosuppression conversion and in one of these the dose of prednisolone was reduced to 5 mg daily 4 months after commencing tacrolimus. The remainder of the patients had discontinued steroids prior to the study period.

Statistical analysis

Results are expressed as means ± standard deviation, except for serum triglyceride which is given as the median and range. Comparisons between patients prior to and after conversion to tacrolimus were performed using Student's t-test or McNemar's test as appropriate. A P value of less than 0.05 was considered to represent statistical significance.

Results

Patient characteristics are shown in Table 5.2. One patient developed intense pruritus within weeks of commencing tacrolimus, which had to be discontinued. Cyclosporin was restarted and this patient has been excluded from further statistical analysis.

Table 5.2. Patient Characteristics (N = 25)

Age (years)	48 ± 3	
Sex (M : F	7 : 18	
Median time from transplant to conversion (months)	29 (range 6 – 54)	
Indication for Liver Transplant	Primary biliary cirrhosis	6
	Alcoholic cirrhosis	5
	Fulminant hepatic failure	4
	Primary sclerosing cholangitis	3
	Hepatitis C cirrhosis	1
	Others	6

There was a small reduction of no clinical relevance in serum bilirubin after conversion, from 14.1 to 10.6 mmol/l ($P<0.05$). Aside from this, conversion to tacrolimus had no effect on hepatic or renal function. Thus, there were no significant differences in serum alanine aminotransferase (ALT), albumin, prothrombin time or serum creatinine after conversion to tacrolimus (Table 5.3). No cardiovascular events occurred during the follow-up period. Changes in the cardiovascular risk factors are summarised in Table 5.4.

Table 5.3. Graft and Renal Function Before and After Conversion to Tacrolimus

	Cyclosporin	Tacrolimus	P-Value
Prothrombin Time (s)	14.2 ± 2	14.4 ± 2	NS
ALT (IU/l)	76 ± 50	68 ± 40	NS
Bilirubin (μmol/l)	14 ± 4	11 ± 3	0.004
Albumin (g/dl)	38 ± 5	39 ± 5	NS
Creatinine (μmol/l)	125 ± 34	121 ± 31	NS

NS = not significant to the 5 % level

Table 5.4. Cardiovascular Risk Factors After Conversion to Tacrolimus

	Cyclosporin	Tacrolimus	P-value
Systolic Blood Pressure	158 ± 25	148 ± 22	0.015
Cholesterol (mmol/l)	5.3 ± 0.9	4.9 ± 0.9	0.01
Triglyceride (mmol/l)	1.2 (0.7 – 5.2)	1.2 (0.7 – 4.0)	NS
Weight (kg)	79.4 ± 22.6	76.1 ± 20.1	0.024
Glucose (mmol/l)	6.0 ± 2.7	6.1 ± 2.7	NS

NS = not significant

Results expressed as mean ± standard deviation except triglyceride for which the median and range are shown.

Blood Pressure

One patient was excluded from the blood pressure analysis because atenolol had been prescribed inadvertently for hypertension one week before starting tacrolimus. 10 of the remaining 24 patients were already taking antihypertensive drugs; 5 were on a beta-adrenoceptor antagonist, 2 were on an alpha-adrenoceptor antagonist, one on a calcium channel blocker and 2 patients were receiving three antihypertensive agents. Mean systolic blood pressure fell from 158 ± 25 mmHg to 148 ± 22 mmHg when patients were converted to tacrolimus (P = 0.015). Nineteen patients (79 %) were hypertensive (SBP ≥ 140 mmHg) on cyclosporin whereas 15 (63 %) remained hypertensive on tacrolimus (P = 0.063). There were no new prescriptions or increases in antihypertensive drug dosages during the period of follow-up after conversion to tacrolimus. Systolic blood pressure in the two patients who were converted purely because of hypertension fell by 44 and 18 mmHg in each case.

Serum Lipids

A cholesterol lowering drug was commenced inadvertently in one patient shortly after conversion to tacrolimus and this patient was excluded from statistical analysis, so that cholesterol measurements were available for 24 out of 25 patients. Mean serum cholesterol fell from 5.3 ± 0.9 mmol/l to 4.9 ± 0.9 mmol/l after conversion to tacrolimus (P = 0.01). Hypercholesterolaemia was defined as a serum cholesterol of 5.2 mmol/l (200 mg/dl) or greater. 12 of the 24 patients (50 %) had hypercholesterolaemia when taking cyclosporin; 7 patients (29 %) remained hypercholesterolaemic on tacrolimus (P = 0.063).

Serial data on serum triglyceride levels were available for 22 out of 25 patients. Hypertriglyceridaemia was defined as a serum triglyceride of 1.9 mmol/l (167 mg/dl) or greater. 5 patients (23 %) had hypertriglyceridaemia on cyclosporin compared to 3 (14 %) on tacrolimus, a non-significant change. Median serum triglyceride did not change after conversion, being 1.2 (range 0.7 to 5.2) mmol/l on cyclosporin compared with 1.2 (range 0.7 – 4.0) mmol/l on tacrolimus.

Weight

68 % of patients lost weight on tacrolimus. The mean weight of the patients at the time of conversion was 79.4 kg ± 22.6 kg. A median time of 11 months after commencing tacrolimus the mean weight was 76.1 ± 20.1 kg (P = 0.024). Mean BMI prior to conversion was 29.0 ± 7.8 kg/m^2 compared with 27.8 ± 6.9 kg/m^2 eleven months after commencing tacrolimus (P= 0.02). 16 patients had a BMI over 25 kg/m^2 prior to conversion compared to 13 patients afterwards. In the subgroup of 8 patients who were converted to tacrolimus solely because of post-transplant weight gain, 6 lost weight with mean weight falling from 100.2 ± 24.1 kg at the time of conversion to 92.9 ± 22.2 kg 11 months later. BMI in this group fell from 36.9 ± 7.8 kg/m^2 to 34.2 ± 6.8 kg/m^2.

Association between serum cholesterol, weight loss and blood pressure

Of those patients who lost weight after conversion, 65 % also had a reduction in blood pressure and 76 % had a reduction in serum cholesterol. The fall in serum cholesterol was however, small. Considering only those patients who lost weight, prior to conversion mean serum cholesterol was 5.3 ± 0.4 mmol/l and post conversion it was 5.1 ± 0.5 mmol/l (P=0.302). This compares with a drop in serum cholesterol in those patients who did not lose weight of 0.6 mmol/l from 5.5 ± 0.2 to 4.9 ± 0.2 mmol/l (P=0.001). There was no correlation between weight loss and the reductions in serum cholesterol and blood pressure (P=0.85 and P=0.55 respectively). In the subgroup who were converted because of weight gain, there was a weak correlation between weight loss and cholesterol reduction (Pearson correlation coefficient = 0.708, P=0.049) but no correlation between weight loss and blood pressure reduction.

Blood Glucose

One patient had diabetes mellitus prior to conversion and which predated liver transplantation. No changes in insulin requirements were necessary for this patient, and there was no difference in glycosylated haemoglobin concentration

(HbA1c) after conversion to tacrolimus. There was no difference in mean blood glucose for the remaining patients between cyclosporin and tacrolimus (Table 5.4) and no new cases of diabetes mellitus developed after conversion.

The changes in blood pressure, serum cholesterol, weight and blood glucose are illustrated in Figures 5a, 5b, 5c and 5d.

Fig.5a. Systolic blood pressure pre and post conversion to tacrolimus

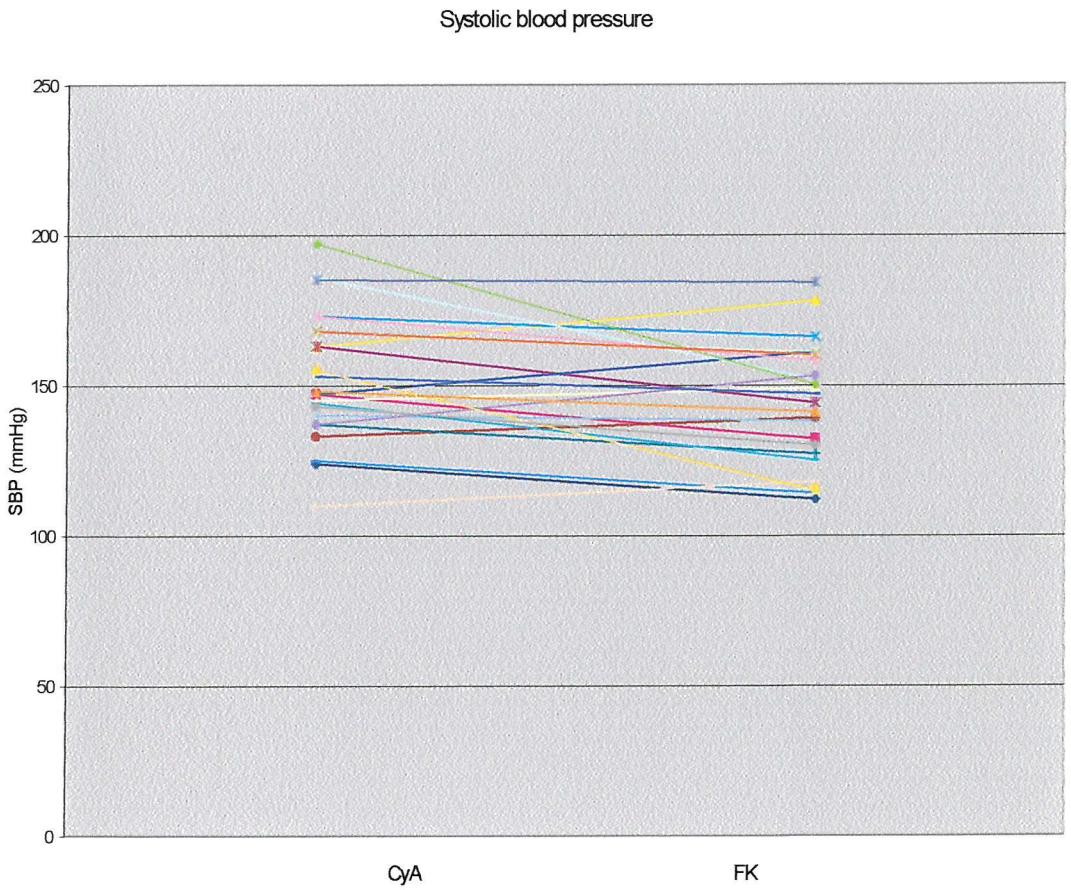


Fig.5.b. Serum cholesterol pre and post conversion to tacrolimus

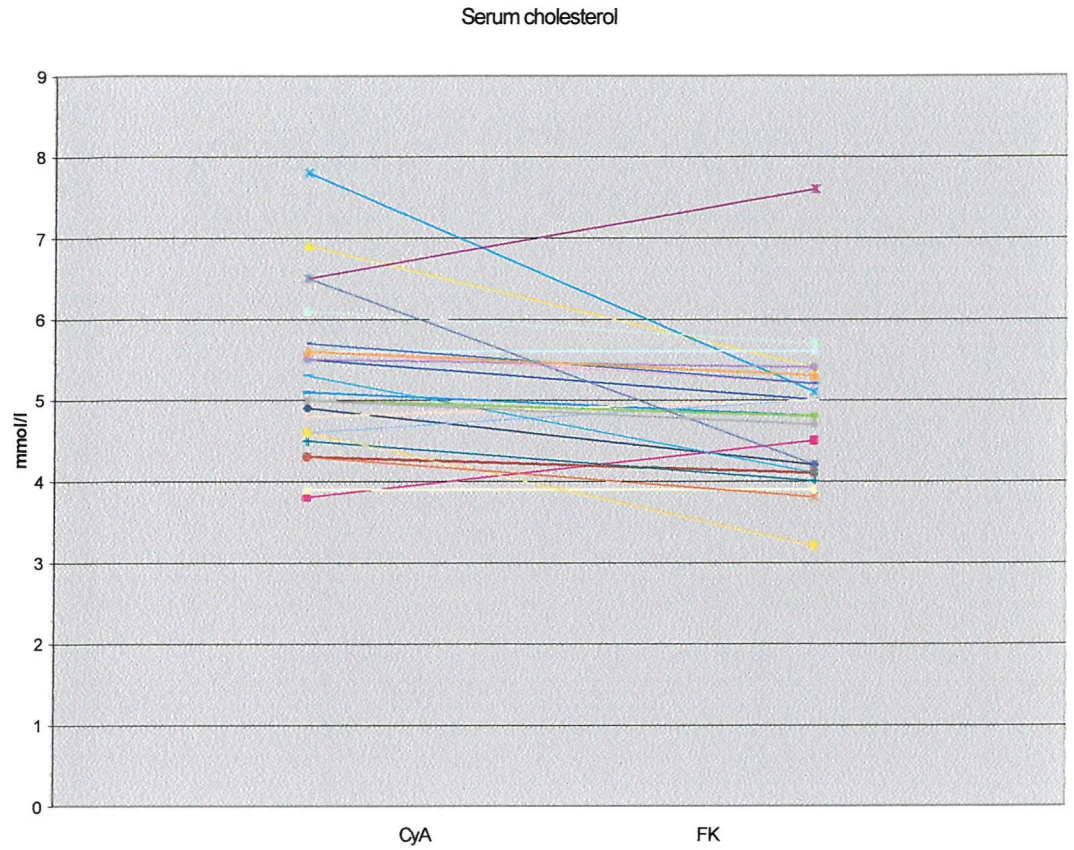


Fig. 5.c. Weight pre and post conversion to tacrolimus

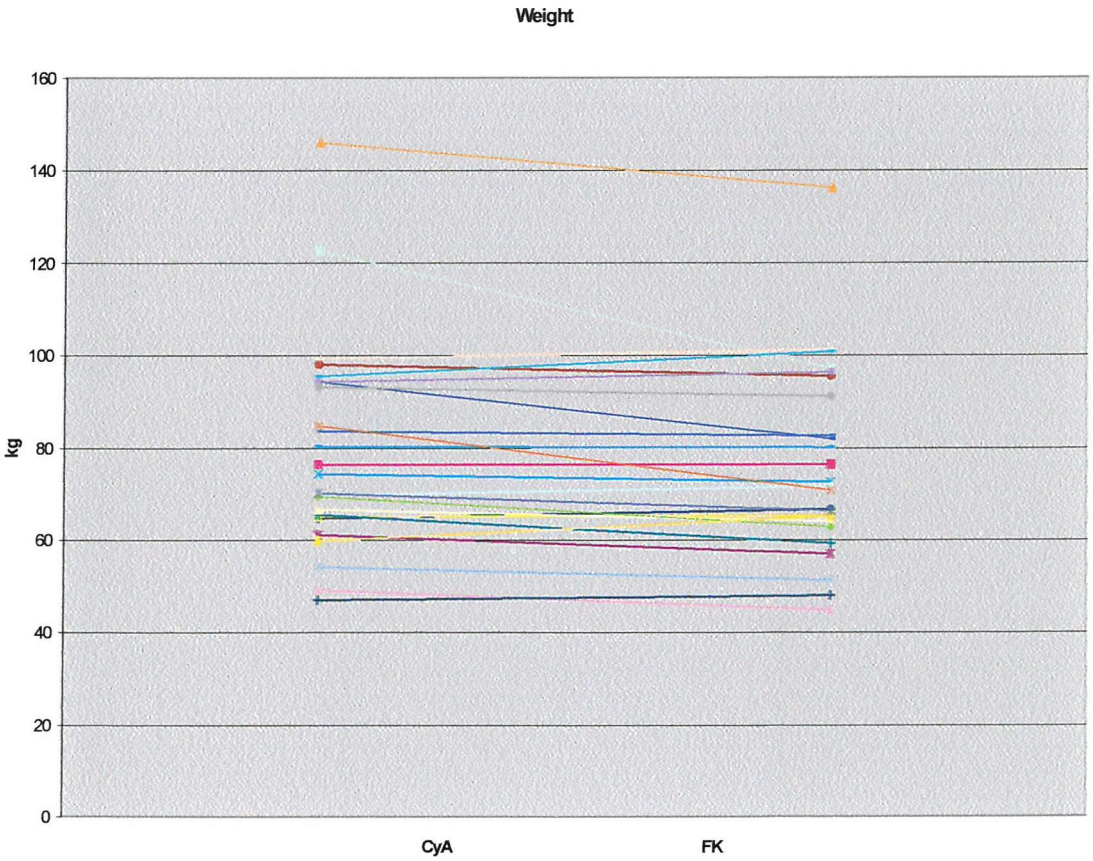
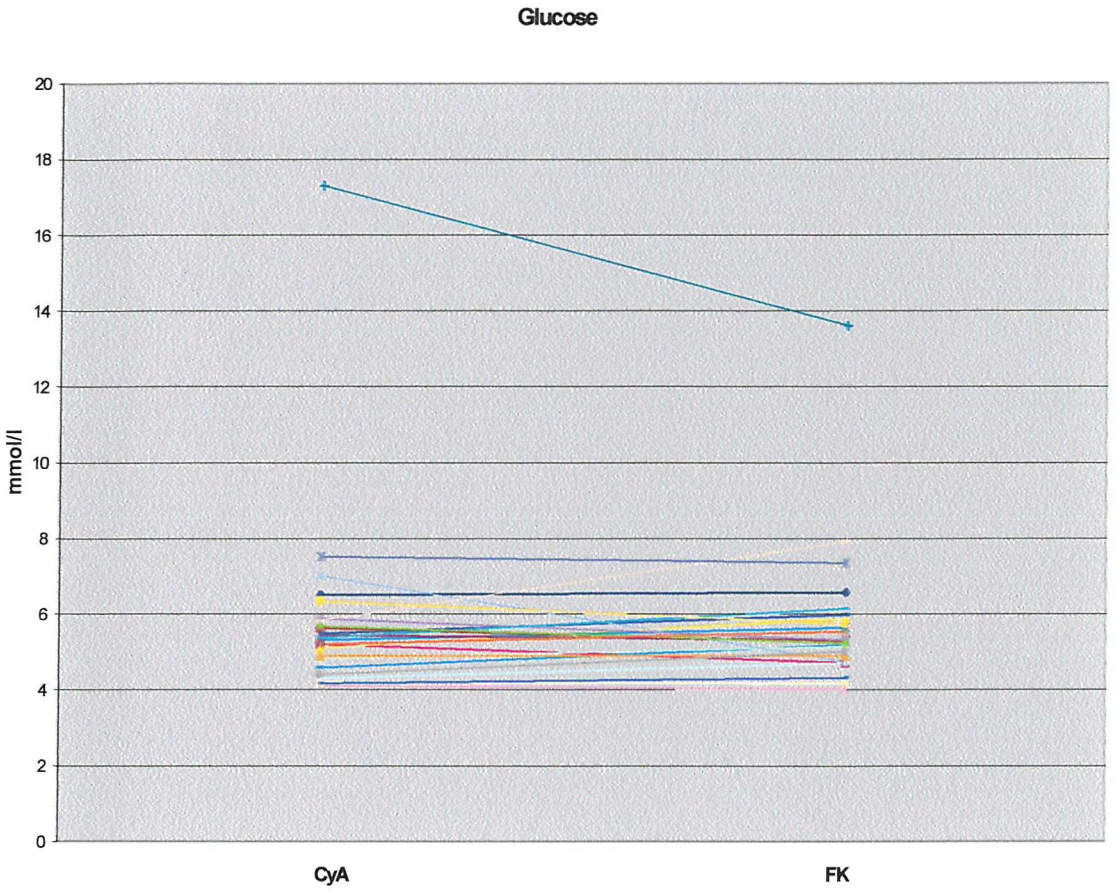


Fig.5.d. Blood glucose pre and post conversion to tacrolimus



Coronary Risk Prediction

Using the Framingham coronary risk prediction equations and inputting systolic blood pressure the mean 10-year coronary risk expressed as a percentage pre-conversion was 13.1 ± 2.1 %. Following conversion to tacrolimus the risk fell to 11 ± 1.8 % ($P < 0.001$).

Discussion

I have shown that conversion to tacrolimus is well tolerated and resulted in significant improvements in a number of cardiovascular risk factors. It has long been recognised that cyclosporin is associated with post-transplant hypertension [208, 223]. More recently it has emerged that tacrolimus based immunosuppressive regimens are associated with hypertension less frequently than cyclosporin [16-21, 114]. Canzanello *et al* reported a prevalence of hypertension at 12 months post-liver transplant of 81 % in cyclosporin and 30 % in tacrolimus treated patients [20]. The same group reported two-year prevalence rates of hypertension of 82 % with cyclosporin and 64 % with tacrolimus [18]. 48 % of cyclosporin treated patients were hypertensive at one year contrasting with 33 % of the tacrolimus group in a series from Fung *et al* [114]. Guckelberger *et al* reported that hypertension occurred in 57 % of long term survivors after liver transplantation treated with cyclosporin compared with 33 % of tacrolimus treated patients [19]. A similar difference at 3 years after transplant was observed in a recent paper by Rabkin *et al* [190].

The mechanisms of post-transplant hypertension are not fully understood and are discussed elsewhere in this thesis. Similarly it is not known why there are differences between tacrolimus and cyclosporin with regard to prevalence of hypertension. There are however, some potential differences between the drugs that could account for the varied effects on blood pressure.

Both drugs cause systemic vasoconstriction, although during the first month after liver transplantation cyclosporin was associated with a progressive and greater rise in systemic resistance index (SVRI) than tacrolimus, and a correspondingly greater rise in blood pressure [28]. Cyclosporin and tacrolimus may interfere with local regulation of vascular tone. Administration of cyclosporin and tacrolimus may be associated with increased circulating levels of endothelin-1, as has been discussed, that may be implicated in transplant hypertension. It is not known whether cyclosporin causes a greater rise in ET-1 than tacrolimus. Cyclosporin may also inhibit endothelial nitric oxide synthesis, which would favour

vasoconstriction [28] and in hypertensive transplant recipients it also alters endothelium-mediated vasodilatation [224]. Altered endothelial function may be important in transplant hypertension and could theoretically account for some of the difference in frequency of hypertension between cyclosporin and tacrolimus [28].

Abnormalities in lipid profiles with elevated serum cholesterol and triglyceride after liver transplant are well documented [23, 33, 36]. The cause of post-transplant dyslipidaemia is multifactorial and includes genetic predisposition, post-transplant diabetes mellitus and chronic renal dysfunction, as well as the effects of corticosteroids and immunosuppressant drugs [24]. Cyclosporin binds to the LDL-cholesterol receptor and may interfere with feedback control of cholesterol synthesis [225] and cyclosporin may also limit cholesterol degradation by reducing bile acid synthesis [226].

As is the case with hypertension, hypercholesterolaemia and hypertriglyceridaemia are observed more frequently with cyclosporin than with tacrolimus. In the long-term follow up of the US Multicenter KF506 Liver Study Group report, there were significance increases in total cholesterol, LDL cholesterol and triglycerides in patients treated with cyclosporin compared to tacrolimus [30]. Similar findings are described at 6 and 12 months post-transplantation by Jindal *et al* [34]. Guckelberger *et al* showed that patients receiving cyclosporin had a significantly higher prevalence of hypercholesterolaemia than patients treated with tacrolimus (76.4 % versus 53.3 %), although there was no significant difference in the prevalence of hypertriglyceridaemia [19]. In a report from the Mayo Clinic, total cholesterol and triglyceride were both significantly lower at 4 and 12 months in patients treated with tacrolimus compared to cyclosporin [20].

The choice of immunosuppression may influence the degree of weight gain after liver transplantation. Canzanello *et al* reported a significant increase in BMI at 12 months with both cyclosporin and tacrolimus compared with pre-transplant BMI

[20]. The relative increase in BMI was slightly greater in patients treated with cyclosporin although not significantly different to tacrolimus. The studies of Canzanello *et al* and Guckelberger *et al* both showed a trend towards an increased prevalence of obesity in patients treated with cyclosporin over tacrolimus although in neither study was the difference between immunosuppression statistically significant [19, 20]. It is not clear why tacrolimus may result in less weight gain than cyclosporin.

Whilst the evidence is in favour of tacrolimus having a more favourable cardiovascular profile than cyclosporin, the impact of changing immunosuppression from cyclosporin to tacrolimus on cardiovascular risk factors in patients with stable graft function has only recently begun to receive attention. Fung *et al* [117] studied 20 patients who were converted to tacrolimus from cyclosporin because of (i) complications relating to cyclosporin, including renal failure and hypertension secondary to cyclosporin toxicity, and/or (ii) uncontrolled liver allograft rejection. Those patients who were hypertensive on cyclosporin were able to discontinue or reduce their antihypertensive medication. Similarly, Pratschke *et al* report that six out of nine patients converted from cyclosporin because of hypertension were able to reduce or discontinue antihypertensive drugs in the 3 months after conversion to tacrolimus [118]. In contrast, it has been recently reported that conversion from cyclosporin to tacrolimus did not result in improvements in blood pressure in any of 16 liver transplant recipients with hypertension [227]. In that study, mean systolic blood pressure was 141 ± 19 mmHg before and 141 ± 18 mmHg after conversion.

In the present study I found that systolic blood pressure fell significantly after conversion to tacrolimus. This change occurred in the absence of any additional antihypertensive therapy and in those patients still on corticosteroids, no significant change in cumulative steroid dose. Furthermore, improvement in blood pressure occurred independently of any effect upon serum creatinine, which did not change after conversion. For those patients with hypertension, the reduction in blood pressure after substituting tacrolimus for cyclosporin could have clinical importance in reducing the need to initiate antihypertensive drugs or to increase

existing treatment. The mean fall in systolic blood pressure of 10 mmHg may appear small, but is comparable to what would be expected by the introduction of an antihypertensive agent.

Pratschke *et al* studied serum lipids in 31 patients with stable graft function who were converted from cyclosporin to tacrolimus because of cyclosporin related side effects [118]. Three months after conversion mean cholesterol and triglyceride levels had fallen significantly. Selzner *et al* noted normalisation of serum cholesterol and triglyceride after 6 months in two patients with hyperlipidaemia converted from cyclosporin to tacrolimus [227]. In a study of 21 patients with hyperlipidaemia a mean of 33 months after transplant, mean serum cholesterol and triglyceride fell, with 55 % of hypercholesterolaemic patients achieving normal serum cholesterol at 3 months [228]. I observed a significant reduction in serum cholesterol but there was no effect upon triglyceride levels. Reduction in serum cholesterol can be influenced by associated weight loss. This is unlikely to have been a factor in the patients studied for there was no correlation between the two parameters in patients who lost weight after conversion.

Of interest in the present study was the effect switching to tacrolimus had upon weight. BMI fell significantly over a median period of 11 months of tacrolimus treatment. Of the eight patients who were converted to tacrolimus solely because of recent weight gain, the mean weight fell from 100.2 kg to 92.9 kg. Although this was a non-significant difference the number of patients is small. In two patients the weight loss was dramatic. A male patient transplanted two years previously whose weight had increased by 30 kg since transplantation lost 22 kg in the twelve months following commencement of tacrolimus. The second patient was a female transplanted 3 years previously. In the 12 months prior to starting tacrolimus her weight had risen from 134 kg to 146 kg. In the 11 months after conversion her weight fell from 146 kg to 136 kg.

The reasons for the observed weight reduction with tacrolimus are not clear. By the time that immunosuppression was changed, patients had already been assessed by a dietician and appropriate weight reducing measures had been attempted. Only

after such measures were undertaken was cyclosporin changed to tacrolimus. No weight reducing measures or formal dietary manipulation were reported by any patient during the period of this study. Only 4 of the 25 patients were receiving corticosteroids (but at a maximum dose of 10 mg prednisolone) and dose reduction occurred in just one patient after conversion, four months after commencing tacrolimus. The daily dose of corticosteroids in the other three patients did not differ between the periods of cyclosporin and tacrolimus treatment. Differences in steroid exposure with cyclosporin and tacrolimus would not appear to account for the observed weight reduction. No patients reported any new gastrointestinal symptoms such as anorexia which could account for weight loss. Whilst it is not clear why such marked weight loss occurs in certain patients, I have shown that for patients on cyclosporin whose weight has increased excessively after transplant switching to tacrolimus is a useful therapeutic manoeuvre to achieve weight reduction.

Recent studies have not reported any difference in the rates of new-onset diabetes between cyclosporin and tacrolimus treated liver transplant recipients [18, 20, 22]. There were no new cases of diabetes mellitus in the patients studied here and no difference in blood glucose when patients were converted to tacrolimus. These findings are in agreement with those previously reported [118] and suggest that converting patients to tacrolimus does not have any diabetogenic effects.

The decrease in 10-year predicted risk of coronary heart disease is an important observation. It suggests that conversion to tacrolimus could have a longer term impact upon CHD and also potentially on survival. Interestingly it has recently been shown that patients with tacrolimus as their primary immunosuppressant have fewer cardiovascular events and a reduced cardiovascular mortality than those treated with cyclosporin after liver transplant [190]. It is plausible therefore that a switch to tacrolimus could impact upon future development of CHD. The main treatable factors governing coronary risk in the transplant recipients are hypertension and/or a high ratio of total to LDL-cholesterol. Switching immunosuppression is clearly one option aimed at reducing predicted and one hopes actual risk of coronary disease. Other options include initiation of

antihypertensive therapy or statins for cholesterol lowering. The means that one employs to reduce coronary risk should be tailored to each individual patient.

In summary, I have demonstrated that switching immunosuppression was well tolerated, with no significant changes in allograft function or in renal function. Only one patient did not tolerate tacrolimus. There was a significant fall in predicted risk of coronary heart disease when patients were converted from cyclosporin to tacrolimus. In addition there were significant benefits realised in reduction of blood pressure, serum cholesterol and weight. Conversion to tacrolimus may eliminate the need for additional drug treatment of hypertension or hypercholesterolaemia, or may allow discontinuation of existing medication. In patients whose weight is increasing I have shown that stopping cyclosporin and commencing tacrolimus can achieve impressive weight loss.

Chapter 6. Hyperuricaemia after Liver Transplantation

Introduction

Hyperuricaemia is a recognised complication of renal and cardiac transplantation [144-151]. Renal dysfunction, such as may occur with cyclosporin, can result in impaired clearance of uric acid by the kidneys and be a cause of hyperuricaemia, whilst hyperuricaemia per se can result in urate nephropathy and worsening renal function [150, 229]. Very few studies have reported hyperuricaemia after liver transplantation. In one of these, transient hyperuricaemia occurred in 14 % during the first year [154]. Gout has been reported after cardiac and renal transplantation and occurs in up to 28 % of renal transplant recipients [145-150]. By contrast, in a series of liver transplant recipients, no cases of gout were reported with cyclosporin immunosuppression despite the presence of hyperuricaemia [154]. At Addenbrooke's Hospital it has been noted that a number of cases of acute gout have occurred in liver transplant recipients. This has led to the study presented here which consists of the findings of a retrospective analysis of 134 consecutive liver transplants examining the incidence of gout but in particular exploring the prevalence of hyperuricaemia in transplant patients. As has been discussed in the general introduction, uric acid is a risk factor for coronary heart disease in the general population. Investigation of hyperuricaemia after liver transplant will throw light on the existence of another coronary risk factor in transplant patients. I have also examined whether patients with hyperuricaemia are at greater risk for coronary heart disease than those with normal serum uric acid levels.

Methods

Patient analysis

The outpatient records of 134 consecutive liver transplant recipients with a functioning allograft beyond six months who received liver transplants between 01/01/94 and 25/11/98 at Addenbrooke's Hospital were evaluated. The three peak serum uric acid levels at any time point after transplant and the corresponding serum creatinine were recorded for all patients. For those patients treated with the xanthine oxidase inhibitor allopurinol, the mean serum creatinine was documented

during the three months prior to and three months after commencement of the drug. Development of gout, a documented previous history of gout, the type of immunosuppression and treatment with diuretics or other drugs known to contribute to hyperuricaemia were recorded. Serum urate and creatinine concentrations were measured by an automated chemistry analyser (Dimension RXL, Dade Behring). Hyperuricaemia was defined, according to the Addenbrooke's biochemistry laboratory reference values, as a serum urate concentration above 0.36 mmol/l for women and 0.45 mmol/l in men on two or more occasions.

Using the Framingham coronary risk prediction equations [46] I have calculated the predicted 10-year risk of coronary artery disease in patients with hyperuricaemia and compared this to the risk in patients with normal serum urate.

Immunosuppression protocol

Cyclosporin was used as maintenance immunosuppression in 75 patients. Cyclosporin was given twice daily in a dose which was adjusted to maintain trough blood cyclosporin levels at 150 – 225 μg/l for the first three months after transplantation, and then at 80 – 150 μg/l subsequently. Tacrolimus was used as maintenance immunosuppression in 59 patients. Tacrolimus was given twice daily in a dose sufficient to maintain trough blood concentrations between 10 – 15 μg/l for the first three months and then at 5 – 15 μg/l subsequently. 3 patients were treated with rapamycin instead of tacrolimus or cyclosporin.

Prednisolone was given in a daily dose of 20 mg for one month and subsequently reducing by 5mg monthly over the next three months. If a satisfactory response to stimulation with adrenocorticotrophic hormone was achieved prednisolone was then discontinued. Azathioprine was commenced at a once daily dose of 75 mg and withdrawn at one year. In patients transplanted for autoimmune hepatitis or primary sclerosing cholangitis with ulcerative colitis, azathioprine was continued long-term.

Statistical analysis

Data are reported as means ± 1 standard error of the mean. The significance of differences between the study populations was analysed with Student's t-test. Fisher's exact test and Pearson's correlation coefficient was used where appropriate. A P value less than 0.05 was considered as significant.

Results

134 patients were included in the analysis. 75 patients (56 %) received cyclosporin and 59 (44 %) received tacrolimus. The male female ratio was 70 : 64. The mean length of time from transplant to data analysis was 39.6 ± 1.4 months

Hyperuricaemia

The overall prevalence of hyperuricaemia was 47 %. An equal percentage of men and women developed hyperuricaemia (Table 6.1). 67 % of patients with hyperuricaemia and 21 % of patients with normal urate levels had a serum creatinine above 125 μmol/l (P<0.001). Serum creatinine was significantly higher in hyperuricaemic than in non-hyperuricaemic patients at 147.5 +/- 6.1 μmol/l and 106.4 +/- 2.9 μmmol/l respectively (P<0.01). The peak uric acid correlated significantly with corresponding serum creatinine with a Spearman Rank correlation coefficient of 0.694 (P<0.001).

The effect of differing immunosuppression was compared. 51 % of cyclosporin treated patients were hyperuricaemic compared to 42 % on tacrolimus (P=not significant) (Table 6.2). In those patients with hyperuricaemia, tacrolimus treated patients had significantly higher serum creatinine than those treated with cyclosporin (175.8 ± 17.4 μmol/l versus 136.2 ± 4.1 μmol/l) (P = 0.039). The number of patients treated with rapamycin was too small for meaningful comparisons to be made, but uric acid levels were normal in all three patients ranging from 0.22 to 0.29 mmol/l.

Table 6.1. Clinical features of hyperuricaemia versus non-hyperuricaemia in liver transplant recipients

	Hyperuricaemia (n=63)	Non-hyperuricaemia (n=71)	P value
Sex (M : F)	33 : 30	37 : 34	NS
Age (year)	52.3 ± 1.4	48.6 ± 1.6	NS
Serum creatinine (μmol/l)	147.5 ± 6.1	106.4 ± 2.9	< 0.01
% pts with creatinine > 125 μmol/l	67	21	< 0.001

NS = not significant

Table 6.2. Effect of immunosuppression on prevalence of hyperuricaemia and on renal function

	Cyclosporin (n = 88)	Tacrolimus (n = 43)	P value
Hyperuricaemic patients (%)	45 (51%)	18 (42%)	NS
Serum creatinine(μmol/l)	136.2 ± 4.1	175.8 ± 17.4	0.039

NS = not significant

This table illustrates that hyperuricaemia affects patients treated with cyclosporin and tacrolimus equally. The serum creatinine was higher in hyperuricaemic patients on tacrolimus.

Gout

Gout was diagnosed according to clinical criteria with hyperuricaemia occurring in the setting of monoarticular arthritis [230, 231]. 8 cases of gout were observed in a total of 134 patients (6%). The male : female ratio was 7:1. 4 occurred in patients taking tacrolimus and 4 with cyclosporin. All eight had hyperuricaemia. Affected joints included wrists (2 patients), knees (2 patients), ankle (2 patients), elbow (1 patient) and metatarsophalangeal (1 patient). The mean time from transplant to the first episode of gout was 25 +/- 5 months. Only one patient with gout had a pre-transplant history of gout. None of these patients were treated with diuretics or other drugs which are known to cause hyperuricaemia.

The patients with gout demonstrated a trend towards higher serum levels of urate and creatinine than the patients with hyperuricaemia who did not have gout, but the differences were not statistically significant (Table 6.3).

Table 6.3. Clinical features of liver transplant recipients with gout versus those with hyperuricaemia but without gout

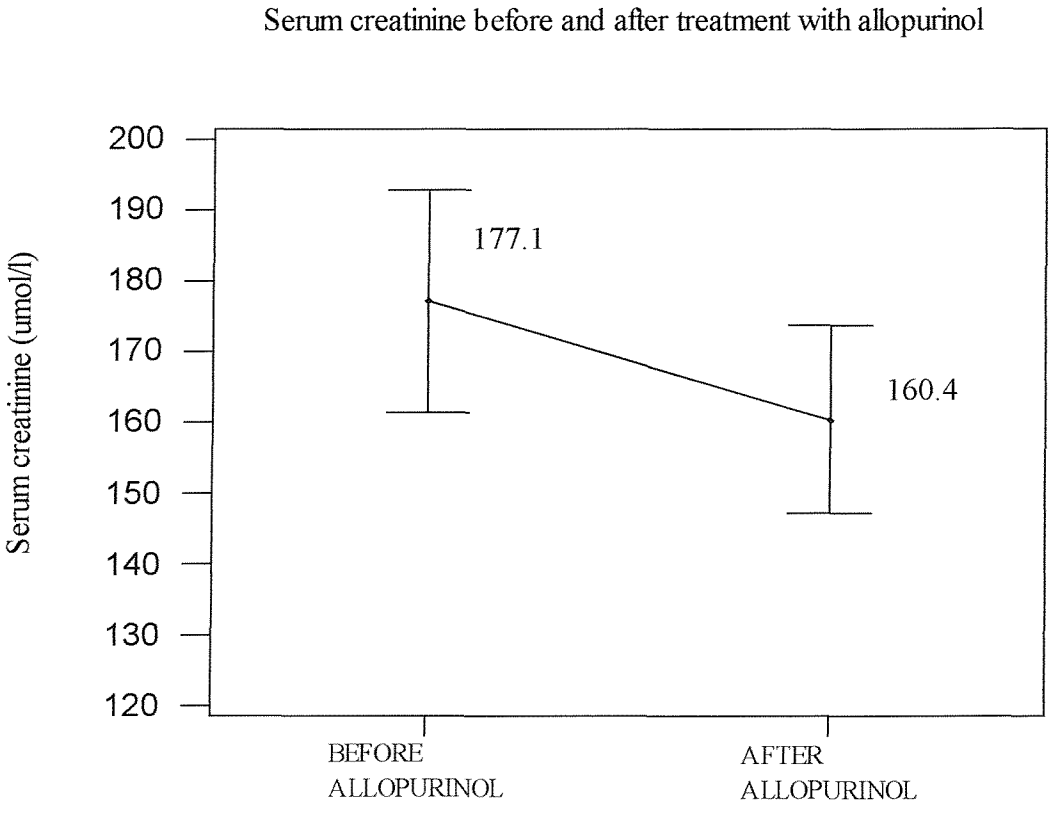
	Gout (n = 8)	Hyperuricaemia without gout (n = 55)	P value
Age (year)	53.9 +/- 0.8	52 +/- 1.6	NS
Sex (M : F)	7 : 1	29 : 26	NS
Serum urate (mmol/l)	0.63 +/- 0.07	0.49 +/- 0.01	NS
Serum creatinine (μmol/l)	191.9 ± 31.4	141 ± 5	NS

NS = not significant

Treatment with allopurinol

All 8 patients with gout were treated with the xanthine oxidase inhibitor allopurinol. These patients all had elevated serum creatinine prior to commencing allopurinol. 10 patients (9 M: 1 F) with hyperuricaemia and high serum creatinine but without gout were also treated with allopurinol. Uric acid levels returned to normal in all patients. Serum creatinine fell in 15 out of the 18 patients. Over a median period of three months treatment with allopurinol the mean serum creatinine fell from 177.1 ± 15.6 μmoll/l to 160.4 ± 13.2 μmol/l (P=0.01) (Figure 6). No significant changes in cumulative dosage of immunosuppression nor in the type of immunosuppression were made during this time. One patient was taking azathioprine and the dose of this drug was halved before allopurinol was commenced.

Figure 6. Serum creatinine in hyperuricaemic patients with and without gout before and after treatment with allopurinol (n = 18)



CHD Risk

The mean 10-year predicted coronary heart disease risk score for patients with hyperuricaemia was 14.1 ± 1 %, whereas that for patients with normal serum urate was 10.1 ± 1 % ($P<0.01$). The risk score was also calculated for patients before and after treatment with allopurinol. This did not change, being 14.2 ± 1 % before and 13.8 ± 1 % after treatment (P=NS).

Cardiovascular events

There were 2 cardiac events during the period of follow-up: one patient had a non-fatal myocardial infarct and one developed angina. Each patient had hyperuricaemia. 2 patients had cerebrovascular accidents, both non-fatal. One of these had hyperuricaemia.

Discussion

I found hyperuricaemia to be common after liver transplantation, occurring in 47 % of patients. This is the largest study to date and has uncovered a far higher prevalence of hyperuricaemia than previous work suggested. There are two principal bodies of work looking into serum uric acid levels after liver transplant. In a study of 59 cyclosporin treated liver transplant recipients Taillandier *et al* found that although serum urate increased by 30 % after transplant only 8 patients developed a transient hyperuricaemia in the first year after transplantation [154]. Van Thiel *et al* showed that serum urate levels increase after liver transplantation, with both cyclosporin and tacrolimus [155]. There was no significant difference between the two immunosuppressants in terms of elevation in serum urate. However, the forty patients were only followed for 20 days after transplant.

The paucity of data for liver transplantation contrasts with renal and cardiac transplants where it has long been recognised that hyperuricaemia occurs as a complication of cyclosporin therapy. Studies report that 30 to 84 % of patients are affected, the prevalence depending somewhat on the definition of hyperuricaemia [144-153]. That almost half the liver transplant recipients had hyperuricaemia is, in my opinion, an important complication of liver transplantation. In common with studies of renal transplantation, we found that hyperuricaemia was associated with renal impairment as suggested by raised serum creatinine [146, 150, 152, 153]. There was a significant correlation between peak uric acid and corresponding serum creatinine. Interestingly tacrolimus treated hyperuricaemic patients had significantly higher serum creatinine than those treated with cyclosporin. I have

not investigated the mechanisms of hyperuricaemia in these patients. Whilst these may be similar to those in renal transplantation additional work should be undertaken investigating uric acid metabolism, not only with cyclosporin as immunosuppression but also with tacrolimus which was associated with hyperuricaemia in 42 % of patients.

An important observation in this study was the effect of treatment of hyperuricaemia on renal function. Of the 18 patients treated with allopurinol, comprising patients with gout and patients with asymptomatic hyperuricaemia, serum uric acid returned to normal in all 18 patients. 15 of these showed a fall in serum creatinine during the first three months of allopurinol treatment, and the mean creatinine of the group fell significantly during this time period. This suggests that hyperuricaemia contributed to the elevation in serum creatinine.

As a complication of hyperuricaemia, it is relevant also to consider the incidence of gout in these patients. Rather like with hyperuricaemia there are few documented cases of gout in liver transplant recipients. No cases of gout were recorded in Taillandier *et al*'s study of 59 liver transplant recipients during the first post-transplant year [154]. Gout has been described in a long term survivor of liver transplantation for glycogen storage disease type 1a [232] and in 4 of 31 patients who reported developing gout by means of a postal questionnaire at three years after transplant [233].

Gout occurred in 6 % of the study population. The mean length of time from transplantation to the presentation of gout was 25 months, similar to that reported following renal and cardiac transplant. None of the patients with gout received treatment with diuretics and only 2 of the hyperuricaemic patients received diuretics during their outpatient follow-up whilst one further patient was treated with hydroxyurea. The relative lack of diuretic use after liver transplantation, as illustrated here, could explain why gout has not been reported as readily as after cardiac or renal transplantation where the use of such drugs is greater [145, 147, 150]. Another factor could be that the doses of cyclosporin used following liver transplantation are less than those used after renal or cardiac transplantation.

Another interesting observation was that the coronary heart disease risk was significantly higher in hyperuricaemic patients than in those with normal serum urate. The simplest interpretation of this is that hyperuricaemia serves as a marker of patients who are at higher risk for coronary disease. It is also conceivable that hyperuricaemia contributes *directly* to the overall cardiovascular risk, particularly given the evidence that elevated serum uric acid is an independent risk factor for not only coronary heart disease but also cerebrovascular disease [157, 164, 165]. There are grounds to speculate on mechanisms for the increased cardiovascular risk seen with hyperuricaemia and these are discussed below.

Uric acid is known to promote low-density lipoprotein oxidation in vitro [234, 235]. Xanthine oxidase has been shown to be an important source of superoxide free radicals and increased uric acid levels are associated with increased production of oxygen free radicals [236], this being an important factor in atherogenesis. Uric acid has been shown to stimulate granulocyte adherence to the endothelium [237] which again serves as an important step in development of atherosclerosis. Elevated uric acid levels are associated with increased platelet adhesiveness and this could contribute to thrombus formation [238]. Uric acid migrates across dysfunctional endothelial cells and accumulates as urate crystals in atherosclerotic plaques [239]. The crystals may then contribute to inflammation and progression of the plaque.

Whether these factors have a bearing on the increased cardiovascular risk of elevated serum uric acid is as yet undetermined. Interestingly, as far as liver transplantation is concerned, hyperuricaemia is recognised as a feature of the metabolic syndrome which was mentioned in Chapter 1. Patients with metabolic syndrome are at heightened cardiovascular risk through the effects of insulin resistance. Serum uric acid does not of course feature in the CHD risk prediction models that are currently in use. Furthermore it was not possible in this study, given the number of patients under follow-up and the small number of observed cardiovascular events, to investigate whether hyperuricaemia is an independent risk factor for cardiovascular disease after liver transplant.

I have reported an important association between liver transplantation and hyperuricaemia. Both cyclosporin and tacrolimus treated patients are affected. In patients with gout and in those with hyperuricaemia and renal impairment, I have shown that treatment with allopurinol results in a significant reduction in serum creatinine. Hyperuricaemia is also a risk factor for cardiovascular disease. I cannot comment at this stage whether hyperuricaemia in liver transplant recipients serves simply as a useful serum marker in individuals at heightened cardiovascular risk or whether it is in fact an independent risk factor for CHD. I suggest that serum uric acid should be monitored after liver transplantation. In those patients found to be hyperuricaemic, close attention should be paid to the existence of other risk factors for CHD.

Final Discussion

One of the most important challenges facing those caring for liver transplant patients in the 21st century is the control of risk factors for cardiovascular disease. Patients are dying less often from infections or graft dysfunction such that 5 - year survival is now 75 % with 10 - year survival not far behind. The effects of ageing as transplant recipients live longer will be such as to increase their risk of cardiovascular disease. Superimposed on this is the high prevalence of risk factors that liver transplantation exposes the recipient too.

There are data emerging to suggest that patients are succumbing to CHD and cerebrovascular disease although the lack of a control population in several of these studies makes it unclear how this mortality differs from the general population. Although the incidence of CHD in patients at Addenbrookes does not differ from the general population during the first 4.5 years after transplant, the number of cardiovascular events was small. However, the predicted probability of developing CHD has been shown to be higher than a matched non-transplant population. I suspect that with more than 10 years of follow-up, the incidence of CHD will increase, perhaps reflecting the increase in risk that I have demonstrated.

Apart from the effects of advancing age, the two principal factors that account for the increase in potential risk of CHD after transplant are hypertension and hypercholesterolaemia, or more precisely a high total cholesterol: HDL-cholesterol ratio. I have demonstrated that hypertension is the most common risk factor for CHD after transplant. Through my study on treatment of hypertension I have identified a batting order for antihypertensive agents, backed up not only by evidence of effective blood pressure control but also through effects upon augmentation index. Thus patients should be started on a calcium channel antagonist such as amlodipine. For those intolerant to this an alternative drug such as lercanidipine may be tolerated. The majority of patients will respond to single agent treatment. Those that do not or in those persistently intolerant of calcium channel blockers, I have shown that lisinopril is more effective at lowering peripheral and central aortic blood pressure than bisoprolol. This could have important implications for long-term left ventricular function. This is an area for further research.

I have shown that plasma endothelin-1 levels are increased in hypertensive transplant recipients at 6 months. With the introduction of endothelin antagonists into the clinical arena, there are exciting possibilities for trialing such drugs in hypertensive patients following liver transplantation.

One area that has not received much attention is that of the loss of nocturnal fall in blood pressure that is observed after liver transplant [209]. By maintaining a higher blood pressure overnight the contribution to risk of CHD may be important. Simple clinic measurements of blood pressure may be insufficient and we possibly should be moving towards 24 hour ambulatory blood pressure monitoring with a view to treatment being directed as much at the nocturnal blood pressure as it currently is to daytime readings. Research into the value of reducing nocturnal blood pressure in transplant recipients with the aim of reduction in cardiovascular risk is required before we can embrace the notion of 24 hour blood pressure monitoring for all our patients.

Elevated serum cholesterol is an important risk factor for CHD in the general population. Hypercholesterolaemia is common after liver transplant and the management of hypercholesterolaemia as a means of reducing CHD risk deserves consideration. Efforts to treat hypercholesterolaemia have focused on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) which inhibit a key rate-limiting enzyme in the pathway for cholesterol biosynthesis in the liver. Several large trials show that statins are effective at lowering cholesterol and reducing mortality from coronary artery disease [240-243]. Such benefits are seen in patients with existing coronary disease but statins can also prevent coronary heart disease in those with risk factors for CHD but who have not yet developed overt disease.

The use of statins after organ transplantation may have been limited by early reports, from heart transplant recipients using high doses of statins, of an increased incidence of myositis and rhabdomyolysis due to interaction with cyclosporin. Perhaps as a result there have been few published reports of the safety and efficacy of statins after liver transplant. More recent studies in both

heart [244-247] and kidney transplant [247-254] recipients have used lower drug doses and have confirmed that statins are well tolerated and effective, with reductions in LDL-cholesterol ranging from 15 % to 42 % in the post-transplant population. There have been only two published trials in liver transplant recipients [255, 256]. In the study of Imagawa [256], pravastatin 20 mg daily given to patients with serum cholesterol above 225mg/dl (5.85mmol/l) was well tolerated, serum cholesterol was reduced by 11 – 17 % at one year and there were no reports of hepatotoxicity. A more recent study looked at just 6 weeks of treatment in which cerivastatin and pravastatin were compared. Both were effective in controlling serum cholesterol [255]. Ideally more data are required to confirm the safety profile of statins in liver transplant recipients in the era of tacrolimus although statins would be expected to interact similarly with cyclosporin and tacrolimus. During the undertaking of this thesis I have also co-ordinated a trial of the safety and efficacy of statins after liver transplant. The results will be available by the end of 2004.

Should patients after liver transplant who develop elevated serum cholesterol be treated with a statin? The majority of transplant patients do not have established CHD as this to a certain extent precludes them from being listed for transplantation. Thus when considering transplant patients with hypercholesterolaemia for treatment with a statin, it is likely that statins would be used as primary prevention against development of CHD. It is recommended that a statin be used for primary prevention in the general population when the 10-year probability of developing CHD is 15 % or greater [257]. The Framingham coronary risk prediction equations [46] or the Joint British Societies Coronary Risk Prediction Charts [258] that are found in the British National Formulary can be used to calculate risk of CHD. I think these guidelines should be employed in the management of transplant patients as a means of identifying and treating those who are at risk from CHD.

By controlling blood pressure to a target of a systolic of < 140 mm Hg and maintaining the serum cholesterol below 5 mmol/l [258] with statin drugs, it is hoped that the risk of CHD in the liver transplant population as a whole will be

minimised. Added to this are the benefits in CHD risk reduction I have shown in converting patients from cyclosporin to tacrolimus, and newer immunosuppressive regimens that require lower doses and shorter duration of corticosteroids. However, it is likely that other factors play a part in the potential for developing CHD after transplant. These include obesity, which is very common after transplant, and possibly elevated serum uric acid. Rather than consider these as separate entities I propose to view them together as features of the metabolic syndrome and suggest that liver transplant recipients manifest features of said syndrome.

The importance of insulin resistance as a risk factor for CHD was first described in 1988 and syndrome X was coined to designate the abnormalities associated with insulin resistance [259]. The syndrome has been renamed and in 2001 The Adult Treatment Panel III designated the constellation of lipid and non-lipid risk factors of metabolic origin the 'metabolic syndrome' [178]. This syndrome is closely linked to insulin resistance and confers an increased risk of CHD. The diagnostic criteria for the metabolic syndrome are listed in Table 1.9. It has recently been estimated that 25 % of adults in the USA meet the criteria for diagnosis [260].

The principle features of the metabolic syndrome are abdominal obesity, dyslipidaemia manifest as high triglyceride and low HDL-cholesterol, hypertension and elevated fasting glucose. However, not having an elevated plasma glucose does not exclude a diagnosis of metabolic syndrome. Indeed, the abnormalities most likely to identify insulin resistance are the changes in triglyceride and HDL-cholesterol [183]. The combination of hypertension and the above dyslipidaemia is strongly suggestive of insulin resistance and most insulin resistant patients will have a fasting glucose below 110mg/dl, the cut off level in the ATP III criteria. Hypertensive patients with the highest ratio of triglyceride to HDL-cholesterol have the greatest risk of CHD [185]. The importance of abdominal obesity is that obesity accentuates the degree of insulin resistance.

A variety of other abnormalities are associated with insulin resistance. These include increased plasma uric acid and reduced renal urate clearance, increased fibrinogen and plasminogen activator inhibitor-1, endothelial dysfunction and polycystic ovary syndrome. The relevance to liver transplantation can be inferred from the data I have acquired. On the basis of hypertension and dyslipidaemia, approximately one third of the patients I studied probably have insulin resistance. The data in chapters 1 and 6 illustrates that a large proportion of patients develop obesity after transplant and almost half have hyperuricaemia. This clustering of abnormalities is certainly supportive of a large number of liver transplant recipients having insulin resistance. Ideally one would have measurements of fasting glucose and insulin as supportive evidence. This is clearly an area for further study.

At present, I would suggest that there is evidence suggesting a link between the cluster of abnormalities encountered in the transplant recipient and insulin resistance. This could have important implications if we are to try and reduce the burden of CHD after liver transplant. Although each component of the syndrome of insulin resistance increases the cardiovascular risk it is the combination of factors that accounts for the heightened risk. If patients with hypertension also have features of insulin resistance, a substantial part of the risk associated with high blood pressure is in fact caused by the other components of the metabolic syndrome, and in particular the lipid abnormalities [186]. Hypertension is the most common risk factor for CHD encountered after liver transplant, but to simply treat the elevated blood pressure may not necessarily achieve the expected benefits in reduction of CHD risk. It is important to address the other features of the metabolic syndrome to have the best chance of reducing the risk of CHD.

A central feature in addressing the metabolic syndrome in liver transplant recipients will be to try and tackle obesity. Excess adiposity and physical inactivity are important lifestyle factors that have an untoward effect on insulin action [183]. It is probable that weight loss accompanied by increased physical exercise will enhance insulin sensitivity and consequently reduce the associated CHD risk factors that are a feature of insulin resistance. It is likely that a

combined approach directed at increasing insulin sensitivity with exercise together with pharmacological intervention to reduce blood pressure and lipid abnormalities will be required to reduce the risk of cardiovascular disease after liver transplantation.

Finally I would suggest that the following studies be undertaken to continue the work presented in this thesis. Firstly, a 10-year follow- up study of cardiovascular morbidity and mortality of the patients presented in Chapter 1. Not only would this provide important data but also the predicted 10-year risk of developing CHD could be then compared with the actual risk. Could calcineurin inhibitors protect against CHD by their possible stimulation of BNP release from the heart? This question could be addressed by extending the work into BNP utilising echocardiography and which has been outlined in Chapter 4. Finally, a clinical trial of endothelin antagonists is warranted. The patients whose hypertension is unresponsive to treatment with calcium channel antagonists and ACE-inhibitors could be targeted for such a trial.

References

1. National Institutes of Health Consensus Development Conference Statement. Liver Transplantation. JAMA 1983; 250:2961-2964.
2. Prince MI, Hudson M. Liver transplantation for chronic liver disease: advances and controversies in an era of organ shortages. Postgrad Med J 2002; 78:135-141.
3. Keeffe EB. Summary of guidelines on organ allocation and patient listing for liver transplantation. Liver Transpl Surg 1998; 4:S108-S114.
4. Lerut JP, Ciccarelli O, Mauel E, et al. Adult liver transplantation and steroid-azathioprine withdrawal in cyclosporine (Sandimmun)-based immunsuppression - 5 year results of a prospective study. Transpl Int 2001; 14:420-428.
5. Keeffe BG, Valantine H, Keeffe EB. Detection and treatment of coronary artery disease in liver transplant candidates. Liver Transpl 2001; 7:755-761.
6. Grant WC, Wasserman F, Rodensky PL, Thomson RV. The incidence of myocardial infarction in portal cirrhosis. Ann Intern Med 1959; 51:774-779.
7. Cigognani C, Malavolti M, Morselli-Labate AM, Zamboni L, Sama C, Barbara L. Serum lipid and lipoprotein patterns in patients with liver cirrhosis and chronic active hepatitis. Arch Intern Med 1997; 157:792-796.
8. Carey W, Dumot J, Pimentel R, et al. The prevalence of coronary artery disease in liver transplant candidates over age 50. Transplantation 1995; 59:859-864.
9. Donovan CL, Marcovitz PA, Punch JD, et al. Two-dimensional and dobutamine stress echocardiography. Transplantation 1996; 61:1180-1188.
10. Plotkin JS, Johnson LB, Rustgi V, Kuo PC. Coronary artery disease and liver transplantation: the state of the art. Liver Transpl 2000; 6(Suppl 1):S53-S56.
11. Froelicher VF, Thompson AJ, Wolthius R, et al. Angiographic findings in asymptomatic aircrew men with electrocardiographic abnormalities. Am J Cardiol 1977; 39:32-38.

12. Johnston SD, Morris JK, Cramb R, Gunson BK, Neuberger J. Cardiovascular morbidity and mortality after orthotopic liver transplantation. Transplantation 2002; 73:901-906.
13. Pruthi J, Medkiff KA, Esrason KT, et al. Analysis of causes of death in liver transplant recipients who survived more than 3 years. Liver Transpl 2001; 7:811-815.
14. Rabkin JM, de la Melena V, Orloff SL, Corless CL, Rosen HR, Olyaei AJ. Late mortality after orthotopic liver transplantation. Am J Surg 2001; 181:475-479.
15. Abbasoglu O, Levy MF, Brkic BB, et al. Ten years of liver transplantation: an evolving understanding of late graft loss. Transplantation 1997; 64:1801-1807.
16. Group EFMLS. Randomised trial comparing tacrolimus and cyclsporin in prevention of liver allograft rejection. Lancet 1994; 344:423-428.
17. Group TUMFLS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994; 331:1110-1115.
18. Canzanello VJ, Textor SC, Taler SJ, et al. Late hypertension after Liver transplantation: A comparison of cyclosporine and tacrolimus (FK 506). Liver Transpl Surg 1998; 4:328-334.
19. Guckelberger O, Bechstein WO, Neuhaus R, et al. Cardiovascular risk factors in lomg-term follow-up after orthotopic liver transplantation. Clin Transplantation 1997; 11:60-65.
20. Canzanello VJ, Schwartz L, Taler SJ, et al. Evolution of cardiovascular risk after liver transplantation: a comparison of cyclosporine A and tacrolimus (FK506). Liver Transpl Surg 1997; 3:1-9.
21. Jain A, Reyes J, Kashyap R, et al. What have we learned about primary liver transplantation under tacrolimus immunosuppression? Ann Surg 1999; 230:441-449.
22. Sheiner PA, Magliocca JF, Bodian CA, et al. Long-term medical complications in patients surviving > 5 years after liver transplant. Transplantation 2000; 69:781-789.

23. Stegall MD, Everson G, Schroter G, Bilir B, Karrer F, Kam I. Metabolic complications after liver transplantation. Diabetes, hypercholesterolaemia, hypertension, and obesity. Transplantation 1995; 60:1057-1060.
24. Munoz SJ. Hyperlipidaemia and other coronary risk factors after orthotopic liver transplantation: Pathogenesis, diagnosis, and management. Liver Transpl Surg 1995; 1(Suppl 1):29-38.
25. Pichlmayr R, Winkler M, Neuhaus P, et al. Three-year follow-up of the European Multicenter Tacrolimus (FK506) Liver Study. Transpl Proc 1997; 29:2499-2502.
26. Ramsay LE, Williams B, Johnston GD, et al. Guidelines for management of hypertension: report of the third working party of the British Hypertension Society. Journal of Human Hypertension 1999; 13:569-592.
27. Textor SC, Canzanello VJ, Taler SJ, et al. Cyclosporine-induced hypertension after transplantation. Mayo Clin Proc 1994; 69:1182-1193.
28. Textor SC, Wiesner R, Wilson DJ, et al. Systemic and renal hemodynamic differences between FK506 and cyclosporine in liver transplant recipients. Transplantation 1993; 55:1332-1339.
29. Textor SC, Taler SJ, Canzanello VJ, Schwartz L, Augustine JE. Posttransplantation hypertension related to calcineurin inhibitors. Liver Transpl 2000; 6:521-530.
30. Abouljoud MS, Levy MF, Klintmalm GB, and the US Multicenter Study Group. Hyperlipidaemia after liver transplantation: long-term results of the FK506/Cyclosporine a US Multicenter Trial. Transpl Proc 1995; 27:1121-1123.
31. Munoz SJ. Progress in posttransplant hyperlipidaemia. Liver Transpl Surg 1997; 3:439-442.
32. Charco R, Cantarell C, Vargas V, et al. Serum cholesterol changes in long-term survivors of liver transplantation: a comparison between cyclosporine and tacrolimus therapy. Liver Transpl Surg 1999; 5:204-208.
33. Munoz SJ, Deems RO, Moritz MJ, Martin P, Jarrell BE, Maddrey WC. Hyperlipidaemia and obesity after orthotopic liver transplantation. Transplant Proc 1991; 23:1480-1483.

34. Jindal RM, Popescu I, Emre S, et al. Serum lipid changes in liver transplant recipients in a prospective trial of cyclosporine versus FK506. Transplantation. Transplantation 1994; 57:1395-1398.
35. Steinmuller TM, Graf K-J, Schleicher J, et al. The effect of FK506 versus cyclosporine on glucose and lipid metabolism - a randomised trial. Transplantation 1994; 58:669-674.
36. Gisbert C, Prieto M, Berenguer M, et al. Hyperlipidaemia in liver transplant recipients: prevalence and risk factors. Liver Transpl Surg 1997; 3:416-422.
37. Taler SJ TS, Canzanello VJ, Schwartz L, Porayko M, Wiesner RH, Krom RA. Role of steroid dose in hypertension early after liver transplantation with tacrolimus (FK 506) and cyclosporine. Transplantation 1996; 62:1588-1592.
38. Stegall MD, Everson GT, Schroter G, et al. Prednisolone withdrawal late after adult liver transplantation reduces diabetes, hypertension, and hypercholesterolaemia without causing graft loss. Hepatology 1997; 25:173-177.
39. Everhart JE, Lombardero M, Lake JR, Wiesner RH, Zetterman RK, Hoofnagle JH. Weight change and obesity after liver transplantation. Liver Transpl Surg 1998; 4:285-296.
40. Palmer M, Schaffner F, Thung SN. Excessive weight gain after liver transplantation. Transplantation 1991; 51:797.
41. Mor E, Facklam D, Hasse J, et al. Weight gain and lipid profile changes in liver transplant recipients: long-term results of the American FK506 multicentre study. Transplant Proc 1995; 27:1126.
42. Navasa M, Bustamante J, Marroni C, et al. Diabetes mellitus after liver transplantation: prevalence and predictive factors. J Hepatol 1996; 25:64-71.
43. Baid S, Cosimi AB, Farrell ML, et al. Posttransplant diabetes mellitus in liver transplant recipients: risk factors, temporal relationship with hepatitis C virus allograft hepatitis, and impact on mortality. Transplantation 2001; 72:1066-1072.
44. Everson G, Trouillot T, Wachs M, et al. Early steroid withdrawal in liver transplantation is safe and beneficial. Liver Transpl Surg 1999; 5:328-334.

45. Trotter JF, Wachs M, Bak T, et al. Liver Transplantation Using Sirolimus and Minimal Corticosteroids (3-Day Taper). Liver Transpl 2001; 7:343-351.
46. Anderson KM, Odell PM, Wilson PWF, Kannel WB. Cardiovascular disease risk profiles. Am Heart J 1991; 121:293-298.
47. Kowalski HJ, Abelmann WH. Cardiac output in Laennec cirrhosis. J Clin Invest 1953; 32:1356-1358.
48. Golub MS, Berger ME. Direct augmentation by cyclosporine A of vascular contractile response to nerve stimulation. Hypertension 1987; 9:96-100.
49. Brown NJ, Vaughn DE. Angiotensin-converting enzyme inhibitors. Circulation 1998; 97:1411-1420.
50. Kurtz A, Della Bruna R, Kuhn KW. Cyclospoine A enhances renin secretion and production in isolated juxtaglomerular cells. Kidney Int 1988; 33:947.
51. Murray BM, Paller MS, Ferris TE. Effect of cyclosporine administration on renal haemodynamics in the conscious rat. Kidney Int 1985; 28:767-774.
52. Barroe E, Boim MA, Ajzarn H, Ramos OL, Schor N. Glomerular haemodynamics and hormonal participation in cyclosporine nephrotoxicity. Kidney Int 1987; 32:19-25.
53. Nahman NSJ, Cosio FG, Henry ML, Ferguson RM. Cyclosporine nephrotoxicity in spontaneously hypertensive rats. Transplantation 1988; 45:768-772.
54. Avdonin PV, Cottet-Marie F, Afanasjeva GV, Loktionova SA, Lhote P, Ruegg UT. Cyclosporine A upregulates angiotensin II receptors and calcium responses in human vascular smooth muscle cells. Kidney Int 1999; 55:2407-2414.
55. Navasa M, Feu F, Garcia-Pagan JC, et al. Haemodynamic and humoural changes after liver transplantation in patients with cirrhosis. Hepatology 1993; 17:355-360.
56. Lee DB. Cyclosporine and the renin-angiotensin axis. Kidney Int 1997; 52:248-260.

57. Textor SC, Canzanello VJ, Taler SJ, et al. Activation of the renin-angiotensin system and sustained endothelin release two years after liver transplantation. Circulation 1994; 90:I-73.

58. Julien J, Farge D, Kreft-Jais C, et al. Cyclosporine-induced stimulation of the renin-angiotensin system after liver and heart transplantation. Transplantation 1993; 56:885-891.

59. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332:411-415.

60. Vierhapper H, Wagner O, Nowotny P, Waldhausl W. Effect of endothelin-1 in man. Circulation 1990; 81:1415-1418.

61. Maguire JJ, Davenport AP. ETA(subscript) receptor-mediated constrictor responses to endothelin peptides in human blood vessels in vitro. Br J Pharmacol 1995; 115:191-197.

62. Watschinger B, Sayegh MH. Endothelin in organ transplantation. American Journal of Kidney Diseases 1996; 27:151-161.

63. Bacon CR, Cary NR, Davenport AP. Distribution of endothelin receptors in atherosclerotic human coronary arteries. J Cardiovasc Pharmacol 1995; 26:S439-S441.

64. Murray BM, Paller MS, Ferris TF. Effect of cyc;losporine administration on renal haemodynamics in conscious rats. Kidney Int 1985; 28:767-774.

65. Min DI, Chen HY, Lee MK, Ashton K, Martin MF. Time-dependent disposition of tacrolimus and its effects on endothelin-1 in liver allograft recipients. Pharmacotherapy 1997; 17:457-463.

66. Grieff M, Loertscher R, Shohaib SA, Stewart DJ. Cyclosporine-induced elevation in circulating endothelin-1 in patients with solid-organ transplants. Transplantation 1993; 56:880-884.

67. Bokemeyer D, Friedrichs U, Baecker A, Drechsler S, Kramer HJ, Meyer-Lehnert H. Atrial natriuretic peptide inhibits cyclosporine-A induced endothelin production and calcium accumulation in rat vascular smooth muscle cells. Clin Sci 1994; 87:383-387.

68. Bunchman TE, Brookshire CA. Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. J Clin Invest 1991; 88:310-314.

69. Deray G, Carayon A, Le Hoang P. Increased endothelin level after cyclosporine therapy. Ann Intern Med 1991; 114:809-809.
70. Fabrega E, Figols J, Duenas C, et al. Value of endothelin in cellular rejection after liver transplantation. Clin Transplant 1997; 11:25-28.
71. Lerman A, Click RL, Narr BJ, et al. Elevation of plasma endothelin associated with systemic hypertension in humans following orthotopic liver transplantation. Transplantation 1991; 51:646-650.
72. Textor SC, Burnett JCJ, Romero JC, et al. Urinary endothelin and renal vasoconstriction with cyclosporine or FK506 after liver transplantation. Kidney Int 1995; 47:1426-1433.
73. Denucci G, Thomas R, D'Orleans-Juste P, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc Natl Acad Sci USA 1988; 85:9797-9800.
74. Cifkova R, Pit'ha J, Trunecka P, et al. Blood pressure, endothelial function and circulating endothelin concentrations in liver transplant recipients. J Hypertens 2001; 19:1359-1367.
75. Lanese DM, Conger JD. Effects of endothelin receptor antagonists on cyclosporine-induced vasoconstriction in isolated rat renal arterioles. J Clin Invest 1993; 91:2144-2149.
76. Kivlighn SD, Gabel RA, Siegl PKS. Effects of BQ-123 on renal function and acute cyclosporine-induced renal dysfunction. Kidney International 1994; 45:131-136.
77. Nakov R, Pfarr E, Eberle S, Investigators H. Darusentan: an effective endothelin A receptor antagonist for treatment of hypertension. Am J Hypertens 2002; 15:583-589.
78. O'Rourke MF. Mechanical principles. Arterial stiffness and wave reflection. Pathol Biol 1999; 47:623-633.
79. Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. Hypertension 1999; 33:1111-1117.
80. Wilkinson IB, Fuchs SA, Jansen IM, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. Journal of Hypertension 1998; 16:2079-2084.

81. Benetos A, Rudnichi A, Safar M, Guize L. Pulse pressure and cardiovascular mortality in normotensive and hypertensive subjects. Hypertension 1998; 32:560-564.

82. Wilkinson IB, MacCallum H, Hupperetz PC, Van Thoor CJ, Cockcroft JR, Webb DJ. Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man. J Physiol 2001; 530:541-550.

83. Mahomed FA. The physiological and clinical use of the sphygmograph. Medical Times Gazette 1872; 1:62-64.

84. O'Rourke MF, Gallagher DE. Pulse wave analysis. Journal of Hypertension 1996; 14:S147-S157.

85. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Eur Heart J 1993; 14:160-167.

86. Latham RD, Westerhof N, Sipkema P, Rubal BJ, Reuderink P, Murgo J. Regional wave travel and reflections along the human aorta: a study with six simultaneousmicromanometric pressures. Circulation 1985; 72:1257-1269.

87. Nichols WW, O'Rourke M. McDonald's blood flow in arteries. Theoretical, experimental and clinical principles. London: Arnold, 1998.

88. Pauca AL, Wallenhaupt ST, Kon ND, Tucker WY. Does radial artery pressure accurately reflect aortic pressure? Chest 1992; 102:1193-1198.

89. Kelly R, Hayward C, Avolio AP, O'Rourke MF. Non-invasive determination of age-related changes in the human arterial pulse. Circulation 1989; 80:1652-1659.

90. Avolio AP, Fa-Quan D, Wei-Qiang L. Effects of ageing on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. Circulation 1985; 71:202-210.

91. O'Rourke MF, Kelly MP. Wave reflection in the systemic circulation and its implications in ventricular function. Journal of Hypertension 1993; 11:327-337.

92. McVeigh GE, Brennan G, Cohn JN, Finkelstein S, Hayes R, Johnston D. Reduced vascular compliance as a marker for essential hypertension. Am J Hypertens 1991; 4:245-251.
93. Westerhof N, O'Rourke MF. Haemodynamic basis for the development of left ventricular failure in systolic hypertension and for its logical treatment. Journal of Hypertension 1995; 13:943-952.
94. Saba PS, Roman MJ, Pini R, Spitzer M, Ganu A, Devereux RB. Relation of arterial pressure waveform to left ventricular and carotid anatomy in normotensive subjects. Journal of the American College of Cardiology 1993; 22:1873-1880.
95. Lakatta EG. Similar myocardial effects of ageing and hypertension. European Heart Journal 1991; 11:29-38.
96. Heintz B, Door R, Gillessen T. Do arterial endothelin-1 levels affect local arterial stiffness? Am Heart J 1993; 126:987-989.
97. Vuurmans TJL, Boer P, Koomans HA. Effects of endothelin-1 and endothelin-1 receptor blockade on cardiac output, aortic pressure, and pulse wave velocity in humans. Hypertension 2003; 41:1253-1258.
98. Ting CT, Brin KP, Lin SJ, et al. Arterial haemodynamics in human hypertension. J Clin Invest 1986; 78:1462-1471.
99. Ting CT, Chou CY, Chang MS, Wang SP, Chiang BN, Yin FCP. Arterial haemodynamics in human hypertension. Circulation 1991; 84:1049-1057.
100. Ting CT, Chen JW, Chang MS, Yin FCP. Arterial haemodynamics in human hypertension. Effects of the calcium channel antagonist nifedipine. Hypertension 1995; 25:1326-1332.
101. Ting CT, Yang TM, Chen JW, Chang MS, Yin FC. Arterial haemodynamics in human hypertension. Effects of angiotensin converting enzyme inhibition. Hypertension 1993; 22:839-846.
102. Chen CH, Ting CT, Lin SJ, et al. Different effects of fosinopril and atenolol on wave reflections in hypertensive patients. Hypertension 1995; 25:1034-1041.
103. Gonwa TA. Hypertension and renal dysfunction in long-term liver transplant recipients. Liver Transplantation 2001; 7:S22-S26.

104. Duvoux C, Cherqui D, Di Martino V, et al. Nicardipine as antihypertensive therapy in liver transplant recipients: results of long-term use. Hepatology 1997; 25:430-433.
105. Lindholm A, Henricsson S. Verapamil inhibits cyclosporine metabolism. Lancet 1987; 1:1262-1263.
106. Pochet JM, Pirson Y. Cyclosporin-diltiazem interaction. Lancet 1986; 1:979.
107. Taler SJ, Textor SC, Canzanello VJ, et al. Hypertension after liver transplantation. American Journal of Hypertension 2000; 13:231-239.
108. Textor SC, Schwartz L, Augustine J, Wilson DJ, Wiesner RH. Hypertension after transplantation: haemodynamic and renal effects of labetalol and nifedipine monotherapy. Am J Hypertens 1992; 5:9A.
109. Slapak GI, Saxena R, Portmann B, et al. Graft and systemic disease in long-term survivors of liver transplantation. Hepatology 1997; 25:195-202.
110. O'Grady JG, Forbes A, Rolles K, Calne RY, Williams R. An analysis of cyclosporine efficacy and toxicity after liver transplantation. Transplantation 1988; 45:575-579.
111. Williams R, Blackburn A, Neuberger J, Calne RY. Long-term use of cyclosporin in liver grafting. QJM 1985; 224:897-905.
112. Mervaala E, Lassila M, Vaskonen T, et al. Effects of ACE inhibition on cyclosporine A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats on a high-sodium diet. Blood Press 1999; 8:49-56.
113. Busuttil RW, Holt CD. Tacrolimus is superior to cyclosporine in liver transplantation. Transpl Proc 1998; 30:2174-2178.
114. Fung J, Abu-Elmagd K, Jain A, et al. A randomised trial of primary liver transplantation under immunosuppression with FK506 vs cyclosporin. N Engl J Med 1991; 23:2977-2983.
115. Sher LS, Cosenza CA, Michel J, et al. Efficacy of tacrolimus as rescue therapy for chronic rejection in orthotopic liver transplantation. Transplantation 1997; 64:258-263.
116. Mor E, Facklam D, Hasse J, et al. Weight gain and lipid profile changes in liver transplant recipients: long-term results of the American FK506 multicenter study. Transplantation Proceedings 1995; 27:1126.

117. Fung JJ, Todo S, Jain A, et al. Conversion from cyclosporine to FK506 in liver allograft recipients with cyclosporine-related complications. Transpl Proc 1990; 22(Suppl 1):S6-S12.
118. Pratschke J, Neuhaus R, Tullius SG, et al. Treatment of cyclosporine-related adverse effects by conversion to tacrolimus after liver transplantation. Transplantation 1997; 64:938-940.
119. Malekkiani N, Durand F, Bernuau J, R, et al. Conversion from cyclosporin to FK506 in adult liver transplantation: results from a North American and European series. Hepatology 2000; 32:741(Abstract).
120. Levy D, Garrison RJ, Savage DD. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham heart study. N Engl J Med 1990; 322:1561-1566.
121. Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. Annals of Internal Medicine 1991; 114:345-352.
122. Nishikimi T, Yoshihara F, Morimoto A, et al. Relationship between left ventricular geometry and natriuretic peptide levels in essential hypertension. Hypertension 1996; 28:22-30.
123. Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. Nature 1988; 332:78-81.
124. Kambayashi Y, Nakao K, Mukoyama M. Isolation and sequence determination of human brain natriuretic peptide in human atrium. FEBS Lett 1990; 259:341-345.
125. Mukoyama M, Nakao K, Saito Y. Human brain natriuretic peptide, a novel cardiac hormone. Lancet 1990; 335:801-802.
126. Yasue H, Yoshimura M, Sumida H, et al. Localisation and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994; 90:195-203.
127. Burnett Jr JC, Kao PC, Hu DC. Atrial natriuretic peptide elevation in congestive heart failure in man. Science 1986; 231:1145-1147.

128. Omland T, Aakvaag A, Bonarjee VVS. Plasma brain natriuretic peptide as an indicator of left ventricular systolic function and long-term survival after acute myocardial infarction. Circulation 1996; 93:1963-1969.
129. de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. Lancet 2003; 362:316-322.
130. Kohno M, Horio T, Yokokawa K, et al. Brain natriuretic peptide as a marker for hypertensive left ventricular hypertrophy: changes during 1-year antihypertensive therapy with angiotensin-converting enzyme inhibitor. Am J Med 1995; 98:257-265.
131. Kohno M, Horio T, Yokokawa K, et al. Brain natriuretic peptide as a cardiac hormone in essential hypertension. Am J Med 1992; 92:29-34.
132. Yamamoto K, Burnett JCJ, Jougasaki M, et al. Superiority of brain natriuretic peptide as a hormonal marker of ventricular systolic and diastolic dysfunction and ventricular hypertrophy. Hypertension 1996; 28:988-994.
133. McDonagh TA, Robb SD, Murdoch DR, et al. Biochemical detection of left-ventricular systolic dysfunction. Lancet 1998; 351:9-13.
134. Berger R, Huelsman M, Strecker K, et al. B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. Circulation 2002; 105:2392-2397.
135. Richards AM, Nicholls MG, Yandle TG, et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. Circulation 1998; 97:1921-1929.
136. Hirata Y, Matsumoto A, Aoyagi T, et al. Measurement of plasma brain natriuretic peptide level as a guide for cardiac overload. Cardiovasc Res 2001; 15:585-591.
137. Cowie MR, Struthers AD, Wood DA. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. Lancet 1997; 350:1349-1353.
138. Suzuki M, Yamamoto K, Watanabe S, Iwata T, Hamada M, Hiwada K. Association between elevated brain natriuretic peptide levels and the development of left ventricular hypertrophy in patients with hypertension. Am J Med 2000; 108:627-633.

139. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002; 347:161-167.
140. Kohn PM, Prozan GB. Hyperuricaemia: relationship to hypercholesterolaemia and acute myocardial infarction. JAMA 1959; 170:1909-1915.
141. Dobson A. Is raised serum uric acid a cause of cardiovascular disease or death? Lancet 1999; 354:1578.
142. Emmerson BT. The management of gout. N Engl J Med 1996; 334:445-451.
143. Steele TH. Hyperuricaemic nephropathies. Nephron 1999; 81(Suppl 1):45-49.
144. Najarian JS, Fryd DS, Strand M, Canafax DM, Ascher NL, Payne WD, et al. A single institution, randomised, prospective trial of cyclosporin versus azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. Ann Surg 1985; 201:142-157.
145. Burack DA, Griffith BP, Thompson ME, Kahl LE. Hyperuricaemia and gout among heart transplant recipients receiving cyclosporine. Am J Med 1992; 92:141-146.
146. Delaney V, Sumrani N, Daskalakis P, Hong JH, Sommer BG. Hyperuricemia and gout in renal allograft recipients. 1992.
147. West C, Carpenter BJ, Hakala TR. The incidence of gout in renal transplant recipients. Am J Kid Dis 1987; 10:369-372.
148. Tiller DJ, Hall BM, Horvarth JS, Duggin GG, Thompson JF, Sheil AG. Gout and hyperuricaemia in patients on cyclosporin and diuretics. Lancet 1985; 1:453.
149. Noordzij TC, Leunissen KML, Van Hooff JP. Renal handling of urate and the incidence of gouty arthritis during cyclosporine and diuretic use. Transplantation 1991; 52:64-67.
150. Lin HY, Rocher LL, McQuillan MA, Schmaltz S, Palella TD, Fox IH. Cyclosporine-induced hyperuricemia and gout. N Engl J Med 1989; 321:287-292.
151. Kahan BD, Flechner SM, Lorber MI, Golden D, Conley S, Van Buren CT. Complication of cyclosporin-prednisone immunosuppression in 402 renal

allograft recipients exclusively followed at a single center from one to five years. Transplantation 1987; 43:197-204.

152. Marcen R, Gallego N, Orofino L, et al. Impairment of tubular secretion of urate in renal transplant patients on cyclosporine. Nephron 1995; 70:307-313.

153. Zurcher RM, Bock HA, Thiel G. Hyperuricaemia in cyclosporin-treated patients: a GFR-related effect. Nephrol Dial Transplant 1996; 11:153-158.

154. Taillandier J, Alemanni M, Liote F, Rucay P, Samuel D, Bismuth H. Serum uric acid and liver transplantation. Transpl Proc 1995; 27:2189-2190.

155. Van Thiel DH, Iqbal M, Jain A, Fung J, Todo S, Starzl TE. Gastrointestinal and metabolic problems associated with immunosuppression with either CyA or FK 506 in liver transplantation. Transpl Proc 1990; 22:37-40.

156. Torun M, Yardim S, Simsek B, Burgaz S. Serum uric acid levels in cardiovascular diseases. J Clin Pharm Ther 1998; 23:25-29.

157. Alderman MH, Cohen H, Madhavan S, Kivlighn S. Serum uric acid and cardiovascular events in successfully treated hypertensive patients. Hypertens 1999; 34:144-150.

158. Ward HJ. Uric acid as an independent risk factor in the treatment of hypertension. Lancet 1998; 352:670-671.

159. Tykarski A. Evaluation of renal handling of uric acid in essential hypertension: hyperuricaemia related to decreased urate secretion. Nephron 1991; 59:364-368.

160. Selby JV, Friedman GD, Quesenberry CPJ. Precursors of essential hypertension: pulmonary function, heart rate, uric acid, serum cholesterol, and other serum chemistries. Am J Epidemiol 1990; 131:1017-1027.

161. Jossa F, Farinaro E, Panico S, et al. Serum uric acid and hypertension: the Olivetti Heart Study. J Hum Hypertens 1994; 8:677-681.

162. Waring WS, Webb DJ, Maxwell SRJ. Uric acid as a risk factor for cardiovascular disease. Q J Med 2000; 93:707-713.

163. Johnson RJ, Kivlighn SD, Kim YG, Suga S, Fogo AB. Reappraisal of the pathogenesis and consequences of hyperuricaemia in hypertension,

cardiovascular disease, and renal disease. Am J Kidney Dis 1999; 33:225-234.

164. Bengtsson C, Lapidus L, Stendahl C, Waldenstrom J. Hyperuricaemia and risk of cardiovascular disease and overall death. A 12-year follow-up of participants in the population study of women in Gothenburg, Sweden. Acta Med Scand 1988; 224:549-555.

165. Bickel C, Rupprecht HJ, Blankenberg S, et al. Serum uric acid as an independent predictor of mortality in patients with angiographically proven coronary artery disease. Am J Cardiol 2002; 89:12-17.

166. Liese AD, Hense HW, Lowel H, Doring A, Tietze M, Keil U. Association of serum uric acid with all-cause and cardiovascular disease mortality and incident myocardial infarction in the MONICA Augsberg cohort. World Health Organisation Monitoring Trends and Determinants in Cardiovascular Diseases. Epidemiology 1999; 10:391-397.

167. Yano K, Reed DM, McGee DL. Ten-year incidence of coronary heart disease in the Honolulu Heart Programme. Am J Epidemiol 1984; 119:653-666.

168. Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Ann Intern Med 1999; 131:7-13.

169. Wannamethee SG, Shaper AG, Whincup PH. Serum urate and the risk of major coronary heart disease events. Heart 1997; 78:147-153.

170. Abbott RD, Brand FN, Kannel WB, Castelli WP. Gout and coronary heart disease: The Framingham Study. J Clin Epidemiol 1988; 41:237-242.

171. Pete H. World Health Organisation. Report of a WHO consultation on obesity. Obesity: preventing and managing the global epidemic. Geneva: World Health Organisation. 1998.

172. Williams DRR, Wareham NJ, Brown DC, et al. Undiagnosed glucose intolerance in the community: the Isle of Ely diabetes project. Diabetic Medicine 1995; 12:30-35.

173. Volmink JA, Newton JN, Hicks NR, Sleight P, Fowler GH, Neil HAW. Coronary event and case fatality rates in an English population: results of the Oxford myocardial infarction incidence study. Heart 1998; 80:40-44.

174. Wolfe CDA, Giroud M, Kolominsky-Rabas P, et al. Variations in stroke incidence and survival in 3 areas of Europe. Stroke 2000; 31:2074-???

175. Primatesta P, Poulter NR. Lipid concentrations and the use of lipid lowering drugs: evidence from a national cross sectional survey. BMJ 2000; 321:1322-1325.

176. Colhoun HM, Prescott-Clarke P. The Health Survey for England 1994. London: HMSO, 1996.

177. Gaziano JM, Hennekens C, O'Donnell C, Breslow J, Buring J. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 1997; 96:2520-2525.

178. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 2001; 285:2846-2497.

179. Loss M, Winkler A, Schneider C, et al. Influence of long-term cyclosporine or FK506 therapy on glucose and lipid metabolism in stable liver graft recipients. Transplant Proc 1995; 27:1136-1139.

180. Neal DAJ, Tom BDM, Gimson AES, Gibbs P, Alexander GJM. Hyperuricaemia, gout and renal function after liver transplantation. Transplantation 2001; 72:1689-1691.

181. Herrero JI, Quiroga J, Sangro B, et al. Hyperhomocysteinaemia in liver transplant recipients: prevalence and multivariate analysis of predisposing factors. Liver Transpl 2000; 6:614-618.

182. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities: the role of insulin resistance and the sympathoadrenal system. N Eng J Med 1996; 334:374-381.

183. Reaven G. Metabolic syndrome. Pathophysiology and implications for management of cardiovascular disease. Circulation 2002; 106:286-288.

184. Sheu WHH, Jeng CY, Shieh SM, et al. Insulin resistance and abnormal electrocardiograms in patients with high blood pressure. Am J Hypertens 1992; 5:444-448.

185. Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Low triglycerides-high high-density lipoprotein cholesterol and risk of ischaemic heart disease. Arch Int Med 2001; 161:361-366.

186. Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. High triglycerides and low HDL cholesterol and blood pressure and risk of ischaemic heart disease. Hypertension 2000; 36:226-232.
187. Reaven GM. Are insulin resistance and/or compensatory hyperinsulinaemia involved in the aetiology and clinical course of patients with hypertension. Int J Obesity 1995; 19(suppl 1):2-5.
188. Asfar S, Metrakos P, Fryer J, et al. An analysis of late deaths after liver transplantation. Transplantation 1996; 61:1377-1381.
189. Wheeler DC, Steiger J. Evolution and aetiology of cardiovascular diseases in renal transplant recipients. Transplantation 2000; 70:SS41-SS45.
190. Rabkin JM, Corless CL, Rosen HR, Olyaei AJ. Immunosuppression impact on long-term cardiovascular complications after liver transplantation. Am J Surg 2002; 183:595-599.
191. Bueno OF, van Rooij E, Molkentin JD, Doevendans PA, De Windt LJ. Calcineurin and hypertrophic heart disease: novel insights and remaining questions. Cardiovascular Research 2002; 53:806-821.
192. Shimoyama M, Hayashi D, Takimoto E, et al. Calcineurin plays a critical role in pressure overload-induced cardiac hypertrophy. Circulation 1999; 100:2449-2454.
193. Lim HW, De Windt LJ, Steinberg L, et al. Calcineurin expression, activation, and function in cardiac pressure-overload hypertrophy. Circulation 2000; 101:2431-2437.
194. Ding B, Price RL, Borg TK, Weinberg EO, Halloran PF, Lorell BH. Pressure overload induces severe hypertrophy in mice treated with cyclosporine, an inhibitor of calcineurin. Circ Res 1999; 84:729-734.
195. Zhang W, Kowal RC, Rusnak F, Sikkink RA, Olson EN, Victor RG. Failure of calcineurin inhibitors to prevent pressure-overload left ventricular hypertrophy in rats. Circ Res 1999; 84:722-728.
196. Roberts CA, Stern DL, Radio SJ. Asymmetric cardiac hypertrophy at autopsy in patients who received FK506 (tacrolimus) or cyclosporine A after liver transplant. Transplantation 2002; 74:817-821.
197. Wilkinson IB, MacCallum H, Rooijmans DF, et al. Increased augmentation index and systolic stress in type 1 diabetes mellitus. Q J Med 2000; 93:441-448.

198. Derkx FH, de Bruin RJ, van Gool JM, et al. Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance prorenin immunoreactivity. Clinical Chemistry 1996; 42:1051-1063.
199. Kubasik NP, Warren K, Sine HE. Evaluation of a new commercial radioassay kit for aldosterone using an iodinated tracer. Clin Biochem 1979; 12.
200. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997; 25:658-663.
201. Moller S, Emmeluth C, Henriksen JH. Elevated circulating plasma endothelin-1 concentrations in cirrhosis. J Hepatol 1993; 19:285-290.
202. Uchihara M, Izumi N, Sato C, Marumo F. Clinical significance of elevated plasma endothelin concentration in patients with cirrhosis. Hepatology 1992; 16:95-99.
203. Uemasu J, Matsumoto H, Kawasaki H. Increased plasma endothelin levels in patients with liver cirrhosis. Nephron 1992; 60:380.
204. Textor SC WD, Lerman A, Romero JC, Burnett JC Jr, Wiesner R, et al. Renal hemodynamics, urinary eicosanoids, and endothelin after liver transplantation. Transplantation 1992; 54:74-80.
205. Bartholomeusz B, Hardy KJ, Nelson AS, Phillips PA. Bosentan ameliorates cyclosporin A-induced hypertension in rats and primates. Hypertension 1996; 27:1341-1345.
206. Kannel WB. Elevated systolic blood pressure as a cardiovascular risk factor. Am J Cardiol 2000; 85:251-255.
207. Cushman WC. The clinical significance of systolic hypertension. Am J Hypertens 1998; 11:182S-185S.
208. Eid A, Steffen R, Porayko MK, et al. Beyond 1 year after liver transplantation. Mayo Clin Proc 1989; 64:446-450.
209. Taler SJ, Textor SC, Canzanello VJ, Wilson DJ, Wiesner RH, Krom RA. Loss of nocturnal blood pressure fall after liver transplantation during immunosuppressive therapy. Am J Hypertens 1995; 8:598-605.
210. Shimada K, Kawamoto A, Matsubayashi K, Nishinaga M, Kimura S, Ozawa T. Diurnal blood pressure variations and silent cerebrovascular

damage in elderly patients with hypertension. J Hypertens 1992; 10:875-878.

211. Bianchi S, Bigazzi R, Baldari G, Sgherri G, Campese V. Diurnal variations of blood pressure and microalbuminuria in essential hypertension. Am J Hypertens 1994; 7:23-29.

212. Holmer S, Hense H, Danser A, Mayer B, Riegger G, Schunkert H. Beta adrenergic blockers lower renin in patients treated with ACE inhibitors and diuretics. Heart 1998; 80:45-48.

213. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol 2000; 525:263-270.

214. Deary AJ, Schumann AL, Murfet H, Haydock S, Foo RS, Brown MJ. Influence of drugs and gender on the arterial pulse wave and natriuretic peptide secretion in untreated patients with essential hypertension. Clin Sci 2002; 103:493-499.

215. De Carlis L, Belli LS, Rondinara GF, et al. Early steroid withdrawal in liver transplant patients: final report of a prospective randomised trial. Transplant Proc 1997; 29:539-542.

216. Punch JD, Shieck VL, Campbell DA, Bromberg JS, Turcotte JG, Merion RM. Corticosteroid withdrawal after liver transplantation. Surgery 1995; 118:783-786.

217. Suzuki M, Hamada M, Yamamoto K, Kazatani Y, Hiwada K. Brain natriuretic peptide as a risk marker for incident hypertensive cardiovascular events. Hypertens Res 2002; 25:669-676.

218. Wang TJ, Larson MG, Levy D, et al. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. Am J Cardiol 2002; 90:254-258.

219. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. Am J Epidemiol 1979; 110:281-290.

220. Clark BA, Elahi D, Shannon RP, Wei JY, Epstein FH. Influence of age and dose on the end-organ responses to atrial natriuretic peptide in humans. Am J Hypertens 1991; 4:500-507.

221. Cheung BM, Dickerson JE, Ashby MJ, Brown MJ, Brown J. Effects of physiological increments in human alpha-atrial natriuretic peptide and human brain natriuretic peptide in normal male subjects. Clin Sci 1994; 86:723-730.

222. Kohno M, Minami M, Kano H, et al. Effect of angiotensin-converting enzyme inhibitor on left ventricular parameters and circulating brain natriuretic peptide in elderly hypertensives with left ventricular hypertrophy. Metabolism 2000; 49:1356-1360.

223. Porter GA, Bennett WM, Sheps SG. Cyclosporine-associated hypertension. Arch Intern Med 1990; 150:280-283.

224. Albillos A, Cacho G, Barrios C, et al. Selective impairment of endothelium-mediated vasodilation in liver transplant recipients with cyclosporin A-induced hypertension. Hepatology 1998; 27:332-338.

225. DeGroen P. Cyclosporine, low density lipoprotein , and cholesterol. Mayo Clinic Proc 1988; 63:1012-1021.

226. Princen HMG, Meijer P, Hofstee B, Havekes LM, Kuipers F, Vonk RJ. Effects of cyclosporine A on LDL receptor activity and bile acid synthesis in hepatocyte monolayer cultures and in vivo in the rat. Hepatology 1987; 7:1109.

227. Selzner N, Durand F, Bernuau J, et al. Conversion from cyclosporine to FK506 in adult liver transplant recipients: a combined North American and European experience. Transplantation 2001; 72:1061-1065.

228. Manzarbeitia C, Reich DJ, Rothstein KD, Braitman LE, Levin S, Munoz S. Tacrolimus conversion improves hyperlipidaemic states in stable liver transplant recipients. Liver Transpl 2001; 7:93-99.

229. German DC, Holmes EW. Hyperuricaemia and gout. Med Clin North Am 1986; 70:419-436.

230. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yu TF. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977; 20:895-900.

231. Perkins P, Jones AC. Gout. Ann Rheum Dis 1999; 58:611-616.

232. Faivre L, Houssin D, Valayer J, Brouard J, Hadchouel M, Bernard O. Long-term outcome of liver transplantation in patients with glycogen storage disease type Ia. J Inherit Metab Dis 1999; 22:723-732.

233. Robinson LR, Switala J, Tarter RE, Nicholas JJ. Functional outcome after liver transplantation: a preliminary report. Arch Phys Med Rehabil 1990; 71:426-427.

234. Bagnati M, Perugini C, Cau C, Bordone R, Albano E, Bellomo G. When and why a water-soluble antioxidant becomes pro-oxidant during copper-induced low-density lipoprotein oxidation: a study using uric acid. Biochem J 1999; 340:143-152.

235. Schlotte V, Sevanian A, Hochstein P, Weithmann KU. Effect of uric acid and chemical analogues on oxidation of human low density lipoprotein in vitro. Free Rad Biol Med 1998; 25:839-847.

236. DeScheeder IK, van de Kraay AM, Lamers JM, Koster JF, deJong JW, Serruys PW. Myocardial malodialdehyde and uric acid release after short-lasting coronary occlusions during angioplasty: potential mechanisms for free radical generation. Am J Cardiol 1991; 68:392-395.

237. Boogaerts MA, Hammerschmidt DE, Roelant C, Verwilghen RL, Jacob HS. Mechanisms of vascular damage in gout and oxalosis: crystal induced, granulocyte mediated, endothelial injury. Thromb Haemost 1983; 50:576-580.

238. Ginsberg MH, Kozin F, O'Malley M, McCarty DJ. Release of platelet constituents by monosodium urate crystals. J Clin Invest 1997; 60:999-1007.

239. Patetsios P, Rodino W, Wisselink W, Bryan D, Kirwin JD, Panetta TF. Identification of uric acid in aortic aneurysms and atherosclerotic artery. Ann N Y Acad Sci 1996; 800:243-245.

240. Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996; 335:1001-1009.

241. Group SSSS. Baseline serum cholesterol and treatment effect in the Scandinavian Simvastatin Survival Study (4S). Lancet 1995; 345:1274-1275.

242. Group WoSPS. Influence of pravastatin and plasma lipids on clinical events in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation 1998; 97:1440-1445.

243. Group TL-TIwPiIDLS. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. N Engl J Med 1998; 339:1349-1357.

244. Ballantyne CM, Radovancevic B, Framer JA. Hyperlipidaemia after heart transplantation: report of a 6-year experience, with treatment recommendations. J Am Coll Cardiol 1992; 19:1315-1321.

245. Barbir M, Rose M, Kushwaha S, Akl S, Mitchell A, Yacoub M. Low dose simvastatin for the treatment of hypercholesterolaemia in recipients of cardiac transplantation. Int J Cardiol 1991; 33:241-246.

246. Campana C, Iacona I, Regazzi MB, et al. Efficacy and pharmacokinetics of simvastatin in heart transplant recipients. Ann Pharmacother 1995; 29:235-239.

247. Kliem V, Wanner C, Eisenhauer D, et al. Comparison of pravastatin and lovastatin in renal transplant patients receiving cyclosporine. Transplant Proc 1996; 28:3126-3128.

248. Holdaas H, Hartmann A, Stenstrom J, et al. Effect of fluvastatin for safely lowering atherogenic lipids in renal transplant patients receiving cyclosporine. Am J Cardiol 1995; 76:102A-106A.

249. Martinez-Hernandez BE, Persaud JW, Varghese Z, Moorhead JF. Low-dose simvastatin is safe in hyperlipidaemic renal transplant patients. Nephrol Dial Transplant 1993; 8:637-641.

250. Arnadottir M, Eriksson LO, Germershausen JI, Thysell H. Low-dose simvastatin is a well tolerated and efficacious cholesterol-lowering agent in cyclosporine-treated kidney transplant recipients: double-blind, randomised placebo-controlled study in 40 patients. Nephron 1994; 68:57-62.

251. Yoshimura N, Oka T, Okamoto M, Ohmori Y. The effects of pravastatin on hyperlipidaemia in renal transplant recipients. Transplantation 1992; 53:94-99.

252. Lye WC, Hughes K, Leong SO, Tan CC, Lee EJ. Abnormal lipoprotein (a) and lipid profiles in renal allograft recipients: effects of treatment with pravastatin. Transplant Proc 1995; 27:977-978.

253. Goldberg RB, Roth D. A preliminary report of the safety and efficacy of fluvastatin for hypercholesterolaemia in renal transplant patients receiving cyclosporine. Am J Cardiol 1995; 76:107A-109A.

254. Ghods AJ, Milanian I, Arghani H, Ghadiri G. The efficacy and safety of fluvastatin in hypercholesterolaemia in renal transplant recipients. Transplant Proc 1995; 27:2579-2580.

255. Zachoval R, Gerbes AL, Schwandt P, Parhofer KG. Short-term weffects of statin therapy in patients with hyperlipoproteinaemia after liver transplantation: results of a randomized cross-over trial. J Hepatol 2001; 35:86-91.

256. Imagawa DK, Dawson III S, Holt CD, et al. Hyperlipidaemia after liver transplantation. Transplantation 1996; 62:934-942.

257. Wood D, De Backer G, Faergeman O, Graham I, Mancia G, Pyorala K. Prevention of coronary heart disease in clinical practice: recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention. Atherosclerosis 1998; 140:199-270.

258. Wood D, Durrington P, Poulter N, McInnes G, Rees A, Wray R. Joint British recommendations on prevention of coronary heart disease in clinical practice. Heart 1998 1998; 80(Suppl 2):S1-S29.

259. Reaven GM. Role of insulin resistance in human disease. Diabetes 1988; 37:1595-1600.

260. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 2002; 287:356-359.