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Effect of Immunosuppressive Agents on Long-Term Survival of Renal Transplant Recipients

Focus on the Cardiovascular Risk

Johannes M.M. Boots,[1,2] *Maarten H.L. Christiaans*[1] and *Johannes P. van Hooff*[1]

1 Department of Nephrology, University Hospital Maastricht, Maastricht, The Netherlands
2 Department of Nephrology, Rijmond-Zuid Medical Centre, Rotterdam, The Netherlands

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Abstract

In the control of acute rejection, attention is being focused more and more on the long-term adverse effects of the immunosuppressive agents used. Since cardiovascular disease is the main cause of death in renal transplant recipients, optimal control of cardiovascular risk factors is essential in the long-term management of these patients. Unfortunately, several commonly used immunosuppressive drugs interfere with the cardiovascular system. In this review, the cardiovascular adverse effects of the immunosuppressive agents currently used for maintenance immunosuppression are thoroughly discussed.

Optimising immunosuppression means finding a balance between efficacy and safety. Corticosteroids induce endothelial dysfunction, hypertension, hyperlipidaemia and diabetes mellitus, and impair fibrinolysis. The use of corticosteroids in transplant recipients is undesirable, not only because of their cardiovascular effects, but also because they induce such adverse effects as osteoporosis, obesity, and atrophy of the skin and vessel wall. Calcineurin inhibitors are the most powerful agents for maintenance immunosuppression. The calcineurin inhibitor ciclosporin (cyclosporine) not only induces these same adverse effects as corticosteroids but is also nephrotoxic. Tacrolimus has a more favourable cardiovascular risk profile than ciclosporin and is also less nephrotox-

ic. It has little or no effect on blood pressure and serum lipids; however, its diabetogenic effect is more prominent in the period immediately following transplantation, although at maintenance dosages, the diabetogenic effect appears to be comparable to that of ciclosporin. The diabetogenic effect of tacrolimus can be managed by reducing the dose of tacrolimus and early corticosteroid withdrawal. The effect of tacrolimus on endothelial function has not been completely elucidated. The proliferation inhibitors azathioprine and mycophenolate mofetil (MMF) have little effect on the cardiovascular system. Yet, indirectly, by inducing anaemia, they may lead to left ventricular hypertrophy. MMF is an attractive alternative to azathioprine because of its higher potency and possibly lower risk of malignancies. Sirolimus also induces anaemia, but may be promising because of its antiproliferative features. Whether the hyperlipidaemia induced by sirolimus counteracts its beneficial effects is, as yet, unknown. It may be combined with MMF, however, initial attempts resulted in severe mouth ulcers.

When evaluating the success of organ transplantation, many studies focus on the initial results, especially the risk of acute rejection and the short-term patient and graft survival. For most transplanted organs, such as the heart, the lung or the liver, this approach seems reasonable, since the survival of the patient is almost completely dependent on the survival of the graft. For transplantation of the small bowel or the pancreas this is not the case, but experience with small bowel transplantation is limited and pancreas transplant recipients belong to a selected risk group with an increased risk of mortality from diabetes mellitus. Therefore, this review focuses on renal transplant recipients.

It has long been debated whether renal transplantation, in addition to improving patient quality of life, also offers a survival benefit over continuation of dialysis. Since transplantation is usually performed in the best candidates, a proper control group is difficult to compose. Nevertheless, recent studies have made it clear that transplantation improves survival,[1,2] even in recipients of kidneys derived from marginal donors.[3] The main causes of mortality after renal transplantation are infections, malignancies and cardiovascular disease.[4,5] The development of infections and malignancies is, in general, more dependent on the immunosuppressive load than on the individual agents administered. Since cardiovascular disease is the main cause of mortality in renal transplantation,[4,5] this review focuses on the cardiovascular risk posed by the different immunosuppressive agents commonly used in renal transplantation.

The immunosuppressive agents currently used to prevent rejection after transplantation include corticosteroids, inhibitors of lymphocyte proliferation (azathioprine and mycophenolate mofetil [MMF]), calcineurin inhibitors (ciclosporin [cyclosporine] and tacrolimus), target of rapamycin inhibitors (sirolimus), mono- or polyclonal antibodies directed against T lymphocytes (antithymocyte globulin [ATG]) or the T-cell receptor CD3 (OKT-3), and antibodies directed against the α-chain of the interleukin (IL)-2 receptor CD25 (daclizumab and basiliximab). At present, a number of other agents are being developed, such as leflunomide, which is used in rheumatoid arthritis, FK 778 (meflunomide; an active metabolite of leflunomide), everolimus (a derivative of sirolimus), and FTY 720. At the moment, experience with these compounds is limited in renal transplantation; therefore, they are not discussed further in this review. Mizoribine is used only in Japan[6] and is, therefore, not considered either.

Antibodies are used either as induction therapy or for treatment of acute rejections, especially in the case of corticosteroid resistance. There are no data available on these compounds with regard to their effect on cardiovascular risk factors. However, in their retrospective analysis of the US Renal Data System (USRDS), Meier-Kriesche et al.[7] observed an increased risk of cardiovascular mortality with both ATG and OKT-3 for antibody induction in the

initial 6 months after transplantation (relative risk [RR] 1.27) and beyond this period (RR 1.17).

This review is restricted to the drugs commonly used for maintenance therapy in renal transplantation today.

1. The Process of Atherosclerosis and Arteriosclerosis

An extensive review of the process of atherosclerosis is beyond the scope of this article. Interesting reviews in this field can be found elsewhere.[8-11]

Atherosclerosis is a multifactorial disease. The initial step in the process is endothelial injury, presently referred to as 'endothelial dysfunction'. Endothelial dysfunction is characterised by an imbalance between relaxing and contracting factors, between anti- and pro-coagulant mediators, and between growth inhibiting and proliferating factors.[12] Differences in blood flow patterns, laminar flow versus turbulent flow, result in different so-called shear stresses on the endothelium. Early atherosclerotic lesions correlate with regions of turbulent blood flow near branch points or bifurcations.[11,13] Under normal circumstances, the endothelium can react to stressors by generating vasodilating compounds such as prostacyclin and nitric oxide (NO),[14] the latter being the more important of the two.[15] It is generated from arginine by stimulation of endothelial NO-synthase (eNOS).[16] NO diffuses into the vascular smooth muscle cells, where it stimulates the enzyme guanylate-cyclase. Consequently, the intracellular concentration of cyclic guanylate-monophosphate is raised, resulting in a reduction of intracellular calcium and relaxation of the smooth muscle cells.[17]

Because of the disturbed endothelium, the permeability of the endothelium for atherogenic lipid particles, especially low-density lipoprotein (LDL), increases and lipid particles accumulate in the vessel wall.[8] The lipid particles undergo metabolic processes, most notably oxidation.[10] This, and also the direct injury to the endothelium, stimulate the expression of adhesion molecules, such as intracellular adhesion molecule-1 and vascular cell adhesion molecule-1,[8,18] and the production of the chemotactic factor monocyte chemotactic protein-1.[19] As a result, the influx of inflammatory cells, monocytes and T cells[20,21] is stimulated, and monocytes differentiate in the vessel wall into macrophages, which phagocytose the lipid material and form foam cells.

This inflammation is clinically known as fatty streaks, the initial stage in the process of atherosclerosis, which is already present in children and adolescents.[22,23] Oxidised LDL, in itself, facilitates the progression of atherosclerosis by being chemotactic for monocytes[24] and T cells,[25] and by impairing the mobility of macrophages.[24] Moreover, oxidised LDL is immunogenic and induces the production of antibodies[26,27] and the formation of immune complexes, which facilitate the phagocytoses of LDL by macrophages.[28,29] The endothelium expresses procoagulant, rather than anticoagulant, properties, and forms vasoactive molecules, cytokines and growth factors. The migration and proliferation of vascular smooth muscle cells is stimulated by these growth factors. At that point, the intermediate stage is reached, which is characterised not only by inflammation but also by proliferation.[8]

As the process continues, the arterial wall thickens. First, the arterial wall can compensate for narrowing the lumen by gradual dilation, a phenomenon called remodelling.[30] Subsequently, increased numbers of macrophages and lymphocytes enter the lesions and proliferate within; activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines and growth factors. Further damage is thus induced and, in the end, focal necrosis and fibrosis disturb the normal vessel wall. This is called an advanced or complicated or mature lesion, characterised by a fibrous cap, consisting of extracellular matrix, smooth muscle cells and collagen, overlying a core of lipid material and necrotic tissue.[10] At some point in the process, the plaque compromises the vessel lumen and may rupture through the endothelium and promote thrombosis.

Two growth patterns of atherosclerotic plaques have been recognised. The first develops in small- and medium-sized lesions (<40% diameter reduction) and is characterised by slow and continuous plaque extension, in which the diameter of the vessel is relatively spared. This type of atherosclerotic lesion is correlated to a constant and cumulative burden of the known risk factors. A second type of growth pattern is characterised by occasional acceleration as a result of plaque fissuring, thrombosis

and fibrous organisation of the thrombus. This growth pattern becomes more prominent in advanced lesion sites (>40% diameter reduction) and is characterised by pro-coagulant risk factors, and is dependent on peak levels of risk factors rather than on the cumulative burden.[31]

A different mechanism of vascular damage is referred to as arteriosclerosis. In contrast to atherosclerosis, which is characterised by inflammation, arteriosclerosis is primarily a degenerative disorder. It is characterised by diffuse fibro-elastic intima thickening, an increase in medial ground substance and collagen, and fragmentation of elastic lamellae with secondary fibrosis and calcification of the media. Unlike atherosclerosis, which occurs mostly in arteries with high shear stresses, arteriosclerosis is generalised throughout the aorta and major arteries.[32] Despite their differences, the two arterial disorders frequently coexist. Atherosclerosis can be visualised by measuring the intima-media thickness (IMT) with ultrasound, usually in the common carotid artery. This IMT shows a clear correlation with cardiovascular endpoints.[33] Using ultrasound, arteriosclerosis can also be visualised by a diminished distensibility or compliance, or by increased stiffening of the artery. An increase in arterial stiffening is associated with an increase in systolic blood pressure (BP) and with an increase in pulse pressure. It leads to an increased velocity of the pulse wave over the arteries. This so-called pulse wave velocity is highly correlated with cardiovascular and overall mortality.[34,35] The structural abnormalities in the arteries parallel alterations in the left ventricle that result in left ventricle hypertrophy (LVH).[36] Both disorders are the result of adaptation to the mechanical shear stress of pressure and volume overload.[37] In renal insufficiency, disturbances of the calcium-phosphate metabolism and hyperparathyroidism are contributory factors.

The arterial system has two distinct and separate functions. The first is to deliver blood to the tissues with minimal loss of mean pressure, the so-called conduit function; this function is mainly impaired by atherosclerotic narrowing of the arteries. Secondly, the arteries have to smooth the pulsations resulting from the cardiac ejection and to transform this pulsatile flow into an almost steady flow through the organs, the so-called cushion function.[32] The latter function is predominantly impaired by arteriosclerosis.[38]

Endothelial function is influenced by all of the classical risk factors known from epidemiological studies, that is, hypertension,[39,40] hyperlipidaemia,[41] diabetes mellitus,[42] smoking,[43] renal failure[44-46] and hyperhomocysteinaemia.[47,48] Hypertension is thought to impair endothelial function by increasing shear stresses to the endothelium. It impairs the steady laminar flow that up-regulates vasoprotective genes such as eNOS, cyclo-oxygenase-2 and superoxide dismutase (SOD).[11] Furthermore, cardiovascular risk factors induce the formation of toxic radicals such as superoxide (O_2^-). The latter inactivates NO by the formation of peroxynitrite ($ONOO^-$). Peroxynitrite is a potent oxidant and is capable of inducing lipid peroxidation.[49] The burden of the formation of toxic radicals and its effect on the endothelium and vasculature is indicated in the term 'oxidative stress'. Superoxide can be neutralised by SOD; thus, SOD protects the formation of NO.[10,14] Homocysteine may induce endothelial dysfunction by accumulation of asymmetric dimethylarginine (ADMA). ADMA is an analogue of the NO precursor L-arginine and inhibits the enzyme NOS. A possible interaction of homocysteine with the functional sulfhydryl moiety of the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which metabolises ADMA, may be responsible for the accumulation of ADMA.[48] Reduced homocysteine, which has a free sulfhydryl group, is the most deleterious form of homocysteine for the vascular function.[50]

Endothelial function can be tested by stimulating eNOS in two ways. First, shear stress on the endothelium can be enhanced by increasing blood flow. This is usually performed in the forearm after releasing a period of blockade of the blood flow. The result is flow-mediated vasodilation. Secondly, chemical substances can be infused that stimulate endothelial receptors. The most common of these substances is acetylcholine.

2. Cardiovascular Risk and Renal Transplantation

Epidemiological studies have helped to establish several independent risk factors for the development of cardiovascular disease. One of the pivotal studies

was the Framingham Heart Study.[51] To date, prospective trials estimating the cardiovascular risk based on the known population-based risk factors in the transplant population have not been performed; however, it is highly unlikely that these risk factors would have less impact after transplantation. On the contrary, the incidence of cardiovascular disease in the renal transplant population is more than four times higher than in the general population.[52] Extrapolation of the risk associated with diabetes, smoking and increasing age, as established in the general population, will underestimate these risks in the renal transplant population.[53] An additional risk factor for cardiovascular disease after transplantation is pre-existing disease.[52,54,55] Renal transplant patients have all gone through the stage of end-stage renal failure, and cardiovascular mortality in this population is 10–20 times higher than in the general population.[56] Some of the known risk factors are influenced by adverse effects of immunosuppressive therapy. In this review, all known risk factors are discussed, with special attention to the effects that the different immunosuppressive agents have on them.

3. Firm Cardiovascular Endpoints

Prospective studies evaluating the effect of the different immunosuppressive drugs on firm cardiovascular endpoints, such as (cardiovascular) mortality, ischaemic heart disease and stroke, are scarce. Hollander et al.[57] reported an 8% higher incidence of cardiovascular death 8 years after transplantation in the patients treated with ciclosporin and corticosteroids than in patients who were converted to azathioprine and corticosteroids 3 months after transplantation. Recently, the 15-year follow-up data of the same study were published, revealing an incidence of cardiovascular mortality and an incidence of at least one cardiovascular event that were quite comparable between the groups.[58] In the initial period after transplantation, tacrolimus posed an increased risk of angina pectoris compared with ciclosporin, but the overall incidence of cardiovascular disorders did not differ between both drugs.[59,60] Recently, in a retrospective analysis of the USRDS database, Schnitzler et al.[61] observed a 21–24% reduction in the risk of death with a functioning graft with the use of MMF compared with azathioprine 3 years after transplantation.[61]

Since there is little evidence thus far about the effects of modern immunosuppressive drugs on firm cardiovascular endpoints, it is important to determine the effects of these drugs on the known cardiovascular risk factors as surrogate endpoints in order to determine what strategy to use.

4. The Effect of Immunosuppression on Cardiovascular Risk Factors

4.1 Endothelial Function

Recently, it has been shown that endothelial function improves after renal transplantation compared with haemodialysis.[62,63]

Studies evaluating the effect of corticosteroids on endothelial function in renal transplant recipients have never been performed. However, it was recently shown that corticosteroids impair endothelial function by increasing reactive oxygen species such as superoxide.[64]

Renal transplant recipients treated with ciclosporin had a significant impairment of endothelium-dependent vasodilation compared with azathioprine-treated patients. The latter group had results comparable with those of controls.[65] However, after conversion from ciclosporin to azathioprine, no improvement was observed in hypertensive ciclosporin-treated recipients, from whom all antihypertensive medication was withdrawn at least 3 days before the measurements were taken.[66] Since hypertension also impairs endothelial-dependent vasodilation,[39,40] the persistent hypertension may be responsible for the lack of improvement in this study. The mechanism by which ciclosporin impairs endothelial function is probably superoxide production mediated by endothelin-1 (ET-1).[67]

Studies evaluating the effect of tacrolimus on endothelial function are scarce. One study in a relatively small group of patients showed impaired endothelium-dependent vasodilation in tacrolimus-treated renal transplant recipients compared with healthy controls. The results obtained with tacrolimus were similar to those of patients treated with ciclosporin.[68] Recently, a second cross-sectional

study showed that endothelial function was better preserved with tacrolimus than with ciclosporin.[69]

No studies evaluating sirolimus or MMF on endothelial function have yet been published.

In an *in vitro* capillary tube assay, the effect of the different immunosuppressive drugs was tested on endothelial cells. Ciclosporin showed a pronounced injurious effect on the morphology of the *in vitro* capillaries. In addition, ciclosporin increased the release of ET-1. Neither effect occurred with tacrolimus. MMF also showed a moderate injurious effect on the capillary morphology, whereas sirolimus and dexamethasone had no effect. Except for dexamethasone, all agents increased the release of prostacyclin.[70] Antagonising ET-1 prevented the injury evoked by ciclosporin.[71]

4.2 Hypertension

Hypertension was one of the first risk factors of cardiovascular disease to be recognised. The Framingham Heart Study studied the relationship between BP and cardiovascular disease. Compared with patients with a BP <130/85mm Hg, the RR for cardiovascular disease increased to 1.84 for males and to 2.12 for females with a BP >160/100mm Hg. Some 28% of cardiovascular events in males and 29% in females could be attributed to a BP that exceeded 130/85mm Hg.[72] Since these data are derived from middle-aged, predominantly Caucasian Americans, evaluation in other populations may reveal some differences, but the outcome will generally be the same. After transplantation, hypertension, although treated, was independently associated with all vascular events; however, this was not the case when ischaemic heart disease or cerebral vascular disease were analysed separately.[52] The cardiovascular risk of hypertension in the transplant population seems more or less comparable with the risk in the general population.[53] In addition, hypertension negatively influences graft survival.[73,74] Treatment of hypertension in the general population lowered the risk of cardiovascular disease by 20–30%, depending on the drug used or the outcome parameter: stroke, coronary heart disease, major cardiovascular events or cardiovascular death. The mean decrease in systolic BP for ACE inhibitors to achieve this reduction was only 3mm Hg.[75] More intensive treatment improved the cardiovascular outcome.[75] Treatment of mild-to-moderate hypertension also prolonged survival in patients with an increased risk of cardiovascular mortality.[76]

The prevalence of hypertension after renal transplantation is high and is related to the immunosuppressive agents used and the timepoint after transplantation.[77] In patients receiving ciclosporin and corticosteroids, the prevalence ranges between 71% and 78%.[78,79] As well as the immunosuppressive agents, the presence of the end-stage native kidneys and the nature of the original disease, renal allograft dysfunction, body mass index, renal artery stenosis and essential or pre-existing hypertension are also likely to contribute to the hypertension.[77,78,80-83] In addition, a graft derived from a female, elderly or hypertensive donor, or the use of a right-sided donor kidney are associated with post-transplant hypertension.[81]

Corticosteroids have been known to induce hypertension since Cushing described the glucocorticoid excess syndrome. The underlying mechanism has still not been completely elucidated. It was once thought that corticosteroids raised BP as a result of water and salt retention via an effect on the mineralocorticoid receptor. It has since become clear that blockade of NO formation by inhibition of both inducible and eNOS, inhibition of transmembrane arginine transport and inhibition of the synthesis of the NOS cofactor BH_4 play a prominent role.[84] Corticosteroids are also associated with a disturbed circadian BP pattern.[85] Conventional BP-lowering treatment has only a moderate effect in Cushing's syndrome in contrast to inhibition of corticosteroid production.[86] Therefore, hypertension after transplantation can be best controlled by avoiding corticosteroids.

It is unclear whether azathioprine in itself has an effect on BP since it is always used in combination with corticosteroids or ciclosporin. However, the incidence of hypertension, as defined by the use of antihypertensive drugs, was higher in a group treated with ciclosporin, azathioprine and corticosteroids than in a group treated with ciclosporin and corticosteroids.[87]

MMF has no effect on the BP. The incidence of hypertension was similar between patients treated with MMF 2g, MMF 3g or azathioprine in combination with ciclosporin and corticosteroids.[88] In addi-

tion, BP remained unchanged after withdrawal of MMF from a triple regimen with ciclosporin and corticosteroids.[89]

Ciclosporin monotherapy induces hypertension to the same extent as corticosteroids in combination with azathioprine.[90] The incidence of hypertension with ciclosporin and corticosteroids has been found to be even higher than with azathioprine and corticosteroids.[79,91,92] After lowering the dose of ciclosporin,[93] or withdrawing it after conversion to either azathioprine[90,94-96] or MMF,[97] BP improves significantly. The withdrawal of corticosteroids or conversion from ciclosporin to azathioprine results in a similar decrease in BP.[96] The complete withdrawal of ciclosporin from a triple drug regimen with MMF and corticosteroids[89] or with sirolimus and corticosteroids[98,99] resulted in a mean decrease in BP of 11/6mm Hg and 6/3mm Hg, respectively. The latter figure was diminished because of the use of fewer antihypertensive drugs in the withdrawal patients. The mechanism by which ciclosporin induces hypertension is complex and has still not been completely elucidated. Ciclosporin stimulates transmembrane influxes of calcium. This facilitates vascular smooth muscle cell contraction and vasoconstriction. Indeed, calcium channel antagonists appear to inhibit ciclosporin-mediated vasoconstriction.[100] However, enhancement of the production of ET-1,[101] transforming growth factor (TGF)-β, and renin,[102] inhibition of NO production, increased sympathetic nerve activity, augmentation of noradrenaline-induced vasoconstriction, increased sodium reabsorption, which could be the result of intra-renal vasoconstriction and subsequent proximal tubular anomaly, and, finally, hypomagnesaemia all play a role in the induction of hypertension by ciclosporin.[103] Ciclosporin also impairs the decrease in nocturnal BP,[95,104] which is restored by conversion to azathioprine.[95]

Tacrolimus can induce vasoconstriction and expression of TGFβ, ET-1 and renin transcription to a comparable extent as ciclosporin.[102] In the pivotal trials comparing tacrolimus and ciclosporin in combination with azathioprine and corticosteroids, the incidence of hypertension was comparable.[59,60] However, in liver transplant recipients, hypertension was less frequent in those treated with tacrolimus, although the differences tended to decrease with time after transplantation.[105,106] In more recent reports, tacrolimus resulted in less renal vasoconstriction[107] and renal transplant patients treated with tacrolimus and equivalent dosages of corticosteroids needed fewer antihypertensive drugs than patients treated with ciclosporin.[107,108] Margreiter[109] observed a lower incidence of hypertension with tacrolimus than with ciclosporin as the Neoral® [1] formulation. After conversion from ciclosporin to tacrolimus because of hyperlipidaemia, BP did not improve, but converted patients needed fewer antihypertensive drugs.[110] In a crossover design, Ligtenberg et al.[111] showed that BP decreased after conversion from ciclosporin to tacrolimus and returned to baseline after reintroduction of ciclosporin. Recently, Klein et al.[112] showed that tacrolimus had no effect on BP and renal haemodynamic parameters in healthy volunteers, whereas ciclosporin raised BP by a mean of 15mm Hg and decreased the glomerular filtration rate (GFR) by a mean of 13 mL/min.[112] In a prospective, randomised conversion trial from ciclosporin to tacrolimus in stable renal transplant recipients, BP dropped by a mean of 5mm Hg after conversion to tacrolimus.[113] These results were sustained up to 2 years after the intervention.[114] The improvement in BP after conversion was even remarkably higher in a smaller Spanish study, in which both systolic and diastolic BP improved by a mean of 20mm Hg 6 months after the intervention.[115]

In the study of Kahan et al.,[116] increasing doses of sirolimus combined with ciclosporin and corticosteroids did not increase the incidence of hypertension. Compared with ciclosporin, azathioprine and corticosteroid triple therapy, the incidence of hypertension was 50% lower with sirolimus, azathioprine and corticosteroids.[117] Recently, Gonwa et al.[118] showed that diastolic BP was significantly higher (mean of 3mm Hg) in patients treated with tacrolimus, sirolimus and corticosteroids than in those treated with tacrolimus, MMF and corticosteroids in the initial 6 months after transplantation. Systolic

1 The use of trade names is for product identification purposes only and does not imply endorsement.

BP was also 4mm Hg higher but this did not reach significance.[118]

4.3 Left Ventricular Hypertrophy

LVH is related to hypertension,[119] but it is also recognised as an independent risk factor of cardiovascular morbidity and mortality.[120,121] The prevalence among patients with renal insufficiency is high and is related to age, the degree of renal insufficiency and anaemia.[119] Other risk factors include arteriovenous connections, hypertension, extracellular fluid overload, arterial stiffening and disturbances of the calcium-phosphate metabolism.[122] LVH is associated with an increased cardiovascular mortality after renal transplantation.[123] LVH often improves up to 2 years after transplantation, but failure to regress has been associated with older age, persisting hypertension, high pulse pressure in normal-size hearts and low pulse pressure in dilated hearts.[124,125] Since hypertension is the risk factor that can be modified the most, many aspects of immunosuppressive drugs that account for hypertension will account for LVH as well.

However, since calcineurin activity is enhanced in cardiac hypertrophy,[126] both calcineurin inhibitors, ciclosporin and tacrolimus, may influence LVH. Indeed, in different animal models of cardiac hypertrophy, both ciclosporin and tacrolimus induced regression of ventricular mass.[127-130] In contrast, in children, higher tacrolimus trough concentrations were associated with reversible, concentric, hypertrophic cardiomyopathy that could not be accounted for by the concomitant use of corticosteroids.[131,132]

In adult bone marrow transplant recipients treated with tacrolimus or ciclosporin to prevent graft-versus-host disease, left ventricular mass increased slightly, but not significantly, with tacrolimus. The increase in left ventricular mass with ciclosporin was higher and reached statistical significance. In addition, significantly more patients developed hypertrophic cardiomyopathy with ciclosporin. A rise in BP with ciclosporin and the concomitant use of corticosteroids may account for the observed difference.[133] In heart transplant recipients, the development of LVH post-transplantation was positively correlated with ciclosporin trough concentrations.[134] In a cross-sectional study, LVH tended to be higher with a ciclosporin-based regimen than with azathioprine-based immunosuppression in renal transplant patients.[104] The direct influence of sirolimus on LVH is unknown at the moment.

Since anaemia is also a risk factor for LVH, MMF[135] and azathioprine,[136] which impair bone marrow function, may also have an indirect negative effect on left ventricular mass. Sirolimus also induces anaemia. A greater number of patients developed anaemia with sirolimus than with MMF, both in combination with tacrolimus and corticosteroids.[118] Moreover, the haemoglobin level with sirolimus 5mg was lower than with azathioprine but similar to azathioprine with sirolimus 2mg.[137]

Hyperparathyroidism is also associated with LVH. Thus far, no data exist that indicate how immunosuppressive drugs influence the parathyroid hormone. However, because of their nephrotoxicity,[138] both tacrolimus and ciclosporin may have an indirect negative effect.

4.4 Hyperlipidaemia

Hypercholesterolaemia is another recognised risk factor for cardiovascular disease. Since the main intervention trials with HMG-CoA reductase inhibitors (statins), it has become clear that, in the general population, an increase in cholesterol increases the risk of cardiovascular disease.[139] Some 27% of all cardiovascular events in males and 34% in females were attributable to a total cholesterol level >5.2 mmol/L (200 mg/dL), the cut-off level of the National Cholesterol Education Program (NCEP) in the US.[72] In the 4S (Scandinavian Simvastatin Survival Study), a reduction of 1% in total and LDL cholesterol reduced the risk of major coronary events by 1.9% and 1.7%, respectively. An increase of 1% in high-density lipoprotein (HDL)-cholesterol reduced the risk by 0.8%.[140]

Hypercholesterolaemia as a risk factor for cardiovascular disease in renal transplant patients has mainly been evaluated in retrospective and cross-sectional studies. The latter have shown an association between hypercholesterolaemia and cardiovascular disease.[52,141] In a retrospective analysis, cholesterol was negatively associated with patient survival but the effect diminished with increasing age,[142] while, in another study, a significantly im-

proved survival was only observed in recipients with a pre-transplant cholesterol level of <5.5 mmol/L.[143] The risk associated with hypercholesterolaemia resembles that in the general population.[53] In a retrospective analysis, intervention with statins reduced all-cause mortality by 24% in renal transplant recipients when controlling for age, cholesterol level before the start of statins and transplant year.[144] In addition, hypercholesterolaemia also negatively influenced graft survival.[142,145,146] Recently, the results of a prospective intervention study, the ALERT (Assessment of Lescol in Renal Transplantation) trial, were published.[147] Intervention with fluvastatin 40mg in ciclosporin-treated transplant recipients with a baseline total cholesterol between 4 and 9 mmol/L showed a significant 35% risk reduction on the composite endpoint of cardiac death and definite nonfatal myocardial infarction after 5 years of follow-up. However, on the primary endpoint of overall cardiac incidents (cardiac death, nonfatal myocardial infarction, coronary artery bypass grafting and percutaneous coronary interventions) only a trend towards a 17% risk reduction was observed. *Post hoc* risk factor analysis in the placebo-treated patients revealed a 41% increase in the risk of the primary endpoint and the composite endpoint of cardiac death and nonfatal myocardial infarction per 1.0 mmol/L increase in LDL-cholesterol. Remarkably, no influence of the intervention was observed on all cause death or graft survival. In addition, statin therapy did not protect against cerebrovascular accidents.[147] Whether more aggressive lipid-lowering therapy would have an effect on cerebrovascular incidents similar to the results in the ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial),[148] in which the patient population consisted of hypertensive patients with an average or lower than average cholesterol level, is still unclear.

The prevalence of hyperlipidaemia after transplantation is high and about 60% of all transplant patients have cholesterol levels in the high-risk category (>6.3 mmol/L), according to the guidelines of the NCEP.[77] Not only the immunosuppression but also other drugs, such as β-adrenoceptor antagonists, and genetic and environmental factors, such as diet, contribute to hypercholesterolaemia.[149]

Corticosteroids increase both total and LDL-cholesterol,[150-152] but they also increase HDL-cholesterol.[150,151,153] Corticosteroids are also positively correlated with serum triglycerides.[154-157]

Corticosteroids increase the hepatic synthesis of very low-density lipoprotein (VLDL) by enhancing the activity of the rate-limiting enzymes acetyl-coenzyme A carboxylase and free fatty acid (FFA) synthetase.[158] The effect on the first enzyme results from a concomitant hyperinsulinaemia.[158] Hyperinsulinaemia may be an important factor in corticosteroid-induced hyperlipidaemia since the correlation between serum triglycerides and plasma insulin levels is highly significant in renal allograft recipients.[154] Corticosteroid-induced hyperinsulinaemia may increase the uptake of FFAs in the liver, the main substrate for the synthesis of VLDL, but this mechanism may be of minor importance.[159] Insulin resistance results in a decreased action of peripheral lipoprotein lipase (LPL), which is responsible for the clearance of triglycerides.[160] Insulin resistance may also promote the oxidation of LDL.[161] The conversion from VLDL to intermediate density lipoprotein (IDL) and subsequently LDL, which is unaffected by corticosteroids, may account for the hypercholesterolaemia.[162] The uptake and degradation of LDL is reduced by corticosteroids,[163] and this results from a decrease in LDL receptor synthesis and subsequent expression.[164,165] The increase in HDL probably results from the depression of plasma cholesteryl-ester transfer protein.[166-168]

Ciclosporin also induces hyperlipidaemia in a dose-dependent way.[169] In patients with amyotrophic lateral sclerosis, without confounding comedication or impaired renal function, ciclosporin increased total cholesterol by 21%, LDL-cholesterol by 31% and, although not significantly, triglycerides by 17%.[170] After renal transplantation, total and LDL cholesterol increased in patients treated with ciclosporin compared with in those receiving azathioprine.[171] In addition, ciclosporin was negatively correlated with HDL-cholesterol levels.[169] Hyperlipidaemia was reversible and total cholesterol decreased by 0.5–1 mmol/L after conversion from ciclosporin to azathioprine,[94,172,173] after conversion from ciclosporin to MMF[97] or after withdrawal of ciclosporin.[174] The decrease in total cholesterol was the result of a decrease in LDL-cholesterol.[172,173] However, subfraction determination of the lipoproteins revealed a decrease in IDL-cholesterol

in the study with MMF.[97] HDL-cholesterol did not change after conversion to azathioprine,[172,173] but it decreased after conversion to MMF.[97] Ciclosporin augmented the hyperlipidaemia induced by corticosteroids.[175] Triglycerides were higher in patients treated with ciclosporin than in patients treated with azathioprine and corticosteroids or MMF and corticosteroids.[94,97,172,176,177] In rats treated with ciclosporin, plasma levels of triglycerides, VLDL triglycerides, total cholesterol and LDL-cholesterol were increased, whereas HDL-cholesterol was decreased.[175,178,179]

Ciclosporin interferes with lipid metabolism in several ways. It decreases the activity of LPL, which is probably the basis for the observed hypertriglyceridaemia.[175,178] Ciclosporin impairs the clearance of LDL.[179] *In vitro* studies have shown that this effect of ciclosporin is mediated by a decreased LDL-receptor synthesis in a way similar to corticosteroids.[164,180] Both drugs act synergistically in this field.[164] Ciclosporin blocks the 27-hydroxylation of cholesterol in mitochondria by a mitochondrial cytochrome P450 enzyme system, thereby enhancing the intracellular concentration of free cholesterol and subsequent down-regulation of LDL-receptor activity.[181] However, *in vivo*, in rats treated with ciclosporin, no reduction in LDL-receptor mass was observed.[178] Ciclosporin also reduces the activity of 7α-hydroxylase, the enzyme that is the rate-limiting step in the conversion of cholesterol into bile acid, which is the principal pathway of cholesterol catabolism.[178] Hyperlipidaemia caused by ciclosporin is not mediated by interference with the rate-limiting enzyme of cholesterol synthesis, HMG-CoA reductase.[178] Nor does the presence of ciclosporin in the LDL particle interfere with the binding of LDL to its receptor and the subsequent uptake.[179,180]

In addition to its effects on total cholesterol and LDL-cholesterol, ciclosporin enhances lipoprotein(a) [Lp(a)], as evidenced by ciclosporin-treated patients having significantly higher levels than patients converted to azathioprine.[153] Lp(a) is a lipid particle comparable to LDL but is composed of different lipoproteins. Other studies have not been able to confirm this observation.[97,182]

Ciclosporin increases the oxidation of LDL,[172,183,184] although Devaraj et al.[185] did not observe a direct effect of ciclosporin on LDL oxidation. Thus, the former observation may be the result of a decrease in plasma triglycerides, resulting in a different, increased and less dense composition of the LDL-particle after ciclosporin withdrawal.[185]

Although at first it was not known that tacrolimus might improve hypertension, in the main US trial comparing tacrolimus with ciclosporin it became clear that hyperlipidaemia, especially hypercholesterolaemia, was less frequent in tacrolimus-treated patients.[59] In a later report from the main European trial, both total and LDL cholesterol were lower in tacrolimus-treated patients than in ciclosporin-treated patients at 1 year. Cholesterol levels with tacrolimus were no different than baseline levels prior to transplantation, whereas there was an increase in the ciclosporin group.[186] Triglycerides were comparable in tacrolimus- versus ciclosporin-treated patients.[187] Conversion from ciclosporin to tacrolimus because of hyperlipidaemia resulted in a 16% decrease in total cholesterol and a 25% decrease in LDL-cholesterol. Triglycerides improved slightly but not significantly after conversion, whereas HDL-cholesterol did not change.[188] Another group published similar results, with a significant decrease in serum triglycerides after conversion to tacrolimus.[110] In a randomised, prospective trial evaluating conversion from ciclosporin to tacrolimus in stable transplant patients, total cholesterol improved significantly by a mean of 0.5 mmol/L, LDL-cholesterol by 0.35 mmol/L and triglycerides by 0.4 mmol/L.[113] These results were sustained up to 2 years after conversion.[114] Similar results were reported by a Spanish group.[115]

Lp(a) was also found to be lower with tacrolimus compared with ciclosporin.[187] However, after conversion from ciclosporin to tacrolimus, Lp(a) did not change.[189]

Besides the improvement in serum lipid levels, the oxidation of LDL from tacrolimus-treated, but not ciclosporin-treated, patients was comparable with that of healthy controls.[183,184] Varghese et al.,[190,191] on the other hand, observed an enhanced susceptibility to oxidation of LDL isolated from tacrolimus-treated transplant patients, compared with LDL isolated from Neoral®-treated patients. The fortification of the Neoral® formulation with tocopherol (vitamin E), which has antioxidant

properties, may account for this remarkable difference.[190,191] Morena et al.[192] observed a similar susceptibility to oxidation of LDL derived from both ciclosporin-treated and tacrolimus-treated patients. However, the best evidence of the difference between tacrolimus and ciclosporin originates from the study of randomised conversion from ciclosporin to tacrolimus. In that study, LDL oxidation clearly improved after conversion to tacrolimus.[189] The data from the Dutch trial[113] are completely in line with the results of a Spanish conversion study.[115]

It was already clear in phase I and II studies that sirolimus raised both total cholesterol and triglycerides to high levels, and the increase appeared to be dose dependent.[116,193-195] When sirolimus was added to ciclosporin in a phase III trial, total cholesterol increased by 0.8 (sirolimus 2mg) to 1.4 mmol/L (sirolimus 5mg) and triglycerides increased by 1.1 (sirolimus 2mg) to 2.0 mmol/L (sirolimus 5mg), respectively, after 6 months of therapy, compared with the addition of azathioprine.[137] The addition of sirolimus 10mg to ciclosporin and corticosteroids for 6 weeks in trials focusing on serum lipids raised both total and LDL-cholesterol by 50% and triglycerides by almost 100%. The effects were fully reversible after discontinuation of sirolimus.[196,197]

Sirolimus is a stronger inducer of hyperlipidaemia than ciclosporin, as shown in phase III trials. Six months post-transplantation, total cholesterol and triglycerides were 1.5 and 0.8 mmol/l, respectively, higher in patients treated with sirolimus (target trough concentration of sirolimus 15 μg/L) in combination with MMF and corticosteroids than in patients treated with ciclosporin (target trough concentration of ciclosporin 100–200 μg/L), MMF and corticosteroids.[198] At sirolimus trough concentrations around 30 μg/L 2 months post-transplantation, the difference with ciclosporin (trough levels 200–400 μg/L), in combination with azathioprine and corticosteroids, was maximally 2.8 mmol/L for total cholesterol and 3.2 mmol/L for triglycerides, respectively.[117] In combination with tacrolimus and corticosteroids, even low dosages of sirolimus 0.5mg increased the incidence of hypercholesterolaemia by 15%, although the effects were less prominent than the combination with ciclosporin and corticosteroids. The increase in total cholesterol ranged between 0.4 (sirolimus 0.5mg) and 1.0 mmol/L (sirolimus 2mg).[199] At 6 months post-transplantation, total cholesterol, LDL-cholesterol and triglycerides were significantly higher (0.67, 0.34 and 0.59 mmol/L, respectively) with sirolimus (target trough concentrations 4–12 μg/L) in combination with tacrolimus and corticosteroids than in the combination of MMF, tacrolimus and corticosteroids.[118]

The mechanism by which sirolimus induces changes in lipid levels has still not been completely elucidated. In studies in transplant recipients with prominent hyperlipidaemia while receiving sirolimus therapy, the VLDL, IDL and LDL fractions and accompanying apoproteins were increased. Initially, it was shown that the catabolism of these lipoproteins was reduced.[200] An additional study showed that the FFA pool was expanded by sirolimus and that the hepatic production of triglycerides and secretion of VLDL was increased.[196,197] Sirolimus enhanced the action of hormone-sensitive lipase and may have inhibited LPL, although the effects on the latter enzyme were not unequivocal.[196,197,200] The effects of sirolimus on lipid metabolism appear to be quite opposite to the effects induced by insulin. Therefore, interference by sirolimus of an insulin-dependent signalling pathway has been suggested as the mechanism underlying the disturbance of lipid metabolism.[196,197] Thus far, no data have been published about the effect of sirolimus on Lp(a) or on the oxidation of LDL.

MMF has no effect on serum lipids. Cholesterol and triglycerides were unchanged after discontinuation of MMF from the combination of tacrolimus, MMF and corticosteroids,[152] and total cholesterol was similar between patients treated with tacrolimus, MMF and corticosteroids and patients treated with tacrolimus and corticosteroids.[201] The effects of azathioprine on cholesterol are similar to those of MMF. Total cholesterol was comparable between patients treated with tacrolimus, azathioprine and corticosteroids and patients treated with only tacrolimus and corticosteroids.[136] Total and LDL-cholesterol and triglycerides were all similar between patients treated with ciclosporin, azathioprine and corticosteroids and patients who had azathioprine withdrawn from the same regimen.[202] There are no

published data about the effect of either drug on Lp(a) or oxidised LDL to date.

4.5 Diabetes Mellitus

Diabetes is also strongly associated with an increased cardiovascular mortality and morbidity. In the Framingham Heart Study, diabetes increased the risk of coronary heart disease by 53% in men and as much as 82% in women.[72] In renal transplantation, the risk is dramatically higher in both males (3-fold) and females (5-fold).[53] Furthermore, the development of diabetes is associated with an impaired graft survival.[203,204]

Corticosteroids disturb glucose tolerance by enhancing insulin resistance[205] and play a significant role in the development of post-transplant diabetes mellitus (PTDM).[206,207] Withdrawal of corticosteroids reduces the incidence of PTDM and improves glycaemic control.[208] Early corticosteroid withdrawal after transplantation may be an important factor in the prevention of PTDM.[209]

Ciclosporin also has an effect on glucose metabolism. In liver transplant recipients treated with ciclosporin, glucose tolerance was impaired compared with control patients (hepatitis C-negative patients with chronic liver disease).[210] Renal transplant patients receiving ciclosporin and corticosteroids had a higher incidence of PTDM than patients treated with azathioprine and corticosteroids,[204,211] even when corticosteroid dosages were higher.[211] Reducing the dose of ciclosporin reversed the diabetes in the majority of affected patients.[211] Glucose tolerance also improved after conversion from ciclosporin to azathioprine.[173] The source of the diabetic effect of ciclosporin is an impairment in pancreatic β-cell secretory function.[210,212] In addition, glucagon production by the α cells is enhanced.[210] In *in vitro* experiments, ciclosporin dose-dependently inhibited insulin secretion.[213,214] However, no effect on glucagon secretion was observed.[214]

In the initial report comparing ciclosporin and tacrolimus, it was mentioned that the incidence of PTDM was comparable between the drugs.[215] However, the main trials showed a significantly higher incidence with tacrolimus;[59,60] with an incidence of PTDM of around 20%[59,216] (versus an incidence of 4% with ciclosporin in the US trial[59]). In a majority of patients, PTDM is reversible after reducing the dose of tacrolimus and withdrawing corticosteroids.[216,217] Subsequently, after the first 6 months post-transplantation, the incidence of PTDM decreases to 5.7% with tacrolimus.[218] In particular, patients who already had subclinical impairment of glucose tolerance were at risk of developing PTDM with tacrolimus.[219] Despite the higher incidence of PTDM with tacrolimus, maintenance levels of both calcineurin inhibitors showed no differences with regard to glucose metabolism.[210,220] Cumulative toxicity was absent.[220] Tacrolimus decreased insulin secretion by pancreatic β cells in a dose-dependent way,[210,219,221,222] and glucagon expression was also enhanced.[210] However, others observed an increase in insulin resistance.[223] In *in vitro* experiments, tacrolimus: (i) dose-dependently and completely reversibly inhibited insulin gene transcription after 24 hours; (ii) had no acute effects on insulin secretion; and (iii) did not affect glucose uptake by insulin. Since calcineurin and FK-binding protein (FKBP)-12 are also present in β-cell lines, the inhibition of insulin secretion by tacrolimus probably occurs along the same pathway as the interference of IL-2 production.[224,225] Although in different concentrations, both calcineurin and FKBP-12 are found in α cells,[225] which may explain the up-regulation of glucagon.[210] The stronger potency of tacrolimus, compared with ciclosporin, to block calcineurin[226] may account for the higher incidence of PTDM observed in tacrolimus-treated patients than in ciclosporin-treated patients in the initial period after transplantation.[59,60,109]

Sirolimus has no known effect on glucose metabolism. The incidence of PTDM was unchanged with the addition of sirolimus to either ciclosporin-[116] or tacrolimus-based regimens.[118]

4.6 Renal Function

Impaired renal function is strongly associated with an increased risk of cardiovascular death, also after renal transplantation. Cardiovascular mortality 10 years post-transplantation increased from 4% for recipients with a normal serum creatinine (<115 μmol/L) at 1 year post-transplantation to 9% for recipients with a serum creatinine >230 μmol/

L.[227] Moreover, recipients with a compromised transplant function at 1 year post-transplantation (GFR <44.8 mL/min/1.73m^2) had an increased risk of both acute coronary syndromes (adjusted hazard ratio [HR] 2.16) and congestive heart failure (adjusted HR 2.95).[228]

Both calcineurin inhibitors are nephrotoxic agents. This nephrotoxicity can be divided into two parts: (i) an initially reversibly dose-dependent renal vasoconstriction;[229] and (ii) in the chronic progressive changes. Histologically, no differences can be shown between the two drugs. Both induce tubulopathy, characterised by vacuolation of epithelial cytoplasm with or without microcalcifications, arteriolopathy and glomerulopathy. Initially, arterioles show prominent vacuolation of smooth muscle cells. Later on, these smooth muscle cells may undergo necrosis and necrotic cells are replaced by proteinaceous material. Glomerular lesions evolve to focal segmental glomerulosclerosis. Finally, calcineurin-induced nephrotoxicity results in tubular atrophy and interstitial fibrosis.[138]

The vasoconstriction is mediated by ET-1 in the afferent arteriole.[230,231] Ciclosporin augments the action of ET-converting enzyme and augments the production of ET-1 by endothelial cells mediated by the enhanced production of TGFβ.[232] ET-1 also plays a role in the development of vasculopathy.[233-235] TGFβ is a potent inducer of fibrosis formation, and ciclosporin not only upregulates TGFβ-1 expression but also its receptors in rat mesangial cells.[236] The chronic tubulointerstitial fibrosis induced by ciclosporin is thought to be mediated by TGFβ[237-239] and by inducing apoptosis in renal tubular cells.[240] However, recently, no toxic effect of ciclosporin on tubular cells could be demonstrated *in vitro* and the *in vivo* observed results may be induced by renal vasoconstriction.[241] Ciclosporin may accumulate in the kidney,[242] enhancing its nephrotoxicity. The combination of ciclosporin with sirolimus results in a serious interaction, leading to accumulation of ciclosporin in the kidney and, thus, aggravating its nephrotoxicity.[243] This interaction has not been observed between sirolimus and tacrolimus,[244] but there are indications that this combination may result in an increased risk of delayed graft function.[245] Moreover, patients treated with tacrolimus and MMF had a better renal function than patients treated with tacrolimus and sirolimus.[118]

Although both calcineurin inhibitors are histologically equally nephrotoxic, more and more evidence has arisen that tacrolimus may be less nephrotoxic. There is less renal vasoconstriction in healthy volunteers with tacrolimus than with ciclosporin.[112] Gene expression of fibrosis formation is lower in both tacrolimus-treated mice[246] and humans[247] than with ciclosporin treatment. Furthermore, TGFβ expression in kidney biopsies is lower in those taken from tacrolimus recipients than ciclosporin recipients.[248] FKBP-12 binds to the TGFβ-1 receptor,[249,250] and it also acts as an anchor of another cytoplasmic protein to that receptor, probably calcineurin. The complex of FKBP-12 and calcineurin prevents the receptor from being activated.[249] Macrolide derivatives of tacrolimus, which can bind to FKBP-12 but have no effect on calcineurin, can remove the FKBP-12/calcineurin complex from the TGFβ-1 receptor and enhance the activation of the TGFβ receptor.[249] However, tacrolimus, which binds to FKBP-12 and inactivates calcineurin, appears to exhibit growth inhibition mediated by TGFβ.[249] The downstream blockade of calcineurin and subsequent gene transcription is, therefore, essential for interruption of the effects of TGFβ. In contrast, ciclosporin induces the expression of TGFβ-1 receptors.[236] In serum, both tacrolimus and ciclosporin enhance the production of TGFβ.[251] Besides their different effects on TGFβ expression, ciclosporin and tacrolimus may also have different effects on ET-1 secretion. Whereas ciclosporin enhanced the production, tacrolimus appeared to have no effect in a cultured renal cell line[252] or in a capillary tube assay.[70] However, another study did not find any difference in ET mRNA expression in renal biopsies from patients receiving either ciclosporin or tacrolimus.[102] Recently, it has prospectively been shown that fibrosis formation was indeed less in tacrolimus-treated recipients than in ciclosporin-treated patients.[253,254]

Clinically, the main prospective trials observed a comparable graft function both with ciclosporin-Sandimmune®[59,60] and with ciclosporin-Neoral®,[109] despite a significant reduction in the incidence of acute rejection. However, in their retrospective analysis of the USRDS, Gjertson et al.[255]

observed that in the long-term tacrolimus-treated patients had a better graft survival. In the past year, the 5-year follow-up data of the main trials comparing tacrolimus with ciclosporin-Sandimmune® were published. In the US trial, graft function was comparable between the two groups on an intention-to-treat analysis.[256] However, a substantial proportion of the ciclosporin-treated patients were converted to tacrolimus, predominantly as a result of lack of efficacy. After censoring for these converted patients, it was shown that patients treated with tacrolimus had a better graft survival. Moreover, creatinine clearance was significantly higher in the tacrolimus-treated patients. The 5-year results of the European trial showed a comparable graft survival, but the incidence of chronic rejection was significantly lower in the tacrolimus group and the projected graft half-life was higher in the tacrolimus-treated patients.[257] Remarkably, not only patients who receive tacrolimus from transplantation onwards may profit from a better renal function with tacrolimus. Serum creatinine decreased 6 months after conversion from ciclosporin to tacrolimus in patients with a stable graft function more than a median of 5 years after a successful transplantation.[113] The 2-year follow-up data of that trial showed a stable transplant function in the conversion group in contrast to a gradual decline in the group that stayed on ciclosporin.[114] Kaplan et al.[258] compared kidneys derived from the same donor, where one kidney was allocated to a recipient with initial treatment with microemulsion ciclosporin (formulation not specified) and the other kidney to a recipient with initial treatment with tacrolimus. Recipients with tacrolimus-based immunosuppression showed lower creatinine values over the whole 5-year study period. However, 5-year graft survival and the rate of decline in renal function were similar in both groups.[258]

4.7 Homocysteine

Hyperhomocysteinaemia is recognised as a cardiovascular risk factor by the extreme juvenile atherosclerosis observed in patients with classically homozygote homocystinuria.[259] These patients lack one of the enzymes involved in homocysteine metabolism: methylene-tetrahydrofolate reductase (MTHFR), cystathionine β-synthase or methionine synthase. They have high fasting homocysteine levels of >50 μmol/L. The incidence of this disease is rare, approximately 1 per 100 000 population.[260,261] More subtle elevations of the homocysteine level of >15 μmol/L, which is proposed as the cut-off level,[262] are more frequent and are present in about 25% of patients with cardiovascular disease versus 5–10% of control populations.[263] These patients do not lack one of the enzymes but rather these enzymes have mutations. The most common is the C677T mutation in the *MTHFR* gene. Some 10–13% of Caucasian populations are homozygous for this mutation.[261,264] The function of these mutated enzymes is decreased. The vitamin status, especially folate, of the person determines whether they will have hyperhomocysteinaemia.[265,266] Homocysteine levels are higher in men than in women and increase with age.[267] More and more evidence has arisen that these less dramatic increases in serum homocysteine are also associated with an increased risk of cardiovascular disease. A meta-analysis of retrospective studies showed that the odds ratio (OR) of coronary artery disease was 1.6 in men for each 5 μmol/L increase in homocysteine level versus 1.8 in women. Some 10% of the risk of coronary artery disease in the population could be attributed to increased homocysteine levels. The risk of a 5 μmol/L increase in homocysteine was comparable with the risk of a 0.5 mmol/L increase in cholesterol.[263] Prospective studies revealed lower risks than the case control studies. Meta-analyses of the prospective trials revealed ORs for ischaemic heart disease for every 5 μmol/L increase in homocysteine of 1.2[268] and 1.23,[269] respectively.

Serum homocysteine levels are dependent on renal function.[270-273] In dialysis patients, the remethylation and the transmethylation of homocysteine are disturbed. The other metabolic route (transsulfuration) is unaffected.[274] In normal kidneys, no significant renal extraction of either total or free homocysteine was demonstrated. Therefore, less renal elimination as the result of the decline in GFR can not explain the higher homocysteine levels in chronic kidney disease.[275] Although homocysteine levels are correlated to the GFR, higher homocysteine levels were not related to the decline in renal function in chronic kidney disease in one study.[276]

The prevalence of hyperhomocysteinaemia after renal transplantation is high and ranges from 50%[277-280] to 80%[281,282] of the transplant recipients. Homocysteine levels are 2- to 6-fold higher in renal transplant recipients compared with healthy controls.[281] Compared with the dialysis status, homocysteine levels improve by a mean of 14% 6 months after transplantation.[283] In patients with immediate good graft function, homocysteine levels decrease directly after transplantation.[284] After transplantation, homocysteine levels are related to the GFR,[277,280,282,283,285] genetic factors,[278,286] the nutritional status[282] and age,[287] and are higher in males than in females.[280,285] Hyperhomocysteinaemia is also associated with an increased risk of cardiovascular disease after renal transplantation,[287-289] with a RR of 1.06 for each 1 μmol/L increase in plasma homocysteine.[287] However, other studies have shown no such relationship.[290,291] Despite the relationship between homocysteine and cardiovascular disease and renal function, no negative effect of hyperhomocysteinaemia on either patient or graft survival has been demonstrated.[292,293]

A limited number of studies evaluating the effect of immunosuppressive drugs on homocysteine levels have been published in the last decade. Arnadottir et al.[285] observed higher homocysteine levels in recipients treated with ciclosporin-based immunosuppression than in patients that were not treated with ciclosporin. These latter patients had homocysteine levels comparable to patients with chronic renal failure with a similar GFR. Despite the higher homocysteine levels, no correlation with ciclosporin trough concentrations was observed.[285] However, in cardiac transplant patients, ciclosporin trough concentrations correlated more to homocysteine levels than serum creatinine.[294] Mor et al.[295] also observed lower homocysteine levels in patients treated with azathioprine and corticosteroids compared with those treated with ciclosporin, azathioprine and corticosteroids, but the ciclosporin-treated patients had a significantly higher serum creatinine. On the other hand, Ducloux et al.[296,297] showed that after matching for confounding variables, ciclosporin did not increase homocysteine levels, and that homocysteine was more related to transplant function and folate status. In addition, in an *in vitro* experiment, Ignatescu et al.[298] showed that ciclosporin had no influence on homocysteine formation in proximal renal tubular cells.

Several studies evaluated homocysteine levels between ciclosporin- and tacrolimus-treated patients. All studies observed similar homocysteine levels in both ciclosporin- and tacrolimus-treated recipients.[281,295,299] Mehra et al.[300] showed the same results in heart transplant recipients. After randomised conversion from ciclosporin to tacrolimus, Artz et al.[189] also observed no improvement in homocysteine levels. Similarly to ciclosporin, tacrolimus showed no effect on homocysteine formation in an *in vitro* experiment with proximal renal tubular cells.[301] On the other hand, Quiroga et al.[302] observed significantly lower homocysteine levels 12 months post-transplantation in tacrolimus-treated patients than in ciclosporin-treated patients, but data on renal function were not mentioned.

In contrast to ciclosporin and tacrolimus, MMF decreased homocysteine formation in the same *in vitro* experiment.[301] In male transplant recipients, homocysteine levels were 1 μmol/L lower in patients treated with ciclosporin, MMF and corticosteroids than in patients treated with ciclosporin, azathioprine and corticosteroids. However, in females, no differences were observed.[301]

There are currently no data about the effect of corticosteroids or sirolimus on homocysteine levels.

4.8 Thrombosis and Fibrinolysis

In the last decade, it has become clear that acute thrombus formation is responsible for acute coronary syndromes. Therefore, fibrinolytic therapy has become an important part of the treatment of myocardial infarction. Several proteins involved in coagulation or fibrinolysis, such as tissue factor (factor VII), fibrinogen, von Willebrand factor (vWF), tissue plasminogen activator (tPA) and plasminogen-activator inhibitor type 1 (PAI-1), have been related to the development of coronary events.[303-308] The increased risk of coronary events associated with tPA reflects increased tPA-PAI-1 complexes and results in a reduced fibrinolytic capacity.[304] The relative risk of coronary events of the highest quintiles versus the lowest quintiles was 2.89 for fibrinogen, 1.85 for vWF and 2.10 for tPA.[304] PAI-1 levels

are associated with insulin levels and insulin resistance.[309,310]

PAI-1 has also shown to be an independent risk factor of coronary artery disease in haemodialysis patients,[311] and reduced fibrinolytic capacity has been related to the development of coronary artery disease in heart transplant recipients.[312] A reduced fibrinolytic potential was demonstrated in nearly 70% in corticosteroid-treated transplant recipients.[313] Moreover, renal transplant recipients have high levels (above a mean of 200%) of both vWF and factor VIII.[314]

Patients with Cushing's disease often develop thromboembolic complications. These patients have been shown to have a hypercoagulable and hypofibrinolytic state due to increased levels of several clotting factors, vWF, α_2-antiplasmine and PAI-1.[315-317] Heart transplant recipients treated with corticosteroids had higher PAI-1 levels and less fibrinolytic capacity than recipients not administered corticosteroids.[318,319] Fibrinolytic capacity in renal transplant recipients treated with corticosteroids was comparable with patients with Cushing's disease.[313] After withdrawal of corticosteroids from a triple therapy regimen with ciclosporin and azathioprine, fibrinolytic capacity improved with lower PAI-1 levels.[320,321] The effect of corticosteroids on PAI-1 may be mediated by their effect on glucose metabolism[205] because PAI-1 levels are related to insulin resistance.[309,310] *In vitro*, corticosteroids were shown to inhibit the production of urokinase type plasminogen activator (u-PA) in a cell culture of aortic endothelial cells.[322] In a cell culture of umbilical vein endothelial cells, corticosteroids increased thrombin-stimulated release of both vWF and PAI-1.[323] On the other hand, corticosteroids inhibited endotoxin-induced coagulation resulting in a hypocoagulable state. It was suggested that this was mediated by inhibition of monocyte-stimulated release of tissue factor.[324]

Ciclosporin is also associated with an impairment of fibrinolytic capacity. Fibrinolysis was impaired in ciclosporin-treated patients compared with both azathioprine-treated patients[325] and healthy controls,[326] by either a defective release of plasminogen activator from the vessel wall or high plasma levels of PAI-1.[325] After conversion from ciclosporin to azathioprine, PAI-I activity decreased significantly.[327] Patients treated with ciclosporin and corticosteroids had higher levels of vWF[328] and fibrinogen[329] than patients treated with azathioprine and corticosteroids. In addition, stepwise conversion from ciclosporin to MMF lowered plasma vWF.[330] Ciclosporin-treated patients with aplastic anaemia had higher levels of fibrinogen, vWF and PAI-1 than aplastic anaemia patients not treated with ciclosporin.[331] Ciclosporin may also negatively influence haemostasis. Retrospectively, thromboembolic complications occurred more often in patients treated with ciclosporin and corticosteroids than in patients treated with azathioprine and corticosteroids, and ciclosporin-treated patients showed higher levels of factor VIII and fibrinogen.[332] However, these results could not be confirmed in a prospective design.[333] *In vitro*, haemostasis was increased in transplant recipients treated with ciclosporin and corticosteroids, with or without azathioprine, compared with those receiving azathioprine and corticosteroids alone.[334] Ciclosporin enhanced the thrombin-stimulated release of both vWF and PAI-1 in a human umbilical vein endothelial cell culture[323,335] and acted synergistically with corticosteroids in this experiment.[323]

Data on the other immunosuppressive drugs are very scarce. Fibrinogen levels were lower in tacrolimus-treated patients than in ciclosporin-treated patients in one study.[187] In another, conversion from ciclosporin to tacrolimus resulted in a significant decrease in plasma fibrinogen levels.[113] Conversion showed no effect on fibrinolysis parameters except for a slight but significant decrease in u-PA.[113]

5. Conclusions

An overview of the cardiovascular adverse effects of currently used immunosuppressive drugs is presented in table I.

Optimising immunosuppression implies finding the optimal balance between efficacy and safety. Thus far, calcineurin inhibitors appear to be the most potent immunosuppressive drugs for maintenance immunosuppression. However, they produce a number of undesirable adverse effects not only on renal function but also on the cardiovascular system. Tacrolimus has several advantages over ciclosporin. First, tacrolimus is more potent than ciclosporin in

Table I. The effect of current immunosuppressive agents on cardiovascular risk factors. Lipoprotein (a) is not included because there are only a small number of studies and no clear conclusions can be drawn

Immunosuppressive agent	Cardiovascular risk factor											
	endothelial function	hypertension	LVH	cholesterol	HDL-C	LDL-C	triglycerides	ox-LDL	diabetes mellitus	renal function	homo-cysteine	fibrinolysis
Corticosteroids	Decrease	Increase	Induced via hyper-tension	Increase	Increase	Increase	Increase	[a]	Increase[b]	Neutral	Lack of data	Decrease
Azathioprine	Lack of data	Neutral or slight increase	Induced via anaemia	Neutral	Neutral	Neutral	Neutral	Lack of data	Neutral	Neutral	Lack of data	Lack of data
Mycophenolate mofetil	Neutral?[c]	Neutral	Induced via anaemia	Neutral	Neutral	Neutral	Neutral	Lack of data	Neutral	Neutral	Decrease?[d]	Lack of data
Ciclosporin	Decrease	Increase	Induced via hyper-tension[e]	Increase	Neutral?[f]	Increase	Increase	Increase	Increase[g]	Decrease	Increased by effect on renal function	Decrease
Tacrolimus	May be less toxic than ciclosporin	Neutral? Less than ciclosporin	[eh]	Neutral? Less than ciclosporin	Neutral	Neutral? Less than ciclosporin	Neutral? Less than ciclosporin	Less than ciclosporin	Increase[gi]	Decrease[j]	Increased by effect on renal function	Decrease?[k]
Sirolimus	Lack of data	Neutral or slight increase	Induced via anaemia	Increase	Neutral	Increase	Increase	Lack of data	Neutral	Neutral	Lack of data	Lack of data

a No direct data. May be negative by inducing insulin resistance.

b By inducing insulin resistance.

c Mycophenolate mofetil showed *in vitro* toxicity against endothelial cells.

d Mycophenolate mofetil inhibited homocysteine formation *in vitro* in renal tubular cells.

e Indirectly by nephrotoxicity and secondary hyperparathyroidism. Possibly beneficial in hypertrophic obstructive cardiomyopathy.

f Some data suggest a decrease.

g By inhibition of insulin secretion.

h Less influence than ciclosporin. Reversible concentric hypertrophic cardiomyopathy reported in children.

i The incidence of diabetes is higher initially after transplantation compared with ciclosporin. At maintenance dosages, the incidence is comparable between both calcineurin inhibitors.

j Tacrolimus may be less nephrotoxic than ciclosporin.

k Tacrolimus results in lower levels of fibrinogen.

HDL-C = high-density lipoprotein-cholesterol; **LDL-C** = low-density lipoprotein-cholesterol; **LVH** = left ventricular hypertrophy; **ox-LDL** = oxidised LDL.

preventing acute rejections.[59,60,109,217] Although tacrolimus-induced nephrotoxicity is histologically identical to that of ciclosporin, more and more evidence is accumulating that, clinically, tacrolimus may be less nephrotoxic. Moreover, tacrolimus has a superior cardiovascular risk profile with regard to hypertension and hyperlipidaemia. The enhanced risk of diabetes is clinically not apparent at commonly used maintenance dosages and can be managed by reducing the dose of tacrolimus and early corticosteroid withdrawal.

Corticosteroids are undesirable for long-term immunosuppression. They induce endothelial dysfunction, hypertension, hyperlipidaemia, diabetes, and result in a decreased fibrinolytic state. The benefit of an increase in HDL-cholesterol does not outweigh these adverse effects. Furthermore, they have several other undesirable adverse effects, such as weight gain and Cushing habitus, atrophy of the skin and vessel wall, and osteoporosis. The fear of an increase in acute or chronic rejection after withdrawal, which was demonstrated with ciclosporin,[336] may be less with the more potent tacrolimus.

Inhibitors of lymphocyte proliferation do not pose the risk of nephrotoxicity and have only minimal adverse cardiovascular effects. Prevention of anaemia may be necessary in order to reduce LVH. However, the immunosuppressive power of these agents is less than that of calcineurin inhibitors. Therefore, they are almost always used in combination with the less desirable corticosteroids. MMF is more powerful than azathioprine, and because it does not interfere with DNA, the risk of developing malignancies may be less. Recently, the use of MMF was indeed associated with a reduction in the number of post-transplant lymphoproliferative disorders.[337] In selected series of low-risk transplant recipients, MMF as baseline maintenance immunosuppression has shown favourable results;[338,339] however, its real value still needs to be determined.

Sirolimus is another promising agent because of its beneficial effects on intimal proliferation.[340-346] However, data on firm cardiovascular endpoints are lacking. In addition, it is still unclear whether the induced hyperlipidaemia counteracts the potential benefits. Care should be taken with regard to the development of drug-induced pneumonia with sirolimus.[347,348] Another advantage of the antiproliferative characteristics of this compound may be a reduced risk of malignancies.[349-354] The combination of sirolimus and MMF seems an attractive alternative for avoiding calcineurin-induced nephrotoxicity. However, with this combination the incidence of acute rejection is higher[198] than with tacrolimus-based immunosuppression,[109,118,355] initial attempts resulted in the development of severe mouth ulcers,[356] and long-term data are lacking. Therefore, more data are necessary to establish the safety and efficacy of the combination of sirolimus and MMF.

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Correspondence and offprints: Dr *Johannes M.M. Boots*, Department of Nephrology, Rijnmond-Zuid Medical Centre, PO Box 9119, Rotterdam, 3007 AC, The Netherlands.
E-mail: BootsJ@mcrz.nl