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Risk factors for renal and cardiovascular endpoints in renal transplantation

Analyses from ALERT study
(**A**ssessment of **LE**scol in **R**enal **T**ransplantation)

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Preface

This thesis includes 4 papers that have reported *post-hoc* analyses from the ALERT study. The analyses involve 2,102 renal transplant recipients (RTRs) who were randomized to receive fluvastatin or placebo and followed up for 7-8 years. Paper 1 uses data from the ALERT core study and Papers 2-4 use data from the ALERT extension study.

Papers

1. Bengt Fellström, Sadollah Abedini, Hallvard Holdaas, Alan G Jardine, Beatrix Staffler, Claudio Gimpelewicz. **No detrimental effect on renal function during long-term use of fluvastatin in renal transplant recipients in the Assessment of Lescol in Renal Transplantation (ALERT) study.** Clinical Transplantation 2006;20(6):732-9.

2. Sadollah Abedini, Ingar Holme, Bengt Fellström, Alan Jardine, Edward Cole, Bart Maes, Hallvard Holdaas. **Cerebrovascular events in renal transplantation.** Transplantation 2009;87(1):112-7.

3. Sadollah Abedini, Ingar Holme, Winfried März, Gisela Weihrauch, Bengt Fellström, Alan Jardine, Edward Cole, Bart Maes, Hans-Hellmut Neumayer, Carola Grønhagen-Riska, Patrice Ambühl, Hallvard Holdaas on behalf of the ALERT study group. **Inflammation in Renal Transplantation**. Clin J Am Soc Nephrol 2009;4:1246-1254.

4. Sadollah Abedini, Andreas Meinitzer, Ingar Holme, Winfried März, Gisela Weihrauch, Bengt Fellstrøm, Alan Jardine and Hallvard Holdaas. **Asymmetrical dimethylarginine is associated with renal and cardiovascular outcomes and all-cause mortality in renal transplant recipients.** Kidney Int. 2010 Jan;77(1):44-50.

Tables and figures

Abbreviations

ACE	angiotensin-converting enzyme
ADMA	asymmetric dimethylarginine
ALERT	The Assessment of Lescol in Renal Transplantation
ALG	antilymphocyte globulin
ARB	angiotensin receptor blockers
ATG	antithymocyte globulin
AZA	azathioprine
CBV	cerebrovascular
CHD	coronary heart disease
CHF	chronic heart failure
CKD	chronic kidney disease
CsA	cyclosporine A
CVD	cardiovascular disease
DWFG	death with functioning graft
ECD	expanded criteria donors
eGFR	estimated glomerular filtration rate
ESRD	end stage renal disease
HD	hemodialysis
HDL	high density lipoprotein
HR	hazard ratio
hsCRP	high sensitivity c-reactive protein
IL-6	interluekin-6
IHD	ischemic heart disease
LDL	low density lipoprotein
L-NMMA	N-monomethyl L-arginine
LVH	left ventricular hypertrophy
MACE	major cardiac events
MI	myocardial infarction
MMF	mycophenolate mofetil
NO	nitric oxide
NOS	nitric oxide synthase
OKT3	Muromonab CD-3
PRMT	protein arginine methyltransferases
RCT	randomized controlled trail
RRT	renal transplant therapy
RTR	renal transplant recipients
SD	standard deviation
SDMA	symmetric dimethylarginine
TAC	tacrolimus

1. The ALERT study (The Assessment of Lescol in Renal Transplantation).

It is well established that renal transplant recipients (RTRs) suffer premature cardiac disease [6;10;72;73;83;91]. In renal transplant patients, declining kidney function results in a yearly graft loss of 3-4% [4;5]. Prior to the ALERT trial, the role of lipids and other risk factors for cardiovascular (CV) disease had not been fully established in this patient population. Although several studies have tried to correlate dyslipidemia with increased risk for CV events in renal transplant patients, results have been mixed.

Drüeke et al examined the effects of lipid levels in a small cohort of 54 kidney recipients over a 7-year period [43]. Of these patients, 25 experienced CV events during the study. These patients had increased atherogenic lipids, and smoking and antihypertensive use were significantly higher. Vathsala et al. found that CV episodes occurred in nearly 10% (46/500) of patients during a 36 month post-renal transplantation follow up [128]. Lipid values were measured serially at 3, 6, 12, 24 and 36 months after transplantation. Patients with a CV event had elevated cholesterol levels at some time points, but not all. Aakhus et al followed prospectively 406 renal recipients for 5 years [6]. During this time, 88 patients died; 65 of these deaths were classified as CV deaths. A correlation was found between total serum cholesterol and ischemic heart disease in a multivariate analysis, but not between total cholesterol, cerebrovascular (CBV), peripheral vascular disease, or cardiac death. Aker et al. demonstrated an association between atherosclerotic CV disease post-transplant and elevated cholesterol levels [12]. Roodnat et al found a correlation between cholesterol 1 year after transplantation and cardiac death in younger recipients [108] . Kasiske et al. examined the risk factors for ischemic heart disease and cerebral and peripheral vascular disease in 706 renal transplant patients over 7 years [73]. In this study, total cholesterol and LDL-cholesterol were not associated with ischemic heart disease in either univariate or multivariate Cox analysis. Another study compared the actual CV risk with that predicted from Framingham CV risk factor data [72]. The results indicated that smoking, diabetes, total and LDL-cholesterol, and blood pressure were all associated with adverse outcome.

In contrast to the studies that demonstrated a potential association between hyperlipidemia and CV events, Pollock et al found no such link in a follow-up study of 192 RTRs [99]. Other studies have found no correlation between post-transplantation hyperlipidemia and patient or graft survival [25;61]. Indeed, one study found that there was an inverse relationship [61].

Clearly, therefore, there was a need for a further randomized, controlled trial (RCT) to establish the relationship between dyslipidemia and CV disease in RTRs.

The ALERT (Assessment of LEscol in Renal Transplantation) is the first, and only, large-scale interventional clinical trial to evaluate CV complications following renal transplantation [63]. The study was initiated and coordinated by the Renal Section, Oslo University Hospital, Rikshospitalet. The core study assessed the effects of fluvastatin on cardiac outcomes in 2,102 RTRs over a 5-6 year period. In an extension of the core study, all patients were offered fluvastatin therapy for an additional 2 years [62-64].

The key findings of the ALERT study were:

a) Fluvastatin reduced major cardiac events (MACE; the primary endpoint) by 17% (p=NS)

b) Fluvastatin reduced LDL-cholesterol by 32% and reduced cardiac death and non-fatal myocardial infarction (MI) by 30%

c) Statin use was found to be safe in this complex population that requires multiple drugs

d) In the 2-year extension, MACE was reduced significantly by 21%, and cardiac death and non-fatal MI by 29%. The extension confirmed the cardioprotective effect of statin therapy in RTRs

The basis for the power calculation in the ALERT study was a survey (national and local registry data) from participating countries in 1994, which estimated a primary endpoint rate of around 5% per year. The investigators realised early on in the study that the event rate was lower than expected, and as a consequence increased the number of patients enrolled in the study. Moreover, at the recommendation of the data safety and monitoring board, the study Steering Committee implemented an amendment to the study protocol approximately 2 – 2 ½ years into the study that doubled the fluvastatin dose.

In the ALERT study population around 50% of deaths were CV, considerably less than reported in a Norwegian epidemiological study conducted by Aakhus et al in transplant patients (75%) [6]. The Aakhus et al study recruited patients in 1991 before the widespread use of statins. The first lipid lowering trials to be published were 4S in 1994 [2], WOSCOP in 1995 [116], and CARE in 1996 [109]. Awareness of the potential of lipid-lowering in

preventing CV events disseminated to the transplant community at this time. Furthermore, patients with pre-existing cardiac disease and overt high atherogenic lipids were probably excluded. There might also have been a reluctance to include patients shortly after transplantation owing to complex therapeutic regimes. In the last year of the ALERT trial, 32% of patients in the placebo arm were taking statins. This may have contributed to the lower than expected CV event rate. In a post-hoc analysis of the ALERT trial, based on a 17% reduction in the primary events, it was calculated that 6800 patients followed for 5 years would have been required to provide 80% power at a significance level of 0.05 (two-tailed).

The 4S [98] and LIPID [124] extension trials were published in 2000 and 2002, respectively. Both trials demonstrated the long-term effectiveness of statins in reducing CV events. In 2002, based on these findings, the ALERT Steering Committee implemented an extension to the core trial and offered all participants statin therapy for a further 2 years. Ninety-two percent of the patients participated in the 2-year extension. The findings of the ALERT core and extension studies changed clinical practice and the guidelines for treating CV disease in RTRs. As a consequence statin therapy has become standard practice in RTRs [71].

2. Introduction

The number of patients with reduced kidney function and chronic kidney disease (CKD) is increasing worldwide [78]. Table 1 shows the prevalence of CKD (categorized according to the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation definitions [81] taken from Hunt study [57] and the Vestfold County population. I undertook a survey in 2007 (unpublished work) to determine the prevalence of CKD in Vestfold County, Norway using blood samples analyzed by the hospital laboratory. Blood samples had been submitted to the laboratory by general practitioners for any reason. In Vestfold County, the prevalence of severe CKD (estimated glomerular filtration rate [eGFR] 15-29 ml/min per 1.73 m^2) was found to be slightly higher than reported by HUNT study [57]. Overall, however, the findings were consistent with the Hunt data and other studies, and showed that some degree of CKD is present in around 10-15% of the general population [36;56;57;78].

In my opinion, any CKD preventive strategies are dependent on regional epidemiological knowledge. Patients in stages 3 and 4 are at particular risk of developing end stage renal disease (ESRD) and will potentially require a renal transplant.

Stages of CKD	eGFR (ml/min per 1.73m^2)	Prevalence of CKD in HUNT study	Prevalence of CKD in Vestfold Norway
1	$\geq$90	3.1 %	NA
2	60-89	3.4 %	NA
3	30-59	4.5 %	12.3 %
4	15-29	0.16 %	0.80 %
Total		11.2 %	13 %

Table 1) Prevalence of CKD based on eGFR and corresponding CKD stages in the HUNT study and in Vestfold County in Norway

Hypertension and diabetes are the two leading causes of CKD. In addition, an aging population and increased patient survival after chronic diseases also contributes to the increasing number of patients with CKD requiring renal replacement therapy (RRT)[36;81;112].

Patients with CKD, even those with a minimal reduction in renal function, experience accelerated atherosclerosis and are at greater risk of CV morbidity and mortality, compared with the general population [14;52]. Indeed, the prevalence of CV diseases such as ischemic heart disease (IHD), left ventricular hypertrophy (LVH) and chronic heart failure (CHF) is several times higher in patients with CKD and ESRD than in the general population [52].

In CKD patients, mortality rates rise exponentially with decreasing GFR. Furthermore, in patients with ESRD receiving hemodialysis (HD), the mortality risk is five times higher in elderly, and more than 100 times higher in younger patients, compared with the general population [52]. Patients receiving HD also have a very high risk of mortality following a MI; 75% will die within two years post-MI. This is higher than for patients with diabetes (without CKD) after MI [59].

Patients with CKD have multiple co-morbidities. As a consequence, CKD patients are five times more likely to die of CV-related diseases than to reach ESRD [3;75]. Patients with CKD are also five times more likely to be hospitalized for any reason than individuals without CKD

[5]. The prevalence of CKD is escalating and HD alone accounts for about 2% of healthcare budgets in Europe [78].

Renal transplantation in patients with ESRD has led to substantial improvements in survival rates [133], better quality of life [118], decreased morbidity, and fewer hospitalizations [3;95]. In addition to improved survival, kidney transplantation is also the most cost-effective treatment option for patients with ESRD [45;115]. Even after a successful renal transplantation, however, patients are still at increased risk of CV morbidity and mortality [3;52]. Traditional CVD risk factors such as age, gender, cholesterol, smoking and systolic blood pressure, as well as the Framingham risk score, cannot fully explain the higher CVD morbidity/mortality rates in RTRs [91]. Consequently, there has been a quest to identify other potentially modifiable CVD risk factors in these patients. In addition, the incidence of CBV events and possible risk factors are also poorly defined in RTRs. Estimates are currently based on retrospective data from national registries. One study found that the prevalence of stroke was approximately 8% in RTRs, with CBV mortality making up 17% of total mortality [7;10;92].

2.1 Developments in immunosuppression

Cyclosporine (CsA) was introduced in the early 1980s. CsA-based immunosuppression, in combination with azathioprine (AZA) and prednisolone, has been the most commonly used regimen in Norway since 1983. The introduction of CsA substantially improved short-term graft survival. Prior to 1983, combination therapy with AZA and prednisolone was used [17;18;26;27;87]. Tacrolimus (TAC) was discovered in 1984 and approved in the USA in 1994. It was initially used in liver transplantation and then subsequently in renal and other solid organ transplantation. Studies have shown a reduced rate of acute rejection with TAC, compared with CsA, although graft survival rates are similar between the two drugs [23].

Mycofenolate mofetil (MMF) was introduced in the late 1990s and from 2001 it replaced AZA in standard maintenance immunosuppression regimens [69]. In 1970s, polyclonal antibody preparations such as antithymocyte globulin (ATG) and antilymphocyte globulin (ALG) were introduced for induction immunosuppression and for the treatment of steroid resistant acute rejections [117]. The use of these agents for induction is still widely used in the USA. A monoclonal antibody (Muromonab CD-3 [OKT-3]) was introduced in the 1980s for treatment of steroid resistant rejection [1;88].

Registry data show that graft function following deceased kidney transplants has improved significantly since 1987; almost two thirds of kidneys transplanted between 1995 and 1999 in the USA were still functioning 5 years later [3;4]. There are several reasons for this improvement. One is the reduction in acute rejections. Acute rejection within the first year of transplantation is a negative predictor of long-term renal allograft survival. Based on data collected from 93,934 patients receiving a renal transplant in the US between 1988 and 1996, allograft half-life more than doubled from 8.8 years to 17.9 years in patients who did not experience acute rejection within the first year. The allograft half-life remained relatively unchanged in patients experiencing at least 1 acute rejection episode [58].

A study of 66,774 RTRs showed that those receiving MMF plus CsA with or without steroids had significantly fewer acute rejections, compared with those receiving CsA plus AZA with or without steroids, during the first 6 months post-transplant. Four-year, death-censored graft survival and patient survival were also significantly improved in MMF-treated patients, compared with AZA [91]. Other newer immunosuppressive drugs are available for clinical use in solid organ transplants. These include sirolimus and everolimus, which act on the mammalian rapamycin target [132]. These drugs, however, are still not widely used as part of standard immunosuppression therapy.

2.2 Patient and graft survival in renal transplantation - historical perspective

The introduction of CsA in the 1980s led to dramatic increases in graft and patient survival [52;110]. During the last decade there has also been a decline in infection-related deaths [60]. Graft and patient survival rates are highest for RTRs receiving kidneys from living donors, followed by those from deceased donors. The lowest survival rates occur in patients receiving kidneys from deceased donors with suboptimal organs; so called expanded criteria donor (ECD) kidneys [3;4]. Figure 1 shows the survival data obtained from The Scientific Registry of Transplant Recipients [4]. In 1996, adjusted short-term patient survival was almost 99%. Adjusted 10-year patient survival was 65.6 %, an increase of about 14% since 1987.

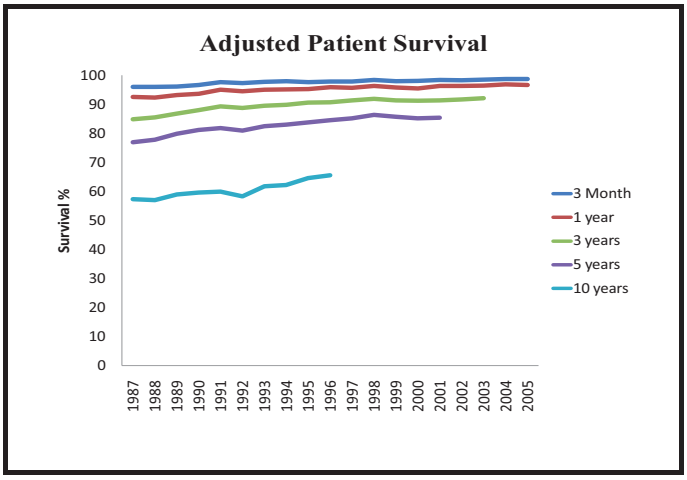

Fig. 1) Adjusted patient survival by year of transplant at 3 months, 1year, 3 years, 5 years and 10 years (deceased donor non-ECD kidney transplants).

Figure 2 shows the adjusted survival data from the European renal registry, categorized according to the RRT used, for dialysis patients, and patients receiving a first transplant (from day 91). Survival data are adjusted for age, gender and primary diagnosis. The data shows that RTRs have better survival shortly after transplantation. At five years, the survival rate is around 40% higher for RTRs, compared with patients on HD, and the survival gap increases with time [3].

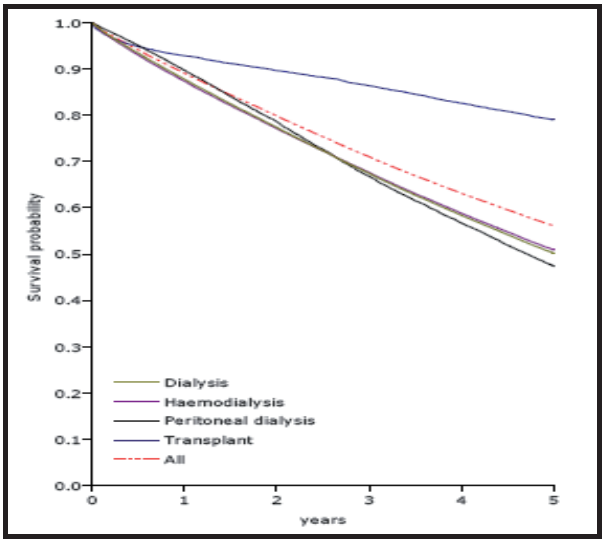

Fig. 2) Patients survival according to different RRT. (European renal registry annual report 2006)

These data are observational and the differences in survival rates between RTRs and ESRD patients on HD may be explained by several factors, i.e. selection bias [131]. Nevertheless, the survival benefit of renal transplantation after day 106 is clear [133].

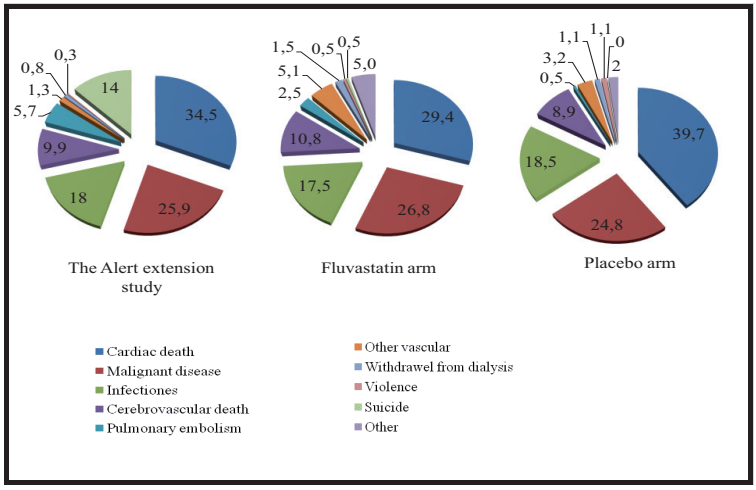

Fig. 3) Cause of death in the ALERT extension study (number in %)

Figure 3 shows all causes of mortality in the ALERT extension study. Cardiac death alone accounted for around 40% of all deaths in placebo-treated patients, and 29.4% in fluvastatin-treated patients. Malignancy and infections were the second and third leading causes of death [62]. CV and CBV death accounted for around 44% of the total mortality [62;64].

Even after successful renal transplantation, RTRs have a higher rate of CV events than the general population. The risk of CVD mortality is highest in younger patients, 10 times that of similar aged individuals in the general population [3;52;72;83;91]. Reduced graft function is an independent risk factor for CV events and mortality in RTRs [49;111]. Graft loss is also associated with increased mortality in RTRs. Rao and colleges analyzed data from the Scientific registry of Transplant Recipients and found that overall mortality was 78% higher in patients with primary graft failure, compared with those who had not yet undergone transplantation [101;102]. Based on existing survival data, between 80-90% of patients transplanted in 2006 are expected to survive for 10 years (Figure 4) [4].

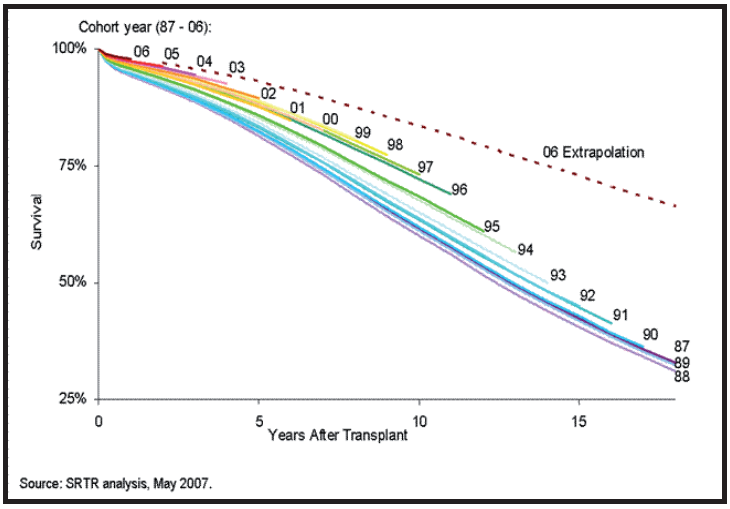

Fig 4) Patterns of mortality by cohort year of transplant (SRTR data)

Several atherosclerotic risk factors are more common in RTRs, and patients with CKD and ESRD (Figure 5) and this may explain, in part, the increased CV mortality. To further improve survival in RTRs it is necessary to minimize CV risk factors, maximize graft function, and avoid a return to hemodialysis [54]. Death with functioning graft (DWFG) is a frequent cause of mortality in RTRs. Cardiac death is the largest single cause of DWFG and accounts for 50% of deaths in these patients [31;96].

2. 3 Cardiovascular risk factors in RTRs

Traditional and novel CV risk factors/markers are summarized in Table 2.

Established risk factors	Novel risk factors
Age	Reduced GFR
Gender	Albuminuria / Proteinuria
High LDL-cholesterol	Inflammation
Low HDL-cholesterol	Cytomegalovirus disease
Diabetes	Anemia
Smoking	Lipoprotein disorders
Previous history of CHD	Electrolyte, Calcium and phosphor disorders
Left ventricle hypertrophy	Malnutrition
Obesity	ADMA
	Acute rejection
	Immunosuppressive medication

Table 2

Traditional CV risk factors include age, hypertension, one or more lipid abnormality (especially increased low-density lipoprotein [LDL]), history of coronary heart disease (CHD) or other vascular disease, smoking, diabetes and obesity. All of these risk factors, except age, are modifiable and should be targets for treatment [6;29;42;62;64;79;80].

Figure 5 shows the prevalence of established CV risk factors found in the ALERT study. As seen in earlier studies [15;72;83], these risk factors were very common in this patient population. About 90% of patients had hypercholesterolemia and high LDL levels. Hypertension was also common, as was obesity, diabetes and smoking. Other known CV risk factors including LVH, reduced renal function and previous history of CHD were evident in 15-20% of patients [63].

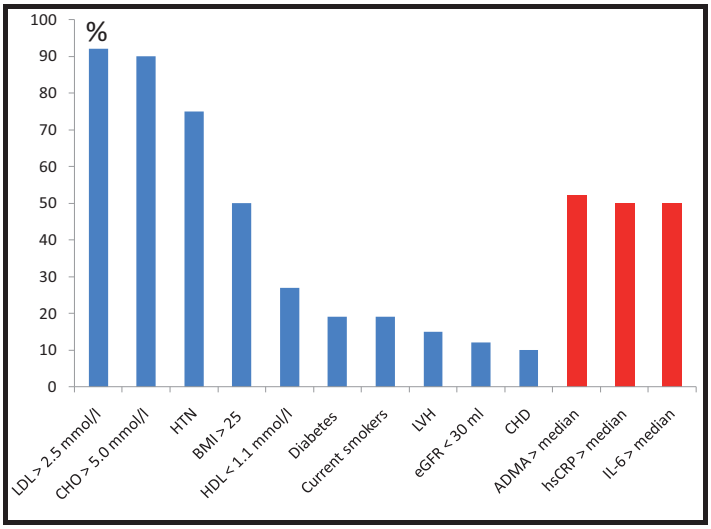

Figure 5) Prevalence of traditional and novel CV risk markers at inclusion to the ALERT study

A) Cardiac death in the placebo arm

Risk factor	HR	P value	95% CI
Age	**1.064**	**0.000**	**1.028-1.101**
Creatinine	**1.008**	**0.015**	**1.001-1.014**
Systolic BP	0.996	0.628	0.981-1.012
CHD	2.119	0.055	0.984-4.563
DM	**2.865**	**0.003**	**1.431-5.734**
LDL	1.249	0.156	0.919-1.698
Smoking	0.991	0.983	0.405-2.420
hsCRP	1.034	0.186	0.984-1.087
IL-6	**1.199**	**0.030**	**1.018-1.414**
ADMA	0.271	0.333	0.019-3.811

B) Mace in the placebo arm

Risk factor	HR	P value	95% CI
Age	**1.040**	**0.000**	**1.017-1.062**
Creatinine	**1.007**	**0.001**	**1.003-1.011**
Systolic BP	1.000	0.940	0..990-1.011
CHD	1.385	0.260	0.768-2.440
DM	**2.015**	**0.003**	**1.265-3.212**
LDL	**1.375**	**0.001**	**1.139-1.661**
Smoking	0.869	0.673	0.453-1.668
hsCRP	1.011	0.575	0.972-1.052
IL-6	**1.132**	**0.033**	**1.010-1.268**
ADMA	0.452	0.351	0.085-2.398

C) All cause of death in the placebo arm

Risk factor	HR	P value	95% CI
Age	**1.065**	**0.000**	**1.043-1.088**
Creatinine	**1.008**	**0.000**	**1.004-1.012**
Systolic BP	1.001	0.792	0.992-1.011
CHD	**1.909**	**0.013**	**1.144-3.187**
DM	**2.446**	**0.000**	**1.576-3.797**
LDL	1.126	0.239	0.924-1.373
Smoking	**1.723**	**0.048**	**1.005-2.952**
hsCRP	1.018	0.303	0.984-1.053
IL-6	**1.162**	**0.003**	**1.051-1.285**
ADMA	3.039	0.145	0.681-13.567

D) CBV events in the placebo arm

Risk factor	HR	P value	95% CI
Age	**1.051**	**0.001**	**1.019-1.083**
Creatinine	0.994	0.138	0.986-1.002
Systolic BP	**1.022**	**0.002**	**1.008-1.036**
CHD	1.910	0.085	0.916-3.984
DM	**3.408**	**0.000**	**1.900-6.113**
LDL	1.164	0.290	0.879-1.541
Smoking	**2.117**	**0.049**	**1.003-4.468**
hsCRP	0.976	0.554	0.901-1.057
IL-6	1.080	0.926	0.956-1.259
ADMA	3.774	0.269	0.358-39.794

Table 3) Adjusted multivariate hazard ratios for traditional and novel CV risk markers in the placebo arm of the ALERT core study (A: Cardiac death, B: Mace, C: All cause of death and D: Cerebrovascular events)

The effect of traditional risk factors and novel risk markers (high sensitivity C-reactive protein [hsCRP], interleukin-6 [IL-6] and asymmetric dimethylarginine [ADMA]) on CV events, CBV events and all-cause mortality was assessed in the placebo arm of the ALERT core study by multivariate Cox survival analysis. Serum creatinine was included in the multivariate model since almost all RTRs suffer some degree of reduced renal graft function. Results adjusted for gender are shown in Table 3. The analysis confirmed that age, serum creatinine, previous history of CHD, and diabetes are the most important traditional risk factors. All were independently associated with cardiac death, MACE, and CBV. In addition LDL predicted MACE, and smoking predicted all-cause mortality. Systolic blood pressure

was only associated with CBV events and IL-6 was associated with CV events and all-cause mortality.

2.4 Non-traditional and novel risk factors/markers in RTRs

Post-hoc analysis of the ALERT study confirmed that traditional CV risk factors are common in RTRs. The pattern of risk factors, however, and their relationship with outcomes, is somewhat atypical, highlighting the unique nature of CV risk in transplant recipients [68].

There is much confusion and/or divergent opinion as to which terms should be used in risk research [50]. Commonly used terms include:

- risk - the probability of an outcome occurring
- correlate - the association between a measure and an outcome
- risk factor - a correlate shown to precede the outcome
- causal risk factor - a risk factor that when modified can change outcomes

LDL-cholesterol is an established risk factor for CV events in most, though not all, patient populations. Lowering LDL-cholesterol reduces the risk of CV events. Numerous other biomarkers of CV outcomes have been proposed. The definitions used, however, are ambiguous. For example a recent paper described a risk factor as being associated with the disease because it is in the causal pathway leading to the disease [127]. In another paper, risk factor was defined as a variable with a significant statistical association with a clinical outcome, but not causal [24].

The Framingham Risk Score has proved to be reasonably accurate in assessing CV risk in most patient populations. It has, however, been less successful in estimating risk in renal transplant patients [65;68;72]. Based on the ALERT study population, work is underway to develop a risk factor calculator for renal transplant patients [48].

Three of the papers included in this thesis have addressed risk in relation to CV outcomes. In these papers, a risk factor is defined as a variable with a statistically significant association with a clinical outcome. An independent risk factor is defined as a risk factor that retains its statistical association with the outcome when other risk factors for the outcome are included in a statistical model. A casual risk factor is considered as to have a casual relationship with

an outcome, either directly or indirectly. It is important to note that casual risk is not defined statistically, but experimentally i.e. a statistical association is necessary but not sufficient.

Another consideration is to differentiate between risk factor and risk marker (biomarker), and in a broader sense to understand whether risk factors inter-relate i.e. mediators, moderators, overlapping or proxy [77]? This is a complex issue and will be touched upon in the discussion of the separate papers.

RTR-specific risk factors include time on HD, and post-transplant-related factors such as organ cold ischemia time, immunosuppression exposure, number of HLA mismatches between recipient and donor, and donor age. None of these factors are modifiable as we currently understand, but should be optimized.

As mentioned earlier, another important CV mortality risk factor is renal function. Fellström et al demonstrated that increased creatinine level is a strong predictor of all-cause mortality [49]. The problem is that even after a successful renal transplantation, most RTRs have reduced renal function. In an analysis of 459 patients, 90% showed some degree of CKD, with at least 60% having CKD stage 3 i.e. GFR between 30 and 59 mL/min/1.73 m^2 [70]. Using nephrotoxic drugs is, therefore, an important issue in transplantation.

Concerns have been raised regarding the potential harmful effect of statins on renal function [11;40]. Paper I of this thesis examined whether fluvastatin had a detrimental effect on renal graft function. In fact, fluvastatin did not have an unfavorable effect on renal function in RTRs, with or without diabetes [47].

In Paper II, risk factors for CBV events were evaluated. The only non-traditional risk factor assessed was polycystic kidney disease. It was found that polycystic kidney disease is a risk factor for hemorrhagic stroke but not for ischemic cerebral events. Although this is a new finding, the pathophysiology supports a plausible mechanistic link.

Much attention has been focussed on identifying other risk factors that could explain the increased CV morbidity/mortality in CKD patients and RTRs. Hyperhomocysteinemia is associated with CV morbidity and mortality in the general population. Interventional RCTs, however, have failed to show any benefit of homocysteine-lowering therapy [34;35]. Small retrospective studies have found an association between homocysteine and CV outcomes in RTRs [44;86]. A large RCT was started to determine whether homocysteine was a risk factor

for CV disease in renal transplant patients [22]. The trial, however, was discontinued prematurely due to futility (Presentation at American Society of Nephrology, San Diego November 2009). Based on the outcome of this trial and other large trials, it seems prudent not to categorize homocysteine as a risk marker for CV events. Other researchers have proposed that homocysteine as an inflammatory marker [13] or a dietary marker [130].

CRP is regarded as a risk factor for CV events and all-cause mortality. In a recent paper, it was demonstrated that in apparently healthy individuals without hyperlipidemia but with elevated high-sensitivity CRP, statin treatment significantly reduced the incidence of major CV events by reducing both CRP and LDL-cholesterol [104;106]. In patients with chronic renal failure, inflammatory markers are elevated [16]. An association between malnutrition, inflammation and atherosclerosis has been demonstrated in these patients [120]. Also, inflammation and activation of the immune system may play an important role in atherogenesis [74]. This may be important in RTRs as their immune system is activated in response to receiving an allograft [37;55;129].

2.5 Asymmetric dimethylarginine, Nitric oxide and NO synthase

ADMA is an endogenous, competitive inhibitor of 3 forms of nitric oxide synthase (NOS): neural, inducible and endothelial. In doing so it reduces nitric oxide (NO) generation [89;126]. Symmetric dimethylarginine (SDMA) and N-monomethyl L-arginine (L-NMMA) are two endogenous compounds related to ADMA. L-NMMA is as potent as ADMA in decreasing NOS but its concentration in plasma is about 10 times lower. It is suggested that the intracellular concentration of L-NMMA and ADMA may be comparable at least in some tissues, indicating that both are important NO synthase regulators[30]. SDMA is present at similar plasma concentrations as ADMA, but it has no effect on NOS activity [125].

ADMA is synthesized during the methylation of protein arginine residues by protein arginine methyltransferases (PRMT), and released during proteolysis. SDMA is eliminated exclusively by renal excretion. More than 90% of ADMA and L-NMMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH), and less than 10% is eliminated by the kidneys (Figure 6)[19]. The plasma level of ADAM in healthy subjects is about 1.0 micromol/L. It is increased by up to 10-fold in CKD patients, and by 2-3- fold in many other conditions [126] .

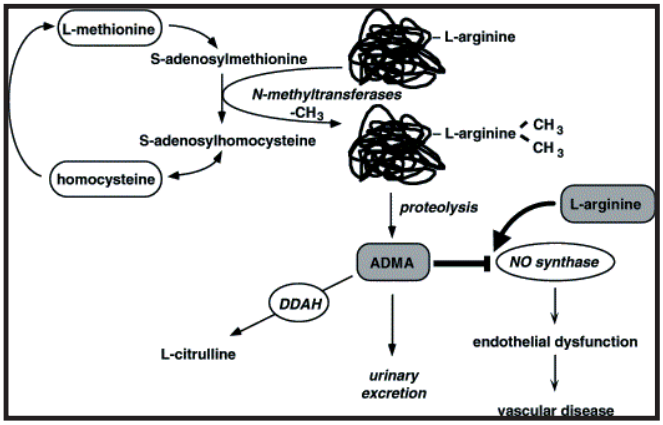

Fig. 6) Pathways related to ADMA. ADMA is formed by methylating arginine residues in proteins through the activity of protein-N-methyltransferase type 1 which uses S-adenosylmethionine, an intermediate of homocysteine metabolism, as a methyl group donor. Proteinases involved in physiological protein turnover release ADMA in to the plasma. Homocysteine, oxidative stress, inflammatory cytokines and hyperglycemia can inhibit DDAH activity. Accumulation of ADMA inhibits NOS by competing with L-arginine. *Modified from [Böger, et al. 2003)[126].*

NO is an important mediator/neurotransmitter and is synthesized from L-arginine by NOS. The availability of NO depends on many factors including expression and activity of NOS, presence of NOS substrate L-arginine, and reactive oxygen species. NO is involved in the regulation of vascular tone, neurotransmission, macrophage function, and mitochondrial respiration [119]. The level of NOS inhibitors may change under certain physiological and pathological conditions leading to NO deficiency. There is comprehensive evidence to support that NO is an important regulator of arterial stiffness and endothelial function [53;94;125]. Vallance et al demonstrated that an infusion of ADMA raised mean arterial blood pressure by 35% in guinea pigs [125]. ADMA also causes atherosclerotic lesions in endogenous NO synthase-deficient mice [122].

The long-term vascular effects of ADMA are not solely mediated by simple inhibition of endothelial NO synthesis. Upregulation of angiotensin-converting enzyme (ACE) and increased oxidative stress via the angiotensin I receptor, appear to be involved in the long-term vascular effects of ADMA [122]. ADMA infusions lead to about a 28% decrease in forearm blood flow in healthy volunteers [28;125]. Using human cerebral arteries obtained during autopsy in 26 patients, Segarra et al demonstrated that ADMA increased vascular tone, which could be prevented by L-arginine [114]. In a double-blind, placebo-controlled trial, 20

healthy men were infused with ADMA or vehicle over a 40-minute period. This was the first study to demonstrate that ADMA increases vascular stiffness and decreases cerebral perfusion in healthy subjects [76].

Data from several epidemiological and prospective trials in various patient populations, including patients with CKD, suggest that ADMA is a CV risk factor [20;123]. In non-transplanted patients with CKD, ADMA has been associated with progression to ESRD, and all-cause mortality [137]. ADMA is also significantly associated with worsening of renal function in patients with mild to moderate CKD [51;103]. There are, however, no data supporting ADMA as predictor of CVD/mortality in RTRs.

The potential to reduce CV morbidity/mortality by modulating ADMA is of great interest. Although no selective ADMA-lowering drugs are currently available, some therapeutic agents have shown ADMA-lowering properties in both animal and human studies. ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are widely used for treating arterial hypertension and heart failure. ACEIs and ARBs have been shown to decrease plasma ADMA levels by 14-24%, by an as yet unknown mechanism [33;38;66;67;90].

Statins are competitive inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A reductase. It is well known that statins reduce cholesterol biosynthesis [2]. Statins also increase endothelial NOS activity, and reduce inflammation by inhibiting leukocyte function and platelet adhesion to the endothelium [100]. These multiple properties would suggest that statins may modulate and/or decrease ADMA levels. Clinical studies in this area, however, have been disappointing. In one RCT no ADMA-lowering effect was seen in patients with normal or moderately elevated LDL-cholesterol treated with simvastatin or atorvastatin [93]. Another study demonstrated a non-significant reduction (9%) in ADMA in 32 hypercholesterolemic patients without ischemic heart disease treated with 40 mg/day pravastatin for 8 weeks [46]. It is important to note that these were only small, short-term studies. No long-term, large-scale, prospective trials have been conducted to assess the effect of statins on ADMA levels.

A few studies have shown that other drugs, e.g. fenofibrates [41;136], rosuvastatin [135], aspirin [39], antioxidants [21] and rosiglitazone [121] have ADMA-lowering/modulating properties. The problem is that these effects are not selective. No long-term RCTs have been conducted to confirm these effects. Several compounds including arylazoamidoximes, NO modulators and DDAH activators, which degrade ADMA, have already been synthesized

[113]. Currently, these agents are not commercially available and no efficacy and safety trials have been conducted.

It is important to keep in mind that ADMA also has favorable effects in some circumstances. In particular, ADMA may help to counter the neural injury caused by neural NOS-generated NO during brain ischemia and in neurodegenerative diseases such as Alzheimer's disease [32]. Increased ADMA may also be beneficial in patients with liver cirrhosis as it may prevent the peripheral vasodilatation that results from increased NO [85]. It will be a challenge, however, for pharmaceutical companies to develop selective ADMA-lowering drugs.

2.6 Inflammation markers - high sensitivity CRP and IL-6

Inflammation is an important determinant of atherosclerosis. hsCRP is recognized as the best biomarker of inflammation and CVD risk [82;105;106;127]. IL-6 is also a biomarker of inflammation, and elevated levels are associated with increased risk of future MI in healthy men [107]. Elevated CRP [138] and IL-6 have been associated with increased CVD risk and mortality in CKD patients [97]. There is, however, scarce data on the effects of hsCRP and IL-6 in RTRs.

2.7 ADMA, hsCRP and IL-6 - ALERT study findings

ADMA, hsCRP and IL-6 were assessed as possible CV risk factors in the ALERT study. Continuous co-variables, including systolic blood pressure > 140 mmHg, LDL > 2.5 mmol/l, age over 64-years, hsCRP, IL6 and ADMA were dichotomized by median. Around 50% of patients had raised ADMA, hsCRP and IL-6 plasma levels. These dichotomized variables were assessed alongside other traditional risk factors including diabetes, CHD, and smoking. Figure 7 shows the prevalence of the traditional risk factors in combination with ADMA, hsCRP, and/or IL-6.

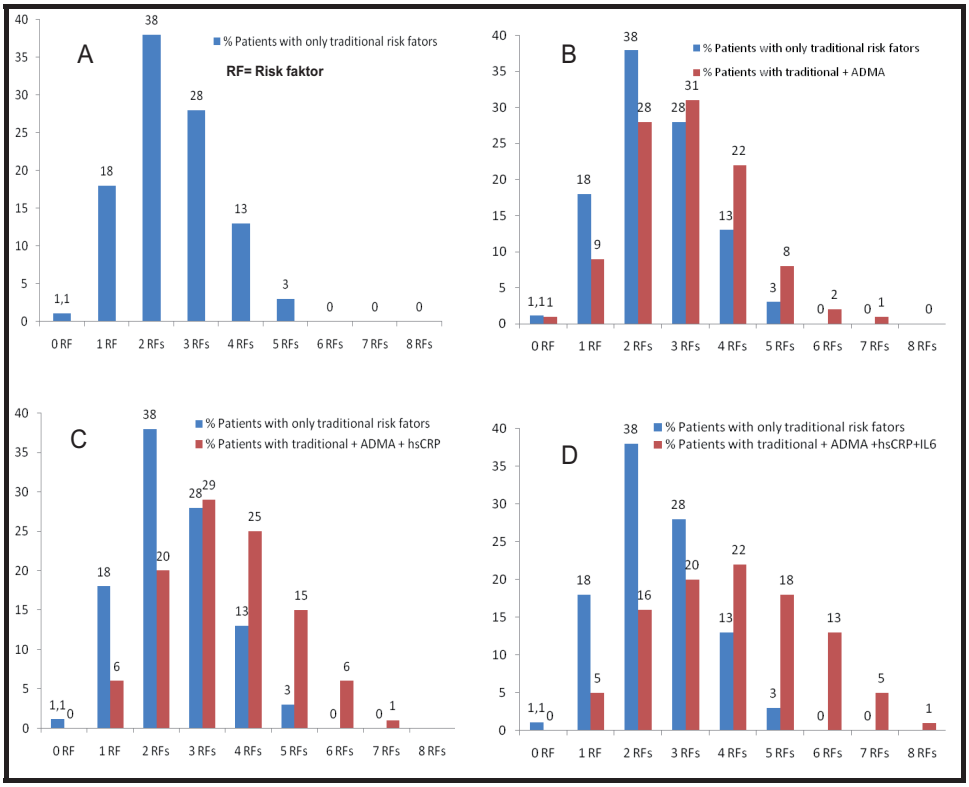

Fig. 7) Prevalence of traditional risk factors (A) and in combination with novel risk markers (B-D) at inclusion to the ALERT study

Figure 7 shows that by including novel risk markers alongside the traditional risk factors, the proportion of patients with more than 4 risk factors increases dramatically suggesting increasing CV morbidity and mortality risk [7-9].

3. Thesis aims

There is a need to identify and address the risk factors that impact on mortality, morbidity and graft function in RTRs. In the ALERT study, both traditional CV risk factors and potentially important non-traditional CV risk factors were assessed in 2,102 RTRs [62-64].

The following questions, which have not previously been evaluated in RCTs, are addressed in this thesis:

I. Does fluvastatin have a negative effect on graft function in RTRs?

II. What are the risk factors for CBV events in RTRs?

III. What role does inflammation play in CV outcomes in RTRs?

IV. Is ADMA a predictor of renal and CV outcomes and all-cause mortality in RTRs?

3.1 Material and methods

All baseline data from the ALERT study database were available.

3.2 Laboratory measurements

Patients (males and females aged 30 – 75 years) who had received a renal transplant more than six months before study enrolment and had a total serum cholesterol level between 4 and 9 mmol/L were eligible to enter the study. Patients with a MI more than 6 months prior to randomization were excluded from the study if their total cholesterol levels ranged between 4 and 7 mmol/L. Patients were assessed 1.5 months after randomization and at 6-monthly intervals throughout the core and extension study.

Laboratory measurements of lipids, serum creatinine, creatinine kinase, and hepatic enzymes were performed at a central laboratory (Eurofins Medinet). This commercial company has many years' experience in analyzing samples for large clinical trials, and is evaluated by several different national and international agencies. The ALERT Steering Committee did not evaluate the analytic process or validate the data.

At baseline, serum, plasma and blood samples were taken. These were frozen at minus 70º C and stored at Medinet. At the end of the study, a range of biomarkers were measured in the baseline samples at a central laboratory (Synlab laboratories in Heidelberg, Germany, Head Winfried März). This laboratory specializes in analyzing a wide range of diverse biomarkers in frozen samples. The ALERT Steering Committee did not independently validate the samples. The assessment of laboratory techniques is beyond the scope of this thesis.

The "new" biomarkers analyzed in this thesis are hsCRP, IL-6 and ADMA. The methodology is described in the respective papers. These biomarkers were measured at baseline only and not throughout the trial. Ideally, it would have been preferable to measure these biomarkers throughout the study. Such an approach, however, was financially prohibitive in this large study. The reported values for hsCRP, IL-6 and ADMA in the ALERT trial corresponded to levels reported previously in other studies in RTRs. Having only one sample taken at baseline

is problematical. It is well known that within-person variability can bias a biomarker's association with disease [134]. The extent of regression dilution bias in prospective trials has been assessed in several methodological meta-analyses [84;134], and has been shown to be a potential problem in trials with few sampling points. The ALERT trial investigators accept that there is potential for bias.

4. Statistical analysis

SPSS versions 15 and 16.0 (© 2007 SPSS Inc.) was used for statistical analysis. For normally distributed variables, mean and standard deviation (SD) are presented. For continuous variables that were not normally distributed, for example hsCRP and IL-6, logarithmic transformation was used to obtain a normal distribution. If the assumption was met, the transformed values were used in the statistical analyses. Demographic and clinical baseline characteristics were compared between the groups using the independent-samples *t*-test and the chi-square test, according to the type of variable analyzed. The Cox proportional hazard model was used to analyze the relationship between risk factors and time to event for study endpoints. All covariates were carefully examined to asses if the assumptions for the Cox hazard model were met.

Aware of the potential limitations of Cox proportional hazard models, we carefully assessed the number of covariates in every model in relation to the number of events and patients at risk. There were, however, no major concerns owing to the relatively large number of events and subjects, and the long-term follow-up time in the ALERT extension trial.

Based on the literature and our clinical judgment, covariates were used in the model as they have been shown to be important for outcomes. The role of other potentially important covariates was also assessed, initially by univariate analysis then by multivariate analysis. The relation between the covariates was also examined by assessing correlations between them, so as to avoid including covariates with important interactions in the same model. Univariate and multivariate analyses were carried out on the outcome risk factors. Hazard ratios (HR) for group comparisons were calculated with 95% confidence limits. Kaplan-Meier plots were used for survival analysis. Log Rank test was applied to compare survival distributions of groups.

A Cox proportional hazard model was used to assess risk factors and/or risk markers (i.e. age, gender smoking, previous CHD, systolic blood pressure, LDL, diabetes mellitus, creatinine, ADMA, hsCRP and IL-6). In total, 1,776 patients were isolated with full datasets. Initially, analysis was performed based on all variables included in the model and the value of the total -2* log- likelihood was recorded. Then one variable was removed at a time and the -2*log-likelihood value was recorded. This variable was then put into the model again and another was taken out until all the included variables had been examined. Percentage contributions for each risk factor/risk marker were given as a fraction of the difference in-2*log-likelihood in a full model and in a model where age, sex and CHD were included.

5. Predictive values of novel risk markers compared with traditional risk factors

Our results (Papers 3-4) show that there was an independent association between the novel risk markers and important clinical outcomes in the ALERT study population. This may raise the question: how much do these findings add to the predictive value of known traditional risk factors? To try and answer this, a multivariate Cox proportional hazard model was used. The results are summarized in Table 4a-d. HRs are given per SD for the parametric variables.

Table 4a) Graft failure or a doubling of serum creatinine (GFDSC)

Risk factors	HR pr SD (95% CI)	P-Value Multivariate	Contribution to reduction in -2*log-likelihood when variable is removed §
Sys BP mmHg	1.32 (1.19-1.46)	0.000	9.2
Smoking	1.38 (1.04-1.80)	0.027	2.0
LDL mmol/l	1.20 (0.98-1.02)	0.109	0.70
Diabetes	1.36 (1.03-1.80)	0.033	1.4
Creatinine umol/l	1.88 (1.79-2.09)	0.000	61
ADMA umol/l	1.12 (1.01-1.24)	0.046	1.4
Ln hsCRP	1.09 (1.02-1.24)	0.155	1.1
Ln IL6	1.21 (1.07-1.37)	0.002	3.2

§ % contributions given as a fraction of the difference in-2*Log-likelihood in a full model and a model where age, sex and CHD are included.

Table 4b) Cardiac death or non fatal-myocardial infarction (CDNFMI)

Risk factors	HR pr SD (95% CI)	P-Value Multivariate	Contribution to reduction in -2*log-likelihood when variable is removed §
Sys BP mmHg	1.08 (0.92-1.25)	0.367	1.5
Smoking	1.31 (0.85-2.01)	0.219	12
LDL mmol/l	1.37 (1.18-1.59)	0.000	31.0
Diabetes	2.12 (1.51-2.98)	0.000	31.0
Creatinine umol/l	1.16 (1.05-1.35)	0.022	7.5
ADMA umol/l	1.17 (1.01-1.35)	0.039	6.0
Ln hsCRP	1.19 (1.01-1.42)	0.049	6.0
Ln IL6	1.17 (0.97-1.08)	0.086	4.5

Table 4c) Death

Risk factors	HR pr SD (95% CI)	P-Value Multivariate	Contribution to reduction in -2*log-likelihood when variable is removed §
Sys BP mmHg	1.20 (0.96-1.23)	0.142	2.3
Smoking	1.83 (1.33-2.50)	0.000	16.1
LDL mmol/l	1.06 (0.94-1.20)	0.346	0.67
Diabetes	1.93 (1.50-2.50)	0.000	23.7
Creatinine umol/l	1.22 (1.10-1.35)	0.000	12.6
ADMA umol/l	1.16 (1.04-1.31)	0.011	6.8
Ln hsCRP	1.12 (0.98-1.25)	0.105	3.5
Ln IL6	1.06 (0.99-1.13)	0.023	5.7

Table 4d) cerebrovascular events

Risk factors	HR pr SD (95% CI)	P-Value Multivariate	Contribution to reduction in -2*log-likelihood when variable is removed §
Sys BP mmHg	1.16 (0.98-1.35)	0.069	4.4
Smoking	1.62 (1.05-2.49)	0.029	7.1
LDL mmol/l	1.08 (0.91-1.27)	0.372	1.6
Diabetes	3.17 (2.27-4.43)	0.000	60.0
Creatinine umol/l	1.00 (0.99-1.00)	0.994	0.0
ADMA umol/l	1.29 (1.11-1.49)	0.001	15.0
Ln hsCRP	0.97 (0.80-1.16)	0.722	0.0
Ln IL6	1.11 (0.92-1.34)	0.268	1.4

6. Results

6.1 Paper I

Long-term fluvastatin use had no detrimental effect on renal function in RTRs.

This study investigated the effect of fluvastatin on graft loss, changes in serum creatinine, calculated creatinine clearance, proteinuria, and renal adverse events versus placebo.

Compared with placebo, fluvastatin treatment had no significant effect on renal function, assessed by serum creatinine (fluvastatin, 175.4±2.20 μmol/L; placebo, 172.7±2.20 μmol/L; P = 0.39), creatinine clearance (fluvastatin, 55.3±0.30 mL/min; placebo, 55.8±0.30 mL/min; P = 0.26) or proteinuria (fluvastatin, 0.58±0.03 g/24h; placebo, 0.53±0.03 g/24h; P = 0.31). Fluvastatin also had no detrimental effect on creatinine clearance or proteinuria in a subgroup of 340 diabetic patients.

6.2 Paper II

Cerebrovascular events in RTRs

This paper investigated the incidence of stroke, and risk factors for ischemic and hemorrhagic CBV events in 2,102 renal graft recipients participating in the ALERT extension trial. The incidence of different CBV event subtypes was compared between placebo and fluvastatin to evaluate any potential influence of lipid-lowering therapy. The incidence and type of CBV events did not differ between the treatment groups so the pooled treatment arm data were analyzed.

A total of 184 (8.8%, 95% CI 4.6-12.9) of the 2,102 patients experienced a CBV event during follow-up, corresponding to an incidence of 1.3% CBV event per year. The mortality rate for patients experiencing a hemorrhagic stroke was 48% (13/27) and for ischemic stroke 6% (8/133). Diabetes, previous CBV event, age, and serum creatinine were independent risk factors for cerebral ischemic events. The risk of a hemorrhagic cerebral event was increased by diabetes, previous polycystic kidney disease, LVH and systolic blood pressure. These results show that the risk factors for CBV events in RTRs differ according to the type of the event.

6.3 Paper III

Inflammation in renal transplantation

The association between inflammation and all-cause mortality and CV events was investigated in the ALERT extension study. Mean baseline IL-6 was 2.9±1.9 pg/ml (n=1,751) and hsCRP 3.8±6.7 mg/l (n=1,910). After adjustment for baseline risk factors, the hazard ratio for a major CV event and all-cause mortality for IL-6 was 1.08 (95% CI, 1.01 – 1.15, P= 0.018) and 1.11 (95% CI, 1.05 – 1.18, P<0.001), respectively. The adjusted HR for hsCRP was 1.10 (95% CI, 1.01 – 1.20, P =0.027) for a CV event and 1.15 (95% CI, 1.06 – 1.1.25, P=0.049) for all-cause mortality. These results suggest that IL-6 and hsCRP are independently associated with major CV events and all-cause mortality in RTRs.

6.4 Paper IV

ADMA is associated with renal and CV outcomes and all-cause mortality in RTRs

The effect of ADMA on graft function (graft failure or doubling of serum creatinine [GFDSC]), MACE, CBV and all-cause mortality was investigated in the ALERT extension trial.

ADMA was analyzed from blood samples obtained at baseline in 1,847 patients. Minimum, maximum and mean values for ADMA were 0.50 umol/l, 1.50 umol/l and 0.78 umol/l, respectively. The fluvastatin and placebo arms were initially analyzed separately for clinical events. As there was no significant difference between the treatment arms, a subsequent analysis was performed on the pooled patient data.

After adjustment for baseline values for established risk factors, ADMA was found to be a significant risk factor for GFDSC: HR 3.71 (95% CI 1.76-7.81, $p<0.001$), MACE: HR 2.53 (CI 1.01-6.34, $p=0.048$), CBV events: HR 7.74 (CI 2.59-23.12, $p<0.001$), and all-cause mortality: HR 4.74 (CI 2.11-10.64, $p<0.001$). The occurrence of clinical endpoints increased with increasing quartiles of ADMA. All endpoints were significantly more likely in the fourth quartile, compared to the first quartile.

This study is the first to demonstrate that plasma ADMA levels are associated with increased incidence of MACE, CBV events, all-cause mortality, and deterioration of graft function in RTRs.

7. Discussion

All 4 papers discussed in this thesis are based on *post-hoc* analyses of the ALERT core and extension study data. Although there are inherent limitations in performing *post-hoc* analyses, the outcomes/biomarkers analyzed were pre-planned.

7.1 The effect of fluvastatin on renal function

The ALERT core study showed that fluvastatin had no adverse effect on GFDSC or on changes in GFR, compared with placebo [31]. A subgroup analysis of the LIPS trial has also shown similar findings in patients undergoing a first successful percutaneous coronary intervention [54]. The ALERT study has also shown that fluvastatin had no detrimental effect

on serum creatinine, creatinine clearance or proteinuria, compared with placebo [30]. There was an overall trend towards a decrease in creatinine clearance over the course of the study, an effect that would be expected with increasing age in this patient population [59]. Patients with diabetes are at increased risk of renal dysfunction [85], and hence may be more susceptible to any potential adverse effect of statin therapy on renal function. The ALERT study showed that fluvastatin had no effect on creatinine clearance or proteinuria in RTRs with diabetes. Patients suffering a graft loss were excluded from the study. This reduced the study sample size and, consequently, the statistical power of the analysis. The finding that fluvastatin has no adverse effect on renal function in RTRs cannot necessarily be extrapolated to other statins or to other patient groups.

7.2 CBV events

Cerebral outcome was assessed based on stroke subtype i.e. ischemic or hemorrhagic. In the ALERT study, risk factors/risk markers differed according to the stroke subtype. An important finding was that polycystic kidney disease was associated with hemorrhagic stroke. The effect of medications on CBV events was also evaluated. In total, 18.4% (9/49) of patients not taking warfarin and/or anti-platelet therapy experienced a hemorrhagic stroke. Although hemorrhagic stroke was lower in patients taking warfarin/anti-platelet therapy, the difference was not statistically different (p=0.537).

Twenty-three percent (43/184) of patients with CBV events had atrial fibrillation. Of those, 26 were treated with coumarin/warfarin. Five (19.2%) of the coumarin-treated patients with atrial fibrillation, and 3 (17.6%) not receiving coumarin/warfarin had a lethal hemorrhagic stroke (p=1.000). These findings do not suggest that lethal hemorrhagic stroke is more frequent in coumarin/warfarin-treated patients in this population. Patients with atrial fibrillation were under-treated with anticoagulation therapy. Greater use of coumarin/warfarin may have influenced the outcome.

Forty-eight percent (13/27) of patients with hemorrhagic stroke died. Only two (7%) of these patients were being treated with coumarin/warfarin. Thus anticoagulation therapy was not associated with the deaths of patients with hemorrhagic stroke. Eighty-three percent (152/184) of the patients with a CBV event were not on warfarin treatment; 32 (21%) of these had a fatal stroke. Of the 32 patients treated with warfarin, 6 (19%) experienced a fatal stroke. This suggests that RTRs do not have an increased mortality risk when treated with coumarin/warfarin.

7.3 Inflammation

The ALERT study showed that inflammation predicts major CV events and all-cause mortality in RTRs [6]. This analysis used pooled data from both treatment arms. As statin therapy may impact on inflammation, a multivariate Cox regression on the ALERT core and extension study was performed separately for the placebo and fluvastatin arms to examine a possible effect of statin therapy on inflammation and clinical outcomes (MACE, death and CBV events).

By looking at each treatment arm separately in the core study, where follow-up time was shorter than in the extension study, we observed substantial loss of endpoints. Despite these limitations, hsCRP was shown to be a significant predictor of all-cause mortality in both the placebo and fluvastatin arms (ALERT core and extension populations), but not for MACE in the core study. HsCRP did not predict CVB events in the ALERT core study.

Similar results were found for IL-6, with the exception that IL-6 did not predict death in the placebo arm of the ALERT core population. It is not possible, based on this analysis, to assess the effect of statin therapy on the inflammation markers; this would require continuous measurements of hsCRP and IL-6. A beneficial effect of statin therapy, however, cannot be excluded.

It is also difficult to assess what value the extension study adds regarding the HR calculations for the inflammation markers hsCRP and IL-6 compared to the corresponding HRs in the core ALERT, but the extension does contribute substantially to the statistical power in terms of longer follow-up time and number of events. This parallels what was found for the primary outcomes in the core ALERT study.

Data from renal registries have shown that better graft survival is achieved with pre-emptive transplantation [3;4]. One possible explanation is less exposure to inflammation associated with prior dialysis. Of the patients enrolled in the ALERT trial, 9.3% (n= 195) had a pre-emptive transplantation. At inclusion, the hsCRP was 2.76 mg/L (95% CI: 2.7-3.46) in pre-emptive patients, and 3.14 (95% CI: 2.84-3.41) in patients with prior dialysis (n=1904). Although hsCRP was numerically higher in dialysis patients, the difference was not statistically significant. IL-6 values were also slightly higher in patients who had received dialysis, compared with a pre-emptive transplantation (2.93 $\pm$ 2.84 pg/ml and 2.79 $\pm$ 2.52 pg/ml, respectively); however, the difference was not statistically significant.

These findings suggest that statin therapy does not have an effect on inflammation even after 7-8 years of therapy, as both inflammation markers were associated with major CV events and all-cause mortality, despite of use of statins.

7.4 ADMA

The effect of ADMA on GFDSC, MACE, CBV events and all-cause mortality was evaluated in the ALERT study. Patients were categorized in to quartiles based on ADMA levels. All endpoints were significantly increased in patients in the 4th quartile, compared with the 1st quartile.

ADMA can accumulate in patients undergoing dialysis therapy. Time on dialysis, therefore, was included in the multivariate analysis along with the other traditional risk factors. No significant association between ADMA level and pre-transplant dialysis duration was found. The analysis used baseline ADMA measurements only, so a casual relationship between ADMA and outcome is not substantiated by this analysis, only an independent association.

The results suggests that the negative effects of ADMA on CV complications are not limited to patients with CKD, but also extend to relatively low risk RTRs with stable renal function.

8. Conclusions and interpretation

- Fluvastatin had no detrimental effect on renal function in RTRs with or without diabetes. Fluvastatin may, therefore, be used without fear of jeopardizing renal function

- Risk factors for CBV events differed depending on stroke subtype. A total of 184 (8.8%, 95% CI 4.6-12.9) patients experienced a CBV event during follow-up, corresponding to an incidence of 1.3% per year. Mortality following a hemorrhagic stroke was 48% (13/27), compared with 6% (8/133) for ischemic stroke. Diabetes, previous CBV event, age, and serum-creatinine were independent risk factors for cerebral ischemic events. Risk factors for a hemorrhagic cerebral event included diabetes, polycystic kidney disease, LVH and systolic blood pressure

- The inflammation markers, IL-6 and hsCRP, are independently associated with major CV events and all-cause mortality in RTRs

- Increased ADMA plasma level is associated with increased incidence of MACE, CBV events, all-cause mortality, and deterioration of graft function in RTRs
- ADMA, hsCRP and IL-6 contributed substantially in the prediction of the clinical outcomes in the ALERT study population. Further studies, however, are needed to confirm these results

9. Clinical implications and future studies

This thesis has evaluated the following:

- The safety of statin therapy
- Risk factors for CBV events
- The role of inflammation as a risk factor
- The role of ADMA as a risk factor

The ALERT study failed to show any beneficial effect of fluvastatin therapy on renal endpoints. Paper 1 shows that fluvastatin is safe to use in RTRs. Paper 2 highlights the risk factors associated with stroke. In particular, in addition to non-modifiable risk factors, modifiable risk factors for different stroke subtypes were identified. This will enable clinicians to initiate strategies for stroke prevention. Although statin therapy reduces CV events in RTRs, there is still considerable residual CV risk in this population. Paper 3 shows that inflammation increases the risk of CV events and mortality. Hopefully, in the future, a randomized, controlled trial will be conducted to further assess this finding.

Paper 4 demonstrates that ADMA is a powerful risk marker for CV morbidity and mortality. This novel risk marker may be used for risk stratification in this high-risk population. This is the first time that a strong association between ADMA and CV outcomes/mortality has been demonstrated in RTRs. It is not yet known, however, if the link between inflammation, ADMA and clinical outcomes is causal. RCTs are required to establish whether anti-inflammatory and ADMA-lowering strategies definitely reduce the risk of CV morbidity/mortality in RTRs.

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Appendix

Paper I-IV

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