CHAPTER 1

INTRODUCTION AND AIMS OF THIS THESIS
INTRODUCTION

The number of patients with end-stage renal disease (ESRD) treated with renal replacement therapy by dialysis or transplantation is rapidly increasing worldwide (figure 1). According to the United States Renal Data System (USRDS), the prevalence of ESRD in the United States of America (USA) reached 1,569 per million subjects in 2005 with prevalent dialysis and transplanted populations of 341,000 and 143,693, respectively. It is projected that there will be nearly 800,000 prevalent ESRD patients receiving renal replacement therapy in 2020 in the USA with a total cost of $54 billion (figure 1). Although current prevalence of ESRD in Europe is below that of the USA, the trend in rise in number of patients with ESRD in Europe is similar to that of the USA.

Renal transplantation is the preferred therapy for patients with ESRD, because of much lower costs compared to dialysis and significant benefits in terms of quality of life and likelihood of survival. During the last three decades, one-year graft survival has impressively improved from approximately 40% in the 1970's to more than 90% for

Figure 1. Prevalent ESRD, dialysis, and transplantation populations from 1980 through 2020 and expenditures of ESRD from 1991 through 2020 in the USA. Solid lines show actual and dashed lines show predicted values.

Figure 2. Graft survival of first cadaver kidney transplants according to different years of transplantation. Figure is available online at: http://www.ctstransplant.org/ (Figure: K-14101-0208).
deceased donors and more than 97% for living donors nowadays (figure 2).\textsuperscript{8–10} This improvement is due to the introduction of a multitude of variables. One of the most important improvements was the introduction of cyclosporine in the early 1980’s for the prevention of acute and chronic rejection.\textsuperscript{11,12} The introduction of newer immunosuppressive drugs such as mycophenolate mofetil and tacrolimus has further improved the first year survival rate.\textsuperscript{13,14} Long-term graft survival has also improved over the last few decades. However, this improvement in long-term graft survival is mostly a consequence of improved short-term graft survival as can be seen in figure 2. This figure shows that the slope of the lines for graft survival are almost similar once transplant survival has surpassed three months. Approximately half of all cadaveric renal allografts are still lost within 10–12 years after transplantation.\textsuperscript{8}

The two main reasons why long-term graft survival is lagging behind short-term graft survival are chronic transplant dysfunction (CTD) and high mortality (figure 3).\textsuperscript{2,8,9} These two reasons will be discussed in more detail in the following paragraphs.

**Figure 3. Causes of death in RTR, from 1993 to 2004.** Although one quarter of RTR die of unknown causes, the major known cause of death in RTR reported by UNOS as of June 1, 2005 is cardiovascular disease. Adapted from Adams, PL. Long-term patient survival: strategies to improve overall health. Am J Kidney Dis 2006; 47; 565-585.\textsuperscript{2}

**CHRONIC TRANSPLANT DYSFUNCTION (CTD)**

CTD is a nonspecific term describing a clinical syndrome which is defined as progressive renal dysfunction occurring beyond three months after transplantation which is independent of acute rejection and specific disease entities, with typical features on biopsy.\textsuperscript{9} Clinically CTD is characterized by a gradual decline in renal function with slowly rising serum creatinine. The decline in renal function is often found in combination with proteinuria and de novo or accelerated hypertension.\textsuperscript{15–17} The prevalence of CTD varies, ranging from 23% at 5 years after transplantation to up to 60% of grafts at 10 yr after transplantation.\textsuperscript{18} CTD is often the functional consequence of chronic allograft nephropathy (CAN) which is a descriptive term for histological changes consisting of interstitial fibrosis (IF), tubular atrophy (TA), atherosclerosis, and glomerulosclerosis.\textsuperscript{16,19}
CTD is a very complex and multifactorial disorder with both alloantigen-dependent and alloantigen-independent factors involved in its development. Alloantigen-dependent factors include acute rejection episodes, HLA matching, donor-specific antibodies and inadequate immunosuppression or noncompliance. Alloantigen-independent risk factors include donor age, brain death, ischemia/reperfusion injury, hypertension, lipid abnormalities and calcineurin inhibitors (CNI)-related nephrotoxicity.\textsuperscript{9,17}

The role of inflammation as a final common pathway in CTD is increasingly acknowledged. During the 9\textsuperscript{th} Banff conference held in 2007 a new lesion score, termed ‘ti’ (total interstitial inflammation), was added to the Banff schema. This lesion score uses quantitative criteria for mononuclear cell interstitial inflammation in total parenchyma (scarred and unscarred) scores.\textsuperscript{20} However, many centers are reluctant to perform surveillance biopsies, as this is an invasive procedure, with an ever existing risk of complications, including intractable bleeding necessitating removal of the graft. As a consequence, currently, noninvasive estimates of glomerular filtration rate (e.g., creatinine clearance or plasma creatinine) and proteinuria are used for the identification of renal transplant recipients (RTR) at increased risk for CTD.\textsuperscript{21-23} However, once serum creatinine starts to rise or proteinuria develops, chronic structural lesions are already present and it is usually too late for intervention.\textsuperscript{9,24} So, there is great need for biomarkers for earlier identification or prediction of CTD.\textsuperscript{25}

**Mortality**

The two major identified causes of mortality among RTR are cardiovascular disease and infection (figure 3).\textsuperscript{2,26} In the next paragraphs these causes will be discussed in more detail.

*Cardiovascular disease*

The main cause of death after renal transplantation is cardiovascular disease. Although cardiovascular mortality is much lower in RTR than in patients on dialysis,\textsuperscript{27,28} RTR are still at higher risk for cardiovascular mortality compared to the general population.\textsuperscript{29,30} The risk factors for cardiovascular disease are at least in part similar to those in the general population and include age, hypertension, diabetes, hyperlipidemia, renal insufficiency, albuminuria, and smoking.\textsuperscript{7,31}

Cardiovascular disease is usually the consequence of the process of atherosclerosis, which is a progressive disease of large arteries, characterized by accumulation of lipids and fibrous elements.\textsuperscript{32} In the past decades, understanding of the pathophysiology of atherosclerosis has evolved enormously. Current evidence supports a central role for chronic inflammation in mediating all stages of this disease from initiation through progression and, ultimately thrombotic complications.\textsuperscript{33,34}
The inflammatory cascade is very complex and, in detail, beyond the scope of this thesis. In summary, the inflammatory cascade can supposedly be triggered by oxidized lipoproteins, dyslipidemia, hypertension, diabetes, obesity, toxins after smoking, and infection.\textsuperscript{34,35} The activated inflammatory cascade subsequently induces endothelial dysfunction, which sets the stage for both initiation and progression of atherosclerotic lesions.\textsuperscript{36} Endothelial dysfunction is supposed to lead to expression of adhesion molecules (such as vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1)) on the luminal surface. The expression of adhesion molecules and secretion of chemokines (such as monocyte chemoattractant protein-1) results in the attachment and migration of circulating leukocytes into the intima. Here the leukocytes take residence and divide. Differentiation of migrated monocytes into macrophages and subsequent uptake of lipids by these cells results in generation of foam cells and fatty streaks. Further recruitment of inflammatory cells and proliferation of smooth muscle cells lead to the development of an atherosclerotic plaque.\textsuperscript{33,34} Localized inflammation also contributes to plaque instability and rupture, predisposing to acute clinical syndromes.\textsuperscript{37}

The important role of inflammation in atherosclerosis has been confirmed by prospective epidemiological studies that have demonstrated increased vascular risk in individuals with elevated levels of a wide range of biomarkers. These include cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-\(\alpha\), cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin and acute-phase proteins such as high sensitivity C-reactive protein (hsCRP), fibrinogen and serum amyloid A (SAA).\textsuperscript{38}

**Infection**

Infection has long been one of the most important medical complications of transplantation of all types. Prior to 1980, 60\% of the RTR developed at least one serious infection during the first year post-transplant, with mortality rates approaching 50\%.\textsuperscript{39} Although the current infection-related one year mortality has been reduced to less than 5\%, infectious complications remain serious threats to successful outcomes following transplantation.\textsuperscript{39} The risk of infection in renal transplant recipients is determined primarily by two factors: the intensity of exposure to potential pathogens (epidemiologic exposure) and the combined effect of all of the factors that contribute to a patient’s susceptibility to infection (the net state of immunosuppression).\textsuperscript{40}

Cytomegalovirus (CMV) is the most occurring opportunistic pathogen after renal transplantation.\textsuperscript{26,40-42} For the purpose of developing consistent reporting of CMV in clinical trials, definitions of CMV infection and disease have been developed. Primary CMV infection is defined as the detection of active CMV replication in an individual previously found to be CMV seronegative, a secondary CMV infections is defined as active CMV replication of the endogenous strain in an already seropositive individual, and CMV
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disease is defined as detection of CMV in a clinical specimen, accompanied by either CMV syndrome with fever, muscle pain, leukopenia and/or thrombocytopenia (other causes excluded), or by organ involvement, such as hepatitis, gastrointestinal ulceration, pneumonitis or retinitis. CMV infection in RTR is highly prevalent (around 80% in the western countries) and 25-33% of the infected RTR develop clinically overt disease after renal transplantation. The typical onset of disease is within the first 1 to 4 months after transplantation. Transmission can occur through transplantation of a CMV seropositive organ. In former days this could also be through transfusion of blood products from a CMV seropositive donor, but this cause has virtually disappeared after widespread introduction in clinical practice of transfusion of blood from which leukocytes have been filtered away. CMV disease can evolve in two ways: (1) primary infection can occur when a CMV seronegative recipient receives a CMV seropositive organ, (2) latent CMV can be reactivated in seropositive recipients as a consequence of, for example, treatment with immunosuppression.

Numerous studies have shown that both CMV disease and CMV reactivation early after transplantation are risk factors for rejection and mortality. Although the exact mechanisms by which CMV causes decreased graft and recipient survival remains unknown, several potential mechanisms have been proposed. CMV causes both direct, including tissue injury and clinical disease, and indirect effects. Indirect effects are diverse and include, for example, increased graft rejection with manifestations such as accelerated coronary atherosclerosis following heart transplantation, development of post-transplant diabetes mellitus, and decreased graft and patient survival. Furthermore, since CMV itself contributes to the net-immunosuppression, superinfections with other pathogens can been seen during an active CMV infection, such as infections with Pneumocystis Jirovecii (PCP) and Aspergillus species.


There is an emerging notion in renal transplantation that CTD and cardiovascular disease share inflammation and accelerated atherogenesis in their pathogenesis. This notion is supported by the histological features of CTD, in which equivalents of atherosclerosis are prominent. These equivalents of atherosclerosis are glomerulosclerosis, hyalinosis, and perivascular inflammation. This notion is further supported by the fact that CTD and cardiovascular disease share many risk factors, including age (of the donor for CTD and of the recipient for cardiovascular disease), hypertension, hyperlipidemia, and obesity.

Inflammation also plays a role in the (re)activation of CMV (figure 4). The pro-inflammatory cytokine TNF-α (re)activates CMV via the TNF receptor I (p55), and the
subsequent activation of protein kinase C and NFκB. In CMV infection, on the other hand, increases the production of cytokines and chemokines such as TNF-α and IL-6.

<table>
<thead>
<tr>
<th>Infections with all kind of micro-organisms e.g. urinary tract infections</th>
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<tbody>
<tr>
<td>&quot;Inflammation&quot;</td>
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<td>&quot;NFκB&quot;</td>
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<td>&quot;CMV (re)activation&quot;</td>
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Figure 4. The interrelationship of inflammation and CMV (re)activation. See text for information. TNF-α: tumor necrosis factor α, PKC: protein kinase C, CMV: cytomegalovirus.

AIM OF THIS THESIS

The main aim of this thesis is to explore the relation of chronic low-grade inflammation with CTD and mortality after renal transplantation.

The prototypical acute-phase reactant marker of inflammation, hsCRP, has been suggested to reflect vascular low-grade inflammation characteristic of atherosclerosis. This suggestion is supported by the finding that hsCRP predicts cardiovascular morbidity and mortality in patients with type 2 diabetes mellitus, in the general population, and in the renal transplant population. The determinants of hsCRP have been investigated in the general population, but not in RTR. In Chapter 2, we investigated the factors associated with hsCRP after renal transplantation in a cross-sectional study.

Chronic low grade inflammation and atherosclerosis are likely to be involved in CTD. However, it is unknown whether hsCRP is a predictor of deterioration of renal function in RTR. In Chapter 3, we investigated prospectively whether hsCRP could be of use as a clinical marker for early identification of RTR at increased risk of deterioration of graft function.

Procalcitonin (PCT) has been suggested as a biomarker for infection-driven inflammation, in bacterial infections and sepsis. Several animal and human studies have shown that, in septic and infectious conditions, parenchymal cells (including kidney, liver, lung, muscle, and adipocytes) are the principal source of circulating PCT.
Chapter 4, we investigated whether steady-state circulating PCT concentrations predict CTD in RTR.

Hemopexin is the plasma protein with the highest binding affinity to heme among known proteins. It is mainly expressed in liver, and belongs to the acute phase reactants, the synthesis of which is induced after inflammation. In Chapter 5, we investigated whether plasma hemopexin activity might be of use as a marker of non-infectious inflammation in renal allografts, leading to deterioration of renal allograft function and graft failure.

Hypoaalbuminemia is common after renal transplantation. Serum albumin is a negative acute phase protein, and hypoalbuminemia may therefore be a reflection of ongoing inflammation. Low serum albumin has been shown to be a predictor for both graft failure and mortality in RTR. In Chapter 6, we investigated whether the associations of serum albumin concentration with graft failure and mortality are independent of hsCRP concentration.

Proteinuria is an established predictor of mortality, in particular cardiovascular mortality, in several populations. Proteinuria and albuminuria are to a certain extent considered a reflection of generalized endothelial dysfunction (ED) in the vascular tree, manifesting itself as an increased tendency for glomerular leakage of albumin into the urine. In Chapter 7, we investigated whether urinary protein excretion is associated with markers of ED after renal transplantation and whether markers of ED affect the association of proteinuria with increased risk for mortality in RTR.

CMV has been established as the single most important pathogen after transplantation. Following primary CMV replication in seronegative individuals CMV establishes non-replicative infection (latency). Reactivation from latency commonly occurs short after transplantation. Hence, most studies investigated the impact of CMV, particularly CMV disease, occurring shortly after transplantation on graft and recipient survival. However, it has been shown that latent CMV can be locally active in a transplanted organ with ongoing low-grade alloreactivity, without systemic signs of activity in the chronic phase after transplantation. As a consequence, investigation of CMV short after transplantation as a risk factor for graft loss or mortality may have negated the possibility that latent CMV is accompanied by ongoing CMV-related inflammation, in the transplanted kidney in particular. In Chapter 8, we investigated the impact of CMV serology determined more than one year after transplantation on graft failure and mortality late after renal transplantation in.

Finally, the results from the above studies are summarized in Chapter 9. Their potential implications are discussed in this same chapter.
REFERENCES


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