Novel diagnostics and therapeutics to prevent injury in native and transplanted kidneys

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Chapter 1

General introduction and outline

The kidneys and development of injury

In physiological conditions, kidneys excrete waste products from the circulation, regulate blood pressure, balance of body fluids, and electrolytes, activate vitamin D for adequate bone mineralization and produce erythropoietin to stimulate red blood cell formation. In case of progressive kidney injury, these functions can become compromised.

Kidney injury is divided into acute and chronic injury. Acute kidney injury is caused by a diversity of conditions, including pre-renal (insufficient perfusion of the kidney), post-renal and renal causes. Chronic kidney disease is defined by the presence of decreased kidney function or kidney damage for at least three months.[1] The assessment of patients with newly diagnosed chronic kidney disease consists of a calculated estimation of the glomerular filtration rate (eGFR), examination of the urine (qualitive tests and microscopic), and, if necessary, serologic testing, radiologic imaging of the kidneys, and kidney biopsy examination. In the majority of cases, chronic kidney injury is irreversible and histological changes are characterized by presence of fibrosis in the kidney.[2] In the United States, 6.7% of the population has a diminished eGFR (<60 ml/min/1.73m2).[3] The most common causes of chronic kidney disease are, amongst others, poorly controlled diabetes mellitus and hypertension. Diabetes mellitus accounts for 30 to 50 percent of patients with end stage renal disease.[4] Consequently, the main aim of treatment for diabetes in an earlier stage is restraining the progression of such complications, by improved glycemic and blood pressure regulation, cardiovascular risk reduction and inhibition of the renin-angiotensin system.

Kidney transplantation and simultaneous pancreas kidney transplantation

In case end-stage renal disease develops, different treatment options are possible; dialysis and kidney transplantation as two renal replacement therapies and conservative treatment consisting of drug and dietary therapy, primarily aiming for optimization of the quality during the final stage of life. The choice for renal replacement therapy depends upon the overall prognosis and condition of the patient. Kidney transplantation is the preferred renal replacement therapy, since kidney transplantation results in better patient survival and improved quality of life.[5,6] Indeed, patient survival is significantly higher in renal recipients, compared to patients on the waiting list that are on dialysis treatment. However, a shortage exists of deceased kidney donors after circulatory death (DCD) or brain death (DBD) and prolongs time on the waiting list for a renal allograft. The number of transplanted kidney grafts from living donors has increased and countervails the shortage of deceased donation. In The Netherlands, the proportion of living kidney donation increased in the last decades to 50% of the 900-1,000 annual kidney transplants.[7] The advantage of living-donor kidney donation is the planning reliability of the transplantation trajectory and a superior graft function and graft survival.[5,8]

In patients with end-stage renal disease due to diabetic nephropathy, simultaneous pancreas-kidney transplantation (SPKT) is the preferred treatment, since SPKT offers

superior long-term survival in patients with diabetes type 1, compared with kidney transplantation alone.[9] SPKT has a deceased donor, since pancreas donation is not possible during life. Next to replacement of kidney function, SPKT restores endogenous insulin secretion and decreases microvascular complications.[10] On a yearly basis, 20-30 SPKTs are performed in the Netherlands.[7]

Although transplantation is the preferred option in many patients with end-stage renal disease, transplantation has disadvantages as well, including a decreased patient survival in the first year after transplantation.[11] Additionally, several factors may limit graft survival. The most prominent causes of graft loss are studied in this thesis, namely (1) rejection and (2) fibrosis and atrophy. In the last decades, the overall graft survival has increased in both the short and long term,[12] although the improvement is mostly caused by better short-term results.[13]

- Rejection is divided into hyperacute, acute and chronic rejection.[14] Hyperacute rejection is due to preformed donor-specific antibodies at the time of transplantation.[15] Frequently, primary non-function is observed and graft loss occurs within 24 hours after transplantation. Hyperacute rejection is rare nowadays, because of improved screening for antibodies. Acute rejection is divided into T cell-mediated rejection and antibody-mediated rejection (ABMR). T cell-mediated rejection is the most common form of acute rejection and is characterized by interstitial inflammation, tubulitis, and sometimes arteritis[16] and is often treated with corticosteroids and/or ATG initially. ABMR is characterized by microvascular inflammation, evidence of antibody interaction with the vascular endothelium and serologic evidence of circulating donor-specific antibodies (DSAs).[16] Although ABMR encompasses only a small proportion of the total number of rejections, the severe decline in kidney function makes ABMR a condition to take into account in the screening and follow up of kidney recipients.[17-19] The presence of preformed DSAs (i.e. DSAs present before transplantation) are associated with the development of ABMR[20] after transplantation and a lower overall graft survival.[21] On the other hand, the evidence for treatment for ABMR is scarce. Plasma exchange, intravenous immune globulin, and glucocorticoids are possible prescribed in patients with ABMR. However, the choice of treatment is based on small studies and expert consensus.[22]

- Chronic damage in a transplanted kidney graft is characterized by interstitial fibrosis and tubular atrophy (IFTA). The process is poorly understood and is typically accompanied by slowly rising serum creatinine concentration and increasing proteinuria. Immunologic factors, such as inflammatory cytokines and cell-mediated and humoral immune responses, are considered to play an important role.[23] An episode of acute rejection would be prognostic for IFTA,[24] although there is no consensus on this subject. Another important factor is the immunosuppressive regimen, since calcineurin inhibitors in

particular, are associated with chronic kidney injury after transplantation.[25] Other factors are glomerular hyperfiltration, delayed graft function, and hyperlipidemia.[26-28]

Other causes, not studied in this thesis, are recurrence of the primary kidney disease (frequently seen in patients with focal segmental glomerulosclerosis, IgA nephropathy, and membranoproliferative glomerulonephritis[29]) and peri-operative complications in the first months after transplantation (i.e. vascular thrombosis, fluid collections, and impaired wound healing[30]).[31,32]

Determination and prevention of kidney injury in native and transplanted kidneys in an early stage

The progression to end-stage renal disease in patients with chronic kidney injury and progression of kidney injury in renal recipients is difficult to predict. In diabetes patients for example, 30 to 40 percent of patients develop diabetic nephropathy, while others show a milder course of deterioration of the kidney function.[4] In transplantation, events that cause kidney injury, such as rejection, occur in a small proportion of recipients and some patients develop more IFTA than others. Identification of these patients is of great importance to personalize prevention and treatment of disease progression. In current monitoring strategies, creatinine clearance and proteinuria play an important role. These, however, are late signs of kidney injury and do not predict further progression of kidney injury. New biomarkers are required to increase understanding of the pathogenesis of kidney injury and thereby recognize progressive injury in an early stage. In addition, different treatment regimens may prevent the progression of injury. In this thesis, we aimed to identify novel markers of vascular and tubular injury in patients with chronic kidney injury and in transplant recipients and improve current diagnostic and therapeutic approaches to prevent kidney injury.

Markers of vascular injury

Regardless of the etiology, both acute and chronic kidney injury involve cellular changes that disturb the delicate renal vasculature.[33] In particular, diabetes mellitus is associated with microvascular injury. In transplantation, microvascular endothelial injury is one of the main features of both acute rejection and chronic injury, previously known as chronic allograft nephropathy.[33] Microvascular injury in the context of kidney disease and cardiovascular diseases have been previously linked to altered levels of specific long noncoding RNAs.[34,35] It is recognized that noncoding RNAs play an important role in molecular mechanisms, such as transcription, splicing and translation.[36] In human, only 1-2% of the genome codes for proteins. The remaining part of the genome does not code for proteins and RNA transcribed from this part is therefore called noncoding RNA[37] while being considered as 'junk' in the past. Several types of noncoding RNA are described, among which microRNA, circular RNA and long noncoding RNA (lncRNA). The latter is the largest group of noncoding RNAs and is characterized by a length of more than 200 nucleotides.

LncRNAs are increasingly described in the context of both glomerular and tubulointerstitial kidney diseases.[38] Next to kidney diseases, lncRNAs are suggested to play an active role in several other vascular diseases.[34] As such, lncRNAs may provide interesting candidates for the detection of early vascular injury in the context of kidney diseases and the vascular status of transplanted renal recipients.

Markers of tubular injury

Next to vascular damage, tubular injury is one of the hallmarks of kidney injury. In order to restrain the negative effects of kidney injury, senescence of cells is induced by the initiation of cell cycle arrest, in particular in tubular cells.[39]

Two rate-limiting factors that regulate the process of cell division and induction of apoptosis are p53 and p27. P53 regulates apoptosis and DNA repair and p27 inhibits cyclins that are necessary for progression through the cell cycle by activation of cyclin-dependent kinase enzymes.[40,41] Interestingly, two novel proteins have been identified that affect p53 and p27. Insulin-like growth factor-binding protein 7 (IGFBP7) is assumed to increase the expression of p53, while IGFBP7 and Tissue inhibitor of metalloproteinase 2 (TIMP-2) increase de novo synthesis and binding capacity of p27, a cyclin-dependent kinase inhibitor.[40,42,43] During episodes of kidney cell injury, G1 cell cycle arrest can be initiated, in order to avoid increased damage due to cell division. Both IGFBP7 and TIMP-2 are approved by the U.S. Food and Drug Administration as urinary biomarkers for prediction of kidney function. However, the potentially added value of these markers in the circulation in systemic diseases is still largely unknown.

Prevention of injury in kidney transplant recipients

In order to improve the prognosis of kidney function, prevention of kidney injury formation is of key importance. In kidney transplantation, adequate screening of the immunological risk before transplantation is one of the strategies to prevent events that induce kidney injury, such as rejection. As described above, ABMR is characterized by the presence of DSAs and is accompanied by severe kidney injury and impaired long term kidney graft function. A large proportion of these patients are immunized before transplantation and have preformed DSAs present before transplantation. A more sensitive screening method may identify high risk patients better and can therefor guide to alternatives in these patients, such as cross-over transplantations, lower the risk for rejection, and subsequently prevent kidney injury.

Novel therapeutics after renal transplantation

Next to improved screening methods, prevention of kidney injury may be achieved by improved immunosuppressive treatment of renal recipients. As previously mentioned, immunosuppressive agents, such as tacrolimus, can induce kidney injury. However, CNI withdrawal translates in higher rejection rates.[44] In this context, mesenchymal stromal cell

(MSC) therapy may be an interesting approach to reduce the load of traditional immunosuppressives, because of the presumed immune regulatory response of this cellular therapy.[45-47] MSC's are a heterogeneous population of multipotent cells, that can be obtained from the bone marrow, umbilical cord or adipose tissue. MSCs can condition the immune system, that can lead to self-sustaining tolerogenic activity. Currently, studies in the field of solid organ transplantation are predominantly phase I trials and frequency and dosage of administration are variable between studies.[48] The implementation of MSC therapy in renal recipients may act as an alternative for CNI use and lower the amount of kidney injury, caused by the immunosuppressive regimen.

Outline of this thesis

This thesis studies the development of injury in native kidneys, kidney grafts and the accompanied vascular injury. Mechanisms of cellular processes involved in kidney injury may clarify the pathophysiology and can offer possibilities to useful diagnostic strategies, as well as therapeutic approaches. In addition, optimization of diagnostic strategies may further improve the prognosis and prevent the need for more advanced treatment.

In **chapter 2**, the circulating and urinary levels of the cell cycle biomarkers IGFBP7 and TIMP-2 are assessed in the context of diabetic nephropathy and SPKT. In addition, a subpopulation was followed longitudinally after SPKT.

Chapter 3 describes four vascular-specific circulating lncRNAs in patients with diabetic nephropathy. LncRNAs were correlated with the vascular markers angiopoietin-2 (Ang-2) and soluble thrombomodulin (sTM). Patients with diabetic nephropathy were followed longitudinally after receiving an SPKT.

In **chapter 4**, vascular-specific lncRNAs are determined in kidney transplant recipients with acute rejection and with a stable kidney function. Patients with acute rejection were followed longitudinally and the correlation was assessed between the mentioned vascular-specific lncRNAs and vascular markers Ang-2 and sTM.

Chapter 5 describes an observational cohort study of living unrelated kidney transplant recipients. The incidence of early acute ABMR was assessed and possible risk factors for ABMR are analyzed. A group of patients that have a high risk of developing ABMR (i.e. female recipients who receive a kidney from their male spouse) are investigated in detail and the current pre-transplant screening for preformed donor-specific antibodies in the context of ABMR risk is studied.

In **chapter 6**, MSC therapy is presented as an interesting alternative approach to induce immune suppression, in order to reduce kidney injury. MSC therapy was offered to kidney transplant recipients as a substitute for the calcineurin inhibitor tacrolimus.

Lastly, **chapter 7** discusses the research presented in this thesis and places the conclusions in the broader context of kidney injury and kidney transplantation. A Dutch summary of this thesis is presented in **chapter 8**, next to a curriculum vitae of the author, a list of publications, and dankwoord.

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