Protocol biopsies after kidney transplantation

PROTOCOL BIOPSIES AFTER KIDNEY TRANSPLANTATION

emphasis on interstitial fibrosis/tubular atrophy and peritubular capillary loss in relation to clinical parameters

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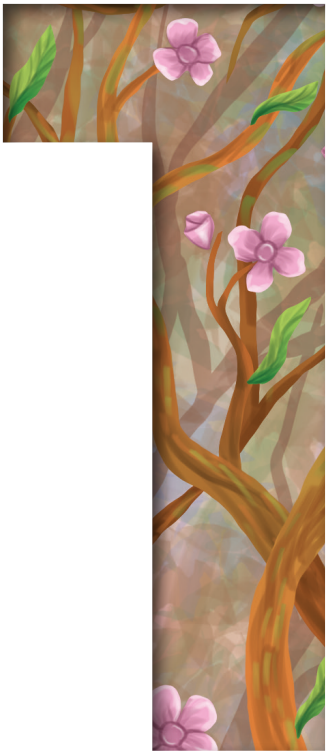

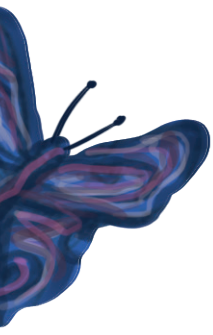

GENERAL INTRODUCTION

Chronic kidney disease and End Stage Renal Disease

Chronic kidney disease (CKD) is a common disease affecting 7-15% of the general population (1, 2), which comes down to approximately 2 million people in the Netherlands (3). In the Netherlands, the incidence of treated end-stage renal disease, the last stage of CKD, in the general population is 117 patients per million people and the prevalence is 1047 patients per million (4). The most common primary renal diseases underlying end-stage renal disease in the Netherlands are displayed in Table 1. According to the WHO, kidney diseases are globally the tenth leading cause of death in 2019 (6). The 5-year patient survival of patients on dialysis is worse than for patients with prostate-, breast-, or colorectal cancer (7). Chronic kidney disease has a major impact on patients daily life: patients report lower health related quality of life than the general population (8). There are many factors that decrease health related quality of life such as symptom burden, comorbidities, side effects of medication and frailty (9). High pill burden is also associated with lower quality of life: one study in dialysis patients showed a median daily pill burden of 19, which is one of the highest in any chronic disease (10).

Table 1. Prevalence of primary disease underlying end-stage renal disease in the Netherlands in 2019 (5)

Primary kidney disease	Patients per million people	Percentage (%)
Glomerulonephritis/sclerosis	175.0	16.3%
Pyelonephritis	67.1	6.2%
Polycystic kidney disease	93.3	8.7%
Diabetes mellitus (overall)	143.3	13.3%
Diabetes mellitus type 1	38.9	3.6%
Diabetes mellitus type 2	104.4	9.7%
Hypertension	127.3	11.9%
Renal vascular disease	65.8	6.1%
Miscellaneous	226.4	21.1%
Unknown	115.9	10.8%

Kidney transplantation

The treatment options for end-stage renal disease are renal replacement therapy through peritoneal, - or hemodialysis, or a kidney transplantation. Compared to patients on dialysis and on the waiting list for renal transplantation, transplanted patients have a significant lower mortality risk (11-13). Furthermore, health related quality of life, and rates of life participation for example physical function and recreation, are considerably higher for patients with a renal transplant compared to dialysis (12, 14, 15). Additionally, transplantation is less expensive than dialysis (16, 17). For these reasons, kidney transplantation is the treatment of choice for most patients and currently the number of patients with a functioning renal transplant exceeds that on a dialysis modality (18).

In 2019, in the Netherlands 831 patients were on the active waiting list of Eurotransplant for a postmortal kidney transplantation (19) while in that year more than half of the 954 transplanted kidneys originate from living donors: 501 kidneys from living donors (LD) versus 453 kidneys from deceased donors (19). De deceased donors are more often cardiac death donors (DCD, 59%) than brain death donors (DBD, 41%) (19).

The qualitatively best kidneys for transplantation are kidneys donated by living donors. LD graft recipients have a better long-term survival than recipients of a deceased donor kidney (11, 20). In Europe, 5 and 10 year graft survival after living donor kidney transplantation were 88% and 72%, respectively, while after deceased donor kidney transplantation it was 77% and 57%, respectively (21). Since the waiting list for kidney transplantation does not decline and patients die on the waiting list for transplantation, also grafts with less favourable quality e.g. from DCD or extended criteria donors, (ECD) are used for transplantation. The latter is defined as a postmortal donor aged ≥ 60, or a donor from 50-59 years old with at least two of the following characteristics:

- History of systemic hypertension,
- Cerebrovascular accident as cause of death,
- Terminal serum creatinine >133 µmol/L (22-24).

These criteria are based on the presence of variables with a 70% increased the risk of graft failure compared to standard criteria donor (22, 23). A recent Dutch study confirmed that extended criteria donor kidney transplantation is associated with higher risk of graft failure and death especially in younger patients, when compared to standard criteria donor kidney transplantations (25). Although DCD grafts have

more short term complication compared to DBD grafts, including higher incidence of primary non-function and delayed graft function (DGF) (26-28), graft- and patient survival of DCD grafts after the first year is comparable to DBD grafts (26-28) and recipients of both DCD and extended criteria donor grafts have a lower mortality risk compared to patients on the waiting list (29-31).

Despite the long kidney transplant waiting list, still 20% of the deceased kidneys reported for allocation in the Eurotransplant region were not transplanted in 2019 (19). Reasons for discard include several donor, organ, and recipient variables, mismatch between donor and recipient, and lack of a suitable recipient on the waiting list for a donor kidney (32, 33). Optimising strategies for allocation of donor kidneys, and with that a reduction of kidney discard, may also help to expand the donor pool.

Complications after transplantation

Renal transplantation and its medical treatment are not without risks on complications. Some complications occur early after transplantation (for example haemorrhage, bacterial infection, and acute rejection), while other complications occur late after transplantation (e.g. malignancy, BK infection) and causes for complications are diverse (for instance surgical, immunological, medical). Complications are reviewed in detail by Thiruchelvam *et al.* in (34). In this thesis, the studies mainly focussed on the following complications: primary non-function and delayed graft function, acute rejections and, on long term deterioration of renal function which finally results in graft loss (chronic transplant dysfunction).

Immunosuppressive regimen

Immunosuppressive treatment after transplantation is always necessary (except in HLA-identical twins) to avoid acute rejection and consequently loss of the renal graft (35). In general, direct posttransplant one starts with a combination of immunosuppressives, each in a high dosage with lowering of dosage (and often the number of immunosuppressives) to maintenance therapy (35). Currently, the most used immunosuppressive maintenance regimen after transplantation is the combination of Tacrolimus (TAC), mycophenolate mofetil (MMF) and steroids (36).

In the studies presented in this thesis, all patients start with triple therapy: Tacrolimus and steroids with either mycophenolate mofetil or sirolimus. The combinations of tacrolimus with sirolimus or mycophenolate mofetil are both effective and safe (37). There are no clear differences regarding delayed graft function, acute rejection rates and graft survival (37). In immunological low-risk patients steroid therapy is halted ten days after transplantation. When there was no rejection either clinical or

in the 3 month protocol biopsy, the sirolimus or mycophenolate mofetil is stopped at three months after transplantation and from then on the patients are on tacrolimus monotherapy. A second protocol biopsy is taken at twelve months to assess possible subclinical rejections. In high-risk patients, that is highly immunised patients or patients who have had early immunological failures of a previous transplant, steroids are continued after ten days. Protocol biopsies are taken at three and twelve months after transplantation. Depending on the results of these protocol biopsies, steroids are withdrawn. In case of a pathological diagnosis of rejection Banff grade 1B or higher in the protocol biopsy, this is treated with additional immunosuppression. Borderline and Banff grade 1A rejections in protocol biopsies are not treated.

Renal transplant biopsies

Protocol and indication biopsies

After transplantation biopsies can be taken on two grounds; protocol biopsies in stable grafts or indication ('for cause') biopsies. Cortical needle biopsies are preferentially used to assess histological injury in transplant kidneys since it has been shown that interstitial fibrosis and arteriosclerosis can be evaluated more accurately in needle core biopsies than in wedge biopsies (38, 39).

At MUMC 'for cause' biopsies are taken about one week after transplantation in case of DGF to evaluate whether there is an underlying rejection. Secondly, indication biopsies are taken with increased creatinine to assess whether function decline results from acute rejection or from another underlying cause.

Protocol transplant renal biopsies are biopsies taken as a routine follow-up procedure in well-functioning grafts (40). These biopsies can be used to detect subclinical rejections, while there is no deterioration of eGFR (yet) and for (monitoring of) tailoring immunosuppressive therapy (40, 41). Furthermore, protocol biopsies can be used in clinic and in studies to evaluate the development of histological injury over time (41). Several studies showed that these biopsies can be safely performed with major complications occurring in 0.2-2% (42-47). In the MUMC$^+$ protocol renal needle core biopsies are taken post-reperfusion, at three, and at twelve months after transplantation. In our centre the 3 and 12 month biopsies are used as a guide to adapt the immunosuppressive therapy as described in the immunosuppression paragraph. The post-reperfusion biopsy is used to rule out hyperacute rejection and to assess the baseline histology of the donor graft.

Banff criteria for scoring histology

Assessment of renal transplantation biopsies are worldwide performed using the Banff criteria. This classification system was designed to standardise evaluation of renal transplant biopsies and it includes both parameters for acute damage and parameters for chronic renal transplant damage (48). The following compartments of the renal tissue are evaluated in the biopsies: glomeruli, tubuli, interstitial compartment, and the vessels. In 2018 a comprehensive overview of the current Banff classification was published by Roufosse *et al.*(49). A distinction is made between acute and chronic injury parameters.

The acute damage parameters in the Banff scheme (Table 2) are mostly seen in the context of acute rejection and include inflammation in glomeruli, tubulointerstitial inflammation and vascular inflammation (49).

Table 2. Overview of acute Banff parameters (49)

Banff lesion score	Abbreviation	0
Glomerulitis	g	0% of glomeruli
Tubulitis	t	0 mononuclear cells in tubules
Interstitial inflammation	i	Inflammation in <10% of unscarred cortical parenchyma
Total inflammation	ti	<10% of total cortical parenchyma
Intimal arteritis	v	0%
Peritubular capillaritis	ptc	<3 leukocytes/PTC

Abbreviations: PTC peritubular capillary

Table 3 shows the Banff chronic injury parameters. Some parameters are discussed in more detail below as they provide an important basis for the questions in this thesis. To assess chronic injury in the transplant biopsies two parameters for glomerular damage are scored: *chronic glomerulopathy (cg)* and *mesangial matrix expansion (mm)* (49). These two parameters are not used in the analyses in this thesis. There are also two parameters to score chronic injury in the vessels: *vascular fibrous intimal thickening (cv)* and *arteriolar hyalinosis (ah)*. Cv scores the extent of arterial intimal thickening in the most severely affected artery and ah gives the presence of hyaline thickening in arterioles (48, 49).

The chronic injury in the interstitial compartment is scored by the parameters *interstitial fibrosis (ci)* and *tubular atrophy (ct)*. Ci reflects the extent of interstitial fibrosis in the renal cortex: up to 5% interstitial fibrosis is considered normal (49). Ct scores the extent of tubular atrophy in the cortical region (48, 49). Ci and ct are usually strongly correlated with each other. Often a combined score is given i.e. the *interstitial fibrosis and tubular atrophy (*IF/TA) score, where the highest score of ci or ct determines the composite IF/TA score as shown in Table 4 (48, 49). As can be seen from Table 3, both ci and ct scores are based on the relative damage (i.e. percentage of area affected) in the renal biopsy. Nevertheless, valuable information from the

1	2	3
<25% of glomeruli	25-75% glomeruli	>75% glomeruli
1-4 mononuclear cells/ tubular cross section or 10 tubular epithelial cells	5-10 mononuclear cells/ tubular cross section (or 10 tubular cells)	>10 mononuclear cells/tubular cross section or foci of tubular basement membrane destruction with i ≥ 2 and t2 elsewhere
10-25% of unscarred cortical parenchyma	26-50% of unscarred cortical parenchyma	>50% of unscarred cortical parenchyma
10-25% of total cortical parenchyma	26-50% of total cortical parenchyma	>50% of total cortical parenchyma
<25% luminal area lost in at least 1 arterial cross section	≥25% luminal area lost in at least 1 arterial cross section	Transmural and/or fibrinoid change and medial smooth muscle necrosis
≥1 leukocyte in ≥10% of PTCs with max. of 3-4/PTC	≥1 leukocyte in ≥10% of PTCs with max. of 5-10/PTC	≥1 leukocyte in ≥10% of PTCs with max. of >10/PTC

biopsy may get lost, when using scores instead of percentages. Especially in studies where progression of IF/TA is studied, scoring IF/TA in percentages may be useful. However, it has not been studied well whether IF/TA on a continuous scale could serve as an reliable alternative for IF/TA in categories.

Besides visual scoring of ci, there are various techniques available to study interstitial fibrosis in the renal biopsy on a continuous scale, such as computerised quantification of renal biopsies stained with Sirius red or immunohistochemical staining of collagen III. It has been shown that non-polarised Sirius red positive staining and collagen type III staining are associated with visual assessment of renal fibrosis (categorical ci scores) (50-52) and with eGFR (50, 52-55). There is hardly any data available on the comparison of these computerised fibrosis assessments and visual scoring of fibrosis on a continuous scale. There is only a single study in a small-sized transplantation cohort of 33 living donor transplants, that reported a significant correlation between Sirius red based and histopathological (visual) assessment of fibrosis on a continuous scale (%) (56). Therefore, larger studies that assess the validity of visual assessment on a continuous scale and secondly compare visual scoring on a continuous scale to computerised scoring are needed.

Table 3. Overview of chronic Banff parameters (49)

Banff lesion score	Abbreviation	0
Chronic glomurolopathy	cg	0% GBM double contour by LM
Mesangial matrix expansion	mm	0% in any glomerulus
Tubular atrophy	ct	0% of the area of cortical tubuli
Interstitial fibrosis	ci	≤5% interstitial fibrosis in cortical area
Vascular fibrous Intimal thickening	cv	0% narrowing of luminal area by fibrointimal thickening
Arteriolar hyalinosis	ah	0

Abbreviations GBM glomerular basement membrane, LM light microscopy

Table 4. Scoring of interstitial fibrosis and tubular atrophy (IF/TA) according to the Banff criteria

	ci 0 (0-5%)	ci 1 (6-25%)	ci 2 (26-50%)	ci 3 (>50%)
ct 0 (0%)	IF/TA 0	IF/TA 1	IF/TA 2	IF/TA 3
ct 1 (1-25%)	IF/TA 1	IF/TA 1	IF/TA 2	IF/TA 3
ct 2 (26-50%)	IF/TA 2	IF/TA 2	IF/TA 2	IF/TA 3
ct 3 (>50%)	IF/TA 3	IF/TA 3	IF/TA 3	IF/TA 3

Abbreviations ct tubular atrophy, ci interstitial fibrosis, IF/TA interstitial fibrosis and tubular atrophy. Green colour indicates the combination of ci and ct leading to IF/TA score 0, yellow indicates combinations that make IF/TA 1, orange the combinations that make IF/TA 2 and red the combinations that make IF/TA 3.

Development of chronic histological injury after transplantation

Initially, chronic histological injury lesions were supposed to occur only late after transplant, however, also in the first year after transplantation, there is an increase of injury score of several histological parameters including globally sclerosed glomeruli and vascular damage (ah and cv) (57). There is also an increase of IF/TA in the first year after transplantation (54, 57-62). In a study comparing biopsies at 4 and at 14 months after transplantation, IF/TA increase was observed, while creatinine levels were stable during that time period (63).

IF/TA is reflecting an irreversible final common pathway in renal injury and therefore, it may serve as a surrogate endpoint in clinical trials (41, 64). It is associated with worse renal function (54, 60, 65-67). Furthermore, higher IF/TA scores in the first

1	2	3
≤25% GBM double contour by LM	26-50% GBM double contour by LM	>50% GBM double contour by LM
≤25% of non-sclerotic glomeruli	26-50% of non-sclerotic glomeruli	>50% of non-sclerotic glomeruli
≤25% of the area of cortical tubuli	26-50% of the area of cortical tubuli	>50% of the area of cortical tubuli
6-25% interstitial fibrosis in cortical area	26-50% interstitial fibrosis in cortical area	>50% interstitial fibrosis in cortical area
≤25% narrowing of luminal area by fibrointimal thickening	26-50% narrowing of luminal area by fibrointimal thickening	>50% narrowing of luminal area by fibrointimal thickening
Mild to moderate in ≥1	Moderate to severe in >1	Severe in many

year after transplantation are associated with reduced long-term graft survival (65, 67-70), also when corrected for other risk factors for graft loss, including rejection, recipient age and serum creatinine (67, 69).

Although the late post-transplant effects of IF/TA have been studied in detail, there is still a lot unknown early after transplant, for example which clinical and baseline histological factors drive IF/TA development and progression. Baseline histological damage and (sub)clinical rejection are shown to be associated with the presence of IF/TA (57, 71) and the progression in IF/TA (58). A few studies have investigated the role of donortype in IF/TA. Our group has previously shown that IF/TA score at one year posttransplant is higher in DCD donors than in LD donors (58). On the other hand, Cosio *et al*. was not able to show a difference between LD and deceased donors (65). Only one study focused on difference in IF/TA score posttransplant between DBD (n=75) and DCD (n=37) donors. They could not find significant differences at six and twelve months after transplantation, albeit that the power of this study was limited (especially due to low number of DCD) (72).

Ischemia reperfusion injury

The process of ischemia and reperfusion during donation and transplantation causes injury that may also be observed in post-reperfusion biopsies. Already in the 1970s, Solez *et al*. described morphological changes in native renal biopsies of patients with acute renal failure, which included among other factors: *tubular necrosis*, *inflammation*, *oedema*, *tubular casts*, and *loss of brush border* (73).

Several studies in the last decade have investigated acute tubular necrosis or acute tubular injury in preimplantation or reperfusion biopsies (74, 75), but a clear histological scoring definition of acute tubular injury is lacking. The lack of clear histological scoring system for ischemia reperfusion injury may hamper comparison of study results. Indeed, several studies reported an association of acute tubular injury with DGF (59, 76, 77), while others did not find such an association (74, 78-80). In addition, data of a possible effect of ischemia reperfusion injury on IF/TA development is lacking.

In 2016 the Banff working group proposed criteria to score preimplantation biopsies (81). In addition to scoring 'traditional' Banff parameters such as IF/TA and interstitial inflammation, they suggested scoring of acute tubular injury as mild, moderate or severe in these biopsies. Mild acute tubular injury was defined as presence of: epithelial flattening, tubule dilation, nuclear dropout, and loss of brush border. Moderate acute tubular injury was defined as focal coagulative type necrosis and severe as infarction (81). Despite the given definition for acute tubular injury, the inter-observer agreement in this study was poor (ICC 0.172) (81), possibly due to the descriptive definition of acute tubular injury. More research is needed for refinement of the acute tubular injury scoring in order to strive for higher inter-observer agreement and secondly to elucidate the effect of ischemia reperfusion injury parameters on renal graft function and development of IF/TA.

Delayed graft function and early graft loss including primary nonfunction

The definition of delayed graft function is not uniform in literature (82, 83). In our studies, the definition of the Dutch Organ Transplant Registry (NOTR) is used: DGF is any need for dialysis in the first week after transplantation. DGF is a multifactorial complication with risk factors being dependent on characteristics of donor, recipient, preservation technique, and transplant procedure (83, 84); examples of risk factors that are donor related are 'higher age' and 'higher terminal creatinine' (84, 85); recipient related risk factor are 'male gender' and 'retransplantation' (84, 85). Examples of preservation and transplant-related risk factors are longer '1st warm' and 'cold ischemia time', 'cold storage' (instead of machine perfusion) and 'more HLA mismatches' (84-86). However, the most important risk factor for DGF is 'donortype': in uncomplicated living donor transplants, the incidence of DGF is very low (83). These risks are higher in deceased donor grafts, with a reported incidence of 17-25% in DBD donor grafts and 42-72% in DCD donor grafts (26-28). When a kidney graft never becomes dialysis-independent is this called 'primary nonfunction' (83). Again the incidence of primary nonfunction in living donor grafts is very low, whereas that in deceased grafts is higher (3-8% in DBD grafts and 3-23% in DCD grafts) (26-28).

Early graft loss is usually defined as graft loss up to 90 days after transplantation and included are graft losses due to primary nonfunction, thrombosis and infarction, technical or operative problems, (hyper)acute rejections, infections and recurrent primary disease (87-89). In the Netherlands it occurs in about 8% of all deceased renal transplantations, and 25% of the early graft failure was caused by primary nonfunction (87). Early graft failure not only necessitates reinstitution of renal replacement therapy, but also leads to lower patient survival compared to patients with a functioning graft (87-89).

Acute rejections

In renal transplantation, the recipient immune system contacts the donor cells on the kidney and may be activated due to immunologically differences between donor and recipient. This can lead to inflammation and to destruction of the kidney tissue and (if untreated) eventually to graft loss (90). Acute rejection can be suspected in case of an acute deterioration of graft function, which cannot be explained otherwise and the gold standard for diagnosis is a kidney biopsy (91). Acute rejections are divided in subtypes, based on the pathophysiological mechanism and histological characteristics, in T-cell and antibody mediated rejection (90, 91). The pathological criteria and description of T-cell and antibody mediated rejection grades are comprehensively described in the Banff classification (92) of which several versions have been made over the years.

T-cell mediated rejection obviously involves a T-cell response to HLA antigens on the kidney graft (90). T-cell mediated rejection is histological characterised by inflammation in tubuli (Banff parameter 't') and inflammation of interstitium (Banff parameter 'i') and it can be accompanied by inflammation of the vasculature (intimal arteritis: Banff parameter 'v') (48). As described in Table 5, dependent of the severity, T-cell mediated rejection is graded from IA to III (48). Treatment is dependent on the severity of the acute rejection (91). International guidelines recommend corticosteroids as the initial treatment of lower grades (93). When associated with vasculitis (T-cell mediated rejection grade II and III) it requires (additional) treatment with T-cell depleting antibodies (91). In MUMC, Banff IA and Banff IB rejections were treated with three pulse doses of 1 gram methylprednisolone on alternate days and steroids were reintroduced at a dose of 10mg/day for 30 days and thereafter tapered to 5mg/d in the following 2 months after treatment of an acute rejection. Vascular rejections, with a Banff grade IIA or higher were treated with a 10-day course of rabbit anti-thymocyte globulin (ATG) and (re-)introduction of steroids.

Antibody mediated rejection has been added as a new category to the Banff guidelines in 2003 (94) and diagnostic criteria for this diagnosis have changed over time (95). In antibody mediated rejections, circulating antibodies against donor HLA bind vascular endothelium in the kidney graft and induce inflammation, cell death and rejection (90). In contrast to T-cell mediated rejection, diagnosis is not based on histological parameters alone. The following three features must be present for diagnosis of antibody mediated rejection:

- Histological evidence of acute tissue injury
- Evidence of antibody interaction with vascular endothelium
- Serologic evidence of donor specific antibodies

Table 5 shows the diagnostic criteria for ABMR diagnosis according to the Banff guidelines of 2013, as used in this thesis. The treatment of antibody mediated rejection is aimed at removing antibody producing B-cells or plasma cells, removing donor specific antibodies and/or inhibiting the subsequent complement-regulated graft injury (91). International guidelines recommend treatment with one or more of the following treatment options alone, or in combination with corticosteroids: plasma exchange, intravenous immunoglobulins (IVIG), anti CD20 antibody, and/or lymphocyte-depleting therapy (93). In our centre, pure humoral rejections were treated with pulse methylprednisolone and a 4-day course of intravenous IgG (IVIG; total 2 g/kg) supplemented with plasmapheresis in case of presence of donor specific antibodies.

Irreversible graft deterioration

Common causes for late graft loss include recipient death with a functioning graft and chronic deterioration of renal function, also called chronic transplant dysfunction, which eventually leads to graft loss (96, 97). Clinically, it is characterised by a worsening of renal function and development of proteinuria (97). Chronic transplant dysfunction is a multifactorial process in which both immunological and non-immunological factors play a role (96-98). Immunological processes that contribute are: episodes of acute rejections, and subacute and chronic alloimmune response (caused by suboptimal immunosuppression or noncompliance of the patient). Non-immunological factors that lead to chronic transplant dysfunction include for example, ischemia reperfusion injury, older donor age or poor graft quality, hypertension, hyperlipidemia and chronic toxic effects of immunosuppressives (97). Since it is a multifactorial process, strategies for prevention of chronic transplant dysfunction and with that improvement of long-term graft function should target the specific underlying causes (97, 99).

Table 5. Histological criteria for diagnosis acute rejections according to the Banff classification

Acute T-Cell mediated rejection	
Grade IA	'i' grade 2 or 3 with 't' grade 2
Grade IB	'i' grade 2 or 3 with 't' grade 3
Grade IIA	'v' grade 1, with or without 'i' and/or 't'
Grade IIB	'v' grade 2, with or without 'i' and/or 't'
Grade III	'v' grade 3), with or without 'i' and/or 't'.
Acute Antibody mediated rejection	
All three features must be present for diagnosis	
1	Histologic evidence of acute tissue injury, including at least one of the following • Microvascular inflammation ('g'>0 and/or 'ptc' >0) • Intimal or transmural arteritis ('v'>0) • Acute thrombotic microangiopathy, • Acute tubular injury
2	Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following • Linear C4d staining in peritubular capillaries • Moderate microvascular inflammation (['g' + 'ptc'] ≥2) • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury
3	Serological evidence of donor-specific antibodies (DSAs) (HLA or other antigens)

Used Banff parameters: 'i' interstitial inflammation, 'v' intimal arteritis, 't' tubulitis, 'g' glomerulitis, 'ptc' peritubular capillaritis.

Histological, chronic transplant dysfunction is characterised by interstitial fibrosis and tubular atrophy (IF/TA), which can be accompanied by vascular changes (fibro-intimal thickening and arteriolar hyalinosis) and glomerular changes (mesangial matrix increase or glomerular capillary basement membrane multilayering) (57, 68, 99). IF/TA is seen in 27-45% of late graft losses and can be seen as a final common pathway leading to graft failure (99, 100). A range of alloimmune, ischemic and inflammatory events causes permanent damage to the kidney which results in loss of nephrons and accumulation IF/TA, and clinically in a decline in renal function (99).

Peritubular capillaries after renal transplantation

In the kidney the peritubular capillaries (PTC) surround the tubuli and form a vast network as depicted in Figure 1. In several renal diseases decrease in PTC density, also called PTC rarefaction, has been described, for example in diabetic nephropathy (101), glomerulonephritis (102, 103), and chronic transplant dysfunction (104-106). Two forms of PTC rarefaction can be distinguished: functional rarefaction, in which there is a pathologically decreased number of perfused PTCs and structural rarefaction which is defined as an anatomically decrease in PTCs resulting in lower PTC density (107). In renal biopsies only structural rarefaction can be studied. There are several methods to score PTC density, for example: vessels/μm^2, % cortical area covered by PTCs, PTC/field or PTC/tubule. In our studies, PTC density was assessed by visually counting PTC and tubule numbers in renal biopsy tissue and expressed as PTC/tubule, as our group has done previously (108).

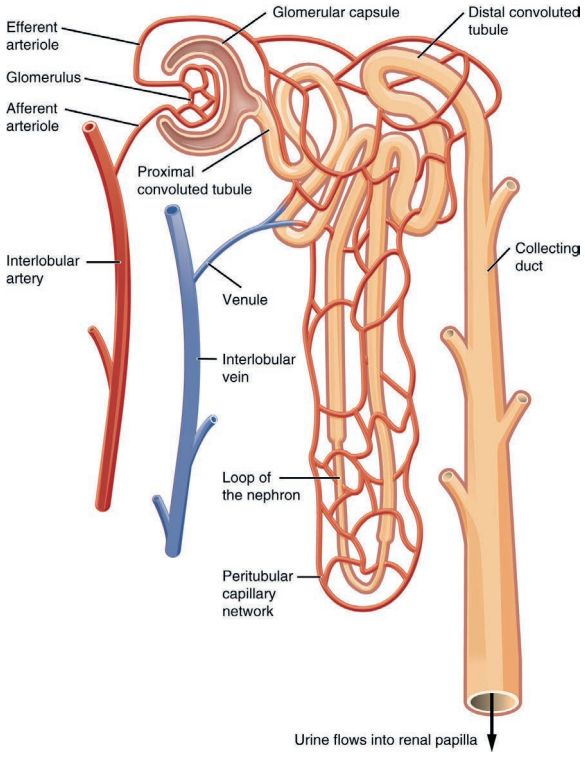

Figure 1. Schematic overview of a nephron and its surrounding vessels (109)

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Animal models for studying peritubular capillary density

In animal studies, PTC density has been studied in early time points after induction of renal injury. In rodents, there are hardly any transplantation models available. Nevertheless, there are models that mimic injury during donation and transplantation, such as ischemia reperfusion injury model.

In studies with rodents, decrease in PTC density occurred after ischemia reperfusion injury (110-114), and more severe injury was associated with more decrease in PTC density (115). This may also be important for the human situation since DCD graft undergo more ischemic injury than DBD and LD grafts.

Besides ischemia reperfusion injury models, also in other experimental models for chronic kidney injury, including unilateral ureteral obstruction, renal mass reduction, and folic acid induced nephropathy, PTC rarefaction has been observed (116-118). Many studies have reported development of interstitial fibrosis alongside decrease in PTC density (110, 111, 113-115, 117-119). Basile *et al.* studied interstitial fibrosis and PTC density at 4, 8, and 40 weeks after induction of ischemia reperfusion injury in rats. They observed neither recovery nor a further decrease of PTC density after 4 weeks, while fibrosis was progressive (111). These data suggest that decrease in PTC density precedes progression of IF/TA and that they may be both part of a 'final common pathway'.

Human studies of peritubular capillary density

Studies of PTC rarefaction after renal transplantation are summarised in Table 6. Three cross-sectional studies have shown lower PTC density in renal biopsies taken from patients suffering from chronic transplant dysfunction compared to PTC density in implantation biopsies or tissue from tumour nephrectomy (104-106). Both Ishii *et al*. and Modelli de Andare *et al*. showed that PTC density was negatively correlated with severity of chronic transplant dysfunction (105, 106). Furthermore, Ishii *et al.* showed an association of lower PTC density with higher IF/TA scores and with worse renal function (105).

Previously, our group has shown in a pilot study of 48 renal graft recipients a decrease of PTC density in the first three months after transplantation in postmortal donor grafts (108). In addition, this decrease was associated with more immunological events. Chapal *et al.* confirmed our data in a study with 42 recipients of DBD grafts (120). In our pilot study, decrease in PTC density during the first 3 months posttransplant was also associated with higher grade of IF/TA and with lower eGFR one year after transplantation. This indicates that early loss may be a marker for later deterioration of graft function (108). Due to the relative small patient population in the pilot study, only few possible contributing factors for decrease in PTC density could be tested.

Table 6. Overview of reported evidence for decreased peritubular capillary density after renal transplantation

Studied groups	Used anti-body	PTC Readout
N=79 CTD N=20 control (pre-Tx)	CD34	PTC/field
N=47 CTD, N= 9, CNI toxicity, N=26 control (post reperfusion)	CD34	PTC/field
N=29 CTD N=19 control (tumour nephrectomy)	CD31	PTC/field
N=30 TG N=12 (control Tx with IF/TA)	CD31	PTC/um^2
N=32 TG, n=23 IF/TA, N=15 control (SG),	CD31	PTC/field
N=8 DGF N=8 control	CD31	PTC/field
n=48 LD, DBD and DCD	CD31/CD34	PTC/tubule
N=42 DBD	CD34	PTC/tubule
N= 18 ABMR N=13 TCMR N=14 control (SG)	CD31	PTC/field

Abbreviations: PTC peritubular capillary, CTD chronic transplant dysfunction, CNI calcineurin inhibitor, IF/TA interstitial fibrosis and tubular atrophy, TG transplant glomerulopathy, SG stable graft, ABMR antibody mediated rejection, TCMR, T-cell mediated rejection, DGF delayed graft function.

Biopsy (Time after Tx)	PTC density	Associations	Authors
mean 6.8 ±4.4 year	Lower in CTD (19.9 ± 9.6 vs. 36.4 ± 2.4 in controls)	Interstitial fibrosis, proteinuria and serum creatinine	Ishii et al. 2005 (105)
not specified	Lower in CTD (mild CTD 226.3 ± 44.1, severe CTD 147.7 ± 94.2 vs. 330.1 ± 45.8 in control)	Acute rejection episodes associated with lower PTC density. Lower graft survival with lower PTC density	Modelli de Anadare et al. 2009 (106)
time to failure median 47.5 months (3.6-169)	Lower counts in both cortex and medulla (cortex: 5.10 ± 0.2 vs. 7.85 ± 0.2 in controls)	more inflammation in CTD	Adair et al 2007(104)
8.8 ± 6 years	No difference between TG and control.	Lower PTC density in regions with IF/TA	John et al. 2010 (123)
TG: 4.0 year (1-11) IF/TA: 4.3 year (1-11)	In IF/TA but not in TG group lower PTC density was observed	IF/TA	Sun et al. 2012 (124)
not specified	15.9 +/- 3.2 PTC/field vs. 26.9 +/- 3.3 capillaries/ field in controls		Wanga et al. 2015(121)
Consecutive protocol biopsies 0-3-12 months	Loss in first three months after tx in DCD grafts	More IF/TA at 12 months Lower renal function at 12 months	Steegh et al. 2011 (108)
Protocol biopsies 0-3 months	23.8% PTC density decrease	More PTC decrease in high sFLT-1 levels	Chapal et al. 2013(120)
ABMR mean day 8 (5-20), TCMR day 15 (11-25) and control day 14 (12-15) post Tx	In ABMR lower density (22.22 ± 2.51 vs. 25.64 ± 1.82 in control) No difference in TCMR (27.23 ± 2.49) vs. control		Li et al. 2014 (122)

Cross-sectional studies addressing PTC density in a specific subgroup of renal transplant recipients are scarce and often small scaled. One study showed lower PTC density in 8 recipients with DGF compared to 8 control patients, but it was not specified when the biopsies were taken (121). Another study showed that patients with antibody mediated rejection (n=18), have lower PTC density compared to patients with a stable graft function (n=14), which is not the case in patients with T-cell mediated rejection (n=13) (122). Up till now, larger cohorts with consecutive (protocol) biopsies in which PTC density early after transplantation and its contributing factors/ pathophysiological pathways could be studied, are lacking.

Aim and outline of the thesis

Aim

As summarized above renal biopsies taken before and after transplantation give insight into the reasons underlying complications after transplantation (such as rejection), but they can also contribute to assessment of the risk to develop chronic transplant dysfunction. As there is a shortage of donor kidneys there is an increase in the use of kidneys from DCD donors, and histological injury after transplantation of DCD grafts is not well known. In Maastricht, there is a long standing experience with DCD kidney transplantation (27). We therefore retrospectively developed and studied a consecutive cohort from our own centre of all patients transplanted between March 2003 and December 2009, of whom protocol biopsies were taken during, and 3 and 12 months after transplantation, and focussed on injury development in relation to donortype. In this single centre cohort we made a distinction between histological injury (including an analysis of methods for interstitial fibrosis development), and injury to peritubular capillaries as we hypothesize that ischemia-related capillary injury is an important pathway in CTD development. We additionally used data from a national cohort of patients receiving a transplant from a deceased donor between January 2000 and December 2015. We specifically focussed on the added value of histological parameters and on macro- and microvascular parameters.

Questions addressed in this thesis

What is the prognostic value of histological parameters in the renal biopsy for transplantation outcome?

- What factors govern IF/TA progression in DBD and DCD donors? (Chapter 3)
- Is histological assessment of ischemia reperfusion parameters in implantation biopsies related to pre- and posttransplant characteristics? (Chapter 4)
- Can visual fibrosis assessment in protocol renal biopsies be ameliorated (Chapter 5)?

What is the prognostic value of macro- and microvascular parameters on transplantation outcome?

- Does macrovascular pathology assessment pre-transplantation aid in the selection of deceased donor kidneys? (Chapter 2)
- What factors govern microvascular decrease in PTC density the first three months after transplantation? (Chapter 6)
- Does microvascular decrease in PTC density occur in indication biopsies taken the first month after transplantation? (Chapter 7)

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THE ASSOCIATION BETWEEN MACROSCOPIC ARTERIOSCLEROSIS OF THE RENAL ARTERY, MICROSCOPIC ARTERIOSCLEROSIS, ORGAN DISCARD AND KIDNEY TRANSPLANT OUTCOME

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Abstract

Background: During organ retrieval, surgeons estimate the degree of arteriosclerosis and this plays an important role in decisions on organ acceptance. Our study aimed to elucidate the association between macroscopic renal artery arteriosclerosis, donor kidney discard and transplant outcome.

Methods: We selected all transplanted and discarded kidneys in the Netherlands between 01-01-2000 and 31-12-2015, from deceased donors aged 50 years and older, for which data on renal artery arteriosclerosis were available (n=2610). The association between arteriosclerosis and kidney discard, the relation between arteriosclerosis and outcome and the correlation between macroscopic and microscopic arteriosclerosis were explored.

Results: Macroscopic arteriosclerosis was independently associated with kidney discard (OR 1.36 95% CI 1.02-1.80 p=0.03). Arteriosclerosis (any degree) was not significantly associated with delayed graft function (OR 1.16 95% CI 0.94-1.43 p=0.16), eGFR 1 year posttransplant (B 0.58 95% CI -2.07-3.22 p=0.67) and long-term graft survival (HR 1.07 95% CI 0.86-1.33 p=0.55). There was a significant association between mild arteriosclerosis and primary nonfunction (OR 2.14 95% CI 1.19-3.84 p=0.01). We found no correlation between macroscopic and histological arteriosclerosis, nor between histological arteriosclerosis and transplant outcome.

Conclusions: Macroscopic arteriosclerosis of the renal artery was independently associated with kidney discard and somewhat associated with primary nonfunction posttransplant. However, there was no effect of arteriosclerosis on delayed graft function, eGFR at 1 year, or long-term graft survival. Our results are valid only after inevitable exclusion of discarded kidneys that had on average more arteriosclerosis. Hence, conclusions should be interpreted in the light of this potential bias.

Introduction

The past few decades have seen a steady increase in the average deceased donor age[1]. A typical donor today is over 50 years old and is likely to have several relevant comorbidities[2]. Indeed, the once called "expanded criteria" donor has gradually become our standard donor. With rising donor age and associated medical conditions, it will be more likely that a substantial amount of arteriosclerosis is encountered during organ retrieval. Arteriosclerosis of the renal artery may cause technical challenges to construct an arterial anastomosis in the recipient. Also, the condition is often believed to be associated with worse renal transplant outcome, either in the short term due to technical/thrombotic vascular complications, or in the long term as a result of a presumed lower functional capacity of the graft[3].

At organ retrieval, the surgical team estimate the degree of macroscopic arteriosclerosis of the renal artery and this information is communicated to the designated recipient centre, along with other donor and organ procurement data. The transplant centre's medical team will base their initial decision to accept or decline a kidney offer mainly on these parameters. The aforementioned macroscopic arteriosclerosis grading is a mandatory organ quality parameter in the whole Eurotransplant procurement region (8 European countries, among which the Netherlands) and entirely depends on the subjective opinion of individual retrieval surgeons. Surgeons receive no specific training or protocol for scoring the degree of renal artery arteriosclerosis.

The aim of this study was to investigate whether kidney grafts with macroscopic arteriosclerosis were discarded more often than those with unaffected renal arteries. In addition, we studied the association between macroscopic renal artery arteriosclerosis and outcome of those kidneys that were transplanted and whether macroscopic surgical assessment of the renal artery correlated with histological signs of arteriosclerosis.

Methods

Data retrieval

We utilized data from the Dutch Organ Transplantation Registry (NOTR) and from Eurotransplant to perform a retrospective cohort study. The NOTR data management committee approved anonymised data usage for this study. No Institutional Review Board approval was required. All kidneys initially offered for transplantation between 1 January 2000 and 31 December 2015, from deceased donors aged 50 years and older, carried out in any of the 8 transplant centres in the Netherlands were selected. We only included kidneys of those retrieval procedures that were actually commenced and for which data on the macroscopic degree of renal artery arteriosclerosis were available. Baseline demographic variables of the donor, recipient and organ preservation, as well as relevant posttransplant outcome measures were collected (Table 1). For occasional missing values (variable <5% incomplete), the overall median was imputed in case of continuous variables, or a negative value ("none" / "no" / "absent") was imputed in case of binary variables. The database was thoroughly checked for inconsistencies and any errors encountered were corrected after consultation of the NOTR data managers.

Macroscopic arteriosclerosis

In Eurotransplant, the procurement surgeon is required to estimate the degree of macroscopic renal artery arteriosclerosis immediately after organ retrieval. Along with other macroscopic organ characteristics, this assessment is digitally stored and made available to the designated recipient's medical team when a kidney is offered for transplantation. The presence of macroscopic renal artery arteriosclerosis can be scored as "none", "mild", "moderate", or "massive". We utilized this score as the variable which conveys the subjective assessment of macroscopic renal artery arteriosclerosis by the procurement surgeon.

Histological degree of arteriosclerosis

In a subcohort of kidneys that were transplanted in the University Medical Centre Groningen and for which a preimplantation renal needle biopsy was available, an experienced renal pathologist reread all histological slides on light microscopy and, specifically for this study, scored the degree of vascular fibrous intimal thickening (*cv*) and arterial hyalinosis (*ah*). Both could be either 0, 1, 2, or 3, based on the *cv* and *ah* chronicity parameters of the Banff scoring system for renal allografts[4–6]. These two Banff subscores are generally considered to be histological indicators of intrarenal arteriosclerosis.

Statistical analysis

Differences between baseline characteristics of transplanted and discarded kidneys were characterized by means of Mann-Whitney U tests and Pearson's chi-square tests, where appropriate.

First, we explored the association between macroscopic renal artery arteriosclerosis and kidney discard. Univariable analysis consisted of a Pearson's chi-square test. In multivariable logistic regression models, we tested whether (degree of) macroscopic arteriosclerosis was an independent risk factor for organ discard. The choice of other covariates in these models was based on literature and presumed clinical relevance. No further selection was applied; hence, all covariates were left in the models, regardless of their significance level. Full models, including all covariates, are listed in the supplementary appendix.

Second, we investigated the relation between macroscopic renal artery arteriosclerosis and delayed graft function (DGF), primary nonfunction (PNF), the (CKD-EPI) estimated glomerular filtration rate (eGFR) at 1 year after transplantation and death-censored graft survival up to 10 years posttransplant. DGF was defined as any dialysis requirement in the first week after transplantation. Univariable analyses of the association between arteriosclerosis and the incidence of DGF and PNF were performed with Pearson chi square tests. Univariable analyses for eGFR consisted of a one-way ANOVA. Univariable analysis of graft survival was performed with a log-rank test. We also conducted multivariable logistic, linear, or Cox regression analyses to explore whether renal artery arteriosclerosis was an independent risk factor for DGF, PNF, a lower eGFR at one year and death-censored graft failure. Selection of covariates for these regression models was done as described in the previous paragraph. Full models are listed in the supplementary appendix.

Third, we studied how well the subjective surgical assessment of macroscopic renal artery arteriosclerosis correlated with Banff histological surrogates for arteriosclerosis in the subgroup of transplanted kidneys for which such data were available. These associations were quantified as Spearman rank correlation coefficients and by means of Pearson chi square tests, with associated p-values. In addition, we investigated whether in this subcohort Banff *ah* and *cv* scores were associated with DGF, PNF, eGFR at 1 year and 10-year graft survival, utilizing univariable logistic, linear and Cox regression.

For all statistical tests and models, a two-sided p-value <0.05 was assumed to indicate a statistically significant association.

Results

Between 1 January 2000 and 31 December 2015, 4034 kidneys from deceased donors aged 50 years and older were offered for transplantation in the Netherlands, of which 3505 (87%) were transplanted and 529 (13%) were discarded during or after organ retrieval. For 2610 kidneys that were considered for a transplant, data on macroscopic arteriosclerosis of the renal artery were available. In this subset, the division between actually transplanted and discarded kidneys was comparable to that of the whole group: 2239 (86%) vs. 371 (14%). In 70% of all 50+ deceased donor kidneys transplanted in the Netherlands, both kidneys of a pair were transplanted nationally (in two different recipients) and thus, both kidneys of those pairs were available in the NOTR database. For 96% of those pairs, macroscopic arteriosclerosis scores of the left and the right kidney were identical. Supplementary Table S1 presents details of this pairwise comparison of arteriosclerosis grading. Demographics of transplanted and discarded kidneys are provided in Table 1. Table 2 shows an overview of the time point in the donor-to-recipient cascade at which kidneys were discarded, as well as grouped reasons for discard. Presumed inferior organ quality was by far the most abundant reason for discard (94.3% of cases). The database did not specifically record when renal artery arteriosclerosis had been the main reason for organ discard. Hence, we could only indirectly determine the extent to which arteriosclerosis might have played a role in the decision to discard a kidney, by means of univariable and multivariable regression analysis exploring risk factors for organ discard. This analysis is presented in the next paragraph.

The association between macroscopic renal artery arteriosclerosis and kidney discard

Sixteen percent of kidneys with any degree of macroscopic arteriosclerosis were discarded, compared to 10% of kidneys without arteriosclerosis ($p<0.0005$). In a multivariable logistic regression model, any macroscopic arteriosclerosis was independently associated with more discard (OR 1.36 95% CI 1.02-1.80 $p=0.03$, Table 3). When the degree of arteriosclerosis was also modelled, mild arteriosclerosis was significantly associated with discard (OR 1.72 95% CI 1.13-2.60 $p<0.0005$, Table 3) and massive arteriosclerosis had an even stronger independent association with discard (OR 3.50 95% CI 2.48-4.93 $p<0.0005$, Table 3) (see supplementary Table S2 for full models).

Table 1. Donor, organ, recipient, and transplant demographics for the whole study cohort for which data on macroscopic arteriosclerosis were available (n = 2610 deceased donor kidneys offered, of which 2239 were transplanted and 371 were discarded).

Donor demographics	***Transplanted kidneys***	***Discarded kidneys***	**p-value**
Donor age[a] (yr)	59 (50–86)	61 (50–85)	0.001
DCD donor (%)	59	74	<0.0005
Donor BMI[a] (kg/m^2)	25 (15–67)	26 (14–53)	<0.0005
Traumatic cause of death (%)	14	11	0.001
Donor history of hypertension (%)	33	33	0.47
Donor history of diabetes mellitus (%)	6	13	<0.0005
Donor terminal serum creatinine[a] (µmol/l)	69 (24–1185)	79 (24–566)	<0.0005
Organ demographics			
Left kidney (%)	51	51	0.96
No macroscopic arteriosclerosis (%)	31	22	<0.0005
Mild macroscopic arteriosclerosis (%)	9	13	0.02
Moderate macroscopic arteriosclerosis (%)	46	31	<0.0005
Massive macroscopic arteriosclerosis (%)	13	35	<0.0005
Recipient demographics			
Recipient age[a] (yr)	60 (3–85)		
Recipient BMI[a] (kg/m^2)	26 (11–45)		
Total time spent on the waiting list[a] (yr)	3.6 (0–20)		
Previous transplants ≥1 (%)	10		
PRA level >5% (%)	6		
Transplant demographics			
HLA mismatches (% of 0 mismatches)	3		
Cold ischemic time[a] (h)	16 (1–47)		

[a]Median (range).

BMI, body mass index; DCD donation after circulatory death; PRA, panel reactive antibodies.

Of transplanted kidneys, 31% had no macroscopic arteriosclerosis and 9% had mild, 46% moderate and 13% massive arteriosclerosis. Of discarded kidneys, 22% had no macroscopic arteriosclerosis and 13% had mild, 31% moderate and 35% massive arteriosclerosis (Table 1).

Table 2. Timing of and reasons for discard of those 371 kidneys that were discarded during or after organ retrieval and for which data on macroscopic renal artery arteriosclerosis were available.

Variable	Number of kidneys, n (%)
Timing of discard	
Discarded before being offered (organ not offered)	13 (3.5)
Discarded after being offered (organ offer not accepted anywhere)	193 (52)
Discarded after initial acceptance	165 (44.5)
Reasons for discard (reported by retrieval team, recipient center, or Eurotransplant)	
Reasons related to presumed inferior organ quality	350 (94.3)
Recipient related medical problems	8 (2.2)
No suitable recipients found	4 (1.1)
Logistical problems	1 (0.6)
Other reasons	8 (2.2)

There was no database field specific for arteriosclerosis-related discard.

Table 3. Logistic regression analysis for the risk of deceased donor kidney discard.

Variable	Odds ratio (95% CI)	p-value
Risk of kidney discard (arteriosclerosis as binary variable)		
Any macroscopic renal artery arteriosclerosis	1.36 (1.02–1.80)	<0.0005
Risk of kidney discard (arteriosclerosis as categorical variable with 4 levels)		
Any macroscopic renal artery arteriosclerosis		<0.0005
Mild vs. no renal artery atherosclerosis	1.72 (1.13–2.60)	0.01
Moderate vs. no renal artery atherosclerosis	0.79 (0.57–1.08)	0.14
Massive vs. no renal artery atherosclerosis	3.50 (2.48–4.93)	<0.0005

Full models, listing all covariates and their respective odds ratios, can be found in table S1 of the supplementary appendix.
CI, confidence interval

The relation between macroscopic renal artery arteriosclerosis and posttransplant outcome

Of transplanted kidneys without macroscopic arteriosclerosis, 46% developed DGF, and in kidneys with any degree of arteriosclerosis the incidence of DGF was 50%. In a univariable analysis, this difference was not statistically significant (p=0.19). In a multivariable logistic regression model, macroscopic arteriosclerosis was also not significantly associated with the occurrence of DGF (OR 1.18 95% CI 0.96-1.46 p=0.12, Table 4).

Of transplanted kidneys without macroscopic arteriosclerosis, 5.3% developed PNF and in kidneys with any degree of arteriosclerosis, the incidence of PNF was 7.3%. In a univariable analysis, this difference was not statistically significant (p=0.08). In a multivariable logistic regression model, any degree of macroscopic arteriosclerosis was significantly associated with the occurrence of PNF (OR 1.53 95% CI 1.01-2.32 p=0.04, Table 4). However, when the various degrees of arteriosclerosis were tested in a multivariable logistic regression model, only kidneys with mild arteriosclerosis had significantly more PNF than grafts with unaffected renal arteries. Moderate or massive arteriosclerosis were not associated with more PNF (Table 4). In addition, we explored whether PNF in kidneys with renal artery arteriosclerosis was more often related to perioperative or postoperative vascular/thrombotic complications, compared to kidneys without macroscopic arteriosclerosis. However, this was not the case: In kidneys without arteriosclerosis, 32% of PNF cases were related to vascular or thrombotic complications, whereas in kidneys with any macroscopic arteriosclerosis this percentage was even lower: 24%.

CKD-EPI calculated eGFR values at 1-year posttransplant (Figure 1) were comparable for kidneys with none, mild, moderate and massive arteriosclerosis (one-way ANOVA p=0.28). In a multivariable linear regression model, arteriosclerosis was also not significantly associated with eGFR one year after transplantation (B 0.02 95% CI -1.49-3.69 p=0.40, Table 4).

Death-censored graft survival (Figure 2) was similar for kidneys with none, mild, moderate and massive arteriosclerosis (log-rank test; p=0.27). In a multivariable Cox proportional hazards model, arteriosclerosis was also not significantly associated with the risk of graft failure after transplantation (HR 1.08 95% CI 0.86-1.36 p=0.49, Table 4).

Full models, listing all covariates and their respective odds/hazard ratios and regression coefficients, can be found in table S3 of the supplementary appendix).

The correlation between macroscopic renal artery arteriosclerosis and histological surrogates for intrarenal arteriosclerosis

For a total of 129 transplanted kidneys, pretransplant biopsies were available. For 109 of these kidneys, macroscopic renal artery arteriosclerosis scores were also available. Table 5 presents an overview of how macroscopic scores correlated with renal histology. The Spearman correlation between macroscopic assessment of arteriosclerosis and Banff microscopic *cv* score was -0.02 (p=0.82) and the Spearman correlation between macroscopic arteriosclerosis and Banff microscopic *ah* score was -0.07 (p=0.50).

In addition, any macroscopic arteriosclerosis was not significantly associated with any positive score on the Banff *cv* and *ah* scales (Pearson Chi-Square test p=0.80 and p=0.54). These results indicate that there was no sign of any relation between macroscopically observed renal artery arteriosclerosis and histological indicators of intragraft arteriosclerosis. In addition, univariable regression analyses indicated that, in this n=129 subcohort, there was no statistically significant association between *cv* or *ah* scores and DGF, PNF, 1-year eGFR and 10-year graft survival.

Table 4. Multivariable risk analysis[a] for delayed graft function, primary non-function, eGFR at 1 year posttransplant and death-censored graft failure.

Variable	Odds ratio / Linear regression coefficient / Hazard ratio (95% CI)[b]	p-value
Risk of delayed graft function		
Any macroscopic renal artery arteriosclerosis	1.18 (0.96–1.46)	0.12
Risk of primary non-function (arteriosclerosis as binary variable)		
Any macroscopic renal artery arteriosclerosis	1.53 (1.01–2.32)	0.04
Risk of primary non-function (arteriosclerosis as categorical variable with 4 levels)		
Any macroscopic renal artery arteriosclerosis		0.09
Mild vs. no renal artery atherosclerosis	2.14 (1.19–3.84)	0.01
Moderate vs. no renal artery atherosclerosis	1.41 (0.91–2.20)	0.13
Massive vs. no renal artery atherosclerosis	1.46 (0.80–2.65)	0.22
Influence on eGFR (CKD-EPI) at 1 year posttransplant		
Any macroscopic renal artery arteriosclerosis	0.02 (-1.49 – 3.69)	0.40
Risk of death-censored graft failure		
Any macroscopic renal artery arteriosclerosis	1.08 (0.86–1.36)	0.49

Full models, listing all covariates and their respective odds/hazard ratios and regression coefficients, can be found in table S2 of the supplementary appendix.

[a]Logistic regression models for delayed graft function and for primary nonfunction, linear regression model for eGFR at 1 year posttransplant and Cox proportional hazards model for death-censored graft failure.

[b]Odds ratios apply to the logistic regression models, linear regression coefficients apply to the linear regression model and hazard ratios apply to the Cox proportional hazards models.

CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR estimated glomerular filtration rate.

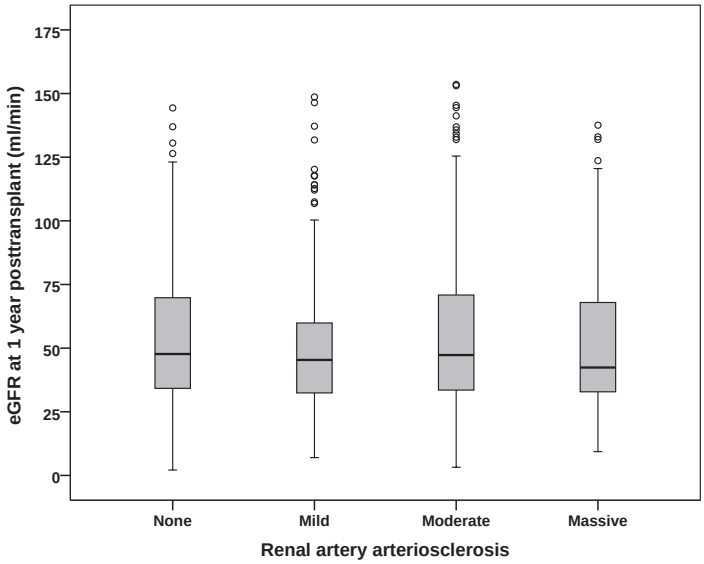

Figure 1. Renal function at one year post-transplant for kidneys with various degrees of renal arteriosclerosis

eGFR (CKD-EPI formula) at 1 year post-transplant for kidneys with various estimated degrees of renal artery arteriosclerosis (medians, interquartile and full ranges).

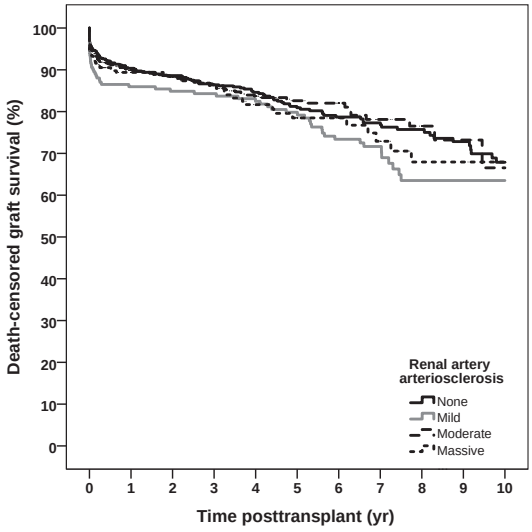

Figure 2. Death-censored graft survival

Death-censored graft survival of transplanted kidneys with various estimated degrees of renal artery arteriosclerosis.

Table 5. Cross-tabulation of macroscopic renal arteriosclerosis score estimated by the procurement surgeon and histological surrogates of intrarenal microvascular arteriosclerosis, scored by an experienced renal pathologist.

Histology score		**Macroscopic renal artery arteriosclerosis**				**Total**
		None	**Mild**	**Moderate**	**Massive**	
Vascular fibrous intimal thickening (*cv*-score)	**0**	5	0	10	0	15
	1	20	0	50	11	81
	2	7	0	3	2	12
	3	0	0	1	0	1
Arteriolar hyaline thickening (*ah*-score)	**0**	28	0	59	12	99
	1	3	0	5	0	8
	2	1	0	0	1	2
	3	0	0	0	0	0
Total		32	0	64	13	109

Values represent the number of kidneys with the respective scores in our local (single-centre) subcohort of 109 allografts for which a pre-implantation biopsy was available.
ah, arteriolar hylainosis; *cv*, vascular fibrous intimal thickening.

Discussion

The present study clearly shows that in the Netherlands, the decision to accept or discard a deceased donor kidney is independently influenced by the reported macroscopic degree of arteriosclerosis of the renal artery. This is interesting, as no traceable literature exists on how macroscopic arteriosclerosis affects graft quality and transplant outcome. Neither do any guidelines exist on how to accurately visually assess the degree of renal artery arteriosclerosis. Intuitively, the practice seems wise, as arteriosclerosis could theoretically lead to a variety of intraoperative and posttransplant complications. First, the vascular anastomosis may be technically challenging, potentially resulting in a higher risk of bleeding or thrombosis, which in turn could compromise transplant outcome. Second, macroscopic arteriosclerosis may also be bad news for overall graft quality. In the past, it has been assumed that if the main renal artery is affected, smaller intrarenal vessels may be diseased as well and this could result in inferior posttransplant function and graft survival. The latter has been shown to some extent in small histopathological series, in which variations of the Banff *ah* score were associated with outcome[3,7]. However, our data showed no correlation whatsoever between histological analogues of arteriosclerosis and reported macroscopic renal artery arteriosclerosis. Although this was done in a small subseries of our main data set, there was not even a trend towards a relevant correlation in these results. Our findings suggest that macroscopic renal artery arteriosclerosis, at least the way it is assessed within Eurotransplant, does not reflect microscopic graft arteriosclerosis in any way. Hence, macroscopic arteriosclerosis cannot be regarded as a surrogate marker for microscopic arteriosclerosis, which is sometimes associated with transplant outcome. This might be explained by the fact that the main renal artery is very different from and quite distant to smaller intrarenal vessels. It seems plausible that intrarenal vascular lesions contribute more to allograft dysfunction than extrarenal arteriosclerotic disease. In addition, an in our experience common – albeit undocumented – observation is that donor surgeons tend to score the degree of renal artery arteriosclerosis looking at the aspect of the aortic patch near the ostium of the renal artery. It is our consistent observation that the patch often has a much higher degree of visible arteriosclerosis than the renal artery itself. An arteriosclerotic patch can easily be removed, which leaves the kidney with an often pristine renal artery that can be anastomosed end-to-side to the recipient iliac artery in a similar fashion as commonly performed for renal grafts retrieved from living donors. Perhaps, if donor surgeons would consistently report only macroscopic arteriosclerosis, which is inside the renal artery itself, a different picture could emerge about the occurrence of macroscopic arteriosclerosis and also of its association with transplant outcome. Nevertheless, in our current study, we also did not find significant associations between histopathologic analogues of intragraft arteriosclerosis and post-transplant outcome. However, this could be due to the limited

sample size in this subcohort, which may not have provided adequate power to detect relatively subtle effects of microscopic arteriosclerosis on outcome. In addition, discarded kidneys are likely to have on average more microscopic arteriosclerosis than organs that were transplanted. An inherit shortcoming of analyses such as the present one is that it remains unknown what posttransplant outcome of discarded allografts would have been and, consequently, relevant bias could be introduced in conclusions on the influence of arteriosclerosis on outcome.

In Eurotransplant, no standardised scoring system, protocol, or guideline for the assessment of macroscopic arteriosclerosis of the renal artery is implemented. It is left to the individual retrieval surgeon to grade and report on the extent of arteriosclerosis per organ. Yet, this very subjective assessment is a compulsory field in the organ report that is passed on to the potential recipient centre at organ offer. Our study suggests that recipient centres do take this score into serious account when judging an organ offer. Given the fact that the current subjective scoring system did not show a relevant association with transplant outcome, our results could stimulate the development of a more standardised and objective assessment methodology, which might perhaps have a predictive value for aspects of renal posttransplant results.

Within the current Eurotransplant context of how donor surgeons score arteriosclerosis and keeping in mind potential selection bias as described before, our data largely contradict the existence of an association between macroscopic renal artery arteriosclerosis and transplant outcome. The only significant association we could find was that transplanted kidneys with mild arteriosclerosis had more PNF. It is tempting to hypothesize that this may indeed be due to more technical complications following a suboptimal arterial anastomosis, leading to graft failure as a result of bleeding or thrombosis. However, vascular reasons for graft failure were even less frequent in those kidneys with PNF and arteriosclerosis, compared to grafts with PNF that had unaffected renal arteries. Moreover, more severe degrees of renal artery arteriosclerosis were not associated with an elevated risk of PNF. This finding cannot be attributed to a too small number of cases in the higher degree of arteriosclerosis subgroups. To the contrary: The subgroup with "mild" arteriosclerosis was the smallest of all four categories, comprising only 9% of all transplants. Each of the other three sub-groups consisted of substantially more cases.

The apparent misconception among transplant clinicians that subjectively graded macroscopic arteriosclerosis of the renal artery would have a relevant influence on outcome could originate from common observations in Nephrology. After all, many studies have reported a clear relationship between chronic native or graft renal artery

stenosis and the development of progressive renal (graft) failure[8-11]. Our present study carefully suggests that this mechanism does not seem to play an important role in kidney grafts accepted for transplantation that have various degrees of renal artery arteriosclerosis already before implantation.

We feel that it is no great surprise that retrieval surgeons' eyes, superficially examining the renal artery, are not the most reliable tool to judge the real condition of the renal graft's (micro)vasculature. Apart from the fact that such macroscopic judgment is very subjective, with likely relevant interobserver variability, reliable vascular quality assessment calls for more advanced diagnostic tools. As mentioned before, histological surrogates for intragraft arteriosclerosis might offer a more predictive pretransplant scoring instrument, but this will have to be demonstrated in a larger cohort with systematically scored preimplantation biopsies. Also, estimating the degree of luminal narrowing in renal arteries on CT-angiographies that deceased donors often routinely undergo could contribute to a more objective rating of actual arteriosclerotic disease, as has been established for coronary artery imaging[12]. Moreover, such an assessment can be done noninvasively and well in advance of a retrieval procedure. The latter would allow the clinical team more time to decide whether kidneys will be procured for transplantation. However, more studies into the relation between radiological scoring of arteriosclerosis and transplant outcome are required before routine donor CT scans can become part of the pretransplant decision-making process.

Our current study has several important limitations. First, our analysis is based on retrospectively collected data. We did not have data on the macroscopic degree of renal artery arteriosclerosis for all transplanted renal grafts in the time period studied and this could have caused bias. Second, this study was only performed on data from transplant centres in The Netherlands. In other countries, scoring, reporting and subsequently interpreting the macroscopic degree of arteriosclerosis could be different. Third, our series of histologically scored preimplantation biopsies was single-centre and quite small. Therefore, we could not reliably determine whether in those biopsies, histopathologic scores for intragraft arteriosclerosis did correlate significantly with posttransplant outcome, as other studies have suggested. Finally, many kidneys with a "massive" arteriosclerosis score were discarded, and we do not know what the outcome would have been when these organs had been transplanted. This may have introduced a bias in our data. Therefore, it is important to note that our results are valid only after inevitable exclusion of discarded kidneys that had on average more arteriosclerosis than those that were transplanted. Conclusions should be interpreted in the light of this potential bias. There was still a considerable number

of "massively" arteriosclerotic kidneys that *were* transplanted (509, 13%). Such a large number should have enabled us to pick up a relevant negative effect of massive arteriosclerosis on transplant outcome. Nevertheless, we cannot entirely rule out that "massively" arteriosclerotic kidneys that were discarded had on average even more severe arteriosclerosis than those that were scored "massive" and transplanted.

In conclusion, our large multicentre retrospective study found that transplant clinicians are much more inclined to discard a 50+ deceased donor kidney when any degree of macroscopic arteriosclerosis of the renal artery is reported by the retrieval team. Subjectively graded macroscopic arteriosclerosis was somewhat associated with PNF, but there was no effect on DGF, eGFR at 1 year, or long-term graft survival. Our analyses also suggest that subjective and nonprotocolled macroscopic assessment of arteriosclerosis is not a good measure of intragraft microscopic arteriosclerosis. Given these data, we feel that kidney discard based on a very subjective macroscopic assessment of renal artery arteriosclerosis – which is currently the only documented and communicated assessment of arteriosclerosis in Eurotransplant – should be discouraged. The implementation of a more structured and objective macroscopic assessment method of renal artery arteriosclerosis could be an opportunity to improve its predictive capacity for renal transplant outcome.

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Supplementary Appendix

Table S1. Overview of (dis)agreement in macroscopic arteriosclerosis grading between the left and the right kidney of each transplanted pair from the same donor (in our data, there were 904 kidney pairs from the same donor that were both transplanted in the Netherlands).

		Macroscopic arteriosclerosis RIGHT kidney				Total
		None	Mild	Moderate	Massive	
Macroscopic arteriosclerosis LEFT kidney	None	277	1	7	0	285
	Mild	2	76	2	1	81
	Moderate	8	2	407	4	421
	Massive	2	0	11	104	117
Total		289	79	427	109	904

Table S2. Logistic regression analysis for the risk of deceased donor kidney discard.

Variable	Odds ratio (95% CI)	p-value
a) Risk of kidney discard (arteriosclerosis as binary variable)		
Donor age (yr)	1.03 (1.01–1.05)	0.02
DCD donor vs. DBD donor	3.60 (2.71–4.78)	<0.0005
Donor BMI (kg/m^2)	1.01 (0.99–1.04)	0.29
Donor cause of death: CVA	0.91 (0.70–1.19)	0.50
Donor cause of death: trauma	0.43 (0.28–0.67)	<0.0005
Donor terminal serum creatinine (µmol/l)	1.02 (1.01–1.02)	<0.0005
Donor history of hypertension	0.99 (0.76–1.28)	0.93
Donor history of diabetes mellitus	1.83 (1.27–2.65)	0.01
Left vs. right kidney	1.00 (0.79–1.25)	0.97
Any macroscopic renal artery arteriosclerosis	1.36 (1.02–1.80)	<0.0005
b) Risk of kidney discard (arteriosclerosis as categorical variable with 4 levels)		
Donor age (yr)	1.02 (1.00–1.04)	0.03
DCD donor vs. DBD donor	3.91 (2.92–5.25)	<0.0005
Donor BMI (kg/m^2)	1.02 (0.99–1.04)	0.20
Donor cause of death: CVA	0.91 (0.69–1.20)	0.50
Donor cause of death: trauma	0.41 (0.26–0.65)	<0.0005
Donor terminal serum creatinine (µmol/l)	1.02 (1.01–1.02)	<0.0005
Donor history of hypertension	0.87 (0.66–1.14)	0.31
Donor history of diabetes mellitus	1.76 (1.20–2.57)	0.004
Left vs. right kidney	0.98 (0.77–1.24)	0.87
Any macroscopic renal artery arteriosclerosis		<0.0005
Mild vs. no renal artery atherosclerosis	1.72 (1.13–2.60)	0.01
Moderate vs. no renal artery atherosclerosis	0.79 (0.57–1.08)	0.14
Massive vs. no renal artery atherosclerosis	3.50 (2.48–4.93)	<0.0005

Table S3. Multivariate risk analysis[a] for delayed graft function, primary non-function, eGFR at 1 year posttransplant and death-censored graft failure.

Variable	Odds ratio / Linear regression coefficient / Hazard ratio (95% CI)[b]	p-value
a) Delayed graft function		
Donor age (yr)	1.01 (1.00–1.03)	0.14
DCD donor vs. DBD donor	2.78 (1.76–4.41)	<0.0005
Donor BMI (kg/m^2)	1.03 (1.01–1.06)	0.004
Donor cause of death: CVA	1.22 (0.95–1.56)	0.12
Donor cause of death: trauma	1.42 (1.03–1.96)	0.04
Donor terminal serum creatinine (µmol/l)	1.01 (1.01–1.01)	<0.0005
Donor history of diabetes mellitus	1.09 (0.75–1.59)	0.66
Donor warm ischemic time (min)	1.04 (1.02–1.07)	0.001
Any macroscopic renal artery arteriosclerosis	1.18 (0.96–1.46)	0.12
Cold ischemic time (hrs)	1.00 (1.00–1.00)*	<0.0005
Number of HLA mismatches	1.02 (0.94–1.10)	0.64
Recipient age (yr)	1.00 (0.99–1.01)	0.42
Recipient BMI (kg/m^2)	1.04 (1.02–1.07)	<0.0005
Recipient history of diabetes mellitus	1.17 (0.91–1.52)	0.22
Total time spent on the waiting list (days)	1.00 (1.00–1.00)*	<0.0005
Most recent PRA level (%)	1.01 (0.99–1.02)	0.39
Number of previous kidney transplants	0.98 (0.74–1.31)	0.91
b) Primary non-function (arteriosclerosis as binary variable)		
Donor age (yr)	1.00 (0.97–1.04)	0.83
DCD donor vs. DBD donor	0.59 (0.28–1.22)	0.15
Donor BMI (kg/m^2)	1.02 (0.99–1.06)	0.21
Donor cause of death: CVA	1.09 (0.71–1.68)	0.69
Donor cause of death: trauma	0.48 (0.24–0.95)	0.03
Donor terminal serum creatinine (µmol/l)	1.01 (1.00–1.01)	0.01
Donor history of diabetes mellitus	1.86 (1.07–3.23)	0.03
Donor warm ischemic time (min)	1.07 (1.03–1.10)	<0.0005
Any macroscopic renal artery arteriosclerosis	1.53 (1.01–2.32)	0.04
Cold ischemic time (hrs)	1.00 (1.00–1.00)*	0.01
Number of HLA mismatches	1.01 (0.88–1.17)	0.86
Recipient age (yr)	1.00 (0.99–1.02)	0.74
Recipient BMI (kg/m^2)	1.05 (1.01–1.10)	0.01
Recipient history of diabetes mellitus	1.15 (0.74–1.79)	0.54
Total time spent on the waiting list (days)	1.00 (1.00–1.00)*	0.30

Table S3. Continued.

Variable	Odds ratio / Linear regression coefficient / Hazard ratio (95% CI)[b]	p-value
Most recent PRA level (%)	1.02 (1.00–1.03)	0.05
Number of previous kidney transplants	0.95 (0.57–1.61)	0.86
c) Primary non-function (arteriosclerosis as categorical variable with 4 levels)		
Donor age (yr)	1.00 (0.97–1.04)	0.82
DCD donor vs. DBD donor	0.60 (0.30–1.24)	0.17
Donor BMI (kg/m^2)	1.02 (0.99–1.06)	0.22
Donor cause of death: CVA	1.08 (0.70–1.66)	0.73
Donor cause of death: trauma	0.50 (0.24–0.93)	0.03
Donor terminal serum creatinine (µmol/l)	1.01 (1.00–1.01)	0.02
Donor history of diabetes mellitus	1.91 (1.10–3.32)	0.02
Donor warm ischemic time (min)	1.06 (1.03–1.10)	<0.0005
Any macroscopic renal artery arteriosclerosis		0.09
Mild vs. no renal artery atherosclerosis	2.14 (1.19–3.84)	0.01
Moderate vs. no renal artery atherosclerosis	1.41 (0.91–2.20)	0.13
Massive vs. no renal artery atherosclerosis	1.46 (0.80–2.65)	0.22
Cold ischemic time (hrs)	1.00 (1.00–1.00)*	0.02
Number of HLA mismatches	1.01 (0.88–1.17)	0.85
Recipient age (yr)	1.00 (0.99–1.02)	0.75
Recipient BMI (kg/m^2)	1.06 (1.01–1.10)	0.01
Recipient history of diabetes mellitus	1.17 (0.75–1.82)	0.49
Total time spent on the waiting list (days)	1.00 (1.00–1.00)*	0.35
Most recent PRA level (%)	1.02 (1.00–1.03)	0.05
Number of previous kidney transplants	0.96 (0.88–1.17)	0.89
d) eGFR (CKD-EPI) at 1 year posttransplant		
Donor age (yr)	-0.61 (-0.83 – -0.39)	<0.0005
DCD donor vs. DBD donor	-2.87 (-8.38 – 2.63)	0.31
Donor BMI (kg/m^2)	0.37 (0.11 – 0.64)	0.01
Donor cause of death: CVA	-3.60 (-6.72 – -0.48)	0.02
Donor cause of death: trauma	1.78 (-2.25 – 5.81)	0.39
Donor terminal serum creatinine (µmol/l)	-0.25 (-0.21 – -0.20)	<0.0005
Donor history of diabetes mellitus	1.00 (-3.64 – 5.63)	0.67
Donor warm ischemic time (min)	-0.12 (-0.38 – 0.15)	0.39
Any macroscopic renal artery arteriosclerosis	0.02 (-1.49 – 3.69)	0.40
Cold ischemic time (hrs)	-0.001 (-0.004 – 0.003)	0.67

Table S3. Continued.

Variable	Odds ratio / Linear regression coefficient / Hazard ratio (95% CI)[b]	p-value
Number of HLA mismatches	1.77 (0.80 – 2.74)	<0.0005
Recipient age (yr)	-0.20 (-0.30 – -0.09)	<0.0005
Recipient BMI (kg/m^2)	-0.05 (-0.34 – 0.23)	0.71
Recipient history of diabetes mellitus	2.23 (-0.92 – 5.38)	0.17
Total time spent on the waiting list (days)	-0.002 (-0.004 – -0.001)	0.01
Most recent PRA level (%)	0.002 (-0.12 – 0.14)	0.93
Number of previous kidney transplants	0.01 (-2.06 – 4.94)	0.42
e) Death-censored graft failure		
Donor age (yr)	1.03 (1.01–1.05)	0.004
DCD donor vs. DBD donor	0.64 (0.42–0.98)	0.04
Donor BMI (kg/m^2)	1.02 (0.99–1.04)	0.19
Donor cause of death: CVA	1.09 (0.83–1.43)	0.55
Donor cause of death: trauma	0.66 (0.45–0.97)	0.03
Donor terminal serum creatinine (µmol/l)	1.00 (1.00–1.01)	0.15
Donor history of diabetes mellitus	1.83 (1.32–2.54)	<0.0005
Donor warm ischemic time (min)	1.04 (1.02–1.05)	<0.0005
Any macroscopic renal artery arteriosclerosis	1.08 (0.86–1.36)	0.49
Cold ischemic time (hrs)	1.00 (1.00–1.00)*	<0.0005
Number of HLA mismatches	1.03 (0.95–1.13)	0.48
Vascular anastomosis time (min)	1.01 (1.01–1.02)	<0.0005
Recipient age (yr)	0.99 (0.98–1.00)	0.02
Recipient BMI (kg/m^2)	1.03 (1.01–1.06)	0.01
Total time spent on the waiting list (days)	1.00 (1.00–1.00)*	0.91
Most recent PRA level (%)	1.01 (1.00–1.01)	0.33
Number of previous kidney transplants	1.05 (0.78–1.42)	0.76

[a]Logistic regression models for delayed graft function and for primary non-function, linear regression model for eGFR at 1 year post-transplant and Cox proportional hazards model for death-censored graft failure.

[b]Odds ratios apply to the logistic regression models, linear regression coefficients apply to the linear regression model and hazard ratios apply to the Cox proportional hazards models.

*Due to the units chosen, odds / hazard ratios and their 95% confidence intervals are very close to 1, but covariates may still be significant predictors in the model.

INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY (IF/TA) PROGRESSION IN THE FIRST YEAR AFTER KIDNEY TRANSPLANTATION IN POSTMORTAL DONORS: ROLE FOR DONOR TYPE AND IMMUNOSUPPRESSIVE REGIMEN

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In preparation

ISCHEMIA REPERFUSION INJURY PARAMETERS IN REPERFUSION BIOPSIES: ASSOCIATION WITH IMPAIRED GRAFT RECOVERY AND MORE IF/TA AT YEAR 1 POST RENAL TRANSPLANTATION

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VISUAL INTERSTITIAL FIBROSIS ASSESSMENT AS CONTINUOUS VARIABLE IN PROTOCOL RENAL TRANSPLANT BIOPSIES

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Abstract

Introduction: In current renal transplant pathology practice interstitial fibrosis is visually assessed in categories according to the Banff classification. As this has a moderate reproducibility, which is little ameliorated by morphometrical analysis, we investigated whether visual renal fibrosis assessment is feasible on a continuous scale, i.e. as percentage affected area of the cortex.

Methods: Protocol renal biopsies taken at transplantation (n=125), three (n=73) and twelve months (n=88) after transplantation were visually scored in categories (Banff), and according to percentages (%) for interstitial fibrosis (ci) and interstitial fibrosis and tubular atrophy (IF/TA). Interobserver variation was assessed, and morphometrical analysis on Sirius Red stained sections was performed. Correlations between the different methods, and their association with donor age and renal function (eGFR) 1 year post transplant were analysed.

Results: Interobserver agreement was equivalent for Banffci and %ci (0.713 vs 0.792), and for BanffIF/TA and %IF/TA (0.615 vs 0.743). Both Banffci and %ci were associated with Sirius red morphometry in three- and twelve-month biopsies. With all three methods, a significant correlation was found between donor age and fibrosis in the implantation biopsy, and between eGFR at 1 year and fibrosis in the 3- and 12-months biopsies. In comparison with Sirius red morphometry the discriminative capacity of %ci was higher for eGFR at 1 year, as well as for fibrosis progression the first year after transplantation.

Conclusion: Interstitial fibrosis assessment on a continuous scale can be used next to scoring in categories according to the Banff classification in protocol renal transplant biopsies.

Introduction

Kidney transplantation is the treatment of choice for patients with end stage renal disease, however allograft survival is limited because of the development of chronic transplant dysfunction (1, 2). Interstitial fibrosis and tubular atrophy (IF/TA) is the histological hallmark lesion of chronic transplant dysfunction, and IF/TA in protocol renal biopsies correlates with renal function and long-term outcome (3, 4). In the Banff classification (5) severity of IF/TA is scored in categories according to the extent of the cortex affected (see Table 1), which is also done for the separate parameters interstitial fibrosis (Banff ci) and tubular atrophy (Banff ct). Interobserver agreement for Banff ci is fair to substantial (6-11), and differs in relation to biopsy type (wedge vs. core and frozen vs. paraffin) (7, 8) and pathologist' experience (11). As interstitial fibrosis assessment in renal transplant biopsies is used on a daily basis in clinical practice, and may even serve as a surrogate end point in clinical trials (3, 12), there is a need to enhance its accuracy and reproducibility.

Automated morphometrical methods using Sirius Red stained sections have been applied in renal transplant pathology (summarized in Table 2). With this technique a percentage of affected cortex is given, and there is substantial interassay (10, 13, 14) and interobserver (10) correlation. Sirius red positivity is correlated with renal function (10, 13-15), and with Banff ci in some (10, 13, 14) but not all (16) studies. Despite these advantages, morphometrical fibrosis assessment has not yet widely reached clinical practice as additional stains, digitalization of images and morphometry software are needed. We hypothesized that visual assessment of interstitial fibrosis as a percentage (i.e. on a continuous scale) can combine the benefits of visual assessment with the quantitative approach of morphometry. Visual assessment of fibrosis was performed on a categorical and continuous scale, and compared with Sirius red morphometry in protocol renal transplant biopsies.

Table 1. Visual assessment of interstitial fibrosis (ci), tubular atrophy (ct) and interstitial fibrosis and tubular atrophy (IF/TA) according to the Banff classification

Banff lesion	Abbreviation	Score			
		0	1	2	3
Interstitial fibrosis	Ci	0-5%	6-25%	26-50%	>50%
Tubular atrophy	Ct	0%	1-25%	26-50%	>50%
Interstitial fibrosis and tubular atrophy	IF/TA	ci 0 and ct 0	ci1 and/or ct 1	ci 2 and/or ct 2	ci 3 and/or ct3

Table 2. Overview of studies using Sirius Red morphometry for interstitial fibrosis assessment in renal biopsies

Study	Patients	Results
Diaz Encarnacion *et al*. 2004 (13)	CAN n=49	Interassay correlation SR: ICC 0.84 SR is associated with Banff ci (ρ0.57, p<0.01), and eGFR (ρ-0.29, p=0.05).
Farris *et al*. 2011 (14)	Native kidney disease (n=14) and postTx (n=1)	Inter assay correlation SR: R^2=0.96, p<0.001 SR associated with: ci% (R^2=0.86, p<0.001), and eGFR (R^2=0.45, p<0.05)
Nara *et al*. 2017 (16)	N=144 Protocol post transplant biopsies at M0 and M12 (mainly living donors)	No association Banff ci and SR at M12 (ρ=-0.112, p=0.927)
Scholten *et al*. 2006 (20)	Protocol post transplant M6 (n=94), M12 (n=97)	SR is associated with IF/TA score (ρ not given, p<0.001)
Rowshani *et al*. 2006 (15)	Protocol post transplant M6 (n=94) and M12 (n=97)	SR M6 and M12 associated with renal function M6 and M12 (ρ not given, p=0.03 and p=0.05, respectively)
Dao *et al*. 2020 (10)	Post transplant biopsies M0 (n=43), d15-20 (n=20), M3 (n=28), and M12 (n=28). DCD type 2	SR Inter observer correlation ICC 0.75 (95%CI 0.67-0.81) (n=151) SR Intra observer correlation ICC 0.88 (95%CI 0.72-0.95) (n=21) SR is associated with Banff ci (ρ=0.62, p<0.001) SR and serum creatinine are correlated at 1 year (R^2=0.32, p=0.013)

Abbreviations: CAN chronic allograft nephropathy, ci interstitial fibrosis, eGFR estimated glomerular filtration rate, SR Sirius Red, TIF tubulointerstitial fibrosis

Material and methods

Patients and clinical data

Patients transplanted in Maastricht UMC+ between April 2003 and December 2009, who received a tacrolimus based immunosuppressive regimen, and of whom a Sirius red stain of a representative protocol biopsy at reperfusion, and/or 3 and 12 months after transplantation was available, were included in this study. Donor characteristics and follow up data were retrieved from patient files. As additional immunosuppression all patients received either sirolimus or mycophenolate mofetil, with an early withdrawal of steroids (17). Estimated glomerular filtration rate (eGFR) was calculated by the Chronic kidney disease epidemiology collaboration (CKD-epi) formula (18).

Collection, storage and use of tissue and patient data were performed in agreement with the Code of conduct for 'Proper Secondary Use of Human Tissue', as described by 'The Federation of Dutch medical scientific societies' (http://www.federa.org). Permission for this study was obtained from the Medical Ethical Committee of the MUMC+ (MEC 09-4-002).

Renal biopsies and analysis

Paraffin tissue for light microscopy was cut at 3µm and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and methenamine silver periodic acid-Schiff (Jones) according to standard staining protocols. Sirius red staining was performed on archival paraffin tissue. In short: deparaffinised tissue was incubated in 0.2% molybdatophosphoric acid (Merck, Kennilworth, New Jersey) for 5 minutes at room temperature followed by 90 minutes incubation at room temperature in 0.1% Sirius red in picric acid solution (both Sirius Red and picric acid from: Klinipath, Duiven, the Netherlands). Slides were then rinsed in 0.01M hydrochloric acid, and tissue was dehydrated using subsequent ethanol steps.

For this study, renal biopsies were considered representative if they contained at least 7 glomeruli and 1 interlobular artery, according to Banff criteria (19). All renal biopsies were rescored for interstitial fibrosis in accordance with the Banff classification (5) by a blinded renal pathologist (LH). Banff parameters were scored in percentages as well as in categories. For interobserver variability, a subset of biopsies was scored for the same parameters by a second, blinded renal pathologist (CPK).

Computerised evaluation of non-polarised Sirius red positive tissue was performed to assess interstitial fibrosis by one blinded observer (AR). Per biopsy, ten images of the renal cortex were taken in a serpentine manner. All pictures were taken with a

non-polarised light microscope (Leica DM3000) at objective 40x. Medullary tissue, blood vessels and glomeruli were excluded when images were acquired. All images were processed by an image processing and analysis system (QWin, Leica's Windows-based image analysis tool kit, Leica, Cambridge, United Kingdom): Sirius red positive tissue area was quantified by a custom-made macro. The software identified Sirius red positive tissue and Sirius red-positive tissue was expressed as percentage of total analysed cortical tissue. See Figure 1 for representative examples.

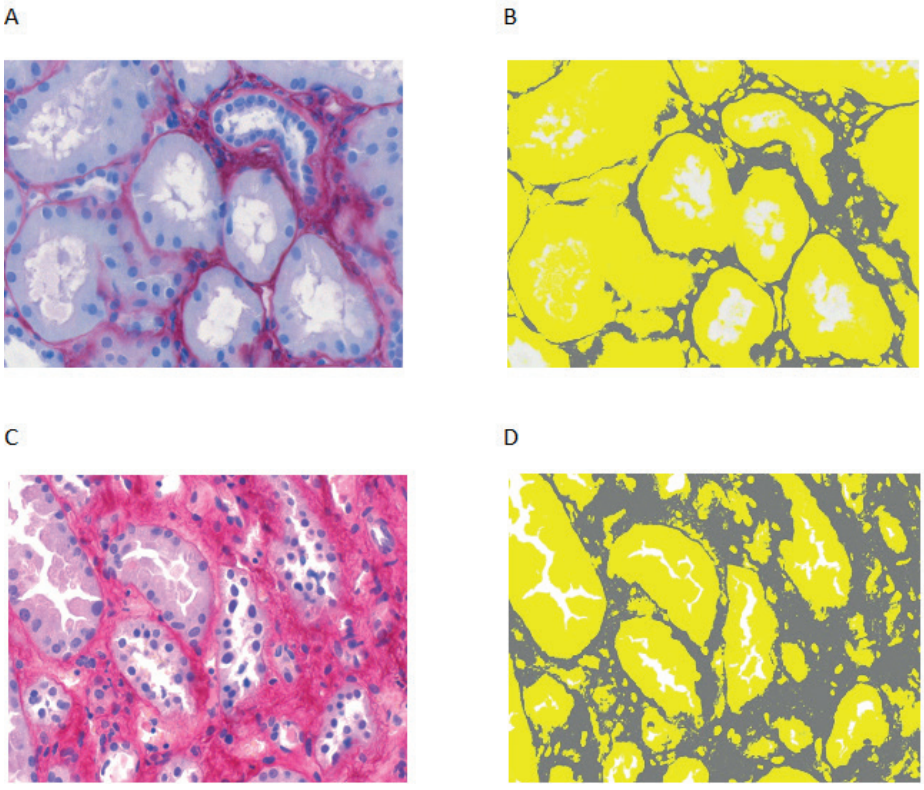

Figure 1. Sirius Red staining of renal biopsy

Figure 1A is a representative example of a picture at objective 40x of a Sirius Red stained renal biopsy taken at twelve months with mild fibrosis (15% in total biopsy). Picture 1B is the overlay generated by the Leica Qwin software of the image shown in 1A. All dark grey area is classified as Sirius Red positive tissue. Figure 1C is a Sirius Red stained month twelve (M12) biopsy with moderate fibrosis (35% in total biopsy) with in Figure 1D its overlay.

Statistics

Continuous data are presented as mean with standard deviation or median and range, where appropriate. Categorical data are given as number with percentage. Interobserver agreement of continuous parameters was tested using intraclass correlation coefficient (ICC), with two-way random effects model with absolute agreement definition. ICC with 95% confidence interval (95% CI) are presented. Interobserver agreement of ordered categorical parameters was tested using weighted kappa, with squared weights; kappa values are given. Association between Sirius red and ci% or IF/TA%, and between Sirius red and renal function was tested by Pearson's rho test. Association between Sirius red and Banff IF/TA or Banff ci score, and the association between Banff ci score and renal function was tested using the Spearman's rho test. A p-value < 0.05 was considered statistically significant. All analyses were executed using SPSS version 25.0 (IBM SPSS, Chicago).

Results

Patients

A total of 144 renal transplant recipients were included for analysis. Of these patients 286 biopsies were scored: 125 implantation biopsies, 73 biopsies taken at three months, and 88 biopsies taken at twelve months. Grafts were from living (21.5%), deceased after brain death (37.5%), and deceased after cardiac death (41%) donors. Mean donor age was 51.2 ± 13.9 years, and 81 (56.3%) of donors was male.

Interobserver agreement of visual interstitial fibrosis assessment as categorial and continuous variable

To address interobserver variability, a subset of 147 biopsies was available (n= 48 implantation, n= 43 at 3 months, and n= 56 at 12 months), and data are given in Table 3. For the categorical assessment on a 0-3 scale overall interobserver agreement for Banff ci is κ0.713 and for Banff IF/TA κ0.615. For fibrosis as continuous parameter (ci% and IF/TA%), ICCs also show substantial agreement. For both categorial Banff scorings and continuous % scoring methods, ICCs for Banff ci are higher than for Banff IF/TA, which is especially apparent in the 12 month protocol biopsy.

Table 3. Interobserver agreement of two blinded pathologists for visual interstitial fibrosis (ci) and interstitial fibrosis and tubular atrophy (IF/TA) assessment

	Overall (n=147)	0 months (n=48)	3 months (n=43)	12 months (n=56)
Banff ci#	0.713	0.522	0.555	0.684
Banff IF/TA#	0.615	0.448	0.521	0.546
% ci*	0.792 (0.720 - 0.846)	0.810 (0.684 - 0.889)	0.751 (0.586 - 0.857)	0.723 (0.514 - 0.843)
% IF/TA *	0.743 (0.623 - 0.823)	0.763 (0.602 - 0.862)	0.770 (0.615 - 0.869)	0.620 (0.280 - 0.794)

Abbreviations: ci interstitial fibrosis, IF/TA interstitial fibrosis and tubular atrophy

weighted kappa

* ICC (95% CI)

Comparison of visual fibrosis assessment on a continuous scale with Sirius Red morphometry

As Sirius red morphometry gives interstitial fibrosis assessment as a percentage, a comparison of Sirius red with %ci was made. As expected, higher %ci is associated with more Sirius red (ρ 0.437, p<0.01). Fibrosis measurements correlate better in biopsies taken three and twelve months after transplantation, than in the implantation biopsies (implantation ρ 0.198, p=0.027, three months ρ 0.602 p<0.001 and twelve months ρ 0.506 p<0.001) (Table 4, Figure 2). As can be seen from Figure 2, in the implantation biopsies % Sirius red was higher than %ci.

A significant correlation with donor age is found with both assessment methods for interstitial fibrosis in the implantation biopsy (ci% ρ0.290, p=0.001, Sirius Red ρ0.220, p=0.013). Both methods showed a significant negative correlation with eGFR after 1 year, i.e. for the 3 month biopsy (ci% ρ-0.337 p=0.004; Sirius red p-0.446 p<0.001) as well as at 12 months (ci%: ρ-0.393 p<0.001, Sirius Red: ρ-0.487 p<0.001). As depicted in Figure 3 the slope of ci% and eGFR at M12 is -0.38 while the slope of Sirius Red and eGFR at M12 is -0.16.

For 51 patients biopsies from all 3 timepoints were available, and progression of fibrosis within the first year post transplant was studied. The median increase in fibrosis assessed by %ci is 10.0% (range -5.0% - 65.0%) while with Sirius red this increase is less pronounced (median + 1.4% (range -15.0% - 19.4%). The progression of %ci significantly correlates with Sirius red progression (ρ0.435 p=0.001) the first year after transplantation.

Table 4. Associations between Sirius Red morphometry and visual interstitial fibrosis (ci) and interstitial fibrosis and tubular atrophy (IF/TA) assessment

	0 months	3 months	12 months
Banff ci and SR†	ρ 0.164	ρ 0.451**	ρ 0.491**
Banff IF/TA and SR†	ρ 0.106	ρ 0.441**	ρ 0.441**
ci% and SR#	ρ 0.198*	ρ 0.602**	ρ 0.506**
IF/TA% and SR#	ρ 0.203*	ρ 0.594**	ρ 0.500**

Abbreviations: ci interstitial fibrosis, SR Sirius Red, IF/TA interstitial fibrosis and tubular atrophy
*p<0.05, **p<0.001
Pearson's rho, † spearman's rho

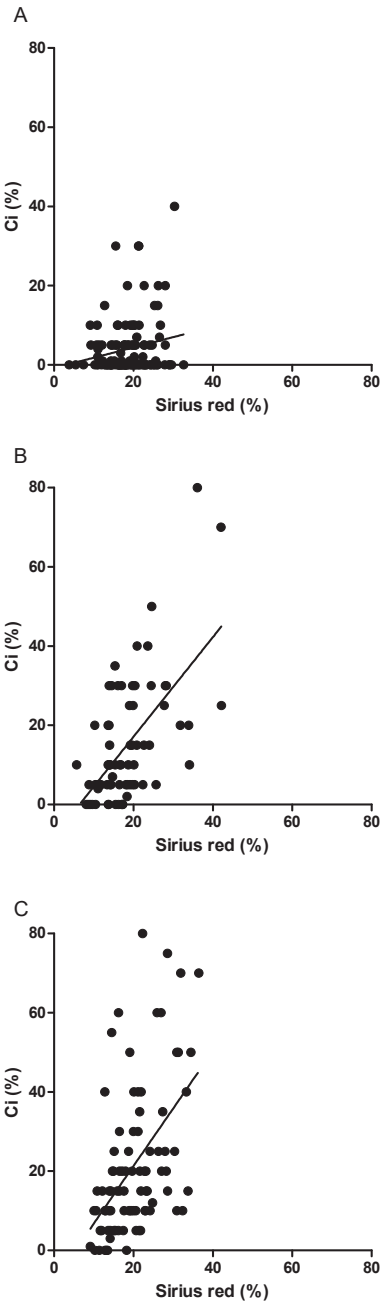

Figure 2. Association between interstitial fibrosis (ci%) and Sirius Red

Association between ci% and SR at A) time of transplantation (ρ0.198, p=0.027), B) three months after transplantation (ρ0.602, p<0.001), and C) twelve months after transplantation, (ρ0.506, p<0.001).

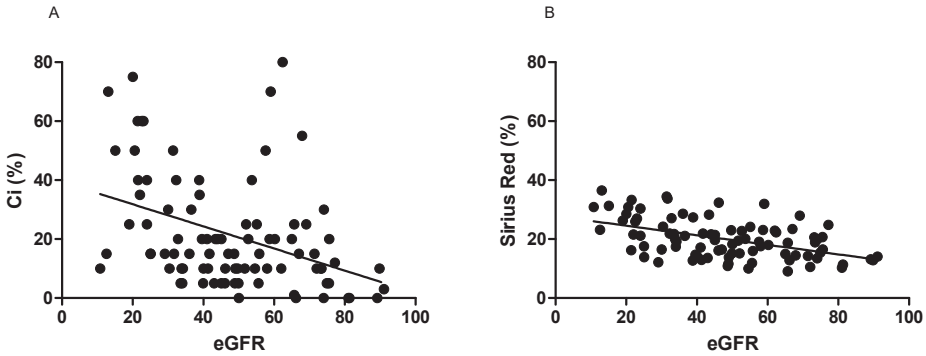

Figure 3. Association of interstitial fibrosis (ci%) and Sirius Red with eGFR twelve months after transplantation

A) Association between Ci %) and renal function (eGFR) twelve months after transplantation (ρ-0.393, p<0.001; slope -0.38); B) association of SR (%) and renal function (eGFR) at twelve months after transplantation (ρ-0.487, p<0.001; slope -0.16).

Discussion

We confirm that interstitial fibrosis in protocol post-transplant biopsies correlates with renal function (3, 4). Furthermore, interstitial fibrosis in the implantation biopsy correlates with donor age, in line with literature (20-22). Visual scoring of the percentage of affected cortical area might give a more precise estimate of interstitial fibrosis as compared to current categorical Banff scoring, however for clinical application it needs to be reproducible and representative. Our comparison of three different methods shows that visual assessment of interstitial fibrosis as continuous parameter (%ci) performs equal to the categorical Banff scoring and morphometrical analysis of Sirius red stained sections.

Interobserver variation for ci% and IF/TA% is at least equivalent to scoring categorical according to Banff. In our setting of retrospectively scored paraffin-embedded needle biopsies, interobserver agreement for ci is as expected from literature (6-11). At all timepoints, there was more agreement between the two pathologists for ci than for IF/TA. This may be explained by the differing cut-offs and definitions, as ci is scored as 1 if 5% -25% of the cortex is affected by interstitial fibrosis, while IF/TA is already scored as 1 if 1-25% of the cortex in the biopsy is affected (5). Especially in the 12 months protocol biopsy, scoring of Banff ci may be preferable over scoring Banff IF/TA if interobserver variation needs minimization. Of note, we scored percentages as a number with differences on a 5% scale, done by eyeballing. Some pathologists prefer to use an ocular grid to quantify the extent of fibrosis, while both methods have not yet been compared (23).

Morphometrical interstitial fibrosis assessment by Sirius red is associated with visual %ci scoring in protocol transplant biopsies, ranging from a weak association in the implantation biopsies to an excellent association at 3 and 12 months. This study therefore extends the earlier observation by Farris *et al*. who investigated predominantly native kidney biopsies from 15 patients (14). The weak association in the implantation biopsy may be explained by the presence of oedema in the implantation biopsy which may give an overestimation of the Sirius red positive tissue. The observation that ci correlates with Sirius red morphometry is in line with most studies performed on renal transplant biopsies (summarized in Table 2). Only Nara *et al*.(16) did not observe a correlation between Banff ci and Sirius red morphometry, however they only studied grafts form living donors, who have less extensive IF/TA than grafts from post-mortal donors (17, 20). At present, morphometrical fibrosis assessment is not feasible in day-to-day clinical practice, which may alter with the ongoing introduction of digital pathology and artificial intelligence software (24). Therefore, it may be beneficial to compare visual fibrosis assessment as a continuous parameter with these innovative techniques in future studies.

It remains to be established whether our findings can be extrapolated to biopsies taken because of clinical deterioration of graft function, and/or to kidney biopsies with primary renal disease, as we only studied protocol renal biopsies. In tumour nephrectomy specimens, scoring of IF/TA density (number of IF/TA foci per area cortex) predicts progression of chronic kidney disease, independent of IF/TA percentage (25). Whether scoring of IF/TA foci in renal transplant biopsies is feasible and useful, also remains to be established. We could only study %ci and Sirius red progression in a subgroup of 51 patients in our cohort. In line with literature (22), visual fibrosis assessment discriminates histological disease progression better than Sirius red morphometry. Further studies, with multivariable models, are needed to investigate whether IF/TA or ci as continuous variable has added value over current Banff classification in the prediction of renal function decline and graft failure. In summary, our data support the introduction of scoring ci and IF/TA on a continuous scale in clinical practice.

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PERITUBULAR CAPILLARY DENSITY IN THE FIRST YEAR AFTER RENAL TRANSPLANTATION: RELATIONSHIP WITH DONOR TYPE, HISTOLOGY AND RENAL FUNCTION

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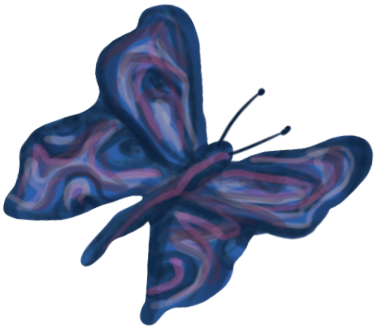

DECREASE IN PERITUBULAR CAPILLARY DENSITY IN THE FIRST MONTH AFTER HUMAN KIDNEY TRANSPLANTATION: RELATIONSHIP WITH REJECTION AND DELAYED GRAFT FUNCTION

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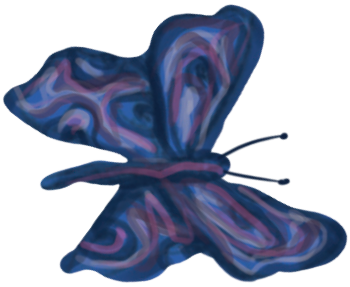

GENERAL DISCUSSION

General discussion

The aim of the studies in this thesis was to validate (histo)pathological parameters in the assessment of kidney injury after renal transplantation in relation to each other and to clinical outcome parameters. Furthermore, we aimed to clarify the post-transplant course of PTC density and its link to fibrosis and renal function. The main conclusions are:

- Assessment of macroscopic arteriosclerosis leads to higher discard rates, while degree of macroscopic arteriosclerosis is not correlated to outcome in transplanted kidneys
- Histological ischemia reperfusion injury parameters in the reperfusion biopsy and IF/TA development in the first year after transplantation are important parameters for evaluation of short- and long-term graft outcome
- Decrease in PTC density occurs very early after transplantation grafts that experience DGF and/or an acute rejection
- Decrease in PTC density precedes IF/TA development.

Below, these points will be discussed and interpreted in the context of the reported literature in more detail and outstanding questions will be defined.

Organ quality assessment

Prompted by the long waiting list for kidney transplantation and the clinical urgency of such intervention, kidneys from older donors, donation after cardiac death (DCDs) and extended criteria donors are increasingly used for transplantation (1, 2). The quality of these kidneys is lower, resulting in lower graft survival of extended criteria donor grafts in younger recipients compared to older recipients and to younger recipients of a standard criterium DBD kidney (3). However, recipients of both DCD and extended criteria donor grafts have a lower mortality risk over patients on the waiting list (4-6). Additionally, health related quality of life is higher for patients with a renal transplant compared to patients on dialysis (7-9). Even though these lower quality donor kidneys are accepted for transplantation, 20% of the kidneys reported to Eurotransplant for allocation were not transplanted in the Eurotransplant region in 2019 (1). The decision whether or not to transplant the donor kidney depends on organ quality as assessed by the following approaches:

- Pre-transplant biopsies for quality assessment are used especially in the USA (10) with a reported discard rate because of biopsy findings of 28%-39% (11). Assessment of pre-transplant biopsies leads to unnecessary discard; a recent study showed that transplanted kidneys in France and Belgium, with similar histology as the discarded kidneys in the USA, had acceptable graft survival rates (12).
- Evaluation of the risk of graft failure, by the use of a clinical risk score, such as the Kidney Donor Risk Index (KDRI). KDRI uses 14 donor and transplant parameters, already known before transplantation, to calculate the risk score for graft failure: donor age, height, weight, history of diabetes and hypertension, ethnicity, cause of death, serum creatinine, hepatitis C virus status, HLA B and DR mismatch, cold ischemia time, double or en block transplantation and being a DCD donor (13). Originally the KDRI was developed in the USA, but it was also validated in the Netherlands; where it was shown to perform comparable to the USA (14).
- Inspection of the organ by the surgeon, who examines the vascular and anatomical quality and variations and identifies renal abnormalities, like tumours and cysts after retrieval (10). One of the organ quality parameters that are mandatory to be scored at retrieval in the Eurotransplant procurement region is macroscopic arteriosclerosis of the renal artery (scored as none, mild, moderate or massive). However, there is no protocol for the scoring of this parameters nor is there specific training for surgeons.

In **Chapter 2** we investigated whether surgical arteriosclerosis assessment of the renal artery correlates with discard rates and outcome in the transplanted arteriosclerotic kidneys. It turned out that macroscopic arteriosclerosis was associated with a higher discard rate, but not with graft outcome in transplanted kidneys. Due to the nature of the study, it remains unknown what the posttransplant function of the discarded kidneys would have been. Next, there was no association between macroscopic arteriosclerosis and microscopic vascular damage, scored as arteriolar hyalinosis and/or intimal thickening. A potential explanation may be that donor surgeons tend to score arteriosclerosis based on the aortic patch, near the ostium of the renal artery. Often macroscopic arteriosclerosis can be observed near this branching point, while further on the renal artery is clean. Therefore unnecessary discard of donated kidneys might be decreased by adapting the Eurotransplant guidelines for donor surgeons about assessment of donor kidney quality: 1. to restrict the scoring of arteriosclerosis to the artery itself (and not including the aortic patch) and 2. to train surgeons in the scoring of macroscopic renal arteriosclerosis. Only once the scoring protocol is standardised, future studies can be performed to evaluate whether renal arteriosclerosis, scored in that way, is associated with graft function and microscopic vascular damage.

Histological renal injury in the first year after transplantation

Chronic kidney disease is histologically characterised, among other factors, by the formation of interstitial fibrosis and tubular atrophy (IF/TA), regardless of the underlying cause of CKD (15, 16). It can be seen as a final common pathway induced by inadequate wound-healing of kidney tissue after repetitive injury (15, 16). IF/TA is also an early marker for chronic transplant dysfunction, and it can already develop in the first year after transplantation (17-23), even before creatinine levels are rising (24). In general, fibrosis develops in two stages; a first stage in reaction to the primary insult (wound healing stage) which can be followed by a second phase in which fibrosis progresses without a new event (pathological fibrosis stage) (25, 26). Injury of the kidney, which can be observed histologically in any compartment i.e. glomeruli, tubuli or vessels, leads to influx and activation of inflammatory cells. Cytokines, produced by these attracted inflammatory cells stimulate production of pro-fibrotic mediators, such as TGF-β by several cell types, including macrophages, T-cells and tubular epithelial cells. In their turn, pro-fibrotic mediators activate mesenchymal cells such as fibroblasts, pericytes, and fibrocytes, which become contractile and matrix-producing myofibroblasts (25). Secondly, also tubular epithelial cells and PTC endothelial cells could dedifferentiate and transform into myofibroblasts (epithelial to mesenchymal transition (EMT) and endothelial to mesenchymal transition (endo-MT)) (27). Normally, in the wound healing process the matrix is degraded, inflammation is halted and normal tissue architecture is restored, after resolution of the initial damage. However, persistent injury can lead to a point of no return and pathological fibrosis production (25, 27). Progression of fibrosis after the first injury is stimulated by several contributing processes:

- Some tubuli may fail to regenerate after tubular injury resulting in atrophic tubuli which continue to produce pro-fibrotic stimuli (28).
- Arteriolar narrowing and microvascular rarefaction causes chronic hypoxia, which further damages tubuli (25, 28).
- After resolution of the initial renal injury, myofibroblasts can remain activated, with further fibrosis production (25).

Although the mechanism of fibrosis development in general is reasonably well understood, not much is known about histological injury early after transplantation and the link with patient and clinical parameters. These research questions were studied in **Chapters 3 and 4** of this thesis.

Fibrosis assessment technique

Traditionally, in clinical settings fibrosis is scored using the categorical Banff criteria (29, 30). As described in detail in **Chapter 1** (general introduction) the Banff fibrosis score is categorical. The original goal of the Banff fibrosis score is to reflect the amount of cortex composed of fibrous tissue (29). However, from a survey done under 248 participants (87% pathologists, 10% nephrologists and 3% from other fields) it became clear that there is considerable variability among the approaches to assess the Banff fibrosis score(31):

- Stains used for assessment; ranged from commonly used trichrome and PAS staining to less commonly used Sirius Red stain,
- Microscope magnification: differed from low power field (4-10x magnification) to high power fields (20x magnification) or a combination of both low and high power fields,
- Definitions used to assess fibrosis: the most commonly reported were: percentage of abnormal cortex (reported by 58% of participants) and percentage of fibrous tissue in cortex (reported by 36%)).

All these differences in the assessment could contribute to the moderate interobserver agreement for Banff fibrosis scores, which range between 0.30 and 0.78 (32-36). In **Chapter 5** we studied interobserver agreement for Banff ci and Banff IF/TA between two nephropathologists and found good interobserver agreement: the interobserver agreement for ci was 0.713 and for IF/TA 0.615.

Besides the Banff categorical score, several other assessment methods for fibrosis scoring are available and used in studies, including visual assessment on a continuous scale, computerised morphometry of both polarised and non-polarised Sirius red staining, and computerised morphometry of other immunohistochemical stains for fibrosis such as collagen III and αSMA (37-41). There is still discussion about which techniques are the most robust and reproducible (37). In **Chapter 5** we compared categorical visual assessment of fibrosis using the Banff classification (Banff ci) to visual fibrosis assessment on a continuous scale (%ci) and with computerised morphometric evaluation of Sirius red stained kidney biopsies. We have shown that interobserver agreement was equivalent for Banff ci and %ci. Furthermore, there was little difference between %ci and Sirius red evaluation in our cohort.

Visual scoring on a continuous scale, i.e. %ci, has some advantages over the use of computerised morphometric fibrosis assessment: it does not require extra investment of different stains and preparation and analysis time since pathologists

use routinely stained slides. The only activity they have to do is to record the relative fibrosis area instead of, or in addition to the categorical Banff fibrosis score (31). Furthermore, there is no need for investment in hard- and software for slide scanning, analysis and storage of the digital scans, which could make computerised assessment impractical for clinical use (37). However, in an era in which digital pathology and the use of whole slide imaging is getting more common (42), this may become a smaller hurdle. A concern for using percentages is that it may be interpreted as a too precise estimation of the fibrosis in the biopsy, yet, pathologists are able to distinguish differences of 5% between test slides (43). Taken all together, visual assessment of relative fibrotic area in renal biopsy tissue is at this moment the easiest and most accurate method to assess fibrosis in clinical practice. However, in a research setting morphometric evaluation could provide more objective fibrosis measures, especially when consecutive biopsies are studied (37).

Ischemia and reperfusion injury parameters

Ischemia reperfusion injury is inevitable during the process of donation and transplantation and it is associated with the occurrence of non-immediate graft function (delayed graft function (DGF) and primary nonfunction) (44, 45). There are many studies focusing on assessment of ischemia reperfusion injury and predicting short term graft outcome, using biochemical parameters and histological parameters. Biochemical parameters that have been studied include perfusate biomarkers, urinary and serum biomarkers and transcription analysis of implantation biopsy. Nowadays, in the Netherlands, most deceased donor kidneys are preserved using machine perfusion. The predictive capacity of a range of possible biomarkers in the perfusate has been tested. Perfusate lactate dehydrogenase (LDH; a non-specific cellular injury marker) and gluthatione-S-transferase (GST; a marker of renal tubule injury) were univariable associated with DGF (46, 47). In a few studies, the latter enzyme was also in multivariable analysis significantly associated with DGF, but it predictive ability was moderate (47). Furthermore, data on perfusate biomarkers in the prediction of graft function is scarce (47). Until now, no perfusate biomarker can predict post-transplant outcome with enough accuracy to be of use in clinical practice.

Promising urinary or serum biomarkers are neutrophil gelatinase-associated lipocalin (NGAL; a marker for proximal tubule injury) and IL-18. The level of both markers, determined in the first 24 hours after transplantation, were predictive of DGF (48, 49). Pleading for the use of urinary and serum samples for biomarker assessment is that serum and urine samples are easy to obtain. A drawback of the

use of urine samples is that patients with DGF often don't have urine production. Secondly, bias can be introduced when a patient has residual diuresis from the native kidney. The clinical value of urinary and serum biomarkers and whether it may aid in clinical decision making still needs to be determined.

Transcription patters in implantation biopsies have been studied in order to distinguish immediate graft function from DGF kidneys. Genes involved in the complement cascade, immunity, and acute phase response were up-regulated in grafts with DGF or slow graft function vs. kidneys with immediate graft function (50, 51). Also some microRNAs, measured in implantation biopsies were associated with DGF (52). Studies combining transcript data with clinical risk factors could assess risk of DGF with more accuracy (52, 53). These studies show that the use of transcripts analysis could be an addition to histology assessment and clinical risk factors in the (pre-transplant) determination of short term graft function. However, there is no fixed set/ agreed set of transcripts that could be used in clinics, and big trials are still lacking (10, 54).

Ischemia reperfusion injury during donation and transplantation causes damage that may be observed histologically in post-reperfusion biopsies. Several studies have investigated acute tubular injury in pre-implantation or reperfusion biopsies (55, 56) but a clear histological definition is lacking (55). Different histological features are used such as loss of brush border, apoptosis and cytoplasmic vacuolisation. Furthermore acute tubular necrosis is used as assessment for acute tubular injury, without a clear definition or grading system (55). Therefore, it is still not clear whether acute tubular injury is a risk factor for DGF or graft failure (56). Besides acute tubular injury, other parameters, including neutrophil influx in glomerular capillaries and in peritubular capillaries have been observed in post-reperfusion biopsies (57, 58).

The value of histological assessment of acute injury in post-reperfusion biopsies for transplant outcome the first year after transplantation was studied in **Chapter 4**. We studied four histological ischemia reperfusion injury parameters: two parameters for tubular injury ('tubular cell necrosis' and 'loss of brush border') (34), and two parameters that reflect reperfusion parameters ('neutrophil influx in glomerular capillaries', and 'neutrophil influx in peritubular capillaries (PTC)') (57, 58) in reperfusion biopsies. We have shown that these parameters are more prevalent in post-mortal donors than in living donor grafts. 'Loss of brush border', is independently correlated with DGF and decreased eGFR at year one after transplantation. Furthermore, 'neutrophils in PTCs' was an independent risk factor for higher IF/ TA scores at one year after transplantation. These results suggest that scoring for histological ischemia reperfusion injury parameters may aid in the prediction of short

term graft function and development of chronic injury. However, more research is needed before these parameters can be implemented in clinical practice. Scoring of the ischemia reperfusion injury parameters should be standardised; the used parameters in our studies are based on 'eyeballing' of the pathologist and a better definition is needed to provide consistent scoring by different pathologists. Secondly, validation studies are needed whether 'loss of brush border' and 'neutrophils in PTCs' can be used to predict DGF, IF/TA at 1 year and renal function after transplantation.

IF/TA and its association with inflammation

In this thesis we studied which clinical parameters are associated with development of IF/TA and its progression in the first year after transplantation in our single centre cohort, with retrospective analyses of the (protocol) biopsies. Our data show that IF/TA develops after ischemia reperfusion injury and inflammatory events, such as in an acute rejection **(Chapter 3)**. These findings are in line with literature as reviewed elsewhere (25).

Inflammation plays a major role in fibrosis development and can often be seen together with fibrosis in renal biopsies. Inflammation can be observed in the context of a rejection, but also in a beneficial inflammatory repair response to kidney injury (25). Interstitial inflammation in areas of interstitial fibrosis and tubular atrophy (i-IF/TA) has been added to the Banff classification in 2015 as a marker for chronic active T-cell mediated rejection, and since then more studies have focused on this parameter (59). It has been shown that i-IF/TA is associated with IF/TA development (60-62), and a study with consecutive biopsies suggests that i-IF/TA precedes IF/TA development (60). Furthermore, i-IF/TA is associated with graft loss (61, 63). Moreover, graft function (eGFR) and graft survival is better for grafts with IFTA *without* inflammation than for grafts *with* i-IF/TA (and the same grade of IF/TA) (64). Although these studies suggest that i-IF/TA has implications for renal graft function and survival, there is a discussion about the context and role of i-IF/TA. Initially proposed as a parameter for chronic active T-cell mediated rejection (59), it is now more acknowledged that i-IF/TA can be seen as the response to injury as is observed in several disease processes including rejections, BK virus infection, pyelonephritis and glomerulonephritis (65-67). Future studies about fibrosis after renal transplantation should include both i-IF/TA and IF/TA to gain more insight in the histological value of inflammation in fibrosis for graft function.

IF/TA in the first year after renal transplantation and its association to clinical parameters

Our group has previously shown that one year after transplantation IF/TA is higher in DCD grafts compared to LD grafts (17). However, there were too few patients to compare IF/TA in DBD vs. DCD donor grafts. Therefore, we now addressed this

research question in an expanded study cohort. In this thesis we showed that there is significantly more IF/TA at one year post-transplant in DCD compared to DBD donors (**Chapter 3**), which is in line with a recent study who studied 1 year protocol biopsies in 87 DCD and 246 DBD grafts (68). Within the group of the DCD grafts, we have shown that IF/TA development was not different between different DCD types (controlled vs. uncontrolled) or between preservation methods (cold storage vs. machine preservation). We also demonstrated, using a Kaplan Meier analysis, a lower death censored 10 year graft survival in the groups with higher IF/TA score at year one after transplantation; which is in line with literature, (68-73). However, it seems contradictory that DCDs have similar graft outcome than DBD grafts (74-76) despite a higher IF/TA scores at year one. In our study, grafts that failed within the first year after transplantation were not included. Due to the relative limited number of patients in the current cohort, we were only able to perform univariable survival analysis and could not study possible effects or interactions between donor type and IF/TA score. It would also be interesting to compare graft outcome in DBD grafts with low vs. high IF/TA and DCD graft with low vs. high IF/TA and i-IF/TA vs. no i-IF/TA. These proposed analyses may provide more insights into the effect of IF/TA on graft survival.

Already for decades, immunosuppressive therapy in our centre has been tailor-made, depending on individual immunological risk stratification for acute rejection. In **Chapter 3** we confirmed earlier findings of our group that IF/TA progression is dependent on the interaction between immunosuppressive regimen and baseline IF/TA. In recipients without IF/TA at baseline tacrolimus therapy in combination with the mTOR inhibitor sirolimus helps slowing down IF/TA progression, while in patients with baseline IF/TA this combination results in higher IF/TA scores at one year compared to patients on tacrolimus in combination with mycophenolate mofetil. This beneficial effect of sirolimus in grafts without baseline IF/TA might be explained by the reported anti-angiogenic activity (77), either indirectly by reducing mTOR dependent vascular endothelial growth factor (VEGF) production or directly by inhibiting mTOR dependent endothelial proliferation (78). It could be hypothesised that in kidney grafts without baseline IF/TA sirolimus prevents detrimental repair (possibly due to inhibition of angiogenesis) while in kidney grafts with pre-existent IF/TA angiogenesis is needed for the recovery of ischemia reperfusion injury. Inhibition of angiogenesis in grafts with baseline IF/TA might hamper the recovery and lead to more IF/TA development. Tailoring immunosuppression based on the pre-existent IF/TA score may help to prevent progression of IF/TA and in that way might contribute to a better graft survival. To achieve this, a pre-transplant biopsy is necessary so immunosuppressive regimen can be chosen timely.

Microvasculature in the first year after transplantation

Microvasculature assessment technique

Several methods have been applied to score PTC density including presenting PTC density as percentage (%) cortical area covered by PTCs, vessels/ μm^2 or PTC/tubule. In our studies, PTC density was assessed by visually counting PTC and tubule numbers in renal biopsy tissue and expressed as PTC/tubule as our group has done previously (79). Our group has previously shown in a pilot study that a decrease in PTC density occurs already in the first three months after transplantation in deceased donor kidneys (79). This finding was later confirmed by another group (80). As both studies were relative small and therefore only a few number of covariates could be taken into account, we studied change in PTC density early after transplantation in a larger cohort containing 54 LD, 57 DBD and 59 DCD grafts, therefore providing the opportunity to study more covariates and perform multivariable analyses. Secondly, as the transplant inclusion of our cohort was done between 2003 and 2010, and clinical follow-up had been monitored since then, this allowed analysing long term (10 year) performance of these grafts. A drawback might be that effects of recent changes in preservation techniques and selection of donor types on PTC density could not be taken into account. In our cohort, most kidneys were preserved on cold storage, while at present almost all deceased kidneys are preserved with machine preservation. Therefore, not all our results may be generalised to the current transplantation situation and should be validated in a more recent cohort.

The method of counting PTC and tubules, as we have done in our studies, is time-consuming and it has moderate interobserver agreement (own unpublished data), which hampers use of PTC counting in larger (multicentre) studies or in clinical use. Automation of PTC and tubule counting may overcome this problem in part. Furthermore, studying PTC/field and tubuli/field as separate parameters may provide an opportunity to study the course of both parameters independent of each other over time and may aid in providing further insight in the tubulovascular cross-talk. Several groups used algorithms which analysed CD34 stained whole slide images and provided microvascular density in vessels/μm^2 (81) or percentage of efficient cortical area occupied by peritubular capillaries (82). In the future, even more advanced techniques, including computerised recognition of different kidney tissue components by use of deep learning techniques on whole slide images may provide a way for high throughput analysis of renal tissue. Reliable classification of glomeruli and tubuli is already possible, but further subclassification, for example differentiation of healthy and atrophic tubuli, remains difficult (83). Furthermore, there are emerging techniques for PTC assessment; such as whole slide analysis of CD34+ staining as proposed in a recent study on peritubular capillary extent (84).

Decrease in PTC density is also called capillary rarefaction. As described in the general introduction, two types or capillary rarefaction can be distinguished: functional and structural rarefaction (85). In our studies we only assessed PTC density on a structural level. Quantification of PTC density on CD31/CD34 stained renal biopsy slides does not provide insights into functional rarefaction. It has been shown that blood flow early after revascularisation is lower in DCD compared to LD, which suggests that functional rarefaction occurs (86, 87). However, the cameras used in these studies to visualise capillary blood flow need to be put directly on the kidney cortex, which makes them inappropriate for follow up of capillary function after transplantation. Other imaging techniques for non-invasive microvasculature assessment include contrast-enhanced ultrasonography (CEUS) and functional magnetic resonance imaging (fMRI). Contrast-enhanced ultrasonography uses microbubble-based contrast agents that allow visualisation of perfusion in capillaries (88). A few exploratory studies have shown the possibility to use this imaging tool to examine microvascular perfusion in chronic kidney disease (89, 90). This technique may also be useful in investigating microvasculature perfusion post-transplantation. Functional MRI parameters arterial spin labelling and cortical perfusion fraction are reported to be correlated with capillary density (defined as number PTC/mm^2 tubulointerstitium) in renal transplant indication biopsies (91). For future studies, combining (non-invasive) functional imaging techniques with PTC density assessment in biopsies may provide deeper insights into the development of PTC rarefaction and the underlying causes for transition of functional in structural rarefaction.

Decrease in PTC density in the first year after transplantation

In **Chapter 6** we have validated the findings that PTC density is stable in LD kidneys while there is statistically significant decrease in PTC density in the first three months after transplantation in DCD kidneys (-11.6%). In DBD kidneys, there was a small non-significant decrease in PTC density in the first three months after transplantation. There was no significant difference in PTC density in LD at three months or one year compared to baseline. Higher baseline PTC density, higher baseline IF/TA score and Banff subclinical rejection in the month three biopsies were associated with a more pronounced decrease in PTC density between baseline and month 3. These results indicate that the decrease in PTC density is dependent on donor type (and with that possibly extent of ischemia reperfusion injury) and inflammatory events. In line, one group recently showed more decrease in PTC density three months after transplantation in kidneys with DGF compared to kidneys with immediate graft function (82).

In **Chapter 7** we have studied PTC density in 'for cause' biopsies taken within the first month after transplantation and compared the results with the group of patients with only protocol biopsies at three months. In the group without a 'for cause' biopsy, PTC density was stable in the first three months after transplantation, while in the group with a 'for cause' biopsy, PTC density was already reduced in the first weeks after transplantation. The 'for cause' biopsy was on average taken at 9 days after transplantation. The decrease in PTC density was observed regardless of the indication for 'for cause' biopsy: DGF, clinical acute rejection, or the combination of DGF and an acute rejection. Of note: PTC density decreased already within the first month after transplantation while there was at that moment no significant increase of IF/TA in the indication biopsy, thereby showing that decrease in PTC density precedes progression IF/TA. Furthermore, between one and three months after transplantation there was a further decrease in PTC density in the DGF group but not in the rejection, nor the DGF+rejection group.

In contrast to LD donors, DBD donors experience a cytokine storm and DCD donors are subjected to a period of warm ischemia time during the period of asystole in the donor (92). Ischemia reperfusion injury leads to a higher risk of DGF (44) and both cytokine storm and a long warm ischemia time have negative effects on graft survival (92). Several strategies to decrease ischemia reperfusion injury are studied and it has been shown that it can be decreased by reduction of cold ischemia time (92, 93). Another option to decrease ischemia reperfusion injury is the use of machine perfusion instead of cold storage as preservation technique for donor kidneys. There is a reduction of risk of primary nonfunction and DGF in machine perfusion preserved deceased donor kidneys (94, 95). Also other changes in machine perfusion, including normothermic perfusion and oxygenated perfusion are studied (92, 96). Strategies to decrease ischemia reperfusion injury may also lead to stabilisation of PTC density. Although it is tempting to assume that machine perfusion also preserves PTC density, this has not been studied yet. As our cohort does not allow for such analyses, as stated before, hence this will require a newer transplant cohort.

We observed an early decrease in PTC density in patients with an acute rejection. To explore possible differences in the pattern of decrease in PTC density depending on the type of rejection, we divided this group using the Banff criteria (65) into pure T-cell medicated rejections (n=19) and antibody mediated rejections (n= 13, of which 9 with mixed T-cell and antibody mediated rejection). Numerically, there was more decrease in PTC density in the antibody mediated rejection group than the T-cell mediated rejection group, supporting the consensus that endothelium is the target of antibody mediated rejection, including endothelium of the PTCs. However, the groups were too small to draw firm conclusions.

Correlation of peritubular capillaries with IF/TA

In **Chapter 6** we reported an association of lower PTC density with higher IF/TA score. In some experimental studies decrease of PTC density precedes IF/TA progression (97, 98). Our studies with consecutive protocol biopsies gave us the opportunity to study the time course of PTC density decrease and IF/TA development. Decrease in PTC density in the first three months was not associated with IF/TA at one year, while the decrease in the first year was associated with IF/TA at one year. Both progression of IF/TA (**Chapter 3**) and decrease of PTC density (**Chapter 6 and 7**) are dependent on ischemic injury (donor type, DGF) and immunological events (acute rejection, subclinical rejection). In **Chapter 4** we observed a correlation of the histological ischemia reperfusion injury parameter 'neutrophil influx in the PTC' in reperfusion biopsies and IF/TA at one year. Because of the association between IF/TA and PTC density, it would be interesting to study whether 'neutrophils in PTCs' is also associated with PTC density decrease. An unproven hypothesis is that ischemia reperfusion injury leads to neutrophil influx in PTCs, which induces and/or aggravates endothelial cell damage resulting in PTC rarefaction and IF/TA development. This may be of particular relevance in DCD donors as they have more neutrophils in PTCs, more IF/TA in the 3 and 12 month protocol biopsies and more decrease in PTC density compared to living donor grafts.

Our observations that IF/TA progression and PTC density decrease are correlated and that PTC density in the 'for cause' biopsy is already decreased without presence of IF/TA suggests that decrease in PTC density precedes IF/TA development. We also observed that decrease in PTC density and IF/TA development are induced by similar factors which suggests that both are part of a final common pathway which leads to chronic transplant dysfunction. Future studies should aim to unravel the interplay between PTC density and IF/TA development. This may help to pinpoint targets for therapeutic interventions to halt PTC density decrease and IF/TA development. Interventions studies would be helpful to clarify a possible causal relation between PTC density decrease and IF/TA development.

Correlation of PTC density and renal function

Our study shows a lower one year eGFR when the decrease in PTC density in the first year after transplantation is more **(Chapter 6)**. However, there was no association between the decrease in PTC density in the first year with a decrease of eGFR after the first year during the ten years follow up posttransplant. This suggests that PTC density does not influence eGFR directly, but acts indirectly, e.g. by promoting IF/TA.

Proteinuria after transplantation is correlated with graft loss and worse patient survival (99-101). Proteinuria is also associated with endothelial dysfunction (102). We have shown an association between lower PTC density at one year after transplantation and higher cumulative incidence of proteinuria in the years following (**Chapter 6**). PTC density may have clinical implications and may aid in detecting patients at risk for worse renal functioning. However, as there were only 40 cases with proteinuria in the ten-year follow up period in the study cohort, larger studies will be needed to conclusively establish the relationship between PTC density and proteinuria and long term eGFR.

Concluding remarks

The central aims of this thesis were to study the value of histological parameters in the assessment of kidney injury in transplant biopsies taken in the first year after transplantation (both protocol biopsies and 'for cause' biopsies) and the role of decrease of PTC density after renal transplantation. We showed that histological ischemia reperfusion injury parameters in the reperfusion biopsy and IF/TA development in the first year after transplantation are important parameters for evaluation of both short and long-term kidney function. Histological ischemia reperfusion injury parameters may in the future be used as a surrogate marker for short term graft function in studies. Furthermore, we have shown that decrease in PTC density occurs early after transplantation in DCD kidneys (compared to both LD and DBD kidneys) and in recipients who experience DGF and/or an acute rejection. Our studies suggest that decrease in PTC density precedes IF/TA development and that both decrease in PTC density and (later) development of IF/TA are part of a final common pathway which leads to chronic transplant parameters. Therefore, enhancing microvascular stability in the kidney may provide a good strategy to decrease IF/TA progression and with that increase kidney graft longevity.

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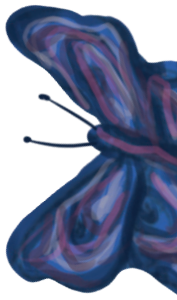
IMPACT PARAGRAPH



Impact paragraph

Renal transplantation is the preferred treatment option for most patients with end stage renal disease (ESRD). However, graft function deteriorates with time posttransplant and eventually retransplantation or dialysis may be needed. Already in the first year after transplantation, histological signs of chronic injury, including interstitial fibrosis and tubular atrophy (IF/TA), can be observed in (protocol) renal biopsies before deterioration of renal function occurs (1). In this thesis, several histological parameters were assessed in protocol biopsies taken at transplant and during the first year after renal transplantation in a single centre cohort in order to study the prognostic value of these parameters for renal transplantation outcome.

Research and clinical implications

The decision of acceptance of a potential postmortal donor kidney is partly based on the macroscopic evaluation of the kidney by the transplant surgeon at time of organ procurement. This evaluation is mandatory in the Eurotransplant region. In our nation-wide study, macroscopic arteriosclerosis of the renal artery is associated with higher discard rate of donor kidneys. However, in kidneys that were transplanted, presence of macroscopic arteriosclerosis was not associated with a worse graft survival or worse renal function compared to kidneys without macroscopic arteriosclerosis. Furthermore, there was no correlation between the grade of macroscopic arteriosclerosis and microscopic intra-renal arteriosclerosis in the donor kidney. Macroscopic arteriosclerosis is usually scored at the aortic patch, near the ostium of the renal artery. At the ostium there is often macroscopic arteriosclerosis present, while further on the renal artery has no sign of macroscopic arteriosclerosis. Therefore, the current method of assessing macroscopic arteriosclerosis seems inadequate to assess the quality of the donor graft. Donor surgeons should be trained to score macroscopic arteriosclerosis in het renal artery and the Eurotransplant guidelines for the assessment of donor kidney quality should be adapted. After standardising scoring of macroscopic arteriosclerosis, future studies can be performed to evaluate whether renal arteriosclerosis is associated with graft function and microscopic vascular damage.

During the process of donation and transplantation, ischemia reperfusion injury is inevitable and it is associated with the occurrence of delayed graft function (DGF) and primary nonfunction (2, 3). However, there is no histological standard scoring for ischemia reperfusion injury (4, 5). We performed an explorative study in which we studied parameters for ischemia reperfusion injury in protocol renal transplant biopsies. We showed that loss of brush border is associated with delayed graft function and decreased renal function at year one after transplantation. Furthermore, presence

of neutrophils in PTCs were associated with higher IF/TA scores at one year after transplantation. There was no association of tubular cell necrosis or presence of neutrophils in glomeruli with outcome. Loss of brush border and presence of neutrophils in PTCs could be an interesting read-out for ischemia reperfusion injury in future studies. However, the parameters to score ischemia reperfusion injury are not well defined neither in our study nor in literature (5). Probably as a result of the poor definition, we and others found low interobserver agreement of these parameters (4). Future research should focus on a better definition and standardisation of the histological ischemia reperfusion injury parameters. This is needed before any conclusion about clinical applicability can be drawn. Additionally, in our study we scored the ischemia reperfusion injury parameters in reperfusion biopsies, i.e. in biopsies taken after the graft is transplanted. Hence, these parameters cannot be used for clinical decision making on suitability of the potential donor graft for transplantation. On the other hand, the parameters may be used in an assessment of baseline quality of the kidney graft.

We studied a cohort of consecutive renal transplants, on a tacrolimus-based immunosuppressive regimen, with available protocol biopsies taken at time of transplantation, and 3 and 12 months after transplantation. We assessed progression of fibrosis in relation to clinical outcome parameters. Our studies confirm that IF/TA is an important predictive parameter for graft function (eGFR) at one year posttransplant, and therefore, IF/TA is an important parameter in the assessment of renal transplant biopsies. We confirm that IF/TA development is associated with ischemia reperfusion injury and inflammatory events (6) and that higher IF/TA scores one year after transplantation are associated with lower graft survival (7-9). We showed that there is more IF/TA progression in DCD vs. DBD in the first year after transplantation, which may be related to more ischemia reperfusion injury. IF/TA progression was also dependent on the immunosuppressive regimen. In kidneys *without* any pre-existent IF/TA, there was less progression of IF/TA when sirolimus was used as additional immunosuppressant to tacrolimus while in kidneys *with* pre-existent IF/TA addition of mycophenolate mofetil to tacrolimus gave less IF/TA progression. A strategy to decrease IF/TA progression in the first year after transplantation, and with that improve graft survival, could be tailoring of immunosuppressive regimen dependent on the IF/TA score at time of transplantation.

Although our studies underline the importance of IF/TA, we have demonstrated, in line with literature, a moderate interobserver agreement for scoring IF/TA in categories according to the Banff classification (10-12). We examined whether visual renal fibrosis assessment is feasible on a continuous scale, i.e. as percentage affected area of the cortex. Interobserver agreement was numerical slightly better for assessment on

continuous scale compared to the Banff classification. Furthermore, we compared visual renal fibrosis assessment with computerised evaluation of fibrosis in percentages on Sirius red stained biopsies. Agreement of assessment on a continuous scale and Sirius red evaluation did not notably differ in our cohort. For clinical use we propose a continuous scoring system, since it does not need extra time of technical investment. However, it needs validation in future (prospective) studies. In research settings computerised evaluation of fibrosis could provide more objective fibrosis data (13).

Decreased peritubular capillary (PTC) density is associated with higher IF/TA scores and lower eGFR in cross sectional studies in chronic kidneys disease and allograft dysfunction (14-16). However, most studies focused on late stages of graft dysfunction and not much in known about PTC stability in early stages post- transplant. Our studies underscore the relation between peritubular capillary (PTC) density and IF/TA in renal transplantation settings. We showed that decrease in PTC density occurs more in DCD than in LD and DBD. Secondly we showed that decrease in PTC density occurs very early (in the first weeks) after transplantation in recipients with complications shortly after transplantation (a rejection and/or delayed graft function). Furthermore, we observed that early decrease in PTC density precedes later progression of IF/TA. Moreover, decrease in PTC density was associated with a lower eGFR at one year after transplantation and more often development of proteinuria up to ten years after transplantation. Decrease in PTC density may therefore serve as a surrogate marker for later graft function. However, assessment of PTC density is still at a too preliminary phase to implement already in clinical settings. There are several assessment methods used to score PTC density in studies and our method is a time-consuming and tedious method. Automated assessment of PTC density might be a solution for using PTC density assessments at a large scale.

Social implications

Compared to patients on the waiting list, transplanted patients have a lower mortality risk and a better quality of life (17-21). Transplanted patients have more quality adjusted life years (QALYs) over ten years than dialysis patients: 5.2-6.3 QALY's versus 4.0 QALY's (22). Furthermore, the costs per QALYs were less for transplantations compared to dialysis (22, 23). Hence, not only ESRD patients but also the society and economy benefit from kidney transplantation. There is however a shortage of donor kidneys: implementation of recommendations from our studies could, in the future, lead to more kidney transplantations with longer graft survival. As described above, we have shown that arteriosclerosis in the renal artery was not associated with worse graft function or survival. Implementing this knowledge in donor quality assessments may decrease unnecessary donor discard and, with that, enlarge the donor pool.

Furthermore, scoring ischemia reperfusion injury parameters provides a new early readout to evaluate interventions before en during donation and transplantation. This could potentially give insight, already early after interventions, which (new) interventions are successful. We have demonstrated that DCD kidneys and grafts with DGF develop more IF/TA, which is associated with worse renal function and shorter graft survival (6). Furthermore, we showed that decrease in PTC density precedes IF/TA progression. Early interventions to stabilise PTC density and interventions that reduce IF/TA progression may protect the graft from decline of function and a longer graft survival, which will increase the gained QALYs in transplantation patients and reduce costs.

Concluding remarks

Protocol biopsies taken after renal transplantation provide a useful tool to study early histological markers for short-term and possibly long-term renal function, which might be implemented in clinical care in the future. A few ischemia reperfusion injury parameters, scored in reperfusion biopsies are associated with clinical course posttransplant. Progression of IF/TA and the decrease of PTC density during the first year posttransplant may be used as indicators for long term renal function. In addition, the findings in this thesis support the development of strategies aimed at prevention of ischemia reperfusion injury and preservation of PTC density, especially in DCD donor kidneys, that may ultimately lead to their increased graft survival and/or an expansion of the donor pool.

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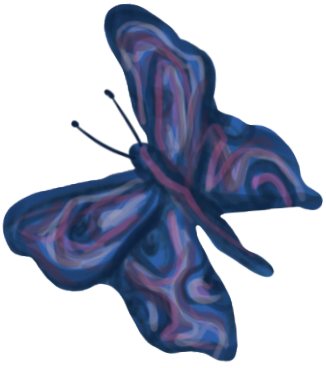
SAMENVATTING



Samenvatting

Bij 7-15% van de populatie, wat neerkomt op ongeveer 2 miljoen mensen in Nederland, werken de nieren onvoldoende en herstelt de nierfunctie niet meer. Dit wordt chronische nierinsufficiëntie genoemd. Als chronische nierinsufficiëntie leidt tot nierfalen is niertransplantatie, naast dialyse, de enige levensreddende en in veel gevallen beste behandeling. Een transplantaatnier gaat echter niet onbeperkt mee. De functie gaat uiteindelijk achteruit, wat chronisch transplantaat dysfunctie wordt genoemd. Chronisch transplantaat dysfunctie maakt dat een patiënt opnieuw dialyse of een nieuwe transplantatie nodig heeft. Donornieren kunnen afkomstig zijn van donoren die bij leven een nier afstaan voor transplantatie of van donoren die overleden zijn. Binnen die laatste groep zijn er donoren die overleden zijn t.g.v. hersenschade (DBD) en donoren die overleden zijn na een hartstilstand (DCD). Tijdens en na niertransplantatie worden vaak nierbiopten afgenomen, onder andere om de oorzaak te onderzoeken van eventuele complicaties na transplantatie (waaronder acute afstotingsreactie). Deze biopten kunnen ook gebruikt worden in onderzoek naar chronisch transplantaat dysfunctie, wat gekenmerkt wordt door littekenvorming in de nier, wat interstitiële fibrose en tubulus atrofie (IF/TA) wordt genoemd.

Er is weinig bekend over de acute schade direct na transplantatie en het effect van deze acute schade op de nierfunctie. Wel is onderzoek gedaan naar de ontwikkeling van IF/TA als marker voor chronisch transplantaat dysfunctie, lange tijd (jaren) na transplantatie. In een aantal van deze studies is ook gekeken naar de kleine bloedvaatjes in de nieren, de peritubulaire capillairen. Daarbij werd geconstateerd dat een lage dichtheid van de kleine bloedvaten samengaat met meer verlittekening (IF/TA). Er is echter slechts weinig bekend of, en zo ja, hoe IF/TA en de kleine bloedvaten zich *kort* na transplantatie ontwikkelen en of dit samenhangt met een slechtere lange termijn prognose.

In het kader van dit proefschrift zijn niertransplantatiebiopten afgenomen in het eerste jaar na transplantatie onderzoek. De biopten zijn onderzocht op diverse pathologische kenmerken. Het doel van dit proefschrift is de pathologische kenmerken te analyseren om te bepalen of er een relatie is/wat de verhouding tussen de mate van nierschade in het biopt en de nierfunctie. We hebben hierbij onder andere de mate van verlittekening (IF/TA) en de hoeveelheid kleine bloedvaten (peritubulaire capillaire dichtheid) onderzocht. Dit hebben we onderzocht in een groep patiënten die tussen maart 2003 en december 2009 een niertransplantaat hebben ontvangen in het MUMC en bij wie na de transplantatie gedurende 12 maanden meerdere nierbiopten

zijn afgenomen. Bijzonder aan deze groep patiënten bij wie transplantaties hebben plaatsgevonden is dat er relatief veel nieren getransplanteerd zijn van DCD donoren. De belangrijkste bevindingen en conclusies van het proefschrift staan hieronder vermeld.

Hoofdstuk 1 is een inleidend hoofdstuk waarin niertransplantaties in zijn algemeen worden besproken. Daarbij is aandacht besteed aan het beloop, de mogelijke complicaties die kunnen optreden na transplantatie en het gebruik van afweeronderdrukkende medicatie die nodig is om afstoting te voorkomen. Hierbij is speciale aandacht gegeven aan de rol van pathologisch onderzoek van niertransplantaatbiopten in de beoordeling van afstotingsreacties en IF/TA en de beoordeling van acute schade na transplantatie (ook wel ischemie reperfusie schade genoemd). Als laatste wordt de dichtheid van de kleine bloedvaten in de nieren besproken en een overzicht gegeven van onderzoek naar de afname hiervan na transplantatie.

Potentiële postmortale donornieren (DBD en DCD) worden door de chirurg van het uitnameteam op het oog beoordeeld op geschiktheid voor transplantatie. Hierbij wordt onder andere gekeken naar mogelijke afwijkingen aan de vaten en bouw van de nier, tumoren en ook naar de mate van aderverkalking in de nierslagader. Op basis van deze beoordeling kan besloten worden dat een donornier ongeschikt is voor niertransplantatie en moet worden afgekeurd. In **Hoofdstuk 2** hebben we onderzocht of de mate van aderverkalking samenhangt met de kans op afkeuring van de potentiële donornieren. Daarnaast is onderzocht of in getransplanteerde nieren de aanwezigheid van aderverkalking samenhangt met een minder goede nierfunctie. Dit hebben we onderzocht in een groep (potentiële) donornieren in alle Nederlandse transplantatiecentra. De conclusie die is getrokken is dat donornieren met meer aderverkalking een grotere kans hadden om afgekeurd te worden. In nieren die getransplanteerd zijn, bestond echter *geen verband* tussen de mate van aderverkalking en de nierfunctie. Bovendien bestond er ook geen verband tussen de mate van aderverkalking die door de chirurg in de nierslagader werd gezien en de mate van aderverkalking in de kleine niervaten, die in het nierbiopt onder de microscoop werd gezien. Dit zou mogelijk kunnen komen omdat de chirurg de mate van aderverkalking vaak aan het begin van de nierslagader beoordeelt, terwijl de nierslagader verderop vrij van aderverkalking kan zijn. Op grond hiervan kunnen we concluderen dat nieren niet afgekeurd zouden moeten worden op basis van de beoordeling van aderverkalking ter hoogte van het begin van de nierslagader. Nader onderzoek moet nog volgen of er wel een verband is tussen aderverkalking verderop in de nierslagader en het functioneren van de transplantaatnier.

In **Hoofdstuk 3** hebben we de ontwikkeling van verlittekening in het eerste jaar na transplantatie onderzocht in nieren die na overlijden zijn gedoneerd (DBD en DCD). Ten tijde van transplantatie was er geen verschil in de hoeveelheid littekenweefsel in de nieren van hersendode donoren (DBD) en nieren van donoren die aan een hartstilstand zijn overleden (DCD). In het eerste jaar na transplantatie was er echter een grotere toename van littekenweefsel in DCD nieren dan in DBD nieren. Er was ook meer progressie bij nieren met een acute afstotingsreactie in het eerste jaar en bij nieren die ten tijde van transplantatie al meer littekenweefsel hadden. Daarnaast was er een verband tussen de mate van verlittekening en het type afweersysteem onderdrukkende medicatie. Bij transplantaatnieren zonder littekenweefsel ten tijde van transplantatie was er minder ontwikkeling van littekenweefsel bij patiënten die een combinatie van de medicijnen tacrolimus en sirolimus kregen. Bij transplantaatnieren waarbij wel al littekenweefsel aanwezig was ten tijde van transplantatie, was de toename van littekenweefsel minder groot dan bij patiënten die een combinatie van tacrolimus en mycophenolaat mofetil gebruikten. Als laatste zagen we dat een grotere hoeveelheid littekenweefsel 1 jaar na transplantatie duidelijk geassocieerd was met een slechtere 10-jaars overleving. Aanpassing van de medicatie die het immuunsysteem onderdrukt op basis van de hoeveelheid littekenweefsel in de nier ten tijde van transplantatie, zou daarom kunnen bijdragen aan minder toename van littekenweefsel en daarmee tot langere transplantaatoverleving.

Tijdens het proces van donatie en transplantatie treedt er onder andere door zuurstoftekort, schade op aan de transplantaatnier. Deze schade kan ervoor zorgen dat een nier vertraagd of helemaal niet gaat functioneren na de transplantatie. In **Hoofdstuk 4** hebben we de waarde van vier histologische parameters onderzocht die gescoord worden in het biopt dat tijdens de transplantatieoperatie afgenomen wordt. Deze 4 parameters zijn: verlies van borstelhaartjes in de cellen van nierbuisjes, het afsterven van niercellen, de aanwezigheid van ontstekingscellen (neutrofielen) in de haarvaten van de nieren en de aanwezigheid van ontstekingscellen in de vaten van de nierfilters (glomeruli). In DBD- en DCD-nieren kwam vaker schade voor dan in nieren van levende donoren. Verlies van borstelhaartjes was een onafhankelijke risicofactor voor het verminderd en/of niet functioneren van de transplantaatnier. Daarnaast was er een verband tussen verlies van borstelhaartjes en een slechtere nierfunctie (“estimated glomerulaire filtratie rate” oftewel eGFR score) op jaar 1. Tevens was er een verband tussen aanwezigheid van ontstekingscellen in de haarvaten (peritubulaire capillairen) met meer littekenweefsel op jaar 1. Dit onderzoek laat zien dat de schade die de nier oploopt tijdens het proces van donatie en transplantatie leidt tot histologisch herkenbare schade in het nierbiopt. Dit komt meer tot uiting in nieren van donoren na

overlijden, dan in nieren die bij leven worden gedoneerd. Het scoren van histologische schade in biopten genomen direct na transplantatie kan in de toekomst mogelijk helpen bij het voorspellen van een slechter klinisch beloop na transplantatie.

Pathologen scoren de hoeveelheid littekenweefsel in niertransplantatie biopten in de regel visueel volgens internationale Banff criteria. Daarbij wordt de hoeveelheid littekenweefsel geclassificeerd in een score van 0 (geen littekenweefsel) tot 3 (>50% littekenweefsel). In **Hoofdstuk 5** hebben we onderzocht wat de beste eenheid is van deze score: het relatieve kleuringsoppervlakte (een score in percentages) dan wel de Banff score in categorieën (0-3). De overeenstemming tussen verschillende beoordelaars was beter voor littekenweefsel gescoord in percentages dan in de Banff score (categorie). Daarnaast hebben we de score van littekenweefsel in percentages vergeleken met computer gestuurde analyse van littekenweefsel op Sirius Rood gekleurde biopten. Er was een goede overeenkomst tussen percentage littekenweefsel gescoord door de patholoog en de Sirius Rood score. Daarnaast was er een verband tussen zowel percentage littekenweefsel als Sirius Rood percentage met nierfunctie een jaar na transplantatie. Wel leek Sirius Rood de hoeveelheid littekenweefsel te overschatten in biopten die tijdens de transplantatie werden genomen. Hierdoor was de toename van littekenweefsel in het eerste jaar na transplantatie kleiner bij de Sirius Rood score dan bij de beoordeling door de patholoog. Dat maakt dat de methode waarbij pathologen het percentage littekenweefsel visueel beoordelen beter geschikt is dan computer gestuurde analyse van het littekenweefsel voor het beoordelen van progressie van littekenweefsel over tijd.

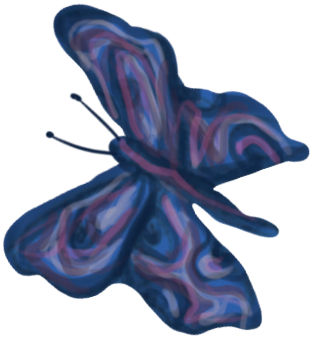
Hoofdstuk 6 en 7 richten zich op de rol van de haarvaten in de nieren (peritubulaire capillaire dichtheid) na niertransplantatie. Hiervoor is de dichtheid van de haarvaten gemeten in de nierbiopten die tijdens transplantatie en 3 en 12 maanden na transplantatie zijn genomen. In **Hoofdstuk 6** concluderen we dat in het eerste jaar na transplantatie de dichtheid van de haarvaten stabiel blijft in nieren van een levende donor, terwijl in nieren die na een hartstilstand werden getransplanteerd een afname van haarvaten in de eerste drie maanden na transplantatie optreedt. In nieren van DBD-donoren is er een minimale afname van haarvaten in de eerste drie maanden na transplantatie. De afname in haarvaatjes in de nier in de eerste drie maanden is daarnaast groter bij nieren die ten tijde van transplantatie meer haarvaten hebben, bij nieren die meer littekenweefsel hebben ten tijde van de transplantatie en indien in het maand 3 biopt tekenen zijn van een afstotingsreactie. Deze resultaten wijzen erop dat afname van het aantal haarvaten afhankelijk is van de donoren van eventuele ontstekingen na transplantatie. Verder hebben we de relatie tussen afname van de dichtheid van haarvaten en de mate van littekenweefsel in latere biopten en

beloop van de nierfunctie onderzocht. De afname van dichtheid van de haarvaten in het eerste jaar houdt verband met een grotere hoeveelheid littekenweefsel en met een slechtere nierfunctie, uitgedrukt als eGFR, op jaar 1. Desondanks is er geen onafhankelijk verband tussen de afname van de dichtheid van de haarvaten in het eerste jaar en de afname van nierfunctie tussen jaar 1 en jaar 10 na transplantatie. Deze bevindingen suggereren dat de dichtheid van de haarvaten indirect de nierfunctie (eGFR) beïnvloedt, mogelijk via de ontwikkeling van littekenweefsel. We vonden een verband tussen de afname de dichtheid van de haarvaten en een verhoogde kans op eiwitten in de urine, wat een uiting is van een verminderde nierfunctie. Gezien het beperkte aantal patiënten in ons onderzoek die in de 10 jaar na transplantatie eiwitten gingen uitplassen, zijn er studies met meer patiënten nodig om de relatie tussen de haarvaten en nierfunctie te onderzoeken.

In **Hoofdstuk 7** hebben we de haarvaten onderzocht in extra biopten die afgenomen zijn in de eerste maand na transplantatie bij patiënten bij wie de transplantaatnier niet op gang kwam, of er een verdenking was op een acute afstotingsreactie. Bij patiënten die geen extra biopt nodig hebben, blijft de dichtheid van de haarvaten in de eerste drie maanden na transplantatie stabiel, terwijl in de patiënten met een extra biopt de dichtheid van de haarvaten in de eerste maand na transplantatie al afneemt. De afname van de dichtheid van de haarvaten zagen we terug in de groep die een nier ontving die vertraagd op gang kwam na transplantatie, de groep met acute afstoting en in de groep van nieren met zowel een afstotingsreactie en een vertraagd herstel van nierfunctie. Dit is in lijn met de bevindingen uit **Hoofdstuk 6** dat afname in dichtheid van haarvaten geassocieerd is met DCD-donoren en afstotingsreacties. De groep patiënten met een acute afstotingsreactie werd verder onderverdeeld op basis van type afstoting: afstoting door lymfocyten of door antilichamen. De groepen waren te klein om de bevindingen diepgaand te kunnen analyseren, maar het leek erop dat antilichaam afhankelijke afstoting gepaard ging met een grotere afname haarvaten. Dit onderschrijft de heersende gedachte dat antilichaam afhankelijke afstoting voornamelijk de vaten aanvalt. In de biopten die afgenomen werden in de eerste maand na transplantatie, zagen we nog geen toename van littekenweefsel in het biopt. Dit suggereert dat afname dichtheid van de haarvaten vooraf gaat aan de ontwikkeling van littekenweefsel in de transplantaatnier. Toekomstige studies die zich richten op het tegengaan van afname van de dichtheid van haarvaten in de nier kunnen bijdragen aan een langere levensduur van de transplantaatnier.

Ten slotte wordt in **Hoofdstuk 8** de belangrijkste bevindingen besproken en in context van de huidige literatuur geplaatst. Tevens worden daar (mogelijke) toekomstige onderzoeksdoelen besproken.

LIST OF ABBREVIATIONS



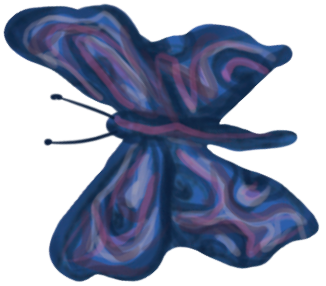
List of abbreviations

ABMR	Antibody mediated rejection
ah	Arteriolar hyalinosis
ANOVA	Analysis of variance
αSMA	alpha smooth muscle actin
ATG	Anti-thymocyte globulin
BMI	Body mass index
BSA	Body surface area
CAN	Chronic allograft nephropathy
CD	Cluster of differentiation
CEUS	Contrast-enhanced ultrasonography
cg	Chronic glomerulopathy
ci	Interstitial fibrosis
CI	Confidence interval
CIT	Cold ischemia time
CKD	Chronic kidney disease
CKD-epi	Chronic kidney disease- Epidemiology Collaberation
CNI	Calcineurin inhibitor
CPD	Clinicopathological diagnosis
ct	Tubular atrophy
CTD	Chronic transplant dysfunction
cv	Vascular fibrosis intimal thickening
DBD	Donation after brain death
DCD	Donation after circulatory death
DGF	Delayed graft function
DSA	Donor specific antibodies
ECD	Extended criteria donors
eGFR	Estimated glomerular filtration rate
EMT	Epithelial to mesenchymal transition
Endo-MT	Endothelial to mesenchymal transition
ESRD	End stage renale disease
ET	Eurotransplant
fMRI	Functional magnetic resonance imaging
g	Glomerulitis
GST	Gluthatione-S-transferase
HE	Haematoxylin-eosin
HLA	Human leucocyte antigen
HR	Hazard ratio

i	Interstitial inflammation
ICC	Intraclass correlation coefficient
IF/TA	Interstitial fibrosis and tubular atrophy
i-IF/TA	Interstitial inflammation in areas of Interstitial fibrosis and tubular atrophy
IGF	Immediate graft function
IL	Interleukin
IRI	Ischemia reperfusion injury
IS	Immunosuppression
IVIG	Intravenous immunoglobulins
KDRI	Kidney Donor Risk Index
LBB	Loss of brush border
LD	Living donor
LDH	Lactate dehydrogenase
M0	Reperfusion/ month 0
M3	Month 3
M12	Month 12
MFI	Mean fluorescence intensity
mm	Mesangial matrix expansion
MMF	Mycophenolate mofetil
mTOR	Mamalian target of rapamycin
NGAL	Neutrophil gelatinase-associated lipcalin
NOTR	Nederlandse Orgaantransplantatie Registratie / Dutch Organ Transplant Registry
OR	Odds ratio
PAS	Periodic acid Schiff
PMN	Polymorphic neutrophils
PNF	Primary nonfunction
PRA	Panel reactive antibodies
PTC	Peritubular capillary
QALY	Quality ajused life years
RNA	Ribonucleic acid
SCR	Subclinical rejection
SD	Standard deviation
SEM	Standard error of mean
SG	Stable graft
SR	Sirius Red
SRL	Sirolimus
t	Tubulitis

TAC	Tacrolimus
ti	Total inflammation
TIF	Tubulointerstitial fibrosis
TCMR	T-cell mediated rejection
TCN	Tubular cell necrosis
TG	Transplant glomerulopathy
Tx	Transplantation
v	Intimal arteritis
VEGF	Vascular endothelial growth factor
WIT	Warm ischemia time

CURRICULUM VITEA AND SCIENTIFIC CONTRIBUTIONS



Curriculum Vitae

Anke Keijbeck werd geboren op 26 september 1990 te Roermond. Zij doorliep het VWO van 2002-2008 op het Connect College te Echt. Hierna startte zij met de studie Geneeskunde aan de Universiteit van Maastricht. In het academische jaar 2009-2010 ontving zij de top 3% studenten prijs. Tijdens de bachelor geneeskunde volgde Anke het Honours programme research en verrichte in het kader daarvan onderzoek naar ADAMs in atherosclerose onder supervisie van Dr. Marjo Donners. In 2014 rondde ze succesvol de studie geneeskunde af, waarna ze startte met promotieonderzoek binnen de onderzoeksschool CARIM, bij de afdelingen pathologie en interne geneeskunde,onderafdeling nefrologie van het MUMC^{+}, onder supervisie van Prof. Dr. Erik Biessen, Dr. Carine Peutz-Kootstra en Dr. Maarten Christiaans. De resultaten behaald tijdens dit traject zijn beschreven in dit proefschrift. In 2018 heeft Anke een jaar als ANIOS interne geneeskunde gewerkt in het VieCuri medisch centrum te Venlo. In september 2020 heeft ze een half jaar als ANIOS reumatologie gewerkt in het Zuyderland Medisch centrum, waar ze daaropvolgend gestart is met de opleiding tot reumatoloog.

Scientific contributions

Publications

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Keijbeck AA, Raaijmaakers AE, Hillen LM, Gelens MACJ, Cleutjens JPM, Peutz-Kootstra CJ*, Christiaans MHL*. Visual interstitial fibrosis assessment as continuous variable in protocol renal transplant biopsies. *Submitted*

Keijbeck AA, van Smaalen TC, Gelens MACJ, van Kuijk SMJ, Hillen LM, Peutz-Kootstra CJ, van Heurn LWE, Christiaans MHL. Ischemia reperfusion injury parameters in reperfusion biopsies: association with impaired graft recovery and more IFTA at year 1 post renal transplantation. *Submitted*

Keijbeck AA, Gelens MACJ, van Kuijk SMJ, Hillen LM, Peutz-Kootstra CJ, Christiaans MHL. Interstitial fibrosis and tubular atrophy (IF/TA) progression in the first year after kidney transplantation in postmortal donors: role for donor type and immunosuppressive regimen. *In preparation*

Keijbeck AA, Steegh FMEG, Gelens MACJ, van Kuijk SMJ, Hillen LM, Cleutjens JPM, Peutz-Kootstra CJ, Christiaans MHL. Peritubular capillary density in the first year after renal transplantation: relationship with donor type, histology and renal function. *In preparation*

Keijbeck AA, Gelens MACJ, Steegh FMEG, Voorter CEM, van Kuijk SMJ, Peutz-Kootstra CJ, Christiaans MHL. Decrease in Peritubular capillary density in the first month after human kidney transplantation: relationship with rejection and delayed graft function. *In preparation*

Oral presentations

Peritubular capillary (PTC) loss occurs in the first month after renal transplantation by ischemic and allo-immune injury.
Maastricht Pathology 2018 joint meeting of the Pathological Society of Great Britain & Ireland and Nederlandse Vereniging voor Pathologie, Maastricht

Peritubular capillary loss occurs in the first month after kidney transplantation.
European Renal Cell Study Group (ERCSG) 2018, Florence, Italië

Early loss of peritubular capillaries after kidney transplantation is associated with later renal function decline: a validation study in 121 patients.
Nederlandse Federatie voor Nefrologie (NFN) najaarssymposium 2016, Lunteren

Interstitial fibrosis in renal biopsy as prognostic marker for renal failure: a comparison of methodology.

- *Pathologie wetenschapsdag 2015, Maastricht*
- *Platform AIO's nefrologie (PLAN) dag 2015, Maastricht*

ADAM activity in regulating macrophage function and polarization in atherosclerosis
Maastricht Medical Students Research Conference (MMSRC) 2012, Maastricht

Poster presentations

Peritubular capillary loss occurs in the first month after kidney transplantation.

- *Bootcongres van Nederlandse Transplantatie Vereniging (NTV) 2018, Rotterdam*
- *American Society of Nephrology (ASN) kidney week 2017, New Orleans, USA*
- *Nederlandse Federatie voor Nefrologie (NFN) najaarssymposium 2017, Utrecht*

Early loss of peritubular capillaries after kidney transplantation is associated with later renal function decline: a validation study in 121 patients.

- *Banff-SCT joint scientific meeting 2017, Barcelona, Spanje*
- *American Society of Nephrology (ASN) kidney week 2016, Chicago, USA*
- *CARIM wetenschapsdag 2016, Maastricht**

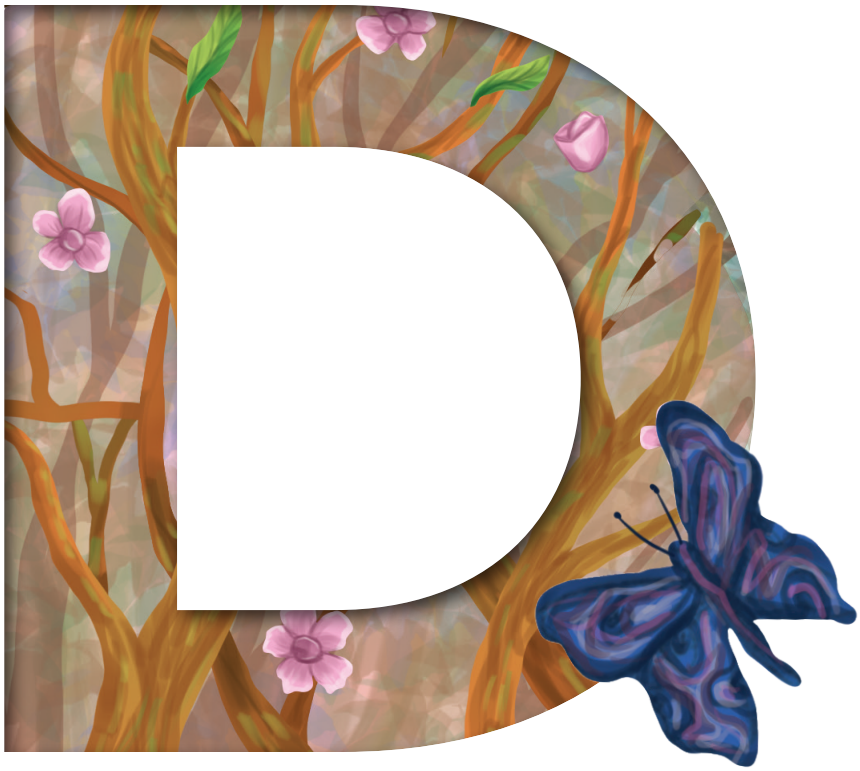
* Award for best poster presentation

Predictive value of histological acute kidney injury parameters in implantation biopsies for delayed graft function.

- *Nederlandse Federatie voor Nefrologie (NFN) naajaarssymposium 2016, Lunteren*

Interstitial fibrosis in renal biopsy as early prognostic marker for CKD: a comparison of methods.

- *Nederlandse Federatie voor Nefrologie (NFN) nefrologendagen 2016, Veldhoven.*



DANKWOORD

Dankwoord

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