# Trends and determinants for graft survival among kidney transplanted patients in Sweden

Salmir Nasic

Department of Molecular and Clinical Medicine
Institute of Medicine
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2024

Trends and determinants for graft survival among kidney transplanted patients in Sweden

salmir.nasic@gu.se

ISBN 978-91-8069-605-0 (PRINT)
ISBN 978-91-8069-606-7 (PDF)
http://hdl.handle.net/2077/79339

Printed in Borås, Sweden 2024
Printed by Stema Specialtryck AB

Read (learn) in the name of your Lord, who created…!

*96th Surah, Al-Alaq*

# Trends and determinants for graft survival among kidney transplanted patients in Sweden.

Salmir Nasic

Department of Molecular and Clinical Medicine, Institute of Medicine
Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden

## ABSTRACT

According to literature, even after a successful kidney transplantation approximately 50% of the patients risk losing the transplant within ≈15 years. A return to dialysis or a new transplantation becomes necessary. Therefore, it is important to identify determinants for graft survival.

This thesis is based on data from two large registries. The linking of the registries and the analyses carried out on the extensive amount of data required an accurate and careful statistical approach.

The overall aim of the thesis was to evaluate and investigate association between recipient characteristics, clinical and histological variables related to graft survival (GS) in a population of kidney transplanted patients. Association between the variables and GS as outcome was analyzed by various survival models.

In study I focus was on GS after kidney transplantations, based on the quality register TIGER, containing data from kidney patients transplanted in the transplantation center in Gothenburg. The study showed that graft survival in general has improved over time but in the last study period 2006– 2017 women had shorter graft survival compared to men.

In study II-IV focus was on different aspects of kidney transplant biopsy findings and associations to GS based on data from both a regional kidney biopsy registry and the TIGER-registry.

The association between biopsy-proven diagnoses and GS was investigated showing that some diagnostic groups were associated with a higher risk of graft

loss. Shorter GS was mainly found in transplants with glomerular diseases, rejections, acute tubular injuries, borderline changes and chronic changes.

Another biopsy-based variable is glomerular macrophage index (GMI) – a biomarker for inflammatory processes in the transplant. Increased levels of GMI were found to be strongly associated with worsened graft survival. Also, the change in GMI between two consecutive biopsies and the magnitude of the change was associated to graft survival. High levels of GMI, and categories where GMI increased, were associated to higher risk of graft loss compared to groups with low or decreasing GMI-level.

The thesis showed that results from transplant biopsies need further attention from clinician in regard to the overall histological results in relation to time of biopsy and presence of high GMI-levels at the first but also at the follow up biopsies. GMI can be very useful even in cases of insufficient other histological findings. Female kidney transplant patients need additional surveillance. Future studies regarding these risk variables will help to reveal how to improve therapy and prolong graft survival.

**Keywords**: kidney transplantations, graft survival, kidney transplant biopsy

ISBN 978-91-8069-605-0 (PRINT)
ISBN 978-91-8069-606-7 (PDF)

# SAMMANFATTNING PÅ SVENSKA

Det krävs dialys eller njurtransplantation för att patienter ska överleva svår njursvikt. Trots en lyckad njurtransplantation så riskerar 50% av patienterna att förlora transplantatet inom cirka 15 år. Återgång till dialys eller ny transplantation blir nödvändig. Det finns prognostiska riskfaktorer, som beaktas inför att patienterna accepteras för njurtransplantation. Emellertid är många frågor om prognostiska faktorer obesvarade. Förbättrad kunskap om biopsidiagnostik, behandlingsstrategier och riskfaktorer kan ge bättre förutsättningar inte enbart för transplantatöverlevnad utan även för möjligheter att överleva med fungerande transplantat.

Syftet med doktorandprojektet var att med hjälp av statistiska analyser av stora datamaterial om njurtransplantationer, härrörande från olika kvalitetsregister, utvärdera olika samband mellan kliniska variabler, biopsifynd, variabler från patientuppföljningar och utfallsvariabler. Studierna fokuserar på diagnostik av njurtransplantatbiopsier relaterat till långtidsutfall registrerat i transplantationsregister.

Material utgörs huvudsakligen av data från ett regionalt njurbiopsiregister och ett transplantationsregister (TIGER). TIGER-registret har funnits sedan 1965 och används vid Transplantationsenheten i Göteborg som verktyg for kvalitetsutvärdering av verksamheten inom upptagnings-området för Transplantationscentrum, Göteborg. I TIGER registreras vikt, längd, samsjuklighet, förekomst av malignitet, avstötningsreaktion, avstötningsbehandling, immunsuppressiv behandling, transplantatfunktion, komplikationer m.m. Fler än 3100 biopsier finns tillgängliga i njurbiopsiregistret med data registrerat sedan 2007. Biopsierna visar bland annat utfall av inflammatoriska celler i vävnad av olika typer (t.ex. immunfärgningar av ytmarkörer på immunceller, C4d-färgning, glomerulär makrofagindex).

Sammanlänkade data avseende ålder, kön och biopsifynd undersöktes i relation till data insamlade under långa uppföljningstider. För att analysera dessa komplexa samband användes både klassiska statistiska metoder som t.ex. Cox-regressionsmodeller och vid behov även så kallade Frailty-modeller.

Transplantatöverlevnad ökade generellt över tid och i sista tidsperioden 2006–2017 var den sämre för kvinnor jämför med män.

Vidare analyserades transplantatöverlevnad efter första biopsi där kortare organöverlevnad observerades för följande diagnosgrupper: Glomerulära

sjukdomar, Rejektioner, Akuta tubulära skador, Borderline förändringar och Kroniska förändringar.

En annan biopsibaserad variabel som undersöktes var glomrulärt makrofagindex (GMI) – en biomarkör för pågående inflammatoriska processer i transplantatet. Ökade nivåer av GMI efter första biopsin kunde påvisas vara starkt associerade till sämre transplantatöverlevnad.

I sista studien studerades GMI–förändring mellan första och andra biopsin och hur det var relaterat till transplantatöverlevnad. GMI var ganska stabilt och oförändrat för de flesta patienterna. Det fanns dock mindre grupper av patienter med större förändringar i form av antingen ökning eller minskning av GMI. Stabilt höga nivåer av GMI och ökande GMI-kategorier kunde kopplas till sämre transplantatöverlevnad medan stabilt låga eller minskande GMI-kategorier kunde kopplas till bättre transplantatöverlevnad.

Att behålla ett fungerande njurtransplantat så länge som möjligt är den kanske enskilt viktigaste faktorn för en njurtransplanterad patients hälsa och livskvalitet, också önskat ur ett samhällsekonomiskt perspektiv. Att hitta variabler och förfina diagnostiken och prognostiska faktorer som i ett tidigt skede kan vända en negativ utveckling är viktigt ur flera aspekter. I denna avhandling har sämre transplantatöverlevnad bland kvinnor observerats. Biopsibaserade fynd, framför allt GMI har visats vara användbart för prognos av transplantatöverlevnad, speciellt när biopsibaserad diagnos är oklar eller ospecifik.

## LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Nasic S, Peters B, Stegmayr B, Kenne Sarenmalm E, Afghahi H, Eriksson M. Sex-specific time trends of long-term graft survival after kidney transplantation – a registry-based study.
*Ren Fail 2023; 45(2):2270078.*

II. Nasic S, Mölne J, Stegmayr B, Peters B. Histological diagnosis from kidney transplant biopsy can contribute to prediction of graft survival.
*Nephrology (Carlton) 2022:27(6):528-536.*

III. Mölne J, Nasic S, Bröcker V, Stegmayr B, Felldin M, Peters B. Glomerular macrophage index (GMI) in kidney transplant biopsies is associated with graft outcome.
*Clin Transplant 2022:36(12):e14816.*

IV. Nasic S, Mölne J, Eriksson M, Stegmayr B, Afghahi H, Peters B. Changes in numbers of glomerular macrophages between two consecutive biopsies and the association to renal transplant graft survival.

*Submitted.*

# CONTENT


1 INTRODUCTION ..... 1
1.1 Kidney function ..... 3
1.2 Kidney transplantations in Sweden ..... 5
1.3 Registries used in the thesis ..... 6
1.3.1 The registry for kidney transplanted patients ..... 6
1.3.2 The regional kidney biopsy registry ..... 9
1.4 Graft survival and prognostic factors ..... 10
1.5 Histopathology ..... 11
1.5.1 C4d ..... 13
1.5.2 Banff classification ..... 13
1.6 Biopsy-based glomerular macrophage index (GMI) ..... 14
1.7 Immunosuppressive therapy (IST) ..... 15
1.8 Epidemiological and statistical concepts ..... 16
2 AIMS ..... 24
3 PATIENTS AND METHODS ..... 25
3.1 Linkage between the registries ..... 25
3.2 Materials ..... 26
3.3 Study design and methods ..... 28
3.4 Statistical analysis ..... 30
3.5 Ethical considerations ..... 33
4 RESULTS ..... 34
4.1 Paper I ..... 34
4.2 Paper II ..... 39
4.3 Paper III ..... 42
4.4 Paper IV ..... 50
5 DISCUSSION ..... 57
5.1 Main findings ..... 57
5.2 Methodological considerations ..... 65
6 FUTURE PERSPECTIVES ..... 70

ACKNOWLEDGEMENT ..... 73
REFERENCES ..... 75

## ABBREVIATIONS

| | |
|---|---|
| AKI | Acute kidney injury |
| ATN | Acute tubular necrosis |
| ABMR | Antibody-mediated rejection |
| CNI | Calcineurin Inhibitors toxicity |
| CI | Confidence interval |
| CKD | Chronic kidney disease |
| DGF | Delayed graft function |
| DSA | Donor-specific antibodies |
| DD | Deceased donor |
| ESKD | End stage kidney disease |
| FSGS | Focal and segmental glomerulosclerosis |
| GS | Graft survival |
| GFR | Glomerular filtration rate |
| GMI | Glomerular macrophage index |
| HR | Hazard ratio |
| HLA | Human leukocyte antigens |
| IST | Immunosuppressive therapy |
| IFTA | Interstitial fibrosis |
| KT | Kidney transplantation |
| KB | Kidney biopsy |
| LD | Living donor |
| MMF | Mycofenolate mofetil |
| RRT | Renal replacement therapy |
| TCMR | T-cell-mediated rejection |
| TIN | Tubulointerstitial nephritis |

# 1 INTRODUCTION

Kidney dysfunction can appear due to acute kidney injury (AKI) or chronic kidney disease (CKD). AKI refer to an abrupt deterioration (within hours) in kidney function, which involves both injury (structural damage) and impairment (loss of function) (1). Acute kidney injury may arise in conjunction with different syndromes or exposure to various environmental factors such as infections, sepsis, toxic agents and rapid glomerulonephritis. Mainly, the incidence of AKI is reported as either community-acquired or hospital-acquired AKI (2). In a meta-analysis of 154 studies, mostly based on high income countries, community-acquired AKI was reported in 8% of ambulatory patients and hospital-acquired AKI was reported in 20–32% of patients in hospital-care (3). Other studies report higher (4) or much lower incidence (5). Considerable variation in estimated incidence numbers in different studies is thought to be related to differences in definitions of AKI (6). The incidence of AKI is related to age and comorbidity. A study done in Italy found that incidence of AKI was 10 times higher in hospitalized patients aged 65 years or more compared to younger patients (7).

Several studies have shown that AKI is associated with adverse events and increased mortality (8-10). Observational studies, based on intensive care unit patients, have shown that 4–5% of the patients develop severe AKI requiring renal replacement therapy (RRT) (11, 12) with high mortality rate. AKI severity has been shown to be a strong predictor for future CKD risk (13).

CKD is a condition where a kidney injury has been present for more than three months with gradual loss of kidney function over time. Commonly reported risk factors for CKD are diabetes, hypertension, smoking, history of acute kidney injury and vascular disease (14-16). Also, obesity, malignancy, chronic lung disease and psychiatric disorder have been associated with an increased risk for CKD (17). Severe CKD, end stage kidney disease (ESKD) most often requires renal replacement therapy (RRT).

Prevalence of Chronic Kidney Disease (CKD) varies between 5–10% in European countries and between 5–12% in the USA (17, 18). In Sweden the prevalence of CKD stage 3–5 (Table 1) is approximately 6% (19).

One of the most important functions of the kidneys is filtering blood by removing waste and excess fluids from the blood to make urine. The glomerular filtration rate (GFR) is a measure of how well the kidneys are filtering. GFR is either measured or estimated (see section 1.1).

According to the international organization Kidney Disease Improving Global Outcomes (KDIGO) CKD is classified based on GFR (20), Table 1.

*Table 1. KDIGO have established 5 stages of CKD based on eGFR (CKD 1-5). Modified from (20)*

| Stage | Description | GFR (ml/min/1.73sqm) |
|---|---|---|
| **1** | Kidney damage with normal GFR | ≥90 |
| **2** | Mild GFR reduction | 60–89 |
| **3** | Moderate GFR reduction | 30–59 |
| **4** | Severe GFR reduction | 15–29 |
| **5** | Kidney failure | <15 or dialysis |

Both acute kidney disease (AKI) and chronic kidney disease (CKD) can lead to decreased kidney function and end stage kidney disease (ESKD). When end stage kidney disease appears after, either acute or chronic episodes, the accumulating waste products and retained fluid must be removed to maintain life. This can be achieved by replacement functions of artificial organs such as hemodialysis, peritoneal dialysis or by transplantation of a kidney from a donor – these methods are collectively called renal replacement therapy (RRT) (19, 21). In hemodialysis, blood is pumped through single use bloodlines out of the body of the patient to an artificial kidney (dialysis machine), passing for rinsing through a dialyzer before returning to the body through plastic bloodlines that are connected to the machine. In peritoneal dialysis, an inside lining is placed in the patient's stomach and by filling up the abdomen with a fluid called dialysate the dialysis is performed. The peritoneum acts as a natural filter for the dialysis. Use of different RRT-models after ESKD variates widely between different countries depending on socioeconomic and cultural factors (21, 22).

Thus, kidney replacement therapy in terms of dialysis or kidney transplantation, is often necessary in case of progressive kidney disease. Today kidney transplantation is a procedure that enables many patients with kidney failure to live basically normal lives (21).

# 1.1 KIDNEY FUNCTION

How well the kidneys are filtering waste products from the blood is expressed by glomerular filtration rate (GFR). Receiving an accurate glomerular filtration rate (GFR) is difficult due to the intricate and time-requiring nature of the measuring process. The process is impractical for both clinicians and patients. GFR is equal to renal clearance rate and is usually registered in units of volume per time e.g. mL/min. The clearance test compares the creatinine level in urine with the creatinine level in blood resulting in a renal clearance rate or GFR (see formula below).

$$GFR = \frac{Urine\ Concentration\ x\ Urine\ volume}{Plasma\ Concentration}$$

Today, the measurement of GFR is usually performed by Iohexol Clearance which has replaced the 51CrEDTA clearance (23). Three to four hours after intravenous administration of Iohexol (a non-ionic x-ray contrast medium that is excreted in the urine), blood samples are taken to measure the amount of not excreted Iohexol. This will be used to calculate the GFR (23). The advantage with measured GFR is a correct measured kidney function for the individual patient, the negative side is that it is a time-consuming procedure for the patients and the health care professionals.

There are different techniques to estimate GFR. One of the techniques often used by health care professionals is an estimation of GFR (eGFR) which is calculated by a formula using just blood test results (serum creatinine and/or cystatin) and data about age and sex. eGFR is typically expressed in milliliters per minute per 1.73 square meters (mL/min/1.73 m²). A higher eGFR indicates better kidney function, while a lower eGFR indicates impaired kidney function. General interpretations of eGFR levels and what they may indicate for a CKD are described in Table 1.

Mathematical formulas used to calculate eGFR consider various factors, including serum creatinine levels, age, gender, and in some cases, weight, and race. The Modification of Diet in Renal Disease (MDRD) equation (24, 25) is the most widely used formula for eGFR calculation:

$$eGFR\ (mL/min/1.73\ m^2) = 175 \times (SCr)^{-1.154} \times (Age)^{-0.203} \times (0.742\ \text{if female}) \times (1.212\ \text{if African American})$$

This formula includes:

- Serum Creatinine (SCr): Creatinine is a chemical waste product of creatine and is excreted by the kidneys. Increased levels of creatinine in the blood can indicate impaired kidney function.
- Age: Age is included into the equation as kidney function tends to decrease with age.
- Sex: A coefficient of 0.742 is applied if the individual is female as women usually have slightly lower creatinine levels than men.
- Race: Another coefficient of 1.212 is applied if the individual is African American. On average, African Americans have a higher creatinine level compared to people of other racial backgrounds.

In recent years, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation has become a popular alternative to the MDRD equation because it is believed to provide a more accurate estimation of eGFR (26, 27). This equation is based on serum creatinine, age and sex. The CKD-EPI equation has been updated several times. The equation from 2009 includes different coefficients for black and white people while the equation from 2021 does not. When eGFR was reported in this thesis the CKD-EPI (2009) equation was used (25, 28, 29), Table 2.

*Table 2. CKD-EPI Equation for estimating GFR for white people, expressed for specific sex and serum creatinine. Modified from (28).*

| **Sex** | **Serum Creatinine µmol/L (mg/dL)** | **Equation** |
|---|---|---|
| Female | ≤62 (≤0.7) | $eGFR=144\times(Scr/0.7)^{-0.329}\times0.993^{Age}$ |
| Female | >62 (>0.7) | $eGFR=144\times(Scr/0.7)^{-1.209}\times0.993^{Age}$ |
| Male | ≤80 (≤0.9) | $eGFR=141\times(Scr/0.9)^{-0.411}\times0.993^{Age}$ |
| Male | >80 (>0.9) | $eGFR=141\times(Scr/0.9)^{-1.209}\times0.993^{Age}$ |

It is important to note that eGFR is an estimation and may not always precisely reflect an individual's true GFR. This is because eGFR relies on population-based data and general assumptions regarding the correlation between creatinine, age, and other factors. Individual variations and unique health circumstances may result in deviations from the estimated values provided by the formula. Therefore, while eGFR serves as a valuable tool for assessing kidney function on a group level, its limitations should be recognized when interpreting results for specific individuals.

It is also important to note that eGFR is just one way of measuring kidney health. Other factors, such as urine tests, medical history, biopsy results etc. are also considered when evaluating kidney function.

## 1.2 KIDNEY TRANSPLANTATIONS IN SWEDEN

In many cases dialysis or kidney transplantation is necessary for patients with advanced kidney disease.

From a patient perspective transplantation is a better and more convenient long-term solution (21). Studies report better health related quality of life for transplanted patients compared to dialysis patients. Several quality-of-life scores among transplanted patients reported similar levels as healthy individuals (21, 30).

The most common organ transplantation in Sweden is kidney transplantation. Between 400 and 500 kidney transplantations have been performed annually in Sweden during the last decade, Figure 1. Kidney transplants originate from a living or deceased donor. During the years 2020–2022, 21–27% of all kidney transplantations were from living donors (31).

In Sweden there are three transplantation centra:

- The transplantation center in Gothenburg administrates the Western, Eastern and Northern Sweden Health Care Regions.
- The transplantation center in Stockholm and Uppsala administrates the Central Sweden and Stockholm Health Care Regions.
- The transplantation center in Malmö and Lund administrates the Southern Sweden Health Care Region.

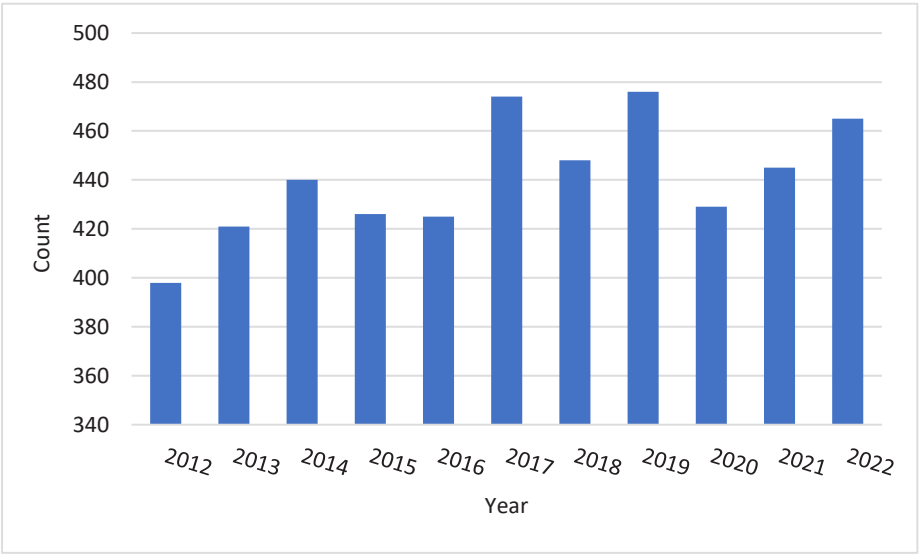
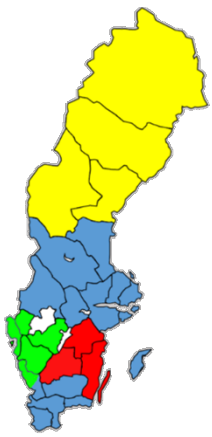



*Figure 1. The number of kidney transplantations in Sweden, years 2012-2022. Modified from Scandiatransplant (31)*

# 1.3 REGISTRIES USED IN THE THESIS

## 1.3.1 The registry for kidney transplanted patients



The quality assessment registry (TIGER), which include all kidney-transplanted patients at the Sahlgrenska University Hospital in Gothenburg (patients from Western, Eastern and Northern Sweden Health Care Regions), was used in all studies in the thesis. The transplantation center serves a population of approximately 3.5 million inhabitants (green, red and yellow areas illustrated in the map to the right). The registrations started 1965 and the average annual number of registered transplantations between 1965–1985 was 61, between 1986–1995 was 110, between 1996–2005 was 96 and between 2006–2017 was 118.

The quality registry has the following aims:

- Enable follow-up of quality parameters and clinically significant results and outcomes after organ transplantation of patients transplanted in the Transplantation center in Gothenburg.
- Enable comparisons between different centra and units in Sweden.
- Enable comparisons between Nordic countries and internationally.
- Enable research, both epidemiological studies and studies with specific clinical issues.

The registry is administrated from the Transplantation center in Gothenburg, stored on a web-server located at Sahlgrenska University hospital in Gothenburg (32). Data entry of baseline data is reported at the Transplantation center while follow-up data is reported by local hospitals in a protocol on a login-required web page. Data is regularly transferred to the TIGER database.

The following variables are entered and calculated:

- Primary kidney disease
- Comorbidity
- Time on ’waiting list’
- Transplant function, rejection and transplant loss
- Patient survival, death and cause of death
- Malignities, infections and other complications
- Immunosuppressive medication (drug)

Data is requested to be reported by the local hospital (unit) at several fixed follow-ups following transplantation: after 3 months, 6 months and thereafter annually. At each follow-up present medication, transplant function and condition, changes and events that have occurred since previous follow-up are registered. There is also a specific protocol that should be used for the reporting of death or graft loss. The persons responsible for registrations at each local unit are most often one or a couple of physicians or nurses that have a personal login and function as contact persons for the TIGER-registry at the local unit.

The number of kidney transplantations in TIGER per year has increased slightly over the years, during the two latest decades it has been around 130 to 170 (Figure 2).

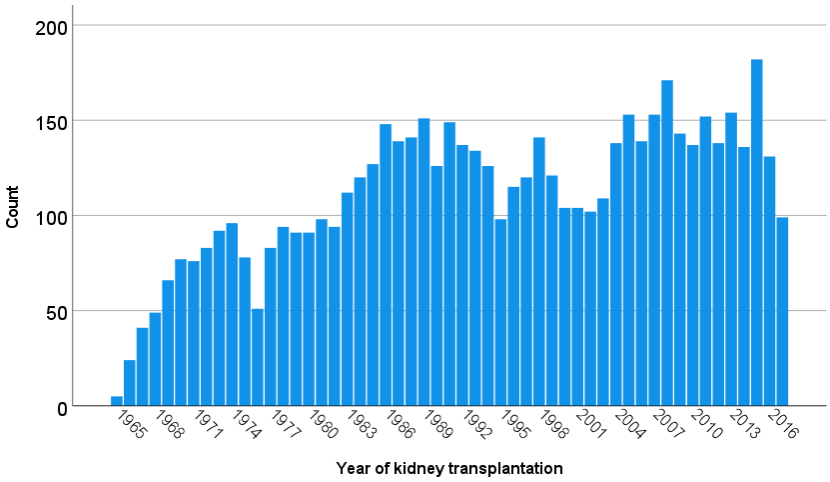



*Figure 2. Annual numbers of kidney transplantations registered in TIGER.*

There are no exact data about registry coverage but according to some reports (33) almost all kidney transplantations are registered in the TIGER baseline form. The follow-ups and annual controls are registered at the local hospitals, the degree of reporting varies between different hospitals and years (Figure 3 and Figure 4), but an average seems to be 60–70%. Missing follow-up data is probably related to lower registration tendency at some hospitals and can sometimes depend on the fact that a patient has moved to another region or country.

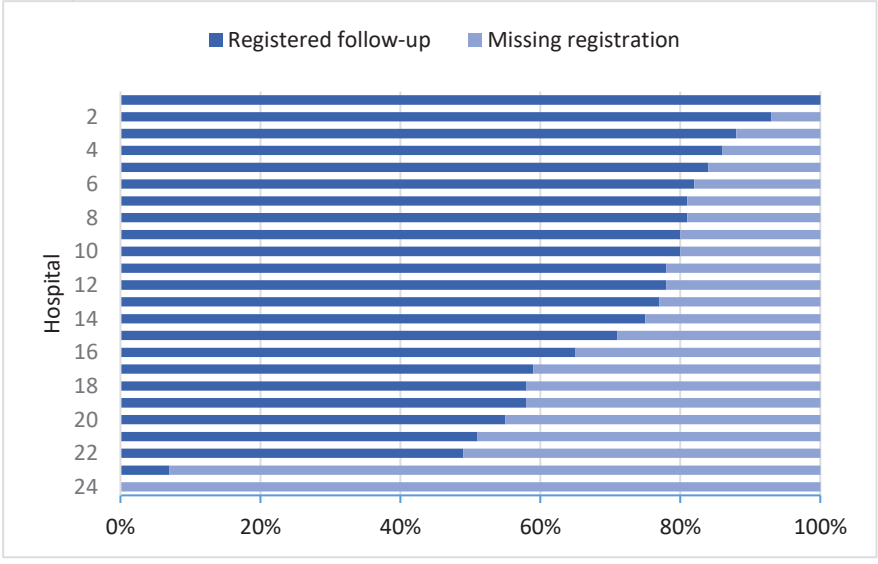



*Figure 3. Adherence to register follow-ups after kidney transplantation according to local units (hospitals), year 2019. Source: Modified from TIGER (32)*

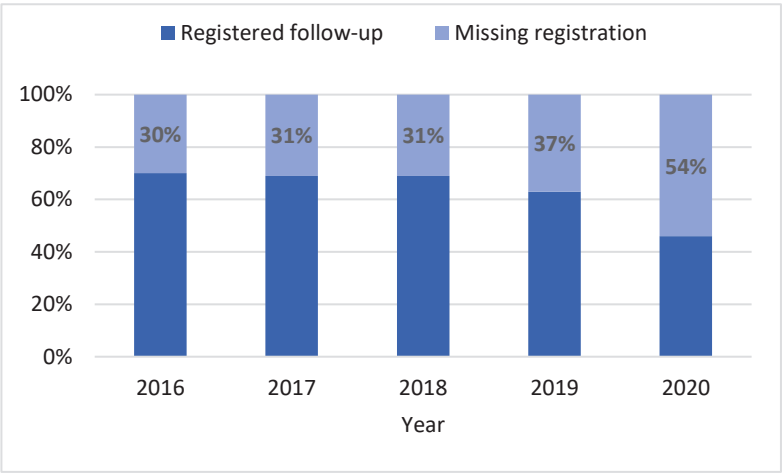



*Figure 4. Adherence to register annual report. Modified from TIGER (32)*

## 1.3.2 The regional kidney biopsy registry

The regional kidney biopsy registry (34) was initiated 2007 in Western Sweden. The catchment area for the kidney biopsy register is Western and Northern Sweden.

The material included in the studies from the regional kidney biopsy registry included 3 130 transplant biopsies performed between January 2007 and September 2017 from 1 542 unique patients. Most of the included biopsies (94%) were indication biopsies and 6% were protocol biopsies performed as part of different studies. Protocol or control biopsies are biopsies performed for study reasons, to examine histological changes over time or effect of different immunosuppressive drugs (35). Indication biopsies are performed when a clinician suspect adverse processes in the transplant, such as rejection, chronic allograft nephropathy and/or recurrent disease (21, 36)

There is a protocol for the registration of kidney biopsies (34), clinical parameters and complications associated with the biopsies. Some basic demographic data such as age, sex, height, and weight are also included in the registry. Histological analysis yields data about biopsy-proven diagnosis and biomarkers for immunological response such as C4d-staining, AB0 blood group and glomerular macrophage index. Some clinical variables as blood pressure, and laboratory values such as hemoglobin, platelets, leukocytes, hematocrit, activated partial thromboplastin time, serum albumin, serum creatinine and urea were requested to be registered– however, there are many missing values in some variables.

## 1.4 GRAFT SURVIVAL AND PROGNOSTIC FACTORS

Even after a successful transplantation 50% of the patients risk losing the transplant (graft) after ≈12 years if the transplant was from a deceased donor, and ≈19 years from a living donor (37). A return to dialysis or a new transplantation is then necessary (21).

Graft survival has significantly increased for kidney transplanted patients over the years (38-40). Most likely due to improved immunosuppressive treatment and health care in general (41).

In all four studies included in the thesis graft survival was defined as time from baseline to graft loss i.e. return to dialysis or re-transplantation. However, baseline was defined differently in the studies. Censoring points were death of other reasons or end of follow-up.

Time on dialysis prior to transplantation, donor age, recipients age, comorbidity, sensitization status, type of donor and some other donor specific variables have been showed to associate with graft survival (21, 42, 43). There are several other factors that may have impact on graft survival, some still sparsely studied, such a sex differences and biopsy-based findings. The focus in this thesis has been on biopsy-based findings and their association to graft survival. In one of the studies, the difference between women and men in graft survival over time was analyzed.

Graft survival is probably the most important and most objective outcome measure for patients, related to patient quality of life and patient survival. Therefore, additional improvements and investigations of explanatory factors are important.

# 1.5 HISTOPATHOLOGY

The gold standard for assessing structural abnormalities in the kidney transplant is evaluating the histopathology of a kidney transplant biopsy. All biopsy assessments in the thesis were performed by pathologists at the department of Pathology at Sahlgrenska University Hospital, Gothenburg University, Sweden, in accordance with the Banff 2007 classification and Banff updates as they became available (44). For more about Banff classification see section 1.5.2.

Biopsies in the studies were included from January 2007 until September 2017. Subspecialized renal pathologists reported all biopsies, and the final diagnoses were retrieved from the local pathology database. Biopsy-proven diagnoses can be grouped in different ways. Below the main groups of diagnoses used in the thesis.

*Infections and tubulointerstitial nephritis*

Healthcare-associated infections occur most often up to 1 month after transplantation. These infections are often of bacterial or fungal etiology. Many infections after transplantation are related to immunosuppression, especially viral infections (polyomavirus etc.) (45, 46). Tubulointerstitial nephritis (TIN) is a group of immune-mediated inflammatory diseases where infiltration of the kidney interstitium and tubules, by inflammatory cells, is involved. It is a common cause of AKI which leads to kidney failure. There are multiple factors that causes TIN such as drug-induced, genetic, infectious etc. (47).

*Acute tubular injuries (ATN and acute CNI-toxicity)*

Calcineurin inhibitors toxicity (acute CNI-toxicity) and Acute tubular necrosis (ATN) are both common cause of Delayed graft function (DGF) shortly after transplantation.

*Chronic changes including IFTA*

The term chronic allograft nephropathy (CAN) was used frequently in the past. Today, the more specific term IFTA (interstitial fibrosis and tubular atrophy) is used for chronic morphological abnormalities (21). These conditions are related to many chronic processes which may affect the graft including chronic rejection, chronic CNI-toxicity, nephrosclerosis, viral and bacterial infections,

among others. Chronic interstitial fibrosis is common after long-term CNI use and is associated with arteriolar lesions (21, 48).

*Hematologic disorders*

Anemia is common both in the early phase post-transplant and in the late post-transplant period (21). In the latter it is most commonly caused by immunosuppression (46) or impaired renal function. More serious hematological diseases, such as posttransplant lymphoproliferative disorders (PTLD) due to long standing immunosuppression are very uncommon but life-threatening events (49).

*Glomerular Diseases*

Overall, 6 to 20% of the kidney transplant recipients develop de novo or recurrent glomerular lesions. Focal and segmental glomerulosclerosis (FSGS) has a 30% to 50% recurrence rate (21, 50). Other common recurrent forms are membranoproliferative glomerulonephritis (MPGN), IgA nephropathy and diabetic nephropathy (21).

FSGS is likely the most common de novo (new disease) glomerular disease (21, 51). De novo membranous nephropathy is found in around 2% of the kidney transplants. Other forms of de novo glomerulonephritis are diabetic glomerulosclerosis (due to post-transplant diabetes) (21).

*Borderline changes*

Borderline changes represent one of the most frequent histological findings early after transplantation. As the name implies, it could be interpreted as an early stage of rejection or as a non-specific inflammation. Thus, the lowest detectable margin for rejection on histology is still unclear (52).

*Rejections*

Rejection refers to a process in which immune system of a transplant recipient attacks the graft (19, 21). Rejections can be classified into acute or chronic. Each has distinctive features, although components of both acute and chronic rejection may be present simultaneously in transplant biopsies after more than 6 months. There are two immunopathologic forms of rejection: T-cell-mediated rejection (TCMR also referred to as cellular rejection) and antibody-mediated rejection (ABMR – humoral rejection) (21, 53, 54). The two forms do not infrequently occur simultaneously and are then called mixed rejections.

There is also evidence that acute TCMR is a risk factor for subsequent ABMR (21).

Acute TCMR is primarily mediated by T lymphocytes. Typical lesions of acute TCMR involve tubules and arteries. Several forms of TCMR also includes involvement of monocytes/macrophages (21).

ABMR is caused by the recipient's antibodies. These are mainly directed at HLA-antigens in the transplanted kidney and react with antigens in the blood vessels of the transplanted kidney (21).

Rejections as group of biopsy-proven diagnoses in this thesis includes histopathological findings of T-cell or antibody-mediated acute or chronic changes or their combinations.

*Minor abnormalities*

Minor abnormalities was used as category for unclear or minimal findings in the biopsy.

### 1.5.1 C4d

ABMR is strongly associated with worsened graft survival after kidney transplantation. C4d is the degradation product of the activated complement factor C4, a component involved in complement cascade initiated by binding antibodies to target molecules. C4d is thus used as a surrogate marker for ABMR (55) and measuring deposition of C4d in peritubular capillaries (C4d staining) is therefore important in clinical practice (56). The presence of C4d (C4d positivity) has been showed in the majority of biopsies with features of chronic, active ABMR although, presence of ABMR without positive C4d staining is also observed. This suggest that C4d is specific but not very sensitive (57).

### 1.5.2 Banff classification

The Banff classification is a schema for classification and grading of kidney transplant pathology, established in Banff, Canada by a group of experts in kidney transplantation (58). The classification has been used for over 3 decades as a standardized approach in diagnosing and grading kidney transplant

pathology. During the years, the Banff classification has been revised and updated several times (59, 60).

The details and different aspects about Banff classification are beyond the scope of this thesis but it could be mentioned that the classification consists mainly of the following categories: normal biopsy findings, active, or chronic antibody-mediated rejection (ABMR), borderline changes, acute or chronic T-cell mediated rejection (TCMR), IFTA, and other changes not considered due to rejection (glomerulonephritis, diabetes, hematological diseases etc.).

## 1.6 BIOPSY-BASED GLOMERULAR MACROPHAGE INDEX (GMI)

Macrophages are important cells involved in both innate and adaptive immune reactions. They have been shown to participate in a wide range of important roles in kidney disease such as surveillance, immune response, tissue injury and repair (61, 62).

Macrophages and dendritic cells capture donor antigens and present them to the immune system, mainly to CD4+ T-helper cells, starting an immune response. Therefore, macrophages have been investigated and monitored in renal transplant biopsies for a long time. It has been observed that a high number of glomerular macrophages correlate with rejections in kidney transplants and imply a worse prognosis (63-66).

A glomerular macrophage index (GMI) as the average number of glomerular macrophages was first established by Magil in glomerular diseases in native kidneys (67) and later in kidney transplants (65, 68). Macrophages were identified using staining for CD68, a pan-macrophage marker (55), by high power field (HPF, 400 x) and a cell was assessed as positive (macrophage) when containing a nucleus or showing a rounded structure in keeping with a cell body (Figure 5).

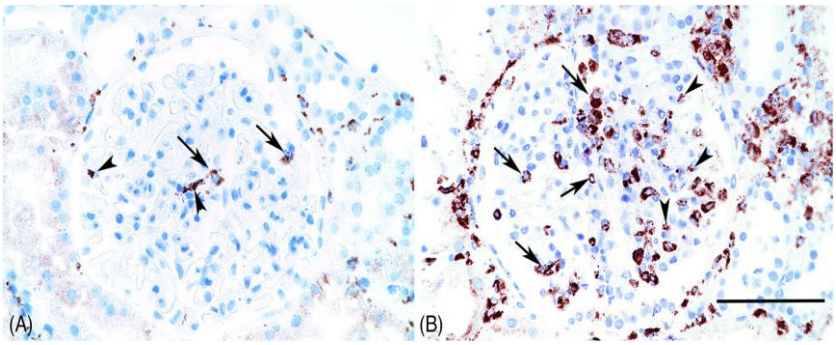



*Figure 5. Light microscopy of CD68 positive macrophages in glomeruli. (A) Biopsy with a low number of positive cells (n = 2) in the glomerular capillaries. (B) Biopsy specimen with a high number of positive cells (n = 28). Arrows = immune-positive cells, arrowheads = cell fragments or processes not identified as positive cells. Bar = 100 micrometer. Reprinted with permission from Mölne et al (69).*

## 1.7 IMMUNOSUPPRESSIVE THERAPY (IST)

Description of different types of immunosuppressive therapy and their models of action is beyond the scope of this thesis though a brief comment is necessary.

Induction therapy is initial immunosuppressive therapy preventively given to patients before, or at the time of the transplantation, to reduce risk of rejection of the transplant (70, 71).

Immunosuppressive therapy covers initial and long-term maintenance therapy (45, 72, 73). Kidney transplant patients are treated with IST according to a standardized protocol recommended by the transplantation center and the therapy dose is controlled by monitoring drug concentration in the blood.

The maintenance immunosuppressive protocols consist mainly of combinations of immunosuppressive medications (72) targeting the immune system to prevent rejections and graft loss. The protocols and medication have evolved over time and common current protocols include combinations of Calcineurin inhibitors (cyclosporine and tacrolimus), azathioprine, mycofenolate mofetil (MMF) and corticosteroids.

Despite the development of new drugs and protocols, 50% of the patients will still lose their transplant within ≈19 years for living donor transplants and ≈12

years for deceased donors (37). This motivates a search for new risk factors for early detection of rejection and other reasons for graft loss, but also markers that help optimize medication to improve the outcome for the patients.

For interpretation of methods and data analyses used in the thesis it is useful to reflect about some methodological concepts.

## 1.8 EPIDEMIOLOGICAL AND STATISTICAL CONCEPTS

### *Sample vs. population of interest*

The set of all subjects, for example people, that a survey concerns and the researcher wants to draw conclusions about is called *population of interest* (or target population). As it most often is difficult to study all subjects from a population almost all epidemiological studies are based on a sample of subjects/individuals. The sample is then used to make inferences about the whole population of interest. In which way the sample is selected is important for the ability to obtain valid results. A random selection of subjects from the population of interest is considered to be the best way to perform sampling as it entails representativity of the whole population and enables good estimates of the true values with the possibility to assess the uncertainty associated with the estimate. In absence of random selection there is a risk for so called *selection bias*. Selection bias might be involved when some individuals are more likely to be selected in the sample. This can compromise the generalizability of the observed results i.e. there is a lack of *external validity* (74, 75).

### *Bias*

Bias is an error that in a systematic way influences the results of a study. There are many different types of bias. Some common bias in epidemiological and clinical studies are selection bias, confounding bias, misclassification bias, information bias, measuring bias etc (74, 75). Conclusions about causality might be biased in presence of a *mediator, moderator* or *confounder* variable, Figure 6. The risk of bias is independent of sample size and is more related to weaknesses in study design and operationalization of the different steps in the study.

## *Causality*

An association between variables does not automatically mean that the change in one variable is the cause of the change in another variable. Causation means that change in a variable X (exposure or intervention) causes change in a variable Y (outcome variable). Conclusions about causality are difficult to draw based on observational studies. Bradford-Hill established nine criteria that can be used to evaluate whether an association is of causal character (76). Some criteria are probably more important for establishing causality. Establishing the time sequence between exposure and outcome is likely essential, meaning that exposure must occur before outcome (74, 77). Some other criteria are about showing dose-response relationship, consistency of the findings, coherency with previous knowledge etc.

Sometimes there is a another variable that is laying in causal sequence between an exposure variable and an outcome variable, a so called *mediator* (mediating variable) (78). When a variable affects the strength and direction of an association it is called *moderating variable* (*moderator* or *modifier*). In statistical terms, if there is a moderator variable Z, this is interpreted as interaction between exposure variable X and mediator Z. Interaction term X*Z is then statistically significant for outcome Y. This means that some combinations of levels from factor X and levels from factor Z have additional effect on the outcome. A *confounding variable* is a variable that influence both the exposure variable and the outcome variable. A confounder may lead to the conclusion that there is an association where no real association exists. It is also possible that a confounder hides an actual association. An illustration of the roll of mediator, moderator and confounder is presented in Figure 6. Confounding and interaction (moderating variable) can be handled in a multivariable model during statistical analysis, but they are not always easy to detect from the beginning. Another method of handling confounding, or moderator is stratified statistical analysis (74, 75).

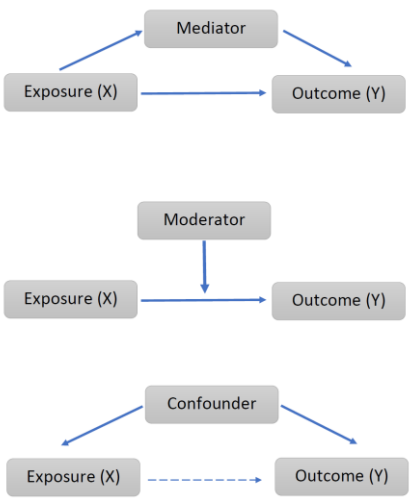



*Figure 6. Illustration of relation between exposure and outcome in presence of mediator, moderator and confounding variable.*

## *Study types*

A crude classification of study type can be done into *experimental* or *observational* studies (74, 79). In an observational study the researcher only observes and collects data but does not influence exposure or events. Most epidemiological studies and registry-based studies are observational studies. On the contrary, in an *experimental study*, the researcher influences the course of events and exposure (or intervention) to be able to investigate the effects of exposure. *Randomized clinical trial* (RCT) is the most typical experimental study design.

Another classification of studies can be done in *prospective* or *retrospective* studies. In prospective studies the data is collected forwards in time in relation to the start of the study, while in retrospective studies data is commonly already collected and events have passed, for example data from sources as medical journals or quality registries. Experimental studies are by nature prospective while observational studies may be prospective or retrospective, depending on whether data is already collected at study start or not.

Studies are called *cross-sectional* when individuals are observed only once, for example screening at a specific point in time or a questionnaire at a specific point in time. On the other hand, *longitudinal studies* are studies which investigate changes over time, for example after an intervention. RTC are usually longitudinal as the researcher is interested in the effect of a treatment or intervention performed at one point in time on an outcome later in time. Populations or groups of individuals followed over time are in epidemiological terms called "cohort" and the study design is then called *cohort study (74, 80)*. Cohort studies are by nature longitudinal (also called *follow-up* studies). Prospective cohort studies are considered the best alternative when RCTs are not possible to perform, for example to study life-style variables as risk-factors at the start of the study and the risk for different diseases later in time. Another type of observational studies that are used to study associations and to find risk-factors for different events are *case-control studies.* Case-control studies are used when it is difficult or resource-requiring to collect data about all individuals in a cohort (74, 79). Case-control design means that data is collected from a sample of cases i.e. individuals having a disease, and the corresponding data is collected from a sample of controls i.e. individuals without disease. Exposure in the past for a possible risk factor of interest for both cases and controls is then compared between cases and controls. If the cases more frequent have reported exposure compared to the controls the researcher may draw conclusion that exposure is likely causally related to the studied disease. For valid results it is important that controls are included

randomly with respect to the exposure of interest. Case-control studies are by nature retrospective as both outcome and exposure have passed in time at the study start.

The study design influences the possibility to draw conclusions about associations and causality (74, 81).

## *Measures of disease frequency*

*Prevalence* is a measure reflecting the number of existing cases of a disease while *incidence* is a measure reflecting the number of new cases of disease during a determined time period (75, 82). Both prevalence and incidence are calculated as proportions of a total population at risk.

*Prevalence = Prevalent cases / Total population*

Prevalent cases are all individuals with the outcome of interest, for example a disease, at a specific point in time. The denominator in the calculation is the total population at risk at the studied point in time, including the prevalent cases.

*Incidence* is usually expressed as *cumulative incidence* i.e. the number of new cases during a specified time interval (incident cases) divided by number of persons at-risk (population at-risk) counted at the start of the study period. Population at-risk includes all persons at risk of developing the outcome of interest at the study start. Both prevalence and incidence are often calculated and reported as standardized, i.e. per 1 000, 10 000 or 100 000 population.

*Cumulative incidence = Incident cases / Population at-risk*

Another more precise way of calculation incidence is *Incidence rate*. In this case the denominator is the total amount of person-time at-risk. Person-time for a participant in a study is an estimate of the actual time at risk that the participant contributed to a study. Total person-time at risk is a sum of all individual person-times at risk.

*Incidence Rate = Incident cases / Total person-time at-risk*

For estimation of prevalence, it is sufficient with cross-sectional studies while estimate of incidence require a cohort study design (follow-up).

*Relative risk (RR)* is used when comparing the risk of developing an outcome in two groups, for example an exposure group (or intervention) and a non-exposure group. It is a measure of risk raise in an exposed group compared to a non-exposed group (74, 75).

$$RR = \frac{Incidence\ rate\ in\ Exposed\ group}{Incidence\ rate\ in\ Unexposed\ group}$$

This ratio (RR) can also be calculated by using prevalence or cumulative incidence, but interpretation of risk may differ and might be more difficult particularly for RR based on prevalence.

In retrospective case-control studies RR is not possible to calculate due to lack of both number of new cases and number in population at risk. In these cases Odds Ratio (OR) is calculated as an approximate of RR.

$$OR = \frac{Exposed\ cases\ /\ Unexposed\ cases}{Exposed\ controls\ /\ Unexposed\ controls}$$

When comparing survival rates between two groups Hazard ratio (HR) is calculated and presented. HR is an estimate of ratio between relative event rates in two groups. HR is defined more precisely in section *Survival analysis* below.

## *Survival analysis*

Survival analysis is a group of statistical models used for comparing survival times or other times of interest (time to recovery, time to recurrent disease etc.) in the presence of censored survival times (74, 83, 84). Time to a defined event is then an outcome variable in the survival model. At the end of the defined follow-up period, there is normally a number of participants where event has not occurred. For these individuals time to event is unknown, *censored.* Censoring means that follow-up is interrupted before event occurrence. Censoring also occurs because of other reasons, for example when a participant emigrates or declines further participance in the study.

A commonly used model for estimating and comparing survival curves is *Kaplan-Meier* survival curves (74, 84). The curves are based on probability of surviving a given length of time and this probability can be calculated by dividing time into many small intervals, for example days or moths. For instance, the probability for an individual to survive two months after

transplantation can be calculated as the probability of surviving one month, multiplied by the probability of surviving a second month given the fact that the patient survived the first month. The most common method for comparing survival between independent groups is *logrank* test (74, 84). The test is based on a number of expected events (E) and observed events (O) and calculating $(O-E)^2/E$ and comparing with a $\chi^2$-distribution.

Kaplan-Meier curves and logrank test can be used for univariable comparisons of survival. *Cox proportional hazard regression (85)* is a model for analyzing survival that can include several explanatory variables and covariates by performing a so-called multivariable model (74, 86, 87). Hazard ratio (HR) can be obtained from Cox-regression and measures difference in survival rates between two groups. Hazard function is closely related to survival probability, representing the risk of dying in a very short time interval after given time. It can be interpreted as the instantaneous risk of dying at time t (hazard rate at time t = h(t)). A hazard ratio is a relative hazard for two rates. If hA(t) is hazard rate in group A at time t and hB(t) is hazard ratio in group B at time t then HR= hA(t)/ hB(t).

Censoring, mentioned above, sometimes occur due to a so-called competing event. Competing risks (CR) is related to events which prevent the occurrence or modify the risk of the primary event or outcome of interest. For example, if death in disease-specific reason is outcome, then death of other reasons could be a competing event. Conventional survival analysis models (Kaplan-Meier method) typically rely on the assumption that censoring occurs randomly, i.e. independently of the risk for the event of interest. Competing risk regression is a model that adjust for the influence of the competing risk on the results. The most used alternative approach to analyze survival data in presence of competing events is cumulative incidence function (CIF). CIF estimates the marginal probability for each competing event and can be used to avoid bias, for example overestimation, using Kaplan-Meier method.

Both Cox proportional hazard regression and Fine and Gray model assume proportional hazard (PH) assumption, but the latter use a so called subdistributional hazard derived from CIF (74, 86, 88). The assumption is that the effect of different variables on survival is constant over time i.e. HR should be constant over time. PH-assumption can be checked through different statistical tests or graphically, for example looking at Schoenfeld residuals or log (-log (survival)) vs log (survival time). When PH-assumption is violated, there are different ways to handle it, e.g. by stratification or by using time dependent Cox-regression. If minor deviation from PH-assumption, the

estimated HR may be interpreted as a weighted average of the time-varying HRs through the follow-up (89).

Another aspect to consider is inclusion of variables in the survival models. Assessment done and variables included after baseline are not eligible to be included in the survival models as it can result in a bias for estimated survival time.

## *Type I and type II error*

In studies researchers usually want to investigate an effect or an association. To be able to test it statistically a null hypothesis and an alternative hypothesis are formulated.

Null hypothesis (H0): "No difference between groups (no effect)"

Alternative hypothesis (H1): "There is a difference (the effect exists)"

Statistically, the power of a hypothesis test is the probability that the test correctly rejects a null hypothesis (H0). The power is denoted as 1- β and represents the chance or probability of detecting a difference that actually exits (74, 90).

The standard level for statistical significance is usually set at 0.05 or 0.01. It is called significance level α (α=5% or α=1%) and is also equivalent to Type I error. A type I error (false positive findings) occurs when researcher rejects a null hypothesis that is actually true, i.e. finding difference that does not exist. Another type of error is β, or so-called Type II-error (false negative findings), which occurs when researchers fail to reject a null hypothesis that in reality is false i.e. not detecting a real difference (74, 75, 90).

## *Missing values*

There are three categories of missing data in epidemiological and clinical studies: *missing completely at random, missing at random and missing not at random* (91, 92). If data is *missing completely at random* (MCAR), the risk of occurrence of missing values is not dependent at all of variables of the study participants i.e. probability of being missing is the same for all participants in the study. In this case the presence of missing data should not affect the results, except from loss of data. If the probability of being missing is the same but only within groups defined by the observed data, the data are *missing at random (MAR)*. When data is missing at random it is possible to decrease

dependency between true value of missing data and risk for missing data by taking into account all the observed variables of the study participants. In case of neither MCAR nor MAR the data is *missing not at random (MNAR).* MNAR means that probability of being missing depends on unknown reasons. MNAR includes possibility that probability of missing data is dependent on outcome or true value of missing data. A MNAR situation is difficult to deal with and can lead to bias in conclusions and results. The type of missing data that requires most consideration is data missing not at random (MNAR).

A MAR situation can be handled through including other observed variables in the model. There are different methods of handling missing data. Exploring the amount of missing data, reasons for why data is missing, and patterns of missing data should be considered. Some common methods are complete case analysis, available case analysis, imputation methods, multiple imputation etc. Complete case analysis is based only on individuals with data on all the relevant variables. This method is unbiased only when missing data is independent of the study outcome. One way of handling missing data in categorical variables is by placing observations with missing data in a separate category (93).

# 2 AIMS

The overall aim of this thesis was to provide insight in associations between variables collected in different registries about kidney transplants and their prognostic value for graft survival. The variables in focus were clinical variables, histology data from kidney transplant biopsies and variables from follow-ups after transplantation. Another aim was to investigate whether extended evaluations of the biopsy data could guide clinicians towards more optimized treatment and in that way improve transplant and patient survival.

The specific aims for each study were as follows:

PAPER I

To investigate sex-specific kidney graft survival over time. If differences existed, the secondary aim was to identify the risk factors.

PAPER II

To examine whether histological results of transplant kidney biopsies can be used as predictors for graft and patient survival. The secondary aim was to investigate association between time since transplantation and biopsy findings.

PAPER III

To explore the level of GMI in a large cohort of post-transplant biopsies and determine the association between GMI and graft survival.

PAPER IV

To investigate histological changes and changes in GMI between two consecutive biopsies, and to investigate whether the magnitude of the change in GMI is associated to graft survival.

# 3 PATIENTS AND METHODS

A summary of the study design and outcome criteria in the four studies is presented in Table 3.

*Table 3. Summary of study design and outcomes for the four studies*

| | Paper | | | |
|---|---|---|---|---|
| | **I** | **II** | **III** | **IV** |
| **Data source** | Regional biopsy registry<br>Quality registry TIGER | Regional biopsy registry<br>Quality registry TIGER | Regional biopsy registry<br>Quality registry TIGER | Regional biopsy registry<br>Quality registry TIGER |
| **Study population** | KT- patients 1965–2017 | KT-patients with first KB 2007–2017 | KT-patients with first KB 2007–2017 | KT-patients with at least two KB 2007–2017 |
| **Exclusion criteria** | Other than first KT | Other than first KB | Other than first KB | Patients with less than two KB |
| | Age<18 years | Age<18 years | Age<18 years | Age<18 years |
| **Baseline**[1] | Date of KT | Date of the first biopsy | Date of the first biopsy | Date of the second biopsy |
| **Primary explanatory variable** | Sex | Histological diagnoses | GMI | GMI-change between two biopsies |
| **Primary outcome** | Graft survival | Graft survival | Graft survival | Graft survival |

KT=kidney transplantation, KB= kidney biopsy; [1]Baseline for graft survival model

## 3.1 LINKAGE BETWEEN THE REGISTRIES

To study data about transplantation together with data about kidney transplant biopsies, a link between the two registries was established by merging data on individual level. However, as TIGER database often includes more than one transplant per patient as well as kidney biopsy registry often includes more than one biopsy per patient, great efforts were required to match the right biopsies with the right transplant. This was done mainly by comparing date of

transplantation and date of biopsy, then the match was done by sorting dates chronologically. An illustration of the linkage is presented in Figure 7.

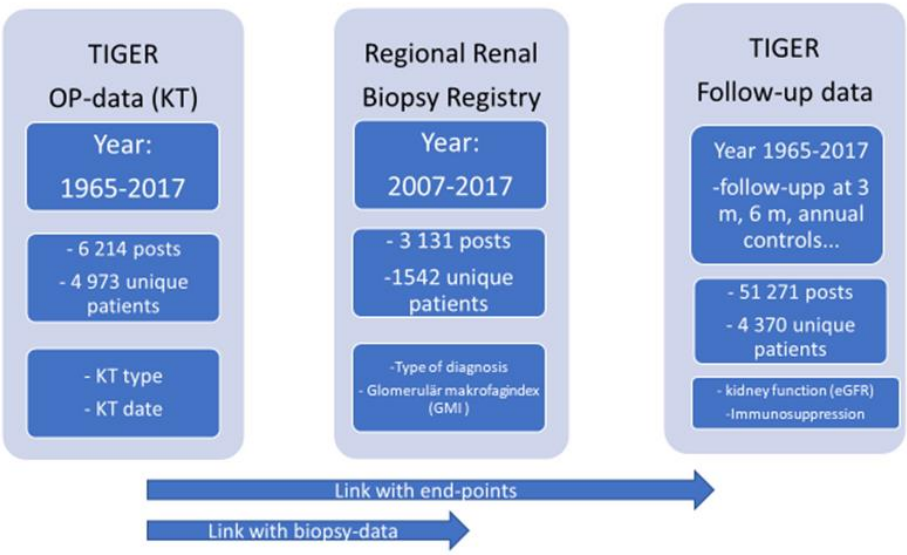



*Figure 7. Description of the TIGER-registry and the kidney biopsy registry and the performed links between the registries.*

## 3.2 MATERIALS

### *Study I*

All kidney transplantations were performed between January 1965 and September 2017 at Sahlgrenska University Hospital in Gothenburg, Sweden and registered in the quality assessment registry TIGER, administrated by the transplantation center in Gothenburg. More about TIGER-registry in section 1.3.1. The first kidney transplant for each adult patient (≥18 years of age) was included in the analysis resulting in a total of 4 698 transplanted patients. Although this was a registry-based study the study design combining transplantation baseline data with graft survival data from follow-up assessments resulted in a pseudo-prospective cohort study.

In this study, all patients that underwent kidney transplantation between the years 1965 and 2017 were included, resulting in 2 956 men (63%) and 1 742 women (37%).

In a sub analysis of the last period, the biopsy-registry data were used to adjust for possible confounders and further explore differences between men and women. For the majority of patients (58%, n=820) in the last time period (2006–2017) there was access to biopsy data which enabled investigation of how biopsy data was related to sex differences.

### *Study II-III*

The Regional kidney biopsy register was described in section 1.3.2. In these two studies the 1 462 kidney transplant biopsies from 1 542 patients were included. Only the first registered biopsy from each patient was included. The biopsies were performed between 1 January 2007 and 30 September 2017. Transplantation data came from the quality registry TIGER described in 1.3.1. In study III glomerular macrophage index (GMI) data was missing in 22 patients resulting in 1 440 biopsies included in the final analysis. Most of the biopsies in the studies were indication biopsies (94%, n=1 371) and the rest were protocol biopsies (6%, n=91). Biopsies were both from deceased and living donors.

### *Study IV*

Data from the Regional kidney biopsy register described in section 1.3.2 was used in this study as well as in study II and III. However, the inclusion criterion for this study was available data about at least two consecutive biopsies performed on the same kidney transplant. The aim was to investigate change between the two biopsies with respect to histological- and GMI-findings, and whether the change was associated to graft survival. Transplantation data came from the quality registry TIGER described in 1.3.1.

A total of 623 patients, with two consecutive biopsies and available data about GMI and graft survival, were included in this study. Majority of the patients (95%) were transplanted after year 2000. There were no transplantations before 1985.

# 3.3 STUDY DESIGN AND METHODS

## *Study I*

Time to graft loss was used as the outcome variable in the survival models. Graft survival between men and women was compared in different time periods.

As the probability of graft survival has increased over time, to a great extent due to improvement and evolution of immunosuppressive treatment (21, 37, 41), the impact of different factors had to be investigated within defined time periods. Immunosuppressive maintenance therapy was generally given as combinations of several drugs but in the analysis, variables were used for the presence of each single drug. To find proper and statistically feasible break points in time for introduction of a drug and when it became predominant, the presence of each drug per year, in the study period 1965–2017, was explored. After considerations, the following four time periods were established as proper for stratifying graft survival: 1965–1985, 1986–1995, 1996–2005, and 2006–2017. There were no distinct breakpoints that would entirely distinguish use of different drugs over time. Although the use of some drugs was overlapping different periods, the suggested division managed to catch greater shifts with respect to predominant drugs in each period (Figure 8).

The biopsy-proven diagnoses were included in a sensitivity analysis based on a multivariate model. The variable containing groups of biopsy-proven diagnoses is described in the next section as it was a variable of primary interest in study II.

## *Study II-IV*

To enable study of the histological material in a systematic way all diagnoses were grouped into nine (9) main groups, including normal biopsy findings as a reference group: Infections and tubulointerstitial nephritis, Acute tubular injuries including both acute tubular necrosis (ATN) and acute calcineurin inhibitor (CNI) toxicity, Chronic changes including interstitial fibrosis and tubular atrophy (IFTA), Hematological diseases, Glomerular diseases, Minor abnormalities, Borderline changes and Rejections (Table 4). The following subgroups of rejections were defined and presented in the studies in this thesis: Acute T-cell mediated rejection (TCMR), Chronic TCMR, Active antibody mediated rejection (ABMR), Chronic ABMR and combinations of these categories. For more about these diagnoses see section 1.5.

*Table 4. Groups of biopsy-proven diagnoses used in the thesis.*

| |
|---|
| Normal biopsy findings |
| Infections and tubulointerstitial nephritis |
| Acute tubular injuries (ATN and acute CNI-toxicity) |
| Chronic changes including IFTA |
| Hematological diseases |
| Glomerular diseases |
| Minor abnormalities |
| Borderline changes |
| Rejections |
| Subgroups of rejections |
| Acute TCMR |
| Chronic TCMR |
| Active ABMR |
| Chronic ABMR |
| Combined active ABMR and acute TCMR |
| Combined chronic ABMR and chronic TCMR |

*TCMR= T-cell mediated rejection; ABMR= antibody-mediated rejection*

Some diagnoses are known to be more common among "older" transplants and some are more common early after transplantation (21, 94). As prevalence of biopsy-proven diagnoses was strongly correlated to time to biopsy post-transplant it was important to investigate the impact of this time variable on the findings in study II and IV. This was done by including "time since transplantation" to biopsy in the multivariable model.

In studies III and IV glomerular macrophage index was investigated. The concept of glomerular macrophage index used in the studies was described in section 1.6. All renal transplant biopsies sent to the pathology unit at Sahlgrenska University hospital, Gothenburg University, Sweden were scored for glomerular macrophage index (GMI) at the time of routine biopsy reporting. When present, ten glomeruli were evaluated in a systematic way,

always starting at the most outer end of the cores until 10 glomeruli were included. Sclerotic glomeruli were not scored. CD68-positivity, using high power field (HPF, 400 x) was assessed as a positive cell (macrophage) when containing a nucleus or appearing as a rounded structure in keeping with a cell body (Figure 5). To obtain the GMI-value as the average number of macrophages, the total number of positive cells were divided by the number of glomeruli scored in a biopsy.

In study IV, the purpose of the analysis was to investigate clinically significant changes in GMI between two biopsies. Clinically significant levels of GMI were defined and cut-offs defined in an earlier study was used (69). Accordingly, GMI ≤1.8 was defined as Low, 1.9-4.5 as Medium, and ≥4.6 as High and switches between the two biopsies were established in Low-Low, Low-Medium, Low-High, Medium-Low, Medium-Medium, Medium-High, High-Low, High-Medium and High-High.

Furthermore, change of histological diagnosis between the two biopsies and association to graft survival was evaluated.

## 3.4 STATISTICAL ANALYSIS

Frequencies and percentages were presented for categorical variables, mean with standard deviations or median with percentiles for continuous variables. The group differences were tested by Chi-square test or Mann-Whitney test depending on the type of data of the compared variables.

In all four studies included in the thesis, graft survival was defined as time from baseline to date of graft loss i.e. return to dialysis or re-transplantation. However, baseline was defined differently in the studies. Baseline in study I was the date of the first transplantation. In study II and III baseline was the date of the first biopsy. In study IV baseline was the date of the second biopsy. In all studies survival models Kaplan-Meier and Cox-regression were used. Hazard ratio (HR) with 95% confidence interval was presented. Censoring points in the models were: time of death of any reason, loss of follow-up due to emigration or the end of the follow up.

Proportional hazard assumption for Cox-regression was tested both graphically and by testing interaction between time and the explanatory variable in a time dependent Cox-model. When multivariable Cox regression was used, the model included the main effects of explanatory variables if nothing else

mentioned. However, in some instances, interactions were explored and then the results are described. Cox regression assumes that continuous explanatory variables have a linear relationship with the log-hazard of the outcome. This assumption was assessed by plotting martingale residuals. When the assumption was not fulfilled then the continuous variable was categorized.

A p-value below 0.05 was considered statistically significant if not otherwise mentioned. IBM SPSS Statistics v. 28.0 (Armonk, NY, USA) and Stata Statistical Software for Windows: Release 17 (College Station, TX, USA) were used for statistical analyses. MedCalc Statistical Software version 14 (MedCalc Software bvba, Ostend, Belgium) was used in some cases for statistical tests based on summarized data.

## *Study I*

Background data and patient characteristics were presented for each period and compared between men and women to detect possible differences that may be confounders for association between sex and graft survival.

Kaplan-Meier survival model was used for initial comparisons between men and women in graft survival in different time periods. The following four time periods were constructed based on major shifts in immunosuppressive treatment regimens: 1965–1985, 1986–1995, 1996–2005, and 2006–2017. The log-rank test was used to compare the survival curves between men and women. A multivariable Cox proportional hazard regression was used to explore risk-factors and to adjust for possible confounders. The Cox-model was restricted to 10-years after transplantation to obtain tenable proportional hazard assumption [21]. The most common censoring point was all-cause death being therefore a so-called competing event for graft loss as the primary outcome. An additional model of competing risk regression (88), was performed to consider the effect of death on main findings.

To explore if differences between men and women variated over time, a sex-by-period interaction term was included in the Cox-model. In an extended multivariable Cox model for patients transplanted between 2006 and 2017 all variables with p-value<0.2 based on univariate analyses were included. For this group of patients, additional data was available: comorbidity, immune-suppressive therapy at discharge, cold ischemia time and induction therapy.

### *Study II*

Graft survival after first biopsy was analyzed according to biopsy-proven diagnoses (9 main groups described in section 3.3). Date of biopsy was the baseline in the survival models. Initially Kaplan-Meier curves were compared. In the next step Cox-regression was applied and graft survival after first biopsy was compared between the groups with normal biopsy findings as reference group and adjusted for age and sex as covariates. In a sensitivity analysis also time from transplantation to biopsy was included in a multivariable Cox-regression.

### *Study III*

The variable containing glomerular macrophage index (GMI) was split into eight evenly distributed classes with respect to number of included biopsies. These classes represented different levels of GMI and were used as explanatory variable for graft survival. Initially graft survival was analyzed by Kaplan-Meier analysis with log-rank test. A Cox-regression model, including covariates, was performed in the next step and results were presented as hazard ratio (HR) with confidence intervals (CI). All variables that were statistically significant associated with GMI in univariable comparisons or p-value $<0.1$ were included in the multivariate Cox-model. Censoring point in all survival models were all-cause death or end of follow-up. Proportional hazard assumption was tested both graphically and by testing interaction between time and diagnosis group or GMI-level. Interaction between time after transplantation and GMI was tested by including an interaction term in the Cox-regression model. Some sensitivity analyses were performed taking into account time elapsed between transplantation and biopsy.

### *Study IV*

As GMI showed positively skewed distribution, non-parametric tests were used for comparisons of GMI (Mann-Whitney between groups and Wilcoxon's signed test between the two biopsies). Changes in frequency of specific histological findings between the two biopsies were tested by McNemar's test. Graft survival after a second biopsy was analyzed by Kaplan-Meier and Cox-regression models, including main effects of GMI-change groups as explanatory variable in the first step and including covariates in further analysis. All variables that were statistically significant or with p-value$<0.1$ in univariable analyses were included in the final model (primary model). Time from transplantation to first biopsy and time between first and second biopsy

were used in analysis as categorized variables based on quartiles as breakpoints. As Kaplan-Meier curves indicated deviation from PH-assumption, the overall HR should be interpreted as a weighted average of the time-varying HRs based on entire follow-up (89).

Some sensitivity analyses were also performed after exclusion of extreme cases. Multivariable Cox-regression, as described above, but with restricted material were applied. In model 1 cases were excluded if the second biopsy was performed 2 years or later after the first biopsy (15% of all cases excluded) and in model 2 cases were excluded if the first biopsy was performed very early (within 1 week) after transplantation (25% of all cases excluded).

## 3.5 ETHICAL CONSIDERATIONS

According to law and regulations in Sweden (Patientdatalagen (SFS 2008:355)), written informed consent is not always required from patients registered in a quality registry. It is assumed that study participants did not object to registry-based research, as register consent was obtained at registration. The Regional Ethical Review Board in Gothenburg, Sweden approved the studies.

Ethical approval DNR: 701-08, EXP 2008-12-18.

Amendment approval DNR: T586-14, EXP 2014-07-24.

All data was anonymized after merging of the registries. The results in all studies were presented on group level making it impossible to identify a specific individual. The database was stored at Skaraborg Hospital on a protected server ensuring no unauthorized access.

# 4 RESULTS

## 4.1 PAPER I. SEX-SPECIFIC TRENDS IN GRAFT SURVIVAL

The median age was 42 years in the first time period (1965–1985) and 52 years in the last time period (2006–2017), (Table 5). The median age was in general similar between men and women but in some periods there was a slight difference and therefore age was included in the multivariate Cox-model. The proportion of living donors was around 25% in the first period and increased to around 35% in the last period. In period 1996–2005 there was a statistical difference in the proportion of living donors between men and women (38% vs 31%, p-value=0.036) and this factor was included in the final multivariate model. Also, distribution of primary renal diseases differed between men and women in each period (Table 5).

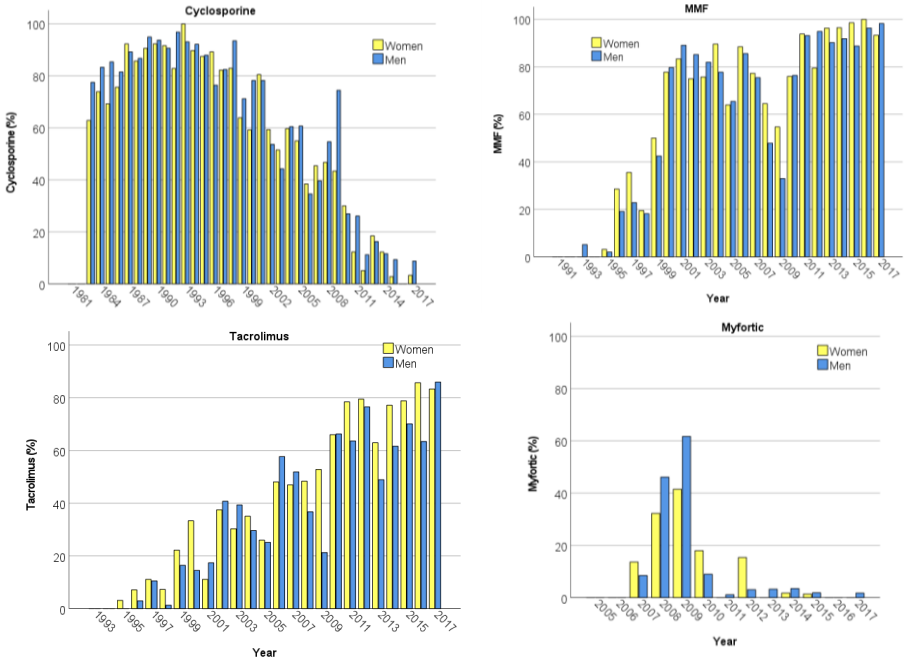



*Figure 8. Year for introduction of different immunosuppressive drugs. Drug prescription at discharge, percentage among women and men each year.*

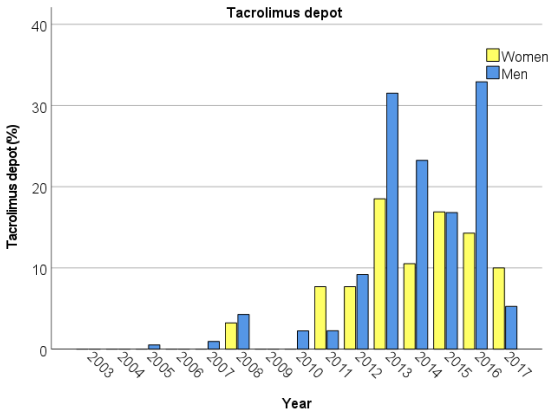



*Figure 9. Year for introduction of tacrolimus-depot. Drug prescription at discharge, percentage among women and men each year.*

Data revealed (Figure 8) that cyclosporine was introduced after 1983 and around 1993 almost 100% of the patients received the drug, both among men and women. Mycophenolate mofetil (MMF) was introduced after 1993. Approximately 80% of the patients received the drug between 1999 and 2005, with a temporary decrease between 2006 and 2010, increasing to 90–100% after 2011 until end of the study. Tacrolimus was introduced 1994 and was prescribed increasingly until the end of study 2017. However, the percentage among women seemed to be higher at some years. Myfortic was introduced in 2007, peaked 2009 (40-60%) but decreased after 2010.

There were no major differences between men and women with respect to single drugs except in the last period where presence of MMF and Tacrolimus was slightly higher among women (85% vs 80.5%, p-value=0.034) and (62% vs 54%, p-value=0.004) (Table 6). Tacrolimus depot was introduced 2007 and increased to ≈30% 2012, Figure 9.

*Table 5. Patient characteristics for kidney transplanted patients according to sex and time period of transplantation. Reprinted with permission (95)*

| Sex | Age and diagnosis | Transplantation period ≤1985 (n=1216) | 1986–1995 (n=1104) | 1996–2005 (n=957) | 2006–2017 (n=1421) |
|---|---|---|---|---|---|
| W | Age, median (IQR) | 43 (33-52) | 48 (35-58) | 51 (38-59) | 51* (40-60) |
| M | Age, median (IQR) | 42 (33-52) | 45 (36-54) | 49 (38-58) | 52 (42-61) |
| W | Deceased donor, n (%) | 339 (72.4) | 314 (74.6) | 236* (68.6) | 324 (63.9) |
| M | Deceased donor, n (%) | 557 (74.5) | 510 (74.7) | 376 (61.8) | 602 (65.9) |
| | **Primary renal disease[1], n (%)** ** | ** | ** | ** | ** |
| W | Glomerulonephritis/ sclerosis | 129 (27.6) | 89 (21.1) | 71 (20.5) | 89 (17.6) |
| W | Pyelonephritis | 158 (33.8) | 74 (17.6) | 40 (11.6) | 13 (2.6) |
| W | Polycystic kidneys. adult type | 59 (12.6) | 61 (14.5) | 66 (19.1) | 116 (22.9) |
| W | Renal vascular diseases due to hypertension | 4 (0.9) | 10 (2.4) | 8 (2.3) | 13 (2.6) |
| W | Renal vascular disease-type unspecific | 1 (0.2) | 3 (0.7) | 2 (0.6) | 7 (1.4) |
| W | Diabetes | 60 (12.8) | 74 (17.6) | 42 (12.1) | 73 (14.4) |
| W | Miscellaneous | 23 (4.9) | 67 (15.9) | 26 (7.5) | 112 (22.1) |
| W | Unknown | 34 (7.3) | 43 (10.2) | 91 (26.3) | 84 (16.6) |
| | **Total** | **468 (100)** | **421 (100)** | **346 (100)** | **507 (100)** |
| M | Glomerulonephritis/ sclerosis | 346 (46.3) | 235 (34.4) | 177 (29.0) | 267 (29.2) |
| M | Pyelonephritis | 107 (14.3) | 57 (8.3) | 36 (5.9) | 23 (2.5) |
| M | Polycystic kidneys. adult type | 84 (11.2) | 88 (12.9) | 94 (15.4) | 117 (12.8) |
| M | Renal vascular diseases due to hypertension | 40 (5.3) | 22 (3.2) | 19 (3.1) | 36 (3.9) |
| M | Renal vascular disease-type unspecific | 2 (0.3) | 4 (0.6) | 5 (0.8) | 9 (1.0) |
| M | Diabetes | 98 (13.1) | 139 (20.4) | 90 (14.7) | 150 (16.4) |
| M | Miscellaneous | 22 (2.9) | 71 (10.4) | 25 (4.1) | 155 (17.0) |
| M | Unknown | 49 (6.6) | 67 (9.8) | 165 (27.0) | 157 (17.2) |
| | **Total** | **748 (100)** | **683 (100)** | **611 (100)** | **914 (100)** |

[1]Grouping according to ERA-EDTA Registry: ERA-EDTA Registry Annual Report 2019. Amsterdam;
*p-value<0.05, **p-value<0.001 W vs M;. W=Women, M=Men, IQR=Interquartile range

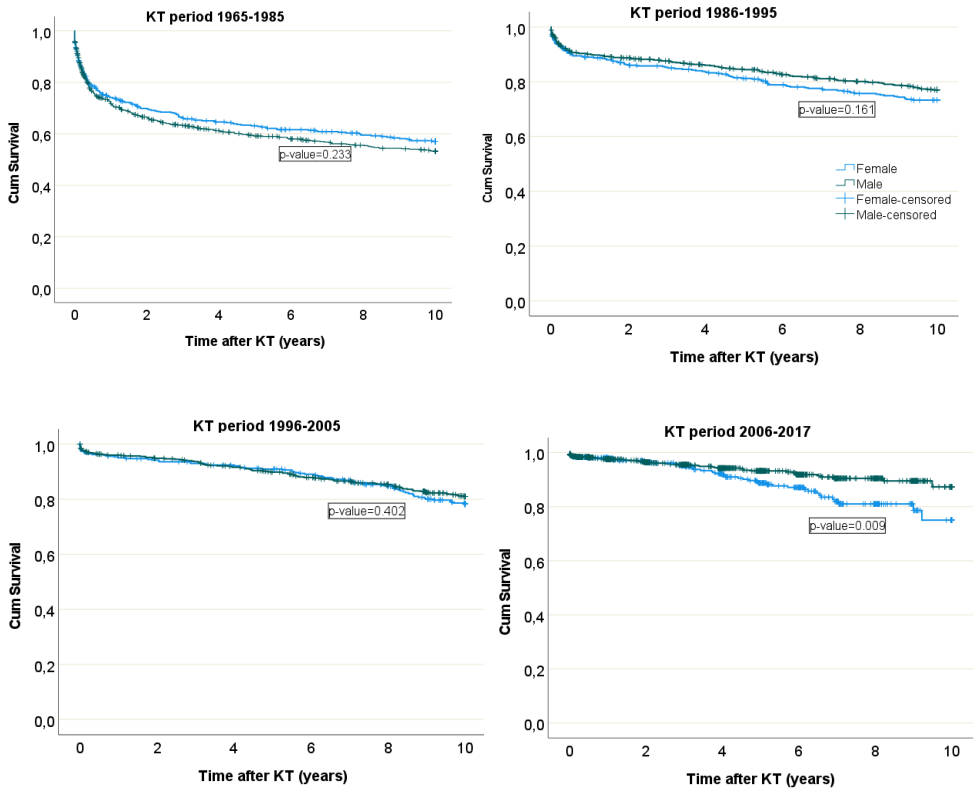



*Figure 10. 10-year graft survival after kidney transplantation (KT) among women and men in four time periods. Kaplan- Meier curves, p-value from log-rank test. Reprinted with permission (95).*

There was no difference between men and women in graft survival during the first three periods. Though, during the last period (2006–2017) 10-year graft survival among women was shorter compared to men (HR 1.68, 95% CI 1.13–2.49, Figure 10). This was also verified by testing interaction sex-by-period in a Cox-regression and interaction term (sex × period), that was statistically significant, p-value=0.026. The risk of graft loss for women remained higher even after including other covariates in the model (HR 1.71, 95% CI 1.08–2.69). The covariates included were age, cold ischemia time, type of donor and primary renal diagnosis.

*Table 6. Immunosuppressive drugs as maintenance therapy based on protocols registered at discharge after kidney transplantation. Occurrence (%) of each drug. 3500 of 4698 transplanted patients had valid protocols at discharge.*

| | Transplantation period (years) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1965–1985 | | 1986–1995 | | 1996–2005 | | 2006–2017 | |
| | Women | Men | Women | Men | Women | Men | Women | Men |
| Total number | n=468 | n=748 | n=421 | n=683 | n=346 | n=611 | n= 507 | n=914 |
| Valid protocols[b] | n=264 | n=405 | n=268 | n=455 | n=264 | n=492 | n=482 | n=870 |
| Azathioprine, n (%)[b] | 216 (81.8) [n.s] | 322 (79.5) | 245 (91.4) [n.s] | 431 (94.7) | 73 (27.7) [n.s] | 163 (33.1) | 3 (0.6) [n.s] | 4 (0.5) |
| Prednisolone, n (%) | 263 (99.6) [n.s] | 402 (99.3) | 268 (100) [n.s] | 453 (99.6) | 261 (98.9) [n.s] | 488 (99.2) | 446 (92.5) [n.s] | 791 (90.9) |
| Cyclosporine, n (%) | 66 (25.0) [n.s] | 93 (23.0) | 266 (99.3) [n.s] | 453 (99.6) | 209 (79.2) [n.s] | 389 (79.1) | 126 (26.1) [n.s] | 266 (30.6) |
| MMF, n (%) | 0 | 0 | 0 | 3 (0.7) | 177 (67.0) [n.s] | 306 (62.2) | 410 (85.1)* | 700 (80.5) |
| Tacrolimus, n (%) | 0 | 0 | 0 | 1 (0.2) | 42 (15.9) [n.s] | 80 (16.3) | 298 (61.8)** | 468 (53.8) |
| Sirolimus, n (%) | 0 | 0 | 0 | 0 | 8 (3.0) [n.s] | 26 (5.3) | 4 (0.8) [n.s] | 4 (0.5) |
| Myfortic, n (%) | 0 | 0 | 0 | 0 | 0 | 0 | 61 (12.7) [n.s] | 130 (14.9) |
| Tacrolimus depot, n (%) | 0 | 0 | 0 | 0 | 0 | 1 (0.2) | 45 (9.3) [n.s] | 106 (12.2) |
| Everolimus, n (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 (0.6) |
| Total number of drugs, *median (quartiles)* | 2 (2–2) | 2 (2–2) | 3 (3–3) | 3(3–3) | 3 (3–3) | 3 (3–3) | 3 (3–3) | 3 (3–3) |
| *median (5-95 perc)* | 2* (2–3) | 2 (2–2) | 3* (2–3) | 3 (3–3) | 3* (2–3) | 3 (3–3) | 3 (2–3) | 3 (2–3) |
| Death or graft loss before discharge.[c], n (%) | 70 (15.0) | 111 (14.8) | 26 (6.2) | 41 (6.0) | 17 (4.9) | 23 (3.8) | 7 (1.4) | 10 (1.1) |
| Protocol missing at discharge, n (%) | 134 (28.6) | 232 (31.0) | 127 (30.0) | 187 (27.3) | 65 (18.8) | 96 (15.7) | 18 (3.5) | 34 (3.7) |

MMF= *mycophenolate mofetil;* [a] For 1198 patients no data were registered at discharge, and for 305 patients there was death or graft loss before discharge - distribution presented in the table above ; [b] Number of valid protocols at discharge. This number was used as the denominator for calculating the percentage of use of each drug.;[c] Majority depending on graft loss (50 patients did not survive until discharge - most of these during the two first periods); n=number; n.s = not significant; * p<0.05 women vs. men; ** p<0.01 women vs men

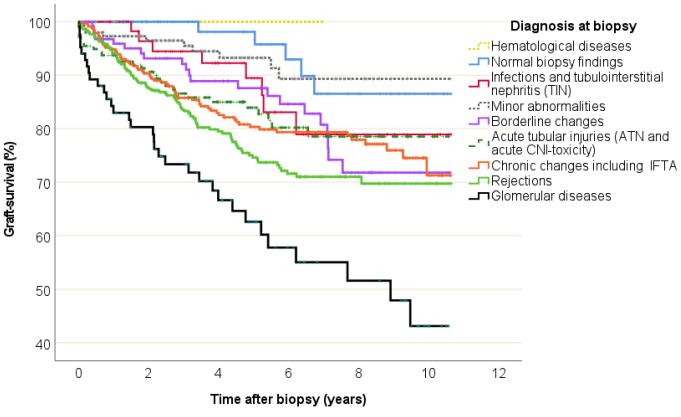


38

## 4.2 PAPER II. HISTOLOGICAL FINDINGS AND GRAFT SURVIVAL

Nine groups of biopsy-proven diagnoses were compared with respect to graft survival by Kaplan-Meier survival curves, Figure 11. Compared to normal biopsy findings (used as reference), shorter graft survival was shown in glomerular diseases (HR 8.2, 95% CI 3.2–21.1), rejections (HR 4.2, 95% CI 1.7–10.3), chronic changes including IFTA (HR 3.2, 95% CI 1.3–7.8), acute tubular injuries (HR 3.0, 95% CI 1.2–7.8) and borderline changes (HR 2.9, 95% CI 1.1-7.6). These HR:s were adjusted for age and sex (Table 7).

Subgroups of rejections were also compared, with respect to graft survival, in a separate analysis with the following results. Compared to acute TCMR as reference group, worse graft survival was detected in chronic TCMR (HR 4.7, 95% CI 1.9–11.3), combined chronic TCMR and chronic ABMR (HR 3.9, 95% 2.3–6.7), active ABMR (HR 3.6, 95% 1.7–7.7) and chronic ABMR (HR 3.5, 95% CI 2.0-6.0), adjusted for age and sex (Table 7).




| Groups of diagnoses | N at risk at start | N of events† | Time at risk (years) ‡ | Inc. Rate§ |
|---|---|---|---|---|
| Hematological diseases | 5 | 0 | 12.1 | 0 |
| Normal biopsy findings | 88 | 5 | 426.6 | 1.2 |
| Infections and TIN | 62 | 8 | 319.0 | 2.5 |
| Minor abnormalities | 149 | 9 | 650.4 | 1.5 |
| Borderline changes | 128 | 21 | 630.2 | 3.3 |
| Acute tubular injuries¶ | 177 | 29 | 820.2 | 3.5 |
| Chronic changes incl. IFTA | 335 | 65 | 1802.3 | 3.6 |
| Rejections | 434 | 96 | 1856.1 | 5.1 |
| Glomerular diseases | 84 | 32 | 347.3 | 9.5 |

N=Number; †Number of graft losses during the whole follow-up period; ‡Sum of all person years at risk; §Inc. Rate=Incidence rates per 100 person years

*Figure 11. Graft survival after biopsy according to biopsy based histological diagnosis. Modified and reprinted with permission (96).*

The analyzes showed that acute tubular injuries were most common (49.4%) among early biopsies (<14 days) in transplants from a deceased donor (DD). Among living donors (LD) within early biopsies (<14 days) rejections (36.2%) and acute tubular injuries (22.8%) were most common. Glomerular diseases occurred later, around 8% within 1–5 years among both LD and DD. It increased to above 15% in biopsies 5–10 years after transplantation, in both LD and DD. Chronic changes including IFTA increased over time in both DD and LD (12% at >14 days and above 40% after 10 years). Prevalence of rejection was between 20 and 32% among DD within the first 5 years and increased to 38% among biopsies 5–10 years after transplantation. Among LD prevalence of rejections was high within 14 days (36%), decreased to 12 % after 1 year and increased again to 38% among biopsies carried out 5–10 years after transplantation (Figure 12).

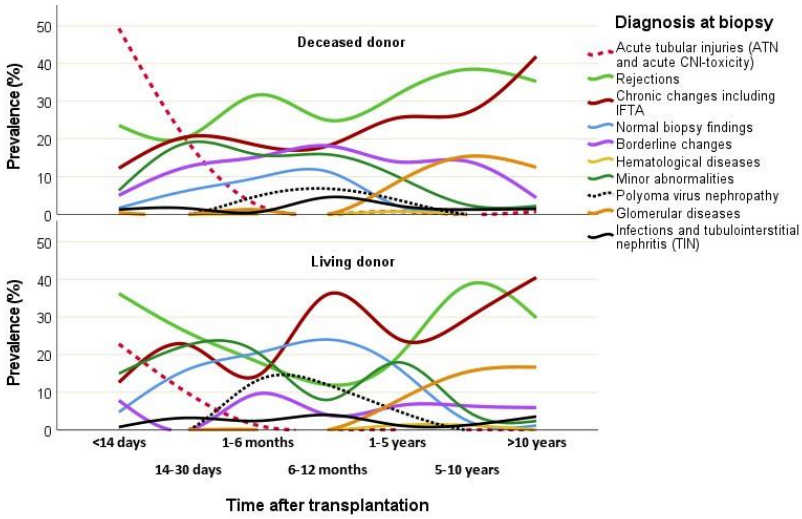



*Figure 12. Prevalence of histological diagnoses over time after transplantation according to type of donor. Prevalences (percentages) are calculated in relation to all biopsy findings at each time point. Reprinted with permission (96).*

*Table 7. Cox regression analysis of death-censored graft survival according to the histological diagnosis of biopsy, in univariate and adjusted model. Reprinted with permission (96).*

| | | Graft survival (time to graft loss) | | | |
|---|---|---|---|---|---|
| | | Crude association | | Adjusted association† | |
| | | HR with 95% CI | p-value | HR with 95% CI | p-value |
| **Main diagnosis** | **(n)** | | | | |
| Normal biopsy findings | (n=88) | ref. | - | ref. | - |
| Infections and TIN ‡ | (n=62) | 2.13 (0.70-6.52) | 0.184 | 2.17 (0.71-6.63) | 0.176 |
| Acute tubular injuries § | (n=177) | 2.96 (1.14-7.64) | 0.025 | 3.01 (1.16-7.78) | 0.023 |
| Chronic changes incl. IFTA | (n=335) | 3.16 (1.27-7.85) | 0.013 | 3.21 (1.29-7.98) | 0.012 |
| Hematological diseases | (n=5) | n.a. | 0.953 | n.a. | 0.954 |
| Glomerular diseases | (n=84) | 7.98 (3.11-20.44) | <0.001 | 8.23 (3.21-21.11) | <0.001 |
| Minor abnormalities | (n=149) | 1.25 (0.43-3.66) | 0.683 | 1.26 (0.43-3.70) | 0.669 |
| Borderline changes | (n=128) | 2.83 (1.07-7.51) | 0.036 | 2.87 (1.08-7.62) | 0.034 |
| Rejections | (n=434) | 4.20 (1.73-10.29) | 0.002 | 4.20 (1.71-10.35) | 0.002 |
| **Subgroups of Rejections** | **(n)** | | | | |
| A) Acute TCMR | (n=235) | ref. | - | ref. | - |
| B) Chronic TCMR | (n=13) | 4.74 (1.97-11.41) | 0.001 | 4.70 (1.95-11.32) | 0.001 |
| C) Active ABMR | (n=28) | 3.64 (1.72-7.67) | 0.001 | 3.65 (1.72-7.72) | 0.001 |
| D) Chronic ABMR¥ | (n=71) | 3.37 (1.95-5.81) | <0.001 | 3.47 (1.99-6.01) | <0.001 |
| Combined A) and C) | (n=5) | n.a. | 0.972 | n.a. | 0.972 |
| Combined B) and D | (n=82) | 3.89 (2.29-6.60) | <0.001 | 3.92 (2.30-6.68) | <0.001 |

n=number; HR=Hazard ratio; CI=confidence interval; ref.=reference category for calculation of HR; n.a.= not applicable- zero events; †Age and gender adjusted; ‡ TIN= tubulointerstitial nephritis; § Acute tubular injuries = ATN (Acute tubular necrosis) and acute CNI-toxicity (Calcineurin inhibitor); ¶ Chronic damages (incl. chronic CNI-toxicity and IFTA/CAN), IFTA=Interstitial fibrosis and tubular atrophy, CAN=Chronic allograft nephropathy); Glomerular diseases=recurrent or de novo disease; TCMR=T-cell-mediated rejections; ABMR=Antibody-mediated rejection; ¥ Chronic ABMR included transplant glomerulopathy (TGP).

The observations showed that several diagnoses are dependent of time elapsed post-transplant until biopsy. However, in an additional model time after transplantation was also included as covariate and the risk remained statistically significant higher for glomerular diseases (HR 5.4, CI 2.1.14.0), rejections (HR 3.7, CI 1.5-9.2), acute tubular injuries (HR 3.6, CI 1.4-9.4) and borderline changes (HR 2.8, CI 1.1-7.4) compared to normal biopsy findings.

Death with a functioning transplant occurred in 8% (121 of 1 462 patients) after a median time of 70 months (mean 84 months) after transplantation. The median age of these patients at time of biopsy was 61 years. The most common causes of death among all patients were cardio-vascular disease (30%), infections (19%), and cancers (14%).

Among patients with graft loss, 15.8% (42 of 265, 42% women, 58% men) died during the follow-up. The median time between the transplant loss and death was 14 months with an interquartile range of 2–28 months. The median age of these patients at time of biopsy was 57 years. Of the 42 patients who died after graft loss, nine (21.4%) died within 30 days after transplant failure. There was no difference between the patients who died within 30 days compared to later than 30 days from graft loss regarding type of transplant kidney (living versus deceased donor transplant) (Table 8).

*Table 8. Number of deaths during follow-up in relation to graft loss, according to type of transplant kidney. N=163 deaths during follow-up.*

| ***N (Column %)*** | **Living donor transplant** | **Deceased donor transplant** | **Total** |
|---|---|---|---|
| Death within 30 days after graft loss | 2 (3.7%) | 7 (6.4%) | 9 |
| Death after 30 days after graft loss | 11 (20.4%) | 22 (20.2%) | 33 |
| Death with functioning graft, N (%) | 41 (75.9%) | 80 (73.4%) | 121 |
| **Total** | **54** | **109** | **163** |

N=Number.

## 4.3 PAPER III. GMI AND ASSOCIATION WITH GRAFT SURVIVAL

GMI was highest among chronic ABMR (median=7), active ABMR (median=6.5) and glomerulonephritis (median=5.2). Also, chronic TCMR (median=3.1), acute TCMR (median=2.3) and other glomerular diseases (median=2.4) had a higher GMI compared to normal biopsy findings (median=1.0). Infections and tubulointerstitial nephritis (median=1) and chronic damages (median=1.5) on the other hand showed low levels of GMI. Age and sex were not associated to GMI (Table 9). There was neither any difference in GMI between living and deceased donors.

Positive C4d (grade 1-3) was found in 13% of the biopsies (n=192), and GMI was higher among these (median 5.1 compared to 1.6 for C4d negative).

The risk for graft loss increased with increasing GMI in a univariable analysis, (Figure 13). Compared to GMI<0.5 the risk for graft loss was higher for biopsies with GMI within 1.9–2.7 (HR 2.92, 95% CI 1.5–5.67), GMI within 2.8–4.5 (HR 3.32, 95% CI 1.70–6.46), GMI 4.6–9.3 (HR 5.39, 95% CI 2.81–10.30) and GMI≥9.4 (HR 6.33, 95 % CI 3.33–12.04), adjusted for age, sex, C4 degree, diagnosis at biopsy and time from KT to biopsy, Table 10.

Also, patient survival was associated to highest GMI-class. Risk for death was significantly higher for GMI≥9.4 compared to GMI<0.5 (HR 2.24, 95% CI 1.22–4.10), adjusted for age, sex, C4 degree, diagnosis at biopsy and time from KT to biopsy, Table 10.

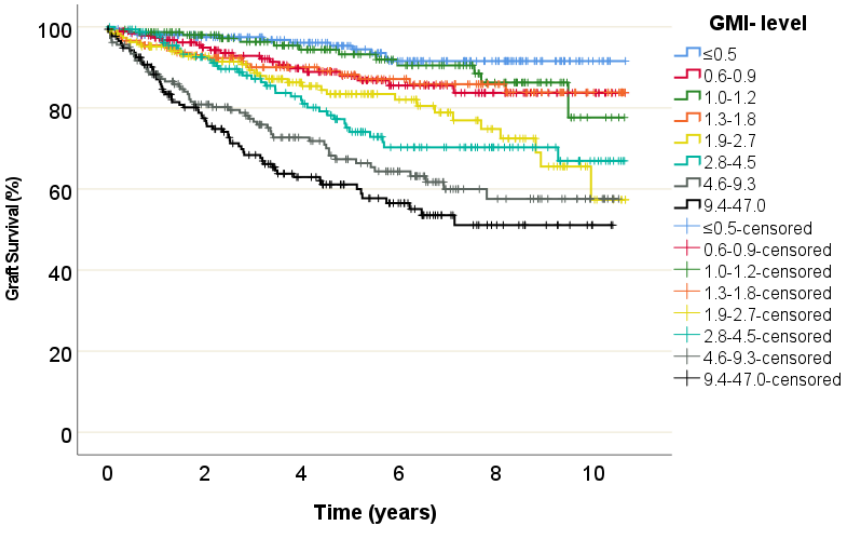
It was observed that interaction between GMI-class and time elapsed after transplantation was significant for graft survival. The effect of increasing GMI was more considerable among patients biopsied after 6 months compared to within 6 months after transplantation. For those with a biopsy performed within 6 months, the effect was moderate and significant only for GMI above 9.4 (Table 11, Figure 14). GMI probably reflects different pathological processes in the transplant also related to time after transplantation.

*Table 9. Levels of GMI according to patient characteristics (continues on page 45). Reprinted with permission (69).*

| Variables | Mean (SD) | Median ($Q_1$-$Q_3$) | p-value[1] |
|---|---|---|---|
| **Sex** | | | |
| *Male, n=913* | 3.6 (5.1) | 1.7 (0.9–3.7) | 0.051 |
| *Female, n=527* | 4.6 (6.2) | 2.0 (0.8–5.9) | |
| ***Age group*** | | | |
| ≤40 years, n=370 | 3.2 (4.2) | 1.7 (0.8–3.6) | 0.065 |
| 41–50 years, n=302 | 4.1 (5.6) | 2.0 (0.9–5.0) | |
| 51–60 years, n=360 | 4.0 (5.6) | 1.8 (0.9–4.5) | |
| ≥60, n=408 | 4.4 (6.3) | 1.9 (0.9–5.0) | |
| **C4d degree** | | | |
| *C4d 0 (negative), n=1248* | 3.5 (5.1) | 1.6 (0.8–3.6) | <0.001* |
| *C4d 1–3, n=192* | 7.0 (7.1) | 5.1 (1.8–10) | |
| **Time from KT to biopsy (years)** | | | |
| <6 months, n=700 | 3.2 (5.2) | 1.4 (0.8–3.0) | reference |
| 6–24 months, n=163 | 2.6 (4.0) | 1.3 (0.7–2.4) | 0.200 |
| 2–6 years, n=145 | 5.4 (7.2) | 2.4 (1.1–6.8) | <0.001* |
| >6 years, n=336 | 5.3 (5.9) | 3.0 (1.4–6.8) | <0.001* |
| Unknown date of transplantation, n=101 | | | – |
| **Diagnostic groups** | | | **p-value[2]** |
| *Normal biopsy findings, n=85* | 1.3 (1.0) | 1.0 (0.5–1.9) | reference |
| *Infections and tubulointerstitial nephritis (TIN), n=61* | 2.1 (3.4) | 1.0 (0.5–2.1) | 0.924 |

| Variables | Mean (SD) | Median ($Q_1$-$Q_3$) | p-value[1] |
|---|---|---|---|
| *Acute tubular injuries[3], n=177* | 2.6 (3.6) | 1.3 (0.8–2.7) | 0.018* |
| *Chronic changes including IFTA[4], n=330* | 2.6 (3.2) | 1.5 (0.8–2.9) | 0.001* |
| *Hematological diseases, n=5* | 2.8 (1.3) | 2.4 (2.0–2.8) | 0.009* |
| *Glomerular diseases, n=82* | 6.3 (6.5) | 4.0 (1.7–8.0) | <0.001* |
| GN, recurrent/de novo, n=48 | 7.5 (6.8) | 5.2 (2.5–12.0) | <0.001* |
| Glom disease, no GN, n=33 | 4.6 (5.8) | 2.4 (1.5–5.5) | <0.001* |
| *Minor abnormalities, n=149* | 1.9 (3.5) | 1.0 (0.6–1.9) | 0.902 |
| *Borderline changes, n=126* | 3.6 (4.7) | 2.0 (0.9–4.4) | <0.001* |
| *Rejections, n=425* | 6.7 (7.5) | 3.9 (1.5–9.2) | <0.001* |
| Acute TCMR, n=234 | 5.2 (6.9) | 2.3 (1.2–6.5) | <0.001* |
| Chronic TCMR, n=12 | 6.3 (9.9) | 3.1 (1.0–7.2) | 0.026* |
| Active ABMR, n=28 | 9.7 (9.1) | 6.5 (3.7–13.2) | <0.001* |
| Chronic ABMR [5], n=69 | 9.4 (7.6) | 7.0 (3.7–13.4) | <0.001* |
| Combined active ABMR and acute TCMR, n=5 | 6.7 (7.7) | 1.6 (1.2–15.0) | 0.186 |
| Combined chronic ABMR and chronic TCMR, n=77 | 7.6 (6.9) | 6.5 (2.0–10.5) | <0.001* |

$Q_1$=first quartile, $Q_3$=third quartile; [1]Comparisons by Mann-Whitney for two groups and Kruskal-Wallis test for more than two groups.
[2] Pairwise comparisons by Mann-Whitney test with normal biopsy findings as the reference group; [3] Acute tubular injuries = acute tubular necrosis (ATN) and acute CNI-toxicity (calcineurin inhibitor); [4] Chronic changes (including chronic CNI-toxicity and IFTA/CAN); IFTA= interstitial fibrosis and tubular atrophy); [5]Including transplant glomerulopathy, n=30; CNI, calcineurin inhibitor; GN, glomerulonephritis
*Statistically significant difference.




**Case Processing Summary**

| GMI-levels/ Class | Total N | N of Events | Censored | |
|---|---|---|---|---|
| | | | N | Percent |
| ≤0.5 | 205 | 12 | 193 | 94,1% |
| 0.6-0.9 | 191 | 21 | 170 | 89,0% |
| 1.0-1.2 | 157 | 13 | 144 | 91,7% |
| 1.3-1.8 | 177 | 21 | 156 | 88,1% |
| 1.9-2.7 | 190 | 34 | 156 | 82,1% |
| 2.8-4.5 | 162 | 36 | 126 | 77,8% |
| 4.6-9.3 | 183 | 57 | 126 | 68,9% |
| 9.4-47.0 | 175 | 66 | 109 | 62,3% |
| Overall | 1440 | 260 | 1180 | 81,9% |

*Figure 13. Graft survival according to GMI-level at first biopsy. Kaplan-Meier curves. Adapted from (69).*

*Table 10. Risk for different endpoints according to GMI-class at biopsy. Cox regression – Hazard ratio (HR) based on crude (univariate) and model adjusted for covariates where time from biopsy to end-point was outcome. Modified and reprinted with permission (69).*

| | | Graft survival (time to graft loss) | | | |
|---|---|---|---|---|---|
| **Diagnosis** | | **Crude association** | | **Adjusted association[1]** | |
| | | **HR with 95% CI** | **p-value** | **HR with 95% CI** | **p-value** |
| ***GMI classes*** | | | | | |
| ≤0.5 | (n=205) | ref. | - | ref. | - |
| 0.6–0.9 | (n=191) | 2.09* (1.034.24) | 0.042 | 1.98 (0.97–4.04) | 0.059 |
| 1.0–1.2 | n=157) | 1.47 (0.67–3.21) | 0.339 | 1.44 (0.66–3.17) | 0.361 |
| 1.3–1.8 | (n=177) | 2.11* (1.04–4.31) | 0.038 | 1.71 (0.83–3.51) | 0.144 |
| 1.9–2.7 | (n=190) | 3.55* (1.84–6.86) | <0.001 | 2.92* (1.50–5.67) | 0.002 |
| 2.8–4.5 | (n=162) | 4.18* (2.17–8.03) | <0.001 | 3.32* (1.70–6.46) | <0.001 |
| 4.6–9.3 | (n=183) | 6.63* (3.56–12.38) | <0.001 | 5.39* (2.81–10.30) | <0.001 |
| 9.4–47.0 | (n=175) | 8.69* (4.69–16.09) | <0.001 | 6.33* (3.33–12.04) | <0.001 |

| | | Survival (time to death) | | | |
|---|---|---|---|---|---|
| | | **Crude association** | | **Adjusted association** | |
| | | **HR with 95% CI** | **p-value** | **HR with 95% CI** | **p-value** |
| ≤0.5 | (n=205) | ref. | - | ref. | - |
| 0.6–0.9 | (n=191) | 1.24 (0.65–2.36) | 0.518 | 1.26 (0.66–2.41) | 0.479 |
| 1.0–1.2 | (n=157) | 0.90 (0.43–1.87) | 0.778 | 0.83 (0.40–1.73) | 0.616 |
| 1.3–1.8 | (n=177) | 0.96 (0.48–1.90) | 0.902 | 0.85 (0.43–1.70) | 0.659 |
| 1.9–2.7 | (n=190) | 1.04 (0.53–2.04) | 0.910 | 0.99 (0.50–1.97) | 0.980 |
| 2.8–4.5 | (n=162) | 1.31 (0.68–2.49) | 0.417 | 1.20 (0.62–2.34) | 0.589 |
| 4.6–9.3 | (n=183) | 1.61 (0.88–2.94) | 0.120 | 1.57 (0.84–2.95) | 0.158 |
| 9.4–47.0 | (n=175) | 2.50*(1.43–4.39) | 0.002 | 2.24*(1.22–4.10) | 0.009 |

N=number; ref.=reference category for calculation of HR; n.a.= not applicable- zero events; [1]Adjusted for age, gender, C4 degree, diagnosis at biopsy and time from KT; * statistically significant increase of risk

*Table 11. Risk for graft loss according to GMI-class at biopsy stratified on time from KT to biopsy. Reprinted with permission (69).*

| | **Time from KT to biopsy** | | | |
|---|---|---|---|---|
| | **≤6 months (n=700)[a]** | | **>6 months (n=644)[a]** | |
| | **HR[b] with 95% CI** | **p-value** | **HR[b] with 95% CI** | **p-value** |
| GMI classes (n within 6m.; >6m.) | | | | |
| ≤0.5 (120; 71) | reference | - | reference | - |
| 0.6–0.9 (113; 69) | 2.14 (0.93–4.90) | 0.073 | 1.98 (0.49–7.97) | 0.335 |
| 1.0–1.2 (95; 54) | 1.24 (0.47–3.22) | 0.665 | 2.53 (0.58–11.0) | 0.214 |
| 1.3–1.8 (85; 80) | 1.05 (0.37–2.99) | 0.922 | 3.51 (0.97–12.70) | 0.055 |
| 1.9–2.7 (95; 89) | 1.47 (0.58–3.76) | 0.413 | 7.06*(2.04–24.43) | 0.002 |
| 2.8–4.5 (68; 78) | 2.07 (0.81–5.32) | 0.129 | 7.20*(2.08–24.88) | 0.002 |
| 4.6–9.3 (55;108) | 2.33 (0.87–6.25) | 0.091 | 12.78*(3.75–43.52) | <0.001 |
| 9.4–47.0 (69; 95) | 5.0* (2.1–11.9) | <0.001 | 12.40*(3.62–42.43) | <0.001 |

[a] 1 344 patients with complete data about date for KT and GMI. [b]Cox regression - Hazard ratio (HR) based on model adjusted for covariates where time from biopsy to endpoint was the outcome. Adjusted for age, gender, C4d degree and diagnosis at biopsy; * statistically significant increase in risk.
n = number; ref = reference category for calculation of HR; KT=transplantation

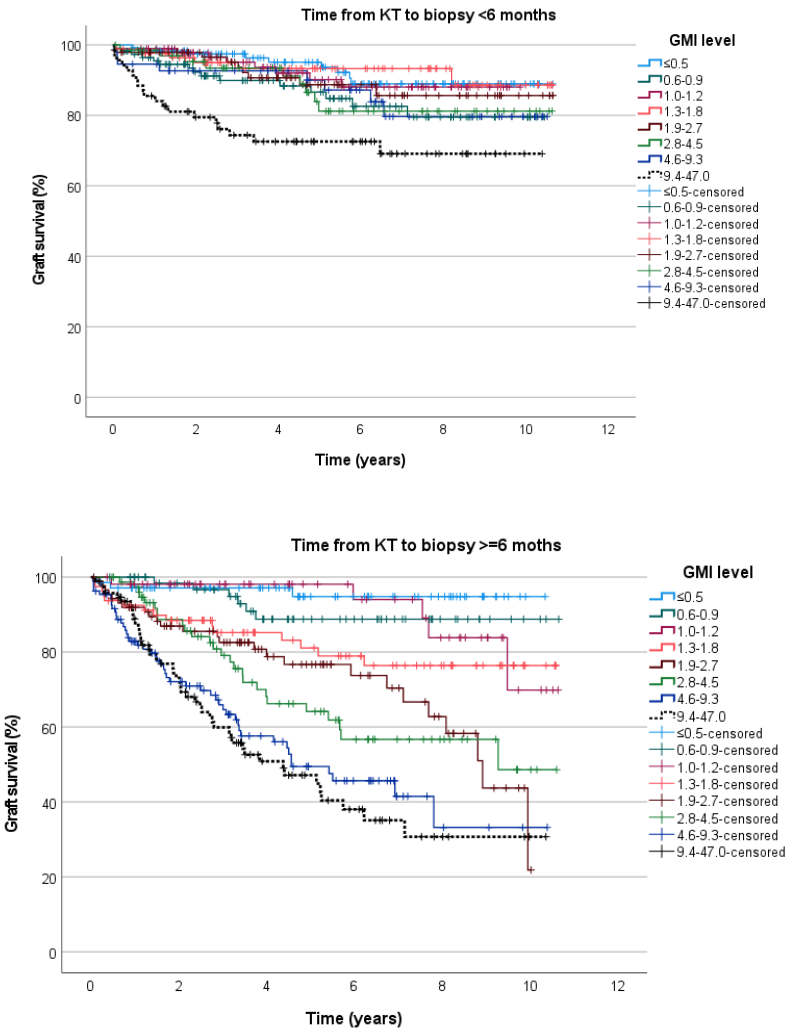



*Figure 14. Kaplan-Meier estimates of death-censored graft survival after biopsy, according to GMI-levels at biopsy and stratified on time from transplantation to biopsy. Reprinted with permission (70).*

## 4.4 PAPER IV. CHANGE IN GMI BETWEEN TWO BIOPSIES AND ASSOCIATION TO GRAFT SURVIVAL

A total of 623 patients with available data from two consecutive biopsies were included in the study. Median time between the first and second biopsy was 86 days. Median time since transplantation until the first biopsy was 34 days (Figure 15).

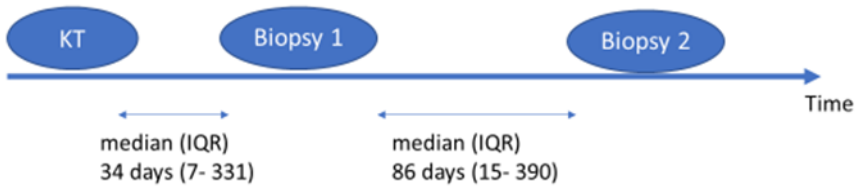



*Figure 15. Time line of performed biopsies in relation to the date of the transplantation. IQR=Interquartile range.*

GMI on the group level was similar in both biopsies (median GMI=1.8). The changes in biopsy-proven diagnoses between biopsy 1 and biopsy 2 are illustrated in Figure 16. The most dominant group was Rejections both at biopsy 1 (32%, n=198) and at biopsy 2 (37%, n=228). Other large groups were Chronic damages (17%, n=106 at biopsy 1 and 19% n=116 at biopsy 2) and Acute tubular injuries (17%, n=105 at biopsy 1 and 6%, n=36 at biopsy 2).

A change in GMI-level was described according to nine GMI-categories and the most common categories were Low-Low GMI (36%, n=227) and High-High (17%, n=104). The categories representing changes Low-High (4%, n=27) and High-Low (4%, n=28) were not very large but are interesting to study from a clinical point of view, (Table 12).

A lower risk for graft loss was observed in almost all GMI-categories compared to High-High as reference category (risk reduction varied between 65% and 80%). The risk reduction remained as statistically significant in Low-Low (HR=0.24, 95% CI 0.13–0.46), Low-Medium (HR=0.25, 95% CI 0.11–0.55), Medium-Low (HR=0.29, 95% CI 0.11–0.77) and High-Low (HR=0.31, 95% CI 0.10–0.98) – adjusted for sex, previous transplantation, days between KT and first biopsy and days between the two biopsies as well as histological diagnosis at biopsy 1 and biopsy 2, (Table 12)

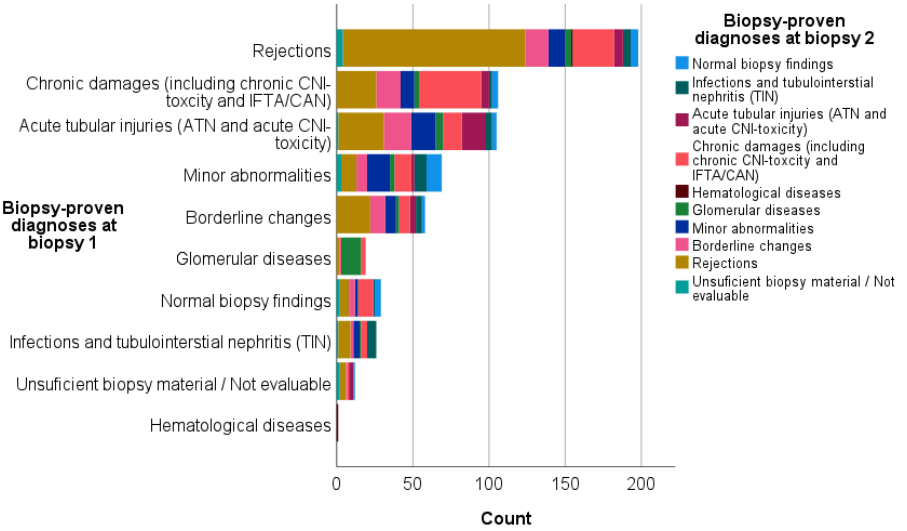



*Figure 16. Distribution and changes of biopsy diagnoses between first and second biopsy. First biopsy (b1) left axis and second biopsy (b2) right axis. n=623*

*Table 12. Graft survival according to GMI-change between first and second biopsy. HR based on Cox-regression (primary model).*

| Change in GMI | N | N events | P-time at risk (years) | Inc. rate (graft-loss)[1] | HR with 95% CI univariable model | HR with 95% CI multivariable model |
|---|---|---|---|---|---|---|
| **L-L** | 227 | 24 | 874 | 2.7 | 0.19[a] (0.12–0.33) | 0.24[a] (0.13–0.46) |
| **L-M** | 63 | 10 | 225 | 4.4 | 0.31[a] (0.15–0.62) | 0.25[a](0.11–0.55) |
| **L-H** | 27 | 7 | 58 | 12.1 | 0.70 (0.31–1.57) | 0.79 (0.32–1.94) |
| **M-L** | 59 | 6 | 188 | 3.2 | 0.23[b] (0.10–0.56) | 0.29[c](0.11–0.77) |
| **M-M** | 60 | 16 | 175 | 9.1 | 0.62 (0.34–1.11) | 0.59 (0.30–1.15) |
| **M-H** | 29 | 7 | 77 | 9.1 | 0.58 (0.26–1.30) | 0.52 (0.22–1.25) |
| **H-L** | 28 | 4 | 110 | 3.6 | 0.25[b] (0.09–0.71) | 0.31[c](0.10–0.98) |
| **H-M** | 26 | 5 | 102 | 4.9 | 0.34[c] (0.13–0.86) | 0.39 (0.14–1.03) |
| **H-H** | 104 | 37 | 246 | 15.0 | reference | reference |

[1]Incidence rate for graft loss per 100 person-years; a: p-value<0.001 , b: p-value<0.01, c: p-value<0.05. Multivariable model adjusted for: age, sex, time between KT and biopsy 1, time between biopsy 1 and biopsy 2, re-transplantation, biopsy-proven diagnosis at biopsy 1 and biopsy-proven diagnosis at biopsy 2; L=Low, M=Medium, H=High; P-time=Person-time, Inc.=incidence; CI=confidence interval

*Sensitivity analyses*

With longer time elapsed between biopsies there was a risk that the second biopsy was not performed to evaluate progress of the same disease/condition that was initiated by the first biopsy. After a longer period of time there was high probability that the second biopsy was taken on other indications and for other reasons. Therefore, a sensitivity analysis was performed without cases where the second biopsy was performed 2 years or later, 15% of the cases were excluded. A Cox-regression model included all covariates that were included in the primary model and the results were confirmed with minor adjustments of HR (model 1 in Table 13)

As early biopsies after transplantation might be related to early complications or problems with delayed graft function, a sensitivity analysis without biopsies within 7 days after transplantation was performed. Due to first biopsy within 7 days after transplantation 25% of the cases were excluded. Cox-regression adjusted for all covariates used in primary model and results were confirmed with minor adjustments of HR (model 2 in Table 13).

*Table 13. Sensitivity analyses: model 1 and model 2 includes all covariates as described in Table 12 but with some restrictions (See the footnote below the table about model 1 and model 2).*

| Change in GMI | HR with 95% CI multivariable model 1 | HR with 95% CI multivariable model 2 |
|---|---|---|
| **Low–Low** | 0.29[a] (0.14–0.58) | 0.23[a] (0.11–0.50) |
| **Low–Medium** | 0.18[a] (0.06–0.49) | 0.24[b](0.09–0.61) |
| **Low–High** | 0.83 (0.29–2.39) | 0.60 (0.19–1.91) |
| **Medium–Low** | 0.33[c](0.12–0.92) | 0.19[c](0.05–0.74) |
| **Medium–Medium** | 0.56 (0.26–1.19) | 0.69 (0.32.1.49) |
| **Medium–High** | 0.56 (0.18–1.71) | 0.60 (0.22–1.62) |
| **High–Low** | 0.29[c] (0.09–0.93) | 0.17[c](0.04–0.85) |
| **High–Medium** | 0.54 (0.19–1.52) | 0.16 (0.02–1.24) |
| **High–High** | reference | reference |

***Model 1:*** *excluding cases where the second biopsy was performed 2 years or later after first biopsy (15% of all cases excluded);* ***Model 2:*** *cases excluded if first biopsy was performed early (within 1 week) after transplantation (25% of all cases excluded).*
*a: p-value<0.001, b: p-value<0.01, c: p-value<0.05*

## SHIFT IN BIOPSY-PROVEN DIAGNOSES BETWEEN FIRST AND SECOND BIOPSY IN RELATION TO GMI-CHANGE

Shift in biopsy-proven diagnoses has been further explored in four extreme GMI-categories: Low-Low, Low-High, High-Low and High-High.

*Low-Low GMI (n=227)*

In this category, with low GMI at both first and second biopsy, there were 48 Acute tubular injuries (21.1%), 45 Rejections (19.8%) and 44 Chronic damages (19.4%) at first biopsy. At second biopsy the number of Acute tubular injuries decreased to 14 (6%), number of Rejections was 51 (22.5%) and Chronic damages 55 (24.2%), Figure 17. A sub analysis of rejections showed that 40 of 45 rejections were acute TCMR (89%) at biopsy 1 and 37 of 51 at biopsy 2 (72.5%).

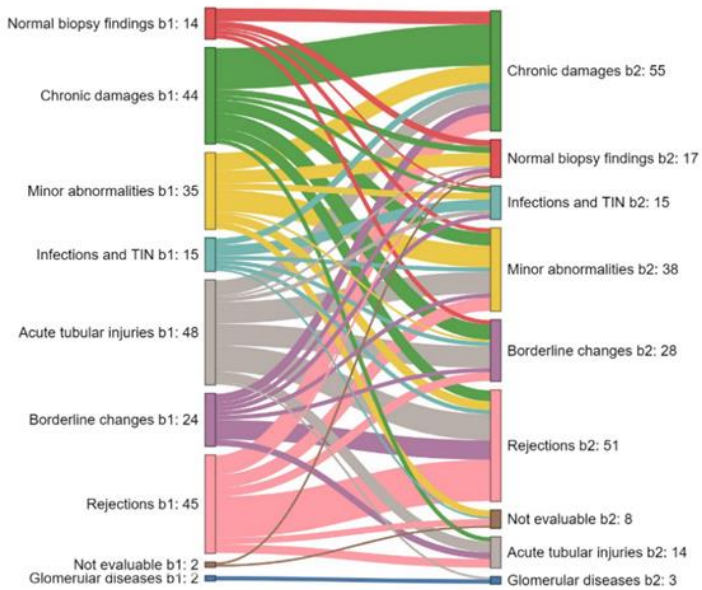



*Figure 17. Distribution and changes of biopsy diagnoses between first and second biopsy. First biopsy (b1) left axis and second biopsy (b2) right axis. GMI-category Low-Low.*

*Low-High GMI (n=27)*

In the Low-High GMI-category, representing worsened GMI, the largest diagnosis group at biopsy 1 was Chronic damages standing for 37% of the

cases (10 of 27) and Minor abnormalities in 14.8% of the cases (4 of 27). Rejections were only 11% (3 of 27) at biopsy 1 and increased to 48% (13 of 27) at biopsy 2, p-value=0.006, Figure 18.

A sub analysis revealed that there were none ABMR rejection at first biopsy and at second biopsy 7 of 13 Rejections were active or chronic ABMR or in combination with chronic TCMR.

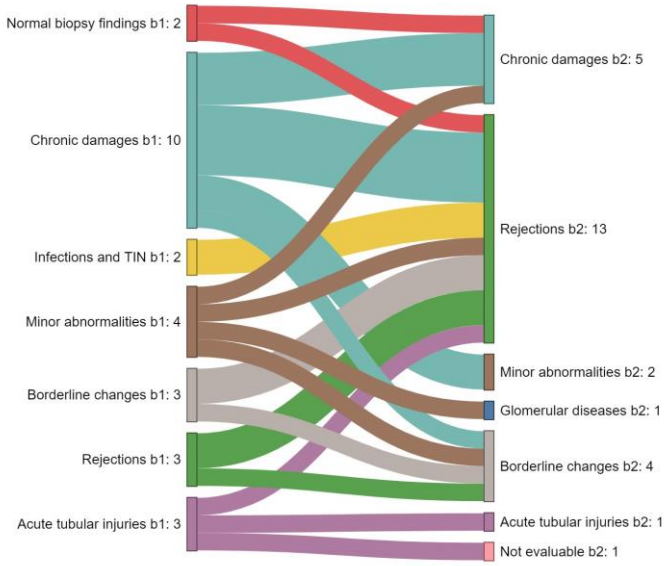



*Figure 18. Distribution and changes of biopsy diagnoses between first and second biopsy. First biopsy (b1) left axis and second biopsy (b2) right axis. GMI-category Low-High.*

*High-Low GMI (n=28)*

The most dominant category at biopsy 1 was Rejections 46% (13 of 28) and at biopsy 2 Rejections decreased to 21% (6 of 28), p-value=0.065. Infections increased from 3.6% (1 of 28) to 18% (5 of 28), Figure 19. A sub analysis revealed that at first biopsy 11 of 13 Rejections (85%) were acute TCMR and 2 (15%) were active ABMR, while 4 of 6 (67%) were acute TCMR and none were active ABMR at second biopsy.

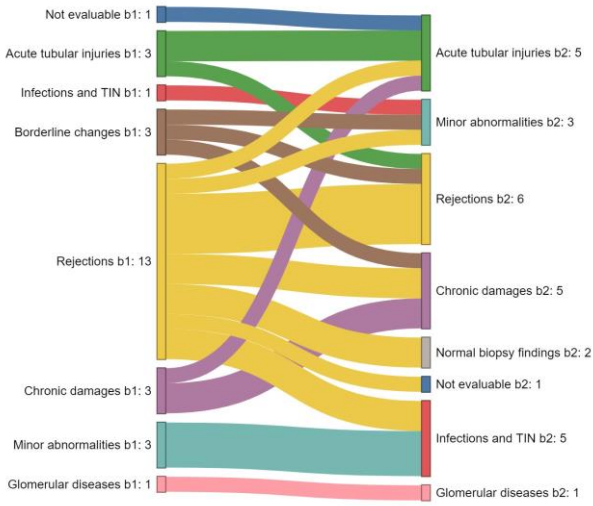



*Figure 19. Distribution and changes of biopsy diagnoses between first and second biopsy. First biopsy (b1) left axis and second biopsy (b2) right axis. GMI-category High-Low.*

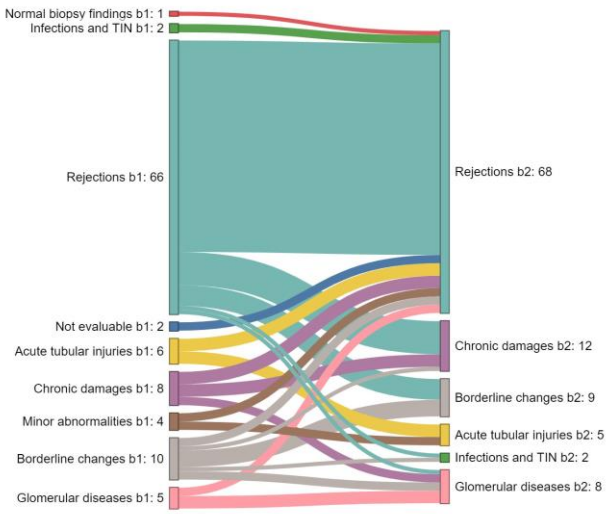



*Figure 20. Distribution and changes of biopsy diagnoses between first and second biopsy. First biopsy (b1) left axis and second biopsy (b2) right axis. GMI-category High-High.*

## *High-High GMI (n=104)*

Rejection was the dominant diagnosis at first biopsy 63% (66 of 104) and second biopsy 65% (68 of 104) and there were only smaller changes among

other groups of diagnoses, Figure 20. A sub analysis showed that among rejections at first biopsy 50 % were acute TCMR (33 of 66), this decreased to 25% (17 of 68) at second biopsy, Figure 21. Active ABMR increased from 9% (6 of 66) to 15% (10 of 68) as well as chronic ABMR from 18% (12 of 66) to 28% (19 of 68).

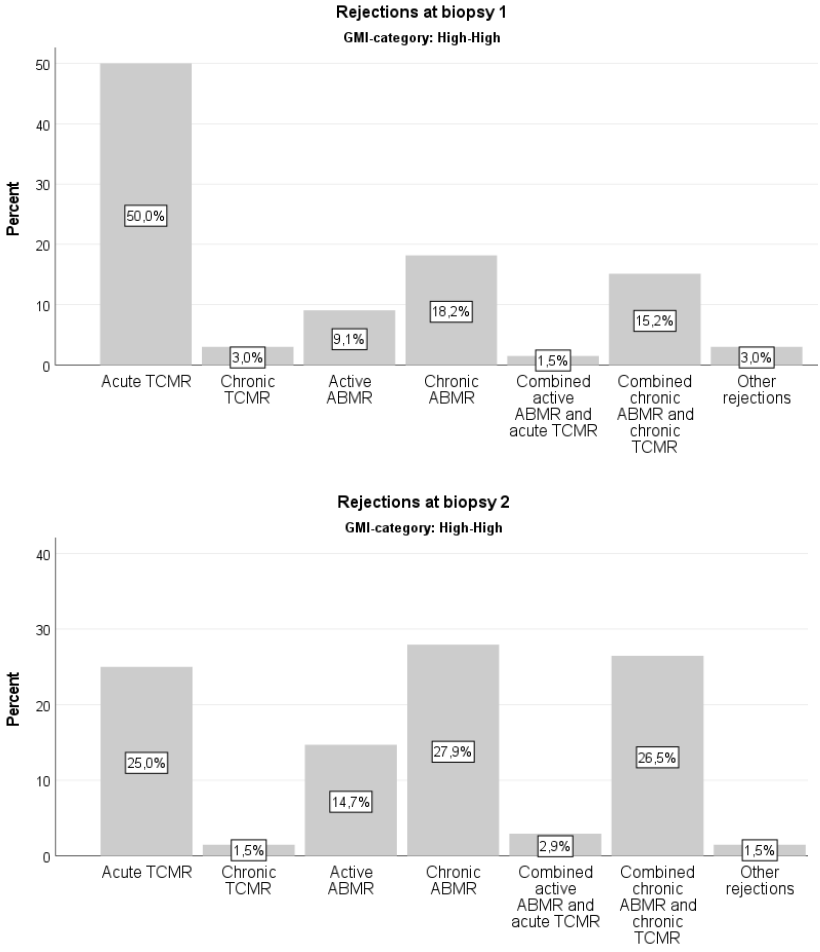



*Figure 21. Distribution of subgroups of rejections at first and second biopsy in GMI-category High-High.*

# 5 DISCUSSION

## 5.1 MAIN FINDINGS

### *Study I*

The presented results about overall improved graft survival over time in study I are consistent with results in earlier studies (37, 41). This improvement is mainly due to development of immunosuppressive therapy (21, 37, 41) .When graft survival was compared between men and women in the entire time period from 1965 to 2017, no difference was observed but when graft survival was compared within different time periods shorter graft survival was observed among women in the last period (2006-2017).

In both kidney transplant (97) and non-kidney transplant recipients (98), sex differences have been observed in earlier studies. In a recent study about kidney transplanted patients in Spain (99), worse graft survival was observed among women in the age group below 60, which is consistent with the present study. In a study from Taiwan, the graft survival was shown to be worse among men and no interaction between recipient and donor sex was found (100). However, the study from Taiwan included only KT between 1988 and 2009 which is earlier than the last period in our study (2006–2017). In other studies, sex disparity between donor and transplant recipient have been shown to play a role for female kidney recipients. In female recipients of kidney from a male donor it has been shown that some females develop antibodies against H-Y antigens from the male donor, which caused acute rejection (101). In the present study a higher proportion of rejections among women (33%) compared to men (21%) was observed. This might be related to sex-disparity between a male donor and a female recipient but as no donor-specific data are included in the registry this could not be investigated. However, the difference in graft survival remained significant even after adjustment for biopsy-based diagnoses in a multivariable model so there are likely other factors except biopsy-based rejections related to the findings. Other factors that were not available in the registry, but might have had impact, on the findings are medication non-adherence, drug concentration in blood, history of pregnancy and history of blood transfusions.

One of the most important causes of rejection and graft loss is medication non-adherence (MNA) (102-106). Factors associated with MNA appear to be low social support, low mental and physical health-related quality of life, and young age (107). The studies about MNA among transplanted patients present

varying findings. Better medication adherence among female transplant patients has been shown in several studies (102, 108). No data about MNA were available in the present study.

There was no difference between men and women in age but there was a general increase over time in mean age of the patients. Although age was not significant for graft survival, increased age of transplanted patients might be a factor related to whether and how often female transplanted patients had been pregnant. Prevalence of pre-transplant history of pregnancy is by natural reasons more common among older women while post-transplant pregnancies are more common among younger women. However, post-transplant pregnancies are in earlier studies described as not affecting the risk of graft failure (109-111). Pre-transplant history of pregnancy is associated to sensitization of the immune system and might therefore be related to higher rates of rejections due to higher panel reactive antibody levels (112). This can be related to worse graft survival among women but as no data about panel-reactivity antibodies was available there was no possibility to investigate it. There is a possibility that some sensitized women, due to previous pregnancies, require more intensive IST. The actual doses of IST were not included in the registry and could not be compared between men and women. Other factors known to be associated with graft survival are donor and recipient characteristics such as age, type of donor, immunologic compatibility, ischemia time, IST and IST dose. Several of these variables were included in the multivariable model in the present study.

As the difference between women and men appeared only during the last time period (2006–2017) the possible confounders that have appeared during the last period are of interest. Some new IST therapies as MMF and tacrolimus were observed to be more frequent among women compared to men (MMF 85% vs 80.5% and tacrolimus 62% vs 54%). MMF and mTOR inhibitors (sirolimus and everolimus) have been associated to higher rate of acute rejections according to some studies (113) but not in the present study.

In an earlier study, differences in graft survival between men and women were shown to be age-dependent (114). Risk of graft loss with a transplant from a male donor was higher among women in age below 44 years but not in women above 44 years. There was no data about donor sex in the present study but during the period 2006–2017 women had slightly worse graft survival in all age groups. The association was statistically significant in age group 45–60 years while in age group <44 years and >60 years there was a similar tendency but not statistically significant.

In conclusion, study I demonstrated worse graft survival after transplantation among women compared to men in 2006–2017. Histological findings of rejection were more frequent among women. The difference in graft survival between women and men persisted after controlling for available covariates but there are likely other variables, not available in the present study, involved in the findings. Therefore, further studies are warranted to confirm, and in next step, explain the findings and improve the outcome for women.

## *Study II*

In this study, an extensive collection of kidney transplant biopsy data concerning graft survival was in focus. The current analysis covers biopsy findings in a more recent transplantation era compared to earlier studies (115, 116). The registry includes both early and late biopsies after transplantation, enabling an examination of the prognostic value of common histological findings in a clinical setting, primarily indication biopsies.

Groups of main diagnoses according to histological findings were compared with respect to graft survival. Compared to normal biopsy findings, shorter graft survival was observed in transplants with glomerular diseases, rejections, acute tubular injuries, borderline changes and chronic changes.

Rejections emerged as the most prevalent finding overall. The time pattern revealed predominant early acute TCMR and a subsequent late occurrence of chronic ABMR, similar to the pattern observed by Sellarés et al. (94) in a Canadian population of patients. Notably, approximately half of all kidney transplant biopsies were conducted within 6 months after transplantation, with the majority occurring within 14 days. During the initial period (< 14 days), acute tubular injuries (ATI) constituted the majority of the findings with 40%, and notably, 20% of these were observed in living donor-related transplants. The relatively large proportion of living donors with ATI suggests potential contributions from pharmacological side effects induced by the induction therapy, evident in both living and deceased donor kidneys.

Within initial 14 days common findings were Rejections (28%) and Chronic changes (12%). Rejection findings persisted in approximately 1/4 of all biopsies until 5 years, increasing to about 1/3 thereafter, aligning with the observations made by Sellarés et al (94). De novo or recurrent glomerulonephritis diagnoses mainly occurred after 5 years or later on, confirming findings by Sellarés et al (94).

Chronic changes in the study, are likely related to donor-derived damages diagnosed in transplant biopsies. Borderline changes and minor abnormalities, including 'normal findings,' represented a notable proportion of the biopsy-proven diagnoses, 20–45% of the findings within 5 years, and decreasing thereafter. These findings may indicate early stages of immunologically or pharmacologically induced changes, with potential implications for chronic damage later. One speculative explanation could be that in these cases the biopsies were conducted to investigate early or non-specific rises in serum creatinine.

Looking at the sub-groups of Rejections, acute TCMR predominated in biopsies taken during the initial years after transplantation, suggesting a more extensive and earlier occurrence compared to previous studies by Sellarés et al. and Arias-Cabrales et al (94, 117). Active ABMR was low prevalent (<2%) at all time points of biopsy, slightly higher the first month (≈4%) and after 5–10 years (2.5%). Single chronic ABMR, and in combination with chronic TCMR, became more frequent a couple years after transplantation, emphasizing the need for focused immunosuppression on the T-cell response during the early post-transplantation period to prevent progression to chronic rejection. Single chronic TCMR was non-existing during first months and low prevalent thereafter (<2%).

Chronic changes, including IFTA, were identified in 12% of the biopsies within 14 days post-transplant and constituted more than 20% of biopsy findings after 6 months, increasing further thereafter. Early IFTA changes may encompass undefined chronic changes in donor kidneys, possibly representing prior TCMR as shown by Nankivell et al. (118). Thus, early IFTA motivates further investigation of the biopsy to clarify if immunological activities are ongoing.

Polyomavirus nephropathy surfaced in biopsies performed after 1 month and up to 5 years after transplantation, similar to findings by Sellarés et al. The impact on graft survival was relatively small and non-significant compared to normal biopsy findings, implying a favorable prognosis, possibly attributed to early diagnosis facilitated by Polymerase Chain Reaction (PCR) monitoring of virus levels in the modern era.

Prevalence of glomerular disease was non-existing within 1 year after transplantation but increased with time, reaching the peak around 5 years.

Among sub-groups of rejections, acute TCMR demonstrated the best outcome concerning graft survival time (approximately nine years in average), whereas

active ABMR, chronic ABMR and chronic TCMR had a shorter graft survival (just over six years in average), stressing the importance of early detection of acute rejection to prevent progression to chronic rejection.

The prevalence of transplant lesions increases with increasing grade according to Banff-criteria (119). Patients with TCMR Banff grade II displayed better graft survival compared to all other rejections in present study, in contrast with findings where Banff grade I showed a significantly better outcome (120). This discrepancy could be attributed to the more intensive therapeutic measures associated with Banff grade II. In our larger study cohort, a relatively large group (n=70) of combined chronic TCMR and chronic ABMR was detected, in contrast to Sellarés et al (94).

In conclusion, transplants with Glomerular diseases, Rejections, Acute tubular injuries, Borderline changes and Chronic changes were associated to shorter graft survival compared to normal biopsy findings, also after controlling for covariates. The study demonstrates the importance of performing indication biopsies at the right time and value of histological findings in planning a future therapeutic course.

## *Study III*

In this investigation, a noteworthy influence of the number of monocytes/macrophages in renal glomeruli, measured as a Glomerular macrophage index (GMI), on transplant outcomes within an extensive cohort of kidney transplant biopsies was presented. The highest values of GMI were observed in biopsies exhibiting active or chronic Antibody-Mediated Rejection (ABMR), intermediate levels in those with T-cell Mediated Rejection (TCMR), and lower levels in cases with borderline changes. Notably, elevated GMIs were also identified in recurrent glomerulonephritis and other glomerular diseases. Moreover, a GMI $\geq$ 4.6, particularly exceeding 9.4, was associated with a significantly poorer graft survival. Additionally, C4d-positivity in peritubular capillaries was significantly associated with a high GMI, exhibiting the correlation between GMI and ABMR. These findings align with prior studies conducted on a more limited number of patients (63-65, 121, 122).

Some early studies examining renal biopsies demonstrated that a high number of macrophages (and T-cells) in kidney transplant biopsies is linked to rejection and an unfavorable prognosis (123, 124). Initial studies focusing on macrophages in glomeruli employed histochemical techniques and demonstrated a substantial link of monocyte infiltration ($\geq$ 2 cells per biopsy

or occurring in at least 50% of glomeruli) and graft failure (125, 126). Magil introduced a GMI for glomerular diseases in native kidney biopsies, later adopted in transplant biopsies (65, 68). Previous studies in transplant biopsies used CD68 as a marker for monocytes/macrophages, with Ozdemir et al. being the pioneers in revealing the high GMI in biopsies with acute and chronic rejection and its lower occurrence in normal biopsies and those with Calcineurin Inhibitor (CNI)-toxicity (68). Magil and Tinckam demonstrated higher GMI (and neutrophils) in C4d positive cases within a series of biopsies with rejection (65). In addition, they showed that C4d-positivity in peritubular capillaries was associated to ABMR, whereas C4d-negativity was associated to TCMR (127). Another study reported that the maximal number of macrophages in glomeruli, G-max, was linked to ABMR in biopsies obtained over a year after transplantation (122). In a subset of biopsies in the present study (n=320) the G-max was evaluated, and the conclusion was that no explanatory contribution to graft survival was added compared to GMI.

Most of above-mentioned studies were conducted on relatively small patient cohorts and lacked subsequent validations. Therefore, GMI was calculated in all transplant biopsies from January 2007 to September 2017, to assess its prognostic value for graft survival. The present study demonstrates that a high GMI in the first transplant biopsy is significantly associated with a poorer graft survival, with variations observed in different biopsy-proven diagnostic groups.

Establishing a clinically significant GMI level proves challenging. Early histochemical studies highlighted significant differences between few monocytes (two per glomerulus) compared to none (125, 126). CD68-based studies have utilized a threshold of <1 versus >1 in GMI, revealing differences in graft function (127) and graft loss (128). Subsequent studies proposed that GMI levels >3 only were observed in ABMR, and a GMI > 1.89 was associated with microvascular inflammation, an important feature of ABMR (129). Lefaucheur et al. calculated a mean GMI being 6.8 in patients with ABMR and worse outcomes (n=8), compared to mean GMI being 3.2 in an ABMR group with a better outcome (n=13) (130). The thesis extensive study III demonstrates that indication biopsies with normal findings have a mean GMI of 1.3, whereas patients with GMI ≥ 1.9 experience impaired graft survival. Furthermore, patients with GMI levels ≥4.6 experience even worse prognosis, escalating to substantially poorer outcomes at levels ≥9.4. Notably, the impact on graft survival is only significant for levels ≥9.4 in early transplant biopsies taken within 6 months. The clinical implication suggests heightened awareness for transplants exhibiting elevated GMI levels. However, determining the

precise GMI threshold requiring increased immunosuppression remains unclear and motivates further research in prospective studies.

The effect of increasing GMI was considerable among patients biopsied after 6 months compared to within 6 months after transplantation. For biopsies performed within 6 months the effect was moderate and significant only for GMI above 9.4. An explanation for this might be that time for exposure to high GMI is a pivotal factor in the sense that being exposed to high GMI for a shorter period of time is not as harmful as being exposed to GMI during a longer period of time. GMI probably reflects different pathological processes ongoing in the transplant also related to time after transplantation.

In conclusion, the findings demonstrate that the GMI in transplant kidney biopsies was highest in ABMR, mixed-rejections and glomerulonephritis. Moreover, increasing GMI-levels were associated with risk for graft-loss, also after controlling for histological diagnoses and time since transplantation. Thus, GMI can guide clinicians to decide need of follow-up and the course and intensity of therapy.

Utilizing histological outcome criteria, including GMI, to facilitate treatment strategies appears justified. The association between a high GMI level and shorter expected graft survival suggests that GMI can be a valuable parameter in therapy decisions. Further investigations should explore the utility of this parameter, correlating GMI with established Banff lesion scores.

### *Study IV*

This study revealed that, in most cases, follow-up GMI levels were comparable with levels at the first biopsy. However, 37% of cases exhibited a clinically relevant change in GMI level. The analysis demonstrated a statistically significant association between GMI-categories, reflecting GMI-changes between the two biopsies, and graft survival. Grafts in the Low-Low, Low-Medium, Medium-Low, High-Low, and High-Medium categories exhibited better survival compared to those in the High-High category. These findings align with our previous study on GMI levels at the first biopsy (69).

Notably, some cases experienced an increase from Low to High GMI, while others decreased from High to Low. These categories are noteworthy, suggesting the possibility of transitioning GMI levels from clinically significant High to Low, thereby improving prognosis, and vice versa for the Low to High category.

The study confirmed that risk groups for macrophage activation included those with a history of transplantation, female recipients, and biopsies performed later in the course. Patients with previous transplantations exhibited an increased risk of graft rejection, possibly due to the development of antibodies against previous grafts (131). Elevated GMI levels in women might be related to higher risk of rejection among women, potentially linked to antibodies developed during previous pregnancies (95, 132).

The findings indicated a significant increase in the risk of graft loss when the time from transplantation to biopsy was longer. This aligns with the increased incidence of ABMR versus TCMR observed later on posttransplant (96) and our earlier findings about higher GMI in ABMR compared to TCMR (69). The effect of the change in GMI between the first and second biopsy on graft survival persisted as statistically significant even after adjusting for other variables.

Sub-analysis of histological findings in relation to GMI category changes revealed that the High-High category was associated with consistent histological findings, showing over 60% rejection in both biopsies. Within the group experiencing rejection, a tendency of shift from TCMR to ABMR was observed, suggesting a need for reassessment of immunosuppressive protocols.

A similar intensified histological pattern was noted for those changing from the GMI category Low to High, with chronic damage and infections in the first biopsy transitioning to rejections in the second biopsy, emphasizing the potential impact of tapered or insufficient immunosuppression.

Conversely, the High to Low category exhibited an inverse histological finding with fewer rejections in the second biopsy, though the relationship to therapy intensity remains unclear without data on immune suppression doses. Those in the Low to Low GMI category had a low proportion of rejections in both biopsies, mainly acute TCMR, consistent with previous data indicating lower GMI levels in TCMR compared to ABMR (69).

The present study demonstrates that extent of macrophage involvement might change in some transplants and when reduced from clinically significant High to Low levels it can positively affect graft survival. In conclusion the findings indicate that high or increasing levels of GMI between first and second biopsy are associated with worse graft survival, while low or decreasing levels are associated with better graft survival. This suggests that clinicians by monitoring GMI and reducing levels might improve prognosis of kidney graft survival.

## 5.2 METHODOLOGICAL CONSIDERATIONS

Prospective studies and randomized clinical trials on transplanted patients are difficult to design and perform requiring great efforts and ethical considerations. When studying effects of treatments, in prospective observational studies, it is often difficult to achieve similar case-mix and comparable groups as treatment is usually prescribed or adjusted on indication. Most research about kidney transplanted patients is registry based as randomized clinical trials and prospective studies are, for various reasons, difficult to perform. However, baseline and follow-up data for transplanted patients are usually included in quality registries administrated by transplant centra, enabling the creating of pseudo-prospective studies as chronologically collected data is available from the registries. Most published studies in the field are observational studies as interventions, change of treatment, drug dose etc. rise ethical issues. Another example of difficulties is when studies need to include kidney biopsies for study reasons, so called protocol biopsies, which may require great practical efforts to achieve an eligible sample size, but it can also be difficult due to ethical considerations related to risk for complications and patient distress. Another aspect that makes it difficult to study biopsy findings or treatment effects is achieving a standardized time of performing biopsy, or standardized time for introducing or changing treatment in relation to different outcomes.

Sparse registrations and extent of unregistered follow-up visits in TIGER may vary across different centers. However, missing data due to loss of follow-up is assumed to be due practical reasons as lack of time or resources at specific hospitals. Hence, the missing data assumes be independent of outcome and therefore “missing at random”. The influence on the results should accordingly be limited.

All four papers are based on registry data (historical cohort data) and have an observational, retrospective study design. Causality between possible risk factors and outcome is difficult to establish based on observational studies but still, it is possible to observe, detect and try to interpret associations between different variables. Criteria for establishing causality were described in section 1.8.

When several statistical tests are performed (multiple testing) there is a higher risk for false findings and type I error. However, the intention in the present studies was to limit the number of statistical tests and to be cautious with drawing conclusions based on a single p-value.

Several additional models and sensitivity analyses were performed to test the robustness of the findings.

## *Study I*

The study is a retrospective cohort study where a historical cohort was selected based on registry data. The transplanted patients included in the cohort were followed for a long time after transplantation and several variables and possible covariates were included in the analysis. Only the first kidney transplant was included for each patient to avoid dependency between observations. Death of any reason was a censoring point in Cox-regression, also investigated as competing event by competing-regression model. The results of the competing regression model confirmed the findings from the primary model (Cox-regression). More variables and possible covariates were included in an additional analysis of the last transplantation period.

TIGER-registry coverage is very high and almost all transplanted patients are registered at baseline containing pre-transplant and transplantation variables. Therefore, selection bias should not be a major issue in this case. However, missing data concerning loss of follow-up is more frequent (varying between 10–40% at some hospitals) but is assumed to be related to organizational problems at some hospitals due to lack of resources for data registration. Thus, this presence of missing data should not be related to patient outcome, it is likely to be "missing at random" and is therefore unlikely to have major impact on the results. However, many such patients often have partial follow-up being included in the survival analyses but are censored at latest known follow-up date.

For additional sub-analysis where biopsy data was used, the possibility of selection bias cannot be precluded as most of the biopsies were performed on indication. Another issue with this biopsy-based variable is that it originated from assessment post baseline (transplantation date) and including of such variables in a survival model might introduce a bias for survival times. However, this biopsy variables were only used in an extended multivariable model that confirmed findings about sex differences based on the entire transplanted population.

Variable about medication (IST) was included in the models based on the discharge protocol, which may not correspond to actual treatment later on. However, it was most convenient for the survival analysis to use a variable

close to baseline as variables including time after baseline are not eligible to be included in the survival models as it can introduce a bias for survival time. There was missing data in some cases regarding treatment at discharge (19% of all patients) but the proportion of missing data was similar among men and women. The proportion of missing data was lower during the last time period (3.5% among women and 3.7% among men). If this missing data is "missing at random" is unknown but as the proportion of missing data was low in the last time period, it is not expected to be involved in any vast impact on the results.

It cannot be precluded that unmeasured confounding factors might have affected the findings, such as organ quality, ethnicity, genetic factors, pregnancy, blood transfusions, donor specific data etc.

### *Study II-IV*

In papers II-IV different variables based on kidney biopsies and their association to graft survival was studied. However, a majority of the biopsies were indication biopsies which are a diagnostic tool used when clinicians suspect renal failure or deterioration of kidney function. Thus, the fact that these biopsies are done on indication might introduce a selection bias. Therefore, the results based on studying this selection of the kidney patients should be interpreted cautiously concerning generalizability. On the other hand, these are real-world data that are observed in clinical settings for patients with increased risk for graft loss. Hence, although external validity of the studies II-IV is questionable the internal validity should be high and the knowledge about biopsy-proven variables and their association to graft survival can guide clinicians and facilitate their decisions to prevent graft loss.

Time passed since transplantation at the time of biopsy was related to both biopsy findings and graft survival which means that it might be a confounder. Therefore, the effect on findings of this variable for time since transplantation was investigated in a sensitivity analysis consisting of a multivariable Cox-regression.

Donor specific data, panel reactivity antibodies (PRA) and donor specific antibodies (DSA) were not registered in the TIGER-registry during the study period. This means that there was no possibility to study these factors or the degree of HLA matches (HLA = human leukocyte antigens) between donors and recipients.

The present studies lack data about history of sensitizing events such as history of pregnancy (pre-transplant and post-transplant) and blood transfusions.

Despite revealing several biopsy-proven diagnostic findings, only the primary diagnosis was used for data analysis, potentially missing interactive pathological mechanisms. The study did not incorporate information on doses and changes in pharmacological management, assuming adherence to established routines at the transplant center.

In study III, increasing GMI was shown to be associated with higher risk for graft loss. As we discussed earlier in section 1.8 the causality is difficult to demonstrate with the present study design. However, according to the Bradford-Hill-criteria mentioned in section 1.8 there are some conditions that can strength assumption about causality. Dose-response association between GMI-levels and graft loss is one such criteria that was observed and fulfilled. Also, some other criteria as time sequence, between the explanatory variable and the outcome and coherency with previous findings, were fulfilled.

Limitations of this study include the extended study period with varied immunosuppression protocols, the absence of biopsy re-evaluations, and reliance on registry data. Concerns may arise regarding the time after transplantation, encompassing biopsies within 2 weeks to several years. Also after adjusting for time after transplantation in a multivariate model, our findings still indicate an elevated risk of graft loss for increasing GMI-levels. The strength of our study lies in the accumulation of a substantial number of consecutive and prospective GMI values over a decade.

In the last study a selection of patients with at least two biopsies was studied. This entails that patients with graft loss after the first biopsy or patients who did not survive are not included in the sample. Thus, the studied population is a selection that have survived and without graft loss after transplantation or after first biopsy introduce a selection bias. Another limitation was that time between the first and second biopsy was not standardized and varied widely, and our assumption was that GMI change between first and second biopsy was linear. However, by investigating this selected patient population we observed some new findings that can facilitate further research and hypothesis to be tested. Hence, although external validity of the study IV is questionable the study has brought some light on histological changes between first and second biopsy and the association to graft survival.

Nevertheless, the strength of the studies lies in the large number of biopsies and transplanted patients, facilitating analyses of sub-diagnoses in relation to both graft survival and the time between transplantation and biopsy.

# 6 FUTURE PERSPECTIVES

In the first study we have observed that graft survival in general has improved over time but in the last study period 2006–2017 women had shorter graft survival compared to men. Further studies should be done to confirm and explore possible explanatory factors for the difference.

Data about type of IST was available in the studies but exact time points of changes in medication, doses, concentration in blood and adherence to treatment were not registered. Future studies should aim at clarifying these variables to improve outcome of graft survival also in women.

The present studies lack data about history of sensitizing events such as: history of pregnancy (pre-transplant and post-transplant) and blood transfusions etc. Therefore, future studies should analyze if worse graft survival among women is related to a higher extent of sensitization among women, in combination with age as a variable, since older patients with grafts are more prevalent during the last decades.

Since prospective studies are difficult to carry out, available registry data for comparison of kidney graft outcome, in combination with data from patient records makes it possible to design pseudo-prospective studies. In our research group we plan a study that includes data from patient records about; type of immunosuppressive treatment; dose; time for insertion and tapering/withdrawal of treatment; the effects on graft survival and kidney function.

In the third study the association between biopsy-based glomerular macrophage index (GMI), a biomarker for inflammatory processes in the transplant, and graft survival was investigated. The increased levels of GMI were found to be strongly associated with worsened graft survival. This marker could, independent of the histological diagnosis, predict outcome. GMI can therefore guide therapeutic decisions, especially if the histological finding is less precise.

In the fourth study a subgroup of patients with two consecutive biopsies were included to study association of change between the first and second biopsy and the association to graft survival. Although there are patients with a considerable change in GMI-levels, for most patients GMI is unchanged. High levels of GMI and categories where GMI increases where associated to higher risk for graft loss compared to groups with low or decreasing GMI-level. The course of GMI-change after a second biopsy might therefore guide therapeutic

decisions. Probably also the efficacy of previous prescription largely independent of the histological finding, especially if it is less precise.

Reliability of GMI-measurements and histology evaluations seems to be high but should be investigated in a future study. A sample of biopsies could be evaluated by two different pathologists to investigate inter-reliability. Also intra-rater reliability (repeatability) in the sense that the same rater evaluates a set of biopsies two times (test-retest) would be possible to perform relatively easy. On the other hand, investigating reliability in the sense of repeating entire procedure for study purpose, including at least two biopsies performed shortly after each other on the same transplant (biopsy and re-biopsy) would likely be controversial due to ethical considerations and risks associated with the biopsies.

Donor specific data, panel reactivity antibodies (PRA) and donor specific antibodies (DSA) were not registered in the TIGER-registry during the study period. This means that there was no possibility to study these factors or degrees of HLA matches (HLA = human leukocyte antigens) between donors and recipients. Future studies should investigate the importance of such variables and relate them to graft survival and the findings in the present studies.

There are several questions suitable for further research – both epidemiological and clinical. In the study we found a higher risk for graft loss among women. As a next step it would be natural to confirm the results with a patient group from another population of kidney transplanted patients, for example from another transplantation centra in Sweden. Also, sensitization issues need to be investigated in more depth as possible confounding factors for the observed sex difference. Research about sensitization due to blood transfusions, previous pregnancies and prior transplantations is sparse as well as association to graft survival. This might be investigated through, for example, a case-control study design with graft loss patients as case group. Merging transplantation data with the Swedish Pregnancy Register can be considered to catch data about previous pregnancies.

Another question that needs to be explored is whether the dose of immunosuppressive drugs and medical concentration in the blood is adequate in similar extent for women and for men. Drug dose and change of type of drug as well as change of dose should be investigated to find possible associations to graft survival and graft function. In a future study we will try to bring some light to these questions. However, this will require the study of medical records requiring a new ethical approvement. Drug dose and change of drugs in

relation to different histological findings as biopsy-proven diagnosis and GMI would also be interesting to study.

The results in last study strengthen the usefulness of GMI as an adjunct predictor for graft survival besides histological classification. GMI gives an estimation of the immunological play between the host and the graft and even better when follow up analysis of GMI are present. The last study indicate that the extent of macrophage involvement may change in some grafts and if reduced from High to Low levels be associated with less graft loss. This indicates that the clinician should aim to lower the extent of macrophage involvement. Whether improvement and deterioration of GMI can be explained by prior changes in treatment is one research question that would be important to investigate.

# ACKNOWLEDGEMENT

First, I want to thank **Björn Peters**, my main supervisor. Thank you for sharing your knowledge, positive attitude to research, generosity with your time, and always prompt feedback. Your never-ending energy is incredible!

**Bernd Stegmayr**, my co-supervisor. Thank you for your supporting and always uplifting manner that helped me when research felt stressing. Your knowledge, clinical experience in the field and supervising skills have been incredibly helpful. Special thanks to you and Birgitta for arranging accommodation for us in your cottage during our research weeks in Umeå.

**Marie Eriksson**, co-supervisor. I am very grateful for your expertise advice and that I had the possibility to discuss statistical and other methodological issues with you. Thank you for prompt feedback and always having time for me. I knew that a cool supervisor from Norrland was right for me.

I am grateful to all supportive colleagues at FoUUI, Skaraborg hospital and in particular my bosses **Elisabeth Kenne Sarenmalm** and **Johanna Låstberg** for always being supportive and encouraging. Elisabeth is also my co-supervisor and have supported me in many ways. Your knowledge about the research- and university world has been priceless. Further, I express my gratefulness to **Johanna**, for language review and cautious reading of the thesis as well as huge help with lay-out in the manuscript.

**Anna-Lena Emanuelsson Loft**, research secretary. Thank you for your help with the manuscript and for solving different practical problems.

**Johan Mölne**, co-author. Thank you for your excellent support and help with interpreting and understandings histological findings but also constructive input and contributions in composing the papers and the thesis.

**Henri Afghahi** and **Anna Wärme**. I appreciate your help, discussions, and explanations regarding clinical issues.

My parents. I try to carry with me your words “You can lose your property, but your skills and knowledge are always with you and are the most valuable”. My mother **Zekija –** I wish you were still here.

Last but not least, thank you my dear wife **Mirzeta Solo Nasic**, for your support, patience and for tolerating my seclusion during some days. Also,

thanks to **Alma**, **Edin** and **Ishak** for being there and reminding me of what really is important in life.

My thesis is based on quality registry data, and I would like to express my gratitude to all the patients who were included in the registries and all medical staff who reported data in the registries. Thanks to Transplantation Centre at Sahlgrenska University Hospital, Gothenburg where quality registry TIGER is administrated and to Regional kidney biopsy registry.

# REFERENCES


1. Makris K, Spanou L. Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes. Clinical biochemist reviews. 2016;37(2):85-98.
2. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders H-J. Acute kidney injury. Nature reviews Disease primers. 2021;7(1):52.
3. Susantitaphong P, Cruz DN, Cerda J, Abulfaraj M, Alqahtani F, Koulouridis I, et al. World Incidence of AKI: A Meta-Analysis. Clinical journal of the American Society of Nephrology. 2013;8(9):1482-93.
4. de Abreu KLS, Silva Júnior GB, Barreto AGC, Melo FM, Oliveira BB, Mota RMS, et al. Acute kidney injury after trauma: Prevalence, clinical characteristics and RIFLE classification. Indian journal of critical care medicine. 2010;14(3):121-8.
5. Mohammed ZA, Suresh AA, Kumar P, Attur RP. Acute Kidney Injury: Prevalence and Outcomes in Southern Indian Population. Journal of clinical and diagnostic research. 2018;12(5):LC05-LC10.
6. Martin-Cleary C, Miguel Molinero-Casares L, Ortiz A, Miguel Arce-Obieta J. Development and internal validation of a prediction model for hospital-acquired acute kidney injury. Clinical kidney journal. 2021;14(1):309-16.
7. Del Giudice A, Aucella F. Acute renal failure in the elderly: epidemiology and clinical features. Journal of nephrology. 2012;25 Suppl 19(Suppl. 19):S48-57.
8. Bagshaw SM. Short- and long-term survival after acute kidney injury. Nephrology, dialysis, transplantation. 2008;23(7):2126-8.
9. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. Kidney international. 2008;73(5):538-46.
10. Sawhney S, Mitchell M, Marks A, Fluck N, Black C. Long-term prognosis after acute kidney injury (AKI): what is the role of baseline kidney function and recovery? A systematic review. BMJ open. 2015;5(1):e006497-e.
11. Metnitz PGH, Krenn CG, Steltzer H, Lang T, Ploder J, Lenz K, et al. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. Critical care medicine. 2002;30(9):2051-8.
12. Uchino S, Bellomo R, Oudemans-Van Straaten H, Ronco C, Kellum JA, Morimatsu H, et al. Continuous renal replacement therapy: A worldwide practice survey : The beginning and ending supportive therapy for the kidney (B.E.S.T. Kidney) investigators. Intensive care medicine. 2007;33(9):1563-70.
13. Heung M, Chawla LS. Acute Kidney Injury: Gateway to Chronic Kidney Disease. Nephron Clinical practice. 2014;127(1-4):30-4.

14. Fox CS, Larson MG, Leip EP, Culleton B, Wilson PWF, Levy D. Predictors of New-Onset Kidney Disease in a Community-Based Population. JAMA : the journal of the American Medical Association. 2004;291(7):844-50.
15. Tuttle KR, Alicic RZ, Duru OK, Jones CR, Daratha KB, Nicholas SB, et al. Clinical Characteristics of and Risk Factors for Chronic Kidney Disease Among Adults and Children: An Analysis of the CURE-CKD Registry. JAMA network open. 2019;2(12):e1918169.
16. Yamagata K, Ishida K, Sairenchi T, Takahashi H, Ohba S, Shiigai T, et al. Risk factors for chronic kidney disease in a community-based population: a 10-year follow-up study. Kidney international. 2007;71(2):159-66.
17. Jonsson AJ, Lund SH, Eriksen BO, Palsson R, Indridason OS. Incidence of and risk factors of chronic kidney disease: results of a nationwide study in Iceland. Clinical kidney journal. 2022;15(7):1290-9.
18. Hallan SI, Coresh J, Astor BC, Asberg A, Powe NR, Romundstad S, et al. International comparison of the relationship of chronic kidney disease prevalence and ESRD risk. Journal of the American Society of Nephrology. 2006;17(8):2275-84.
19. Aurell M, Samuelsson O, Guron G, Saeed A. Njurmedicin. Femte upplagan / under redaktion av Mattias Aurell, Ola Samuelsson, Gregor Guron, Aso Saeed ed: Stockholm : Liber; 2020.
20. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. Kidney international. 2014;85(1):49-61.
21. Locke JE. Handbook of Kidney Transplantation. Sixth Edition. G. M. Danovitch (Editor) Wolters Kluwar, 2017, 606pp. ISBN: 9781496326157. 2018;18(7):1820-.
22. Robinson BMD, Akizawa TP, Jager KJP, Kerr PGP, Saran RP, Pisoni RLP. Factors affecting outcomes in patients reaching end-stage kidney disease worldwide: differences in access to renal replacement therapy, modality use, and haemodialysis practices. The Lancet. 2016;388(10041):294-306.
23. Nilsson-Ehle P. Iohexol Clearance for the Determination of Glomerular Filtration Rate: 15 Years' Experience in Clinical Practice. Ejifcc. 2001;13(2):48-52.
24. Miller G, Myers GL, Eckfeldt JH, Greenberg N. Calculating eGFR using the MDRD equation. QJM : monthly journal of the Association of Physicians. 2007;100(2):142-3.
25. Trans JG, Krogstrup NV, Oltean M, Jespersen B, Nielsen MB, Birn H. A comparison of four established GFR formulas to estimate measured GFR and changes in GFR in adult kidney transplant recipients. Scandinavian journal of clinical and laboratory investigation. 2022;82(4):296-303.

26. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C–Based Equations to Estimate GFR without Race. The New England journal of medicine. 2021;385(19):1737-49.
27. Miller WG, Kaufman HW, Levey AS, Straseski JA, Wilhelms KW, Yu H-Y, et al. National Kidney Foundation Laboratory Engagement Working Group Recommendations for Implementing the CKD-EPI 2021 Race-Free Equations for Estimated Glomerular Filtration Rate: Practical Guidance for Clinical Laboratories. Clinical chemistry (Baltimore, Md). 2022;68(4):511-20.
28. Levey AS, Stevens LA, Coresh J, Schmid CH, Yaping Z, Castro AF, et al. A New Equation to Estimate Glomerular Filtration Rate. Annals of internal medicine. 2009;150(9):604-12.
29. Montañés Bermúdez R, Bover Sanjuán J, Oliver Samper A, Ballarín Castán JA, Gràcia García S. Assessment of the new CKD-EPI equation to estimate the glomerular filtration rate. Nefrología. 2010;30(2):185-94.
30. Iqbal MM, Rahman N, Alam M, Deb Nath PK, Waheed S, Islam K, et al. Quality of Life Is Improved in Renal Transplant Recipients Versus That Shown in Patients With Chronic Kidney Disease With or Without Dialysis. Experimental and clinical transplantation. 2020;18(Suppl 1):64-7.
31. Scandiatransplant 2023 [Available from: http://www.scandiatransplant.org/data/scandiatransplant-figures.
32. Transplantationscentum Göteborg 2022 [Available from: https://www.sahlgrenska.se/omraden/omrade-5/verksamhet-transplantationscentrum/vardgivare/njurtransplantation/.
33. Svenskt Njurregister (SNR) 2023 [Available from: https://www.medscinet.net/snr/page.aspx?id=7.
34. Peters B, Andersson Y, Stegmayr B, Mölne J, Jensen G, Dahlberg P, et al. A study of clinical complications and risk factors in 1001 native and transplant kidney biopsies in Sweden. Acta radiologica (1987). 2014;55(7):890-6.
35. Schwarz A, Gwinner W, Hiss M, Radermacher J, Mengel M, Haller H. Safety and Adequacy of Renal Transplant Protocol Biopsies. American journal of transplantation. 2005;5(8):1992-6.
36. Fiorentino M, Bolignano D, Tesar V, Pisano A, Van Biesen W, D"Arrigo G, et al. Renal Biopsy in 2015 - From Epidemiology to Evidence-Based Indications. American journal of nephrology. 2016;43(1):1-19.
37. Poggio ED, Augustine JJ, Arrigain S, Brennan DC, Schold JD. Long-term kidney transplant graft survival—Making progress when most needed. American journal of transplantation. 2021;21(8):2824-32.
38. Lopez V, Cabello M, Ruiz P, Alonso J, Hernandez D. MP699 GRAFT SURVIVAL IN KIDNEY TRANSPLANT EXPANDED CRITERIA DONORS: ANALYSIS OF TWO PERIODS. Nephrology Dialysis Transplantation. 2016;31(suppl1):i572-i.

39. Meier-Kriesche H-U, Ojo AO, Port FK, Arndorfer JA, Cibrik DM, Kaplan B. Survival improvement among patients with end-stage renal disease: Trends over time for transplant recipients and wait-listed patients. Journal of the American Society of Nephrology. 2001;12(6):1293-6.
40. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bhave N, Bragg-Gresham J, et al. US Renal Data System 2017 Annual Data Report: Epidemiology of Kidney Disease in the United States. American journal of kidney diseases. 2018;71(3):A7-A.
41. Hariharan S, Israni AK, Danovitch G. Long-Term Survival after Kidney Transplantation. N Engl J Med. 2021;385(8):729-43.
42. Lim J-H, Lee S-H, Lee YH, Lee JP, Yang J, Kim MS, et al. Analysis of time trends in preemptive kidney transplantation and effect of pre-transplant dialysis duration on graft survival: a nationwide cohort study. Korean journal of transplantation (Online). 2020;34(1):S42-S.
43. Foroutan F, Friesen EL, Clark KE, Motaghi S, Zyla R, Lee Y, et al. Risk Factors for 1-Year Graft Loss After Kidney Transplantation: Systematic Review and Meta-Analysis. Clinical journal of the American Society of Nephrology. 2019;14(11):1642-50.
44. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, et al. Banff 07 Classification of Renal Allograft Pathology: Updates and Future Directions. American journal of transplantation. 2008;8(4):753-60.
45. Fortun J, Martin-Davila P, Pascual J, Cervera C, Moreno A, Gavalda J, et al. Immunosuppressive therapy and infection after kidney transplantation. Transplant infectious disease. 2010;12(5):397-405.
46. Almeida CC, Silveira MR, de Araújo VE, de Lemos LLP, de Oliveira Costa J, Reis CAL, et al. Safety of immunosuppressive drugs used as maintenance therapy in kidney transplantation: a systematic review and meta-analysis. Pharmaceuticals. 2013;6(10):1170-94.
47. Joyce E, Glasner P, Ranganathan S, Swiatecka-Urban A. Tubulointerstitial nephritis: diagnosis, treatment, and monitoring. Pediatric nephrology (Berlin, West). 2017;32(4):577-87.
48. Farouk SS, Rein JL. The Many Faces of Calcineurin Inhibitor Toxicity-What the FK? Adv Chronic Kidney Dis. 2020;27(1):56-66.
49. Samant H VP, Kothadia JP. Posttransplant Lymphoproliferative Disorders StattPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [Available from: https://www.ncbi.nlm.nih.gov/books/NBK513249/.
50. Uppal NN, Monga D, Shah HH. Glomerular Disease After Kidney Transplantation. Cham: Cham: Springer International Publishing; 2019. p. 787-808.
51. Abbas F, El Kossi M, Jin JK, Sharma A, Halawa A. De novo glomerular diseases after renal transplantation: How is it different from recurrent glomerular diseases? World J Transplant. 2017;7(6):285-300.

52. Park SH, Friedewald JJ. Are borderline changes real rejection? Current viewpoints. Curr Opin Nephrol Hypertens. 2020;29(6):656-62.
53. Loupy A, Haas M, Roufosse C, Naesens M, Adam B, Afrouzian M, et al. The Banff 2019 Kidney Meeting Report (I): Updates on and clarification of criteria for T cell– and antibody-mediated rejection. American journal of transplantation. 2020;20(9):2318-31.
54. Halloran PF, Reeve JP, Pereira AB, Hidalgo LG, Famulski KS. Antibody-mediated rejection, T cell–mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. Kidney international. 2014;85(2):258-64.
55. MÖLne J, Nyberg G, BlohmÉ I, Rydberg L, Breimer ME, Svalander CT. Glomerular C3c deposition and intravascular macrophage accumulation in early humoral renal allograft rejection signifies a poor short-term outcome. APMIS : acta pathologica, microbiologica et immunologica Scandinavica. 2006;114(10):700-11.
56. Nickeleit V, Mihatsch MJ. Kidney transplants, antibodies and rejection: is C4d a magic marker? Nephrology, dialysis, transplantation. 2003;18(11):2232-9.
57. Corrêa RRM, Machado JR, da Silva MV, Helmo FR, Guimarães CSO, Rocha LP, et al. The Importance of C4d in Biopsies of Kidney Transplant Recipients. Clinical & developmental immunology. 2013;2013:678180-8.
58. Solez K, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, et al. International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. Kidney international. 1993;44(2):411-22.
59. Loupy A, Haas M, Solez K, Racusen L, Glotz D, Seron D, et al. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. American journal of transplantation. 2017;17(1):28-41.
60. Roufosse C, Naesens M, Haas M, Lefaucheur C, Mannon RB, Afrouzian M, et al. The Banff 2022 Kidney Meeting Work Plan: Data-driven Refinement of the Banff Classification for Renal Allografts. American journal of transplantation. 2023.
61. Collin M, Bigley V. Monocyte, Macrophage, and Dendritic Cell Development: the Human Perspective. Microbiology spectrum. 2016;4(5).
62. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of Monocytes, Macrophages, and Dendritic Cells. Science (American Association for the Advancement of Science). 2010;327(5966):656-61.
63. Bergler T, Jung B, Bourier F, Kühne L, Banas MC, Rümmele P, et al. Infiltration of Macrophages Correlates with Severity of Allograft Rejection and Outcome in Human Kidney Transplantation. PloS one. 2016;11(6):e0156900-e.

64. Magil AB. Monocytes/macrophages in renal allograft rejection. Transplantation reviews (Philadelphia, Pa). 2009;23(4):199-208.
65. Magil AB, Tinckam K. Monocytes and peritubular capillary C4d deposition in acute renal allograft rejection super(1). Kidney international. 2003;63(5):1888-93.
66. Molne J, Nyberg G, Blohme I, Rydberg L, Breimer ME, Svalander CT. Glomerular C3c deposition and intravascular macrophage accumulation in early humoral renal allograft rejection signifies a poor short-term outcome. APMIS : acta pathologica, microbiologica et immunologica Scandinavica. 2006;114(10):700-11.
67. Magil AB. Monocytes in human glomerulonephritis. An electron microscopic study. Lab Invest. 1981;45:77-81.
68. ÖZdemir BH, Demirhan B, GÜNgen Y. The presence and prognostic importance of glomerular macrophage infiltration in renal allografts. Nephron. 2002;90(4):442-6.
69. Mölne J, Nasic S, Bröcker V, Stegmayr B, Felldin M, Peters B. Glomerular macrophage index (GMI) in kidney transplant biopsies is associated with graft outcome. Clinical transplantation. 2022;36(12):e14816-n/a.
70. Hardinger KL, Brennan DC, Klein CL. Selection of induction therapy in kidney transplantation. Transplant international. 2013;26(7):662-72.
71. Lasmar MF, Dutra RS, Nogueira-Machado JA, Fabreti-Oliveira RA, Siqueira RG, Nascimento E. Effects of immunotherapy induction on outcome and graft survival of kidney-transplanted patients with different immunological risk of rejection. BMC nephrology. 2019;20(1):314-.
72. Muntean A, Lucan M. Immunosuppression in kidney transplantation. Clujul medical. 2013;86(3):177-80.
73. Simmons RG, Abress L, Anderson CR. Quality of life after kidney transplantation: a prospective, randomized comparison of cyclosporine and conventional immunosuppressive therapy. Transplantation. 1988;45(2):415-21.
74. Altman DG. Practical statistics for medical research: London : Chapman and Hall; 1991.
75. Björk J. Praktisk statistik för medicin och hälsa. Första upplagan, [ny tryckning] ed: Stockholm : Liber; 2020.
76. Hill AB. The environment and disease: association or causation? Journal of the Royal Society of Medicine. 2015;108(1):32-7.
77. Rothman KJ, Greenland S. Causation and Causal Inference in Epidemiology. American journal of public health (1971). 2005;95(S1):S144-S50.
78. Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. International journal of epidemiology. 2013;42(5):1511-9.

79. Song JW, Chung KC. Observational Studies: Cohort and Case-Control Studies. Plastic and reconstructive surgery (1963). 2010;126(6):2234-42.
80. Carlson MDA, Morrison RS. Study design, precision, and validity in observational studies. Journal of palliative medicine. 2009;12(1):77-82.
81. Murad MH, Asi N, Alsawas M, Alahdab F. New evidence pyramid. Evidence-based medicine (English ed). 2016;21(4):125-7.
82. Noordzij M, Dekker FW, Zoccali C, Jager KJ. Measures of Disease Frequency: Prevalence and Incidence. Nephron (2015). 2010;115(1).
83. Harrell FE. Regression modeling strategies : with applications to linear models, logistic and ordinal regression, and survival analysis. Second edition. ed: Cham : Springer; 2015.
84. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. British journal of cancer. 1976;34(6):585-612.
85. Cox DR. Analysis of survival data. Oakes D, editor: London : Chapman and Hall; 1984.
86. Katz MH, Hauck WW. Proportional hazards (cox) regression. Journal of general internal medicine : JGIM. 1993;8(12):702-11.
87. Schober P, Vetter TR. Kaplan-Meier Curves, Log-Rank Tests, and Cox Regression for Time-to-Event Data. Anesthesia and analgesia. 2021;132(4):969-70.
88. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.
89. Stensrud MJ, Hernán MA. Why Test for Proportional Hazards? JAMA : the journal of the American Medical Association. 2020;323(14):1401-2.
90. Akobeng AK. Understanding type I and type II errors, statistical power and sample size. Acta Paediatrica. 2016;105(6):605-9.
91. Austin PC, White IR, Lee DS, van Buuren S. Missing Data in Clinical Research: A Tutorial on Multiple Imputation. Canadian journal of cardiology. 2021;37(9):1322-31.
92. Heymans MW, Twisk JWR. Handling missing data in clinical research. Journal of clinical epidemiology. 2022;151:185-8.
93. Bennett DA. How can I deal with missing data in my study? Australian and New Zealand journal of public health. 2001;25(5):464-9.
94. Sellarés J, De Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence. American Journal of Transplantation. 2012;12(2):388-99.
95. Nasic S, Peters B, Stegmayr B, Kenne Sarenmalm E, Afghahi H, Eriksson M. Sex-specific time trends of long-term graft survival after kidney transplantation – a registry-based study. Renal failure. 2023;45(2):2270078-.

96. Nasic S, Mölne J, Stegmayr B, Peters B. Histological diagnosis from kidney transplant biopsy can contribute to prediction of graft survival. Nephrology (Carlton). 2022;27(6):528-36.
97. Momper JD, Misel ML, McKay DB. Sex Differences in Transplantation. Transplantation reviews (Philadelphia, Pa). 2017;31(3):145-50.
98. Melk A, Babitsch B, Borchert-Mörlins B, Claas F, Dipchand AI, Eifert S, et al. Equally Interchangeable? How Sex and Gender Affect Transplantation. Transplantation. 2019;103(6):1094-110.
99. Sancho A, Gavela E, Kanter J, Beltrán S, Castro C, Escudero V, et al. Graft survival differences in kidney transplants related to recipient sex and age. Frontiers in medicine. 2022;9:962094-.
100. Chen P-D, Tsai M-K, Lee C-Y, Yang C-Y, Hu R-H, Lee P-H, et al. Gender differences in renal transplant graft survival. Journal of the Formosan Medical Association. 2013;112(12):783-8.
101. Tan JC, Wadia PP, Coram M, Grumet FC, Kambham N, Miller K, et al. H-Y Antibody Development Associates With Acute Rejection in Female Patients With Male Kidney Transplants. Transplantation. 2008;86(1):75-81.
102. Denhaerynck K, Steiger J, Bock A, Schafer-Keller P, Kofer S, Thannberger N, et al. Prevalence and Risk Factors of Non-Adherence with Immunosuppressive Medication in Kidney Transplant Patients. American journal of transplantation. 2007;7(1):108-16.
103. Gandolfini I, Palmisano A, Fiaccadori E, Cravedi P, Maggiore U. Detecting, preventing and treating non-adherence to immunosuppression after kidney transplantation. Clinical kidney journal. 2022;15(7):1253-74.
104. Gokoel SRM, Gombert-Handoko KB, Zwart TC, van der Boog PJM, Moes DJAR, de Fijter JW. Medication non-adherence after kidney transplantation: A critical appraisal and systematic review. Transplantation reviews (Philadelphia, Pa). 2019;34(1):100511-.
105. Morrissey PE, Flynn ML, Lin S. Medication Noncompliance and its Implications in Transplant Recipients. Drugs. 2007;67(10):1463-81.
106. Vlaminck H, Maes B, Evers G, Verbeke G, Lerut E, Van Damme B, et al. Prospective Study on Late Consequences of Subclinical Non-Compliance with Immunosuppressive Therapy in Renal Transplant Patients. American journal of transplantation. 2004;4(9):1509-13.
107. Scheel JF, Schieber K, Reber S, Stoessel L, Waldmann E, Jank S, et al. Psychosocial Variables Associated with Immunosuppressive Medication Non-Adherence after Renal Transplantation. Frontiers in psychiatry. 2018;9:23-.
108. Boucquemont J, Pai ALH, Dharnidharka VR, Hebert D, Furth SL, Foster BJ. Gender Differences in Medication Adherence Among Adolescent and Young Adult Kidney Transplant Recipients. Transplantation. 2019;103(4):798-806.

109. Kattah AG, Albadri S, Alexander MP, Smith B, Parashuram S, Mai ML, et al. Impact of Pregnancy on GFR Decline and Kidney Histology in Kidney Transplant Recipients. Kidney international reports. 2022;7(1):28-35.
110. Majak GB, Reisæter AV, Weedon-Fekjær H, Henriksen T, Michelsen TM. The Effect of Pregnancy on the Long-term Risk of Graft Loss, Cardiovascular Disease, and Death in Kidney Transplanted Women in Norway: A Retrospective Cohort Study. Transplantation. 2018;102(9):e391-e6.
111. van Buren MC, Schellekens A, Groenhof TKJ, van Reekum F, van de Wetering J, Paauw ND, et al. Long-term Graft Survival and Graft Function Following Pregnancy in Kidney Transplant Recipients: A Systematic Review and Meta-analysis. Transplantation. 2020;104(8):1675-85.
112. Oweira H, Ramouz A, Ghamarnejad O, Khajeh E, Ali-Hasan-Al-Saegh S, Nikbakhsh R, et al. Risk Factors of Rejection in Renal Transplant Recipients: A Narrative Review. Journal of clinical medicine. 2022;11(5):1392.
113. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med. 2007;357(25):2562-75.
114. Vinson AJ, Zhang X, Dahhou M, Süsal C, Döhler B, Sapir-Pichhadze R, et al. Age-dependent Sex Differences in Graft Loss After Kidney Transplantation. Transplantation. 2022;106(7):1473-84.
115. Matas AJ, Fieberg A, Mannon RB, Leduc R, Grande J, Kasiske BL, et al. Long-term follow-up of the DeKAF cross-sectional cohort study. Am J Transplant. 2019;19(5):1432-43.
116. Naesens M, Kuypers DR, De Vusser K, Evenepoel P, Claes K, Bammens B, et al. The histology of kidney transplant failure: a long-term follow-up study. Transplantation. 2014;98(4):427-35.
117. Arias-Cabrales C, Redondo-Pachon D, Perez-Saez MJ, Gimeno J, Sanchez-Guerri I, Bermejo S, et al. Renal graft survival according to Banff 2013 classification in indication biopsies. Nefrologia. 2016;36(6):660-6.
118. Nankivell BJ, Shingde M, Keung KL, Fung CL, Borrows RJ, O'Connell PJ, et al. The causes, significance and consequences of inflammatory fibrosis in kidney transplantation: The Banff i-IFTA lesion. Am J Transplant. 2018;18(2):364-76.
119. Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. Transplantation. 2018;102(11):1795-814.
120. Colvin RB. The renal allograft biopsy. Kidney Int. 1996;50(3):1069-82.

121. Magil AB. Infiltrating Cell Types in Transplant Glomerulitis: Relationship to Peritubular Capillary C4d Deposition. American journal of kidney diseases. 2005;45(6):1084-9.
122. Papadimitriou JC, Drachenberg CB, Munivenkatappa R, Ramos E, Nogueira J, Sailey C, et al. Glomerular Inflammation in Renal Allografts Biopsies After the First Year: Cell Types and Relationship With Antibody-Mediated Rejection and Graft Outcome. Transplantation. 2010;90(12):1478-85.
123. Raftery MJ, Seron D, Koffman G, Hartley B, Janossy G, Cameron JS. The relevance of induced class II HLA antigens and macrophage infiltration in early renal allograft biopsies. Transplantation. 1989;48(2):238-43.
124. Strom TB, Tilney NL, Carpenter CB, Busch GJ. Identity and cytotoxic capacity of cells infiltrating renal allografts. N Engl J Med. 1975;292(24):1257-63.
125. Harry TR, Coles GA, Davies M, Bryant D, Williams GT, Griffin PJ. The significance of monocytes in glomeruli of human renal transplants. Transplantation. 1984;37(1):70-3.
126. Om A, Baquero A, Raja R, Kim P, Bannett AD. The prognostic significance of the presence of monocytes in glomeruli of renal transplant allografts. Transplant Proc. 1987;19(1 Pt 2):1618-22.
127. Tinckam KJ, Djurdjev O, Magil AB. Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status. Kidney international. 2005;68(4):1866-74.
128. Srinivas TR, Kubilis PS, Croker BP. Macrophage index predicts short-term renal allograft function and graft survival. Transplant international. 2004;17(4):195.
129. Sentís A, Kers J, Yapici U, Claessen N, Roelofs JJTH, Bemelman FJ, et al. The prognostic significance of glomerular infiltrating leukocytes during acute renal allograft rejection. Transplant immunology. 2015;33(3):168-75.
130. Lefaucheur C, Nochy D, Hill GS, Suberbielle-Boissel C, Antoine C, Charron D, et al. Determinants of Poor Graft Outcome in Patients with Antibody-Mediated Acute Rejection. American journal of transplantation. 2007;7(4):832-41.
131. Assfalg V, Selig K, Tolksdorf J, Meel M, Vries E, Ramsoebhag AM, et al. Repeated kidney re-transplantation—the Eurotransplant experience: a retrospective multicenter outcome analysis. Transplant international. 2020;33(6):617-31.
132. Porrett PM. Biologic mechanisms and clinical consequences of pregnancy alloimmunization. American journal of transplantation. 2018;18(5):1059-67.