Assessing the Complex Causes of Kidney Allograft Loss

Elisabet Van Loon, MD,[1,2] Aleksandar Senev, MD,[1,3] Evelyne Lerut, MD, PhD,[4,5] Maarten
Coemans,[1,6] Jasper Callemeyn, MD,[1,2] Jan M. Van Keer, MD,[1] Liesbeth Daniëls,[3] Dirk
Kuypers, MD, PhD,[1,2] Ben Sprangers, MD, PhD,[2,7] Marie-Paule Emonds, MD, PhD,[1,3] and
Maarten Naesens, MD, PhD[1,2]

[1] Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven,
Belgium;

[2] Department of Nephrology and Renal Transplantation, University Hospitals Leuven,
Leuven, Belgium;

[3] Histocompatibility and Immunogenetic Laboratory, Red Cross-Flanders, Mechelen,
Belgium;

[4] Department of Imaging and Pathology, KU Leuven, Leuven, Belgium;

[5] Department of Pathology, University Hospitals Leuven, Leuven, Belgium.

[6] Leuven Biostatistics and Statistical Bioinformatics Centre, Department of Public Health and
Primary Care, KU Leuven, Leuven, Belgium;

[7] Department of Microbiology and Immunology, Laboratory of Molecular Immunology, Rega
Institute, KU Leuven, Leuven, Belgium

1

Correspondence: Maarten Naesens, MD, PhD, Department of Nephrology and Renal Transplantation, University Hospitals Leuven , Herestraat 49, 3000 Leuven, Belgium. Tel: +32 16 34 45 80; Fax: +32 16 34 45 99, Email: maarten.naesens@kuleuven.be

Abbreviations

AMR	antibody-mediated rejection
HLA-DSA	anti-HLA donor-specific antibodies
FSGS	focal segmental glomerulosclerosis
GNF	glomerulonephritis
IF/TA	interstitial fibrosis/ tubular atrophy
IQR	interquartile range
PNF	primary nonfunction
PVAN	polyomavirus associated nephropathy
TCMR	T cell-mediated rejection

ABSTRACT

Background

Although graft loss is a primary endpoint in many studies in kidney transplantation and a broad spectrum of risk factors has been identified, the eventual causes of graft failure in individual cases remain ill studied.

Methods

We performed a single-center cohort study in 1000 renal allograft recipients, transplanted between March 2004 and February 2013.

Results

In total, 365 (36.5%) graft losses were identified, of which 211 (57.8%) were due to recipient death with a functioning graft and 154 (42.2%) to graft failure defined as return to dialysis or retransplantation. The main causes of recipient death were malignancy, infections and cardiovascular disease. The main causes of graft failure were distinct for early failures, where structural issues and primary nonfunction prevailed, compared to later failures with a shift towards chronic injury. In contrast to the main focus of current research efforts, pure alloimmune causes accounted for only 17.5% of graft failures and only 7.4% of overall graft losses, although 72.7% of cases with chronic injury as presumed reason for graft failure had prior rejection episodes, potentially suggesting that allo-immune phenomena contributed to the chronic injury.

Conclusion

In conclusion, this study provides better insight in the eventual causes of graft failure, and their relative contribution, highlighting the weight of nonimmune causes. Future efforts aimed to improve outcome after kidney transplantation should align with the relative weight and expected impact of targeting these causes.

Introduction

Despite improvements in short-term survival of kidney allografts, this progress was not matched in long-term graft survival.[1] Registry data show relatively limited improvement in death-censored graft failure rates beyond the first year post transplantation since the late 1980s.[1,2] The high rate of graft loss after kidney transplantation illustrates the need for novel initiatives to improve graft survival. However, in order to target graft loss, its causes need to be elucidated.[3]

Recipient death (i.e. death with a functioning graft) accounts for the majority of graft losses,[4,5] but it remains unclear how much of this is premature death.

Graft failure (loss of graft function), usually defined as return to dialysis or retransplantation, is the second cause of graft loss after death with a functioning graft.[6,7] As graft failure is the hard endpoint of many clinical studies in this field, its risk factors have been identified extensively in literature. However, association does not equal causality and too few studies focused on assessing the eventual causes of individual graft failures.

Several cohort studies have given insights in the possible causes of graft failure. These data suggested that immunological processes accounted for 35% to 64% of late kidney allograft losses, with importance of anti-HLA donor-specific antibodies (HLA-DSA).[6-8] However, often no biopsies are performed for late graft failure, which potentially biases the conclusions. In assessing the causes of graft failure, well-known risk factors for end-stage kidney diseases in native kidneys, like arterial hypertension, diabetes, obesity, ageing, reflux, infections, postrenal problems often remain unexplored. The histology of late biopsies illustrated that chronic damage, especially chronic tubulo-interstitial damage (IF/TA), precedes and predicts graft failure.[8] However, tubular atrophy, interstitial fibrosis and glomerulosclerosis are nonspecific hallmarks of nephron loss, irrespective of the underlying cause, and the consequence of multifactorial processes, both immunological and nonimmunological.

4

In this large cohort study of patients followed longitudinally, we aimed to identify the causes of graft loss, defined as (premature) death with a functioning graft or graft failure, using all available clinical information, detailed data on HLA-DSA, and extensive histological information from protocol-specified and clinically indicated biopsies.

Materials and Methods

Study population and data collection

All consecutive adult recipients of a kidney transplant at the University Hospitals-Leuven between March 2004 and February 2013 were eligible for this study. Recipients of combined transplants or kidney transplants after another solid organ transplant were excluded. All transplants were performed with negative complement-dependent cytotoxicity crossmatches. The clinical data were prospectively collected during routine clinical follow-up. This study was approved by the Ethics Committee of the University Hospitals-Leuven (S53364 and S61971).

Biopsies and histological scoring

All posttransplant renal allograft biopsies performed in this cohort, until time of data extraction on December 2018, were included. Protocol-specified biopsies were performed in addition to clinically indicated biopsies. Protocol kidney transplant biopsies were performed at the time of transplantation, and at 3, 12, and 24 months after transplantation. In addition, patients who were transplanted before October 2005 were invited for a protocol biopsy performed at 48 months, patients transplanted before November 2008 for a protocol biopsy at 36 months, and patients transplanted before January 2010 for a protocol biopsy at 60 months. One pathologist (EL) reviewed all biopsies. The severity of the histological lesions was semiquantitatively scored according to the Banff categories. Diagnosis of the histological phenotypes was strictly based on the criteria as defined by the Banff 2017 consensus.[9]

5

Furthermore, borderline changes were defined as foci of tubulitis (t > 0) with minor interstitial inflammation (i1) or moderate-severe interstitial inflammation (i2 or i3) with mild (t1) tubulitis.

Detection of circulating anti-HLA antibodies and HLA genotyping

The pretransplant and posttransplant follow-up of anti-HLA antibodies was systematically monitored in 1 histocompatibility laboratory (HILA – Belgian Red Cross Flanders); details on this assessment were previously published.[10] All sera were routinely screened using a LIFECODES LifeScreen Deluxe (LMX) kit (Immucor Inc., Norcross,GA) and in case of positive screening, the donor-specificity was assessed using LIFECODES Single Antigen Bead (LSA) kits (Immucor). In order to determine true donor specificity, donor DNA samples were genotyped at high-resolution for the HLA -A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, and -DPB1 loci. A positive result for circulation HLA-DSA was defined as a mean fluorescence intensity (MFI) of the donor-specific bead above 500.

Assessment of causes of graft loss

There is no strict definition of 'early' versus 'late' graft loss. Using 1 year after transplantation seems to be the best substantiated threshold based on current clinical research standards where endpoints of graft survival were used,[11-17] and from epidemiological data that illustrate a fundamental difference in general improvement of graft outcome after 1 year.[1,2] Graft loss encompassed either patient death with a functioning graft or graft failure. Premature death was defined as recipient death earlier than expected based on expected remaining lifetimes. Expected remaining lifetimes were estimated for each patient based on Belgian life expectancy tables (www.statbel.fgov.be) for the general population. To compare with life expectancy when the patient would have remained on dialysis, expected remaining lifetimes were estimated for each patient based on age at transplantation and extrapolated from data on prevalent dialysis patients in the same decade as our cohort.[18] Graft failure was

6

defined as irreversible loss of graft function requiring chronic dialysis or retransplantation.

Eight patients lost more than 1 graft during follow-up and each instance was analyzed

individually.

To assess the causes of graft loss, electronic medical records and letters from referring centers

were systematically reviewed, as well as all biopsies performed prior to graft failure. The

causes of graft failure were divided in 9 groups based on the clinical and histological

information (**Table 1**). In some cases, several injurious processes could have contributed to

graft failure. These cases were classified under 1 etiologic group based on the investigators'

best judgment as of the principal cause of graft failure.

Statistical analysis

Variables with normal distribution are displayed as mean ± standard deviation. For variables

without normal distribution median and interquartile range [IQR] are given. For variance

analysis of continuous clinical variables in different groups, T-test and parametric 1-way

ANOVA were used. Dichotomous variables were compared using the chi-square test. For

plotting cause-specific survival analysis of competing risks we used cumulative incidence

functions. We used SAS software (version 9.4; SAS Institute Inc., Cary, NC) for statistical

analysis and GraphPad Prism (version 8; GraphPad Software, San Diego, CA) for data

presentation.

Results

Study population characteristics

Between 2004 and 2013, 1137 patients were transplanted. Patients with combined transplants

or with a history of another organ transplantation before kidney transplantation were

excluded, leaving 1000 transplantations in 969 recipients available for this study (**Figure 1**).

The median follow-up time was 7.49 [IQR 4.91-10.02] years. Baseline and clinical

characteristics of the entire study population are shown in **Table 2**.

7

In total, 3622 biopsies were performed after transplantation and assessed using the Banff classification: 2775 protocol-specified biopsies and 847 indication biopsies. One year, 2 year and 5 year overall graft survival were 93.4%, 90.7% and 80.5% (**Figure 2**).

During follow-up, 365 grafts (36.5%) were lost in 357 patients: 211 (21.1%) due to death with a functioning graft and 154 (15.4%) due to graft failure. Of the 365 graft losses, posttransplant biopsies were available in 336 cases. Of the last biopsies before graft loss, 234 were protocol-specified biopsies and 102 were indication biopsies.

Baseline characteristics of grafts that were ultimately lost during follow-up were different than grafts that continued functioning over time, characterized by older recipients, older donors, more deceased donors, longer cold ischemia time, more repeat transplantations, more pretransplant diabetes mellitus and presence of HLA-DSA compared to those transplantations where no graft loss occurred during the follow-up period **(Table 2).**

Patient death with a functioning graft

Death with a functioning graft was the main cause of graft loss (211 of 365 grafts lost, 57.8%), representing 21.1% of all transplantations in this cohort. Mean time to death after transplantation was 5.37 [IQR 3.05 – 7.93] years. All of these deaths could be considered premature when compared to expected remaining lifetimes for the general population. When considering death to be premature when compared to the expected remaining lifetimes on dialysis, 47.4% of recipient deaths could not be considered premature.

In 63/211 (29.9%) the cause of death remained unknown despite thorough inspection of medical charts. When a cause could be identified, infection and malignancy were equally common (23.7% and 24.2% respectively), followed by cardiovascular death in 15.6% **(Figure 3A)**. When considering premature deaths compared to expected remaining lifetimes on dialysis this shifted slightly towards more infectious causes (27.0% unknown, 26.1% infectious, 18.9% malignancy, 18.0% cardiovascular and 9.9% other causes). Malignancies

8

consisted of 12 lung cancers, 4 oropharyngeal cancers, 9 gastrointestinal cancers, 4 hematological malignancies, 4 breast cancers, 5 skin cancers, 4 renal cell carcinomas, 4 transitional cell carcinomas, 1 ovarian cancer, 1 neuroendocrine tumor and 3 metastasized processes with unknown primary. Cardiovascular deaths included 14 cardiac arrests, 13 vascular causes and 6 heart failures. In 16 of the 50 (32.0%) infectious causes of recipient death at least 1 opportunistic pathogen was detected. Another cause was identified in 14 patients (6.6%). Mean age at death was 68.6 ± 10.1 years. There was no difference in recipient age at time of death between the different causes of death (mean recipient age at death 68.9 ± 9.6 years for infectious causes, 70.6 ± 8.3 for malignancies, 67.3 ± 10.0 for cardiovascular causes, 65.0 ± 15.3 for other causes and 68.1 ± 10.3 for unknown causes, p=0.33).

Graft failure (loss of graft function)

Graft failure was the cause of graft loss in 154/365 grafts, accounting for 43.3% of all graft losses and representing 15.4% of all transplantations studied. Mean time to graft failure was 3.08 [IQR 0.46 – 6.37] years. In comparison to grafts lost due to recipient death, grafts lost due to graft failure had significantly lower recipient age, shorter time posttransplant, more HLA-A, -B, -DR antigen mismatches, received more induction therapy, more anti-HLA DSA and recipients were more often female **(Table 2).** No differences were noted in donor age, baseline body mass index and cold ischemia time.

At time of the last biopsy before graft loss, eGFR was lower in the patients with graft failure (24.4 ± 18.6 mL/min/1.73m²) compared to death with a functioning graft (44.9 ± 20.2 mL/min/1.73m²; p<0.0001) and also proteinuria was higher (0.86 ± 1.35 vs. 0.25 ± 0.34 g/g creatinine respectively, p<0.0001). The last biopsies were closer to the date of graft loss in cases with graft failure (0.67 [IQR 0.04-2.75] years) compared to the cases with death with a functioning graft (2.76 [IQR 0.85-5.07] years, p<0.0001) and were more often indication

9

biopsies (at time of clinical graft dysfunction) in cases with graft failure (75/140 (53.6%) of last biopsies compared to 27/196 (13.8%) of last biopsies in cases with death with a functioning graft, p<0.0001).

In the evaluation of the causes of graft failure, in many cases no clear cause could be attributed (**Figure 3B**). In 14 cases (9.1%) no cause could be identified ('unknown'), but in 33 cases (21.4%) no clear cause was identified although the last biopsy demonstrated chronic damage. In the remaining cases 1 or more causes could be identified. In 27 (17.5%) this was rejection (both acute and chronic), in 18 (11.7%) this was a structural cause, in 16 (10.4%) PVAN was identified, in 15 (9.7%) a hemodynamic cause, in 12 others (7.8%) primary nonfunction, in 13 (8.4%) recurrent or de novo glomerulonephritis. In 6 (3.9%) cases another cause was identified: 3 intragraft malignancies, 1 thrombotic microangiopathy, 1 nephrocalcinosis, and 1 uncontrollable intragraft mycobacterial infection. Of the rejections, 18 were AMR (6 acute and 12 chronic), 4 acute TCMR and 5 mixed rejections. Next to these specific causes, in 30/107 (28.0%) also nonspecific chronic damage (IF/TA grade 2-3) was present in the last biopsy prior to graft failure.

Between causes of graft failure, differences were seen in time to graft failure (0.00 [IQR 0.00-0.00] years in PNF, 0.13 [IQR 0.02-0.35] years in mechanical causes, 1.41 [IQR 0.72-3.14] years in PVAN, 3.02 [IQR 0.63-4.76] years in rejection, 6.79 [IQR 2.51-9.14] years in glomerulonephritis, 3.25 [IQR 1.03-6.82] years in unknown cases, 3.81 [IQR 2.06-6.66] years in other cases, 5.42 [IQR 3.05-7.89] years in hemodynamic causes and 6.02 [IQR 3.73-8.59] years in chronic injury, p < 0.0001). Also time from last biopsy until graft failure was different (-0.03 [IQR -0.04- -0.02] years in PNF, 0.14 [IQR 0.00-0.30] years in mechanical causes, 0.61 [IQR 0.09-1.94] years in rejection, 0.59 [IQR 0.12-1.31] years in PVAN, 0.79 [IQR 0.44-6.10] years in glomerulonephritis, 1.41 [IQR 0.29-1.82] years in other causes, 1.57

[IQR 0.31-3.85] years in unknown causes, 2.10 [IQR 0.95-3.56] years in chronic injury and 3.09 [IQR 0.32-5.16] years in hemodynamic causes, p < 0.0001).

Of all graft failure cases (N=154, also including the cases without biopsies), 92 (59.7%) had ever experienced an episode of rejection. Of these, 53/92 (57.6%) experienced at least 1 episode of TCMR or borderline changes, 6/92 (6.5%) at least 1 episode of AMR but no TCMR or borderline changes, and 33/92 cases (35.9%) had experienced both AMR and TCMR/borderline changes episodes prior to graft failure. 60 (39.0%) ever had IF/TA grade 2-3 in a biopsy, 21 (13.6%) ever PVAN, and 31 (20.1%) ever glomerular disease. In all cases where chronic injury was identified as the main cause for graft loss, 24 of 33 (72.7%) had ever experienced a rejection episode, all of these 24 had experienced TCMR/borderline changes and 3 also AMR. Eight patients also had a history of glomerular disease in the graft (7 with FSGS lesions, 1 with signs of IgA nephropathy).

Donor specific anti-HLA antibodies

In the overall cohort of 1000 transplantations, 149 had HLA-DSA (mean fluorescence index cutoff 500) of which 101 were pretransplant, 41 were de novo and 7 had both pretransplant and de novo formation of HLA-DSA. Of these 149 transplantations with presence of DSA, 66 (44.3%) eventually lost their graft, of which 42/66 (63.6%) due to graft failure and 24 (36.4%) to recipient death. One fourth (42/149; 28.2%) of all cases with HLA-DSA experienced graft failure, which is 42/154 grafts (27.3%) that failed during follow-up. 30/42 (71.4%) of these graft losses had experienced at least 1 AMR episode, whereas the other 12 never experienced a biopsy-proven AMR episode (1 recurrent light chain deposition disease, 1 hemodynamic cause, 1 primary nonfunction, 5 chronic disease and 3 unknown cause of failure).

Early versus late graft failure

We then evaluated causes separately for early (in the first year after transplantation) versus late after transplantation (after the first year). Graft failure early after transplantation (N=48) was primarily due to surgical issues (16/48; 33.3%) or primary nonfunction (12/48; 25.0%). Rejection was less frequently a cause of early graft failure (8/48; 16.7%). Causes were different for later graft failures (N=106), where the most important cause had shifted to chronic injury (33/106; 31.1%), followed by rejections (19/106; 17.9%), hemodynamic causes (12/106; 11.3%) and PVAN (11/106; 10.4%) (**Figure 4**). A further separation of causes of graft failure according to time groups (<1 month, 1 month - 1 year, 1 year - 5 years, 5 years to 10 years and >10 years) is provided in **Figure S1** (SDC, http://links.lww.com/TP/B887)**.**

The complex multifactorial interplay of causes of graft loss

In graft failures where biopsies were done (N=140, 65 protocol-specified and 75 indication biopsies), we evaluated the histological lesions in the last biopsy before graft failure (N=35 in the early group, N= 105 in the late group). Given the very different causes of early and late graft failure, we considered these groups separately. As expected, chronic lesions (IF/TA grade 2-3 or transplant glomerulopathy) were much more prevalent in the group with late graft failure (64/105 (61.0%) in the late group vs. 1/35 (2.9%) in the early group) **(Figure S2**, SDC, http://links.lww.com/TP/B887)**.** However, also acute lesions like tubulitis and interstitial inflammation were more prevalent in this late group (53/105 (50.5%) with tubulitis score > 0 and 43/105 (41.0%) with interstitial inflammation score > 0 in the late group vs. 9/35 (25.7%) with tubulitis score > 0 and 7/35 (20%) with interstitial inflammation score > 0 in the early group).

More than 1 possible specific cause for graft failure could be identified in 39/154 (25.3%) cases (for example rejection + infection, rejection + PVAN, rejection + donor pathology, rejection + oxalate nephropathy, PVAN + mechanical injury, PVAN + hemodynamic disturbances, etc.). When considering only histological diagnoses by looking at the last biopsy before graft failure, both active diseases (AMR, TCMR, PVAN, GNF) and chronic diseases (IF/TA and transplant glomerulopathy) sometimes overlapped. For example, in the last biopsies of grafts failing between 1 year and 10 years, both active and chronic diseases were present in more than 30% (**Figure 5**).

Discussion

In this observational, single-center study the causes of graft loss were studied by retrospectively reviewing the medical records from each individual graft that was lost. Death with a functioning graft was the main cause of graft loss. The causes of death and graft failure, defined as loss of graft function, were heterogeneous, multifactorial and highly time-dependent. Graft failure early after transplantation was primarily due to surgical issues or primary nonfunction, and less frequently attributed to rejection. Late graft failures were mostly explained by accumulated chronic injury, followed by acute rejections and hemodynamic causes. In the majority of cases with chronic injury as presumed cause of graft failure, chronic injury was preceded by T-cell mediated rejection or borderline changes. Although we confirmed anti-HLA DSA as a major independent risk factor for graft failure, only one fourth of all anti-HLA DSA positive transplantations experienced graft failure during follow-up in this study, and only one fourth of failed grafts had donor-specific anti-HLA antibodies as potential contributor to graft failure.

Strengths of this study are the detailed information on these transplantations, including complete anti-HLA DSA information, and the extension beyond histological assessment of graft loss. By including transplantations without biopsy information, the important share of

13

mechanical causes and primary nonfunction in graft failure was captured. Also, in those patients where biopsy information was present, both protocol and indication biopsies were included, minimizing bias of using only indication biopsies (underestimating slowly progressing diseases that may not lead to acute graft dysfunction) or only protocol biopsies (with an inherent survivor bias at late time points).

Recipient death with a functioning graft was the main cause of graft loss in our cohort.[4,5,19] This finding is known from literature, but is often overshadowed by the attention for (histology of) graft failure. In comparison to the general population, all patients who died in this cohort died prematurely, obviously relating to the risk of death associated with end-stage renal disease and other frequent comorbidities in this population. However, when we defined premature death in relation to the life expectancy of dialysis patients, we found that 47.4% of patient deaths were not premature, and death occurred later than the average life expectancy in dialysis. This may illustrate the ultimate success of transplantation in comparison to dialysis, rather than its failure. Despite extensive documentation of the clinical history of all our patients, the cause of death was not always known, which is analogous to previous studies.[6,20] The main known causes of death in this study are malignancy, infections and cardiovascular disease, confirming what is known from literature.[6,20]

These 3 causes of patient death with a functioning graft are likely enhanced by the immunosuppressive medication given to transplant recipients, although the contribution of immunosuppression to recipient death could not be quantified. Since the choice of the immunosuppressive regimen is a modifiable risk factor, in contrast to recipient age, which is the main risk factor for recipient death, this suggests room for improvement and further efforts should be aimed at more tailored immunosuppression to the risk for death of each individual patient. Reduction of immunosuppressive medication to avoid side effects is often balanced against fear of ensuing rejection episodes. However, as was put in perspective in this

14

study, much more grafts are lost due to recipient death caused by malignancy, infection and cardiovascular disease (N=134), than to rejection episodes, even when considering all nonspecific chronic injury to be due to previous rejection episodes (N=60). Although of course, these causes of death are multifactorial and not solely caused by immunosuppression, and on the other hand the observed risks of insufficient immunosuppression,[21] this observation reinforces the challenging balance between over-and under immunosuppression. Ongoing discussions on new endpoints in clinical trials to improve outcome after kidney transplantation, should therefore include recipient death as main factor hampering long-term success.

Similar to causes of recipient death, causes of graft failure remained often unclear, especially when active and chronic disease processes coincide. Chronic damage accumulates over time as a final common endpoint of both allo-immune and nonimmune injuries and thus remains a nonspecific finding, present in many biopsies beyond 1 year after transplantation. This study cannot exclude that the large group of death-censored graft failures attributed to nonspecific chronic injury, could have been the consequence of earlier allo-immune injury, as was indicated by previous studies.[22,23] A large proportion of these cases of nonspecific chronic injury indeed had history of acute rejection, and other studies have supported the allo-immune nature of chronic injury, called chronic active T-cell mediated rejection in the 2017 Banff classification.[9] However, our observation that IFTA prior to graft failure is often preceded by rejection episodes does not prove causality that allo-immune injury was the sole mechanism behind development of chronic injury (interstitial fibrosis/ tubular atrophy). Better understanding, especially from protocol biopsies, is needed to address whether this chronic injury is possibly mediated by persistent, slowly progressive allo-immune injury, and whether increased immunosuppression could temper the progression of chronic injury. For analytical purposes, 1 main cause is often singled out, although in the biopsy alone already multiple

15

processes can be at play, with further risk contribution of classical risk factors like arterial hypertension, diabetes mellitus, obesity, drug toxicity, etc.[24-27] The complexity of these coinciding risk factors requires complete follow-up data modelled multivariate in time-dependent joint models to correctly capture their true contribution to graft failure or recipient death.[28-30]

Such extensive statistical joint modelling would still not allow assessing the causes of graft failure, but at best yield risk factors for graft failure and improved estimation of the effect sizes of these. However, the identification of risk factors with large effect size, like e.g. anti-HLA DSA, should be interpreted carefully. Although we confirmed anti-HLA DSA as a major independent risk factor for graft failure, only a small minority of patients have or develop anti-HLA DSA (15% in the current cohort), often of low level and transient,[31] such risk factor is only relevant for a minority of patients. The large effect size of HLA-DSA needs to be balanced to the relatively low prevalence of such antibodies in the transplant population. This explains why only one fourth of failed grafts had donor-specific anti-HLA antibodies as potential contributor to graft failure and only one fourth of all anti-HLA DSA positive transplantations experienced graft failure during follow-up.

There are some limitations to this study. This study is descriptive and the complexity of time-dependent factors at play in each individual patient remains difficult to capture. The variable time interval between the last biopsy (and associated observations) and the time of graft failure, calls for caution in the interpretation hereof. We did not assess nonadherence as a potential factor as this information was not prospectively collected and difficult to capture retrospectively with risk of introducing bias by other clinical parameters. Furthermore, our study population consisted largely of Caucasians and most of them were on a tacrolimus-based regimen, limiting generalizability of these findings to other and higher risk populations. Also, the low proportion of living donors in our center could influence the amount of chronic

16

injury and IF/TA.[6] Another limitation is that no morphometric assessment was done, potentially missing the severity of mesangial matrix expansion (related to diabetes and obesity) and intimal fibrosis.[32] The attribution of single primary causes to each graft failure is inherently subject to interpretation bias and needs to be interpreted cautiously. The data come from a well-established transplant program in a Western European country, with a relatively homogeneous and highly educated population with universal access to healthcare and a highly efficient allocation program through Eurotransplant. Extrapolation or comparison of our conclusions to other contexts needs to be done cautiously. Finally, the definition of premature death is not standardized, there is no matched population with the same background risk profile, and follow-up time is not equal in all patients. These factors impact on the percentage of premature death and require caution in the interpretation of the data on premature death. Further work on the definition of premature death after kidney transplantation is warranted.

In conclusion, we demonstrate that recipient death is the most common cause of graft loss, most often related to infections, malignancy and cardiovascular diseases. More emphasis on premature death in research and in clinical trials in kidney transplantation is needed. The causes of graft failure were time-dependent, differing importantly between the first postoperative year post transplantation and later after transplantation. Allo-immunity was only identified as the definite culprit in a minority of graft losses, and HLA-DSA were potentially involved in only 27 % of graft failures in this lower risk population. Chronic lesions are nearly omnipresent late after transplantation but are nonspecific, ill explained and precede graft failure. Chronic injury as presumed cause of graft failure is often preceded by earlier rejection episodes, potentially reflecting an allo-immune component in the chronic injury and graft failure, although causality cannot be inferred from these data. Further research into the evolution, classification and underlying etiology of chronic injury is required to guide preventative measures to improve late allograft survival.

17

Acknowledgments

18

References

1. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant.* 2011;11(3):450–462.

2. Coemans M, Süsal C, Döhler B, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int.* 2018;94(5):964–973.

3. Tong A, Sautenet B, Poggio ED, et al; SONG-Tx Graft Health Workshop Investigators. Establishing a core outcome measure for graft health: a Standardized Outcomes in Nephrology-Kidney Transplantation (SONG-Tx) Consensus Workshop Report. *Transplantation.* 2018;102(8):1358–1366.

4. Gill JS, Rose C, Pereira BJ, et al. The importance of transitions between dialysis and transplantation in the care of end-stage renal disease patients. *Kidney Int.* 2007;71(5):442–447.

5. Cosio FG, Hickson LJ, Griffin MD, et al. Patient survival and cardiovascular risk after kidney transplantation: the challenge of diabetes. *Am J Transplant.* 2008;8(3):593–599.

6. El-Zoghby ZM, Stegall MD, Lager DJ, et al. Identifying specific causes of kidney allograft loss. *Am J Transplant.* 2009;9(3):527–535.

7. Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant.* 2012;12(2):388–399.

8. Naesens M, Kuypers DR, De Vusser K, et al. The histology of kidney transplant failure: a long-term follow-up study. *Transplantation.* 2014;98(4):427–435.

9.   Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 Kidney Meeting Report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* 2018;18(2):293–307.

10. Senev A, Coemans M, Lerut E, et al. Histological picture of antibody-mediated rejection without donor-specific anti-HLA antibodies: clinical presentation and implications for outcome. *Am J Transplant.* 2019;19(3):763–780.

11. Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. *N Engl J Med.* 1983;309(14):809–815.

12. Canadian Multicentre Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. Analysis at three years. *N Engl J Med.* 1986;314(19):1219–1225.

13. Pirsch JD, Miller J, Deierhoi MH, et al. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. *Transplantation.* 1997;63(7):977–983.

14. Vítko S, Margreiter R, Weimar W, et al. Everolimus (Certican) 12-month safety and efficacy versus mycophenolate mofetil in de novo renal transplant recipients. *Transplantation.* 2004;78(10):1532–1540.

15. Durrbach A, Pestana JM, Pearson T, et al. A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). *Am J Transplant.* 2010;10(3):547–557.

16. Vincenti F, Charpentier B, Vanrenterghem Y, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant.* 2010;10(3):535–546.

17.   Sutherland DE, Fryd DS, Strand MH, et al. Results of the Minnesota randomized prospective trial of cyclosporine versus azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. *Am J Kidney Dis.* 1985;5(6):318–327.

18.   Kramer A, Stel VS, Abad Diez JM, et al. Renal replacement therapy in Europe-a summary of the 2010 ERA-EDTA Registry Annual Report. *Clin Kidney J.* 2013;6(1):105–115.

19.   Gaston RS, Fieberg A, Helgeson ES, et al. Late graft loss after kidney transplantation: is "death with function" really death with a functioning allograft? *Transplantation.* 2019. [Epub ahead of print]

20.   Oniscu GC, Brown H, Forsythe JL. How old is old for transplantation? *Am J Transplant.* 2004;4(12):2067–2074.

21.   Hricik DE, Formica RN, Nickerson P, et al. Adverse outcomes of tacrolimus withdrawal in immune-quiescent kidney transplant recipients. *J Am Soc Nephrol.* 2015;26(12):3114–3122.

22.   El Ters M, Grande JP, Keddis MT, et al. Kidney allograft survival after acute rejection, the value of follow-up biopsies. *Am J Transplant.* 2013;13(9):2334–2341.

23.   Gago M, Cornell LD, Kremers WK, et al. Kidney allograft inflammation and fibrosis, causes and consequences. *Am J Transplant.* 2012;12(5):1199–1207.

24.   Wu DA, Robb ML, Forsythe JLR, et al. Recipient comorbidity and survival outcomes after kidney transplantation: a UK-wide prospective cohort study. *Transplantation.* 2019. [Epub ahead of print]

25.   Naik AS, Sakhuja A, Cibrik DM, et al. The impact of obesity on allograft failure after kidney transplantation: a competing risks analysis. *Transplantation.* 2016;100(9):1963–1969.

26.   Hamdani G, Nehus EJ, Hanevold CD, et al. Ambulatory blood pressure, left

ventricular hypertrophy, and allograft function in children and young adults after

kidney transplantation. *Transplantation.* 2017;101(1):150–156.

27.   Schena FP, Pascoe MD, Alberu J, et al. Conversion from calcineurin inhibitors to

sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and

safety results from the CONVERT trial. *Transplantation.* 2009;87(2):233–242.

28.   Vanrenterghem YF, Claes K, Montagnino G, et al. Risk factors for cardiovascular

events after successful renal transplantation. *Transplantation.* 2008;85(2):209–216.

29.   Fieuws S, Verbeke G, Maes B, et al. Predicting renal graft failure using multivariate

longitudinal profiles. *Biostatistics.* 2008;9(3):419–431.

30.   Tomašev N, Glorot X, Rae JW, et al. A clinically applicable approach to continuous

prediction of future acute kidney injury. *Nature.* 2019;572(7767):116–119.

31.   Senev A, Lerut E, Van Sandt V, et al. Specificity, strength, and evolution of

pretransplant donor-specific HLA antibodies determine outcome after kidney

transplantation. *Am J Transplant.* 2019;19(11):3100–3113.

32.   Denic A, Morales MC, Park WD, et al. Using computer-assisted morphometrics of 5-

year biopsies to identify biomarkers of late renal allograft loss. *Am J Transplant.*

2019;19(10):2846–2854.

Figure legends

Figure 1. Study design.

Figure 2. Stacked plot of cumulative incidence functions over time after transplantation showing event-free survival, death-censored graft failure and recipient death with a functioning graft.

Figure 3. Causes of graft loss in 365 kidney transplantations, divided in death with a functioning graft (A) and graft failure defined as return to dialysis or retransplantation (B). For these diagrams causes were simplified to 1 principal cause by investigator's judgment. In some cases overlapping causes were present. Especially chronic injury is a nonspecific finding that could be the final common endpoint of both nonimmune and allo-immune causes such as previous acute rejection episodes which is why the discriminating line between nonspecific injury and rejections could be interpreted as a rather blurry line. DSA positivity is indicated in cases with rejection and nonspecific chronic injury as cause of graft failure. 59.3% (16/27) of all rejections as cause of failure and 15.2% (5/33) of chronic injury as potential cause of graft failure were DSA positive. HLA-DSA was positive in 3/14 (21.4%) of cases not explained by other processes. Nonspecific chronic injury was defined as IF/TA grade 2-3 without evidence of a concomitant specific disease.

Figure 4. Causes of death-censored graft failure in the first year after transplantation (N=48) and after the first year after transplantation (N=106). Nonspecific chronic injury is defined as IF/TA grade 2-3 without evidence of a concomitant specific disease.

Figure 5. Histological diagnoses of 140 last biopsies before graft failure, dependent on time of graft failure. (a) Prevalence of active and/or chronic histological lesions over time. (b) Prevalence of active diseases or chronic lesions only, or overlap of active disease and chronic lesions over time. Cg, transplant glomerulopathy.

23

Tables

Table 1. Classification of causes of death censored graft failure.	
Graft failure group	*Definition*
Primary nonfunction	Permanent lack of graft function from the time of transplantation, without detectable technical or immunological problems, necessitating dialysis after kidney transplantation.
Structural/mechanical issues	Technical surgical complications including arterial and venous thrombosis, vascular or ureteral stenosis (including ensuing postrenal failures) and suture problems leading to graft loss.
Rejection	When the last biopsy or biopsies before failure had pathological evidence of rejection (T cell-mediated rejection and/or acute or chronic antibody-mediated rejection) and no other specific injury (that could be a more probable cause of graft failure).
Glomerulonephritis	Recurrent or de novo glomerular disease that was clinically deemed responsible for graft failure; incidental positive IgA staining or isolated focal segmental glomerulosclerosis (FSGS) lesions without considerable clinical or pathological impact were not considered as principal causes of graft failure.
Polyoma-virus associated nephropathy (PVAN)	When pathological evidence of serious injury hereof was present, combined with positive BK-virus staining in the biopsy, most often present in follow-up biopsies.
Hemodynamic causes	Serious medical conditions leading to severe and/or continuing prerenal injury such as cardiorenal or hepatorenal syndromes and severe septic or hypovolemic shock requiring permanent renal replacement therapy.
Other causes	Specified conditions that were deemed the cause of graft failure that could not be classified as one of the above.
Chronic injury	Cases that displayed severe interstitial fibrosis/tubular atrophy (IF/TA) grade 2-3 in the last biopsy, without evidence of a concomitant specific disease.
Unknown cases	When no clear explanation for graft failure could be assigned despite extensive review of all clinical and pathological information.

Table 2. Demographics for entire cohort (N=1000)							
	Entire cohort (N=1000)	Overall graft loss (N=365)	Remained functioning & alive (N=635)	p-value	Graft failure (N=154)	Death with functioning graft (N=211)	p-value
Recipient characteristics at transplantation							
Age (y), mean ± SD	53.7 ± 13.3	58.58 ± 12.95	50.92 ± 12.60	**<0.0001**	52.70 ± 14.64	62.88 ± 9.52	**<0.0001**
Recipient BMI at time of transplantation (kg/m²), mean ± SD	25.4 ± 4.5	25.56 ± 4.52	25.29 ± 4.43	0.36	25.83 ± 4.87	25.69 ± 4.26	0.53
Sex (male), n (%)	609 (60.9%)	216 (59.18%)	393 (61.89%)	0.40	80 (51.95%)	136 (64.45%)	**0.02**
Caucasian ethnicity, n (%)	984 (98.4%)	358 (98.08%)	626 (98.58%)	0.59	150 (97.40%)	208 (98.58%)	0.43
Diabetes mellitus, n (%)	434 (43.4%)	199 (54.5%)	235 (37.0%)	**<0.0001**	76 (49.4%)	123 (58.3%)	0.09
- Pretransplant diabetes	175 (17.5%)	93 (25.5%)	82 (12.9%)	**<0.0001**	34 (22.1%)	59 (28.0%)	0.20
- New-onset diabetes after transplantation	259 (25.9%)	106 (29.0%)	153 (24.1%)	0.09	42 (27.3%)	64 (30.3%)	0.52
Repeat transplantation, n (%)	154 (15.4%)	72 (19.73%)	82 (12.91%)	**0.004**	34 (22.08%)	38 (18.01%)	0.33
Donor characteristics at transplantation							
Age (y), mean ± SD	47.7 ± 14.8	49.44 ± 15.73	46.77 ± 14.22	**0.008**	49.91 ± 15.67	49.10 ± 15.81	0.63
Sex (male), n (%)	535 (53.5%)	189 (51.78%)	346 (54.49%)	0.41	76 (49.35%)	113 (53.55%)	0.43
Deceased donor, n (%)	941 (94.1%)	356 (97.53%)	585 (92.13%)	**0.0005**	149 (96.75%)	207 (98.10%)	0.41
Donation after brain death, n (%)	780 (78.0%)	307 (84.11%)	473 (74.49%)	**0.0004**	129 (83.77%)	178 (84.36%)	0.88
Transplant characteristics, treatment at transplantation and follow-up							
Cold ischemia time (h), mean ± SD	14.2 ± 5.7	15.09 ± 4.91	13.66 ± 5.99	**<0.0001**	14.87 ± 4.71	15.26 ± 5.06	0.46
Follow-up time after transplant (y), median [IQR]	7.49 [4.91-10.02]	4.72 [1.97-7.47]	8.74 [6.56-10.98]	**<0.0001**	3.08 [0.46-6.37]	9.25 [7.08-11.67]	**<0.0001**
Total HLA-A,-B,-DR mismatches, mean ± SD	2.7 ± 1.3	2.79 ± 1.36	2.65 ± 1.26	0.12	2.98 ± 1.33	2.66 ± 1.37	**0.04**
Immunosuppression regimen: TAC-MPA-CS, n (%)	87.4 (87.4%)	314 (86.03%)	560 (88.19%)	0.32	135 (87.66%)	179 (84.83%)	0.44
Induction therapy, n (%)	416 (41.6%)	146 (40.0%)	270 (42.52%)	0.44	73 (47.40%)	73 (34.60%)	**0.01**
Donor-specific anti-HLA antibodies, n (%)	149 (14.9%)	66 (18.08%)	83 (13.07%)	**0.03**	42 (27.27%)	24 (11.37%)	**<0.0001**
- pretransplant	101 (10.1%)	50 (13.70%)	51 (8.03%)	**0.004**	30 (19.48%)	20 (9.48%)	**0.006**
- de novo	41 (4.1%)	12 (3.29%)	29 (4.57%)	0.33	9 (5.84%)	3 (1.42%)	**0.02**
- pretransplant and de novo	7 (0.7%)	4 (1.10%)	3 (0.47)	0.25	3 (1.95%)	1 (0.47%)	0.18

25

Figure 1

1137 kidney transplantations
(March 2004-February 2013)

137 excluded:
- 113 combined transplants
- 24 history of other transplanted organ

1000 transplantations in 969 patients

365 (36.5%) graft losses in 357 patients

154 (15.4%)
due to
graft failure

211 (21.1%)
due to
recipient death with a
functioning graft

26
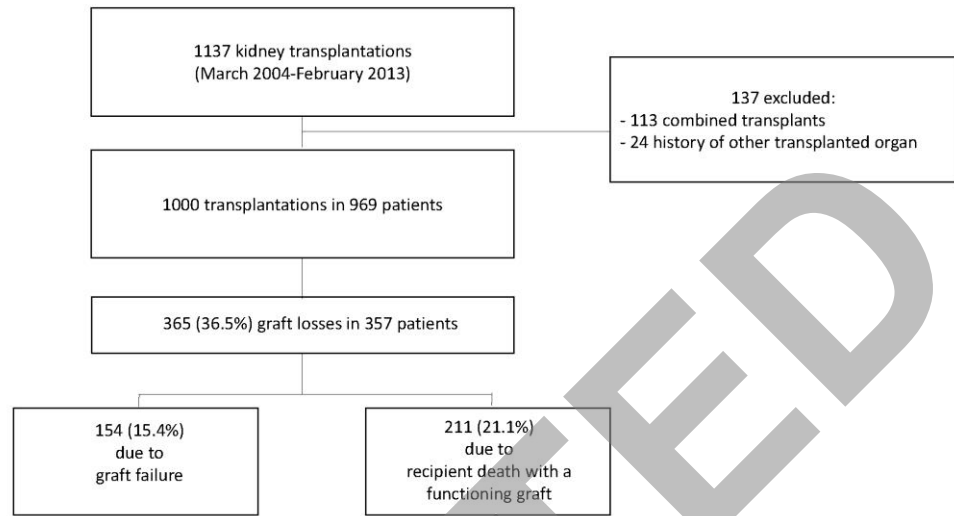
Figure 2

Stacked plot of Cumulative Incidence Functions
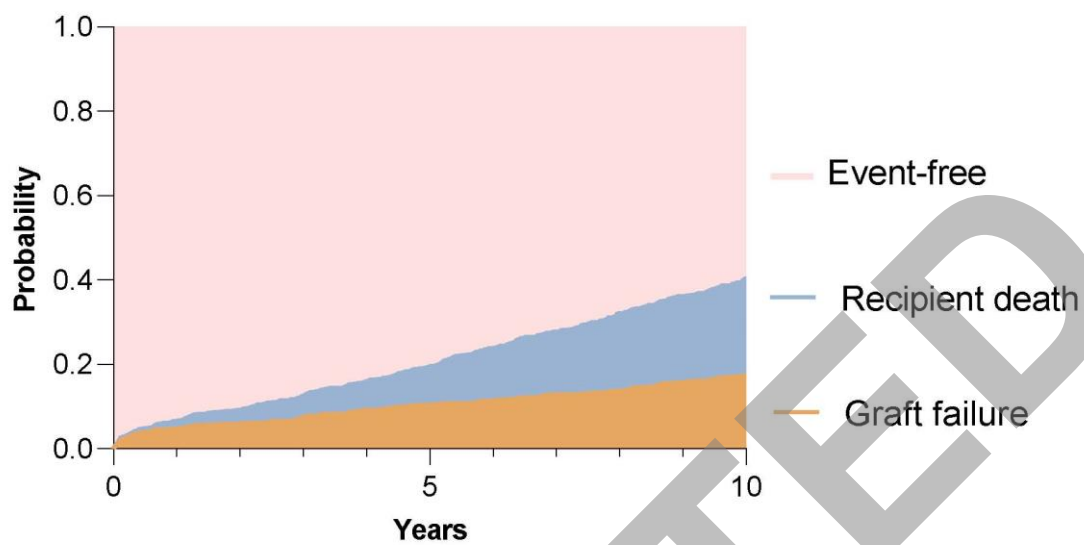
Figure 3

A Causes of death with a functioning graft (N=211)

6.6% Other
15.6% Cardiovascular
29.9% Unknown
57.8%
24.2% Malignancy
23.7% Infectious

B Causes of graft failure (N=154)

3.9% Other
9.7% Hemodynamic
9.1% Unknown
7.8% Primary non function
21.4% Non-specific chronic injury
11.7% Mechanical
15.2%
59.3%
DSA positivity
8.4% GNF
17.5% Acute and chronic rejection
10.4% PVAN
42.2%

28
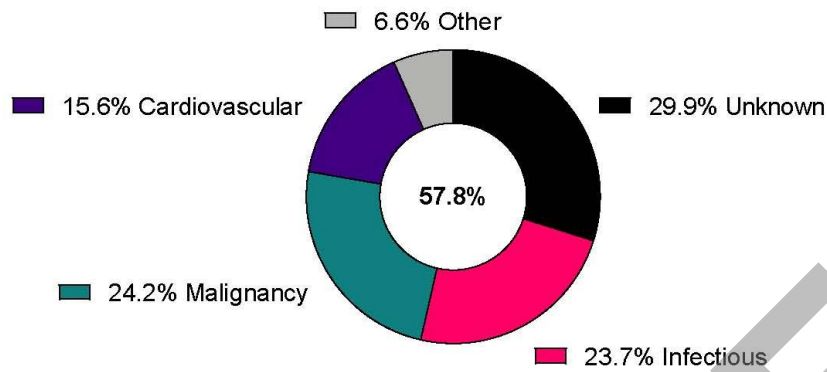
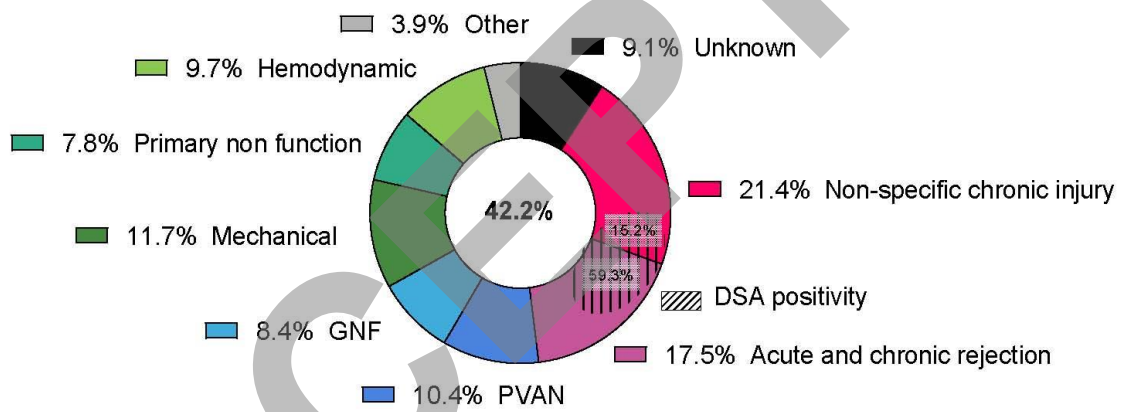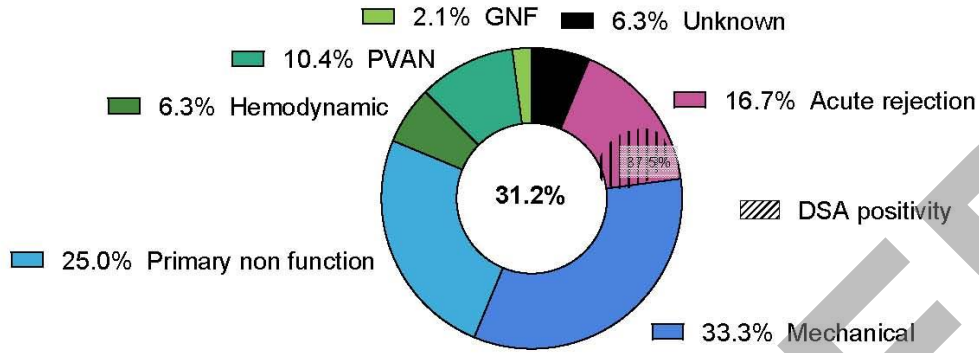# Figure 4

A   Causes of early graft failure (< 1 year, N=48)

- 2.1% GNF
- 6.3% Unknown
- 10.4% PVAN
- 16.7% Acute rejection
- 6.3% Hemodynamic
- 31.2%
- DSA positivity
- 25.0% Primary non function
- 33.3% Mechanical
- 87.5%

B   Causes of late graft failure (> 1 year, N=106)

- 5.7% Other
- 10.4% Unknown
- 11.3% GNF
- 10.4% PVAN
- 68.8%
- 31.1% Non-specific chronic injury
- 11.3% Hemodynamic
- DSA positivity
- 1.9% Mechanical
- 68.4%
- 17.9% Acute and chronic rejection

29

Figure 5