Comparison of kidney transplant outcomes in HLA compatible and incompatible transplantation: a national cohort study.

Running title: HLA compatible and incompatible kidney transplantation outcomes

Key words: HLA incompatible transplantation, Antibody mediated rejection, Flow-cytometry crossmatch, Donor specific antibody

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Summary statement

Compared to HLA compatible transplantation, HLA incompatible kidney transplantation, especially in the presence of a positive FC-XM, is associated with an increased risk of rejection, inferior graft function and death. For many highly sensitised recipients a compatible transplant is not on offer and HLA incompatible transplantation can be an appropriate alternative to remaining on dialysis.

Abstract

Background: Reports of HLA incompatible (HLAi) kidney transplant outcomes are inconclusive, especially in the context of lower level Donor Specific Antibodies (DSA).

Methods: Multi-centre national cohort study of HLAi kidney transplant recipients matched in 1:2 ratio with HLA compatible (HLAc) kidney transplant recipients. HLAi defined as DSA identified by Luminex. Antibody mediated rejection (AMR) and transplant-survival were analysed using Kaplan-Meier plots. Propensity score (PS) matching was used to compare recipient and transplant survival between groups.

Results: We included 61 HLAi and 122 HLAc recipients; mean age 46 years; 60% female. MFI_{T0}: 3327 (IQR 1352 – 6458), 23 (38%) were Flow cytometry crossmatch positive (FC-$XM_{POS)}$. DSA_{POS}/FC-XM_{POS} transplantation carried an increased risk of AMR at 1 year (52%) compared to DSA_{POS}/FC-XM_{NEG} (27%) and HLAc (0%). Unadjusted death censored graft loss at 3 years was 13% (HLAi) and 8% (HLAc). Three-year patient survival was 95% in HLAc, 84% in DSA_{POS}/FC-XM_{NEG} and 69% in DSA_{POS}/FC-XM_{POS} recipients; 58% of HLAi deaths were infection-related. HLA incompatibility was associated with a decreased 3-year survival in our PS-matched cohort.

Conclusion: In kidney transplantation, DSA and positive FC-XM carries an increased risk of AMR. Despite inferior transplant and survival outcomes compared to HLAc transplantation, it remains a realistic option for highly sensitised patients facing prolonged waiting times and reduced survival on dialysis.

Key words: HLA incompatible transplantation, Antibody mediated rejection, Flow-cytometry crossmatch, Donor specific antibody

Introduction

In kidney transplantation, sensitisation of a recipient to donor human leukocyte antigens (HLA) decreases access to compatible donors. This increases waiting time and risk of death while on the waiting list. Transplantation in the presence of Donor Specific Antibodies (DSA), HLA incompatible [HLAi] transplantation) has been associated with adverse outcomes[1,2,3–7] and is reserved for difficult to match recipients when other options for transplantation are limited.

In order of increasing sensitivity; complement dependent cytotoxic crossmatch (CDC-XM), flow cytometry crossmatch (FC-XM) and single antigen bead (SAB) assays are used to semi-quantify the immunological risk of DSA in clinical settings[8]. Due to the evolution of antibody detection technology, immunosuppression, induction therapy and AMR treatment, variability exists between centres which can make comparison of studies difficult. Additionally, cut offs for positivity in FC-XM methods vary between centres. There is consensus not to perform transplantation across a positive CDC-XM because of poor recipient and transplant outcomes[9]. However, reports are inconclusive regarding HLAi transplant outcomes in the context of lower levels of DSA or a positive FC-XM[5,10–12].
To clarify risks and benefits of kidney transplantation in the presence of DSA, with and without a positive FC-XM, we compare outcomes and survival of recipients of all CDC-XM negative, HLA incompatible kidney transplants in Scotland with a carefully matched cohort of HLA compatible recipients.

Methods

Study cohort and data collection

This national multi-centre cohort study included all recipients of HLA incompatible kidney transplants performed within Scotland, UK, between 2011 and 2017 (HLAi group*)*. HLA compatible transplant recipients (HLAc) were selected from a contemporary cohort of patients who received a kidney transplant between 2015 and 2016 (Scottish National Kidney Transplant Service; Edinburgh Royal Infirmary and Queen Elizabeth University Hospital in Glasgow), as previously reported[13]. Two HLAc patients were matched to each HLAi patient. Matching criteria were recipient age (within 5 years), recipient gender and type of transplant (donation after cardiac death (DCD), brain death (DBD) or living donor). If >2 matches for 1 case were found, the optimal distribution of controls was sought, prioritising recipient gender > age > type of transplant for each match. Both cohorts were followed until graft loss, death or until 1 July 2020. Baseline characteristics were retrospectively obtained from the electronic recipient record (VitalDataClient). Co-morbidity data was collected using the electronic recipient record followed by review of recipients' case records. Cumulative mean fluorescence intensity (MFI) of DSA present at time of transplant (T_0) and at 1, 3 ,6 and 12 months post-transplant for HLAi transplant recipients as well as graft function parameters at 3, 6, 12, 24 and 36 months for both HLAi and HLAc transplant recipients were retrospectively collected. Cause of death was recorded using ERA-EDTA classification[14]. Death-censored graft survival was defined as return to dialysis or re-transplantation and was censored for death with a functioning graft. Graft loss included death with a functioning graft. Renal biopsies, performed on indication and reported according to the Banff

Classification criteria[15], were reviewed by the authors and correlated with recipient records reporting clinical rejection.

HLA typing

A full HLA type and antibody screen was performed prior to transplant listing for patients. HLA typing for patients and living donors was performed using medium resolution Luminex PCR-SSO for HLA-A, B, C, DRB1, DQA1, DQB1, DPA1 and DPB1 (Rapid SSO eRes kits, IBG Immucor, UK) . For deceased donors HLA types were determined at medium resolution by UK laboratories as part of organ allocation, with the HLA type being confirmed locally using PCR-SSO, as above.

HLA DSA determination

Patient samples were screened using One Lambda kits (OL, Canoga Park, CA) and specificities were defined using One Lambda LABScreen SAB class I and II. Identification of antibody specificity was performed using a LABScan 200 Flow analyzer (Luminex Corporation, Austin, TX) and analysis was undertaken with OL Fusion software using predefined and validated positive cut-offs. As donors were only typed to medium resolution, assigning MFIs to mismatched donor HLA antigens used the most likely donor HLA allele based on known frequencies in the UK population. HLA-DSA against all loci were assumed to be of equal relevance.

FC-XM

Flow cytometry crossmatch (FC-XM) is routinely used ahead of HLA incompatible transplantation, but not in recipients who are unsensitised. In sensitised recipients, with HLA antibodies present which are not directed to the potential donor, a FC-XM is performed on the day of transplant. If this is inadvertently positive, the transplant will be classed as an HLA incompatible transplantation. This is rare and no such cases are included in our HLAc cohort. The process of FC-XM included isolation of donor T and B lymphocytes from EDTA peripheral blood, which were then incubated with patient serum at 37°C. A fluorescein isothiocyanate labelled anti-human IgG was added for detection. T and B lymphocytes were distinguished using monoclonal CD3 and CD19 antibodies. Fluorescent intensity of the sample was compared to a negative control serum. *Auto* FC-XM was performed using patient lymphocytes and patient serum.

CDC-XM

Recipient serum was added to donor T or B lymphocytes and complement to facilitate antibody dependent cellular cytotoxicity. Cell death was visualised using cellular dyes. A DTT step was included to exclude IgM reactivity.

Definitions

HLA incompatible transplantation was defined as the presence of ≥1 DSA with a mean MFI value ≥1000 identified by Luminex[16]. Where a DSA was present with an MFI≥1000 in an historical sample and a lower degree of positivity was measured at time of transplant (T_0), this was classed as Historical DSA regardless of FC-XM results at time of transplantation. FC-

XM is considered positive in the event of *allo* FC-XM positivity (either B or T-cell), not explained by auto-antibody and in the presence of a relevant DSA. MFI was measured at time of transplantation (MFI_{T0}) and at indication thereafter. Peak level of MFI prior to transplantation (MFI_{MAX}) was an historical measurement or at T_0, whichever was higher.

Induction therapy and maintenance immunosuppression

Immunosuppression protocols are consistent across the Scottish National Kidney Transplant Service for live and deceased donor transplants. In preparation for transplantation, desensitisation with plasma exchange was offered to all high immunological risk patients with a current DSA receiving a living donor transplant[17]. Alternate day plasma-exchange (up to 6 sessions) was provided, with intravenous immunoglobulin (IVIg) at 100mg/kg following each session. No routine post-transplant desensitisation treatment was provided. High immunological risk patients had induction with lymphocyte depleting agents (LDa, usually anti-thymocyte globulin) with basiliximab used in other recipients. Maintenance immunosuppression consisted of prednisolone, tacrolimus (Prograf) and mycophenolate mofetil (MMF) with dosing as per the ELITE-Symphony trial[18]. Initial MMF dosing was equal (1 gram twice daily) in both groups. Following HLAi transplantation a tacrolimus level of 8-12 ng/mL for the first month was targeted, compared to 7-10 ng/mL for HLAc recipients.

Propensity score matching

The decision to proceed with an HLA incompatible transplantation is confounded by subject characteristics, such as renal replacement therapy (RRT)-vintage and transplantation history, which subsequently impacts on level of sensitisation, accepted HLA mismatch and transplant outcomes. To account for systematic differences in baseline characteristics when

estimating the effect of HLA incompatible transplantation on patient and transplant outcomes, propensity score matching was used.

Propensity scores (PS) were estimated using multivariate logistic regression models, predicting the probability of receiving a HLA incompatible transplant based on the independent baseline variables age, graft number, RRT-vintage and HLA mismatch level. Predicted probabilities were sorted (N=183) by increasing magnitude and SPSS Propensity Score Matching command was used to locate the nearest available match, limiting the maximum distance to 0.1. A PS-matched cohort was formed (N=68) consisting of 34 identified matches between both cohorts. Balance of the PS-matched cohort was ascertained using t-tests, evaluating the differences between the matched HLAi and HLAc groups for each propensity score variable. Table A (appendix) describes the original cohort and the PS-matched cohort.

Statistical analysis

Descriptive statistics were used to summarise the baseline recipient characteristics. Recipients who lost their graft or died were censored from analysis from the date of the event. Chi-Square testing and One-Way ANOVA were used to compare categorical variables. Unpaired t-test and non-parametric testing were used to compare continuous variables between groups for normally distributed and non-normally distributed variables, respectively. Risk of death and time until event-analysis for AMR, allograft loss and death censored graft loss were analysed using Kaplan-Meier plots. Log-rank testing was performed for subgroups.

Using the PS-matched cohort, multivariable logistic regression analyses were performed to estimate the relative associations of pre-transplant variables with mortality. Initial selection of these variables was based on clinical experience and literature review[19,20] which included: age, presence of comorbidities, HLA incompatibility and RRT-vintage. Variables were individually entered and where multiple explanatory variables with a p-value <0.1 were detected, these were entered using a conditional forward stepwise entry method into the regression analysis.

All statistical analyses were performed using SPSS version 25 (IBM, Armonk, NY) and $p<0.05$ was considered statistically significant.

Results

Cohort descriptions

The 61 HLAi kidney transplants that were performed between 2011 and 2017 were matched with 122 HLA compatible (HLAc) kidney transplant recipients (2015 – 2016). Recipients were matched for age [mean age in both groups was 46 (SD±11) years], gender (59% female) and donor type (25% received a live donor organ). This was the first kidney transplant for 82% of HLAc recipients compared to 30% of HLAi recipients ($p<0.001$). HLAc recipients had a shorter RRT-vintage (median 2.4 [IQR 0.3 – 5.8] years) compared to HLAi recipients (median 17.3 [IQR 6.4 – 22.4] years, $p<0.001$). A minority of HLAc recipients (7%) received LDa induction, compared to 75% of HLAi recipients ($p<0.001$). Mean duration of follow up was 3.6 (SD±1.4) years.

Thirty-four percent of HLAc recipients had ≥1 comorbidities, compared to 43% of HLAi recipients ($p=0.233$). An overview of characteristics of both cohorts is shown in Table 1.

HLA incompatibility

Forty-nine recipients (80% of HLAi) had one or more DSA with MFI>1000 at T_0 (current DSA), with a median MFI_{T0} of 3760 (IQR 2257 – 7457). Historical DSA were identified in the remaining 12 recipients at a mean of 840 days (SD±586) before transplantation. Median historical MFI_{MAX} was 3667 (IQR 2621 – 7014) and median MFI_{T0} was 653 (IQR 283 – 891, [$p<0.001$]) (Figure 1). Eleven living donor recipients received plasma-exchange and IVIg pre-transplant. Mean MFI prior to desensitisation was 4401, reducing on average 69% post desensitisation. Seven recipients remained FC-XM positive despite desensitisation treatment.

Thirty-two recipients (52%) had more than one DSA; 26 recipients (43%) had only class I DSA, 20 (33%) only class II and 15 (24%) had both class I and II DSA. MFI and cross match details are presented in Table 2.

Rejection

In total 51 recipients developed one or more acute rejection episodes during follow up; 34 in HLAi (56%) and 17 (14%) in HLAc recipients (p<0.001). Twenty five developed acute AMR of which 24 followed HLAi transplantation (39%). Median time to AMR in this group was 14 days (IQR 7-128 days); 22 occurred during the first year. AMR risk within the first 3 years after HLAi transplantation was increased irrespective of whether the DSA was historical or present at time of transplantation and independent of the level of MFI_{T0} and MFI_{MAX} (Figure 2). T-cell mediated rejection (TCMR) rates within the first year post-transplant were 11% (N=13) in the HLAc and 16% (N=10) in the HLAi cohort (p=0.270). Median time until TCMR was 105 days (IQR 6-567) and 137 days (IQR 52-475) in HLAc and HLAi respectively (p=0.860).

Role of the Flow Cytometry Crossmatch

Transplantation in the presence of DSA and a negative FC-XM carried an increased risk of AMR within the first year post-transplantation (27%), compared to HLAc transplantation (0% [p<0.001]). The highest risk of AMR during the first year was seen in recipients with a DSA and positive FC-XM (52% [p<0.001], Figure 3).

In DSA_{POS}/$FC\text{-}XM_{NEG}$ recipients; AMR risk was 43% following IL2-Ra induction and 24% following LDa induction. In DSA_{POS}/$FC\text{-}XM_{POS}$ recipients, AMR risk was 71% following IL2-Ra compared to 44% following LDa induction (p=0.224 and 0.141 respectively)

The role of DSA post-transplantation

In 28 (46%) HLAi recipients the DSA present prior to transplantation was not detectable at 3 years post-transplantation; 28 had a persistent DSA with MFI ≥1000 and no HLAi recipients developed *de novo* DSA. Of note, 1 HLAc transplant recipient developed AMR in the presence of *de novo* DSA >3 years post-transplantation following non-compliance and consequently lost their transplant.

Of the 25 recipients that developed AMR, 22 (88%) had DSA with MFI ≥1000 at time of AMR and 15 (63%) MFI>3000. Twenty HLAi recipients with a current DSA (41%) and 4 recipients with an historical DSA (33%) developed AMR, in all cases this coincided with an increase in MFI of the historical DSA. Eleven of the fifteen recipients that received an HLAi living donor organ had plasma-exchange prior transplantation; 6 of these (55%) developed AMR within the first month post-transplantation, all associated with a rise in MFI of pre-existing DSA.

Transplant function and patient survival

Transplant function

Eighty-eight percent of HLAi recipients were alive with a functioning transplant at 1 year post-transplant, compared to 94% of HLAc recipients. For these HLAi recipients, the mean unadjusted eGFR at 1 year was 50 [SD±21] mL/min/1.73m^2 compared to 58 [SD±21] following HLAc transplantation (p=0.014). The HLAi recipients that did not develop AMR during the first year had similar eGFR at 1 year as HLAc recipients (55 and 58 mL/min/1.73m2, p=0.446), compared to an eGFR of 37 mL/min/1.73m2 in those who did develop AMR (p<0.001).

Graft loss

In total, 26 transplants (14%) failed, 11 within the first year (7%). Death censored graft loss at 3 years was 13% following HLAi transplantation and 8% following HLAc transplantation (p=0.09, Figure 4A). Graft loss at 3 years, defined as return to dialysis or re-transplantation, including death with a functioning graft, was 30% following HLAi transplantation and 12% following HLAc transplantation (p=0.004, Figure 4B).

Mortality

Twenty-one recipients (12%) died during follow up, 19 with a functioning transplant. Mortality at 3 years was 5% in HLAc recipients, compared to 16% in DSA_{POS}/FC-XM_{NEG} and 31% in DSA_{POS}/FC-XM_{POS} recipients (p=0.013). The most common cause of death in the HLAi cohort was infection (58%), compared to cardiovascular events (33%) following HLAc transplantation. Two HLAi recipients died following infection with Pneumocystis jiroveci, one HLAc recipient died following infection with SARS-CoV-2 infection. Table B in the appendix provides details about cause of death.

Aiming to reduce measured confounding, propensity scoring was used to form a new cohort consisting of 34 HLAc and 34 HLAi transplant recipients matched on RRT-vintage, re-transplantation and duration of follow-up (see Appendix Table A). Survival analysis in this PS-matched cohort (N=68) supported the analysis performed in the full cohort; HLAi transplantation was associated with a reduced 3-year patient survival (81% vs 100%, p=0.012) but not graft survival (90% vs 94%, p=0.618). Cohort size and event rate prohibited regression analysis.

Discussion

There remain questions regarding the outcomes, risks and optimal management strategies for HLAi transplantation. This cohort study compared recipient and transplant outcomes in a cohort of CDC-XM negative HLAi recipients matched for age, gender and transplant type with a contemporary cohort of HLA compatible kidney transplant recipients. The consistent approach to HLAi definition, recipient selection and management, in combination with the availability of detailed patient HLA antibody histories, has allowed us to compare the clinical implications of kidney transplantation in the presence of DSA in CDC-XM negative / FC-XM positive and negative recipients with standard HLAc transplants.

Transplantation in the presence of a DSA and a positive FC-XM carried the highest risk of AMR at 1 year, compared to DSA_{POS}/FC-XM_{NEG} and HLA compatible transplantation. Importantly, AMR risk was similar between recipients with a DSA present at time of transplant and those with an historical DSA and was independent of the level of MFI_{T0} and MFI_{MAX}. A persistent or rising MFI of either a current or historical DSA post-transplantation was seen in recipients who developed AMR. This suggests that the presence of pre-existing or historic DSA at any level is a marker of immunological memory.

The patient who developed *de novo* DSA (*dn*DSA) was an HLAc recipient who was non-compliant and consequently lost their graft. This is in line with reports of a rapid decline in transplant function following medication non-adherence leading to *dn*DSA-associated graft loss[21]. The incidence of *dn*DSA development post-transplantation reported in the literature varies between 14% and 50%, which is far higher than we describe[22–25]. Unfortunately, many cohorts have evaluated recipients with a mixture of pre-transplant and *dn*DSAs or used insensitive methods to rule out DSAs at the time of transplantation. This makes it

difficult to determine if DSAs were truly *de novo* or pre-existing[22,24,26]. Vitally, *dn*DSA can only be defined when complete historical data and information on sensitising events is available[16].

Our study suggests that historical DSA are of clinical significance even when the MFI at time of transplantation is low. In our cohort 4 recipients developed AMR which coincided with a rise in MFI of a DSA that was absent at time of transplantation, but had been present historically. Without knowledge of historical sensitising events and detailed immunological assessment this could incorrectly be classified as AMR in the context of *dn*DSA, where in fact this is a memory response of a pre-existing DSA.

This study has shown a statistically significant stepwise increase in risk of AMR at 1 year from HLA compatible transplantation (0%) to DSA_{POS}/FC-XM_{NEG} transplantation (27%) and FC-XM_{POS} transplantation (52%). Transplant function at 1 and 3 years and graft survival were inferior following HLAi transplantation compared to HLAc transplantation and were negatively associated with prior AMR. Similar results of increased graft loss and mortality following incompatible transplantation, especially in the context of acute rejection, were suggested in a large multicenter trial by *Motter et.al.* in 2021[27]. Despite this, 3-year graft survival in our cohort was good (87%), despite a higher risk of AMR following HLAi transplantation. Recipients with a positive FC-XM had the highest 3-year mortality (31%), consistent with results of a large multicentre analysis which reported a two-fold increased mortality risk in DSA_{POS}/FC-XM_{POS} recipients compared to DSA_{POS}/FC-XM_{NEG} recipients[20].

To place this in context; in Scotland, the 10-year patient survival following start of RRT is approximately 34% for patients aged 45-65 years[28] and the hazard of death at 6 months for patients in the same age bracket with a RRT-vintage of >10 years has been estimated as 10%

in the UK[29]. Our HLAi cohort had a median RRT-vintage of 17 years at time of transplantation. Whilst we acknowledge that patients on the kidney transplant waiting list will have a lower *a priori* risk of dying compared to those with ESRD who are deemed unsuitable for transplant, we believe that a patient survival rate of 79% at 3 years as achieved in our HLAi program is better than would have been expected if patients had prolonged their time on dialysis whilst awaiting a compatible transplant.

The limitations of this study lie in its retrospective design and small cohort size which prohibits firm conclusions around causality, specifically when comparing the PS-matched cohorts. We also acknowledge that our HLAc and HLAi cohorts were not matched for all recipient and donor characteristics that may be associated with the reported inferior unadjusted transplant and patient outcomes in the HLAi cohort. For example, although the number of recipients with >1 comorbidities were similar, HLAi recipients had a significantly longer history of ESRD then HLAc recipients, which is known to reduce resilience and negatively impact survival[28–30]. Additional propensity score (PS) matching was used to address the potential effect of observed confounders such as RRT-vintage and comorbidities on mortality. Survival analysis within this PS-matched cohort associated HLAi transplantation with reduced 3-year patient survival compared to HLAc transplantation. This suggests the presence of unmeasured confounders which may include side-effects of prolonged immunosuppression, polypharmacy and subclinical advancing cardiovascular disease post-transplantation impacting in a different fashion on morbidity and mortality in both cohorts.

Lastly, protocol transplant biopsies or DSA monitoring post-transplantation are not routinely performed in recipients with a low immunological risk (i.e. in our HLAc cohort), but are more commonly performed following HLAi transplantation. Attrition bias due to the inherently lower presumed rejection risk in the HLAc group and the lack of protocol biopsies may have resulted in an under-reporting of subclinical (chronic) rejection and transplant glomerulopathy[12]. Transplant function and rejection rates in this group are however comparable to international literature and the consistent approach to clinical management in Scotland, following national and international consensus guidelines, suggests the outcomes of this study are likely to be relevant on a wider scale.

In summary, compared to HLA compatible kidney transplantation, HLA incompatible kidney transplantation, especially in the presence of a positive FC-XM, is associated with an increased risk of AMR, inferior graft function and increased mortality. For many highly sensitised recipients a compatible transplantation is not on offer and in reality, the choice is between remaining on dialysis, which is associated with inferior patient outcomes, and embarking on a kidney transplant journey which may be associated with enhanced risk of rejection and suboptimal graft function. A detailed record of historical DSA and the use of FC-XM prior to transplantation is paramount to inform clinicians and patients of the individualised risk profile ahead of HLAi transplantation.

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Conflict of Interest Statement

The authors declare no conflicts of interest

Ethical Approval

NHS Health Research Authority deemed NHS Research Ethic Committee review not required.

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Tables

Table 1: Recipient characteristics

	HLA compatible N=122	HLA incompatible N=61	P-value
Age; years (mean ± SD)	46 (±11)	46 (±11)	0.957
≥1 Comorbidities^; N (%)	40 (33)	25 (41)	0.275
Diabetes pre-transplant	19 (16)	9 (15)	0.885
Ischaemic heart disease	9 (7)	10 (16)	0.059
Cardiovascular disease*	13 (11)	14 (23)	**0.027**
Cancer pre-transplant	5 (4)	2 (3)	0.750
Cancer post-transplant	2 (2)	2 (3)	0.750
Duration of follow up; years, (mean ± SD)	3.8 (± 1.12)	3.2 (± 1.7)	**0.006**
Female gender; N (%)	72 (59)	36 (59)	0.999
Amount HLA mismatches; N (%)			**0.044**
MM 0	20 (16)	2 (3)	
MM 1 to 3	77 (64)	43 (74)	
MM 4 to 6	24 (20)	47 (23)	
Induction agent; N (%)			**<0.001**
IL2 receptor antagonist	113 (93)	15 (25)	
Lymphocyte depleting agent	9 (7)	46 (75)	
Cold ischaemia time; mins (mean ± SD)			
Living donor recipient	228 (80)	280 (159)	0.187
Deceased donor recipient	777 (298)	963 (242)	**0.001**
Live donor; N (%)	30 (25)	15 (25)	0.999
Plasma-exchange desensitisation; N (%)	0	13 (21)	**<0.001**
Re-transplantation; N (%)	22 (18)	43 (71)	**<0.001**
RRT vintage; years (median, IQR)	2.4 (0.3 – 5.8)	17.3 (6.4 – 22.4)	**<0.001**

^Co-morbidities; all co-morbidities excluding Non-melanoma skin cancer and Hypertension.
* Cardiovascular disease; composite of documented cardiac arrhythmia, peripheral or arterial vascular disease, ischaemic heart disease, congestive heart failure.

Table 2: HLA incompatible transplantation; characteristics

	All HLAi recipients N=61	Current DSA N=49	Historical DSA N=12
Complement-dependent Crossmatch positive; N	0	0	0
Flow cytometry X-match positive; N (%)			
T FC-XM	13 (7)	12 (25)	1 (8)
B FC-XM	25 (41)	21 (43)	4 (33)
FC-XM$_{POS}$ and relevant DSA	23 (38)	23 (47)	0
DSA class present; N(%)			
HLA Class I only	26 (43)	18 (37)	8 (66)
HLA Class II only	20 (33)	18 (37)	2 (17)
HLA Class I and II	15 (24)	13 (26)	2 (17)
Cumulative MFI; median (IQR)			
At time of transplant	3327 (1352 – 6458)	3760 (2257 – 7457)	653 (283 – 892)
Peak MFI	4021 (2704 – 7344)	4030 (2704 – 7778)	3667 (2621 – 7014)
Cumulative MFI, at time of transplant; N (%)			
<3000	27 (44)	15 (31)	12 (100)
3000 – 10000	25 (41)	25 (51)	0
>10000	9 (15)	9 (18)	0
Immunodominant DSA MFI; median (IQR)			
At time of transplant	2087 (1228 – 4361)	3177 (1708 – 5296)	653 (248 – 892)
Peak MFI	3208 (1816 – 5452)	3208 (1816 – 5648)	3175 (1662 – 5455)

DSA; Donor specific antibody
FC-XM$_{POS}$; positive flow cytometry crossmatch
MFI; Mean fluorescence intensity

Figures

Figure 1: Mean fluorescence intensity (MFI) in recipients with current and historic DSA

Box plot of distribution of MFI levels in recipients with an historical DSA and those with a current DSA.

* Median cumulative MFI at time of transplantation for recipients with an historic DSA was significantly lower than those with a current DSA ($p<0.001$).

^ The peak level of cumulative MFI prior to transplantation in recipients with an historic DSA was similar to the level of MFI at time of transplantation for those with a current DSA ($p=0.796$)

Figure 2: Risk of AMR is increased in HLAi transplantation

Risk of AMR is increased in HLAi transplantation (Figure 2A; Log rank testing, CHI^2 57.3, $p<0.001$), regardless of the level of MFI (Figure 2B; Log rank testing, CHI^2 0.212, $p=0.899$) and whether the DSA is historical or current (Figure 2C; Log rank testing, CHI^2 0.078, $p=0.780$).

Figure 3: Risk of AMR is increased in HLAi transplantation with a positive FC-XM

HLA incompatible kidney transplantation in the presence of a negative flow cytometry crossmatch ($FC\text{-}XM_{NEG}$), carries an increased risk of AMR within the first year post-transplantation (27%), compared to HLA compatible transplantation (0%). The highest risk of AMR during the first year post-transplantation is seen following HLA incompatible transplantation with a positive FC-XM (52%), Log rank testing, CHI^2 76.4, $p<0.001$.

Figure 4: Risk of graft loss and death is increased following HLAi transplantation

A: risk of death censored graft loss (Log rank testing, CHI^2 2.89, p=.090). B: risk of graft loss – defined as either return to dialysis / re-transplantation or death with a functioning graft (Log rank testing, CHI^2 8.24, p=0.004). C: risk of death (Log rank testing, CHI^2 6.30, p=0.012).

Appendix

Table A: Comparison of original and PS-matched cohort

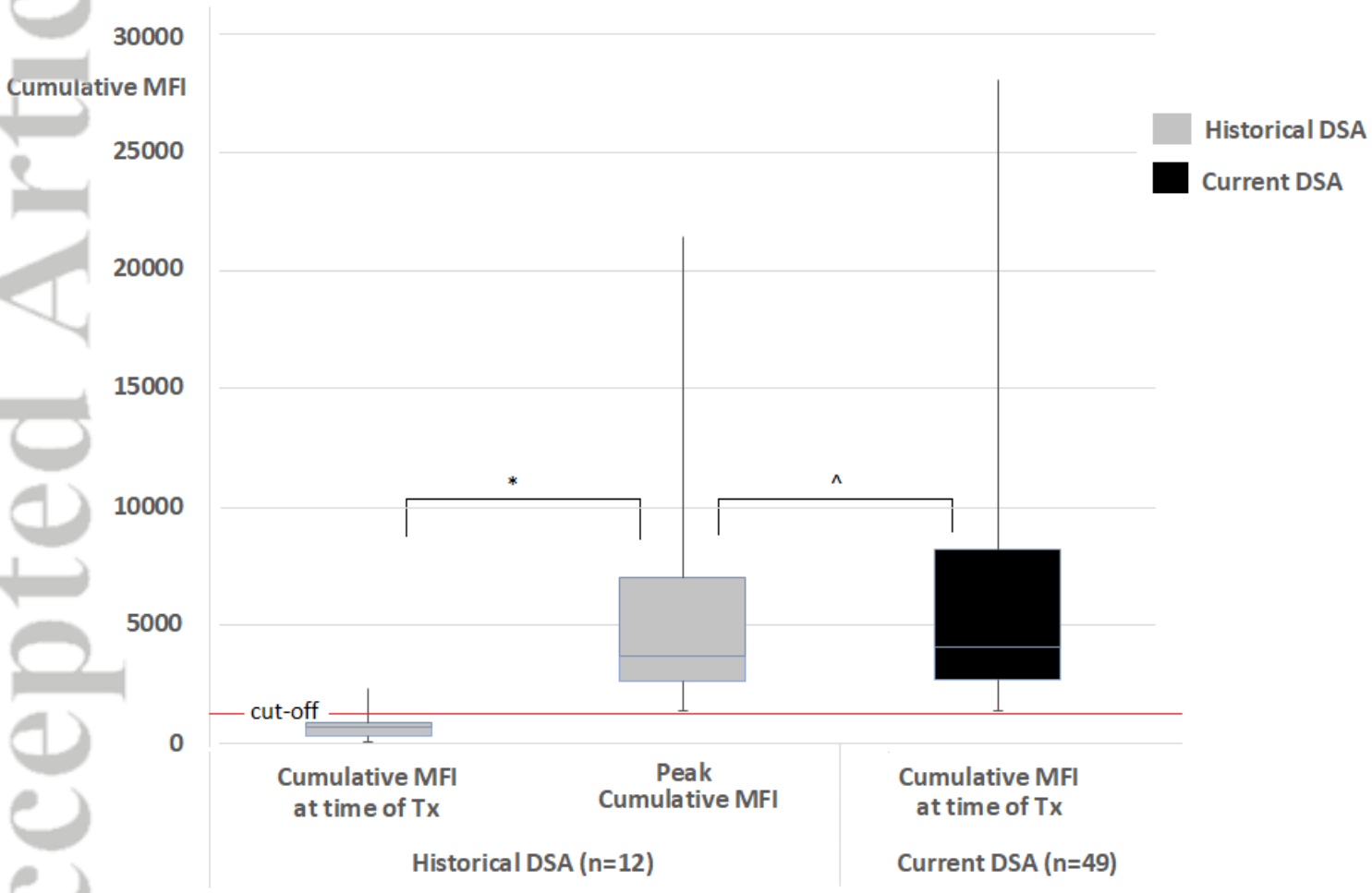
	Original cohort			Propensity Score matched cohort		
	HLA compatible N=122	**HLA incompatible N=61**	**P-value**	**HLA compatible N=34**	**HLA incompatible N=34**	**P-value**
Age; years (mean ± SD)	46 (±11)	46 (±11)	>0.999	44 (±11)	48 (±11)	0.172
≥1 Comorbidities; N (%) Diabetes pre-transplant	40 (33) 19 (16)	25 (41) 9 (15)	0.275 0.885	10 (29) 5 (15)	13 (38) 6 (18)	0.442 0.742
Duration of follow up; years, (mean ± SD)	**3.8 (± 1.1)**	**3.2 (± 1.7)**	**0.006**	4.2 (± 1.2)	3.5 (1.7)	0.061
Female gender; N (%)	72 (59)	36 (59)	>0.999	22 (65)	20 (59)	0.618
Live donor; N (%)	30 (25)	15 (25)	>0.999	6 (18)	10 (29)	0.253
Re-transplantation; N (%)	**22 (18)**	**43 (71)**	**<0.001**	22 (65)	20 (59)	0.618
RRT vintage; years (median, IQR)	**2.4 (0.3 – 5.8)**	**17.3 (6.4 – 22.4)**	**<0.001**	11.0 (3.0 – 19.2)	12.5 (4.0 – 19.0)	0.627
Amount HLA mismatches; N (%) **MM 0** **MM 1 to 3** **MM 4 to 6**	 **20 (16)** **77 (64)** **24 (20)**	 **2 (3)** **43 (74)** **47 (23)**	**0.044**	 5 (15) 22 (67) 6 (18)	 0 (0) 26 (81) 6 (19)	0.070

Table A: Comparison of the original cohort and the propensity score matched cohort of HLA compatible and HLA incompatible transplant recipients. In the original cohort, duration of follow up, re-transplantation, RRT-vintage and ≥4 HLA mismatches were statistically significantly different between both groups. In the Propensity Score matched cohort there were no measurable differences between the groups.

Table B: Cause of death

	HLA compatible N=122	HLA incompatible N=61	P-value
Mortality; N (%)	9 (7)	12 (20)	**0.014**
Mortality at 1 year	0	3 (5)	**0.014**
Mortality at 3 years	6 (5)	11 (18)	**0.004**
Cause of death; N (%)			
Cardiovascular	3 (33)	1 (8)	0.149
Infection	2 (22)	7 (58)	0.098
Malignancy	0	2 (17)	0.198
RRT complication / withdrawal	2 (22)	1 (8)	0.368
Other/unknown	2 (22)	1 (8)	0.368

Table B: Death at 1 and 3 years in the full cohort (N=183) was significantly higher following HLA incompatible transplantation. Most common cause of death following HLA compatible transplantation was cardiovascular disease (33%) whereas infection was most common cause of death following HLA incompatible transplantation (58%).



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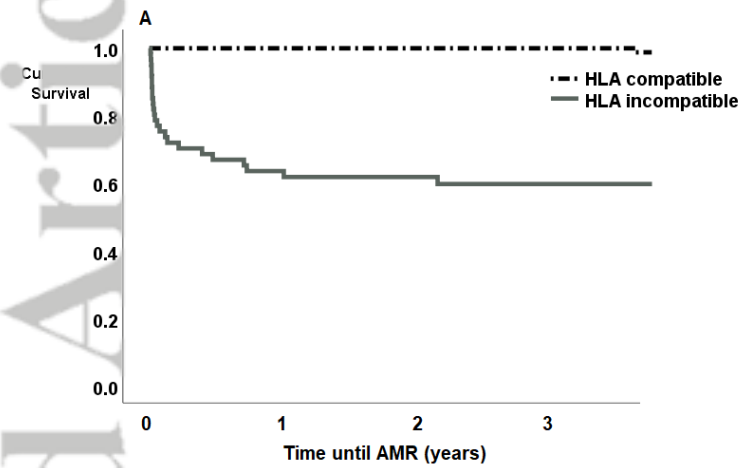
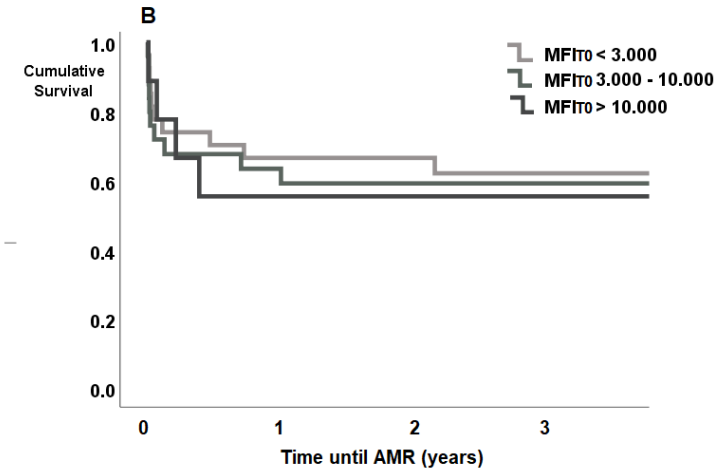
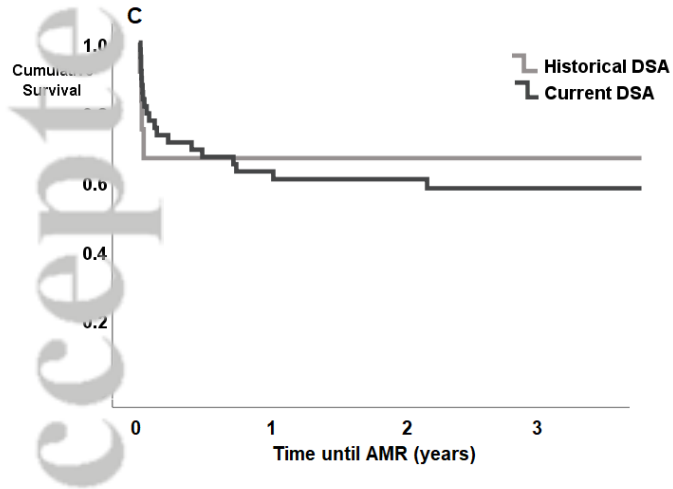
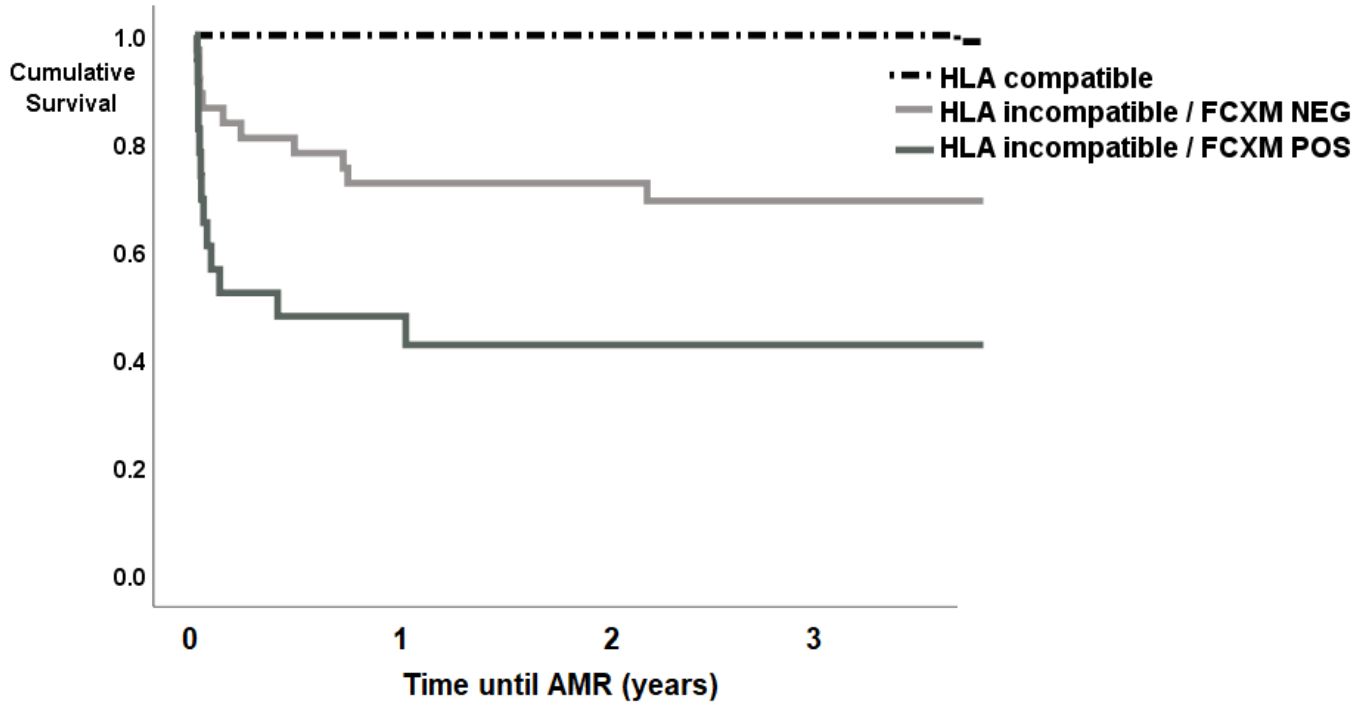






Recipients at risk	Time until AMR (years) 0	1	2	3
HLA compatible	122	115	112	103
HLA incompatible	61	36	32	22
MFIT0 < 3.000	27	17	15	10
MFIT0 3.000 - 10.000	25	15	13	9
MFIT0 > 10.000	9	4	4	3
Historical DSA	12	8	7	6
Current DSA	49	28	25	16

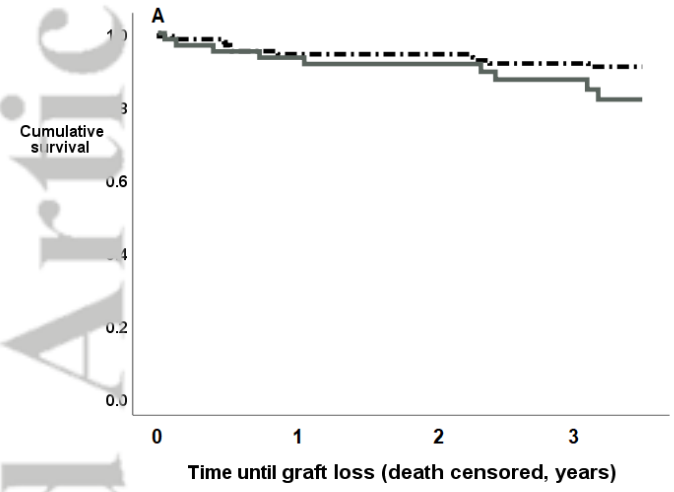
NEP_14102_2 Risk of AMR.tif

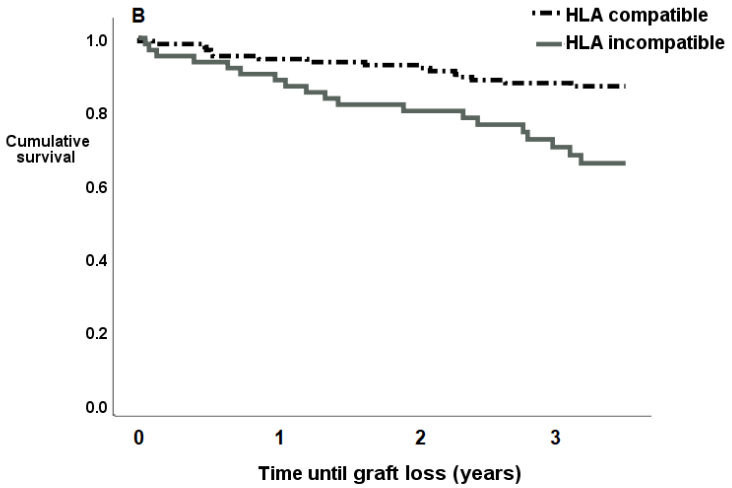


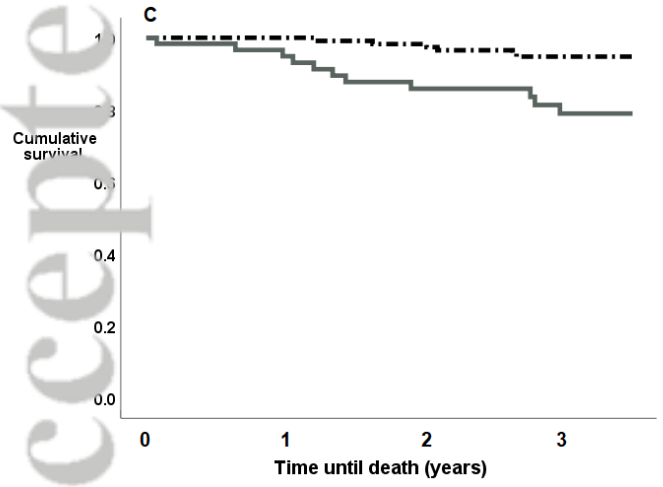
Recipients at risk

	0	1	2	3
HLA compatible	122	115	112	103
HLA incompatible / FCXM NEG	37	26	23	16
HLA incompatible / FCXM POS	23	9	8	5

NEP_14102_3 Risk of AMR FCXM.tif







Recipients at risk

	Time until graft loss / death (years)			
	0	1	2	3
HLA compatible	122	115	112	103
HLA incompatible	61	53	46	33

NEP_14102_4 Risk of GL dc.tif