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Clinical relevance of pretransplant donor-specific and non-donor-specific HLA-antibodies identified using newer sensitive assays in kidney transplant patients

Degree Project in Medicine

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List of abbreviations

ACR	Acute cellular rejection
AHR	Acute humoral rejection
AMR	Antibody-mediated rejection
CCR	Chronic cellular rejection
CDC	Complement-dependent cytotoxicity
CHR	Chronic humoral rejection
cMFI	Cumulative mean fluorescence intensity
DD	Deceased donor
DSA	Donor-specific antibiodies
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
FCM	Flow cytometry
FCXM	Flow cytometry crossmatch
HLA	Human leucocyte antigen
HLA-Abs	Human leucocyte antigen antibodies
Ktx	Kidney transplantation
LD	Living donor
MCS	Mean channel shift
MFI	Mean fluorescence intensity
MHC	Major histocompability complex
NDSA	Non-donor-specific antibodies
PRA	Panel reactive antibody
Pretx	Pretransplant
SAB	Single antigen beads
SD	Standard deviation

Abstract

Title: Clinical relevance of pretransplant donor-specific and non-donor specific HLA-antibodies identified using newer sensitive assays in kidney transplant patients

Degree Project in Medicine, Programme in Medicine, Sahlgrenska Academy at the University of Gothenburg, Sweden 2019.

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Introduction: The presence of anti-HLA-antibodies (HLA-Abs) in serum, both donor-specific (DSA) and non-donor-specific (NDSA) has been increasingly associated with chronic antibody-mediated rejection and decreased long-term kidney graft survival after kidney transplantation. In recent years, more sensitive and specific assays such as single antigen beads and flow cytometry crossmatch (FCXM) have been developed to detect HLA-Abs. However, the clinical significance of low levels of pretransplant HLA-Abs detected by these sensitive assays remains controversial.

Aim: The aims of the study are: 1) to elucidate the clinical relevance of pretransplant HLA-Abs (both DSA and NDSA) detected using newer sensitive assays in kidney transplant recipients on graft function and survival, and 2) to examine the outcomes of recipients with positive FCXM in relation to DSA, using the current sensitive assays.

Methods: This retrospective study comprises patients who underwent kidney transplantation between 2012-2015 at Sahlgrenska University Hospital (n=444). All patients were divided into three subgroups based on their pretransplant HLA-Ab status. All HLA-Abs positive patients (n=88) were further divided into four groups according to DSA and FCXM status.

Results: DSA-positive recipients had a significantly inferior lower estimated glomerular filtration rate and a trend to worse 6-year graft survival compared to HLA-Abs-negative or NDSA groups. Recipients with positive FCXM who also were DSA-positive had significantly worse overall graft survival and function compared to those without DSA as well as NDSA recipients.

Conclusions: Pretransplant DSA were associated with inferior graft function and a trend to lower 6-year graft survival in out kidney transplant population. A positive FCXM had prognostic implications for graft function and survival only in presence of DSA.

Key words: Kidney transplantation - donor specific antibodies - flow cytometry crossmatch - single antigen bead assay – chronic allograft rejection – acute allograft rejection

1. Background

1.1 History about the human leucocyte antigen (HLA)

Major Histocompatibilty Complex (MHC) are cell surface proteins that bind to peptides derived from pathogens and present them to the immune cells of the host (2). In this way, foreign pathogens are recognized and eliminated by the immune cells (2). This was first described by a British physician and pathologist named Peter A. Gorer in 1936 and later by George Snell which later led to the discovery of the human variant of MHC; Human Leucocyte Antigen (HLA) (3).

HLA was first discovered 1958 by Dr. Jean Dausset who later received Nobel Prize in Physiology or Medicine for his great discovery (4). In 1952, Dr. Dausett did an experiment where he mixed a leucopenic patient's blood sample with leucocytes from another individual and observed an unexpected aggregation of enormous leucoagglutinates (3). The antibody produced strong agglutination reactions against the leucocytes from the blood donor but was inactive against the patient's own leucocytes (3). The studies provided evidence for the existence of anti-leucocyte antibodies similar to AB0 blood groups (3). Whilst the anti-AB0 blood group antibodies exist naturally; anti-leucocyte group antibodies arise after immunization (3). The alloantibodies in these sera detected a polymorphic system of antigens on human leucocytes. The leucocyte antigen received the name "MAC" (later HLA), an acronym made up of the initials of three patients who served as volunteers in the laboratory for these experiments. In the original report of these observations, it was suggested that this MAC antigen might be of importance in transplantation. MAC was later assigned the designation Human Leucocyte Antigen-A2 (HLA-A2) (3).

The HLA-system maps to the short arm of chromosome 6. The complete structure and gene map of the HLA region was published 1999 (5). The genomic sequence of the region is approximately 3,3-Mbp whereas more than 200 genes has been identified. The HLA complex is divided into three regions classified as Class I, II and III (Figure 1). HLAs vary between individuals as a result of genetic differences (6, 7).

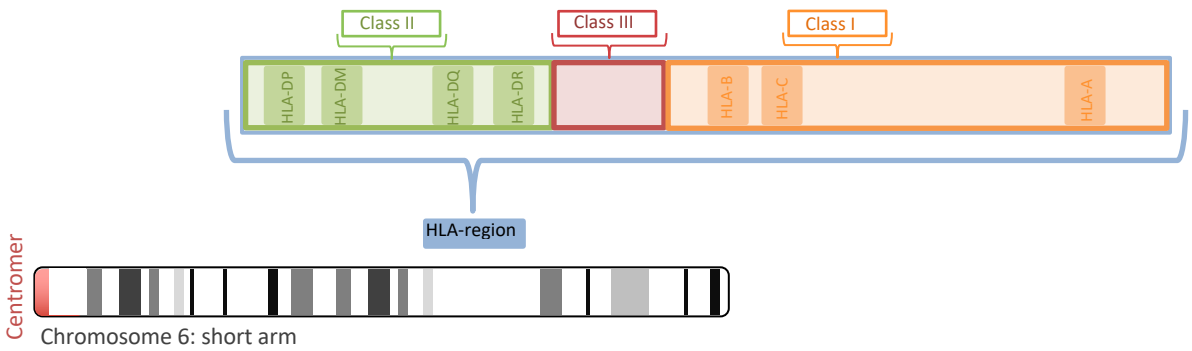


Figure 1
Systematic figure of the human leucocyte antigen (HLA) region on chromosome 6. The HLA-region consists of Class I (HLA-A, B, C), Class II (HLA-DP, DM, DQ, DR) and Class III.

Class I consist of HLA-A, HLA-B and HLA-C and are found on the surface on most of the human cells (7). Class II consist of HLA-DP, HLA-DQ and HLA-DR and are expressed on the surface of cells involved in the immune response (e.g. B cells, activated T cells and macrophages) (6, 7). HLA-DM is compared to the other HLA class II molecules an intracellular protein that help load the foreign peptides onto the HLA class II molecules enabling it forming a HLA-peptide-complex (8). The last region, class III, does not encode HLA molecules but contains complement components such as C2 and C4, tumor necrosis factors (TNFs) and 21-hydroxylase (9).

1.2 Kidney transplantation

In parallel with the process of defining the HLA-antigens, there was a growing interest in the relationship between HLA and transplantation. Candidates for kidney transplant (Ktx) are patients with chronic kidney disease who develop end-stage renal disease (ESRD) needing dialysis immediately, or within a very near future (10). ESRD is a serious health problem worldwide and is associated with considerably higher mortality and a huge socioeconomic burden. Ktx is the treatment of choice for patients with ESRD. It offers significantly improved survival and quality of life as well as substantial reductions in health care costs as compared to dialysis (11).

Many unsuccessful Ktx had been done earlier with the statement that there was a “biological force” that prevented successful transplantation of organs between humans (12). In 1954, the first successful long-term Ktx was done in Boston by Dr. Joseph Murray. The transplantation was done

between two homozygotic twins and survived for 8 years (13). Eight years later, it was showed that this could also be done between genetically nonrelated patients by using immunosuppression, in that time with total body irradiation (14). Dr. Murray was later awarded the Nobel Prize in Medicine for his breakthrough (15). The first Ktx in Sweden was performed 1964 by surgeon Curt Fransson in Stockholm (16). The first Ktx in Gothenburg was performed at Sahlgrenska University Hospital (SU) in 1965 by Professor Lars-Erik Gelin. Fifteen years later, SU became the third hospital in the world with more than 1000 Ktx performed (17).

At present, almost 10 000 patients with ESRD are under active care for ESRD in Sweden of which almost 60% have received Ktx (18). The leading cause of ESRD is glomerulonephritis which represents almost 25% of cases. Approximately 420 Ktx are performed in Sweden every year of which 60% are from deceased donors (DD) and 40% from living donors (LD) (18). At the Transplant center of SU, approximately 150-160 Ktx are performed every year. However, in spite of significantly improved short-term outcomes, the long-term graft loss has remained unchanged leading to a loss of approximately 40% grafts in a time span within 10 years (19). More specifically, approximately 140-150 kidney grafts are lost per year in Sweden (18). Kidney graft failure is now a major cause of ESRD and return to dialysis. This leads to increased morbidity, mortality, increased number of patients on the waiting list incrementing the shortage of organs as well as increased financial burden on the health care system (19). Therefore, maximizing long-term graft survival and reducing the need for retransplantation is paramount. Immune injury caused by chronic active antibody-mediated rejection (cAMR) has been recognized as a leading cause of long-term graft loss and return to dialysis in more than 50% of the kidney grafts (20).

1.3 Immune response and allograft rejection

An allograft is a graft transplanted between nonidentical individuals (allogeneic) of the same species (21). Except monozygotic twins, all individuals from the same species are allogeneic, which makes most allografts recognized as foreign and are destroyed by the immune system of the recipient (21). The donor and recipient are in this case histo-incompatible and the graft is immunologically rejected.

HLA can be seen as a person's fingerprints, and every individual express a unique set of these antigens. The immune response will be activated after a Ktx, both non-specific and specific (21). A rejection occurs as a result of an immune reaction, involving both cell-mediated and antibody-mediated hypersensitivity reactions directed against HLA on the foreign graft (7). The non-specific activation occurs immediately after the Ktx due to the ischemic damage and surgical trauma while the specific activation is initiated by either T cells or B cells (22). The graft from the donor will have foreign HLA-molecules that will activate the T cells and B cells in the recipient (22). The T cells have two different types of recognition: a direct and an indirect pathway (22). In the direct activation, the donor's antigen-presenting cells will present the donor's foreign HLA class II molecules to T cells and activate them (22). The activated T cells will differentiate into different types of T cells that might kill the cells in the graft from the donor. In the indirect activation, the recipient's antigen-presenting cells will degrade the molecules into peptides and present them to T cells that will activate and initiate a response (22). The recipients B cells will recognise the donor's HLA-molecules and will thus be activated (due to stimulation by T cells) and produce a massive amount of antibodies that will have high affinity to the graft and destroy the HLA molecules on the surface (7). This immune response will result in graft loss through either hyperacute, acute or chronic allograft rejection (7).

1.3.1 Human Leucocyte Antigen antibodies (HLA-Abs)

By manipulating the recipient's immune system by immunosuppression, the time to graft rejection is delayed and the time of graft survival increases (2, 21, 23).

When a patient is exposed to cells from another individual, which can happen during transfusion, pregnancy or transplantation, a sensitization can occur against HLA class I and HLA class II molecules (3, 24, 25). A transplant candidate can develop HLA antibodies (HLA-Abs) either before transplantation or develop *de novo* after transplantation. These antibodies can develop against foreign graft HLA from the donor and are for that reason called donor specific antibodies (DSA) (26). Present HLA-Abs that are not developed against the specific donor are therefore called non-DSA (NDSA).

Despite the progress in developing new effective immunosuppression, the presence of HLA-Abs in serum, both DSA and NDSA has been increasingly associated with cAMR and decreased long-term kidney graft survival after Ktx (27). This was observed in a longitudinal study from 2002 where HLA-Abs where shown to appear 6 months to 8 years before rejection (28). The antibodies did not cause an immediate effect and therefore years elapsed before chronic rejection resulted in failure (28). In further publications, it was shown that HLA-Abs were associated in both acute and chronic allograft rejections (29, 30). Fortunately, by increased knowledge in the pathological mechanisms of allograft rejections, the rate in acute rejections has been reduced remarkably. However, chronic graft rejections remain a major barrier to long-term renal graft survival (31, 32).

1.4 HLA antibody detection assays

1.4.1 Complement-dependent cytotoxicity crossmatch (CDC-XM)

In 1964, Paul I. Terasaki and John McClelland developed a lymphocyte microcytotoxicity assay, also called complement-dependent cytotoxicity crossmatch (CDC-XM), that excludes the possibility of preexisting donor-specific antibodies in the recipient (33). CDC-XM was first being used as a detection of antibodies after a rejection to clarify that antibodies were the cause of the graft loss (34). This was described when Terasaki et al. summarized the first 30 cases with hyperacute rejections, 80% of the cases showed positive crossmatches (34). It was suggested that a crossmatch test should be done before and prospectively after a Ktx to avoid immediate rejections. Later on, studies were done where antibodies were detected before and after the transplantation. These antibodies were later shown to be DSA (3, 35, 36).

The recipient's blood, which may contain HLA-Abs, is mixed with the donor's blood which express HLA antigens (Figure 2). Complement factors are then added to the mix, and if the recipients blood contains HLA-Abs, a cell lysis becomes present. If the cell lysis occurs, the CDC-XM is positive which means there is a presence of HLA-Abs which makes the recipient and donor mismatched and a transplantation between these two individuals are contraindicated (34).

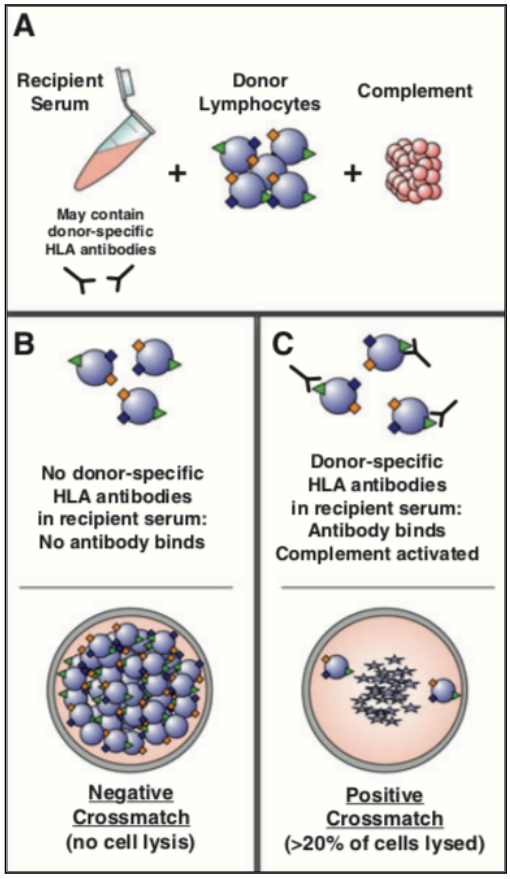


Figure 2. Complement-dependent cytotoxicity crossmatch. Recipient's serum is mixed with donor's lymphocytes and complement factors. If cell lysis occur, crossmatch is positive and indicates detection of donor-specific antibodies (DSA).
Image adapted from Mulley, W. et al. (59)

Since its discovery, CDC-XM has been mandatory used as pretransplant (pretx) detection of complement-binding HLA-Abs in renal donor organ allocation schemes.

To identify sensitized recipients and estimate their likelihood of finding a crossmatch-compatible donor, panel reactive antibody (PRA) test is used. Lymphocytes from a panel of donors that represent the local population (potential donor population) are tested against the recipient's serum which will identify antibodies circulating in the recipient's blood. PRA will in percentage detect the likelihood of finding a crossmatch-compatible unrelated donor. For an example, a patient with 60% PRA would be crossmatch positive with 60% of the donors (37). It has been shown that high PRA levels is associated with poor kidney graft outcomes despite the absence of DSA (38). The test has made it possible to test thousands of alloantibodies against large panels of cells and to type large numbers of patients (3).

1.4.2 Flow Cytometry crossmatch (FCXM)

After the introduction of CDC-XM, early graft loss was still a major problem, suggesting that undetected presensitization was still occurring in transplanted patients and that CDC-XM was not sensitive enough (3). In 1983, Garavoy et al. introduced into clinical practice the flow cytometry crossmatch (FCXM) as a new investigational technique that the standard CDC assay missed (39, 40).

The recipient's serum is mixed with the donor's lymphocytes and additional fluorochrome-labeled antibodies against human IgG are added in the mix. These antibodies have a specific Fc part of IgG and can be further specific through additional antibody subtypes (e.g. IgG2, IgG3).

If there is DSA, they will bind to the donor's lymphocytes and the added anti-human IgG-antibodies thereafter bind to the DSA-lymphocyte-complex. The molecules will thereafter pass a light individually that sorts the molecules due to their size which quantifies the potentially DSAs either against T-cells and/or B-cells (39, 41). The positive results will be further specified using mean channel shift (MCS) to score the intensity of antibodies in the recipient. Generally, a 40 channel shift for T-lymphocytes and an 80 channel shift for B-lymphocytes are considered as positive tests (42).

The FCXM has made it possible to detect low titer of antibodies with more specificity including identification of complement-binding and non-complement-binding antibodies and has the ability to detect developing antibody response weeks to months before the levels can be measured by CDC-XM (39, 43, 44).

1.4.3 Single antigen bead assay (SAB)

Almost thirty years after Terasaki et al. introduced CDC-XM, a single antigen technique that was more sensitive and specific was developed (Figure 3). This single antigen bead assay (SAB) is also called Luminex®. The recipient's serum is incubated with Luminex beads coated with HLA antigens. If the recipient has HLA-Abs present, each individual antibody will recognise and attach to the specific HLA-antigen on the surface of the bead. A secondary antibody, that is fluorescently labeled (anti-human IgG) is added that can allow us to quantify exactly how much antibodies is present on each bead by measuring the degree of fluorescence through flow cytometry (45, 46). This is expressed as the mean fluorescence intensity (MFI), where high MFI indicates high DSA levels (47).

Luminex® can detect low levels of DSA that cannot be detected neither by FCXM or CDC-XM because of its specificity and sensitivity (45). There has been no doubt that presence of DSA in a recipient serum with a positive CDC-XM before transplant has been associated with hyperacute rejections why a positive CDC-XM generally has been considered as a contraindication to Ktx (34, 48). However, the exact clinical significance of low-levels of pretx DSA detected by SAB that do not cause a positive CDC-XM is not well understood. There have been conflicting reports concerning the impact of DSA detected exclusively by SAB on allograft survival (49-57).

Although many studies showed a correlation of DSA detected by SAB and impaired allograft outcome, the presence of HLA-Abs in pretx serum did not necessarily always result in graft loss (53, 58). Thus, the clinical impact of positive pretx HLA-Abs, particularly DSA, detected by more sensitive assays still remains controversial. Starting in 2012, the HLA-Abs and DSA have been assessed pretx using Luminex SAB routinely in all Ktx recipients at SU.

CDC-XM has its limitations that includes false positive and negative results. False positive results can occur since it does not differentiate between different types of complement-binding antibodies (HLA-Abs, non-HLA-Abs and IgM) (59). False negative results may occur when DSA levels are too low to result in activation of the complement cascade or if the antibodies are of the type that does not cause complement activation (59). FCXM is more sensitive since it detects low titer of antibodies and more specific since it only detects IgG antibodies by its anti-human IgG labeling. Lastly, Luminex® will detect only HLA-Abs by its beads with HLA antigens and also the levels of every specific HLA-Ab (45). The comparison between the different histocompatibility methods are summarized in Table 1.

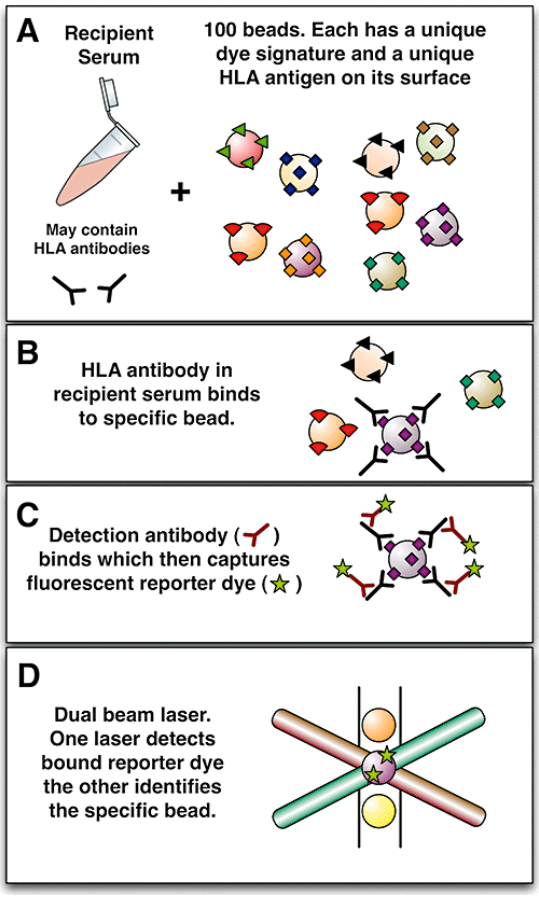


Figure 3. Single antigen bead assay (Luminex®). Recipient's serum is incubated with beads coated with HLA antigens. A recipient with a certain HLA-Ab will recognise the HLA-antigens and attach to them. A flouorescently labeled antibody is added. By flow cytometry the certain HLA-Ab will be indentified and quantified.
Image adapted from Mulley, W. et al. (59)

Table 1 Comparison between different histocompatibility methods and the difference in their specificity and sensitivity. CDC-XM will detect both HLA-Abs, non-HLA-Abs and IgM but will not detect low titers of antibodies why FCXM was developed. By using a labeled anti-human IgG, only HLA and non-HLA-Abs are detected. Low levels of antibodies can be detected using the flow cytometry. The SAB (Luminex®) will by its beads coated with HLA antigens detect specifically HLA-Abs and low titers of HLA-Abs by measuring the degree of fluorescence. Table inspired by Dr. Kathryn Tinckam (1).

Type	*CDC-XM**	*FCXM**	*SAB**
Detects HLA-Abs*	Yes	Yes	Yes
Detects non-HLA-Abs	Yes	Yes	No
Detects IgM	Yes	No	No
Detects low titer Ab	No	Yes	Yes

** CDC-XM=Complement-dependent cytotoxicity crossmatch; FCXM=Flow cytometry crossmatch; SAB= Single antigen bead (Luminex®); HLA-Abs=anti Human Leucocyte Antigen antibodies*

1.5 Highly immunized recipients

A small group of all transplanted recipients are highly immunized meaning they have broadly reactive HLA-Abs known pretx. The probability of finding a matching graft and being transplanted is severely limited, meanwhile the waiting time for transplantation is correspondingly increased (60). To increase the likelihood of transplantation among these recipients, possible donations need to expand by defining acceptable HLA mismatches. In 2009, the Scandiatransplant Acceptable Mismatch Program (STAMP) was initiated to increase the likelihood of transplantation in highly immunized recipients without increasing the risk of acute antibody-mediated rejection (61).

Immunosuppressive treatment in Ktx recipients is today necessity in preventing and treating rejections (21). The immunosuppressive treatment can be divided into induction, maintenance and rejection therapy where there is usually an overlap in the different treatments. The induction therapy is a treatment recipients provide before and during Ktx to stimulate the immune system into developing tolerance. The maintenance treatment is given to prevent rejections from occurring (21).

At SU, recipients that are highly immunized pretx (e.g. DSA-positive recipients or PRA >50%) will receive either i) desensitization therapy prior to Ktx and/or ii) high-risk induction therapy followed by oral high-risk immunosuppressive protocol. Some of these recipients are included in the STAMP-program.

2. Aim

The overall aim with this project was to elucidate the clinical impact of pretx HLA-Abs (both DSA and NDSA) detected using newer sensitive assays in Ktx patients on graft- and patient survival. Furthermore, we wanted to examine the outcomes of patients with positive FCXM (with negative CDC-XM) in relation to DSA.

By improving our understanding of the risk of pretx HLA-Abs, both NDSA and particularly low-level DSA might help improve the Ktx outcomes by allowing personalized risk stratification for graft loss, better organ allocation as well as individualized management of patients in higher risk for immunologic graft loss.

3. Materials and Methods

3.1 Study design and study population

This is a retrospective cohort study conducted on patients who underwent Ktx between January 2012 and December 2015 at SU (n=646).

All adult recipients who were ≥18 years at the time of transplant, both with living and deceased donor Ktx, and residing in Sweden were included in the study. Minors and recipients not residing in Sweden, e.g. those from Iceland who underwent Ktx at SU but live in Iceland, were excluded (n=54). Recipients of multi-organ transplants were also excluded, e.g. kidney-pancreas, kidney-liver (n=44). Moreover, all patients that were ABO-incompatible at the time of transplantation were excluded from the study because of the risk of false-positive FCXM results (n=89). In patients who received more than 1 transplant during the study period, the second transplant was excluded from analysis (n=3), and we excluded patients if baseline SAB results were not available (n=15).

After all the exclusions (n=202), a total of 444 patients were included in the study (Figure 4).

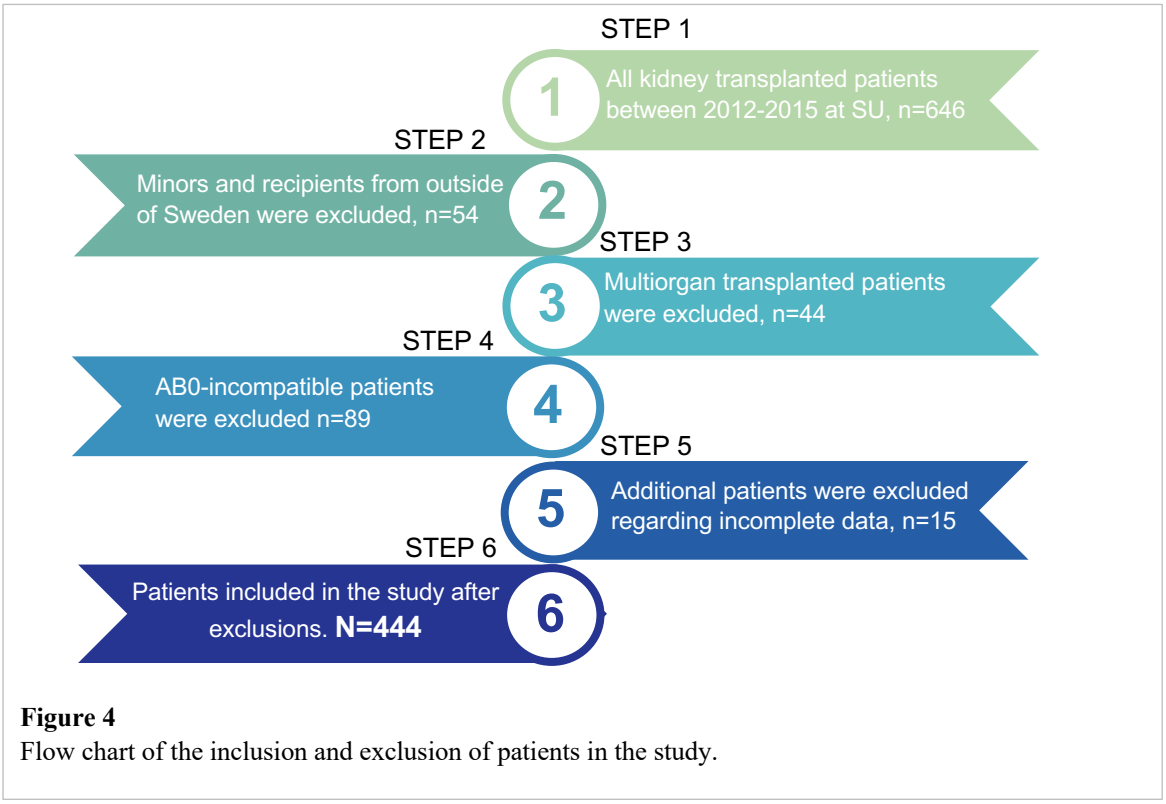


Figure 4
Flow chart of the inclusion and exclusion of patients in the study.

3.2 Pretransplant analyses

Routines before a Ktx can differ between different hospitals in Sweden. The general routine which applies to SU and their patients is described in the following section (Figure 5) (62).

3.2.1 While on the waiting list

All recipients, deceased and potential living donors are tissue typed against HLA-A, B, DR and DQ (Class I & II). HLA-matching is not taken into serious consideration when selecting kidney recipients, partly because of efficient modern immunosuppression, partly because of risk of long ischemia time of the transplant and partly high costs.

After the tissue typing of the recipient, the recipient provides blood samples for screening of HLA-Abs either by a technique called Luminex Screen or flow cytometry (FCM) every third month while waiting for Ktx. The recipient's serum is regularly tested against a panel of cells from volunteer donors, the value provided in a PRA %, which may differ over time. PRA will in percentage detect the likelihood of finding a crossmatch-compatible unrelated donor. For an example, a patient with 60% PRA would be crossmatch positive with 60% of the donors.

When a deceased patient is accepted as an organ donor, suitable recipients from the waiting list are chosen. Blood group, presence of HLA (both DSA and NDSA), age-matched recipient together with recipients with the longest waiting time will be taken as considerations.

3.2.2 Before transplant

The presence of HLA is screened with a fresh serum sample from the recipient using the Luminex Screen or FCM. If there is a positive result, regardless of screening method, the patient's serum is subjected to a second stage of testing by Luminex SAB that will provide information if the HLA-Abs are DSA or NDSA (Table 2). If there is DSA, the SAB will provide an MFI value. Currently, no standard MFI cut-off values exist but a value >1000 is considered positive.

Table 2
All recipients will before Ktx be screened against HLA-Abs either by a technique called Luminex Screen or flow cytometry (FCM). If there is a positive result, regardless of screening method, the patient's serum is subjected to a second stage of testing by Luminex SAB what will provide information if the HLA-Abs are donor-specific or not. If the antibodies are donor-specific, the levels of antibodies will be determined through an MFI value where an MFI >1000 is considered positive. The table demonstrates three different examples of how the analyses are performed due to the HLA-Abs screening results.

	Luminex Screen or FCM*			Luminex SAB*		
Patient	**HLA-Abs* +/-**	**Class I/Class II**	**PRA* (%)**	**DSA* +/-**	**Class I/Class II**	**MFI***
1	+	Class II	76%	+	Class II	4768
2	-	-	-	*SAB* not performed because of negative screen*		
3	+	Class I	43%	-	-	-

**FCM=flow cytometry; SAB=single antigen bead; HLA-Abs=anti Human Leucocyte Antigen antibodies; PRA=panel reactive antibody (0-100%); DSA=donor specific antibodies; MFI=mean fluorescence intensity*

3.2.3 Crossmatch

Crossmatch with a fresh serum sample from the recipient is tested against donor cells right before tx. CDC-XM is the golden standard and is done before every Ktx. A positive CDC-XM indicates that the recipient has strong DSA thus a Ktx is absolutely contraindicated and is not performed. FCXM is more sensitive, and takes longer time, and is performed routinely before transplant with all living donors (LD) transplantations. For deceased donors (DD) transplantations, FCXM is performed only if Luminex screen is positive for HLA-Abs. However, the results arrive after the transplantation has been done. For HLA-negative DD transplantations, the transplantation will be performed on the basis of a negative CDC-XM alone (Figure 5).

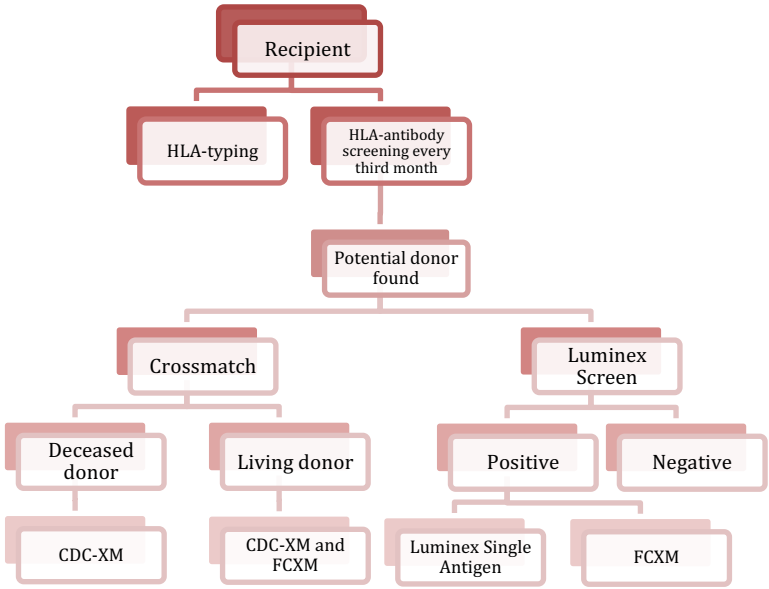


Figure 5
Flow chart summarizing pretx analyses in Ktx recipients at SU. A recipient will be HLA-typed and screened against HLA-Abs every third month. When a potential donor is found (depending on deceased or living), a crossmatch will be performed. Except this, a fresh screening against HLA-Abs will be performed and further characterized if positive.

3.3 Data collection

The patients are anonymized and replaced with an individual Scandianumber. The pretx analyses were extracted and collected from the database of the tissue typing lab at Transfusion Medicine, SU. TIGER is the electronic quality register for Ktx patients at the SU. All recipients are regularly followed-up at their respective outpatients' clinic every three months, four times a year including a physical examination and measurements of creatinine and other laboratory values. Clinical data and laboratory results from respective outpatient clinics in Sweden are entered in the TIGER for every recipient at least once a year. Data regarding the transplantation date, baseline characteristics of the donors and the recipients, the laboratory results and clinical outcome data such as graft/patient status have been extracted from TIGER. In some cases, the follow-up data was missing in TIGER, which was then obtained through fax from each responsible center. In addition, supplementary data was extracted from the electronic journal system Melior and from YASWA (Yet Another Scandia transplant Web Application) via the Transplantation center, SU.

Recipients with no DSA but with no complete typing (n=22) were regarded as negative.

Estimated glomerular filtration (eGFR) was calculated using the CKD-Epi formula (63).

Highly immunized patients (e.g DSA-positive recipients or PRA >50%) received either i) desensitization therapy prior to Ktx and/or ii) high-risk induction therapy followed by oral high-risk immunosuppressive protocol.

3.4 Outcome measures

The primary outcomes were overall graft survival, death-censored graft survival and allograft function. The secondary outcomes were patient survival and allograft rejections.

3.4.1 Subgroups

All patients were divided into three groups based on their pretx analysis (Table 3). The three groups were as follows: i) positive HLA-Abs with DSA [HLA+/DSA+], ii) positive HLA-Abs but no DSA (NDSA) [HLA+/DSA-] and iii) no HLA-Abs [HLA-/DSA-]. The DSA in the [HLA+/DSA+] group was further characterized with both DSA class and MFI.

Patients with positive HLA-Abs were further divided into following four subgroups according to DSA and FCXM status: i) negative FCXM with no DSA, [FCXM-/DSA-], ii) negative FCXM with DSA [FCXM-/DSA+], iii) positive FCXM with no DSA [FCXM+/DSA-] and iv) positive FCXM with DSA [FCXM+/DSA+].

Table 3

All patients were divided into three groups based on their pretx analysis; No HLA-Abs, NDSA (positive HLA-Abs but no DSA) and DSA (positive HLA-Abs with DSA). Patients with positive HLA-Abs were further divided into four subgroups according to DSA and FCXM status. Simplified table of the subgroups depending on their HLA-, DSA- and FCXM status.

Group		FCXM-status
No HLA-Abs	HLA- DSA-	-
NDSA	HLA+ DSA-	FCXM+ DSA-
		FCXM- DSA-
DSA	HLA+ DSA+	FCXM+ DSA+
		FCXM- DSA+

The patient and overall graft survival was compared among the three groups and four subgroups above.

3.5 Statistical methods

Results of continuous measured data are presented as means (standard deviation, SD) and categorical variables are expressed as proportion, if not stated otherwise. To calculate the cumulative incidences of patient and graft survival, the Kaplan and Meier estimator was used through SPSS version 25 (IBM Corp., Armonk, New York).

Time-to-event data were compared with the log-rank test, if applicable. For statistical comparisons, the group with no HLA-Abs [HLA-/DSA-] was the reference group. The starting point for the follow-up of patient was the time of transplantation. The endpoints for death-censored graft failure was need of dialysis or retransplantation. Data was censored for death with functioning allograft and loss of follow-up. Chi-square test was used for the analysis of categorical data, and the

unpaired *t* test or the Wilcoxon rank sum test for continuous data, as appropriate. Comparison of more than two groups was performed using one-way ANOVA. A two-sided P-value <0.05 was considered statistically significant.

For our analysis of eGFR, patients with ESRD were assigned an eGFR of 0 mL/min/1.73m^2 and then censored, meaning subsequent visit-based eGFR values were not included.

3.6 Ethics

The study was conducted according to the Helsinki Declaration and was approved by the Regional Ethical Review in Lund (ethics approval number: 372/16). Before undergoing transplantation, all patients agree on usage of their data in registers and databases. All recipients were anonymized and replaced with an individual Scandianumber.

4. Results

4.1 Patient characteristics among all patients

The patient characteristics are shown in Table 4. Comparison of the three groups (No HLA-Abs, NDSA and DSA) was performed using one-way ANOVA and Chi-square test was used for the analysis of categorical data between the groups. The age range is between 19 to 76, and the mean age at tx is similar among all groups (p=0.73). Compared to no HLA-Abs recipients, recipients in the DSA group were more likely to be female (p<0.001) and transplanted more than one time (p<0.001). Recipients in all groups were more likely to receive a transplant from a deceased donor (p=0.36).

Table 4. Patient characteristics among all patients

	ALL PATIENTS (N=444)	**NO HLA-ABS (N=354)**	**NDSA (N=57)**	**DSA (N=33)**	**P-VALUE**
AGE AT TX* ± SD (RANGE) IN YEARS	51.5 ± 13.9 (19-76)	51.3 ± 14.4 (19-76)	52.8 ± 11.2 (27-75)	50.9 ± 13.9 (22-73)	p=0.73
GENDER WOMEN, N (%) MEN, N (%)	159 (36) 285 (64)	105 (30) 249 (70)	32 (56) 25 (44)	22 (67) 11 (33)	**p<0.001**
PATIENTS WITH >1 KIDNEY TX N (%)	68 (15)	24 (7)	29 (51)	15 (46)	**p<0.001**
DONOR TYPE*, N (%) DD LD	298 (67) 146 (33)	233 (66) 121 (34)	43 (75) 14 (25)	22 (67) 11 (33)	p=0.36
PERCENT PRA* AT TX (MEAN ± SD) CLASS I CLASS II	7.8 ± 21.5 9.0 ± 24.7	-	29.7 ± 30.9 46.7 ± 40.1	53.2 ± 33.2 40.1 ± 34.6	**p<0.001** p=0.43
PRA ≥80% (class I and/or II) N (%)	38 (9)	-	23 (40)	15 (46)	**p<0.001**
DIALYSIS PRIOR TO TRANSPLANTATION N (%) YES NO	355 (80) 89 (20)	277 (78) 76 (22)	51 (90) 6 (10)	27 (82) 6 (18)	p=0.07
TIME ON DIALYSIS PRETX* (MEAN YEARS ± SD)	2.6 ± 2.0	2.6 ± 2.0	2.3 ± 1.5	3.3 ± 2.5	p=0.1

**Tx=transplantation(s); DD=deceased donor; LD=living donor; PRA=panel reactive antibody; Pretx=pretransplant*

Recipients with NDSA were more likely to have received dialysis prior to transplant (p=0.07) but recipients with DSA had dialysis for a longer time (p=0.1).

Recipients sensitized in class I were higher in the DSA group (p<0.001) but lower in class II compared to the NDSA group (p=0.43). Thus, highly sensitized recipients were seen more frequently (46% compared to 40%) in the DSA group (p<0.001).

4.2 Patient outcomes among all patients

The patient outcomes among all patients are shown in Table 5. The follow-up time was slightly shorter in NDSA recipients with a mean of 3.3 ± 1.2 years compared to no HLA-Abs recipients with a mean of 3.8 ± 1.2 years (p=0.012).

Table 5. Patient outcomes among all patients

	ALL PATIENTS (N=444)	**NO HLA-ABS (N=354)**	**NDSA (N=57)**	**DSA (N=33)**	**P-VALUE**
FOLLOW UP TIME (MEAN YEARS ± SD)	3.7 ± 1.2	3.8 ± 1.2	3.3 ± 1.2	3.7 ± 1.2	**p=0.012**
OVERALL GRAFT LOSS N (%)	30 (6.8)	23 (6.5)	2 (3.5)	5 (15.2)	p=0.096
DEATH-CENSORED GRAFT LOSS N (%)	17 (3.8)	14 (4.0)	0 (0)	3 (9.1)	p=0.09
PATIENT MORTALITY N (%)	14 (3.2)	10 (2.8)	2 (3.5)	2 (6.1)	p=0.59
RENAL GRAFT FUNCTION (MEAN ± SD) eGFR*	54.8 ± 23.0	55.8 ± 23.6	54.7 ± 20.3	43.4 ± 20.8	**p=0.013**

**eGFR=estimated glomerular filtration rate*

4.2.1 Patient- and graft survival

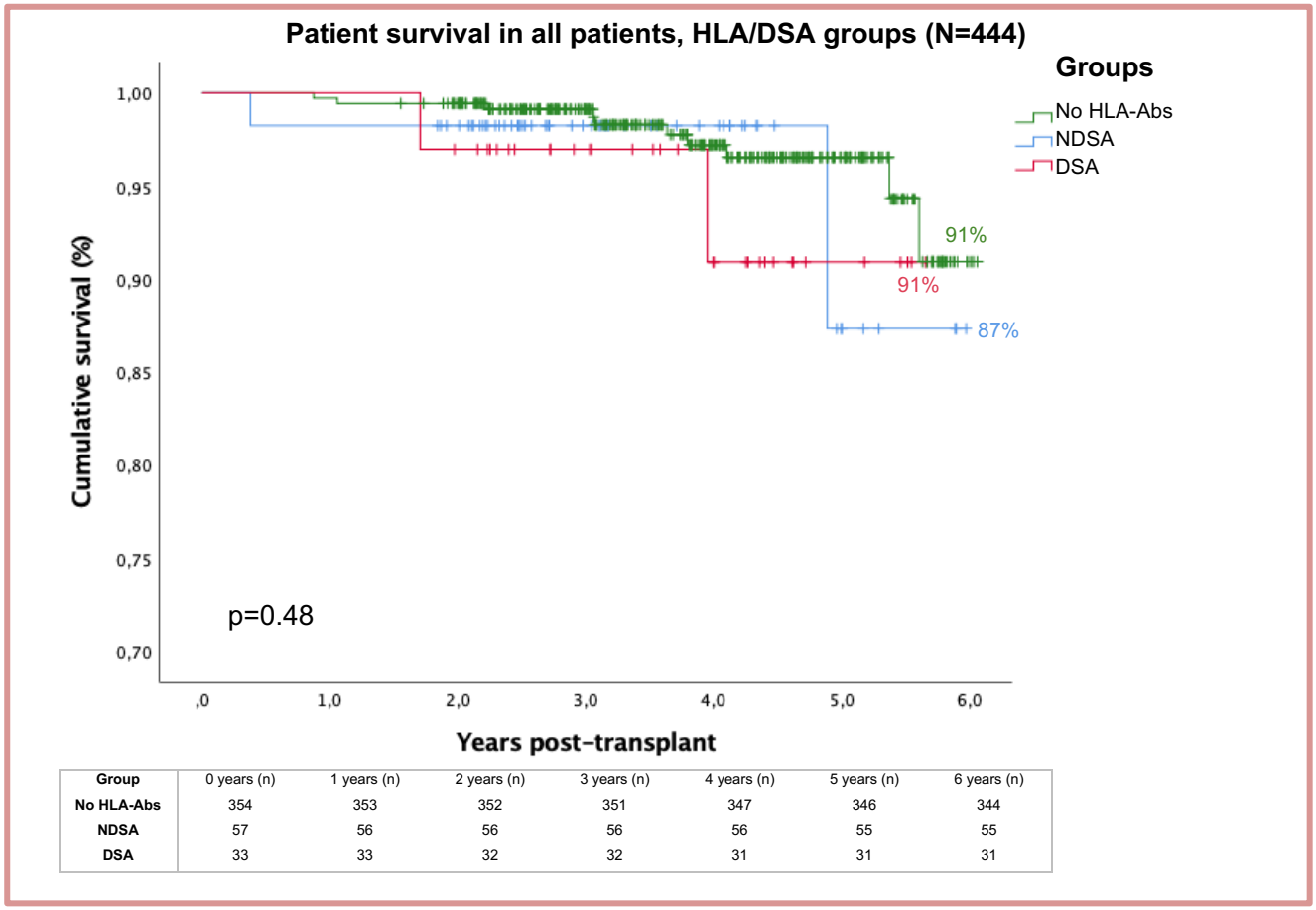
As shown in Table 5, patient survival was similar between the three groups (p=0.59).

There was a trend to worse overall graft survival in recipients with DSA (84.8%) as compared to no HLA-Abs recipients (93.5%) and the NDSA group (96.5%) (p=0.096). There was no death-censored

graft loss in the NDSA group but 4% in no HLA-Abs recipients and 9.1% in the DSA group (p=0.09).

The Kaplan-Meier survival analysis showed a cumulative patient survival of 91% in no HLA-Abs, 87% in NDSA and 91% in DSA which was not significantly different (Figure 6).

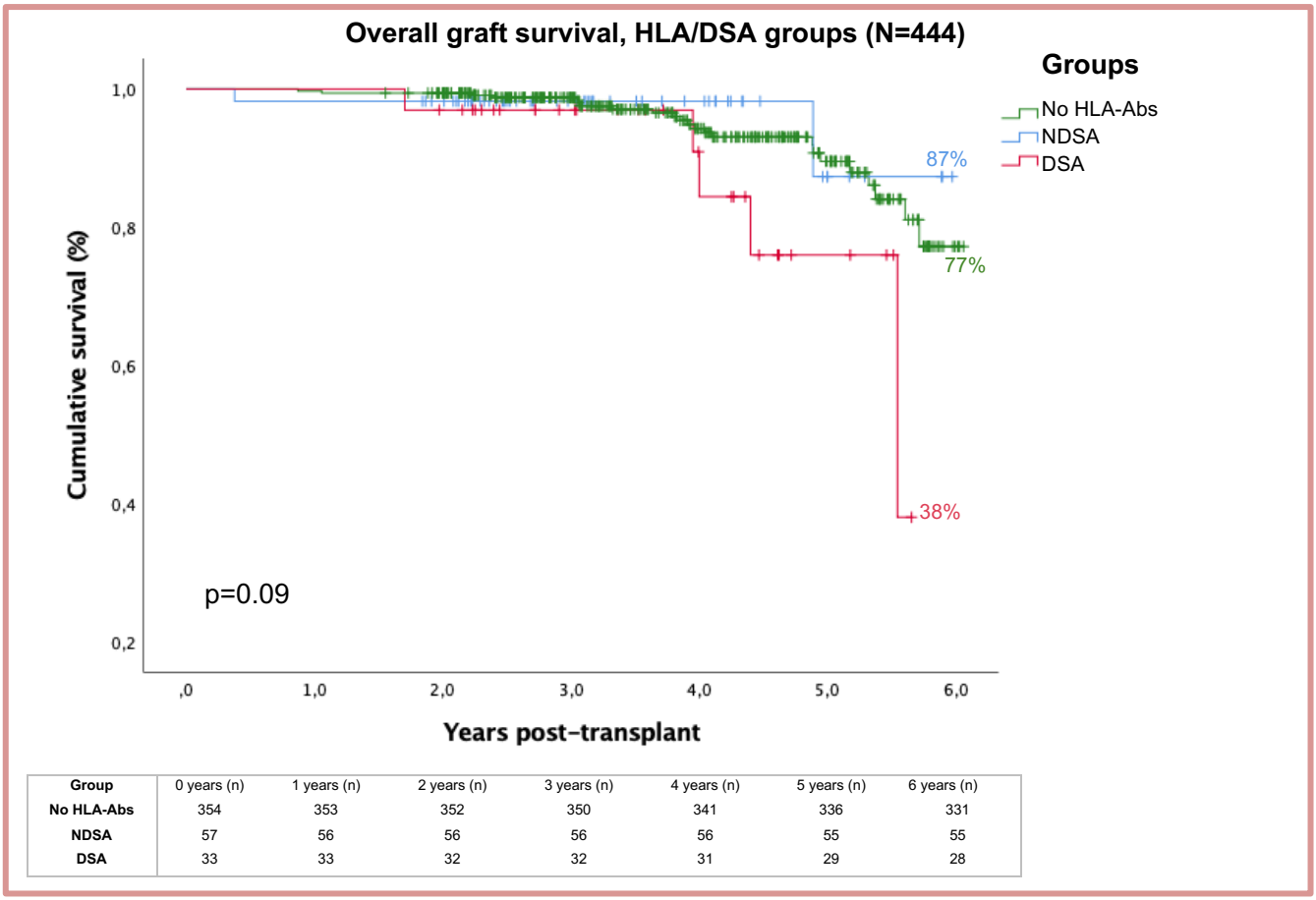
A similar percentage of censored cases were present in no HLA-Abs (97.2%), NDSA (96.5%) and DSA (93.9%).



Group	0 years (n)	1 years (n)	2 years (n)	3 years (n)	4 years (n)	5 years (n)	6 years (n)
No HLA-Abs	354	353	352	351	347	346	344
NDSA	57	56	56	56	56	55	55
DSA	33	33	32	32	31	31	31

Figure 6. Kaplan Meier curve showing patient survival in all patients divided into the three main groups no HLA-Abs, NDSA and DSA. The table below the curve shows the number of patients (n) left in each group after an event, in this case death, had occurred. Notice the y-axis scale. The cumulative patient survival between the groups was not significantly different.

As shown in Figure 7, the overall graft survival curve showed a trend to lower cumulative survival in the DSA group (38%) as compared to no HLA-Abs recipients (77%) and the NDSA group (87%) (p=0.09). A similar percentage of censored cases were present in no HLA-Abs recipients (93.5%) and the NDSA group (96.5%) but not in the DSA group (84.8%).



Group	0 years (n)	1 years (n)	2 years (n)	3 years (n)	4 years (n)	5 years (n)	6 years (n)
No HLA-Abs	354	353	352	350	341	336	331
NDSA	57	56	56	56	56	55	55
DSA	33	33	32	32	31	29	28

Figure 7. Kaplan Meier curve showing overall graft survival in all patients divided into the three main groups no HLA-Abs, NDSA and DSA. The table below the curve shows the number of patients (n) left in each group after an event, in this case either death or return to dialysis had occurred. Notice the y-axis scale. Recipients with DSA showed a trend to lower cumulative survival compared to No HLA-Abs recipients and NDSA group.

4.2.2 Renal graft function

Renal graft function was significantly lower in recipients with DSA with a mean eGFR of 43.4 ± 20.8 compared to recipients with no HLA-Abs with a mean eGFR of 55.8 ± 23.6 (Figure 8) (p=0.013).

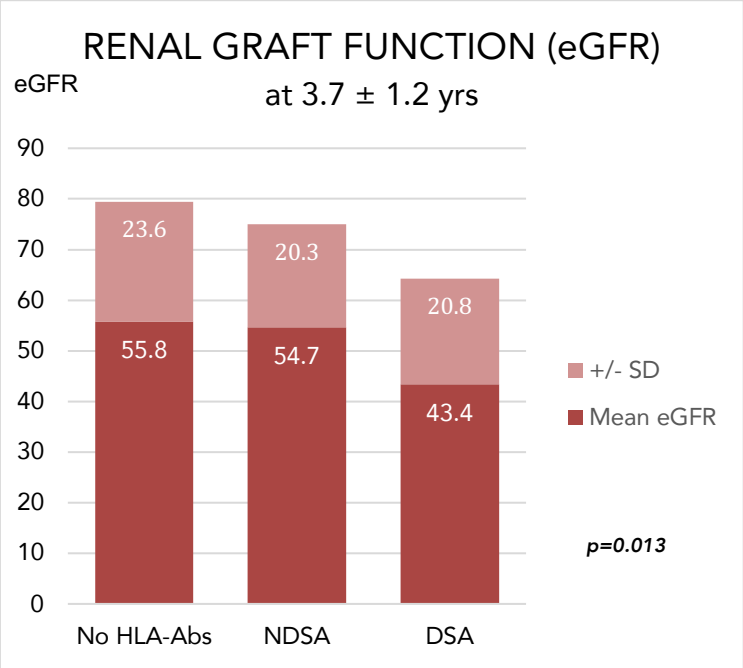


Figure 8. Renal graft function defined by eGFR between the three groups. eGFR was significantly lower in recipients with DSA with a mean eGFR of 43.4 ± 20.8 compared to recipients with no HLA-Abs recipients with a mean eGFR of 55.8 ± 23.6 (p=0.013).

Table 6. Renal graft function among all patients (N=444)

GRAFT FUNCTION eGFR*	eGFR* MEAN ± SD	ALL PATIENTS (N=444) N (%)	DSA- PATIENTS (N=411) N (%)	DSA+ PATIENTS (N=33) N (%)	P-VALUE
<30	15.9 ± 11.8	53 (12)	47 (11)	6 (18)	p=0.039• p=0.25▽
30-59	46.2 ± 9.0	217 (49)	197 (48)	20 (61)	
≥60	77.1 ± 13.6	174 (39)	167 (41)	7 (21)	

**eGFR=estimated glomerular filtration rate; •=calculated p-value of comparing DSA-positive and DSA-negative patients with eGFR <60; ▽=calculated p-value of comparing the different levels of eGFR between DSA-positive and DSA-negative patients*

All recipients were divided in three groups due to their eGFR (<30, 30-59 and ≥60) (Table 6). The majority of recipients (49%) had an eGFR between 30-59 with a mean eGFR of 46.2 ± 9.0. Out of recipients with detected DSA, 61% had an eGFR between 30-59, 21% had an eGFR ≥60 and 18% had an eGFR <30. Thus, a significantly higher proportion of patients with DSA had an eGFR of <60 (79%) as compared to the remaining DSA-negative patients (no HLA-Abs and NDSA) (59%)

(p=0.039). There were no significant differences between the DSA+ and DSA- groups in the different levels of eGFR (p=0.25).

4.3 DSA characteristics

Recipients with detected DSA were grouped by their pretx FCXM status (Table 7). Recipients with positive FCXM [FCXM+/DSA+] were more likely to have class I DSA (50%) compared to recipients with negative FCXM [FCXM-/DSA+] (27%) (p=0.04). Recipients with only class I DSA and positive FCXM had a mean cumulative MFI (cMFI) of 4391 ± 3846 with a median of 3182, compared to negative FCXM recipients that had a mean cMFI of 1788 ± 350 with a median of 1661 (p=0.03). Recipients with negative FCXM were more likely to have class II DSA (64%) compared to positive FCXM (30%) (p=0.08), with a mean cMFI of 2812 ± 1488 compared to positive FCXM with 5995 ± 4837 (p=0.39). The median cMFI was slightly higher in recipients with positive FCXM (4545) compared to recipients with negative FCXM (3047).

Table 7. DSA characteristics grouped by their FCXM status (n=31).

	FCXM- DSA+ (N=11)	**FCXM+ DSA+ (N=20)**	**P-VALUE**
CLASS I DSA ONLY N (%)	3 (27)	10 (50)	**p=0.04**
CLASS II DSA ONLY N (%)	7 (64)	6 (30)	p=0.08
CLASS I + II DSA N (%)	1 (9)	4 (20)	p=0.65
cMFI* CLASS I DSA (MEAN ± SD) MEDIAN (RANGE)	**1788 ± 350** 1661 (1519-2183)	**4391 ± 3846** 3182 (1036-12478)	**p=0.03**
cMFI CLASS II DSA (MEAN ± SD) MEDIAN (RANGE)	**2812 ± 1488** 3047 (1143-5106)	**5995 ± 4837** 4545 (1821-12427)	p=0.39
cMFI CLASS I + II DSA (MEAN ± SD) MEDIAN (RANGE)	**5252 ± 0** 5252 (5252)	**15020 ± 2641** 14856 (11964-18402)	**p=0.02**

**cMFI= cumulative mean fluorescence intensity*

Recipients with positive FCXM were more likely to have both class I and II DSA (20%) compared to recipients with negative FCXM (9%) (p=0.65) and had almost triple as high cMFI

(15020 ± 2641) as the recipients with negative FCXM (5252 ± 0) (p=0.02). The cMFI levels in all recipients (n=31) are demonstrated in Figure 9, divided in class of DSA and FCXM status.

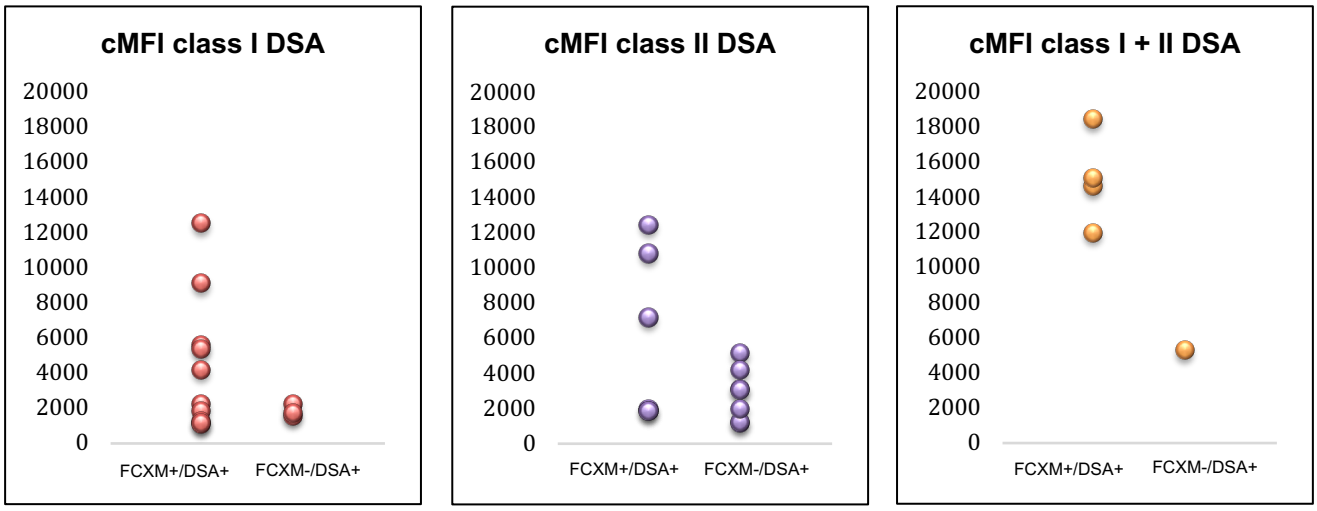


Figure 9. Cumulative mean fluorescence intensity (cMFI) levels in all DSA-positive recipients, grouped due to their pretx FCXM status (n=31). Recipients with positive FCXM (FCXM+/DSA+) had significantly higher cMFI in DSA class I *(p=0.03)* and DSA class I + II *(p=0.02)* compared to recipients with negative FCXM (FCXM-/DSA+).

4.4 Patient outcomes among DSA-positive patients

Outcomes in patient- and graft survival among DSA-positive patients (n=33) are shown in Table 8 and 9.

4.4.1 Patient- and graft survival

Recipients were divided due to their DSA class and survival status (Table 8). In the survival group (n=31), the majority were class II DSA (45%), following by class I (36%) and class I+II (19%). Recipients with no survival (n=2) were only positive in class I DSA (100%). The cMFI in the survival group (mean 5618 ± 4937, median 3078) was similar compared to the recipients with no survival (mean cMFI 1714 ± 716, median 3063) (p=0.28).

Recipients with graft loss (n=5) had a majority of class I DSA only (40%) and class II DSA only (40%) which is similar to recipients with no graft loss (n=31) with 45% having class II DSA only and 36% class I DSA only (Table 9). Recipients with graft loss has a mean cMFI of 4681 ±

5852 with a median of 2220, which was not significantly different than the recipients with no graft loss who had a mean cMFI of 5507 ± 4794 with a median of 4149 (p=0.73).

Table 8. Patient survival among DSA-positive patients

PATIENT SURVIVAL N = 33	**CLASS**	**N (%)**	**CUMULATIVE MFI (cMFI*) MEAN ± SD** MEDIAN (RANGE)	**P-VALUE**
YES N = 31	CLASS I	11 (36)	**5618 ± 4937** 3078 (1036-18402)	p=0.28
	CLASS II	14 (45)		
	CLASS I + II	6 (19)		
NO N = 2	CLASS I	2 (100)	**1714 ± 716** 3063 (1208-2220)	
	CLASS II	0 (0)		
	CLASS I + II	0 (0)		

**cMFI= cumulative mean fluorescence intensity*

Table 9. Graft survival among DSA-positive patients

GRAFT SURVIVAL N = 33	**CLASS**	**N (%)**	**CUMULATIVE MFI (cMFI*) MEAN ± SD** MEDIAN (RANGE)	**P-VALUE**
YES N = 28	CLASS I	11 (39)	**5507 ± 4794** 4149 (1036-18402)	p=0.73
	CLASS II	12 (43)		
	CLASS I + II	5 (18)		
NO N = 5	CLASS I	2 (40)	**4681 ± 5852** 2220 (1208-15079)	
	CLASS II	2 (40)		
	CLASS I + II	1 (20)		

**cMFI= cumulative mean fluorescence intensity*

4.4.2 Renal graft function

All DSA-positive patients (n=33) were divided in three groups due to their eGFR (<30, 30-59 and ≥60) (Table 10). The majority of recipients (61%) had an eGFR between 30-59 with a mean eGFR of 42.3 ± 8.6. 21% of recipients had an eGFR over 60 (72.1 ± 6.7) and 18% had an eGFR under 30 (13.6 ± 15).

The cMFI appeared to be progressively higher in the groups with eGFR <60, however, it was not statistically significant (p=0.77). The cMFI was 4164 ± 4067 in ≥60 group; 5672 ± 5178 in 30-59 group and 5835 ± 5456 in <30 group.

Table 10. Renal graft function among DSA-positive patients

ALL DSA+ PATIENTS N = 33	**eGFR***	**eGFR*** MEAN ± SD	**N (%)**	**CUMULATIVE MFI (cMFI*)** **MEAN ± SD** MEDIAN (RANGE)	**P-VALUE**
RENAL GRAFT FUNCTION	**<30**	13.6 ± 15	6 (18)	**5835 ± 5261** 3616 (1829-15079)	p=0.77
	30-59	42.3 ± 8.6	20 (61)	**5672 ± 5178** 3596 (1036-18402)	
	≥60	72.1 ± 6.7	7 (21)	**4164 ± 4067** 2220 (1143-12478)	

**eGFR=estimated glomerular filtration rate; cMFI= cumulative mean fluorescence intensity*

4.5 Patient characteristics among all HLA-positive patients

The patient characteristics among HLA-positive patients (n=88) are shown in Table 11. Comparison of the four subgroups (FCXM-/DSA-, FCXM+/DSA-, FCXM-/DSA+ and FCXM+/DSA+) was performed using one-way ANOVA and Chi-square test was used for the analysis of categorical data between the groups. The recipients were divided into four subgroups due to their pretx FCXM and DSA status.

The age range is between 22 to 75, and the mean age at Ktx was similar among all groups (p=0.53). The mean age was slightly lower in recipients with detected DSA [FCXM-/DSA+] and [FCXM+/DSA+]. The gender distribution was also similar where >50% were women in each group (p=0.62), so were the recipients with multiple Ktx (p=0.86). Recipients in all groups were more likely to receive a transplant from a deceased donor, whereas 100% of the recipients in [FCXM+/DSA-] compared to 60% in [FCXM+/DSA+] received tx from a deceased donor (p=0.14).

All recipients (100%) in [FCXM-/DSA+] and FCXM+/DSA-] received dialysis prior to transplant compared to 88% respectively 70% in [FCXM-/DSA-] and FCXM+/DSA+] (p=0.07).

Recipients in [FCXM-/DSA+] had dialysis for a longer time (4.1 ± 3.2 years) compared to the other groups (p=0.02).

Recipients sensitized in class I were higher in [FCXM+/DSA+] (p<0.01) but lower in class II compared to [FCXM+/DSA-] (p=0.12). Highly sensitized recipients were seen more frequently in [FCXM+/DSA-] (56%) compared to 30% in [FCXM-/DSA-] (p=0.64).

Table 11. Patient characteristics among HLA-positive patients

	ALL PATIENTS (N=88)	**FCXM- DSA- (N=48)**	**FCXM+ DSA- (N=9)**	**FCXM- DSA+ (N=11)**	**FCXM+ DSA+ (N=20)**	**P-VALUE**
AGE AT TX* ± SD (RANGE) IN YEARS	52.4 ± 12.1 (22-75)	52.1 ± 11.5 (27-75)	56.8 ± 8.8 (46-70)	48.8 ± 16.3 (22-72)	53.0 ± 12.7 (29-73)	p=0.53
GENDER						
WOMEN, N (%)	52 (59)	26 (54)	6 (67)	6 (55)	14 (70)	p=0.62
MEN, N (%)	36 (41)	22 (46)	3 (33)	5 (45)	6 (30)	
PATIENTS WITH >1 KIDNEY TX N (%)	43 (49)	23 (48)	6 (67)	4 (36)	10 (50)	p=0.86
DONOR TYPE*, N (%)						
DD	64 (73)	34 (71)	9 (100)	9 (82)	12 (60)	p=0.14
LD	24 (27)	14 (29)	0 (0)	2 (18)	8 (40)	
PERCENT PRA* AT TX (MEAN ± SD)						
CLASS I	38.3 ± 33.9	30.8 ± 33.0	23.9 ± 15.5	43.0 ± 34.4	60.2 ± 33.2	**p<0.01**
CLASS II	43.9 ± 38.1	41.8 ± 39.3	72.6 ± 36.0	40.2 ± 34.9	37.8 ± 34.3	p=0.12
PRA ≥80% (class I and/or II) N (%)	37 (42)	18 (38)	5 (56)	4 (36)	10 (50)	p=0.64
DIALYSIS PRIOR TO TRANSPLANTATION N (%)						
YES	76 (86)	42 (88)	9 (100)	11 (100)	14 (70)	p=0.07
NO	12 (14)	6 (12)	0 (0)	0 (0)	6 (30)	
TIME ON DIALYSIS PRETX (MEAN YEARS ± SD)	2.6 ± 1.9	2.2 ± 1.4	2.7 ± 1.8	4.1 ± 3.2	2.8 ± 1.7	**p=0.02**

**Tx=transplantation(s); DD=deceased donor; LD=living donor; PRA= panel reactive antibody; Pretx=pretransplant*

4.6 Patient outcomes among all HLA-positive patients

The patient outcomes among all HLA-positive patients are shown in Table 12. The follow-up time was a little higher in [FCXM-/DSA+] with a mean of 4.1 ± 1.3 years compared to the other groups with an approximal mean of 3.3 ± 1 years (p=0.19).

Table 12. Patient outcomes among all HLA-positive patients

	ALL PATIENTS (N=88)	**FCXM- DSA- (N=48)**	**FCXM+ DSA- (N=9)**	**FCXM- DSA+ (N=11)**	**FCXM+ DSA+ (N=20)**	**P-VALUE**
FOLLOW UP TIME (MEAN YEARS ± SD)	3.4 ± 1.2	3.3 ± 1.3	3.1 ± 0.8	4.1 ± 1.3	3.4 ± 1.0	p=0.19
OVERALL GRAFT LOSS N (%)	5 (5.7)	2 (4.2)	0 (0)	1 (9.1)	4 (20)	p=0.13
DEATH-CENSORED GRAFT LOSS N (%)	1 (1.1)	0 (0)	0 (0)	1 (9.1)	2 (10)	p=0.13
PATIENT MORTALITY N (%)	4 (4.5)	2 (4.2)	0 (0)	0 (0)	2 (10)	p=0.51
RENAL GRAFT FUNCTION (MEAN ± SD) eGFR*	51.6 ± 19.8	55.0 ± 20.7	48.2 ± 17.5	48.0 ± 19.2	41.7 ± 21.6	**p=0.07**
ACUTE CELLULAR REJECTION (ACR) N (%)	8 (9.1)	2 (4.2)	1 (11.1)	1 (9.1)	4 (20)	p=0.23
ACUTE HUMORAL REJECTION (AHR) N (%)	6 (6.8)	3 (6.3)	0 (0)	0 (0)	3 (15)	p=0.31
CHRONIC CELLULAR REJECTION (CCR) N (%)	2 (2.3)	1 (2.1)	1 (11.1)	0 (0)	0 (0)	p=0.27
CHRONIC HUMORAL REJECTION (CHR) N (%)	8 (9.1)	3 (6.3)	0 (0)	1 (9.1)	4 (20)	p=0.24

**eGFR=estimated glomerular filtration rate*

4.6.1 Rejections

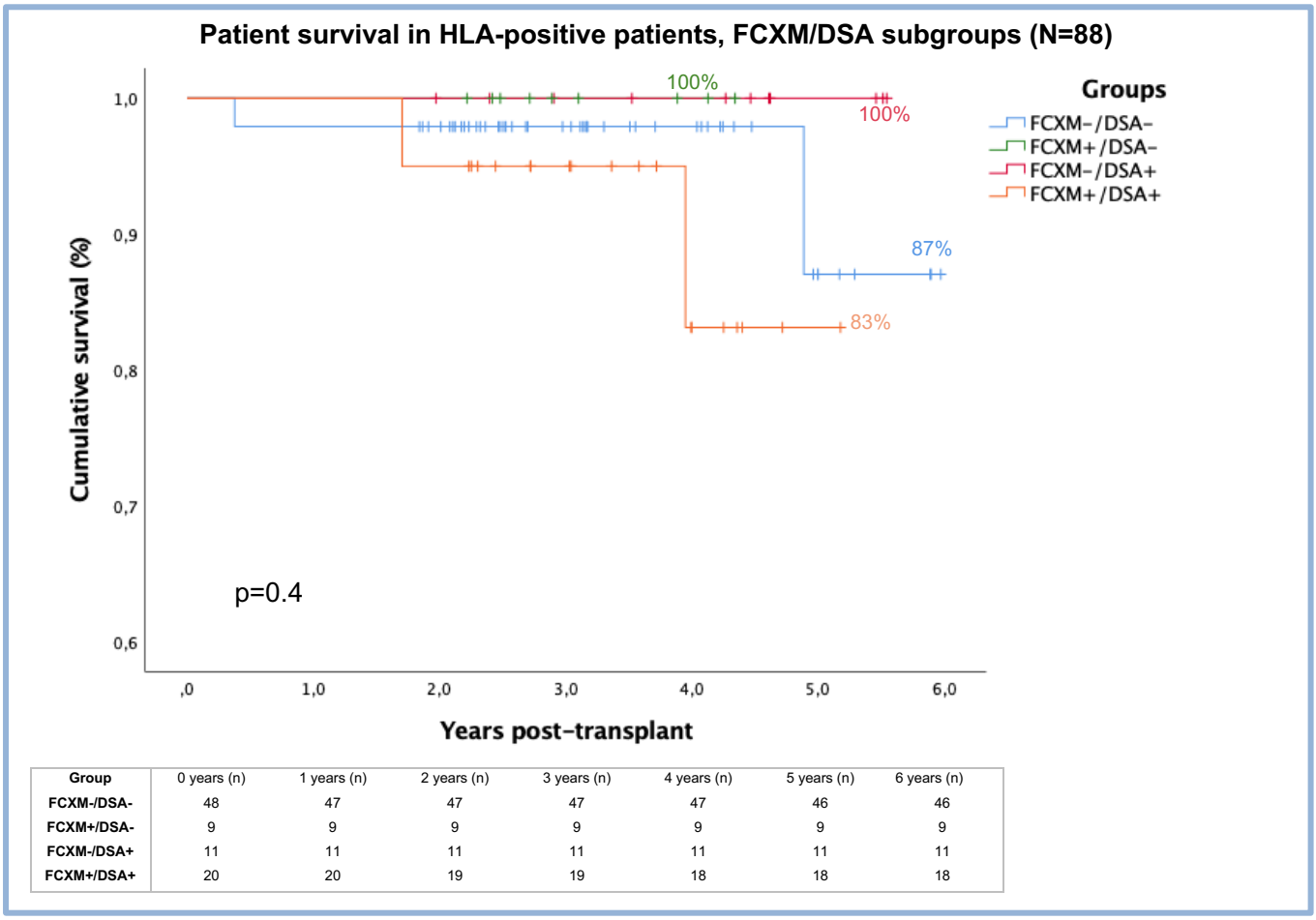
The incidence of different type of biopsy-proven rejections are shown in Table 12. There were no significant differences in any type of rejections between the groups (p=0.23; 0.31; 0.27; 0.24).

The incidence of all types of rejections except for chronic cellular rejections appeared to be higher in recipients with positive FCXM and detected DSA [FCXM+/DSA+], although statistically not significant.

4.6.2 Renal graft function

Renal graft function was higher in recipients in [FCXM-/DSA-] compared to the other groups with a mean eGFR of 55.0 ± 20.7 (p=0.07). Recipient in [FCXM+/DSA+] had a lower eGFR compared to the other groups (41.7 ± 21.6) (Table 12).

4.6.3 Patient- and graft survival

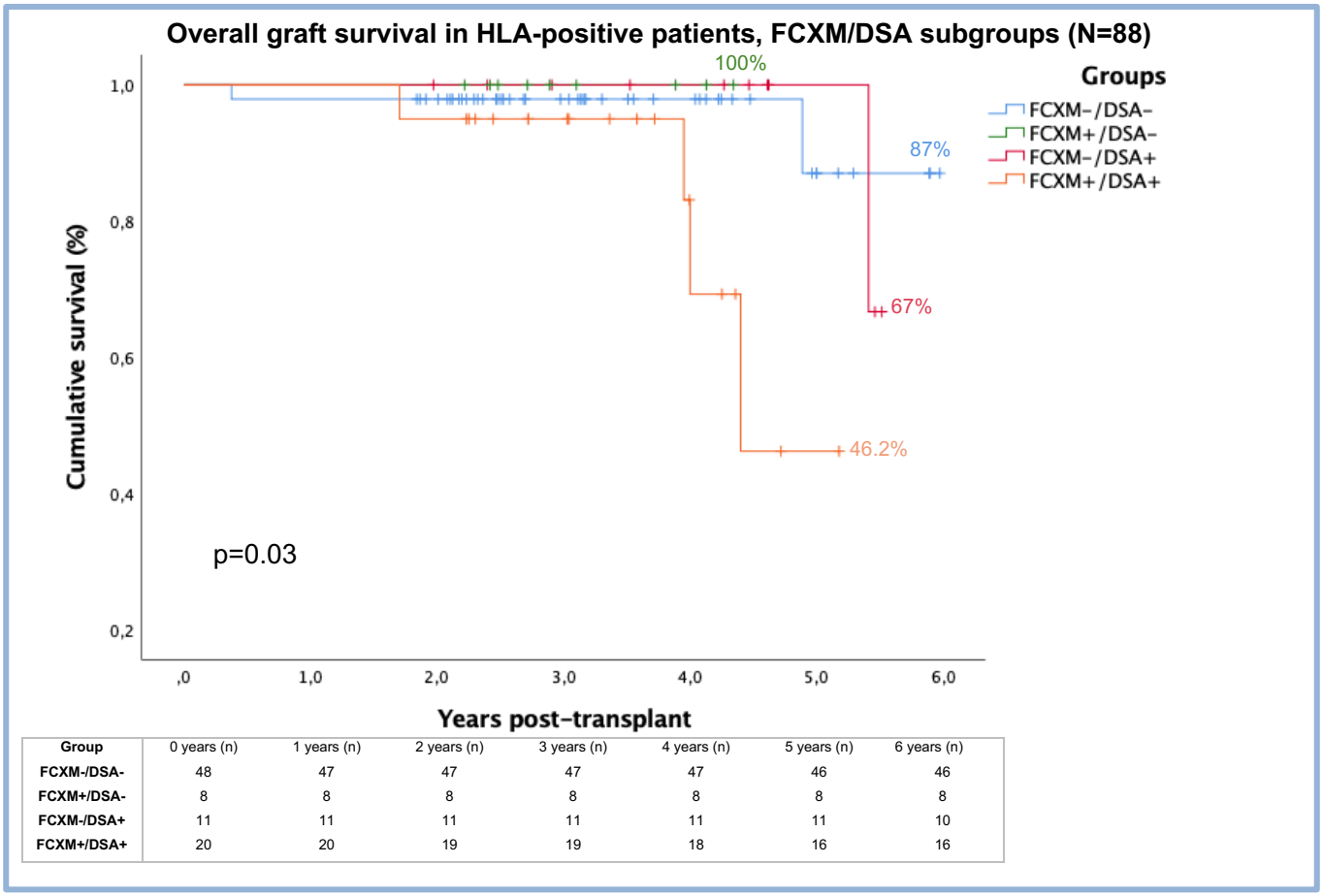


Group	0 years (n)	1 years (n)	2 years (n)	3 years (n)	4 years (n)	5 years (n)	6 years (n)
FCXM-/DSA-	48	47	47	47	47	46	46
FCXM+/DSA-	9	9	9	9	9	9	9
FCXM-/DSA+	11	11	11	11	11	11	11
FCXM+/DSA+	20	20	19	19	18	18	18

Figure 10. Kaplan Meier curve showing similar patient survival in all HLA-positive patients divided into four subgroups [FCXM-/DSA-], [FCXM-/DSA+], [FCXM+/DSA-] and [FCXM+/DSA+]. The table below the curve shows the number of patients (n) left in each group after an event, in this case death, had occurred. Notice the Y-axis scale.

There was a trend to higher overall graft survival in recipients with [FCXM+/DSA-] (100%) and [FCXM-/DSA-] (95.8%) compared to recipients with [FCXM-/DSA+] (90.9%) and [FCXM+/DSA+] (80%) (p=0.13) (Table 12). There were two (10%) death-censored graft loss among recipients in [FCXM+/DSA+] and one (9.1%) in [FCXM-/DSA+] group (p=0.13). There was no significant difference in patient mortality among the groups (p=0.51).

The Kaplan-Meier survival analysis showed a similar cumulative patient survival of 87% in the four groups: 87% in [FCXM-/DSA-], 100% in [FCXM-/DSA+], 100% in [FCXM+/DSA-] and 83% in [FCXM+/DSA+] (Figure 10) (p=0.4), whereas, there were significant differences in the overall graft survival: 87% in [FCXM-/DSA-], 67% in [FCXM-/DSA+], 100% in [FCXM+/DSA-] and 46.2% in [FCXM+/DSA+] (Figure 11) (p=0.03). Thus, the [FCXM+/DSA+] group had the worst overall graft survival.



Group	0 years (n)	1 years (n)	2 years (n)	3 years (n)	4 years (n)	5 years (n)	6 years (n)
FCXM-/DSA-	48	47	47	47	47	46	46
FCXM+/DSA-	8	8	8	8	8	8	8
FCXM-/DSA+	11	11	11	11	11	11	10
FCXM+/DSA+	20	20	19	19	18	16	16

Figure 11. Kaplan Meier curve showing overall graft survival in all HLA-positive patients divided into four subgroups. The table below the curve shows the number of patients (n) left in each group after an event, in this case either death or return to dialysis had occurred. Notice the Y-axis scale. Recipients with DSA and positive FCXM had worst overall graft survival compared to the other groups.

4.6.4 Therapeutic approach in highly immunized patients

Recipients that were highly immunized (n=33) received different types of immunosuppression prior to and after transplant. The different therapeutic approaches, including graft- and patient survival, is seen in Table 13.

All the recipients in group 2 (n=2) and 3 (n=2) had a functioning graft after the mean follow-up time at 3.7 ± 1.2 years. In group 1 (n=3), 2 recipients had a functioning graft and 1 lost its graft. In group 4 (n=26), 22 recipients had a functioning graft. However, 2 lost their graft and 2 had died during the follow-up period.

Table 13. Highly immunized patients (e.g DSA-positive recipients or PRA >50%, n=33) received either i) desensitization therapy prior to tx and/or ii) high-risk induction therapy followed by oral high-risk immunosuppressive protocol. The table also shows the graft- and patient survival between the different groups.

GROUP (N=33) (MEAN FOLLOW-UP TIME 3.7 ± 1.2 YEARS)	**1** **RITUXIMAB PE* + IVIG* ATG*** (N=3)	**2** **RITUXIMAB PE* + IVIG* BASILIXIMAB** (N=2)	**3** **ECULIZUMAB** (N=2)		**4** **RITUXIMAB** (N=26)	
			+	-	+	-
DESENSITIZATION (LD*, N=5)	3	2				
ECULIZUMAB (STUDY) (N=2)			1	1		
OTHERS (N=26)					18	8
FUNCTIONING GRAFT (N=28)	2	2	2		22	
GRAFT LOSS (N=3)	1				2	
DEATH (N=2)					2	

** LD=living donor; PE=plasma exchange; IVIG=Intravenous immunoglobulin-mediated immunosuppression; ATG=Anti-thymocyte globulin*

5. Discussion

In this retrospective study, we wanted to elucidate the clinical relevance of pretx HLA-Abs (both DSA and NDSA) detected with newer sensitive assays in Ktx recipients on graft function and graft survival. In addition, we also wanted to examine the outcomes of recipients with positive FCXM in relation to the DSA, using the current sensitive assays.

We analysed a total of 444 patients whose underwent Ktx at Sahlgrenska University Hospital between 2012-2015 with both living and deceased donors. By grouping the recipients according to their pretx HLA, FCXM and DSA status, we could examine and compare the outcomes in all groups.

5.1 Summary of main results

In total, we had 90 recipients with pretx HLA-Abs in our Ktx population. Out of these, 33 recipients were detected to have pretx DSA (37% of all our HLA-Abs positive recipients). Recipients with positive DSA were more likely to be females and/or have received multiple transplants. These recipients were also more likely to have higher PRA in class I compared to recipients with NDSA. These results are most likely due to exposure to donor antigens during pregnancy or because of earlier transplantations (3, 24).

Remarkably, a gradual decline in renal graft function was seen in correlation with detected antibodies, both DSA and NDSA. Among the HLA-Abs positive recipients, recipients with DSA and positive FCXM [FCXM+/DSA+] had lower eGFR compared to the other subgroups. Considering eGFR is a surrogate marker for the long-term outcome, pretx HLA-Abs (both DSA and NDSA) may have an effect on long-term graft survival. A study published in 2017 by Vimal et al. showed similar findings where DSA-negative recipients had higher eGFR posttransplant compared to DSA-positive recipients (64).

However, the study did not show significant difference in allograft outcomes between DSA-positive and DSA-negative recipients, but only a slight trend, suggesting that pretx DSA should not be seen as an absolute contraindication to Ktx, especially with the availability of current desensitization and high-risk immunosuppressive protocols (64). This has also been described by

Süsal et al. (53) and Kamburova et al. (58), while other studies showed a correlation of DSA and impaired allograft outcome. Richter et al. described that HLA-Abs detected with SAB are a risk factor for long-term kidney graft loss even in the absence of DSA, suggesting both DSA and NDSA have an increased risk of allograft rejection (50). In our Ktx population, (30/444) recipients had either lost their graft or died during the mean follow-up time of 3.7 ± 1.2 years. Among recipients with pretx positive DSA (DSA+) at baseline (n=33), 5/33 (15.2%) had an overall graft loss. Recipients with no HLA or HLA but with NDSA had a similar outcome with an overall graft loss of 6.5% (23/354) and 3.5% (2/57), respectively. The Kaplan-Meier survival analysis showed a lower graft survival in recipients with DSA and positive FCXM, compared to recipients with positive FCXM but negative DSA. No statistical significant differences were found in the different types of rejections or in patient survival among different groups in our study.

Another important finding from our study was that DSA-positive recipients with a positive FCXM had higher cMFI in both class I and/or II compared to FCXM-negative recipients. This has also been described in the study by Schinstock et al. where recipients with DSA and high FCXM had the highest cMFI in all three categories (65). Further, a high sensitization (PRA ≥80%) was found to be associated with cAMR even in the absence of DSA (65). This aspect was not evaluated in our study. Instead, we examined the whole groups according to their DSA-status and the overall outcomes. In total, 15 DSA-positive recipients (15/33) in our study had a PRA ≥80% and whether the 5 recipients with an overall graft loss were out of these 15 recipients will need to be studied further.

Lastly, the role of desensitization and high-risk induction therapy was not directly studied in our study population. Koefoed-Nielsen et al. studied the STAMP-program showing the program proves to be a valid approach for transplanting highly immunized patients (61). Although a small amount of the STAMP-patients were included in our study, they were not further evaluated. Pretransplant desensitization with a combination of Rituximab and Intravenous Immune Globulin (IVIG) has been presented as an effective regimen for recipients awaiting a transplant (66). Highly immunized recipients remain on the waiting list for extended periods of time while undergoing dialysis, which can also be seen in our study. This combination therapy has been partly implicated in

some of our recipients but would need to be further evaluated with a larger study population to study its efficiency. Positive expectations of the pretransplant desensitization would except cost savings also reduce morbidity and mortality and improved quality of life by reducing the time in dialysis.

5.2 Strengths, limitations and future research

The strengths of the study are well characterized, large Ktx cohort who ere prospectively followed up, availability of pre- and posttransplant clinical and laboratory data in electronic registers, pretx DSA testing performed using SAB in a majority of patients and a reasonably long duration of follow-up.

However, there are also some limitations that deserve specific consideration. First, being a retrospective analysis, it suffers from some of the inherent limitations of this kind of study design. Second, even though we had a reasonable size of study population with 444 patients, this sample size may be considered relatively small, especially for the subgroup analysis, thereby limitating the power of the study. Third, we did not take into consideration the different types of immunosuppressive treatment our recipients received posttransplant, which may have an important impact on the outcomes. Only recipients that were highly sensitized that had received a high-risk protocol were further investigated for the type of immunosuppression.

Fourth, we did not characterize further the DSA except for the DSA class, e.g. IgG subclassification and C1q-antibody biding. The clinical impact of different characteristics of DSAs on allograft survival would be an interesting aspect to look at in future research. Fifth, the biopsy diagnosis of all rejections were extracted from the reports, and were not reevaluated for the study by the pathologists.

Last but not the least, an even longer follow-up than the current mean follow-up of approximately four years is needed to better understand the clinical impact of pretx DSA and its association with graft loss in the long-term.

6. Conclusions

Pretx DSA were associated with lower eGFR and a trend to lower 6-year graft survival in our Ktx population. A positive FCXM (with a negative CDC-XM) had prognostic implications for graft function and survival only in presence of DSA. Therefore, positive FCXM in absence of DSA should not represent a barrier to Ktx.

Populärvetenskaplig sammanfattning

Klinisk relevans av HLA-antikroppar detekterade med känsligare analysmetoder innan transplantation hos njurtransplanterade patienter

I Sverige behandlas idag cirka 10 000 patienter för en njursjukdom i slutskedet, varvid cirka 60% av dessa har genomgått en njurtransplantation. Tack vare framstegen inom immunsuppressiv behandling har man de senaste åren kunnat göra signifikanta förbättringar i den kortvariga transplantatöverlevnaden bland njurtransplanterade. Trots detta fortsätter rejektion vara en ledande orsak till transplantatförlust och återgång till dialys i över 50% av alla njurtransplanterade. Närvaron av antikroppar mot human leucocyte antigen (HLA) i serum, som både kan vara donatorspecifika men även icke-donatorspecifika, har alltmer blivit associerade med kronisk antikroppsmedierad rejektion och sämre långsiktig transplantatöverlevnad efter en njurtransplantation.

De senaste åren har man utvecklat mer sensitiva och mer specifika analysmetoder som detekterar dessa HLA-antikroppar. Trots dessa välutvecklade känsliga metoder, som fångar upp mycket låga nivåer av HLA-antikroppar, har man haft delade meningar om den kliniska relevansen av dessa låga nivåer och vad dessa låga nivåer har för påverkan på transplantatet efter en njurtransplantation. Många publicerade studier har påvisat en korrelation mellan donatorspecifika antikroppar (som detekterats innan transplantation) och sämre transplantatöverlevnad, men inte alla donatorspecifika antikroppar har lett till transplantatförlust.

Denna studie syftar till att utvärdera den kliniska relevansen av HLA-antikroppar (både donatorspecifika samt icke-donatorspecifika) detekterade innan transplantation av dessa känsliga analysmetoder genom att titta på transplantatfunktion och överlevnad.

I denna retrospektiva studie inkluderades totalt 444 patienter som genomgick en njurtransplantation på Sahlgrenska Universitetssjukhus mellan 2012-2015. Blodprovsanalyser med SAB och FCXM utfördes innan transplantation och patienterna har fördelats i 3 subgrupper: i) negativ i HLA-

antikroppar, ii) positiv i HLA-antikroppar som inte är donatorspecifika och iii) positiv i HLA-antikroppar som är donatorspecifika.

Resultatet visade att i våran studiepopulation fanns 33 patienter som hade donatorspecifika antikroppar innan transplantation. Denna grupp hade signifikant sämre transplantatfunktion och en trend mot sämre 6-års transplantatöverlevnad jämfört med patienter som var negativa i HLA-antikroppar eller hade antikroppar men som var icke-donatorspecifika. Dessutom, patienter som var positiva i både SAB och FCXM, hade sämre transplantatöverlevnad och funktion jämfört med patienter som endast var positiva i FCXM.

Slutsatsen av denna studie är därför att donatorspecifika antikroppar som detekteras innan transplantation är associerade med sämre njurfunktion och en trend mot sämre 6-års transplantatöverlevnad i vår studiepopulation. En positiv FCXM hade prognostisk implikation på njurfunktionen samt transplantatöverlevnad endast i närvaro av donatorspecifika antikroppar (positiv SAB).

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