Living kidney transplantation: beyond immunological barriers

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Abstract

Although criteria for living kidney donations have greatly evolved in recent years with acceptance of related and unrelated donors, an immunological incompatibility, between a living donor and his intended recipient, could impede up to 40% of such procedures. To avoid refusal of willing and healthy living donors, different strategies have emerged to overcome immunological incompatibilities. ABO incompatible kidney transplantation can now be achieved with identical outcomes to compatible/identical kidney transplantation and kidney paired donation is the safest way for HLA-incompatible patients to undergo kidney transplantation. Implemented with success in many countries either as national or multiple regional independent programs, kidney paired donation could include simple exchanges between any number of incompatible pairs, incorporate compatible pairs and non-directed donors to start a chain of compatible transplantations, lead to acceptance of ABO-incompatible matching, and integrate desensitizing protocols. Incorporating all these variations, kidney paired donation has been able to facilitate kidney transplantation in [...]
PD thesis, December 2014

"LIVING KIDNEY TRANSPLANTATION: BEYOND INNATE AND ACQUIRED IMMUNOLOGICAL BARRIERS"

K. Hadaya
17/11/2014
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A. SUMMARY

Growing incidence of end-stage renal disease, shortage of kidney from deceased donors and better outcomes for recipients from living donor have led many centers worldwide to favour living donor kidney transplantations programs. Although criteria for living donations have greatly evolved in recent years with acceptance of related and unrelated donors, an immunological incompatibility, either due to ABO incompatibility and/or to positive crossmatch, between a living donor and his intended recipient, could impede up to 40% of such procedures. To avoid refusal of willing and healthy living donors, different strategies have emerged to overcome immunological incompatibilities. ABO incompatible kidney transplantation can now be achieved with identical outcomes to compatible/identical kidney transplantation and kidney paired donation is the safest way for HLA-incompatible patients to undergo kidney transplantation. Implemented with success in many countries either as national or multiple regional independent programs, kidney paired donation could include simple exchanges between any number of incompatible pairs, incorporate compatible pairs and non-directed donors to start a chain of compatible transplantations, lead to acceptance of ABO-incompatible matching, and integrate desensitizing protocols. Incorporating all these variations, kidney paired donation has been able to facilitate kidney transplantation in up to 49% of registered patients from incompatible pairs.
B. INTRODUCTION

1. END-STAGE KIDNEY DISEASE

Nowadays, chronic renal disease is recognized as a worldwide public health priority, as it is associated with significant morbidity and mortality. The number of deaths due to chronic renal diseases had risen by 82.3% in the last 2 decades, the highest increase among the top causes of death, behind HIV/AIDS and diabetes\(^1\). In parallel, with a growing incidence of end-stage renal disease, the number of patients receiving renal replacement therapy is estimated > 1.4 million, with an annual growth rate of 8\(^2\)\%.

Kidney transplantation is the treatment of choice for medically suitable patients with end-stage kidney disease; it reduces the mortality risk compared to dialysis in all age groups and improves the quality of life, offering benefits in terms of life expectancy\(^3;4\). To be medically suitable, the patients must have a better than 50% chance of surviving 5 years after transplantation.

However, as the widening gap between organ demand and supply is a worldwide ascertainment associated with death on the waitlist, different strategies felt in place. Regarding deceased donors, acceptance criteria have been expanded as less ideal organ donors are available and donations programs after cardiac arrest have been implemented in many countries\(^4\). Meanwhile, living kidney donation became the main program and the program of choice in many countries.

2. LIVING KIDNEY DONATION

a. Results
Superior long-term recipients and grafts survivals from living donors\(^5;6\), very low kidney donor mortality (0.03\%) and major morbidity (\(< 1\%) rates, and minimal long-term risks for medically suitable living donors, have led many countries to place greater emphasis on living kidney donation programs.

b. Prevalence
Since 2001 and since 2008, in the United States (US) and in the United Kingdom (UK) respectively, living kidney donation accounts for more than one in three of all kidney transplantations performed. In Switzerland, a country in the long tradition of living kidney donations, we observe the same situation since 2002.
c. Selection criteria in Switzerland

According to Swiss Transplantation Law that came into force in July 2007, a living donor could be either genetically or emotionally related to a recipient. Table 1 shows Swiss acceptance living donor’s criteria.

Table 1: Acceptance living donor’s criteria in Switzerland

<table>
<thead>
<tr>
<th>Age limits</th>
<th>&gt;18ans No upper age limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relationships to the recipient</td>
<td></td>
</tr>
<tr>
<td>Genetical</td>
<td>First, second...degree</td>
</tr>
<tr>
<td>Emotional</td>
<td>Partners, spouses, friends</td>
</tr>
<tr>
<td></td>
<td>Crossover</td>
</tr>
<tr>
<td>None</td>
<td>Altruistic donor allocated anonymously to the best matched recipient on the waitlist</td>
</tr>
<tr>
<td>ABO incompatibility</td>
<td>Accepted</td>
</tr>
<tr>
<td>Expanded criteria donors</td>
<td>Well controlled hypertension, &gt;65 years old</td>
</tr>
</tbody>
</table>

d. Immunological barriers

There are two kinds of immunological barriers in renal transplantation: one is innate, related to ABO incompatibility between the donor and the recipient and the other one is acquired, due to circulating donor specific antibodies (DSA) in the recipient against at least one human leukocyte antigen of the donor and leading to positive crossmatch. The DSA are detected during the patient’s workup, as the result of prior transplantations, pregnancies or blood transfusions. In up to 40% of cases, one or both of these immunological barriers can impede a willing and healthy living donor to donate a kidney to his intended recipient. Until recently, an immunological barrier led either to rule out the living donor with the patient remaining on the deceased donor waiting list, or to undergo recipient’s desensitization protocols. Nowadays, different strategies have emerged to overcome successfully these immunological incompatibilities and to allow safe living kidney donation⁷.
3. INNATE AND ACQUIRED IMMUNOLOGICAL BARRIERS IN KIDNEY TRANSPLANTATION

a. ABO incompatibility

ABO blood group barrier has conventionally been considered an absolute contraindication to solid organ transplantation. Until recently, only ABO identical or compatible kidney transplantation (O donor as universal donor could donate to any recipient blood group), whether performed with a living or a deceased donor, was considered safe and therefore allowed.

i. Basics in blood groups: antigens and antibodies

In 1901, Karl Landsteiner (1868-1943) discovered the blood group system while studying coagulation phenomenon. Landsteiner’s law that ensues shows the inverse relationship between antigens expressed at red blood cells surface and the physiologic presence of circulating antibodies, when the corresponding antigen is absent. This law permitted transfusing effectively and without danger, and led to his author the Nobel Prize in Medicine in 1930.

Table 2 shows the number of AB antigens at adult red blood cells surfaces.

Table 2: Expression of ABO antigens at adult red blood cells surface

Since 1959, it is known that ABO antigens are not only expressed on circulating blood cells but also in many tissues such as the heart, the intestines, the liver, the lungs and the pancreas. In the kidney, they are found on glomerular and peri-tubular endothelial capillaries surfaces and...
on tubular basal membranes, where they act as real histocompatibility antigens. In the presence of circulating anti A/B/AB antibodies as it is the case in ABO incompatible (ABOi) kidney transplantation, antigen-antibody binding activates the complement cascade and the coagulation pathway leading to hyperacute humoral rejection and graft loss.

Even so, from 1955 until 1987, 53 ABOi deceased and living donors’ kidney transplantations were performed worldwide. Catastrophic 13% one year graft survival lead up to forget about this method (Table 3).

Table 3: ABOi kidney transplantations between 1955 and 1987

<table>
<thead>
<tr>
<th>Papers</th>
<th>DD/LD</th>
<th>N</th>
<th>Graft survival at 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hume et al. (1955)</td>
<td>DD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Murray et al. (1960)</td>
<td>DD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Starzl et al. (1964)</td>
<td>3 DD/2 LD</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Dunea et al. (1965)</td>
<td>2 DD/1 LD</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Couch et al. (1966)</td>
<td>DD</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Murnaghan et al. (1967)</td>
<td>DD</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sheil et al. (1969)</td>
<td>DD</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Wilbrandt et al. (1969)</td>
<td>DD</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Paul et al. (1978)</td>
<td>DD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cook et al. (1987)</td>
<td>DD</td>
<td>25</td>
<td>1</td>
</tr>
</tbody>
</table>

Total (%) 53 7 (13)

*DD: deceased donor, LD: living donor*

ii. History, techniques and program’s implementation

- in Belgium

In 1981, in Brussels, a mistake in operating room helped restarting the process: A1 deceased donor kidney graft was transplanted into O recipient. Immediate medical reaction consisting of plasma exchanges, polyclonal antibodies infusion and high level triple immunosuppressive therapy maintenance (cyclosporine A, azathioprim and corticosteroids) will allow 22 years graft survival.
Following this unexpected success, Belgium will pioneer an ABOi living kidney program from 1982. A series of 26 intentional ABOi living donor kidney transplant performed after plasmapheresis and splenectomy and under polyclonal antibodies infusion, cyclosporine A, azathioprim and corticosteroids showed 75% one year graft survival. ABOi kidney transplantation was no more absolute contraindication.

- In Japan

In Japan, a country relying on living donors (no brain death legislation until 1997 due to religious culture), the first successful ABOi kidney transplantation will be performed in 1985. By means of regular plasmapheresis or double-filtration plasmapheresis, splenectomy, anticoagulation and intensified immunosuppression, 96% graft survival at 1 year and 91% at 5 years were achieved. Nowadays, 30% of all Japanese living kidney donations are ABOi.

- Elsewhere

ABOi kidney transplantations were noted with interest but splenectomy with its accompanying morbidity and mortality impeded its wider acceptance by patients and physicians.

- In Sweden

In 2001, A/B/AB specific immunoadsorption became possible thanks to Glycosorb® column, Glycorex Transplantation, Lund, Sweden. Glycosorb® is a low-molecular carbohydrate column formed of a sepharose matrix to which are fixed A, B or AB synthetic trisaccarides, identical to blood groups antigenic epitopes. The column is placed in series after a plasmapheresis column or centrifuge, to receive the patient’s plasma and to specifically bind anti-A, B or AB isoimmunoglobulin (IgG) and isoagglutinin (IgM) via covalent attachment of their Fc region. All the plasma is given back to the patient and no substitution liquid is therefore necessary. The major advantage of such a technique is efficient depletion of circulating blood group antibodies without considerable losses of protective antibodies and coagulation factors. On each session, at least two plasma volumes are processed, lowering anti-A/B/AB antibodies by 2-4 steps. When antibody titers against donor erythrocytes are ≤1:8, transplantation is carried out on the following day.

In 2003, Tydén et al from Karolynska Institute, Sweden, reported on this method, and replaced splenectomy by a single dose of rituximab 375mg/m² given 2-4 weeks prior to transplantation, together with 0.5g/kg intravenous immunoglobulins on day -1 and triple drug immunosuppression therapy (tacrolimus, mycophenolate mofetil and corticosteroids) starting 7 days before
surgery. In the princeps paper, 4 ABOi renal transplant recipients experienced this regimen with 100% graft and patient survivals and no humoral rejection at 6 months post-transplant. The same group reported 3 years follow up of 20 adult patients: patient and graft survivals, incidence of acute humoral rejection and graft function were identical to ABO compatible/identical kidney transplantations. The same protocol was given to 10 ABOi children kidney transplantation with the same results.

This elegant approach become widespread and was progressively implemented in many European, Asian and North American countries with great success.

The mean features of ABOi kidney transplantation are summarized in Table 4.

**Table 4: Key points for ABOi kidney transplantation program**

- Need for a living A, B or AB donor
- Need for an O, A or B recipient
- Preconditioning regimen based on single dose rituximab perfusion and immunosuppressive therapy onset before surgery
- Preoperative anti-A/B/AB IgG and IgM titers ≤1:8 thanks to any specific or unspecific apheresis method
- Long-term corticosteroids maintenance

### iii. Immunosuppressive regimen

Conventional immunosuppressive regimen maintenance, the same as ABO compatible/identical kidney transplantation, is based on tacrolimus or cyclosporine A, mycophenolate mofetil and corticosteroids. The routine pre-transplant use of intravenous immunoglobulin was stopped in most centers. Each center is free to give an induction therapy with basiliximab, a monoclonal anti-IL2 activated receptor antibody, thymoglobulins or alentuzumab, polyclonal anti-T lymphocytes antibodies, based on concomitant anti-HLA immunological risk. Different groups evaluated the outcomes following corticosteroids tapering. Rapid steroid withdrawal at day 7 has been associated with 30% early acute humoral rejection episodes, which were even so successfully reversed, and with 100% patient and graft survivals at 1 year post-transplant. When late withdrawal took place between 6.6 and 13.4 months post-transplant, 60% of the recipients experienced subclinical or clinical acute rejection episode. This incidence is much higher than in ABO compatible/identical grafts. Therefore, the need for long term corticos-
steroids maintenance is nowadays accepted in ABOi kidney transplantation, which is a significant difference with ABO compatible/identical kidney transplantation.

iv. Middle and long term results

- In Japan
In 2010, 1'878 ABOi kidney transplantations have been performed in Japan. Patient survival at 3, 5 and 10 years was 97%, 96% and 91%, respectively, identical to ABO compatible/identical kidney transplantations. Graft survival at 3, 5 and 10 years was 93%, 91% and 83%, respectively, identical to ABO compatible/identical transplantations. Incidence of cancer and infections were not increased.

- In Europe
Three-year outcomes of 1'420 ABOi kidney transplantations from 101 centers registered with the Collaborative Transplant Study between 2005 and 2012 were recently published. Overall graft survival death-censored graft survival was identical between ABOi and ABO compatible recipients: 89.9±0.9%, p=0.44 vs 89.7±0.2%, p=0.61. No significant difference in patient survival (95.6±0.6% vs 96.3±0.1%, p=0.11) nor in acute rejection episodes during the first year post-transplant (16.3% vs 17.8%, p=0.36) was seen. Serum creatinine and incidence of cancer revealed similar rates at 3-year.

v. Accommodation state
Paradoxical coexistence between a graft with target antigens and a recipient with circulating antibodies remaining each with its own blood group is due to establishment of an “accommodation” state, cornerstone of ABOi kidney transplantation.

When protocol biopsies of functioning kidney grafts are performed, no microcirculation inflammation is seen under light microscopy, even so circulating low titers anti A/B/AB antibodies are detected and peri-tubular capillaries C4d deposition is positive by immunofluorescence. The presence of C4d positive is the proof that carbohydrate ABO antigens/anti-ABO antibodies binding has occurred on endothelial surfaces, followed by classical complement pathway activation. Thereafter, the fact that the complement cascade does not end in hyperacute or acute humoral rejection and graft destruction implicates some form of regulatory pathway before the terminal membrane attack complex. “Accomodation” is not tolerance because the recipient immune system can rejects fresh tissue from the same donor. The absence of injury in the face of immunity is still incompletely understood but could be allowed by different mechanisms: an acquired and active graft resistance, by low level activation of tissular glycosyltransferase
enzyme leading to lower expression of A/B/AB tissular antigens at the endothelium surface in the graft; resistance to complement activation, by CD59 intragraft induction and expression following binding of antibodies to antigen endothelial cells, leading to complement regulation at the level of C9 \textsuperscript{91,92}; some endothelial chimerism occurring over time \textsuperscript{93}.

Collaboration with Professor Joerg Seebach, Service of Immuno-Allergology, Gisella Puga Yung, scientific collaborator and Tianshu Lan, PhD student, Geneva University Hospitals, has begun. The aims of this project will be to develop:

- ABO antigens’ labeling and quantification at the endothelium surfaces in the graft
- Longitudinal follow-up on day 0, 3 months, 1 year and 5 years post-transplant on graft biopsies in order to analyze the kinetics of ABO antigens expression

In Switzerland and at Geneva University Hospitals

In Switzerland, Basel University Hospital begun ABOi kidney transplantation in 2005, followed by Zurich University Hospital in 2006, Geneva University Hospitals in May 2008, Bern University Hospital in 2009 and St. Gallen Kantonsspital in 2010. All these transplantation centers agreed on a common national protocol. The major differences with the Swedish protocol are reuse of Glycosorb\textsuperscript{®} column and on-demand post-transplant immunoadsorption strategy. In fact, the column is priced 3’900 Euros for unique usage. As a patient could need 1 to 18 immunoadsorption sessions (median 5) prior to surgery, transplantation costs are hugely increased by such a method. The Swiss idea was to develop a regeneration protocol with columns reused for maximally 4 times. Evaluation of reused column performance has been proved safe and efficient, with substantial cost savings of 1’053’000 Euros for 80 ABOi kidney transplantations \textsuperscript{18}.

On August 2014 follow up, 124 ABOi adult kidney transplantations were reported in Switzerland with 97.6% patient survival and 95% graft survival.

From May 2008 to April 2014, 17 adults underwent ABOi kidney transplantations at Geneva University Hospitals. Table 5 shows demographics and results of this procedure.
Table 5: Demographic data of 17 ABOi adult kidney transplantations performed at Geneva University Hospitals

<table>
<thead>
<tr>
<th></th>
<th>Donors</th>
<th>Recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender, N (%)</td>
<td>6 (35)</td>
<td>14 (82)</td>
</tr>
<tr>
<td>Female gender, N (%)</td>
<td>11 (65)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Age years, median (min, max)</td>
<td>54 (22-70)</td>
<td>53.7 (22-77)</td>
</tr>
<tr>
<td>Relationship to the recipient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>partners, friends</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>family (mother, siblings)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Blood groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 A</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2 B</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>1 A</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Preemptive, N (%)</td>
<td>5 (29)</td>
<td></td>
</tr>
<tr>
<td>Number of SIA pre-transplant</td>
<td></td>
<td>4.5 (0-9)</td>
</tr>
<tr>
<td>median (min, max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of SIA post-transplant</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Acute rejection episode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cellular subclinical antibody mediated</td>
<td>1 at 3 month</td>
<td>0</td>
</tr>
<tr>
<td>BK virus nephropathy</td>
<td>N</td>
<td>2 at 3 month</td>
</tr>
<tr>
<td>reversibility</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>Survivals at 01.08.2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>recipients</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>grafts</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

SIA, *specific immunoadsorption, Glycosorb*®

Our paediatrician colleagues are aware of these excellent results and will contact us if ABOi kidney transplantation is needed for a child.
Between 2005 and 2011, 80 adult recipients underwent ABOi kidney transplantations at 5 Swiss centers. The mean follow up was 3.9 years. At 5 years, patient and death-censored graft survivals were 97.5% and 96% respectively, and estimated glomerular filtration rate was 55.8ml/ml (± 18.88). Rejections episodes occurred during the first 3 months in 16 patients (20%); among them, 3 had acute cellular rejection and 13 acute humoral rejection. A/B antibodies were involved in 4 patients.
The Reuse of Immunoadsorption Columns in ABO-Incompatible Kidney Transplantation Is Efficient: The Swiss Experience

Marc Schiesser,1,7 Daniel C. Steinemann,1 Karine Hadaya,2 Uyen Huyen-Do,3 Ute Eisenberger,3 Isabelle Binet,4 Thomas Fehr,5 and Michael Dickenmann6

Background. We developed a multicentric Swiss protocol for ABO-incompatible kidney transplantation including immunoadsorption column reuse. The aim of this study was to assess efficacy and safety of immunoadsorption column reuse in ABO-incompatible kidney transplantation.

Methods. We performed a multicentric prospective trial including all ABO-incompatible kidney transplantations in Switzerland from 2005 to 2011. Patients received rituximab and standardized immunosuppression with tacrolimus, mycophenolate mofetil, and steroids. Antigen-specific perioperative immunoadsorption was performed. Immunoadsorption columns were reused after restoration. Graft survival, patient survival, kidney function, rejections, number of columns, adverse events after column reuse, and anti-A/anti-B antibody titers were assessed.

Results. Seventy-one ABO-incompatible patients underwent antigen-specific immunoadsorption and could be transplanted across the blood group barrier. Kaplan-Meier estimates for both, patient-censored and death-censored graft survivals were both 97.2% at 5 years. Allograft function was excellent with a mean estimated glomerular filtration rate of 54 mL per min after 1 year. The median number of pretransplant immunoadsorptions was 5. All centers performed column reuse. A total of 394 immunoadsorption procedures were performed with reused filters. Patient survival, graft survival, and adverse events did not differ when filters were reused. Column reuse resulted in cost savings of 21,458 USD per patient.

Conclusion. We have introduced a national protocol for ABO-incompatible kidney transplantation including immunoadsorption column reuse. Column reuse was efficient and safe.

Keywords: ABO-incompatible kidney transplantation, Immunoadsorption, Antibody-mediated rejection, Kidney transplantation, Plasmapheresis.

The increasing discrepancy between patients on the waiting list and transplanted kidneys has initiated innovative strategies to decrease organ shortage. Regarding living donor kidney transplantation sophisticated protocols to overcome ABO incompatibility were introduced in the past (1). Initial attempts have been made in the late 1980s in Japan. With improved understanding of the mechanisms of accommodation and antibody-mediated rejection (AMR) and progress in immunosuppression, the results have improved markedly, and are nowadays only slightly inferior or comparable to those of ABO-compatible kidney transplantation (2–5).

However, the pretreatment of the recipient is elaborate and expensive. Different techniques to remove the blood group antibodies are used, such as the double filtration plasma exchange, regular therapeutic plasma exchange, and nonspecific and specific immunoadsorptions (6, 7). A major advantage of specific immunoadsorption is the efficient depletion of circulating blood group antibodies without considerable losses of protective antibodies and other essential plasma constituents (8). On the other
hand, this treatment is expensive if the immunoadsorption columns are used only once as suggested by the manufacturer.

In Switzerland, we have introduced ABO-incompatible transplantation in 2005, and the involved transplantation centers have agreed on a common protocol using specific immunoadsorption to remove the blood group antibodies. Because of the high costs for the immunoadsorption and additional immunosuppressive medication, we have subsequently introduced a protocol to reuse the immunoadsorption columns (9). We report the results of the Swiss multicenter ABO-incompatible kidney transplantation cohort with a focus on safety and efficacy of the reuse of immunoadsorption columns.

RESULTS

Patient Characteristics

From September 2005 until December 2011, a total of 80 patients with an ABO-incompatible donor recipient constellation were transplanted in five Swiss transplant centers using a common protocol. Nine patients had additional donor-specific antibodies and were treated with plasma exchange and were therefore excluded from the study. The remaining 71 patients were treated with selective immunoadsorption and included into the analysis. No patient was lost to follow-up. The mean follow-up was 4.2 years. Fifty-two (73%) recipients were men, 19 (27%) were women. The majority had blood group O (64.7%). The blood group constellations were as follows: 46 patients, A to O; one patient, AB to O, eight patients, A to B; three patients, AB to A, two patients, AB to B, three patients, B to O; and eight patients B to A. The most frequent type of relationship between donor and recipient was married couples, and there was a significantly higher proportion of female donors (45, 63.4%) compared to male donors (26, 36.6%). The mean donor age was 52.5 (±10.7) years, and the mean recipient age was 51.8 (±13.2) years. Fifty (70%) recipients were on dialysis at the time of transplantation, whereas 30% received a preemptive transplant.

Patient Survival, Graft Survival, and Graft Function

The Kaplan-Meier estimate for patient survival was 97.2% at 5 years (Fig. 1A). The Kaplan-Meier estimates for death-censored graft survival after 1, 2, and 5 years were excellent with 100%, 98.6%, and 97.2%, respectively (Fig. 1B).

The estimated glomerular filtration rate (eGFR) calculated by the chronic kidney disease epidemiology collaboration formula after 1, 2, and 5 years were 54.05 mL per min (±16.03), 52.98 mL per min (±14.48), and 54.0 mL per min (±15.87), respectively. The serum creatinine levels at 1, 2, and 5 years were 126 μmol/L (±39.2), 128 μmol/L (±35.9), and 129.4 μmol/L (±54.6), respectively. The percentage of patients with a pathologic albumin-to-creatinine ratio or protein-to-creatinine ratio over time is given in Figure 2.

Rejection Rate

Thirteen (18.3%) patients developed biopsy-proven acute rejection. The Kaplan-Meier curve for rejection-free survival is given in Figure 3. Among the 13 biopsy-proven acute rejection, three patients had a cellular rejection, whereas 10 had an AMR. The cellular rejections could be treated successfully in all but one case with methylprednisolone pulses. One patient needed antithymocyte globulin. Four patients with AMR had to undergo therapeutic plasma exchange. One patient lost his graft because of early sepsis or caecum perforation and subsequent rejection because of immunosuppression reduction. The majority of rejections occurred early at a mean interval of 4.9 months after transplantation; six...
rejections occurred within the first month and seven within the first 3 months. At the end of the follow-up period, 25% of the patients were on a corticosteroid-free immunosuppression regimen.

Complications

The overall perioperative complication rate (<30 days) was 26.8% according to the Clavien/Dindo classification (10). Seventeen percent of all patients experienced severe complications Grad III to IV requiring an intervention or a reoperation. Among these, there were four patients with a septicemia, two patients with a bleeding complication, two patients with a ureteral complication, and four with a lymphocele. All complications occurred exclusively after the transplantation and not during the immunoadsorption period before the transplantation. In the further follow-up, four patients developed cytomegalovirus (CMV) disease and 13 had BK viremia; however, no polyomavirus nephropathy occurred. Two of 71 patients died during the follow-up period.

One patient developed a severe donor-derived herpes simplex virus 2 infection and lost his graft. In the further course, he developed multiple complications and died 199 days after transplantation from a complicated pancreatitis. Another patient died 800 days after transplantation from an E. coli sepsis with a functioning graft. No complication (infection/lever) occurred during the immunoadsorption therapy before transplantation.

Selective Immunoadsorption and Reuse of the Columns

Seventy-one patients underwent antigen-specific immunoadsorption treatment. Four hundred twenty-nine immunoadsorption therapies were performed before transplantation and 30 posttransplantation. The specific immunoadsorption successfully lowered the antibody titers in all patients allowing a subsequent transplantation. The median number of immunoadsorptions performed before transplantation was 5 (range, 3–18; Fig. 4A). Nine (12.7%) patients underwent immunoadsorption after transplantation. The median number of immunoadsorptions after transplantation was zero (range, 0–11). The columns were successfully reused in all centers. Overall, we used one to four columns per patient, but only two patients needed more than three columns. We performed a median of three (range, 1–12) immunoadsorptions per column (Fig. 4B).

At the introduction of the column reuse protocol, we used more columns per patients also for logistical reasons. One hundred fifty-one columns were used for 459 immunoadsorptions. Using this strategy, we could save 308 columns, which corresponds to 1,523,541 USD (average cost per column 4,946 USD). This corresponds to a saving of 21,458 USD per transplantation.

At the beginning, we assessed the efficacy of the column reuse by checking the antibody titers in the eluate after the column. Details about the first 57 column efficacy tests are given in Table 1. Because the filter reuse efficacy was shown to be reliable, we then focused only on the in vivo antibody titers as a surrogate for the filter efficacy.
Furthermore, we performed a subgroup analysis for patients, who were treated with filter reuse. Of the 71 patients, 54 patients (76%) underwent immunoadsorption using filter reuse. A total of 394 immunoadsorption procedures were performed with reused filters. The outcome did not differ from the remaining patients. The patient survival rate was 98% (95% confidence interval [95% CI], 94% to 100%) in the filter reuse subgroup and 91% (95% CI, 75% to 100%) in the other subgroup (P = 0.418). The graft survival was 98% (95% CI, 94% to 100%) in the filter subgroup compared to 91% (95% CI, 75% to 100%) (P = 0.435). The rate of acute rejection was 19% (10/53) in the filter subgroup (95% CI, 11% to 31%) and 17% (3/18) (95% CI, 6% to 39%) in the other group (odds ratio 1.13 with 95% CI, 0.29 to 5.86, P = 0.870). The complication rate was 30% in the filter reuse subgroup (95% CI, 20% to 44%) and 22% (95% CI, 9% to 45%) in the other group (odds ratio 1.48 with 95% CI, 0.44 to 6.04, P = 0.544). No septicemias and reactions to dissolved filter components similar to first-use syndrome were observed in the filter reuse group.

The eGFR at 1, 2, and 5 years in the filter reuse subgroup were 53.4 ± 15.4 mL per min, 52.4 ± 15.0 mL per min, and 52.7 ± 16.2 mL per min, respectively. The eGFR did not differ from the eGFR in the other subgroup at 1 and 2 years and at the end of follow-up with values of 55.1 ± 17.7 mL per min (P = 0.611), 55.2 ± 12.6 mL per min (P = 0.575), and 54 ± 14.0 mL per min (P = 0.763). No patient treated with a reused filter experienced an infectious complication at the time before transplantation.

Course of Blood Group Antibody Titers and B Cells

The IgG and IgM titers were successfully lowered to antibody titers of less or equal 1:8 before transplantation using specific immunoadsorption (Fig. 5A and B). Reuse of the immunoadsorption columns did not compromise the elimination of the blood group antigens, and no patient was deferred from transplantation because of insufficient lowering of anti-A/B titers. The titers remained low in most of the patients throughout the follow-up period. Only two patients presented with an IgG or IgM titer of more than 1:8 during the follow-up period. The mean number of B cells was 98.6 cells/μL (normal range) before transplantation and remained low at a mean of 16.4 cells/μL after 1 year.

**DISCUSSION**

This is the first report on a multicenter protocol reusing specific immunoadsorption columns for ABO-incompatible kidney transplantation. The protocol has been established and

### TABLE 1. IgM and IgG titers for the first use to the third reuse

<table>
<thead>
<tr>
<th></th>
<th>1° use (N=57)</th>
<th>1° reuse (N=57)</th>
<th>2° reuse (N=28)</th>
<th>3° reuse (N=8)</th>
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<tbody>
<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG titer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>46 (80.7%)</td>
<td>22 (78.6%)</td>
<td>7 (87.5%)</td>
</tr>
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<td>1</td>
<td>1 (1.8%)</td>
<td>1 (1.8%)</td>
<td>1 (3.6%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>2</td>
<td>5 (8.8%)</td>
<td>2 (3.5%)</td>
<td>2 (7.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>4</td>
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<td>3 (10.7%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>8</td>
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<td>0 (0.0%)</td>
</tr>
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<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
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<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
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<tr>
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<td>67.6</td>
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<th>1° reuse (N=57)</th>
<th>2° reuse (N=28)</th>
<th>3° reuse (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM titer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6 (10.5%)</td>
<td>19 (33.3%)</td>
<td>9 (32.1%)</td>
<td>6 (75.0%)</td>
</tr>
<tr>
<td>1</td>
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<td>15 (26.3%)</td>
<td>11 (39.3%)</td>
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</tr>
<tr>
<td>2</td>
<td>19 (33.3%)</td>
<td>18 (31.6%)</td>
<td>5 (17.9%)</td>
<td>2 (25.0%)</td>
</tr>
<tr>
<td>4</td>
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<td>2 (3.5%)</td>
<td>2 (7.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>8</td>
<td>5 (8.8%)</td>
<td>1 (1.8%)</td>
<td>1 (3.6%)</td>
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</tr>
<tr>
<td>16</td>
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<td>0 (0.0%)</td>
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<tr>
<td>32</td>
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<tr>
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<td>Rank</td>
<td>78.2</td>
<td>74.3</td>
<td>74.9</td>
<td>67.1</td>
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</tbody>
</table>

a P trend = 0.083 (Spearman ρ over the mean ranks).
b P trend = 0.333 (Spearman ρ over the mean ranks).

N (%) for columns.

There was no significant difference regarding filter efficacy. 57 filters were tested after the first use and first reuse, 28 filters after the second reuse, and eight filters after the third reuse.

IgG, immunoglobulin G; IgM, immunoglobulin M.
approved by the Swiss ABO-incompatible kidney transplantation group and has been standardized in all transplantation centers across Switzerland. We have shown that the protocol is efficient in reducing the antibody titers and is safe to perform in clinical practice. The results of the multicenter ABO-incompatible cohort are comparable to the results of the available ABO-compatible data in the literature (1,11) and the Swiss Transplant Cohort Study established since May 2008.

The reuse of the columns did not result in an increased rate of acute rejection or inefficient elimination of blood group antibodies. We observed that the anti-A/B antibody titers and the peripheral B cell pool remained suppressed up to 1 year after transplantation.

The protocol for the column reuse was solid and could be successfully used even in centers with small patient numbers. The costs of the peritransplant treatment could therefore be reduced substantially by eliminating the costs for additional columns. Using this strategy, we saved the expenditure of over one million Euros in this cohort, which corresponds to a saving of approximately 17,000 Euros per transplantation. This is an important factor in times, where reimbursement for hospitals is becoming more and more restrictive.

In addition to cost savings, this multicenter approach allowed evaluation of protocol minimization. Our data suggest that preoperative treatment with intravenous immunoglobulin (IVIG) and prophylactic postoperative immunoadsorption are not necessary, which has already been shown by others (13).

In conclusion, we have established a national protocol including the reuse of specific immunoadsorption columns in ABO-incompatible kidney transplantation. The protocol and reuse of the columns proved to be efficient and safe and allowed substantial cost savings while maintaining good outcome results.

**MATERIALS AND METHODS**

**Patients**

This is a multicenter prospective trial that included all patients from transplant centers performing ABO-incompatible kidney transplantation in Switzerland from December 28, 2005 to December 31, 2011. A working group for ABO-incompatible kidney transplantation has been established with representatives from all five Swiss transplant centers performing ABO-incompatible kidney transplantation. The working group agreed on one protocol for the desensitization and immunosuppressive treatment. Each center obtained approval for the protocol by the respective local ethical committee (Ref. StV 11-2005). The workup of recipients and donors was performed according to our national and center-specific guidelines.

Male or female patients regardless of age and race, suffering from end-stage renal disease, and fulfilling the general criteria for living donor kidney transplantation were included in the trial. First and repeated kidney transplantations were accepted. All patients gave their written informed consent. Patients were suitable for the protocol if they had a blood group-incompatible living kidney donor with a current negative T-cell and B-cell CDC cross-match (XM) test.

**Desensitization and Immunosuppression**

A single dose of rituximab (375 mg/m²) was given 4 weeks before the transplantation. Maintenance immunosuppression with tacrolimus (0.1 mg/kg twice daily), mycophenolate mofetil (1000 mg twice daily, 500 mg twice daily if body weight was less than 50 kg), and prednisone (25 mg once daily) was started before transplantation. Selective blood group antibody removal was performed with a low-molecular carbohydrate column containing A or B blood group antigens linked to a sepharose matrix (Glycosorb; Glycorex Transplantation, Lund, Sweden). Apheresis sessions were performed daily until the immunoglobulin (IgG) and isoagglutinin (IgM) antibody titers against donor erythrocytes were 1:8 or less. The transplantation was then carried out the following day. With each session, at least two plasma volumes were processed. At the beginning of the study, a single dose of IVIG (0.5 g/kg body weight) on day −1 was given; later, IVIG therapy was discontinued. The participating centers were free to give an induction therapy with 20 mg basiliximab on days 0 and 4.

**Follow-Up**

Blood group antibodies against donor erythrocytes were measured daily for 2 weeks, weekly until day 31 and 6, and 12 months thereafter. At the beginning of study 3, prophylactic apheresis sessions were scheduled after transplantation. Later, apheresis sessions were only performed on demand in case of graft dysfunction and a titer increase greater than 1:8 within the first week or greater than 1:16 within the second week after transplantation. A graft biopsy was performed, and daily immunoadsorption or therapeutic plasma exchange was started in these cases. An isolated increase of blood group
antibody titers in the absence of clinical signs of graft dysfunction was not mandatory for the start of apheresis sessions.

Target tacrolimus trough levels were 8 to 10 ng/mL from day −14 to 90, 6 to 8 ng/mL from day 90 to 365, and 4 to 6 ng/mL thereafter. Target mycophenolate mofetil trough level was greater than 2 mg/mL. Steroids (methylprednisolone intravenously and prednisone orally), 500 mg IV on day 0, 250 mg IV on day 1, 100 mg IV on day 2, 50 mg orally from day 3 to 6, 0.5 mg/kg body weight orally from day 7 with a reduction by 5 mg every 2 weeks until 15 mg every 2 weeks, then by 2.5 mg every 2 weeks until a maintenance dose of 0.1 mg/kg was given. Steroid withdrawal could be considered if the 1-year protocol biopsy revealed no signs of rejection.

**Graft Biopsies**

Protocol biopsies were performed at 12 months or more frequent according to center policy. Diagnostic biopsies were performed in case of otherwise not explained graft dysfunction using formalin fixation and fresh frozen technique. Staining was performed according to previously described procedures (14). Biopsies were judged according to the revised Banff criteria 2007 and 2009 (15, 16).

**Treatment of Acute Rejection**

Acute cellular rejection was treated according to local practice with methylprednisolone pulses. In case of suspected or biopsy-proven AMR methylprednisolone (0.5 g intravenously on three consecutive days) was administered. Daily selective immunoadsorption or therapeutic plasma exchange and IVIG could be applied at the discretion of the treating physician.

**Detection of Anti–Human Leukocyte Antigen Antibodies and Assignment as Human Leukocyte Antigen–Donor–Specific Antibodies**

All sera were tested for class I (i.e., human leukocyte antigen [HLA]-A/B/C) and class II (i.e., HLA-DR/DQ/DP) anti-HLA antibodies using single antigen flow bead assay on a Luminex platform (LabScreen; One Lambda, Canoga Park, CA). A positive result was defined as a baseline normalized mean fluorescence intensity greater than 500. Donor specificity of anti-HLA antibodies was determined by comparison of the anti-HLA antibody specificities with the HLA typing of the donor as previously reported (5). The CDC-XM assay was performed as reported previously (17). No prospective flow cytometry-XM was performed. Typing of HLA-antigens was determined by serology (A/B/DR) and/or by sequence specific primer DNA typing (A/B/DR/DQ).

**Detection of Blood Group Antibody Titers**

Blood group antibody titers were determined against donor-specific erythrocytes by the saline method (IgM) and the indirect Coombs test (IgG) as described previously (3). The CDC-XM assay was performed on an emergency base. The authors also thank R. Warschkow for statistical assistance.

**Regeneration of the Columns**

Patients with filter reuse were analyzed in a subgroup (filter reuse group). Immunoadsorption columns were regenerated using solutions from Fresenius Medical Care (Redmond, WA). Immediately after the immunoadsorption procedure, the plasma was rinsed out of the column using 1000 mL Buffer PA pH 7.0. After rinsing, the antibodies were eluted with a citrate solution: 1000 mL eluate PA pH 2.2. After elution, the column was neutralized with 1,000 mL buffer PA pH 7.0. Finally, the column (volume, 70 mL) was rinsed and filled with 250 mL Immunosorbs Preservation Solution containing 0.04% polyhexamethylenebiguanide. The columns were stored in the dark at +2°C to +8°C.

**Evaluation of Column Performance After Regeneration**

In a subgroup of 57 columns, the performance of the column was systematically evaluated at the end of every immunoadsorption. Blood group antibody titers against the donor’s blood group were estimated in the blood taken 10 min before the end of the procedure from the line immediately after the column (Table 1). Negative or low antibody titers indicate efficient antibody removal even at the end of treatment and despite of reuse of the columns. One column revealed a lack of efficacy after the first regeneration in the ex vivo testing. The mean IgG titers of blood group A antibodies were 1.6 after the first reuse (median, 0; range, 0 to 64) and 0.36 (median, 0; range, 0 to 4) after the second reuse. Columns were reused for maximally four times and remained efficient even after multiple reuses. Immunoglobulin G levels appeared to be lower than IgM titers.

**Complications**

Perioperative complications were graded according the Clavien/Dindo classification (10). Infectious complications were monitored throughout the whole follow-up period and graded as severe, when a hospitalization was needed. BK and CMV replication were monitored on a routine basis. The CMV disease was defined according to Ljungman et al. (18).

**Statistics**

Statistical analysis was performed using SPSS for Windows, version 15 (SPSS Inc., Chicago, IL). Kaplan-Meier estimates were calculated, Log rank test and chi-square test to compare groups were applied as appropriate. A two-sided P value was considered as statistically significant if less than 0.05.

**ACKNOWLEDGMENTS**

The authors thank the hemodialysis and therapeutic plasma exchange units of each hospital for performing the immunoadsorption treatments and for providing the respective data, and the blood banks of each hospital for performing blood group antibody titers on an emergency base. The authors also thank R. Warschkow for statistical assistance.

**REFERENCES**


viii. Conclusions

Based on blood group frequencies in the general population, there is 20-30% risk that a willing living donor is ABOi with his intended recipient (Table 6). Eliminating up to one third of potential living donors is no more a fatality as overcoming ABOi in kidney transplantation has now become an established and effective practice in many centers worldwide, thanks to preconditioning, immunoadsorption and long term triple immunosuppressive drug maintenance. The accommodation state achievement enables identical short, middle and long term outcomes to compatible/identical kidney transplantation.

In the future, preconditioning strategies will probably be tailored according to baseline A/B/AB blood group antibodies titers.

Table 6: Blood groups frequency in Swiss population

<table>
<thead>
<tr>
<th>ABO blood groups</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>41</td>
</tr>
<tr>
<td>A</td>
<td>47</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
</tr>
</tbody>
</table>
b. Donor specific antibodies
   i. Prevalence and consequences

Preformed DSA, either class I or II, are major barriers to living donor kidney transplantation ruling up to 54% of otherwise appropriate pairs. They result in positive cell-based crossmatch (either by flow cytometry or complement-dependent cytotoxicity (CDC)), are associated with high rates of all forms of antibody-mediated rejection, early graft loss and reduced long-term graft survival 19. Despite desensitization strategies using a combination of plasmapheresis, intravenous immunoglobulin and T and B-cell depleting agents 20-22 in order to reverse positive crossmatches and allow transplantation, up to 50% of surviving grafts show chronic active antibody-mediated rejection and premature graft failure after 5-year post transplant 19;23. Unlike ABOi transplantation, accommodation has been rarely seen in patients with DSA. In line with this, highly immunized patients with a panel-reactive antibody (PRA) level of 80% or more are particularly disadvantaged, translating into a waiting time in excess of 10 years on the transplant waitlist.

   ii. Single-antigen beads assay: the Luminex technology

Histocompatibility laboratories resort Luminex™ technology as a routine procedure for anti-HLA antibodies detection and follow up. This solid-phase assay uses color-coded microbeads coated with purified single HLA antigens and defined by a specific fluorescent color tone, to detect fluorescent emission after incubation of the patient’s serum. The intensity of the fluorescent signal correlates with the amount of anti-HLA antibodies, expressed as mean fluorescence intensity, MFI. Luminex™ provides assessment of the immunological risk between a recipient and a potential donor. As a high-sensitivity technology, it allows the detection of low to very low titers of DSA, previously undetectable by former assays, and in the presence of a negative cross-match. The relevance of these isolated DSA (i.e., with a negative crossmatch), detected by means of high sensitivity technology (i.e., single-antigen beads (SAB)) on renal transplantation outcomes is less clear 24.

   iii. Paper

In a retrospective study between Zurich and Geneva University Hospitals, the outcomes of 37 living donor kidney recipients with isolated pretransplant DSA (i.e., with a negative CDC crossmatch) were analysed. The aim of this study was to determine the prognostic impact of such DSA (class and strength as detected by a mean fluorescence intensity, MFI) detected by SAB and to compare 3 generations of crossmatch: the cell-based assays, CDC and flow cytometry.
crossmatch (FXM), in which HLA antigens are expressed on the intact T and/or B lymphocytes, and to a solid-phase assay, Luminex crossmatch, in which isolated HLA antigens, A, B and DR, are bound to a matrix (Figure 1).

**Figure 1:** 3 assays for anti-HLA antibodies’ detection

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Pre-transplant DSA class I alone above 900 MFI or DSA class I at lower strength in combination with Luminex crossmatch or T-cell CDC crossmatch were predictive of antibody-mediated rejection and reduced graft function.

Following these results, the 6 Swiss kidney transplant centers considered a DSA value equal or greater than 1’000 MFI clinically relevant for antibody-mediated rejection and graft survival, and introduced this parameter in the new Swiss allocation system implemented in 2012. Furthermore, these data and a consensus between HLA laboratories in Europe and in United States led to international adoption of 1’000 MFI DSA cut-off for donor refusal, for all but highly immunized patients. For these patients, DSA with MFI lower than 10’000 MFI should not contraindicate kidney transplantation, as their chance of finding a donor would be too low.

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*Current issues and future direction in kidney transplantation, chapter 5, 2013*
Donor-Specific Antibody Levels and Three Generations of Crossmatches to Predict Antibody-Mediated Rejection in Kidney Transplantation

Sebastian Riethmüller,1,8 Sylvie Ferrari-Lacraz,2,3 Markus K. Müller,1 Dimitri A. Raptis,1 Karine Hadaya,4 Barbara Rüsi,5 Guido Laube,6 Gregory Schneider,2,3 Thomas Fehr,7 and Jean Villard2,3,8

Background. This study evaluated the prognostic impact of pretransplant donor-specific anti-human leukocyte antigen antibodies (DSA) detected by single-antigen beads and compared the three generations of crossmatch (XM) tests in kidney transplantation.

Methods. Thirty-seven T-cell complement-dependent cytotoxicity crossmatch (CXM) negative living donor kidney recipients with a retrospectively positive antihuman leukocyte antigen antibody screening assay were included. A single-antigen bead test, a flow cytometry XM, and a Luminex XM (LXM) were retrospectively performed, and the results were correlated with the occurrence of antibody-mediated rejections (AMRs) and graft function.

Results. We found that (1) pretransplant DSA against class I (DSA-I), but not against class II, are predictive for AMR, resulting in a sensitivity of 75% and a specificity of 90% at a level of 900 mean fluorescence intensity (MFI); (2) with increasing strength of DSA-I, the sensitivity for AMR is decreasing to 50% and the specificity is increasing to 100% at 5200 MFI; (3) the LXM for class I, but not for class II, provides a higher accuracy than the flow cytometry XM and the B-cell CXM. The specificity of all XMs is increased greatly in combination with DSA-I values more than or equal to 900 MFI.

Conclusions. In sensitized recipients, the best prediction of AMR and consecutively reduced graft function is delivered by DSA-I alone at high strength or by DSA-I at low strength in combination with the LXM or CXM.

Keywords: Kidney transplantation, Luminex crossmatch, Solid-phase assay, Anti-HLA antibodies, Donor-specific antibodies.

(Humoral sensitization to human leukocyte antigens (HLA) is a barrier for solid organ transplantation, which can occur because of pregnancy, blood transfusion, previous transplants, or sensitization to cross-reactive epitopes found on microorganisms and ingested proteins (1). The presence of donor-specific anti-HLA antibodies (DSA) is associated with all forms of antibody (Ab)-mediated rejection (AMR) (2). Screening for preformed DSA has evolved from complement-dependent cytotoxicity crossmatch (CXM) (3) to the more sensitive flow cytometry crossmatch (FXM) (4, 5). In contrast to these “cell-based” or “membrane-dependent” assays, in which the DSA target is the HLA expressed on the intact lymphocytes, advances in HLA purification technology have facilitated the development of “solid-phase assays” (SPA), whereby the isolated (membrane-independent) HLA are bound to a solid matrix. Assays with both mixed and single HLA antigens directly linked to fluorescent-labeled beads are available (6–8), which detect complement-binding and noncomplement-binding anti-HLA Abs using fluorescent anti-human immunoglobulin G as a secondary Ab. Recently, a crossmatch (XM) test using the solid-phase technology has been introduced, namely the Luminex crossmatch (LXM; LIFECODES DSA, Tepnel Lifecodes, Stamford, CT) (9, 10).

Preexisting or de novo DSA correlate with a higher risk of graft failure (11–14). However, the relevance of DSA de-
leukocyte antibody tests. DSA, donor-specific antihuman assay and three different crossmatch for evaluation of the single-antigen bead positive and represent our target population those, 37 were found to be positive and represent our target population for evaluation of the single-antigen bead assay and three different crossmatch tests. DSA, donor-specific antihuman leukocyte antibody tests.

Among those, 37 were found to be positive and represent our target population for evaluation of the single-antigen bead assay and three different crossmatch tests. DSA, donor-specific antihuman leukocyte antibodies.

PATIENTS AND METHODS

Patients
In the University Hospitals of Zurich (ZH) and Geneva (GE), 155 (ZH: 113; GE: 42) living donor kidney transplantations with a negative T-CXM were performed between January 2005 and June 2008. Thirty-seven patients (ZH: 30; GE: 7) had a positive anti-HLA Ab screening assay (LABScreen Mixed, One Lambda Inc., Canoga Park, CA) on the day of transplantation and were included in this retrospective study (study design, see Fig. 1). In addition to the anti-HLA Ab screening assay, our procedure included panel reactive antibody (PRA), LABScreen Single Antigen assay, and three different XMs in the same serum. All recipients and donors were typed for HLA-A, -B, and -DR by serology and polymerase chain reaction with sequence-specific primer and for HLA-DQ by serology only. Follow-up was evaluated until 1 year posttransplant.

Standard immunosuppression in all patients consisted of prednisone, mycophenolate, and—all according to physician’s judgment—cyclosporine or tacrolimus. Ab induction therapy including rituximab, basiliximab, or Thymoglobulin (Genzyme, Cambridge, MA) was used if one or more of the following conditions were fulfilled: B-cell CXM (B-CXM) positive, current or peak PRA more than 10%, retransplantation, and six HLA-mismatches. In addition to these parameters and the T-CXM, there was no additional information about the risk of AMR available at the time of transplantation.

Panel Reactive Antibodies
All serum samples were tested in a microlymphocytotoxicity assay against a T-lymphocyte panel of 30 donors. Cells were incubated with serum for 30 min at 21°C and then with complement for 30 min.

Anti-HLA Antibody Screening Assays
Patients were screened with LABScreen Mixed (LSM12, One Lambda Inc., Canoga Park, CA). This assay contains a panel of color-coded microbeads coated with multiple HLA antigens to identify class I or II anti-HLA IgG Abs and was performed according to the manufacturer’s instructions (21). Test interpretation was performed using HLA Visual software (One Lambda Inc.) on the LABScan100 flow cytometer (Luminex Inc., Austin, TX), and the positive cutoff was at 3.0.

LABScreen® Mixed
LABScreen® Single Antigen
LABScan® Mixed

37 HLA-sensitized recipients
118 non HLA-sensitized recipients
20 DSA positive recipients
17 DSA negative recipients

CXM and FXM
CXM was performed by the classical complement-dependent cytotoxicity method. Patient sera were tested with and without dithiothreitol according to the European Federation for Immunogenetics standards. XMs turning negative after dithiothreitol treatment were considered negative for further analysis.

FXM was retrospectively performed for all patients in one center (GE) with a two-color fluorescence technique as described previously (22, 23). Cell staining was analyzed using FACScanibur and Cell Quest softwares (BD PharMingen, San Jose, CA). T- and B-cell flow XM (T-FXM and B-FXM, respectively; 1024-channel log scale) were reported positive, if median channel shifts with a value of more than 3 standard deviations of control serum values were observed.

Luminex Crossmatch
Donor lymphocytes (30 x 10^6) were lysed with lysis buffer according to the manufacturer’s instruction (LIFECODES DSA, Tepnel Lifecodes). Eight microtiter plates of donor lysates were incubated for 30 min with 5 μl of anti-HLA class I- or II-specific beads, which capture donor HLA antigens. Fifty-five microliters of these donor HLA antigen-loaded beads were added to 96-well plates and washed three times. Patient sera were cleaned with SeraClean (Tepnel Lifecodes) before incubation for 30 min with donor HLA antigen-loaded beads. Revelation was performed with 5 μl of anti-human phycoerythrin-conjugated IgG. Detection of the patient anti-HLA IgG Abs and result interpretation were performed using LifeMatch software (Tepnel Lifecodes) on the LABScan100 flow cytometer (Luminex). Positivity was defined according to the manufacturer’s rules: the raw MFI value of each capture bead is compared with three cutoff values (background adjustment factors [BAFs]). The three BAF values are calculated from the background measured on the three negative control beads (CON) in each test well. Each CON has a separate and lot-specific equation for calculating the BAF values. The BAF calculated for a CON is subtracted from the raw MFI value of the capture bead. The process is repeated for each of the remaining two CONs to obtain three adjusted MFI values. A sample is considered to be positive for DSA if at least two adjusted MFI values are positive.

Single-Antigen Bead Assay
To identify the specificity of anti-HLA IgG Abs, we retrospectively performed a high-definition LABScreen Single Antigen (One Lambda) class I assay in LABScreen Mixed class I positive individuals and a class II assay in LABScreen Mixed class II positive individuals (7, 24). Twenty microliters of
serum were added to 5 μl of class I panel (LS1A01, LAT1-HD) or class II panel (LS2A01, LAT2-HD) microbeads, and the mixture was processed according to the manufacturer’s instructions. Result interpretation was performed using LABScan 100 software (OneLambda) on the LABScan 100 flow cytometer (Luminex). A positive result was defined as more than 500 MFI, which was calculated as (fluorescence of beads coated with HLA and incubated with patient serum) – (fluorescence of beads without HLA and incubated with patient serum) – (fluorescence of beads with and without HLA and incubated with negative control serum).

**Outcome Parameters**

The primary outcome parameter was AMR during the first year posttransplant. All clinically suspected rejections were confirmed by needle core biopsy. Biopsy specimens were evaluated by light microscopy and immunofluorescence including C4d staining. The histologic classification followed the Banff ’97 classification with its updates (25, 26). One case with moderate transplant glomerulitis and peritubular capillaritis without C4d positivity was also assumed as AMR.

Secondary outcome parameters were the incidence of T-cell–mediated rejections (TMR) and allograft function, measured as absolute and relative decline of the creatinine clearance (CrCl) according to Cockcroft-Gault (27). The decline was defined as the difference between the best graft function in the outpatient setting during the first 3 months posttransplant and the graft function 1 year posttransplant.

**Statistics**

For comparison of continuous variables, Student’s t test were used as appropriate. Categorical data and differences among groups. Nineteen patients received any Ab induction (nine Thymoglobuline, nine basiliximab, and one rituximab). The DSA + group received more Ab induction compared with the DSA − group (14 vs. 5 patients, Table 1).

No difference was observed with regards to the type of Ab induction between the DSA ± and AMR ± groups.

Of the 37 sensitized living donor kidney recipients, 20 had DSA (nine against class I only, seven against class II only, and four against classes I and II). Of all patients, 18 (49%) had at least one rejection episode during the first year posttransplant, and 22% had at least one AMR episode during the first year posttransplant.

**RESULTS**

**Patient Characteristics, Type, and Frequency of Rejections**

Patient characteristics are given in Table 1. Diagnoses leading to need of transplantation were glomerulonephritis (27% patients), pyelonephritis or interstitial nephritis (24%), polycystic kidney disease (16%), vascular and hypertensive nephropathy (5%), diabetic nephropathy (5%), and “other” (22%). There were no differences between the DSA ± and AMR ± groups. Nineteen patients received any Ab induction (nine Thymoglobuline, nine basiliximab, and one rituximab). The DSA + group received more Ab induction compared with the DSA − group (14 vs. 5 patients, Table 1).

Patient characteristics are given in Table 1. Diagnoses leading to need of transplantation were glomerulonephritis (27% patients), pyelonephritis or interstitial nephritis (24%), polycystic kidney disease (16%), vascular and hypertensive nephropathy (5%), diabetic nephropathy (5%), and “other” (22%). There were no differences between the DSA ± and AMR ± groups. Nineteen patients received any Ab induction (nine Thymoglobuline, nine basiliximab, and one rituximab). The DSA + group received more Ab induction compared with the DSA − group (14 vs. 5 patients, Table 1). No difference was observed with regards to the type of Ab induction between the DSA ± and AMR ± groups.

**Table 1. General characteristics of all patients, those with and without AMR and with and without DSA**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=37)</th>
<th>No AMR (n=29)</th>
<th>AMR (n=8)</th>
<th>P</th>
<th>DSA (n=17)</th>
<th>DSA+ (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age (yr), mean (SD)</td>
<td>38 (±17)</td>
<td>37 (±19)</td>
<td>40 (±9)</td>
<td>0.738</td>
<td>35 (±17)</td>
<td>40 (±17)</td>
<td>0.392</td>
</tr>
<tr>
<td>Male sex</td>
<td>21 (57%)</td>
<td>16 (55%)</td>
<td>5 (62%)</td>
<td>0.806</td>
<td>9 (53%)</td>
<td>12 (60%)</td>
<td>0.746</td>
</tr>
<tr>
<td>Donor age (yr), mean (SD)</td>
<td>49 (±9)</td>
<td>50 (±10)</td>
<td>46 (±6)</td>
<td>0.246</td>
<td>50 (±10)</td>
<td>48 (±9)</td>
<td>0.498</td>
</tr>
<tr>
<td>No. transplants, n (%)</td>
<td>24 (65)</td>
<td>21 (72)</td>
<td>3 (38)</td>
<td>0.100</td>
<td>14 (82)</td>
<td>10 (50)</td>
<td>0.082</td>
</tr>
<tr>
<td>HLA mismatch (%)</td>
<td>13/66/21</td>
<td>14/65/21</td>
<td>11/67/22</td>
<td>0.447</td>
<td>18/70/12</td>
<td>5/65/30</td>
<td>0.177</td>
</tr>
<tr>
<td>A: 0/1/2</td>
<td>8/50/42</td>
<td>10/48/42</td>
<td>0/56/44</td>
<td>0.510</td>
<td>6/70/12</td>
<td>10/30/60</td>
<td>0.110</td>
</tr>
<tr>
<td>B: 0/1/2</td>
<td>24/50/26</td>
<td>24/48/28</td>
<td>22/56/22</td>
<td>0.905</td>
<td>35/47/18</td>
<td>15/50/35</td>
<td>0.149</td>
</tr>
<tr>
<td>CR (mL/min) at 1 yr, mean (SD)</td>
<td>64 (±19)</td>
<td>64 (±20)</td>
<td>64 (±14)</td>
<td>0.042</td>
<td>66 (±21)</td>
<td>63 (±17)</td>
<td>0.607</td>
</tr>
</tbody>
</table>

**Table 1. General characteristics of all patients, those with and without AMR and with and without DSA**

*Any antibody induction therapy (rituximab or basiliximab or Thymoglobuline).

AMR, antibody-mediated rejection; DSA, donor-specific anti-human leukocyte antigen antibody; SD, standard deviation; CrCl, creatinine clearance; IS, immunosuppression; CyA, cyclosporine A; MPA, mycophenolate.
Frequency of AMR and TMR according to anti-HLA antibody and DSA status

<table>
<thead>
<tr>
<th>Rejection type</th>
<th>Anti-HLA ab− “a” (n=118)</th>
<th>Anti-HLA ab+ “a” (n=37)</th>
<th>P</th>
<th>DSA− “a” (n=17)</th>
<th>DSA+ “a” (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMR</td>
<td>0</td>
<td>8 (22%)</td>
<td>&lt;0.001</td>
<td>1 (6%)</td>
<td>7 (35%)</td>
<td>0.048</td>
</tr>
<tr>
<td>TMR</td>
<td>24 (20%)</td>
<td>15 (40%)</td>
<td>0.018</td>
<td>7 (41%)</td>
<td>8 (40%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

“a” Screened with LABScreen Mixed assay; our study population consisted only of the 37 screen positive patients; but for comparative purposes, the results for the screen negative group are also listed here.

AMR, antibody-mediated rejection; TMR, T-cell-mediated rejection; HLA, human leukocyte antigen; DSA, donor-specific anti-human leukocyte antigen antibody; ab, antibody.

Receiver operator characteristic (ROC) curve analysis for prediction of antibody-mediated rejection (AMR) by single-antigen bead (SAB). The ROC curve for the predictability of AMR by SAB using varying cutoff levels based on mean fluorescence intensity (MFI) shows a significant result only for donor-specific antihuman leukocyte antibody (DSA) against class I (DSA-I; area under the curve 0.828, \( P = 0.005 \)), whereas the result for DSA against class II (DSA-II) was not significant. The circle marks the point on the curve, where sensitivity and specificity are equally weighted and corresponds to a DSA value of 900 MFI. (DSA-I: n=13; DSA-II: n=11).

Two patients had two episodes. Eight patients had AMR (44% of the patients with rejection; 22% of all patients), of which two of them were hyperacute and three were chronic. Only one patient had an isolated chronic AMR, and in two other patients, chronic AMR was accompanied by acute AMR and TMR. Fifteen patients had acute TMR, of which five of them had AMR at the same time. Only 7 of 37 patients had a time-of-transplant PRA class I value more than 0% (3%, 7%, 27%, 63%, 70%, 82%, and 93%), and two of them had AMR (the patient with 27% PRA had hyperacute AMR, and the patient with 70% PRA had acute and chronic AMR and acute TMR).

AMR and DSA Analysis by SAB

The incidence of AMR was significantly higher in patients with DSA (7 of 20; 35%) than in patients without DSA (1 of 17; 6%; \( P = 0.048 \), Table 2). Thus, in terms of the predictability of AMR, just the presence or absence of DSA measured by SAB reached a sensitivity of 87.5% and a specificity of 55.2%. In our population, it results in a PPV of 35.0% and a NPV of 94.1%. TMR was not predicted by any humoral sensitization test. Interestingly, during the first year posttransplant, none of the 118 recipients without anti-HLA Abs experienced AMR, and the rate of TMR was much lower (Table 2).

The ROC curve analysis for prediction of AMR by SAB yielded a significant result only for DSA against class I (DSA-I), whereas the result for DSA against class II (DSA-II) was not significant (Fig. 2). Therefore, we evaluated DSA-I separately. For every patient, only the DSA with the highest MFI was used to compute sensitivity and specificity. The cumulative MFI values for all DSA-I or DSA-I and DSA-II yielded no better results (data not shown). Table 3 summarizes sensitivity, specificity, PPV, and NPV of DSA-I at different levels in terms of AMR. If sensitivity and specificity are equally weighted, the cutoff value has to be set at 900 MFI, as revealed by ROC curve analysis. Further cutoff values are 500 MFI (i.e., whether DSA are present or not) and 5200 MFI, where specificity reaches 100%.

We observed only one AMR positive for DSA-II but negative for DSA-I. This was a chronic AMR combined with acute AMR and Banff IIa TMR. The patient’s serum contained a donor-specific anti-DR1 Ab (672 MFI). The one patient with isolated chronic AMR revealed a high-level anti-DQ2 Ab (14,759 MFI) together with an anti-A11 Ab (1022 MFI), both of which were donor-specific.

Three patients had graft loss. One patient (with DSA-II only and no rejection episode) lost his graft after 7 months because of renal vein thrombosis. The other two patients had hyperacute AMR within 24 hr after reperfusion and had pretransplant DSA as follows (MFI in brackets): patient 1 showed activity against A2 (9795), A24 (9420), and B51 (5296); patient 2 against B44 (12791), A1 (12651), DR7 (12927), DR17 (5828), and DQ2 (2901).

Binary logistic regression analysis revealed that only the presence of DSA was an independent risk factor for AMR, but not the recipient’s age, sex, number of transplants, and HLA mismatches (data not shown).

Luminex Crossmatch

In a next step, we compared SAB results with three different XM methods. The correlation between several DSA levels (classes I and II) and the three generations of XMs are presented in Table 4. Then, the prediction of AMR was determined with regards to different XMs alone and in combination with DSA class I (Table 5). Of special interest, there was the newly developed XM with Luminex technique (LXM) because it uses the same bead-based fluorescent technique similar to the SAB assay. The LXM for class I (LXM-I) achieved the highest values for both test characteristics and signifi-
and PPV increased to 100% (by trend, the sensitivity was higher and the specificity was lower than when only DSA class I are considered. Values are presented n (%). DSA, donor-specific anti-human leukocyte antigen antibody; MFI, mean fluorescence intensity; T-CXM, T-cell complement-dependent cytotoxicity crossmatch; B-CXM, B-cell complement-dependent cytotoxicity crossmatch; T-FXM, T-cell flow crossmatch; B-FXM, B-cell flow crossmatch; LXM-I, Luminex crossmatch for class I; LXM-II, Luminex crossmatch for class II.

**TABLE 3.** Test characteristics for prediction of AMR by SAB-derived DSA at different strengths (n=37)

<table>
<thead>
<tr>
<th>DSA class I (MFI)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥500</td>
<td>75</td>
<td>76</td>
<td>46</td>
<td>92</td>
<td>76</td>
<td>0.013</td>
</tr>
<tr>
<td>≥900</td>
<td>75</td>
<td>90</td>
<td>67</td>
<td>93</td>
<td>86</td>
<td>0.001</td>
</tr>
<tr>
<td>≥5200</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>88</td>
<td>89</td>
<td>0.001</td>
</tr>
</tbody>
</table>

AMR, antibody-mediated rejection; SAB, single-antigen bead; DSA, donor-specific anti-human leukocyte antigen antibody; MFI, mean fluorescence intensity; PPV, positive predictive value; NPV, negative predictive value.

**TABLE 4.** DSA class I/II levels and three generations of crossmatches in the 37 recipients with a positive screening assay and a negative T-CXM

<table>
<thead>
<tr>
<th>DSA (MFI)</th>
<th>B-CXM</th>
<th>T-FXM</th>
<th>B-FXM</th>
<th>LXM I</th>
<th>LXM II</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSA 5000</td>
<td>5200</td>
<td>5000</td>
<td>5000</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>B-CXM</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>T-FXM</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>B-FXM</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>LXM I</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>LXM II</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Here DSA classes I and II are given, in contrast to tables 3 and 5, where only DSA class I are considered. Values are presented n (%).

FXM and CXM

The FXM achieved no significant test results in our study. By trend, the sensitivity was higher and the specificity was lower compared with B-CXM (Table 5). The combination of FXM and DSA-I with a cutoff value of 900 MFI was of borderline significance, but predictability of AMR was weak compared with the other two XMs combined with DSA values.

In 7 of 37 patients, the transplant was performed with a positive B-CXM. Four of these patients had DSA-I or DSA-I and -II, which led to hyperacute AMR in two cases and to chronic AMR in one case. By trend, the B-CXM had a low sensitivity of 38% but a high specificity of 86%. In combination with DSA-I more than or equal to 900 MFI, specificity and PPV increased to 100% (P=0.007), that is, all three patients having this combination experienced AMR, and two of them experienced hyperacute AMR.

**Graft Function**

The mean CrCl decline after 1 year was 11 mL/min in all patients, 24 mL/min (P=0.027) in patients with AMR, 13 mL/min in patients with TMR, and 7 mL/min in patients without any rejection episode. Patients with DSA-I more than 500 MFI had a CrCl decline of 15 mL/min, patients with DSA-II more than 500 MFI had a CrCl decline of 12 mL/min, and patients with DSA-I more than or equal to 900 MFI had a significant stronger CrCl decline of 21 mL/min (P=0.025).

The three cases with graft loss because of hyperacute AMR and renal vein thrombosis were excluded from CrCl decline analysis.

**DISCUSSION**

AMR because of preformed DSA is responsible for a large proportion of renal allograft losses and above average CrCl decline within the first year posttransplant (17, 18, 29–31). Thus, in this study, we analyzed the outcomes of 37 HLA-sensitized living donor kidney recipients to define the predictability of AMR by cell-based assays and by Luminex SPAs performed with day-of-transplant sera. The main findings were as follows: (1) pretransplant DSA-I, but not DSA-II, are predictive for AMR; (2) with increasing strength of DSA-I, sensitivity is decreasing and specificity is increasing; (3) LXM-I, but not LXM-II, provides a higher accuracy than FXM and B-CXM (in the context of a negative T-CXM), and the specificity of all XMs is greatly increased in combination with DSA-I values above a particular cutoff.

We would like to stress that these MFI values are only valid for LABScreen Single Antigen assays (OneLambda) measured on a LABScan100 flow cytometer platform. In the recent Australian National Association of Testing Authorities quality assurance program, the performances of SAB assays were compared among laboratories and between the two vendors. There was a good correlation of the MFI values among laboratories using products from the same vendor. However, when comparing the test parameters between the two vendors, there were important differences in MFI (32). Furthermore, PPV and NPV are only valid for our HLA-sensitized population.

In the past 2 years, several studies investigated the problem of low-strength pretransplant DSA detected by SAB. The results are conflicting. In one study of 64 living donor kidney recipients with 12 DSA-positive patients, no difference in rejection and graft function was observed (16). Gupta et al. (15) found DSA not to be relevant for rejection, but for long-term graft failure in CXM-negative patients. However, AMR and TMR were not differentiated, and there was no accurate information about DSA levels. Nevertheless, a trend for more rejections in the DSA-positive group was described (32% vs. 23%). Several authors found that DSA went along with an increased risk for AMR but not for long-term graft function (19, 20, 33), and others found DSA to be relevant for both AMR and graft failure (17, 18, 30, 31). Unfortunately, there are no standardized populations. In most studies, DSA detection by SPA was performed in the context of a negative T- and B-CXM (18–20) or even a negative T- and B-FXM (33), but in some studies, a positive B-CXM was accepted (30, 31). In addition to us, one other group found a positive correlation.
between the strength of pretransplant peak DSA and AMR so far. In the study by Lefaucheur et al. (17), including only day-of-transplant CXM-negative patients, 35% of patients with pretransplant DSA experienced AMR, which is similar to our study. The risk for AMR was significantly increased in patients with strongly positive (semiquantitative) peak DSA compared with those with weakly positive peak DSA. The difficulty to draw definitive conclusions came from the absence of standardization of the studies with regard to immunosuppressive therapy and definition of rejection. We believe that the current evidence from the literature argues for a certain degree of relevance of low-strength pretransplant DSA for AMR and presumably also for graft failure. However, we also agree that the CXM remains an invaluable tool in clinical decision making (34).

With our cohort of HLA-sensitized patients, we were able to establish a clear relationship between day-of-transplant DSA-I strength and the probability of AMR. By ROC curve analysis, two MFI cutoff values were defined, which provide highest accuracy. The DSA-I cutoff at 900 MFI provides highest equally weighted sensitivity and specificity, whereas the cutoff at 5200 MFI leads to a specificity and, therefore, a PPV of 100%. We advise special caution for MFI values more than 10,000; both patients in our study having such values have rejected hyperacutely.

It might well be that inclusion of B-CXM positive patients in our study and historic B-CXM positive patients in the study by Lefaucheur et al. have a stake in gaining significance results. However, even when performing the analysis without the seven B-CXM positive patients, the results of AMR prediction by DSA-I with more than or equal to 900 MFI were still significant, as were the results of the LXM.

The 7 patients with DSA-II only and the 11 patients with DSA-II or DSA-I and II did not show a higher risk for AMR than patients without DSA-II. These findings are consistent with the results reported by Ho et al., (31) where patients with DSA-II only showed no increased AMR rate or decreased graft survival, and by Eng et al., (30) where graft survival in B-CXM positive patients with DSA-I only was significantly poorer than in patients with DSA-II only and in patients with a negative B-CXM. Other studies reported that DSA-II were correlated with the development of chronic rejection such as transplant glomerulopathy (35–37). Because of the small number of DSA-II-positive patients, the short follow-up time, and the lack of protocol biopsies, our study failed to show such an effect. The clinical relevance of DSA-II may be related to the HLA-locus and expression level of HLA class II antigens in kidney endothelium, and the latter is in turn dependent from the inflammation status (38, 39). Thus, when determining the risk associated with DSA-II, it could be useful to take into consideration the specificity and strength of DSA-II and the inflammation status of the patient.

The LXM assay uses “capture beads,” which are coated with monoclonal Abs targeted against a fragment shared by almost all HLA antigens. Beads with captured HLA molecules from lyzed donor cells are incubated with patient serum. Because LXM uses the same technology as SAB, it could be a more relevant XM compared with CXM and FXM, when correlation with SAB is performed. Billen et al. (9) demonstrated the validity of the LXM-I (HLA A, B, and C), but not for LXM-II, because most of the HLA-DQ and -DP molecules were not captured by the LXM beads, as also attested by the manufacturer. LXM has several advantages compared with CXM in that (1) no viable donor lymphocytes are necessary and lysate can be stored at minus 80°C for up to 6 months, which is particularly useful for posttransplant monitoring; (2) it only detects DSA of IgG isotype, so it prevents false-positive results because of irrelevant Abs; (3) a clear differentiation between class I and II is guaranteed; and (4) it may detect Abs against rare donor HLA antigens, because the sensitivity does not rely on a bead panel. Unlike CXM, LXM also detects noncomplement-binding Abs but misses non-HLA Abs.

Our study tested for the first time the association between LXM and AMR. We found for LXM-I a sensitivity of 57% and a specificity of 85%. Hence, the specificity is in the same range as the B-CXM and T-FXM, but the sensitivity is higher. In contrast, the LXM-II was not predictive for AMR, similar to that in the studies of Billen et al. (9, 10). Until now FXM was the most sensitive technique, but despite some centers strongly support a broad use of FXM (29), the complexity of the test and a persistent debate about the relevance of positive FXM for graft survival has prevented its larger utilization (40, 41). We did not use pronase in our FXMs, which has been demonstrated to increase the quality of test results (42);
therefore, the interpretation of B-FXM results should be done with caution.

A great advancement is achieved by combining the LXM and the SAB results because of reduction of false-positive results. Patients with a positive LXM-I and DSA-I alone at high strength (>900 MFI experienced AMR with a PPV of 80% by a test specificity of 96%. This is important to avoid inappropriate exclusion of patients from transplantation. Consistent with our results, one recent study showed that 45% of patients with DSA did not experience clinical or subclinical AMR and had no reduced 5-year graft survival (18), emphasizing the clinical importance of a high PPV. Thus, DSA-I at low strength (>900 MFI) in combination with a positive LXM-I or DSA-I alone at high strength (>5200 MFI) are the best predictors of AMR.

To the strengths of our study belong that (1) confounding induction therapies were distributed equally in patients with and without AMR, although more DSA-positive patients received Ab induction therapy, making our results even stronger (Table 1); (2) we included only living donor kidney recipients with a short ischemia time, thereby reducing the possible influence of different inflammation levels on the HLA expression and therewith the probability of early AMR (38, 39, 43, 44); (3) we examined both classes of DSA separately; and (4) we included in the analysis DSA levels in addition to three different XM techniques. However, our study also has shortcomings, because we included relatively few patients, who were followed up only for 1 year, and we only investigated DSA against HLA-A, B, -DR, and -DQ, but not against -C and -DP.

In conclusion, we believe that the best prediction of AMR and consecutively graft function in a T-CXM-negative population is delivered by the SAB test alone or by SAB in combination with LXM or B-CXM. Both the SAB test and the LXM yield only significant results for anti-HLA class I Abs. The decision to transplant or not should not be based solely on a binary XM result, but the clinician should use SPAs, which provide greater sensitivity for detecting DSA and a semiquantitative analysis to enhance the interpretation of XM results. These tests together with patient’s clinical data must be integrated into the decision algorithms for performing a given transplant or not and for guiding immunosuppressive treatment and strategies for desensitization.

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REFERENCES


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4. HOW TO OVERCOME ANTI-HLA IMMUNIZATION IN LIVING KIDNEY TRANSPLANTATION

a. Background
Allowing sensitized patients against their willing living donor to undergo a safely kidney transplantation is only possible through kidney paired donation (KPD). Also known as paired kidney exchange, crossover transplantation or closed-loop kidney swaps, this procedure can overcome immunological barriers and resort to all potential living donors. Variations of KPD incorporate compatible pairs and non-directed donors (NDDs) to start a chain of compatible transplantations.

b. A brief history of kidney paired donation
Although the idea of KPD was originally proposed by Rapaport in 1986, it was not until 1991 that the first successful living donor exchange program was developed in Korea, a country largely dependent on living donation as a result of limited deceased donation. This program, despite being referred to as the Korean KPD program, is managed at a single center in Seoul and does not have the structure of a national program integrating multiple units involved with living donor kidney transplantation. Single center programs have also been reported from several other countries including Romania, Turkey, and India. Since 2000, multiple single centers or regional KPD programs were started in the US leading to 2,095 transplantations. Networked, multi-center, national KPD registries exist in The Netherlands, the UK, Canada, and Australia. Switzerland has played an important role in KPD, since the first crossover transplantation in the Western World was performed on May 23, 1999, at Basel University Hospital.

c. Conventional kidney paired donation
KPD is a strategy that helps patients finding a suitable kidney donor when their only intended living donor is unsuitable for them, due to immunological reasons. In a conventional or balanced crossover procedure, 2 incompatible pairs simply exchange donors (2-way kidney donor exchange), creating 2 compatible matches (Figure 2-A). In a more complex procedure, 3 or more incompatible pairs can be matched with other incompatible pairs such that multiple compatible transplantations can be performed (3-, 4-, N-way KPD exchanges) (Figure 2-B).
**Figure 2:** Exchange strategies in kidney paired donation: A conventional 2-way loop exchange between 2 incompatible donor-recipient pairs (A) a 3-way loop exchange among 3 incompatible donor-recipient pairs (B). Multi-way loops can be arranged with 3, 4, 5 or more pairs.

In any N-way exchange, 3 prerequisites are essential: each patient must have a healthy and willing live donor, each live donor must be compatible with a recipient and all pairs must have agreed to accept indirect living kidney donation from a stranger who is a willing but incompatible donor to his intended recipient. The probability of finding the optimal number of suitable matches depends on the size of the pool of incompatible pairs and the rules and conditions built into the sophisticated matching software\(^4\text{5};4\text{9};5\text{3};5\text{5}\). Withdrawal of a donor from the exchange agreement after his original recipient has been transplanted would harm the remaining recipient on two levels: firstly, the patient would not receive the promised kidney transplant and secondly he/she would also lose their willing, though incompatible living donor as the “bargaining chip” for another alternative KPD. Thus, the only way to ensure that all recipients in
a KPD procedure will be transplanted is to perform all living donor surgical procedures simultaneously. Logistic is therefore the cornerstone of such procedures, requiring high availabilities of surgeons, anaesthetists and operating rooms.

d. Unbalanced kidney paired donation

Another variant of KPD mixing compatible and incompatible donor/recipient pairs is labelled altruistically unbalanced paired donation \(^{56-58}\). In this instance, a transplant candidate with an ABO/HLA compatible living donor may benefit from receiving a transplant either from a younger donor age \(^ {59}\) or with a better HLA match \(^{58;60}\). The latter case is particularly attractive to otherwise compatible pairs who have a high degree of HLA-mismatch, as is the case between spouses, or who are at high immunologic risk combination, as husband-to-wife. While improved HLA matching may \(^ {59}\) or may not \(^ {61}\) be associated with better long-term outcomes, selection of a better matched donor may be important for those likely to require repeat transplantation.

Unbalanced KPD was first proposed as a possible solution to help O recipients in the KDP pool \(^{56-58}\) to find a match. Indeed, as O donors will rarely enter a KPD pool, with the exception being those who have positive crossmatch with their recipient, scarce O donors are available for O recipients. In unbalanced KPD, one ABO-incompatible pair (e.g. A-donor to O-recipient) and one compatible but not identical pair (e.g. O-donor to A-recipient) could exchange (Figure 3), resulting in 2 ABO identical living donor kidney transplants.
**Figure 3:** Exchange strategies in kidney paired donation. Unbalanced N-way loop exchanges between 1 compatible and 1 incompatible donor-recipient pairs (A) or 1 compatible and 2 incompatible donor-recipient pairs (B).

However, there may be a delay of a few months in donation/transplant surgeries of HLA/ABO compatible pairs participating in a KPD program, until finding a better match. Although there are a number of challenges to overcome, this approach is consistent with accepted ethical tenets and it has been shown that inclusion of even a small number of HLA/ABO-compatible pairs in KPD program can result in substantial increase of incompatible pair match rates.  

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e. Non-directed donors chains

Incorporation of NDDs, also known as Good Samaritans or altruistic donors \(^{62,63}\) into a KPD registry can initiate a chain of transplants \(^{64,65}\). As a NDD is not associated with any indented recipient, it results in a minimum of two transplants via “domino” chains. Such chains are either closed when the last donor in the chain gives to a patient on the deceased-donor waitlist \(^{66,67}\) or opened (so called “never ending”) when the final donor, also called “bridge donor” will wait to initiate a future NDD chain (Figure 4).

**Figure 4:** Multi-way exchanges beginning with a non-directed donor (NDD) and including multiple donor-recipient pairs. The closed chain donation model ends with the final donor donating to a patient on deceased-donor wait list (A). The open chain model ends with a bridge donor that will start a new chain of transplants (B).
Allocation of NDD into a KPD registry has been shown to facilitate a much larger number of transplants \cite{62,63}, on average up to 3.5 transplants per NDD chain \cite{27,64,65}. For the NDD, donation of their kidney in a NDD chain amplifies their feelings of self-esteem and well-being \cite{61}. For these reasons, given the optimizing effect associated with NDD chains, some advocate that NDD should preferentially be allocated to KPD registry, an approach that is customary in the US \cite{66}. An important ethical issue regarding NDD participation in KPD is the diversion of NDD kidneys from unpaired highly sensitized patients on the waiting list \cite{68}. Agreeing on a pathway where a NDD is first allocated within a defined group of highly sensitized unpaired recipients on the deceased donor wait list before being included in the KPD registry, as is the case in the UK \cite{45} could help mitigate the ethical issue. The autonomy of each NDD also needs to be taken into consideration, and donors with very specific time constraints should be given the choice to donate directly to a patient on the waiting list. NDDs chains have also the advantage of facilitate transplantations of incompatible pairs who cannot be matched in conventional KPD loops, where donors cannot mutually reciprocate in a closed loop arrangement. Arranging the logistics for multiple transplant surgeries within a NDD chain is also easier, because surgeries can be arranged sequentially. The risk of voluntary donor withdrawal and chain breakdown is modest and is offset by the benefits of leveraging one NDD to enable multiple transplants \cite{64}. Unlike conventional KPD, this modest risk does not irreparably harm the transplant candidate, as he/she will still be able to enter into another match cycle, because their co-registered donor won’t have yet undergone nephrectomy. The risk of donor reneging can be minimized by limiting the waiting periods for sequential donor surgeries to a maximum of 24 hours.

f. Kidney paired donations registries

Because the match probability increases with the number of registered incompatible pairs in any given KPD pool \cite{38,53,55}, countries with a relatively small population like Switzerland, will benefit from a national KPD program, as multiple independent regional registries would not reach a critical mass of registered incompatible pairs. Worldwide there are currently four national KPD programs in The Netherlands, the UK, Canada and Australia \cite{27}, which could be used as models for a Swiss KPD registry. All have an oversight body, which is part of their national government health system or which is managed by a national organization. Matching cycles occur every 3-4 months in each of these registries, unlike other registries that use revolving, real-time computer matching \cite{41,69}. All four countries use a matching algorithm whose primary allocation criteria are based on virtual crossmatch \cite{27}. In The Netherlands and Canada, donors travel to the recipient’s center for surgery, whereas kidneys are transported between centers in the UK and Australia. All
programs endeavour to ensure that surgeries take place on the same day and that anaesthetic induction time is the same. Commonalities and differences of the multiple in the United States (US) programs have been reviewed in detail elsewhere. The undeniable success of some of the American programs has been dependent on the inclusion of compatible pairs and NDD, using bridge donors, and integrating KPD into desensitization protocols.

i. **in Australia**

The Australian KPD program is known as the Australian paired Kidney eXchange (AKX) Program and was established in 2010 following the initial experience of a regional pilot program in Western Australia. From October 2010 until August 2014, the Australian KPD program has facilitated 101 kidney transplants (91 completed, 10 awaiting surgery) among the 207 registered pairs (49% of registered KPD candidates) and 4 waitlist recipients; 89 transplants were achieved using N-way chains and 16 transplants were performed through NDDs chains (15%). This relatively high proportion of transplants was achieved despite a pool consisting primarily of highly sensitized, HLA incompatible pairs (35% of registered patient had cPRA 95-100%), compared to non-sensitized, ABO incompatible pairs. Overall transplant rates have been excellent and the proportion of patients with cPRA 50-96% being transplanted (62%) is fairly similar to the proportion of transplanted patients with cPRA 0-50% (73%), although, not surprisingly, the proportion of transplants among extremely highly sensitized candidates with cPRA ≥97% has been low (25%) (Figure 5).

**Figure 5:** Australian KPD program: patients waiting unmatched by cPRA
NDD chains have been a minor driver in the Australian program due to the low number of NDDs included. The Australian program compares favorably with the Dutch program, in which over a 10-year period 242 kidney transplants have been facilitated among the 655 registered pairs using ≥2-way chains (transplant rate 37%)\(^{27}\). It also outperforms the UK program, in which between April 2007 and 2014, 284 of the 991 registered patients (29%) proceeded to receive a kidney transplant through 2-way and 3-way loops and 36 through NDD chains (3.6%)\(^{27}\). The Canadian program active since 2009 shares many commonalities with the Australian program: its KPD pool is mainly composed of highly sensitized, HLA-incompatible pairs and the proportion of KPD transplant among registered transplant candidates is equally excellent (44%)\(^{27}\). A major driver of the success in Canadian registry is the inclusion of a large number of NDDs (n=54 at the end of 2013), facilitating 62% of the registered recipient transplants versus 38% in N-way exchanges\(^{27,46}\). Comparison between the four national programs\(^ {27}\) would suggest that match and transplant rates from N-way loops does not only rely upon incompatible pairs pool size. Indeed, transplant rate from loops is 41% in the Australian program despite inclusion of only 40 to 50 patients per match cycle, whereas transplant rates from loops were only 25% in the UK program despite inclusion of 160 to 180 patients in each allocation round. The most plausible explanation for this success is wide acceptance of ABO-incompatible matching in the Australian program\(^ {47}\). On the other hand, the power of NDDs domino chains in KPD program is clearly demonstrated by the Canadian program, where 62% of all KPD transplants were facilitated by NDD chains\(^ {27,46}\).

\textit{ii. in Switzerland}

In Switzerland, the first paired kidney exchange procedure took place at Basel University Hospital on the 23\textsuperscript{rd} May 1999 between a Swiss and a German couple\(^ {51}\). Thereafter, it was not until September 2011 that the next crossover procedure between 2 incompatible couples was carried out at Geneva University Hospitals under the leadership of Dr.Karine Hadaya. Then, the first inter-hospital paired kidney exchange was successfully completed through a combined effort of the Zurich and Geneva transplant teams. After the success of the third KPD procedure, a working group was formed with representatives of all Swiss kidney transplant units with support of Swisstransplant and the Council of the Swisstransplant Foundation, convinced of the need for this program. In order implement a successful national KPD program, Dr Karine Hadaya and Dr Jean Villard (head of Transplant Immunology Unit and National Reference laboratory for Histocompatibility) went to the Dutch Transplant Foundation in Leiden, The Netherlands, to meet the leading team in Europe for kidney paired donation. Table 1 shows the key ingredients for a successful national KPD program.
Table 7: Key ingredients for a successful national KPD program

- Agreement for absolute donor criteria between the transplant centers
- Full donor evaluation before registration in a match cycle
- Simultaneous anesthetic induction time for donor surgeries
- A National Coordination Centre organizing KPD activities between transplant centers and HLA laboratories
- A computer allocation system using virtual crossmatch and ranking criteria algorithm

It is worth noting that at the time, a national protocol for ABO incompatible kidney transplantation had already been established in Switzerland with success since 2005. Because the effectiveness of a KPD program depends largely upon the pool’s size of incompatible living donors’ couples, it was important to gauge the likely referral base of HLA and ABO incompatible pairs. Thus, all 6 Swiss kidney transplant centers were surveyed with regard to their own potential incompatible pairs. The survey showed that in 2012 there were at least 38 patients with incompatibilities to their intended donor, either due to preformed DSA with positive crossmatch and/or ABO incompatibility. While this relatively small number may seem discouraging, it is worth noting that in Australia, a country with a population of 22 million, the input of new pairs per match cycle in the last 12 months has averaged 14 added each time on a pool of 32-38 existing pairs. Thus, the projected enrolment of at least 38 incompatible pairs in Switzerland, a country of 7 million inhabitants, was thought adequate to warrant the establishment of a national KPD program. Discussions with the Federal Public Health Office to implement a national platform in accordance to existing Swiss Transplantation Law to allow an effective Swiss national KPD program are currently underway.

While a formal national KPD registry is yet to be finalized, exchange of information between Geneva University Hospitals and Zurich University Hospital transplant teams on incompatible pairs has led to 13 crossover transplantations: two 2-way and three 3-way loop exchanges, between September 2011 and October 2013.
Figures 6A to 6E: All crossover procedures performed in Switzerland

6A

Geneva, 09/2011

Receivers

Mrs 0
blood group A

ABOi, rebound antibodies

Donors

Mr 0
blood group B

ABOi, refusing long term corticosteroids

Mrs 0
blood group A

ABO identical

Mr 0
blood group B

Mrs B
blood group B

Mr B
blood group A

No DSA
XM CDC -
XM Facs -

Mr B
blood group A

No DSA
XM CDC -
XM Facs -
Between Zurich and Geneva, 2011

Mrs S
blood group B

Mr S
blood group A

1 DSA, anti-B MFI 1'97
XM CDC B+
XM Facs B+

Mrs Sch
blood group A

Mrs Sch
blood group B

3 DSA, anti-DR and anti-DQ;
MFI 2'132, 2'714, 1'011
XM CDC -
XM Luminex B+

Thymoglobuline induction

Mrs S
blood group B

Mrs Sch
blood group A

ABO identical

Mr S
blood group A

Mrs Sch
blood group B

1 DSA, low MFI 1'011
XM CDC -
XM Facs -

No DSA
XM CDC -
XM Facs -
Between Zurich and Geneva, 2012

Mr C  
blood group 0

Mrs C  
blood group 0

Mr G  
blood group B

1 DSA  
high MFI

2 DSA  
MFI 12'889, 10'870

4 DSA  
MFI 2'000-6'000

Mrs B  
blood group 0

Mr S  
blood group 0

Mrs P  
blood group 0

XM CDC  
+

XM Facs  
+

Desensitization protocols

Mr C  
blood group 0

Mrs C  
blood group O

Mr G  
blood group B

1 DSA MFI 2'574

3 DSA  
(MFI 8'767, 1'295, 6'567)

Mrs B  
blood group 0

Mr S  
blood group 0

Mrs P  
blood group 0

XM CDC  
-

XM Facs  
-
Between Zurich and Geneva, 2012

Mrs. K
blood group A

Mr T
blood group 0

Mrs d’A
blood group A

4 DSA
MFI 960-3’104

no DSA
ABOi

2 DSA
MFI 9663, 6’121

Mr M
blood group A

Mrs T
blood group A

Mr d’A
blood group 0

XM CDC  +  -  +

Mrs K
blood group A

Mr T
blood group 0

Mrs d’A
blood group 0

no DSA
ABO identical

no DSA

Mrs B
blood group 0

Mr S
blood group 0

Mr d’A
blood group 0

XM CDC  -  -  -
Matching were identified manually using a virtual crossmatch approach that takes into consideration preformed anti-HLA antibodies and donor and recipient blood groups. Immunological suitability of identified matches was confirmed by cell-based crossmatches performed at the
Swiss National Reference Laboratory in Geneva. All donations and all transplantations took place on the same day at the same anaesthetic induction time. All donors travelled to the recipient’s center for their operations. Choice was given to the new donors-recipient’s pairs to meet: 10 over 13 pairs met before surgery. Reasons for crossover procedures are shown in Figure 4, KPD allowed in all cases to overcome the immunological barrier (positive crossmatch and/or ABO incompatibility) each recipient had with his intended donor.

**Figure 7:** Immunological incompatibilities of 13 living donors pairs in Switzerland

One year graft and patient survivals are 100%. Six and 12 month post surgeries’ psychological evaluation of 12 donors and recipients native from Geneva reported no regret. All matched pairs that met are still in touch.

g. Special considerations

i. Allocation algorithms in kidney paired donation

The ability of KPD to match incompatible pairs depends upon the pool size\textsuperscript{38,55}, the ratio of ABO-incompatible to HLA-incompatible pairs, the level of sensitization of transplant candidates and the algorithm-specific allocation rules. Using N-way exchanges, the match probability for ABO-incompatible or sensitized non-O recipients is around 60%, but can be as low as 20% for ABO-incompatible O recipients\textsuperscript{74}. This observation has been used as an argument to preferentially match scarce O donors in KPD registry to O recipients in the interest of fairness\textsuperscript{53,75}. Because many ABO-incompatible pairs may not accept to participate in KPD program to undergo live donor kidney transplantation\textsuperscript{9,76}, an important source of unsensitized recipients is removed
from KPD pool, leading to a high proportion of highly sensitized pairs. When the number of pairs referred for KPD because of HLA incompatibility outnumbers the pairs with ABO incompatibility the match probability decreases. A strategy to minimize this problem would be to offer ABO incompatible pairs to option to be entered first in the KPD registry with the aim of improving HLA matching and to resort to directed ABO-incompatible transplantation if no suitable match is identified within an agreed number of match cycles. This policy is already adopted by the Netherlands.

The key ingredient of any KPD program is the matching algorithm selecting pairs within the pool. The ideal algorithm should identify the maximum number of possible transplants, while minimizing the probability of unexpected post-match positive cell-based crossmatches and simultaneously promoting high quality exchanges. These difficult goals require sophisticated KPD software that takes into account 2 critical elements of priority for matching: blood group matching and negative crossmatch. Virtual crossmatch approach is widely accepted to allocate suitable donors in the pool to registered transplant candidates, although the extent of HLA-antigens included in this virtual crossmatch algorithm varies between registries. The Australian KPD program uses the computer platform of the National Organ Matching System (NOMS), which is also responsible for deceased donor organ allocation, with a purpose-built KPD allocation module. The NOMS computer program matches each recipient with any donors using a 2-step process: 1) ABO-compatibility or acceptable donors matching (in case of approved ABO-incompatibility matching) and 2) HLA virtual crossmatches among these ABO-acceptable donors and recipients. Next, the NOMS program generates ≥2-way exchanges using 6 ranking criteria: 1) prioritizing combinations that maximize the number of potential transplants; 2) selecting matches for recipients with low match probabilities (high cPRA); 3) maximizing the number of ABO identical pairs, giving O-to-O pairs priority; 4) minimizing the number of simultaneous transplants within a chain in a single hospital; 5) maximizing the number of short chains; and 6) promoting matches for patients with longer waiting times.

Despite the relatively small pool of incompatible pairs, the high proportion of sensitized patients and the small number of NDDs, the Australian program has been able to facilitate kidney transplantation in 49% of registered patients.

ii. Highly sensitized recipients: integration of desensitization and ABOi matching

Despite desensitization protocols or inclusion in a KPD registry, highly sensitized patients with cPRA ≥ 97% remain difficult to undergo living kidney transplantation. In the Australian program,
50% of unmatched pairs still waiting in the registry have a cPRA of 99-100%. It is obvious that for these patients either strategy alone will not be able to find a suitable crossmatch-negative donor without any detectable DSA. Several strategies to help these highly sensitized patients include reducing constraints in the matching algorithm such as allowing ABO-incompatible matching, expanding the KPD pool size through international collaboration, including all NDDs and large numbers of compatible pairs and allowing DSA positive KPD transplants in order to let them undergo acceptable, albeit not ideal kidney transplantation. While traditionally most KPD programs use virtual crossmatch criteria to identify fully compatible matches that will avoid even low-strength DSA, some KPD programs have already successfully explored the option of a hybrid strategy combining KPD with low immunologic risk desensitization. With this strategy, although KPD recipients would have a positive virtual crossmatch based on the DSA identified by SAB testing, they can be safely transplanted in most instances in the presence of a negative CDC crossmatch and in many cases even a negative flow cytometric crossmatch. This hybrid strategy used quite extensively at Johns Hopkins Hospital in Baltimore and in a few cases in Australia, was proving effective in transplanting patients who are both hard to match and difficult to desensitize.

### iii. Combination with ABO-incompatible transplantation

Given the excellent outcomes of ABO-incompatible transplantation in the absence of DSA, allowing ABO-incompatible matching results in a “virtual” expansion of the donor pool and increases the match probability for sensitized patients. This strategy has already been applied successfully in the Australian program and has also been adopted, though not widely implemented in the UK program. In the Australian program, each participating centre may register recipients for ABO-incompatible matching based on his expertise in managing ABO-incompatible transplantation and if the recipient’s baseline anti-ABO titers are 1:64 or less by column agglutination technology (Ortho method). Moreover, non-A1 blood group donors are treated as O donors in recipients with low anti-A titers. Of the 115 incompatible registered pairs, an ABO-incompatible donor was accepted for 36 patients with DSA against their original donor, 54% with a cPRA of more than 75% and 36% of more than 90%. Ten recipients found a suitable match and were successfully transplanted. Thanks to incorporation of ABO incompatibility, these 10 recipients were distributed across 2-way (N= 3) and 3-way (N= 5) exchanges, resulting in 21 transplantations.

### iv. Legal framework

Successful kidney transplantation was first reported when a patient with kidney failure received
a kidney from his identical homozygous twin in 1954. Living donor kidney transplantation remained restricted to haploidentical siblings and first degree related donors until introduction of potent immunosuppressive drugs in the mid-80s. Since then, transplantation laws and relationships between living donors and recipients had evolved in line with developments in biology and pharmacotherapy. In directed living donor kidney transplantation, a genetically or emotionally related donor knows the recipient beforehand. When considering a KPD program, which is an expansion of conventional living kidney donation, it is important to be aware of possible legislative barriers and commitment of politicians in changing their national organ transplant act. The fact that the donor and the recipient of the matched pair are strangers to one another could hurdle KPD program establishment. In theory, a KPD could be considered an arrangement akin to a bilateral contract, where a donor would agree to donate a kidney to a stranger, if his/her co-registered recipient would receive a kidney in return; this is generally known as ‘valuable consideration’. In the US, the National Organ Transplant Act (NOTA) of 1984 prohibited “any person to knowingly acquire, receive, or otherwise transfer any human organ for valuable consideration for use in human transplantation.” Although the concept of “valuable consideration” applies to monetary value, an exchange of organs was also considered valuable, as ‘one pair is paying with a kidney in order to receive a kidney”. In 2007, a bill was passed both in the House and in the Senate that amends NOTA to clarify that “kidney exchange shall not be considered to involve the transfer of a human organ for valuable consideration”. In Australia, there is no Federal Legislation on Organ and Tissue Transplants and each state has their own Transplant Act. All states Transplant Act have a prohibition against trading in human tissues, the majority include a clause mandating that "...a person must not enter into, or offer to enter into, a contract or arrangement under which any person agrees, for valuable consideration, whether given or to be given to any such person or to any other person...", which basically translates in a prohibition on live donor kidney exchange. However, all Transplant Acts have a special provision for the Minister for Health to grant exemption to allow KPD to occur. To date, a ministerial exemption is required for each pair participating in the program in all states except Victoria and Queensland. Like most human tissue and transplant acts in developed countries, the Swiss Transplant Law on human organs, tissues and cells, regulates the prohibition on organ trading and similar valuable consideration that could be perceived as trading of organs or tissues for human transplantation (http://www.admin.ch/opc/de/federal-gazette/2004/5453.pdf). In chapter 2, section 1, paragraph 6(d), it is outlined that crossover live donor transplantation is not prohibited as organ trading, because no financial or other gain is being associated. No detail is given regarding how a KPD program should be implemented and whether NDDs could be in-
tegrated to allow initiation of chains.

v. Donor travel or organ transport

In a regional or a national KPD program, it is well accepted that surgeries are performed in the recipient’s center but whether the donors will need to travel or the kidneys will be transported, sometimes long distances by country, is still matter of debate. In The Netherlands, donors travel for surgery but their follow up is monitored in their native center. For the donors, it could mean logistical and financial barriers, separation from the initial intended recipient and being supported by an unfamiliar team. The transport of kidneys is associated to fear of reduction in the benefit that is attributed to the short cold ischemic time (CIT). Using United Network for Organ Sharing data, a study of over 30’000 living donor kidney recipients in the US showed comparable short and long term graft outcomes for that up to 8 hours CIT\(^{85}\). A study by Segev et al. that specifically looked at 56 kidneys transported among 30 transplant centers in the context of KPD demonstrated that median 7.2 hours CIT (interquartile range 5.5-9.7) was associated with excellent early allograft function with no patients experiencing delayed graft function as defined by the need for dialysis in the first week\(^ {86}\). Furthermore, transporting the kidneys allow easier preservation of donor anonymity but prevent the recipient’s centers to refuse a donor for one reason or another. To date, no categorical recommendation can be made that would fit with all countries. In Switzerland, a country of roughly 41’200km\(^2\) and that has a maximum north-south length of 220km and an east-west length of about 350km it is easy for the donors to travel to the matched recipient’s center and related costs are reimbursed. Choice is given either to keep strict anonymity or to meet with the matched recipient. In a country like Australia, with approximately 7’700’000km\(^2\) (185 times the size of Switzerland) extending 3’200km north-south and 4’000km east-west, it was agreed that kidneys would be transported to the recipient’s center for implantation. Among the 100 KPD performed, average CIT has been 6±3 hours with the longest CIT being slightly less than 14 hours. All recipients experienced immediate graft function except for one case who required dialysis and whose CIT was 6.4 hours.

vi. Medical suitability criteria

In KPD, mandatory medical donor criteria, aside from those usual for all live donors\(^ {87}\), must be sufficiently stringent to ensure such kidney’s quality that risk of rejection a matched donor is minimal by the recipient’s center. In Australia, agreement between all transplanting units helped to establish specific guidelines for donor suitability criteria. Following identification of a matched donor, all his medical information is made available to the recipient’s center in an anonymous fashion, for reasons of transparency. To date, only 2 matched donors were thus
refused, due to dual renal arteries which were deemed risky for the recipient. These 2 donors were later on accepted for another matched recipient by another transplanting unit. Agreement on what would be the acceptable upper donor age could perhaps be difficult to reach. Data from the Australian Transplant Registry show that a donor-recipient age difference up to 30 years provide kidneys of excellent quality, with similar 5 and 10 years graft and patient survivals to recipients from organs of similar vintage ⁸⁸. These relevant findings have been implemented in KPD program where a suitable match is not limited by high donor-recipient age difference.

h. Paper

It is time to establish a funded Swiss national KPD program. This view is supported by all 6 transplantations centers, who have agreed to establish a registry, have concurred on a computer software and allocation algorithm and have settled for a central and dedicated coordination through Swisstransplant. Crossover living donor transplantation is not considered as organ trading and thus is not prohibited by the Swiss Transplantation Law. The legal framework is on hold and governmental support is required. This paper is a plea for implementing a national KDP program in Switzerland ⁹⁵.
Kidney Paired Donation: a Plea for a Swiss National Program

Invited review

(Short title: Running Title: Kidney Paired Donation)

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Abstract

Growing incidence of end-stage renal disease, shortage of kidneys from deceased donors and a better outcome for recipients of kidneys from living donor have led many centers worldwide to favour living donor kidney transplantation programs. Although criteria for living donation have greatly evolved in recent years with acceptance of related and unrelated donors, an immunological incompatibility, either due to ABO incompatibility and/or to positive crossmatch, between a living donor and his intended recipient, could impede up to 40% of such procedures. To avoid refusal of willing and healthy living donors, a number of strategies have emerged to overcome immunological incompatibilities. Kidney paired donation is the safest way for such patients to undergo kidney transplantation. Implemented with success in many countries either as national or multiple regional independent programs, it could include simple exchanges between any number of incompatible pairs, incorporate compatible pairs and non-directed anonymous donors (NDADs) to start a chain of compatible transplantations, lead to acceptance of ABO-incompatible matching, and integrate desensitizing protocols. Incorporating all variations of kidney paired donation, the Australian program has been able to facilitate kidney transplantation in 49% of registered incompatible pairs.

This review is a plea for implementing a national kidney paired donation program in Switzerland.

Key words: Living donor kidney transplantation; kidney paired donation; altruistic donation; blood group incompatibility; HLA incompatibility.
Background

Kidney transplantation is the treatment of choice for patients with end-stage renal disease; it reduces the mortality risk compared to dialysis in all age groups and improves the quality of life, offering benefits in terms of life expectancy [1, 2]. Living donor kidney transplantation is associated with superior long-term recipient and graft survivals compared to deceased kidney donors [3], possibly because of the shorter dialysis waiting-time or avoidance of dialysis [4]. The global shortage of deceased donor organs has led to increased reliance on living kidney donation programs [5]. In Switzerland, the number of kidney transplantations from living donors has exceeded that from deceased donors throughout the last decade. Unfortunately, ABO blood group incompatibility or pre-existing donor specific antibodies (DSA) to human leukocyte antigen (HLA) as a result of prior transplants, pregnancies or blood transfusions, are major barriers to living donor kidney transplantation, excluding up to 54% of otherwise appropriate pairs [6]. Different strategies have emerged to overcome these immunologic incompatibilities. ABO-incompatible kidney transplantation with long-term outcomes equivalent to ABO-compatible kidney transplantation can now be achieved using strategies that include removal of anti-blood group antibody using plasmapheresis or specific immunoabsorption, rituximab [7, 8], and long-term standard immunosuppression [9]. In contrast, HLA-incompatible transplantation in the presence of preformed DSA resulting in positive cell-based crossmatch (either by flow cytometry or complement-dependent cytotoxicity CDC) is still associated with high rates of antibody-mediated rejection, early graft loss and reduced long-term graft survival [10]. Despite desensitization strategies using a combination of plasmapheresis, intravenous immunoglobulin and T and B-cell depleting agents [11-13] in order to reverse positive crossmatches [14], up to 50% of surviving grafts show chronic active antibody-mediated rejection and premature graft failure after 5 years [10, 15]. Highly immunised patients with a panel-reactive antibody (PRA) level of 80% or more are particularly disadvantaged translating into a waiting time in excess of 10 years on the transplant waitlist. Allowing sensitized patients against their willing living donor to undergo a safely kidney transplantation is only possible through kidney paired donation (KPD). Also known as paired kidney exchange, crossover transplantation or closed-loop kidney swaps, this procedure can overcome immunological barriers and resort to all potential living donors [16-18]. Variations of KPD incorporate compatible pairs and non-directed anonymous donors (NDADs) to start a chain of compatible transplantations. In this overview we argue in favour of the establishment of a national KPD registry in Switzerland and discuss key ingredients that are critical for a successful program.
A brief history of kidney paired donation

Although the idea of KPD was originally proposed by Rapaport in 1986 [19], it was not until 1991 that the first successful living donor exchange program was developed in Korea, a country largely dependent on living donation as a result of limited deceased donation [20]. This program, despite being referred to as the Korean KPD program, is managed at a single centre in Seoul and does not have the structure of a national program integrating multiple units involved with living donor kidney transplantation [20, 21]. Single centre programs have also been reported from several other countries including Romania [22, 23] Turkey [24, 25] and India [26-28]. Since 2000, multiple single centres or regional KPD programs were started in the United States (US) leading to 2095 transplantations [29-33]. Networked, multi-centre, national KPD registries exist in The Netherlands [16, 34, 35], the United Kingdom (UK) [36], Canada [37] and Australia [38-41]. Switzerland has played an important role in KPD, since the first crossover transplantation in the Western World was performed on May 23, 1999, at Basel University Hospital [42]. One of the most successful national KPD programs, the Australian paired kidney exchange program [17, 38-41, 43] was established in August 2010 and is managed by a Swiss physician and co-author of this review.

Conventional kidney paired donation

KPD is a strategy that helps patients finding a suitable kidney donor when their only intended living donor is unsuitable for them, due to immunological reasons. In a conventional or balanced crossover procedure, 2 incompatible pairs simply exchange donors (2-way kidney donor exchange), creating 2 compatible matches (Figure 1) [19]. In a more complex procedure, 3 or more incompatible pairs can be matched with other incompatible pairs such that multiple compatible transplantations can be performed (3-, 4-, N-way KPD exchanges) (Figure 1). In any N-way exchange, 3 prerequisites are essential: each patient must have a healthy and willing live donor, each live donor must be compatible with a recipient, and all pairs must have agreed to accept indirect living kidney donation from a stranger who is a willing but incompatible donor to his intended recipient. The probability of finding the optimal number of suitable matches depends on the size of the pool of incompatible pairs and the rules and conditions built into the sophisticated matching software [36, 40, 44-46]. Withdrawal of a donor from the exchange agreement after his original recipient has been transplanted would harm the remaining recipient on two levels: firstly, the patient would not receive the promised kidney transplant and secondly he/she would also lose their willing, though incompatible living donor as the “bargaining chip” for another alternative KPD. Thus, the only way to ensure that all recipients in a KPD procedure
will be transplanted is to perform all live donor surgical procedures simultaneously. Logistics is therefore the cornerstone of such procedures requiring high availabilities of surgeons, anaesthetists and operating rooms.

**Unbalanced kidney paired donation**

Another variant of KPD mixing compatible and incompatible donor/recipient pairs is labelled altruistically unbalanced paired donation [47-49]. In this instance, a transplant candidate with an ABO/HLA compatible living donor may benefit from receiving a transplant either from a younger donor age [50] or with a better HLA match [49, 51]. The latter case is particularly attractive to otherwise compatible pairs who have a high degree of HLA-mismatch, as is the case between spouses, or who are at high immunologic risk combination, such as husband-to-wife. While improved HLA matching may or may not [52] be associated with better long-term outcomes, selection of a better matched donor is important for those likely to require repeat transplantation. Unbalanced KPD was first proposed as a possible solution to help O recipients in the KPD pool [47-49] to find a match. Indeed, as O donors will rarely enter a KPD pool, with the exception being those who have positive crossmatch with their recipient, scarce O donors are available for O recipients. In unbalanced KPD, one ABO-incompatible pair (e.g. A-donor to O-recipient) and one compatible but not identical pair (e.g. O-donor to A-recipient) could exchange (Figure 2), resulting in 2 ABO identical living donor kidney transplants. However, there may be a delay of a few months in donation/transplant surgeries of HLA/ABO compatible pairs participating in a KPD program, until they find a better match. Although there are a number of challenges to overcome, this approach is consistent with accepted ethical tenets and it has been shown that inclusion of even a small number of HLA/ABO-compatible pairs in KPD program can result in substantial increase of incompatible pair match rates [53;Bingaman, 2012 #3620].

**Non-directed anonymous donors chains**

Incorporation of NDAD also known as Good Samaritans or altruistic donors [54, 55] into a KPD registry can initiate a chain of transplants [56, 57]. As an NDAD is not associated with any indented recipient, it results in a minimum of two transplants via “domino” chains. Such chains are either closed when the last donor in the chain gives to a patient on the deceased-donor waitlist [58, 59] or opened (so called “never ending”) when the final donor, also called “bridge donor” will wait to initiate a future NDAD chain (Figure 3). Allocation of NDAD into a KPD registry has been shown to facilitate a much larger number of transplants [54, 55], on average up to 3.5 transplants per NDAD chain [18, 56, 57]. For the NDAD, donation of their kidney in an NDAD chain amplifies their feelings of self-esteem and well-being [55]. For these reasons, given the
optimising effect associated with NDAD chains, some advocate that NDAD should preferentially be allocated to KPD registry, an approach that is customary in the United States [58]. An important ethical issue regarding NDAD participation in KPD is the diversion of NDAD kidneys from highly sensitized patients on the waiting list [60]. Agreeing on a pathway where an NDAD is first allocated within a defined group of highly sensitized unpaired recipients on the deceased donor wait list before being included in the KPD registry, as is the case in the UK [36], could help mitigate the ethical issue. The autonomy of each NDAD also needs to be taken into consideration, and donors with very specific time constraints should be given the choice to donate directly to a patient on the waiting list. NDAD chains have also the advantage of facilitating transplantations of incompatible pairs who cannot be matched in conventional KPD loops, where donors cannot mutually reciprocate in a closed loop arrangement. Arranging the logistics for multiple transplant surgeries within an NDAD chain is also easier, because surgeries can be arranged sequentially. The risk of voluntary donor withdrawal and chain breakdown is modest and is offset by the benefits of leveraging one NDAD to enable multiple transplants [56]. Unlike conventional KPD, this modest risk does not irreparably harm the transplant candidate, as he/she will still be able to enter into another match cycle, because his/her co-registered donor has not yet undergone nephrectomy. The risk of donor reneging can be minimized by limiting the waiting periods for sequential donor surgeries to a maximum of 24 hours.

Kidney paired donations registries
Because the match probability increases with the number of registered incompatible pairs in any given KPD pool [29, 44, 46], countries with a relatively small population like Switzerland, will benefit from a national KPD program, as multiple independent regional registries would not reach a critical mass of registered incompatible pairs. Worldwide there are currently four national KPD programs in The Netherlands, the UK, Canada and Australia [18], which could be used as models for a Swiss KPD registry. All have an oversight body, which is part of their national government health system or which is managed by a national organisation. Matching cycles occur every 3-4 months in each of these registries, unlike other registries that use revolving, real-time computer matching [32, 61]. All four countries use a matching algorithm whose primary allocation criteria are based on virtual crossmatch [18]. In The Netherlands and Canada, donors travel to the recipient’s centre for surgery, whereas kidneys are transported between centres in the UK and Australia. All programs endeavour to ensure that surgeries take place on the same day, and that anaesthetic induction time is the same [18]. Commonalities and differences of multiple programs in the US have been reviewed in detail elsewhere [30, 33, 61-64]. The undeniable success of some of the American programs has been dependent on the
inclusion of compatible pairs [33] and NDAD, using bridge donors [64], and integrating KPD into desensitization protocols [63, 65].

Kidney paired donation in Australia
The Australian KPD program is known as the Australian paired Kidney eXchange (AKX) Program and was established in 2010 following the initial experience of a regional pilot program in Western Australia [41]. From October 2010 until August 2014, the Australian KPD program has facilitated 101 kidney transplants (91 completed, 10 awaiting surgery) among the 207 registered pairs (49% of registered KPD candidates) and 4 waitlist recipients; 89 transplants were achieved using N-way chains and 16 transplants were performed through NDAD chains (15%). This relatively high proportion of transplants was achieved despite a pool consisting primarily of highly sensitized, HLA-incompatible pairs (35% of registered patient had cPRA 95-100%), compared to non-sensitized, ABO-incompatible pairs. Overall transplant rates have been excellent, and the proportion of patients with cPRA 50-96% being transplanted (62%) is fairly similar to the proportion of transplanted patients with cPRA 0-50% (73%), although, not surprisingly, the proportion of transplants among extremely highly sensitised candidates with cPRA ≥97% has been low (25%)(Figure 4). NDAD chains have been a minor driver in the Australian program due to the low number of NDADs included. The Australian program compares favourably with the Dutch program, in which over a 10-year period 242 kidney transplants have been facilitated among the 655 registered pairs using ≥2-way chains (transplant rate 37%)[18]. It also outperforms the UK program, in which between April 2007 and 2014, 284 of the 991 registered patients (29%) proceeded to receive a kidney transplant through 2-way and 3-way loops and 36 through NDAD chains (3.6%)[18]. The Canadian program active since 2009 shares many commonalities with the Australian program: its KPD pool is mainly composed of highly sensitized, HLA-incompatible pairs and the proportion of KPD transplant among registered transplant candidates is equally excellent (44%)[18]. A major driver of the success in Canadian registry is the inclusion of a large number of NDADs (n=54 at the end of 2013), facilitating 62% of the registered recipient transplants versus 38% in N-way exchanges [18, 37]. Comparison between the four national programs [18] would suggest that match and transplant rates from N-way loops does not only rely upon incompatible pair pool size. Indeed, transplant rate from loops is 41% in the Australian program despite inclusion of only 40 to 50 patients per match cycle, whereas transplant rates from loops were only 25% in the UK program despite inclusion of 160 to 180 patients in each allocation round. The most plausible explanation for this success is wide acceptance of ABO-incompatible matching in the Australian program [38]. On the other hand, the power of NDAD domino chains in KPD
program is clearly demonstrated by the Canadian program, where 62% of all KPD transplants were facilitated by NDAD chains [18, 37].

Kidney paired donation in Switzerland
In Switzerland, the first paired kidney exchange procedure took place at Basel University Hospital on the 23rd May 1999 between a Swiss and a German couple. Thereafter, it was not until September 2011 that the next crossover procedure between 2 incompatible couples was carried out at Geneva University Hospitals. The first inter-hospital paired kidney exchange in Switzerland was successfully completed through a combined effort of the Zurich and Geneva transplant teams. After the success of the third KPD procedure, representatives of all Swiss kidney transplant units, Swisstransplant and the Council of the Swisstransplant Foundation joined efforts to promote the establishment of a Swiss national KPD program. It is worth noting that at the time, a national protocol for ABO incompatible kidney transplantation had already been established in Switzerland with success since 2005.

Since the effectiveness of a KPD program depends largely upon the pool’s size of incompatible living donors’ couples, it was important to gauge the likely referral base of HLA and ABO incompatible pairs. Thus, all 6 Swiss kidney transplant centres were surveyed with regard to their own potential incompatible pairs. The survey showed that in 2012 there were at least 38 patients with incompatibilities to their intended donor, either due to preformed DSA with positive crossmatch and/or ABO incompatibility. While this relatively small number may seem discouraging, it is worth noting that in Australia, a country with a population of 22 million, the input of new pairs per match cycle in the last 12 months has averaged 14 added each time on a pool of 32-38 existing pairs. Thus, the projected enrolment of at least 38 incompatible pairs in Switzerland, a country of 7 million inhabitants, was thought adequate to warrant the establishment of a national KPD program. Discussions with the Federal Public Health Office to implement such a program and the need for a national platform in accordance to existing Swiss Transplantation Law are currently underway.

While a formal national KPD registry is yet to be finalized, exchange of information between the transplant centres on incompatible pairs has led to 13 crossover transplantations: two 2-way and three 3-way loop exchanges, between September 2011 and October 2013 (Figure 1). Matching was performed manually using a virtual crossmatch approach that takes into consideration preformed anti-HLA antibodies and donor and recipient blood groups. Immunological suitability of identified matches was confirmed by cell-based crossmatches performed at the Swiss National Reference Laboratory in Geneva. All donations and all transplantations took place on the same day at the same anaesthetic induction time. All donors travelled to the recipient’s centre for
their operations. Choice was given to the new donors-recipients pairs to meet: 10 over 13 pairs met before surgery. Reasons for crossover procedures are shown in (Figure 4); KPD allowed in all cases to overcome the immunological barrier (positive crossmatch and/or ABO incompatibility) each recipient had with his intended donor. One year graft and patient survival are 100% and the matched pairs that met are still in touch.

**Special considerations**

*Allocation algorithms in kidney paired donation*

The ability of KPD to match incompatible pairs depends upon the pool size [29, 46], the ratio of ABO-incompatible to HLA-incompatible pairs, the level of sensitisation of transplant candidates and the algorithm-specific allocation rules. Using N-way exchanges, the match probability for ABO-incompatible or sensitised non-O recipients is around 60%, but can be as low as 20% for ABO-incompatible O recipients [66]. This observation has been used as an argument to preferentially match scarce O donors in KPD registry to O recipients in the interest of fairness [44, 67]. Because many ABO-incompatible pairs may not accept participation in KPD program to undergo living donor kidney transplantation [7-9], an important source of unsensitised recipients is removed from KPD pool, leading to a high proportion of highly sensitized pairs [38, 68]. When the number of pairs referred for KPD because of HLA incompatibility outnumbers the pairs with ABO incompatibility the match probability decreases [36]. A strategy to minimize this problem would be to offer ABO incompatible pairs the option to be entered first in the KPD registry with the aim of improving HLA matching and to resort to directed ABO-incompatible transplantation if no suitable match is identified within an agreed number of match cycles.

The key ingredient of any KPD program is the matching algorithm selecting pairs within the pool. The ideal algorithm should identify the maximum number of possible transplants, while minimizing the probability of unexpected post-match positive cell-based crossmatches and simultaneously promoting high quality exchanges. These difficult goals require sophisticated KPD software that takes into account 2 critical elements of priority for matching: blood group matching and negative crossmatch [40, 44]. Virtual crossmatch approach is widely accepted to allocate suitable donors in the pool to registered transplant candidates, although the extent of HLA-antigens included in this virtual crossmatch algorithm varies between registries [18, 32, 36, 37, 40, 44, 56]. The Australian KPD program uses the computer platform of the National Organ Matching System (NOMS), which is also responsible for deceased donor organ allocation, with a purpose-built KPD allocation module [40]. The NOMS computer program matches each recipient with any donors using a 2-step process: 1) ABO-compatibility or acceptable donors matching (in case of approved ABO-incompatibility matching) and 2) HLA virtual crossmatches among these
ABO-acceptable donors and recipients. Next, the NOMS program generates ≥2-way exchanges using 6 ranking criteria: 1) prioritizing combinations that maximize the number of potential transplants; 2) selecting matches for recipients with low match probabilities (high cPRA); 3) maximising the number of ABO identical pairs, giving O-to-O pairs priority; 4) minimizing the number of simultaneous transplants within a chain in a single hospital; 5) maximizing the number of short chains; and 6) promoting matches for patients with longer waiting times. Despite the relatively small pool of incompatible pairs, the high proportion of sensitized patients and the small number of NDADs, the Australian program has been able to facilitate kidney transplantation in 49% of registered patients [39, 43].

**Highly sensitized recipients: integration of desensitization and ABOi matching**

Despite desensitization protocols or inclusion in a KPD registry, highly sensitized patients with cPRA ≥ 97% remain difficult to undergo living kidney transplantation. In the Australian program, 50% of unmatched pairs still waiting in the registry have a cPRA of 99-100% (Figure 5). It is obvious that for these patients either strategy alone will not be able to find a suitable crossmatch-negative donor without any detectable DSA. Several strategies to help these highly sensitized patients include reducing constraints in the matching algorithm such as allowing ABO-incompatible matching [38], expanding the KPD pool size through international collaboration [69], including all NDADs and large numbers of compatible pairs [29, 53] and allowing DSA positive KPD transplants in order to let them undergo acceptable, albeit not ideal kidney transplantation. While traditionally most KPD programs use virtual crossmatch criteria to identify fully compatible matches that will avoid even low-strength DSA [36, 37, 40, 44], some KPD programs have already successfully explored the option of a hybrid strategy combining KPD with low immunologic risk desensitization [11, 63, 65, 70-72]. With this strategy, although KPD recipients would have a positive virtual crossmatch based on the DSA identified by SAB testing, they can be safely transplanted in most instances in the presence of a negative CDC crossmatch and in many cases even a negative flow cytometric crossmatch [72]. This hybrid strategy used quite extensively at Johns Hopkins Hospital in Baltimore [63, 65] and in a few cases in Australia, was proving effective in transplanting patients who are both hard to match and difficult to desensitize.

**Legal framework**

Successful kidney transplantation was first reported when a patient with kidney failure received a kidney from his identical homozygous twin in 1954. Living donor kidney transplantation remained restricted to haploidentical siblings and first degree related donors until introduction
of potent immunosuppressive drugs in the mid 80s. Since then, transplantation laws and relationships between live donors and recipients have evolved in line with developments in biology and pharmacotherapy. In directed living donor kidney transplantation, a genetically or emotionally related donor knows the recipient beforehand. When considering a KPD program, which is an expansion of conventional living kidney donation, it is important to be aware of possible legislative barriers and commitment of politicians in changing their national organ transplant act. The fact that the donor and the recipient of the matched pair are strangers to one another could hurdle KPD program establishment. In theory, a KPD could be considered an arrangement akin to a bilateral contract, where a donor would agree to donate a kidney to a stranger, if his/her co-registered recipient would receive a kidney in return; this is generally known as ‘valuable consideration’. In the US, the National Organ Transplant Act (NOTA) of 1984 prohibited “any person to knowingly acquire, receive, or otherwise transfer any human organ for valuable consideration for use in human transplantation.” Although the concept of “valuable consideration” applies to monetary value, an exchange of organs was also considered valuable, as ‘one pair is paying with a kidney in order to receive a kidney”. In 2007, a bill was passed both in the House and in the Senate that amends NOTA to clarify that “kidney exchange shall not be considered to involve the transfer of a human organ for valuable consideration”. In Australia, there is no Federal Legislation on Organ and Tissue Transplants and each state has their own Transplant Act. All states Transplant Act have a prohibition against trading in human tissues, the majority include a clause mandating that “…a person must not enter into, or offer to enter into, a contract or arrangement under which any person agrees, for valuable consideration, whether given or to be given to any such person or to any other person…”, which basically translates in a prohibition on living donor kidney exchange. However, all Transplant Acts have a special provision for the Minister for Health to grant exemption to allow KPD to occur. To date, a ministerial exemption is required for each pair participating in the program in all states except Victoria and Queensland. Like most human tissue and transplant acts in developed countries, the Swiss Transplant Law on human organs, tissues and cells, regulates the prohibition on organ trading and similar valuable consideration that could be perceived as trading of organs or tissues for human transplantation (http://www.admin.ch/opc/de/federal-gazette/2004/5453.pdf). In chapter 2, section 1, paragraph 6(d), it is outlined that crossover live donor transplantation is not prohibited as organ trading, because no financial or other gain is being associated. No detail is given regarding how a KPD program should be implemented and whether NDADs could be integrated to allow initiation of chains.
Conclusions and perspectives
In many countries, KPD has become the fastest growing source of live donor kidney transplantations. By including all healthy, willing but immunologically incompatible living donors, but also non-directed anonymous donors and compatible pairs it is possible to achieve a sufficiently large pool of donors and recipients in order to find the best match for the highest number of recipients, even in a relatively small country like Switzerland. Facilitating immunologically compatible kidney transplants should remain the primary aim of a KPD program, but for those extremely highly sensitized patients, KPD can enable living donor kidney transplantations with low immunological risk and longer graft survivals. KPD is also beneficial to unpaired patients, as removing all patients with potential living donor from the deceased donor waitlist will lower their waiting time. Finally, increased access to kidney transplantation will help reduce the demand for illegal commercial transplantation.

In our opinion, it is time to establish a funded Swiss national KPD program; this view is supported by all 6 transplantations centres, who have agreed to establish a registry, have concurred on a computer software and allocation algorithm and have settled for a central and dedicated coordination through Swisstransplant. Crossover living donor transplantation is not considered as organ trading and thus is not prohibited by the Swiss transplant law. The legal framework is on hold and governmental support is required.
References


Table 1: Key ingredients for a successful national kidney paired donation (KPD) program

- Agreement for absolute donor criteria between the transplant centers
- Full donor evaluation before registration in a match cycle
- Simultaneous anaesthetic induction time for donor surgeries
- A National Coordination Centre organizing KPD activities between transplant centers and HLA laboratories
- A computer allocation system using virtual crossmatch and ranking criteria algorithm
Figure 1: Exchange strategies in kidney paired donation: A conventional 2-way loop exchange between 2 incompatible donor-recipient pairs (A) a 3-way loop exchange among 3 incompatible donor-recipient pairs (B). Multi-way loops can be arranged with 3, 4, 5 or more pairs.
Figure 2: Exchange strategies in kidney paired donation. Unbalanced N-way loop exchanges between 1 compatible and 1 incompatible donor-recipient pairs (A) or 1 compatible and 2 incompatible donor-recipient pairs (B).
Figure 3: Multi-way exchanges beginning with a non-directed donor (NDAD) and including multiple donor-recipient pairs. The closed chain donation model ends with the final donor donating to a patient on deceased-donor wait list (A). The open chain model ends with a bridge donor that will start a new chain of transplants (B).
**Figure 4:** Immunological incompatibilities reasons of 13 living donor pairs in Switzerland
Figure 5: Level of sensitisation of 38 transplant candidates still waiting unmatched in the Australian KPD program after at least 2 match cycles. Two thirds of patients waiting are highly sensitised with a cPRA >95%.
Kidney transplantation is an ongoing challenge. Although induction of tolerance is still the goal, the main factor limiting its success is the shortage of organs. Pre-transplantation immunological risk assessment is of critical importance in order to determine the ideal/best donor-recipient combination.

To be able to answer to increasing demand, transplant nephrologists and surgeons together with immunologists have developed strategies to try to overcome innate and acquired immunological barriers in order to avoid rejecting willing and healthy living donors, and to prevent antibody mediated rejection episodes.

ABOi kidney transplantation has now become a safe and efficient procedure, widespread worldwide, with long-term morbid-mortality outcomes identical to ABO compatible/identical transplantations. It is well established in Switzerland since 2005 and the working group is meeting regularly to exchange experience and data. In the future, paediatric living kidney and living liver transplantations would benefit from the Swiss adult kidney ABOi transplantation expertise. However, this technique still requires a higher level of immunosuppression, both before and after transplantation, rituximab and low-dose corticosteroids long term maintenance, respectively. The Swiss centers are working on how to carefully reduce this immunosuppression.

Reasons of death with a functioning kidney graft are due to cardio-vascular events, tumors and infections. These causes are in part preventable, as they are more or less associated with the immunosuppressive regimen prescribed at transplantation and maintained throughout the patients’ lives. Reducing the immunosuppressive regimen without taking risk leading to immunological graft failure (acute and chronic rejection episodes) could be achieved by avoiding high immunological risk kidney transplantations. When a patient presents with a living but incompatible donor, kidney paired donation is the answer. In many countries, KPD has become the fastest growing source of live donor kidney transplantations. After ethical and legal concerns, it is more administrative and logistic challenges inherent to organizing cooperative programs between transplant centers, on a regional, national, European or international levels, which dominates. By including all healthy, willing but immunologically incompatible living donors, but also non-directed donors and compatible pairs it is possible to achieve a sufficiently large pool of donors and recipients in order to find the best match for the highest number of recipients, even
in a relatively small country like Switzerland. Facilitating immunologically compatible kidney transplants should remain the primary aim of a KPD program, but for those extremely highly sensitized patients, KPD can enable living donor kidney transplantations with low immunological risk and longer graft survivals. KPD is also beneficial to unpaired patients, as removing all patients with potential living donor from the deceased donor waitlist will lower their waiting time. Finally, increased access to kidney transplantations will help reduce the demand for illegal commercial transplantation.

A Swiss national KDP program is on hold as a legal framework is needed. Regular meeting are organised with the Federal Office of Public Health since 2012. When the national KPD registry will be implemented and the Swiss software developed in accordance to current Transplantation Swiss Law, a significant time saving and efficiency will occur compared with the current method, which is an on-time exchange of immunological information and search for the best donor-recipient pair by transplantation centers.

Another point that I would like to implement in Switzerland is including altruistic donors in the KDP program. Choice should be given to altruistic donors to give either to one patient on the waiting list, or to begin a chain of compatible living kidney transplantations. It is the ethical duty of transplantation teams to ensure the best use of the incomparable gift that altruistic donors are making to society. Optimizing the use of altruistic donors is a challenge with the support of support of the 6 Swiss kidney transplant centers.


