

The background of the slide is a high-magnification light micrograph of a kidney biopsy. It shows several glomeruli, which are clusters of capillaries. The glomeruli are stained with hematoxylin and eosin (H&E), giving them a pinkish-red appearance. The surrounding tissue, including the tubules and interstitium, is also visible, showing some degree of cellular infiltration and structural changes consistent with a kidney biopsy specimen.

Blood Group ABO-incompatible Kidney Transplantation

Annelies de Weerd

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Colofon

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Blood Group ABO-Incompatible Kidney Transplantation

Bloedgroep ABO-incompatibele niertransplantatie

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Erasmus Universiteit Rotterdam
op gezag van de
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Chapter 1

General introduction and aims.

INTRODUCTION

Kidney transplantation

Chronic kidney disease is a major health burden affecting millions of people worldwide. End-stage renal disease (CKD5, eGFR <15 ml/min) has an estimated global prevalence of 0.1% (1). Kidney transplantation is the preferred choice of treatment for patients with end-stage renal disease. Kidney transplantation requires an elaborate medical infrastructure. Even in countries with well-developed deceased donor and living donor transplant programs, access to transplantation is not ubiquitous. For example in the Netherlands only two-thirds of patients on renal replacement therapy have a functioning kidney allograft (2). Barriers to kidney transplantation are medical conditions in the recipient, shortage of donor organs and circulating antibodies against potential donors. These antibodies can be directed against HLA molecules on nucleated cells, non-HLA (endothelial) antigens and against targets on red blood cells. Clinically the most relevant red blood cell antigens are those from the ABO blood group system. Incompatibility in ABO blood group between donor and recipient can cause life-threatening hemolysis after blood transfusion. Blood group A and B antigens are not only expressed on red blood cells, but also on endothelial, tubular and glomerular cells (3). Therefore anti-ABO antibodies are highly relevant in kidney transplantation. Not accounting for different blood groups between the kidney allograft and the recipient may cause hyper-acute rejection of the graft.

The ABO blood group system

Red blood cells carry numerous sugars and proteins on their surface that are antigenic in other humans. These molecules have various functions ranging from structural, channel and transport functions to complement regulation and infection prevention (4). Polymorphisms in red blood cell antigen can result from natural selection. For example the Duffy glycoprotein, involved in clearing inflammatory cytokines, has a West African Duffy variant phenotype Fy (a-b-) that protects the red blood cell from malarial invasion (5). Red blood cell antigens are categorized in 29 blood group systems according to the different encoding genes. Of these, the ABO blood group system is the most notable, for its relevance in transfusion medicine due to the immunogenic nature of its antigens. The different blood groups A, B, AB and O result from the inherited activity of glycosyltransferase enzymes encoded by the ABO gene. People with blood group O express the α -1,2-fucosyltransferase enzyme that forms an α -l-fucose to α -d-galactose carbohydrate backbone bound to glycolipids or proteins, named the H antigen. In people with blood group A, α -1,3-n-acetylgalactosaminyltransferase attaches β -N-acetylgalactosamine (the A antigen) to this H backbone. There are two different transferases expressing either low or high amounts of A antigen: A1 individuals are high expressers and A2 individuals (20% of blood group A) are low expressers. People with blood group B express on their H antigen D-galactose (the B antigen) under the influence of the enzyme α -1,3-galactosyltransferase, while blood group AB individuals express both sugars as depicted in Figure 1 (6).

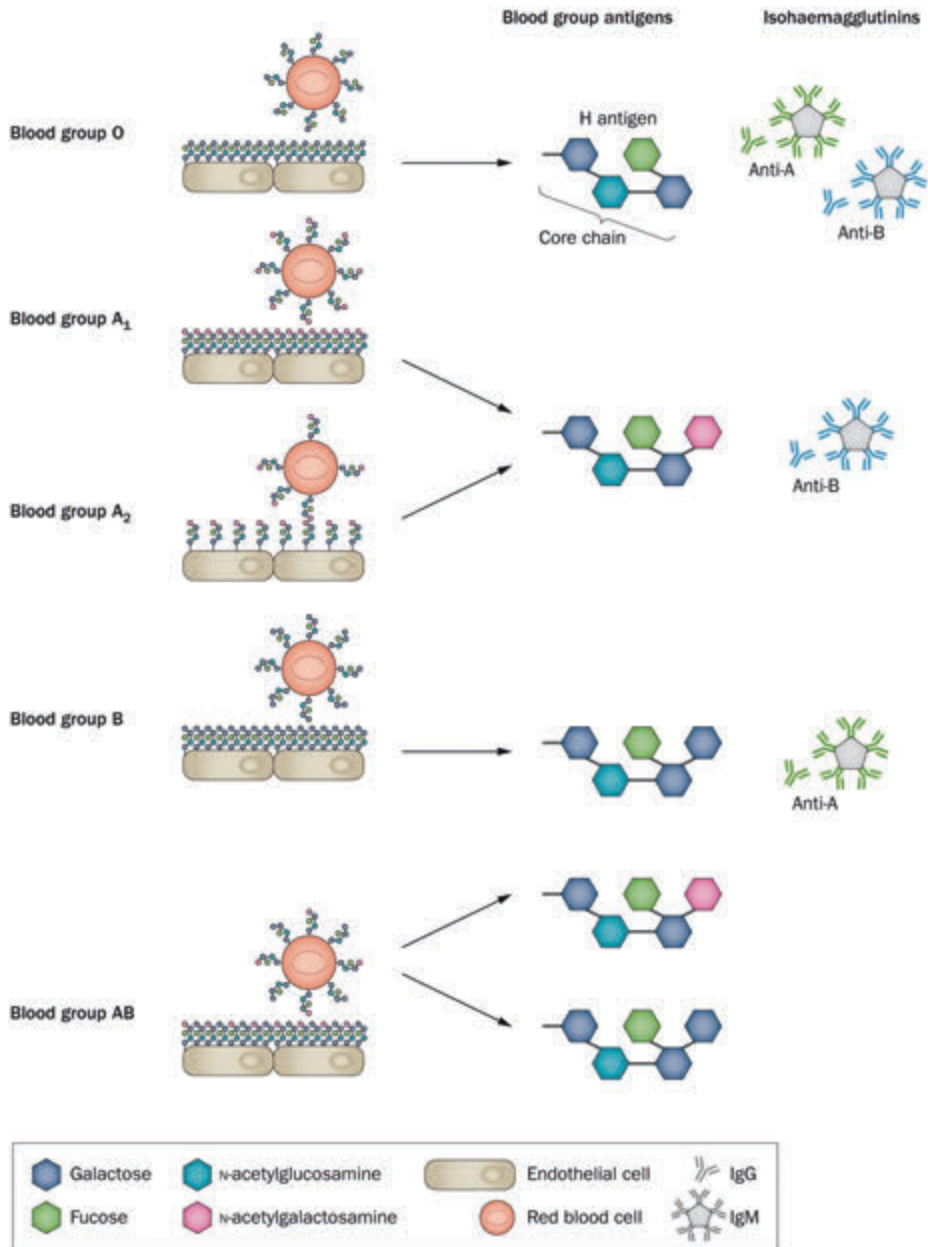


Figure 1. The structure of ABO surface antigens and their corresponding antibodies. Reprinted with permission from reference 6: Böhmgig, Nat. Rev. Nephrology 2015 (6).

The function of the ABO polymorphisms is not fully understood. Blood group distribution varies by ethnicity. A remarkable finding is that blood group O individuals have 25% lower levels of Von Willebrand factor than the other ABO types, and are overrepresented in heavy menstrual bleeding and post-tonsillectomy hemorrhage (7), with a relative protection against venous thrombosis (8, 9).

The ABO blood group system is also defined by the presence or absence of antibodies that can agglutinate blood, as discovered by Karl Landsteiner in 1901. ABO-antibodies are absent at birth. During infancy, when the gut becomes colonized with bacteria, "isoagglutinin" formation starts as a result of similarities in bacterial antigens and blood group AB antigens. Auto-antibodies are eliminated leaving O individuals with anti-AB antibodies, B individuals with anti-A antibodies, A individuals with anti-B antibodies and AB individuals without any AB isoagglutinins (10). These isoagglutinins are the source of serious hemolytic transfusion reactions in transfusion medicine. In solid organ transplantation, non-ABO-identical transplantation can elicit immune responses, because AB antigens are also expressed on other cell types. Because AB antigens are present on parenchymal and vascular kidney cells, anti-A/B antibodies directed against the allograft can result in antibody-mediated rejection in ABO-incompatible kidney transplantation. There are many other blood group systems including Duffy, Kidd and Rhesus. Although the last-named system is highly relevant for avoiding immunization in obstetrical care, it is less relevant for solid organ transplantation as renal cells do not express the Rhesus antigens. Rarely, mismatches for minor blood group systems are related to acute rejection of the transplanted kidney (11).

ABO blood group and kidney transplantation

Blood group O donors are "universal donors" (lacking A and B antigens) because they are compatible with any blood group of the recipient. Vice versa blood group O recipients (with A/B antibodies) are only compatible with blood group identical donors (blood group O donors). Blood group distribution varies by population. A random recipient donor combination will be blood group incompatible in roughly one out of three cases. In the Dutch population, blood group O candidates are compatible with approximately 47% of potential donors (12). Blood group AB individuals are "universal recipients" and compatible with any potential donor. Blood group O candidates have less access to kidney transplantation when kidney allografts are allocated in an ABO-compatible manner instead of an ABO-identical manner. Deceased donor kidneys are mostly allocated by Eurotransplant in a blood group identical manner (standard ETKAS and senior programs). However, a minority of approximately 5% of kidneys is allocated via the Acceptable Mismatch program in a blood group compatible program to prioritize the highly immunized candidates (13). In the living donor programs the vast majority of donations are directed and therefore blood group compatible. The allocation in living donation programs therefore disadvantages blood group O candidates. In 2012, 42% of the Dutch population had blood group O, as against 41% in the recipients of a deceased donor kidney allograft and only 33% in recipients of a living donor graft

(14). This relatively low percentage existed despite operational ABO-incompatible and exchange programs and remains a problem today. In 2018, 57% of all Dutch patients actively waiting for a donor kidney had blood group O (408/719 (13)). Of all transplanted deceased donor kidneys 49% were blood group O, whereas in the living donor recipients 38% had blood group O (13). These numbers underlie the importance of optimal allocation rules and underscore the clinical need for ABO-incompatible transplantation.

History of ABO-incompatible kidney transplantation

During the pioneering years of the first relatively successful human kidney transplantations in the 1950s, ABO blood group incompatibility was deemed a risk for hemolysis in the allograft. In the 1960s, the experiences of Starzl et al. led to the perception that blood group violation could also cause rejection of the allograft (15). In Figure 2 his findings in a series of 16 kidney allograft recipients led to Starzl's reflections on the safety of ABO-incompatible (ABOi) kidney transplantation that still hold true today.

SURGERY			
Volume 55 Number 2		FEBRUARY, 1964	
Clinical surgery			
Renal homografts in patients with major donor-recipient blood group incompatibilities			
T. E. STARZL, M.D.* T. L. MARCHIORO, M.D. J. H. HOLMES, M.D. G. HERMANN, M.D. R. S. BRITTAİN, M.D. O. H. STONINGTON, M.D. D. W. TALMAGE, M.D. W. R. WADDELL, M.D. DENVER, COLO. <i>From the Department of Surgery, University of Colorado Medical Center, and the Denver Veterans Administration Hospital</i>			
Addendum Table I. Blood group mismatches		Addendum Table II. Direction of acceptable mismatched tissue transfer*	
Blood group	No. of mismatches	Good early function	Alive
O to A	4	4	3
A to O	3	1	2*
B to A	1	1	1
A to AB	1	1	0
B to O	1	0	1*
Rh+ to Rh-	2	2	2
Rh- to Rh+	4	4	4
*O is universal donor; AB is universal recipient.			

Figure 2. This series of 16 non-ABO-identical kidney transplant recipients led to Starzl et al.'s speculations on the safety of ABO-incompatible kidney transplantation. Reprinted with permission from reference 15: Starzl, Surgery 1964 (15).

For decades, the presence of ABO antibodies against the blood group of the donor was considered an absolute contra-indication for kidney transplantation. However, as this ABO barrier severely limited the pool of donor organs available for O recipients, and not all unintended ABOi transplantations had a deleterious course, interest in overcoming ABO barriers never faded (16). In the early 1980s, Alexandre and colleagues pioneered ABOi kidney transplantation in Belgium (17). A desensitization treatment was developed consisting of three elements: removal of circulating antibodies, inhibition of antibody production and immunosuppression to inhibit immune responses. Plasmapheresis, splenectomy, infusion of donor platelets and immunosuppression were used to desensitize these ABOi allograft recipients preoperatively (18). ABOi transplantation was further disseminated in Japan and the USA (19, 20). Modifications made to the desensitization treatment in Japan were local graft irradiation and plasmapheresis with or without immunoadsorption. The “gamechanger” in ABOi kidney transplantation was the introduction of rituximab (21). This anti-CD20 monoclonal antibody made splenectomy an obsolete procedure (22). Rituximab and immunoadsorption have been the cornerstones of ABOi kidney transplantation in Europe from the early 21st century onwards. It is now considered a safe procedure, accounting for approximately one fourth of living procedures in some centers in Germany and Japan. Its contribution to kidney transplantation procedures depends on the elaboration of living-donor programs, the presence of kidney exchange programs and the availability of desensitization treatments. In the Netherlands, the first ABOi kidney transplantation took place in 2006 at the Erasmus Medical Center (MC). The applied desensitization treatment was launched by Tyden et al. in Stockholm (23). This “Swedish” protocol consisted of rituximab, selective immunoadsorption (with an A/B antigen-specific adsorption column), IVIG (intravenous immunoglobulins) and immunosuppression preoperatively (23, 24). Subsequent adaptations of the Erasmus MC protocol consisted of the discontinuation of postoperative plasmapheresis and change in the induction agent from B-cell depletion to combined T- and B-cell depletion. Randomized controlled trials are lacking in ABOi kidney transplantation and novel therapies have been launched and modified in pioneering centers. Desensitization elements have been selectively adapted according to patients’ characteristics (such as age and blood group titers) and single center experiences. A difficulty in studying ABOi kidney transplantation is to define an appropriate comparator: should ABOi candidates be compared to dialysis, deceased donor transplantation with or without waiting list time, other types of desensitization treatment or living donor blood group compatible transplantation?

The nature of A/B antibodies, at the center of desensitization protocols, is not fully understood. The damage caused by circulating A/B antibodies differs from the (in general more deleterious) effect of anti-HLA antibodies (25, 26). Ligation of A/B antibodies to endothelial cells has been demonstrated to induce different signaling pathways than HLA-antibodies in experiments with endothelial cell line expressing A/B antigens (27). Interaction of A/B antibodies with vascular cells inhibited the ERK1/2 (extracellular signal-regulated kinases) pathway, resulting in an increase in complement regulatory

proteins via the induction of CD55 and CD59. This led to the acquired resistance of the endothelial cells to complement-mediated cytotoxicity in these experiments. This could be an explanation for the frequent finding of C4d deposition in biopsies of ABOi kidney allografts without clinical signs of antibody-mediated rejection. This apparent accommodation of A/B antibodies without graft injury, is a key concept in ABOi kidney transplantation. Lower A/B antigen expression over time in the ABOi donor kidney versus stable A/B antigen expression in the ABO-compatible (ABOc) donor allograft, as well as blood-type chimerism in the renal endothelium, have also been described (28). This apparent accommodation that may exist in the allograft in the presence of A/B antibodies questions target titers perioperatively.

The overall aim of this thesis is to improve outcomes for kidney transplant candidates who are blood group incompatible with their potential donor. It includes clinical studies in the Erasmus Medical Center as well as in national and international patient cohorts, with the following aims:

AIMS OF THE THESIS

1. To weigh the risk of ABOi kidney transplantation by comparing patient and death-censored graft survival in a meta-analysis, including all single center studies comparing ABOi and (matched) ABOc kidney transplant recipients (chapter 2).
2. To analyze the results of the first cohort of 50 ABOi kidney transplant recipients in the Erasmus Medical Center Rotterdam, in order to evaluate the Swedish desensitization protocol (chapter 3).
3. To explore the clinical observation of a higher bleeding risk after ABOi kidney transplantation and to study its underlying mechanisms (chapter 4).
4. To study the relation between postoperative A/B antibody titers and rejection, in order to clarify the need for postoperative immunoadsorption (chapter 5).
5. To analyze the national cohort of ABOi kidney transplant recipients by comparing this cohort with propensity matched blood group compatible living donor and deceased donor recipients, in order to establish the risk of desensitization and to gain clinical information for counseling incompatible donor-recipient pairs (chapter 6).
6. To analyze outcomes of ABOi kidney transplantation according to different induction therapies, comparing rituximab single agent therapy to alemtuzumab and to rituximab/basiliximab induction therapy (chapter 6).
7. To explore the possible booster effect of a Gram-negative sepsis on A/B antibody formation, by studying a recipient in whom a *Serratia marcescens* sepsis preceded a fulminant antibody-mediated rejection (chapter 7).

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Chapter 2

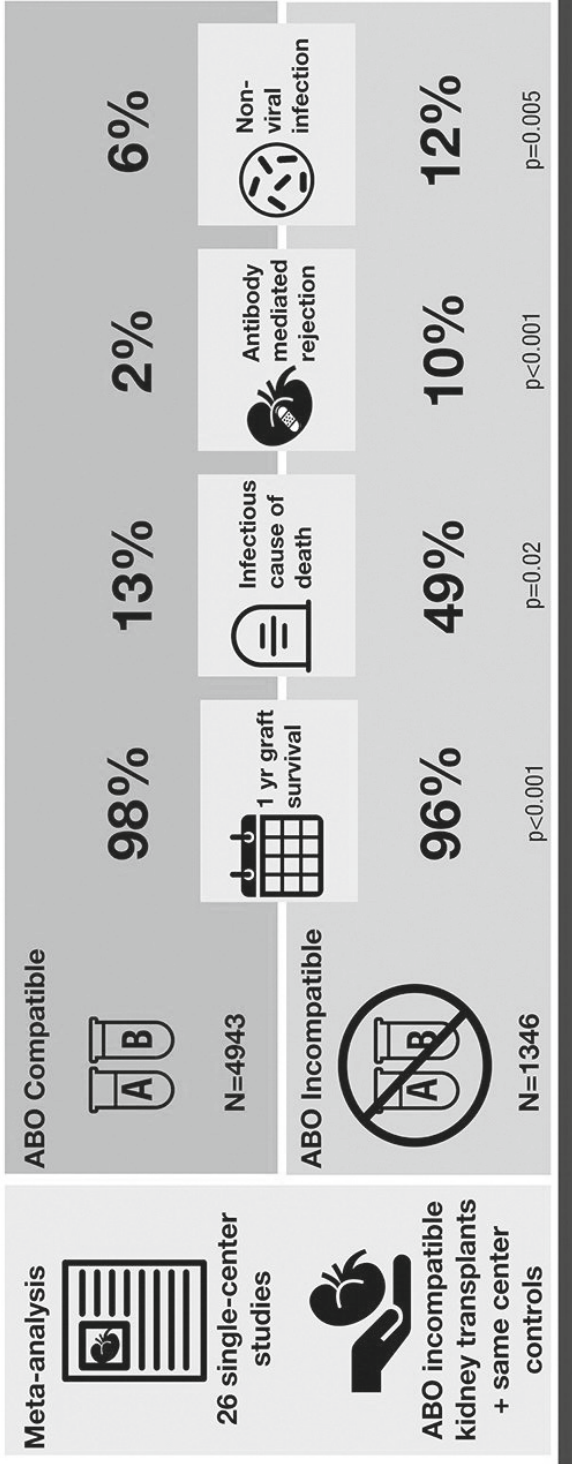
ABO-incompatible kidney transplant outcomes:
A meta-analysis.

*How safe is crossing the ABO blood group
barrier?*

Annelies E de Weerd, Michiel GH Betjes.

Clin J Am Soc Nephrol. 2018 Aug 7;13(8):1234-1243.

How safe is crossing the ABO blood group barrier in kidney transplantation?



Conclusions ABO-incompatible kidney transplant recipients have good outcomes albeit inferior to center-matched ABO-compatible control patients.

Annelies E. de Weerd and Michiel G.H. Betjes. ABO-Incompatible Kidney Transplant Outcomes: A Meta-Analysis. CJASN doi: 10.2215/CJN.00540118

ABSTRACT

Background

ABO blood group-incompatible kidney transplantation is considered a safe procedure, with non-inferior outcomes in large cohort studies. Its contribution to living kidney transplantation programs is substantial and growing. Outcomes compared to center-matched ABO blood group-compatible control patients have not been ascertained.

Design

Comprehensive searches were conducted in Embase, Medline, Cochrane, Web-of-Science and Google Scholar. MOOSE study guidelines for observational studies and Newcastle Ottawa bias scale were implemented to assess studies. Meta-analysis was performed using Review Manager 5.3. A subgroup analysis on antibody removal technique was performed.

Results

After identifying 2728 studies addressing ABO-incompatible kidney transplantation, 26 studies were included, describing 1346 unique ABO-incompatible patients and 4943 ABO-compatible controls. Risk of bias was low (all studies $\geq 7/9$ stars). Baseline patient characteristics revealed no significant differences in immunological risk parameters. Statistical heterogeneity of studies was low (I^2 0% for graft and patient survival). One-year uncensored graft survival of ABO-incompatible patients was 96% versus 98% in ABO-compatible controls (RR 0.97, CI 0.96-0.98, $p < 0.001$). 49% of reported causes of death in ABO-incompatible patients were of infectious origin, versus only 13% in ABO-compatible patients ($p = 0.02$). Antibody-mediated rejection (3.86, CI 2.05-7.29, $p < 0.001$), severe non-viral infection (1.44, CI 1.13-1.82, $p = 0.003$) and bleeding (1.92, CI 1.36-2.72, $p < 0.001$) were also more common after ABO-incompatible transplantation.

Conclusion

ABO-incompatible kidney transplant recipients have good outcomes albeit inferior to center-matched ABO-compatible control patients.

INTRODUCTION

Desensitization protocols have allowed for successful transplantation of kidney allografts across the ABO blood group barrier. Pioneering centers in ABO-incompatible kidney transplantation have published patient and graft survival rates comparable to ABO-compatible transplantations (1-3). These reassuring outcomes combined with long waiting times for deceased donor kidneys and the shortage of available living donors have led to a broader implementation of ABO-incompatible transplantation. It accounts for one fourth of living donor transplantations in German centers (4, 5) and almost one third of procedures in Japanese centers (6, 7).

Therefore, a kidney transplant candidate with an ABO-incompatible living donor has options to wait for a deceased ABO-compatible donor (remain on dialysis), participate in a kidney exchange program (if operative) or proceed with desensitization for ABO-incompatible transplantation. In order to ensure the ability to make an informed decision about which option to choose, the additive risk of desensitization for ABO-incompatible donor-recipient pairs should be known.

Data on the potential additive risk of ABO-incompatible kidney transplantation have been deduced from registry and cohort studies. Recent cohort studies have shown no significant difference in graft and patient survival compared to ABO-compatible kidney transplantation (5, 8, 9). The largest registry study with ABO-compatible controls is from the Collaborative Transplant Study (10, 11). Three-year patient survival was not significantly lower, however there was more infection-related mortality. A registry study from Korea reported comparable graft survival rates, with a trend towards inferior patient survival (12). Registries from the United States revealed somewhat different results with more early graft loss and equal or inferior patient survival (13, 14), but these registries also contained splenectomized patients. A disadvantage of these registries is the uncertainty about completeness and quality of data, and inclusion of patients treated with different desensitization protocols, limiting external validity.

For these reasons we have performed a meta-analysis of single center cohort studies published with a control group consisting of ABO-compatible patients from the same hospital. Information from such a meta-analysis includes a large number of patients allowing the ability to identify risk differences in relatively rare events like patient death and graft loss across different desensitization regimes.

The primary objective of the meta-analysis was to compare the risk for graft and patient survival after ABO-incompatible versus ABO-compatible kidney transplantation. As secondary outcomes the differences in risk for acute rejection, infectious complications, and post-operative bleeding were analyzed.

METHODS

We have performed the meta-analysis according to guidelines for observational studies as described in the MOOSE study (15).

Literature search strategy

Research database

The meta-analysis is a sequel of a systematic literature search to create an ABO-incompatible research database. This original search was carried out in Embase, Medline, Cochrane, Web-of-Science and Google Scholar. The broad index terms are described in Supplemental Table 1, which identifies all studies on ABO-incompatible kidney transplantation published till July 1st 2017. Studies were screened for relevance to this database: eligibility criteria were medical aspects of kidney transplantation (excluding studies on financial aspects). A limitation was English language only and case reports were excluded. Search results were analyzed in Endnote. This database was built in Excel and categorized studies according to topic(s).

Meta-analysis

Next, studies in this database were screened for inclusion in the meta-analysis by two independent researchers, AdW and MB (both nephrologists). Eligible were all single center studies comparing ABO-incompatible patients with ABO-compatible controls reporting patient and graft survival. Conference abstracts were excluded. Reports usually define an era with splenectomy and a “modern” era with rituximab induction, with improved outcomes in the latter (7, 16). For this reason the older studies using splenectomy were excluded. Studies on combined HLA-incompatible and ABO-incompatible transplantation were excluded, as were studies on deceased donor kidney allografts. The final step was to identify unique patients by excluding overlapping reports from the same center. Authors were contacted via email and ResearchGate to ask for missing data and (if available) for 3-year follow-up data.

Data collection and data items

The following items were identified in the included studies and reported in Excel:

Patients and controls: Patients were all living ABO-incompatible kidney transplant patients during the study period of the included study who were treated without splenectomy. Controls were either consecutive (all patients with a living ABO-compatible kidney transplant during the study period) or matched according to the matching criteria of the included study (see quality assessment). In general, studies excluded HLA-incompatible transplantations from their analysis.

Immunoadsorption and plasmapheresis: There is no universal ABO-incompatible desensitization protocol and centers differ in immune suppressive therapy and isoagglutinin removal technique. For analysis, studies were divided into centers using (mainly) plasmapheresis versus (mainly) immunoadsorption.

Baseline patient characteristics: number of patients, recipient and donor age, level of panel-reactive antibodies (PRA), donor-specific antibodies (DSA), percentage of retransplant and pre-emptive transplantations were recorded.

Uncensored-graft survival, patient survival: these outcome were deduced from Kaplan-Meier survival curves and from numbers and percentages described in the results sections. Uncensored graft survival was established as follows: its numerator was determined by either graft loss or patient death and the denominator was the total number of patients censored for loss to follow-up.

Uncensored graft survival was determined for year one and if available year three. In case of availability of year three data, graft survival after year one was determined as follows: the numerator was graft survival at year three and the denominator was the number of patients censored for follow-up and censored for graft loss and patient death at year one.

Cause of death: infectious origin versus non-infectious origin (including unknown) causes of death were extracted from the included studies.

Biopsy-proven acute rejections included all (mainly year one) biopsy-proven rejections, excluding subclinical and borderline rejections.

BK viremia: studies described either *any* viremia, viremia from a cut-off level onwards or BK nephropathy. If various BK outcomes were reported, *any* BK viremia was scored. The outcome BK therefore is heterogeneous, but comparable between patients from the same center. This holds true for cytomegalovirus (CMV) viremia as well.

Severe non-viral infection: for this item heterogeneous outcomes were combined: sepsis; hospitalization for infection for 7 or more days; bacterial infection; sepsis; pneumonia; fungal infection; *Pneumocystis jirovecii* pneumonia; bacterial infection requiring hospitalization. Urinary tract infections were excluded.

Bleeding: this was a combination of postoperative blood transfusion or bleeding leading to surgical intervention, whichever was reported in the included studies.

Follow-up: graft survival was analyzed at year one and if available year three. Rejection, infection and bleeding were censored at year one if this information was provided. If not, these outcomes were reported during total follow-up of the individual studies. Studies reported either mean or median follow-up, making it impossible to determine the mean follow-up of patients in the meta-analysis. This mean follow-up was established by approximation: mean follow-up was determined by multiplying the total number of patients in each study by their corresponding (mean or median) follow-up in months, divided by the total number of patients in all included studies.

Risk of bias assessment

The Newcastle-Ottawa scale was adopted to assess the quality of the retrospective cohort studies (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). Studies were graded according to selection of study groups, comparability of groups and ascertainment of exposure and outcomes. A maximum of nine stars represents the lowest risk of bias (Supplemental Table 2 Newcastle-Ottawa checklist).

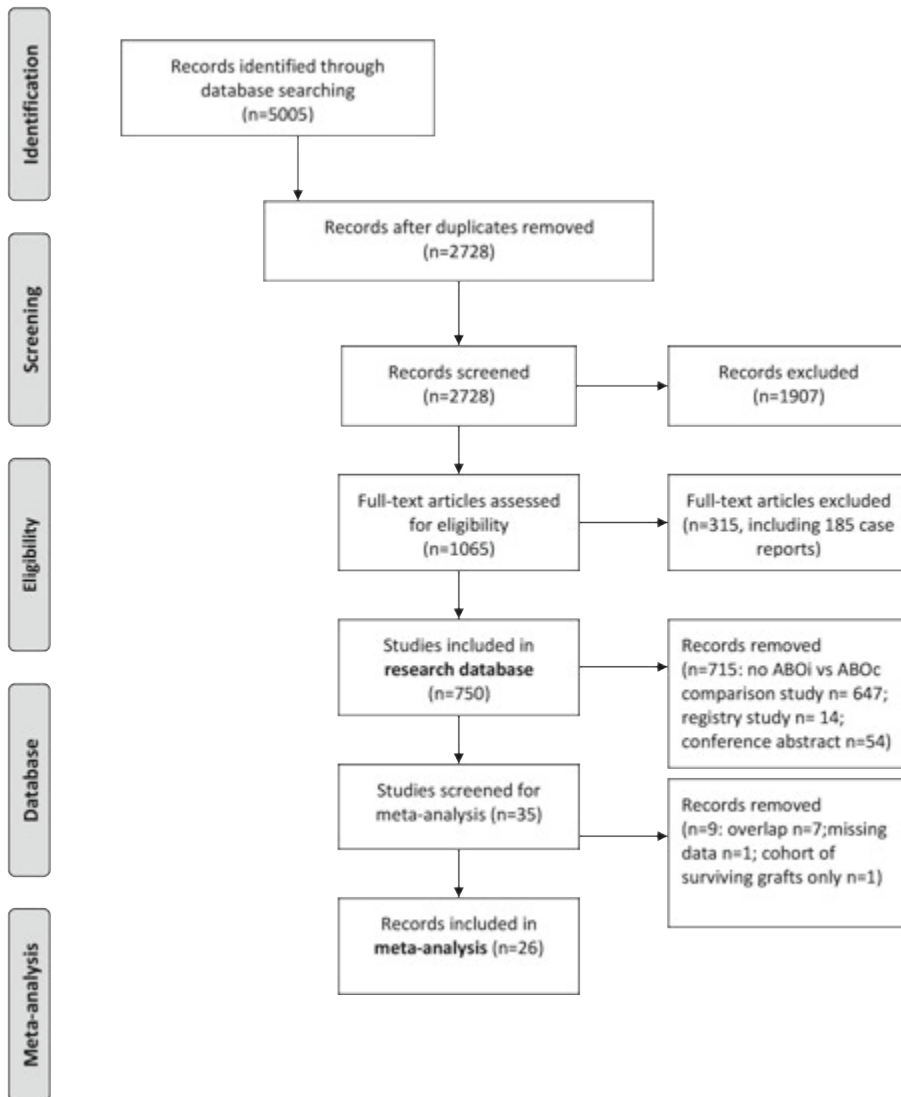
Statistical analysis

The meta-analysis was performed using Review Manager 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark). Baseline characteristics were descriptive for continuous variables and reported group means weighed for number of included patients. For the meta-analysis the Mantel-Haenszel analysis method was used. We divided the included studies into two subgroups in advance, defining studies with (mainly) immunoadsorption versus studies with (mainly) plasmapheresis as antibody removal technique. This subgroup analysis was planned because the only meta-analysis so far about ABO-incompatible kidney transplantation compared these two desensitization techniques (17). Lo et al. demonstrated inferior outcomes after plasmapheresis. Statistical heterogeneity was formally assessed with I^2 (where 0-40% was considered low heterogeneity) and visually by judging overlap in confidence intervals. Differences between groups were analyzed with relative risk (RR) ratios at fixed time spans. Risk ratios were calculated using a fixed-effect model. If heterogeneity was however higher than 40%, a random-effect model was used. Forest plots represent studies in order of year of publication. $P < 0.05$ was considered statistically significant.

RESULTS

Literature search results

Figure 1 presents the PRISMA flow chart. After removing duplicates a total of 2728 unique studies were identified. 750 studies were selected for the ABO-incompatible research database, of which 36 described center-matched ABO-incompatible cohorts. Of these, 9 were excluded because of missing data on patient and graft survival (18), bias due to description of the ABO-incompatible cohort by selecting only surviving grafts (19), and because of overlap in patients (20-26). Finally, 26 studies were included in the meta-analysis. In these 26 articles, a total of 1346 unique ABO-incompatible patients were compared to 4943 center-matched controls. The characteristics of the included studies are shown in Table 1.



ABOi: ABO-incompatible ABOc: ABO-compatible vs: versus

Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the systematic literature search.

Table 1. Overview of the included studies in the meta-analysis.

Study	period	country	ABOI	ABOc	controls	desensitization	induction	follow-up (months)
Ashimine, 2014 (16)	2005-09	Japan	51	228	consecutive	DFPP	<i>all</i> : BAS ABOI with titer >8: RTX 2 x 200 mg	ABOI: 36 ABOc: 52
Barnett, 2013 (27)	2005-11	England	62	167	consecutive	>64: IA 16-64: DFPP ≤8: none <2008: IVIG	<i>all</i> : BAS ABOI with titer ≥8: RTX 375 mg/m ² (n=2 ABOI: ATZ)	ABOI: 26 ABOc: 33
Becker, 2015 (28)	2005-13	Germany	34	86	matched	IA with IVIG	<i>all</i> : BAS ABOI: RTX 375 mg/m ²	ABOI: 22 ABOc: 20
Bennani, 2016 (29)	2011-15	France	44	44	matched	≥128: DFPP and IA 32-64: IA 8-16: PE ≤4 none	<i>all</i> : DSA or PRA>25%: ATG no DSA and PRA ≤25%: BAS ABOI: RTX 375 mg/m ²	ABOI: 6 ABOc: 6
Bentall, 2014 (30)	1999-2006	United States	73	652	consecutive	PE with IVIG	<i>all</i> : ATG	ABOI: 67 ABOc: 73
Flint, 2011 (31)	2005-08	Australia	37	52	matched	PE <2008: IVIG	<i>all</i> : BAS	ABOI: 26 ABOc: 22
Genberg, 2008 (1)	2001-05	Sweden	15	30	matched	IA with IVIG	ABOc: none ABOI: RTX 375 mg/m ²	ABOI: 41 ABOc: 48
Habicht, 2011 (32)	2007-09	Germany	21	47	consecutive	IA	<i>all</i> : BAS ABOI: RTX 375 mg/m ²	ABOI: 17 ABOc: 15
Hatekeyama, 2014 (6)	2006-13	Japan	13	29	consecutive	DFPP	<i>all</i> : BAS ABOI: RTX 100 mg/m ²	ABOI: 28 ABOc: 37
Hwang, 2013 (33)	2009-11	Korea	35	138	matched	PE with IVIG	<i>all</i> : BAS ABOI: RTX 100-375 mg/m ²	ABOI: 24 ABOc: 24

Table 1 (continued). Overview of the included studies in the meta-analysis.

Study	period	country	ABOI	ABOC	controls	desensitization	induction	follow-up (months)
Iwai, 2015 (34)	2001-14	Japan	4	16	consecutive	DFPP or PE	<i>all</i> : BAS ABOI: RTX 150 mg/m ²	ABOI: 39 ABOC: 38
Jha, 2016 (35)	2011-14	India	20	669	consecutive	5 pts: PE with IVIG 12 pts: DFPP 3 pts: none	ABOC: 55% BAS, 5% ATG, 40% none ABOI: BAS and RTX 200 mg	ABOI: 10 ABOC: 17
Kauke, 2016 (36)	2007-12	Germany	26	52	matched	IA	ABOC: PRA>5%: ATG and BAS PRA ≤5%: none ABOI: ATG and RTX	ABOI: 12 ABOC:12
Kim, 2017 (37)	2010-16	Korea	71	726	consecutive	PE and IVIG	<i>all</i> : BAS ABOI: RTX 200 mg	ABOI: 27 ABOC: 42
Kwon, 2016 (38)	2012-15	Korea	234	600	consecutive	PE	<i>all</i> : BAS ABOI: RTX (n=67: 500 mg; n=167: 200 mg)	ABOI: 36 ABOC: 36
Lee, 2016 (39)	2010-14	Korea	97	118	consecutive	PE >128: IVIG	<i>all</i> : BAS ABOI: RTX ≥128: 375 mg/m ² <128: 200 mg RTX	ABOI: 34 ABOC: 36
Melexopoulou, 2015 (40)	2005-13	Greece	30	30	matched	IA or DFPP	<i>all</i> : BAS or dacluzumab ABOI: RTX 375 mg/m ²	ABOI: 74 ABOC: 78
Okumi, 2016 (7)	2005-13	Japan	144	333	consecutive	DFPP	<i>all</i> : BAS ABOI: RTX 200 mg	ABOI: 48 ABOC: 56
Park, 2016 (41)	2011-13	Korea	11	21	consecutive	DFPP and once PE	<i>all</i> : BAS ABOI: RTX 200 mg	ABOI: 15 ABOC: 15

Table 1 (continued). Overview of the included studies in the meta-analysis.

Study	period	country	ABOi	ABOc	controls	desensitization	induction	follow-up (months)
Sanches-Escudero, 2016 (42)	2011-13	Spain	30	146	consecutive	PE or IA	<i>all</i> : BAS or ATG depending on immunological risk <i>ABOi</i> : RTX 2 x 200 mg	ABOi: 21 ABOc: 21
Schachtner, 2015 (4)	2005-12	Germany	35	62	matched	IA, IVIG	<i>all</i> : BAS <i>ABOi</i> : RTX 375 mg/m ²	ABOi: 42 ABOc: 37
Shin, 2015 (8)	2009-12	Korea	73	396	consecutive	PE	<i>all</i> : BAS <i>ABOi</i> : RTX 500 or 200 mg	ABOi: 39 ABOc: 46
Subramanian, 2016 (9)	2007-12	United States	18	45	matched	PE and IVIG	<i>all</i> : ATG <i>ABOi</i> : RTX 200 mg	ABOi: 29 ABOc: 29
Van Agteren, 2014 (43)	2006-12	Netherlands	50	100	matched	IA and IVIG	<i>ABOc</i> : BAS <i>ABOi</i> : RTX 375 mg/m ²	ABOi: 38 ABOc: 38
Yokoyama, 2016 (44)	2008-13	Japan	21	50	consecutive	PE	<i>all</i> : BAS <i>ABOi</i> : RTX	ABOi: 12 ABOc: 12
Zschiedrich, 2016 (5)	2004-14	Germany	97	106	consecutive	IA	<i>all</i> : BAS <i>ABOi</i> : RTX 375 mg/m ²	ABOi: 58 ABOc: 48
TOTAL			1346	4943				

ABOi: ABO-incompatible ABOc: ABO-compatible ATG: anti-thymoglobulin ATZ: alemtuzumab BAS: basiliximab DFPP: double-filtration plasmapheresis DSA: donor-specific antibodies IA: immunoadsorption IVIG: intravenous immunoglobulin PE: plasma exchange PRA: panel-reactive antibodies RTX: rituximab

Baseline characteristics of patients included

Follow-up of ABO-incompatible patients was 37 months versus 40 months in controls (Table 2, Supplemental Table 4). Recipients of ABO-incompatible kidney allografts were slightly older and had younger donors than ABO-compatible controls (recipients 47 vs 45 years and donors 48 vs 49 years in ABO-incompatible vs ABO-compatible). Since the majority of studies lacked standard deviations for these items, no significance level could be determined. This holds true for total number of HLA mismatches on A, B and DR loci (3.6 vs 3.1). There were no differences in level of PRA, DSA, retransplants and pre-emptive transplantations between ABO-incompatible and ABO-compatible patients. As expected, ABO-incompatible patients more often received unrelated transplants, although this characteristic was only reported on in the minority of studies.

Table 2. Baseline characteristics of patients included in the meta-analysis.

	ABO-incompatible	patients/ (studies)	ABO-compatible	patients/ (studies)
follow-up (months)	37	1346 (26)	40	4943 (26)
age	47	1346 (26)	45	4943 (26)
donor age	48	940 (22)	49	4029 (22)
total HLA MM	3.6	1208 (23)	3.1	4083 (23)
PRA “any prespecified” (%)	15	783 (13)	15	2499 (13)
DSA (%)	4	689 (10)	4	2312 (10)
retransplantation (%)	10	1004 (17)	10	3592 (17)
pre-emptive (%)	27	999 (18)	28	4138 (17)
living-related (%)	47	255 (6)	62*	593 (6)

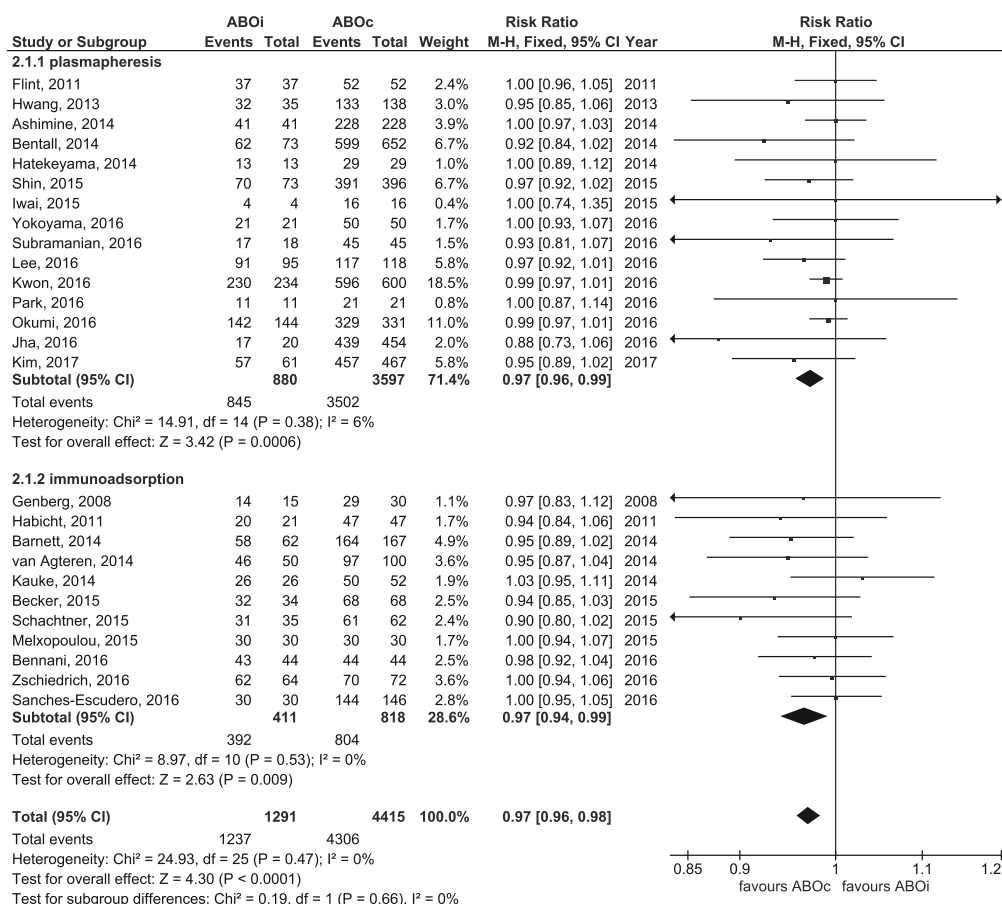
* $p < 0.0005$ HLA: human leucocyte antigen MM: mismatch PRA: panel-reactive antibody DSA: donor-specific antibody

Risk of bias

The risk of bias was low: the majority of studies (13) had no bias item, ten studies had one bias item and three studies had two bias items (Supplemental Table 3). Examples of these bias items were the use of different calcineurin inhibitors and a shorter follow-up of ABO-incompatible patients. Studies were equally divided into consecutive and matched control groups. In the majority of studies criteria for matching were scarcely explained. Supplemental Table 5 provides detailed matching information.

Graft survival (uncensored)

All 26 studies reported one-year graft survival. No study found significant differences between ABO-incompatible and ABO-compatible uncensored graft survival. Combining these data revealed inferior graft survival for ABO-incompatible patients (96% vs 98% for controls, $p=0.002$). The relative risk (RR) for one-year graft survival was decreased in ABO-incompatible patients (RR 0.97, CI 0.96-0.98, $p<0.0001$, $I^2=0\%$; $p=0.47$, Figure 2). The subgroup analysis dividing plasmapheresis and immunoadsorption revealed the same pattern ($I^2=0\%$; $p=0.66$ for subgroup differences). Graft survival remained inferior at three years (92% vs 94%, $p=0.037$, Supplemental Figure 1). However, ABO-incompatible grafts surviving after one year had comparable outcomes to ABO-compatible controls three years after transplantation: uncensored graft survival between one and three years was 97% for both ABO-incompatible and ABO-compatible patients ($p=0.70$, Supplemental Figure 2).

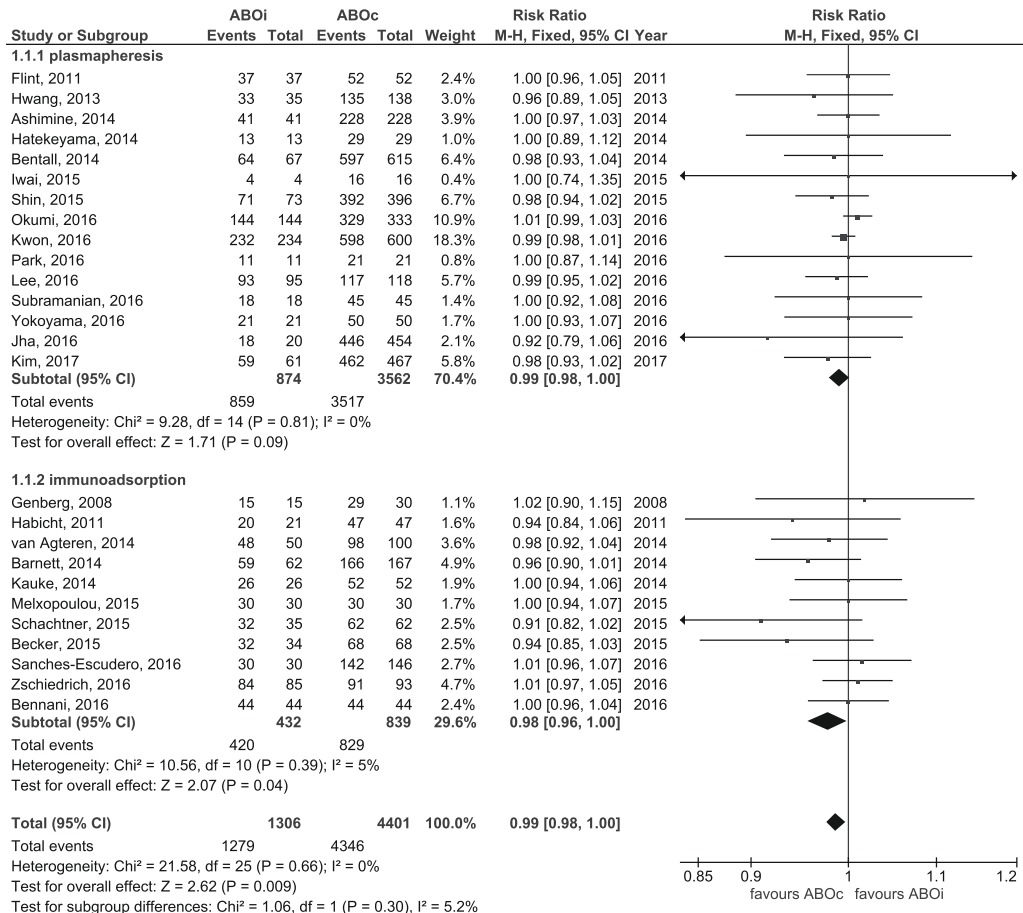


ABOc: ABO-compatible ABOi: ABO-incompatible 95% CI: 95% confidence interval M-H: Mantel-Haenszel.

Figure 2. Forest plot of comparison: ABO-incompatible kidney transplantation versus center-matched ABO-compatible control patients; outcome: One-year uncensored graft survival. Subgroup analysis: plasmapheresis versus immunoadsorption.

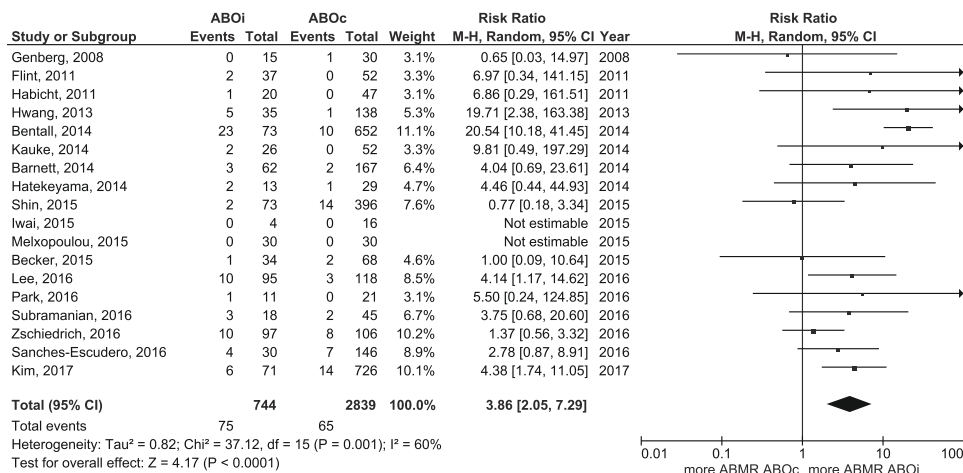
Patient survival

One-year patient survival was lower for ABO-incompatible patients (98% vs 99%, $p=0.03$). The relative risk for one-year patient survival in ABO-incompatible patients was 0.99, CI 0.98-1.00, $p=0.009$, $I^2=0\%$; $p=0.66$ (Figure 3). Fifteen articles reported on causes of death during the follow-up period. In ABO-incompatible patients 49% of reported causes of death were of infectious origin, versus only 13% in ABO-compatible patients ($p=0.02$; ABO-incompatible: 18 infection, 1 malignancy, 15 miscellaneous, 3 unknown versus ABO-compatible: 6 infection, 5 malignancy, 24 miscellaneous, 3 unknown, 9 not reported).



ABOc: ABO-compatible ABOi: ABO-incompatible 95% CI: 95% confidence interval M-H: Mantel-Haenszel.

Figure 3. Forest plot of comparison: ABO-incompatible kidney transplantation versus center-matched ABO-compatible control patients; outcome: One-year patient survival. Subgroup analysis: plasmapheresis versus immunoadsorption.



ABMR: antibody-mediated rejection ABOc: ABO-compatible ABOi: ABO-incompatible 95% CI: 95% confidence interval
M-H: Mantel-Haenszel.

Figure 4. Forest plot of comparison: ABO-incompatible kidney transplantation versus center-matched ABO-compatible control patients; outcome: Antibody-mediated rejection.

Rejection

22 studies reported on rejection. Biopsy-proven acute rejection was more common in ABO-incompatible patients (RR 1.39, CI 1.19-1.61, $p < 0.0001$, $I^2 = 38\%$; $p = 0.05$, Table 3), especially antibody-mediated rejection (RR 3.86, CI 2.05-7.29, $p < 0.00001$, $I^2 = 60\%$; $p = 0.001$, random-effect model, Table 3, Figure 4).

Infection

Severe non-viral infection

17 studies reported infectious episodes. Severe non-viral infections occurred more often in ABO-incompatible patients (RR 1.44, CI 1.13-1.82, $p = 0.003$, $I^2 = 39\%$, $p = 0.06$ Table 3).

Viral infection

18 studies reported CMV, either viremia or disease. CMV viremia was slightly more common in ABO-incompatible patients (RR 1.20, CI 1.04-1.37, $p = 0.01$, $I^2 = 17\%$, $p = 0.26$, Table 3).

BK viremia was more common in ABO-incompatible patients in nine studies, did not occur in two studies and was less common in four studies (RR 1.70, CI 1.14-2.56, $p = 0.01$, $I^2 = 45\%$, $p = 0.03$, random-effect model, Table 3).

Bleeding

Nine studies included a table with bleeding-related parameters. These outcomes occurred almost twice as often in ABO-incompatible versus ABO-compatible patients (RR 1.92, CI 1.36-2.72, $p = 0.0002$, $I^2 = 10\%$, $p = 0.35$, Table 3), both with immunoadsorption and with plasmapheresis ($I^2 = 0\%$, $p = 0.84$ for subgroup differences, Supplemental Figure 3).

Table 3. Comparison of complications after ABO-incompatible versus ABO-compatible kidney transplantation.

	% ABO- incompatible (events/total)	% ABO- compatible (events/total)	RR [CI]	<i>p</i> -value
Infection:				
severe non-viral infection	12 (105/876)	6 (163/2666)	1.44 [1.13 – 1.82]	0.005
CMV	22 (210/976)	19 (465/2462)	1.20 [1.04 – 1.37]	0.01
BK	14 (121/875)	8 (215/2644)	1.70 [1.14 – 2.56]	0.01
Biopsy-proven acute rejection	24 (193/806)	17 (523/3117)	1.39 [1.19 – 1.61]	< 0.001
Antibody-mediated rejection	10 (75/744)	2 65/2839	3.86 [2.05 – 7.29]	< 0.001
Bleeding	11 (63/579)	4 (53/1450)	1.92 [1.36 – 2.72]	< 0.001

CI: confidence interval CMV: cytomegalovirus RR: relative risk

DISCUSSION

ABO-incompatible kidney transplantation, as performed in the last decade, is considered safe as compared to ABO-compatible transplantation. The current meta-analysis shows that ABO-incompatible kidney transplant recipients have very good outcomes, but with a higher risk of losing their allograft within one year after kidney transplantation compared to center-matched ABO-compatible controls. In addition, the risks for severe infection, viral infection, antibody-mediated rejection and post-operative bleeding were all higher in the ABO-incompatible patient group.

Although inferior graft survival in the ABO-incompatible group was a consistent finding in the 26 studies included for analysis, it was not significant in any of these studies, because of insufficient number of patients per study. However, inferior graft survival has been described in some registry studies. United Network for Organ Sharing data for instance reveal inferior graft survival within the first year and, similar to the results of the meta-analysis, grafts surviving thereafter had comparable outcomes to ABO-compatible patients (45). Early graft losses occurred in the direct postoperative period (within 14 days) in a relatively large cohort of ABO-incompatible patients described by Montgomery et al. (13). A Japanese registry also revealed inferior one-year graft survival (46), but both these Japanese and American cohorts contained patients treated with splenectomy.

The largest registry study, especially in the modern era without splenectomy, the Collaborative Transplant Study (CTS) by Opelz et al. and Morath et al. (10, 11) described

1420 ABO-incompatible patients. Their outcomes show similar death-censored graft survival rates for ABO-incompatible versus ABO-compatible transplantation. However, there was a slightly decreased one-year patient survival in the ABO-incompatible group owing to infectious-related deaths. Our data are limited on cause of death but reveal the same pattern, with infection as cause of death in half of the ABO-incompatible patients versus only 13% of ABO-compatible patients. These findings are in accordance with a Korean ABO-incompatible registry reporting inferior patient survival due to 83% infection-related deaths compared to only 27% in ABO-compatible controls (12).

These registry studies lack a comparison of baseline characteristics between the ABO-incompatible and ABO-compatible cohorts but their outcomes strengthen the results of our meta-analysis: ABO-compatible controls in the meta-analysis were either matched or consecutive, leading to similar baseline clinical and demographic characteristics between the groups. Therefore it seems that the inferior outcomes after ABO-incompatible kidney transplantation cannot be contributed to “unchangeable” patient characteristics but to the procedure itself.

The explanation for the higher infection-related one-year mortality after ABO-incompatible kidney transplantation is speculative as data analysis on the single patient level was not possible. For example it could not be deciphered if antibody-mediated rejection and patient death coincided. Other potential factors that may have resulted in a higher risk of severe infection are induction therapy and plasmapheresis. The vast majority of patients in the included studies received rituximab induction. Rituximab induction on top of a standard immunosuppressive regime is considered relatively safe and was not associated with infection in a large randomized trial in kidney transplant recipients (47). In the CTS registry, rituximab resulted in better death-censored graft survival compared to no induction therapy, arguing against a major effect of rituximab on infectious complications (11). The removal of protective immunoglobulins by plasmapheresis may contribute to a higher risk for infection. However, there was no difference in mortality and infectious complications between patients receiving immunoadsorption versus plasmapheresis. This is contrary to a meta-analysis of 4810 ABO-incompatible patients stratified according to desensitization technique (17): in this study by Lo et al., after a mean of 26 months follow-up, overall graft survival was significantly worse after plasmapheresis compared to immunoadsorption.

Of note is the doubled risk of bleeding in ABO-incompatible patients, irrespective of plasmapheresis technique used. This is a consistent finding in Dutch immunoadsorption patients (48).

This meta-analysis reveals that ABO-incompatible transplantation has very good outcomes, albeit inferior than ABO-compatible transplantation: the overall uncensored graft loss at one year post-transplantation was 4.2% in the ABO-incompatible and 2.5% in the ABO-compatible patient group. These outcomes are favorable compared to remaining on dialysis or receiving a deceased donor kidney allograft (49). However, it also indicates that the ABO-incompatible procedure with its specific complications is associated with a higher one-year graft loss and mortality, although the absolute

numbers remain low. In this is the strength of this meta-analysis, since the ABO-incompatible literature lacks randomized controlled trials and guidelines are based on cohort studies generally lacking power to detect low-frequency clinically relevant differences. Statistical heterogeneity for graft and patient survival was very low in this meta-analysis. This information can assist transplant candidates and their doctors in balanced clinical decision making. It is also a further incentive to promote the use of kidney exchange programs allowing for ABO-compatible matches to be made in case of ABO incompatibility.

Publication bias is a limitation of this meta-analysis. The discussions of the included studies generally conclude favorable ABO-incompatible outcomes. Less favorable ABO-incompatible outcomes might be left unpublished and the true additive risk of ABO-incompatibility might be higher. An important consideration is that some patients cannot proceed to transplantation despite ABO-incompatible desensitization. Becker et al. for example report desensitization failures in 5 out of 39 procedures (28). Since this meta-analysis only includes transplanted patients, the true burden of ABO-incompatible desensitization is probably higher. Other limitations are the lack of individual patient data and the inconclusive reporting of match criteria. Completeness of reporting of adverse events and the observation period after transplantation differed between studies. Follow-up of ABO-incompatible patients was shorter than controls. This however may suggest that the higher incidence of complications observed after ABO-incompatible kidney transplantation is a conservative estimation. Given the slightly inferior results of ABO-incompatible kidney transplantation, it is important to optimize the possibilities for compatible transplantation: national or regional kidney exchange programs are therefore of proven value and utmost importance to improve outcomes after living kidney transplantation (50).

In conclusion, ABO-incompatible kidney transplantation has very good outcomes but is associated with a greater risk for graft loss and lower patient survival within the first year after transplantation compared to ABO-compatible controls.

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Chapter 2

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SUPPLEMENTAL MATERIALS

ABO-incompatible kidney transplant outcomes: A meta-analysis. *How safe is crossing the ABO blood group barrier?*

Supplemental Table S1. Search criteria.	
Embase.com	('blood group ABO incompatibility'/de OR 'blood group incompatibility'/de OR (((abo OR ab0 OR 'blood group' OR 'type A' OR 'type A2' OR 'type A1' OR 'type B' OR 'type AB' OR 'type O') NEAR/6 (incompatib* OR mismatch* OR barrier* OR antibod*)):ab,ti) AND ('kidney transplantation'/exp OR 'renal graft dysfunction'/exp OR 'kidney donor'/de OR (transplantation/de AND kidney/exp) OR ((kidney* OR renal*) NEAR/3 (transplant* OR homotransplant* OR autotransplant* OR graft* OR allograft* OR donor* OR donat* OR recipient*)):ab,ti)
Medline (OvidSP)	("Blood Group Incompatibility"/ OR (((abo OR ab0 OR "blood group" OR "type A" OR "type A2" OR "type A1" OR "type B" OR "type AB" OR "type O") ADJ6 (incompatib* OR mismatch* OR barrier* OR antibod*)):ab,ti.) AND ("Kidney Transplantation"/ OR kidney/tr OR ((kidney* OR renal*) ADJ3 (transplant* OR homotransplant* OR autotransplant* OR graft* OR allograft* OR donor* OR donat* OR recipient*)):ab,ti.)
Cochrane	(((abo OR ab0 OR 'blood group' OR 'type A' OR 'type A2' OR 'type A1' OR 'type B' OR 'type AB' OR 'type O') NEAR/6 (incompatib* OR mismatch* OR barrier* OR antibod*)):ab,ti) AND (((kidney* OR renal*) NEAR/3 (transplant* OR homotransplant* OR autotransplant* OR graft* OR allograft* OR donor* OR donat* OR recipient*)):ab,ti)
Web of science	TS=((((abo OR ab0 OR "blood group" OR "type A" OR "type A2" OR "type A1" OR "type B" OR "type AB" OR "type O") NEAR/6 (incompatib* OR mismatch* OR barrier* OR antibod*)))) AND (((kidney* OR renal*) NEAR/3 (transplant* OR homotransplant* OR autotransplant* OR graft* OR allograft* OR donor* OR donat* OR recipient*))))
Google Scholar	"ABO ab0 incompatibility incompatible "kidney renal transplantation graft allograft donor donors donation recipient"

Supplemental Table S2. Newcastle-Ottawa quality assessment scale cohort studies. A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

	1) Representativeness of the exposed cohort
	<input type="checkbox"/> a) truly representative of the average kidney transplant recipient *
	<input type="checkbox"/> b) somewhat representative of the average kidney transplant recipient *
	<input type="checkbox"/> c) selected group
	<input type="checkbox"/> d) no description of the derivation of the cohort
	2) Selection of the control group
Selection	<input type="checkbox"/> a) drawn from the same community as the exposed cohort *
	<input type="checkbox"/> b) drawn from a different source
	<input type="checkbox"/> c) no description of the derivation of the non-exposed cohort
	3) Ascertainment of exposure
	<input type="checkbox"/> a) secure record (eg medical file) *
	<input type="checkbox"/> b) structured interview *
	<input type="checkbox"/> c) written self report
	<input type="checkbox"/> d) no description
	4) Demonstration that outcome of interest was not present at start of study
	<input type="checkbox"/> a) yes *
	<input type="checkbox"/> b) no
Comparability	1) Comparability of cohorts on the basis of the design or analysis
	<input type="checkbox"/> a) study controls for baseline immunosuppression (TAC vs CsA vs mTORI) *
	<input type="checkbox"/> b) study controls contemporaneity *
	1) Assessment of outcome
	<input type="checkbox"/> a) independent blind assessment *
	<input type="checkbox"/> b) record linkage *
	<input type="checkbox"/> c) self report
	<input type="checkbox"/> d) no description
Outcome	2) Was follow-up long enough for outcomes to occur
	<input type="checkbox"/> a) yes (one year) *
	<input type="checkbox"/> b) no
	3) Adequacy of follow-up of cohorts
	<input type="checkbox"/> a) complete follow-up of all subjects accounted for *
	<input type="checkbox"/> b) subjects lost to follow-up unlikely to introduce bias: small number lost > 90% follow-up, or description provided of those lost*
	<input type="checkbox"/> c) follow-up rate < 90% and no description of those lost
	<input type="checkbox"/> d) no statement

TAC: tacrolimus CsA: ciclosporin mTORI: mammalian target of rapamycin

Supplemental Table S3. Bias assessment.

Study maximum score	selection ****	comparability **	outcome ***	bias item(s)
Ashimine, 2014 (16)	****	-	***	calcineurin inhibitor contemporaneity
Barnett, 2013 (27)	****	*	***	calcineurin inhibitor
Becker, 2015 (28)	****	*	***	calcineurin inhibitor
Bennani, 2016 (29)	****	**	**	follow-up
Bentall, 2014 (30)	****	**	***	-
Flint, 2011 (31)	****	**	***	-
Genberg, 2008 (1)	****	**	***	-
Habicht, 2011 (32)	****	**	***	-
Hatekeyama, 2014 (6)	****	**	***	-
Hwang, 2013 (33)	****	**	***	-
Iwai, 2015 (34)	****	**	**	follow-up
Jha, 2016 (35)	****	*	**	calcineurin inhibitor follow-up
Kauke, 2016 (36)	****	*	***	calcineurin inhibitor
Kim, 2017 (37)	****	*	***	contemporaneity
Kwon, 2016 (38)	****	*	***	contemporaneity
Lee, 2016 (39)	****	**	**	follow-up
Melexopoulou, 2015 (40)	****	*	***	calcineurin inhibitor
Okumi, 2016 (7)	****	**	***	-
Park, 2016 (41)	****	**	***	-
Sanches-Escudero, 2016 (42)	****	**	***	-
Schachtner, 2015 (4)	****	**	***	-
Shin, 2015 (8)	****	**	***	-
Subramanian, 2016 (9)	****	**	***	-
Van Agteren, 2014 (43)	****	**	***	-
Yokoyama, 2016 (44)	****	*	***	calcineurin inhibitor
Zschiedrich, 2016 (5)	****	*	**	contemporaneity follow-up

Supplemental Table S4. Follow-up of patients in the included studies and time span of reporting on rejection and infectious complications.

Study	study follow-up (months)	rejection follow-up (months)	infection follow-up (months)
Ashimine, 2014 (16)	ABOi: 36 ABOc: 52	not specified	not specified
Barnett, 2013 (27)	ABOi: 26 ABOc: 33	12	not applicable
Becker, 2015 (28)	ABOi: 22 ABOc: 20	during follow-up	during follow-up
Bennani, 2016 (29)	ABOi: 6 ABOc: 6	6	6
Bentall, 2014 (30)	ABOi: 67 ABOc: 73	12	not applicable
Flint, 2011 (31)	ABOi: 26 ABOc: 22	12	12
Genberg, 2008 (1)	ABOi: 41 ABOc: 48	during follow-up	during follow-up
Habicht, 2011 (32)	ABOi: 17 ABOc: 15	during follow-up	during follow-up
Hatekeyama, 2014 (6)	ABOi: 28 ABOc: 37	during follow-up	during follow-up
Hwang, 2013 (33)	24 (no detailed follow-up information)	during follow-up	during follow-up
Iwai, 2015 (34)	ABOi: 39 ABOc: 38	during follow-up	during follow-up
Jha, 2016 (35)	ABOi: 10 ABOc: 17	during follow-up	during follow-up
Kauke, 2016 (36)	12 (no detailed follow-up information)	12	12
Kim, 2017 (37)	ABOi: 27 ABOc: 42	12	during follow-up
Kwon, 2016 (38)	36 (no detailed follow-up information)	not applicable	during follow-up
Lee, 2016 (39)	ABOi: 34 ABOc: 36	during follow-up	during follow-up
Melexopoulou, 2015 (40)	ABOi: 74 ABOc: 78	during follow-up	during follow-up
Okumi, 2016 (7)	ABOi: 48 ABOc: 56	not applicable	during follow-up
Park, 2016 (41)	ABOi: 15 ABOc: 15	12	during follow-up

Supplemental Table S4 (continued). Follow-up of patients in the included studies and time span of reporting on rejection and infectious complications.

Study	study follow-up (months)	rejection follow-up (months)	infection follow-up (months)
Sanches-Escudero, 2016 (42)	ABOi: 21 ABOc: 21	12	not applicable
Schachtner, 2015 (4)	ABOi: 42 ABOc: 37	12	during follow-up
Shin, 2015 (8)	ABOi: 39 ABOc: 46	during follow-up	during follow-up
Subramanian, 2016 (9)	29 (no detailed follow-up information)	12	not applicable
Van Agteren, 2014 (43)	ABOi: 38 ABOc: 38	not applicable	not applicable
Yokoyama, 2016 (44)	12 (no detailed follow-up information)	12	12
Zschiedrich, 2016 (5)	ABOi: 58 ABOc: 48	during follow-up	during follow-up

ABOc: ABO-compatible ABOi: ABO-incompatible

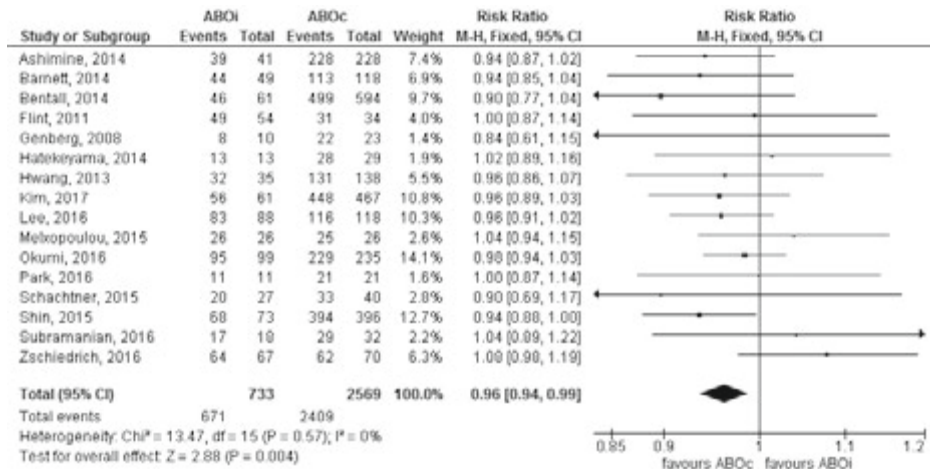
Supplemental Table S5. Selection of study cohort and control group.

Study	period	study group	selection of controls
Ashimine, 2014 (16)	2005-09	DSA-positive patients excluded	consecutive
Barnett, 2013 (27)	2005-11		consecutive
Becker, 2015 (28)	2005-13	DSA-positive patients excluded	matching 2:1 (ABO _c : ABO _i): one transplantation directly before and one directly after ABO _i procedure
Bennani, 2016 (29)	2011-15		matching: 1:1 gender, age, time of transplantation
Bentall, 2014 (30)	1999- 2006	FACS-positive crossmatch excluded	consecutive
Flint, 2011 (31)	2005-08	excluded: pretransplant rituximab donor-specific antibodies	matching: immunosuppression
Genberg, 2008 (1)	2001-05	FACS-positive crossmatch excluded	matching: initial immunosuppressive therapy
Habicht, 2011 (32)	2007-09	FACS-positive crossmatch excluded	consecutive
Hatekeyama, 2014 (6)	2006-13		consecutive
Hwang, 2013 (33)	2009-11		matching: initial immunosuppressive therapy
Iwai, 2015 (34)	2001-14	all recipients aged >60 years with a spousal transplant	consecutive
Jha, 2016 (35)	2011-14		consecutive
Kauke, 2016 (36)	2007-12	DSA-positive patients excluded	matching: maintenance immunosuppressive therapy
Kim, 2017 (37)	2010-16	DSA-positive patients excluded	matching: maintenance immunosuppressive therapy
Kwon, 2016 (38)	2012-15	FACS-positive crossmatch excluded	matching: maintenance immunosuppressive therapy
Lee, 2016 (39)	2010-14	FACS-positive crossmatch excluded 43 ABO _c patients were excluded because of rituximab treatment	matching: maintenance immunosuppressive therapy
Melexopoulou, 2015 (40)	2005-13		matching 1:1: 'randomly selected on the basis of similar baseline demographic and clinical characteristics of donors and recipients'

Supplemental Table S5 (continued). Selection of study cohort and control group.

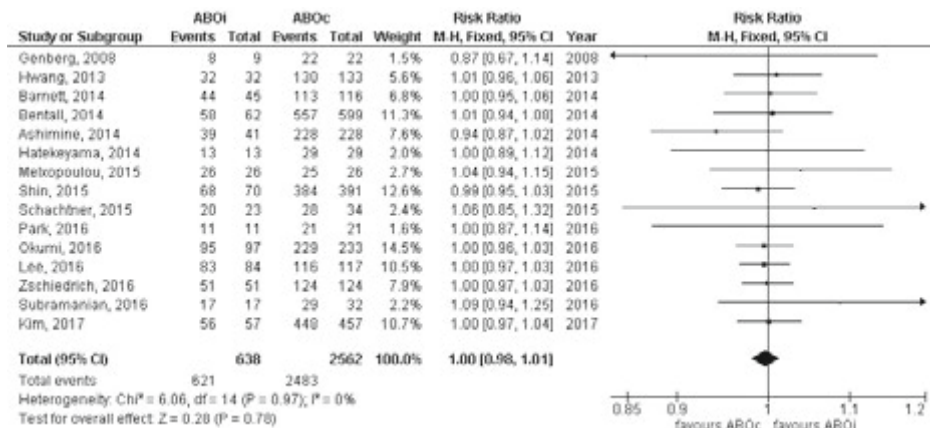
Study	period	study group	selection of controls
Okumi, 2016 (7)	2005-13	FACS-positive crossmatch excluded	consecutive
Park, 2016 (41)	2011-13	all spousal transplants	consecutive
Sanches-Escudero, 2016 (42)	2011-13		consecutive
Schachtner, 2015 (4)	2005-12		matching: basiliximab induction, maintenance immunosuppression, availability of virology screening
Shin, 2015 (8)	2009-12	FACS-positive crossmatch excluded	consecutive
Subramanian, 2016 (9)	2007-12		matching not described
Van Agteren, 2014 (43)	2006-12		matching 2:1 (ABOc : ABOi): age, number of HLA mismatches
Yokoyama, 2016 (44)	2008-13		consecutive
Zschiedrich, 2016 (5)	2004-14	ciclosporin-treated patients excluded	consecutive

ABOc: ABO-compatible ABOi: ABO-incompatible DSA: donor-specific antibodies FACS: flowcytometry



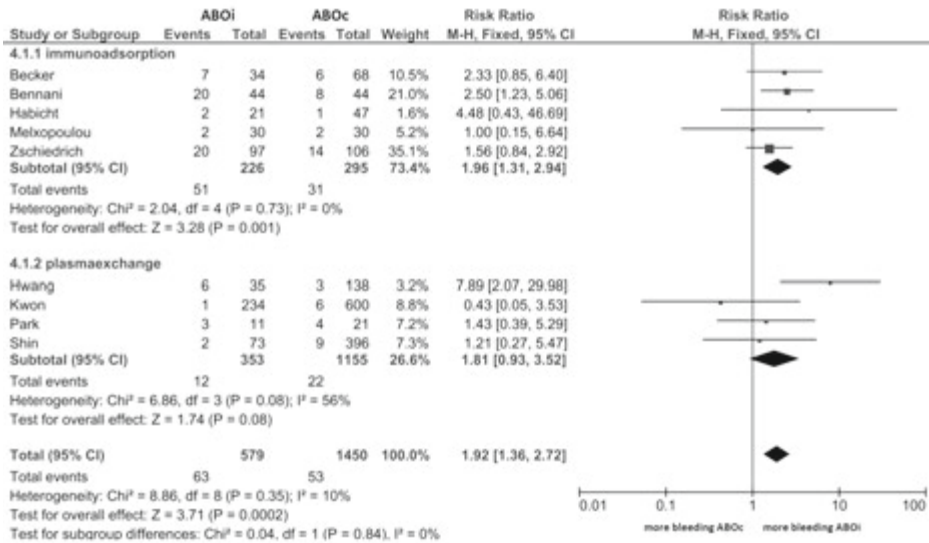
ABOc: ABO-compatible ABOI: ABO-incompatible 95% CI: 95% confidence interval M-H: Mantel-Haenszel.

Supplemental Figure S1. Forest plot of comparison: ABO-incompatible kidney transplantation versus center-matched ABO-compatible control patients; outcome: Graft survival uncensored year 3.



ABOc: ABO-compatible ABOI: ABO-incompatible 95% CI: 95% confidence interval M-H: Mantel-Haenszel.

Supplemental Figure S2. Forest plot of comparison: ABO-incompatible kidney transplantation versus center-matched ABO-compatible control patients; outcome: Graft survival uncensored between year one and year three.



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ABOc: ABO-compatible ABOi: ABO-incompatible 95% CI: 95% confidence interval M-H: Mantel-Haenszel.

Supplemental Figure S3. Forest plot of comparison: ABO-incompatible kidney transplantation versus center-matched ABO-compatible control patients; outcome: Bleeding. Subgroup analysis: plasmapheresis versus immunoadsorption.

REFERENCES

See page 35.



Chapter 3

The first fifty ABO blood group incompatible kidney transplantations; the Rotterdam experience.

Madelon van Agteren, Willem Weimar, Annelies E de Weerd, Peter AW te Boekhorst, Jan NM IJzermans, Jacqueline van de Wetering, Michiel GH Betjes.

J Transplant. 2014; 913902.

ABSTRACT

This study describes the single center experience and long-term results of ABOi kidney transplantation using a pre-transplantation protocol involving immunoadsorption combined with rituximab, intravenous immunoglobulins and triple immune suppression.

Fifty patients received an ABOi kidney transplant in the period from 2006 to 2012 with a follow-up of at least one year. Eleven antibody mediated rejections were noted of which 5 were mixed antibody and cellular mediated rejections. Nine cellular mediated rejections were recorded. Two grafts were lost due to rejection in the first year. One-year graft survival of the ABOi grafts was comparable to 100 matched ABO compatible renal grafts, 96 % vs. 99%. At 5 year follow-up, the graft survival was 90% in the ABOi vs. 97% in the control group.

Post-transplantation immunoadsorption was not an essential part of the protocol and no association was found between antibody titers and subsequent graft rejection. Steroids could be withdrawn safely 3 months after transplantation. Adverse events specifically related to the ABOi protocol were not observed.

The currently used ABOi protocol shows good short- and mid-term results despite a high rate of antibody mediated rejections in the first years after the start of the program.

INTRODUCTION

Matching for the antigens of the human ABO blood group system is necessary when foreign cells or organs are considered for donation. If not matched properly, the circulating anti-A and/or anti-B blood group antibodies of the recipient will bind to the antigenic moieties of the cell surface-bound A and B blood group molecules within the kidney transplant (1). The antibodies attached will activate the complement system leading to local cell damage and eventually cell and organ destruction (2). Therefore, ABO-incompatible (ABOi) kidney transplantation carries a high risk for acute and irreversible antibody mediated rejection and cannot be performed without pretreatment of the recipient (3, 4).

Pretreatment of the recipient is aimed at substantial lowering of the concentration of circulation antibodies before transplantation and reducing the subsequent production of these antibodies. To this end, a number of protocols have been developed that originally included plasmapheresis for antibody removal and splenectomy for permanent reduction of antibody production. In most protocols, high dose intravenous immunoglobulins were also peri-operatively given as this exerts a pleiotropic immune suppressive effect, particularly in the case of antibody mediated immune diseases. However, in recent years new protocols have been developed based on the use of the B cell depleting antibody Rituximab and the availability of an immunoabsorption column that specifically binds anti-A or anti-B antibodies. This column is able to clear efficiently these antibodies from the plasma, thereby obviating the need for plasma exchange. The Swedish ABOi kidney transplantation protocol was among the first that successfully combined these new treatment modalities into a highly effective pretreatment protocol (5). Published data have shown remarkably good short- and long-term acceptance and functioning of the ABOi transplanted kidneys using this protocol (6, 7).

In our transplantation center in the Netherlands we adopted the Swedish protocol and started the ABOi transplantation program in 2006. Over the years we have left out parts of this protocol and the use of steroids was stopped after 3 months in the post-transplantation period, similar to our standard immune suppressive protocol for ABOc patients.

The long-term results of the first 50 ABOi kidney transplants in a period of 5 years are now reported in detail and safety and long-term results are in accordance with other reports. However, early antibody mediated rejections were observed more frequently than previously described although in the majority timely treatment was effective. Post-operative removal of antibodies and continuation of prednisone beyond three months after transplantation did not appear to be essential for the success of the program.

PATIENTS AND PROTOCOL

Living ABOi kidney donor-recipient combinations were evaluated for the ABOi procedure after routine pretransplantation screening. Patients with titers of IgM and IgG antibodies against blood group A or B below or equal to 1:128 were considered eligible for ABOi kidney transplantation. At first, only O recipients and AB donors were included. The remaining ABOi couples participated in the Dutch national kidney exchange program, because of their good chance to find suitable donors. The protocol described by Tyden et al. (5) was followed with the exception that plasma for immunoadsorption was generated from the blood by a plasma separating dialyzer and not by centrifugation. The immunoadsorption was performed using a specific adsorption column for anti-A or anti-B antibodies (Glycorex Transplantation AB, Lund, Sweden). If needed, the routine hemodialysis session was combined with the immunoadsorption procedure. Such a simultaneous session was performed without specific problems and with similar adequacy as immunoadsorption alone. All patients were given a single dose rituximab (375 mg/m²) one month prior to kidney transplantation. Two weeks before transplantation, mycophenolic acid (1000 mg bid), tacrolimus twice daily (target trough level 10-15 µg/L) and prednisone 20 mg once daily were given. The immunoadsorption procedure was performed daily before the transplantation. The number of sessions was dependent on the height of the titer of anti-donor blood group antibodies and the rebound after every session. Therefore, the number of pretransplantation immunoadsorptions varied with a median of 4 sessions (range 0 to 7). During the procedure 6 liters of plasma were passed over the column and antibody titers were assessed before and after the procedure. The kidney transplantation was performed the day after the last immunoadsorption but only if the post-adsorption IgM and IgG antibody titers were below 1:8. In 4 patients no immunoadsorption was performed as the anti-ABO titers were already <1:8. Following the original protocol, we performed 3 immunoadsorptions at days 1, 4 and 7 post-operatively in the first 25 patients. The day before kidney transplantation, after the last immunoadsorption, 0.5 gram/kg IVIG was given. In all patients, prednisone was stopped 3 months after transplantation and tacrolimus trough levels were adjusted to 5-10 µg/L, following our standard post-transplantation treatment protocol. No T-cell depleting agent was used for induction therapy.

The percentage of CMV seropositive patients was 66% and routinely all our transplantation patients, except for CMV-/- combinations, received prophylaxis with valganciclovir during the first 6 months after kidney transplantation. Routine kidney biopsies were performed in the first years of our ABOi kidney transplantation program but this policy was abandoned, as it did not contribute to clinical decision-making (see results section).

A kidney biopsy by indication was performed if rejection was suspected on the grounds of an unexpected halt in improvement or worsening of kidney function. Histological confirmation of rejection was obtained by following the Banff criteria 07

for acute cellular en antibody mediated rejection (AMR). Acute AMR was treated with extra immunoadsorptions, high dose steroids (1000 mg prednisolon/day for 3 days) and IVIG (1 gram/kg). In case of unresponsiveness, T-cell depleting therapy was given according to our local protocol.

The first 25 patients received post-operative immunoadsorptions per protocol and were routinely monitored for isoagglutinin titers after transplantation. As the isoagglutinin titers remained low and no rebound occurred, we abandoned the policy of routine post-transplantation immunoadsorptions.

For the purpose of comparison, 2 cases of ABO compatible kidney transplantation were selected from the same period and matched for age and number of HLA mismatches for every case of ABOi kidney transplantation. The post-transplantation immunosuppressive medication protocol was similar to the ABOi protocol except for induction therapy with basiliximab in all patients from 2007 onwards.

Determination of antibody titers

Patient serum was tested for the presence of IgM and IgG antibodies against the ABO-blood group antigens of the donor using the red blood cells (RBC) of the donor. Hemagglutination was assessed fully automated using the ORTHO BioVue system column agglutination technology. Test RBC, with or without serum, were placed in the chamber above the 6-microcolumn cassette preloaded with diluent and/or reagent and glass beads. Upon centrifugation, RBC are forced through the bead column where agglutinated cells are trapped, while unagglutinated RBC travel to the bottom of the column, forming a discrete pellet. The isohemagglutinin titer is determined by the highest dilution of patient serum that still results in donor RBC agglutination.

Statistical analysis

The SPSS software version 18.0 was used for all statistical tests. Descriptive statistics were used to summarize baseline characteristics. Distribution of data was tested using the Kolmogorov-Smirnov test. Continuous variables with a normal distribution are presented as means (SD) and compared using parametric t-tests. Skewed distributed continuous variables are presented as medians and compared using the non-parametric Mann-Whitney test. Categorical variables are presented as numbers and/or percentages. For selected comparison between two group proportions, the chi-square test was used. Survival percentages were analyzed with the Kaplan-Meier method. A p-value of less than 0.05 was considered significant; all probabilities were 2-tailed.

RESULTS

Patients' characteristics

From the start of the ABOi program in 2006 until March 2012, a total of 50 patients received an ABOi kidney transplant from a living donor. Clinical follow-up was at

least one year with a median follow-up of 38 months. The clinical and demographical patient characteristics are shown in Table 1. The majority of ABOi donor-recipient combinations were from an A and/or B positive donor to an O positive recipient (Table 1). This selection is caused by our national living kidney donor exchange program, which can accommodate an appropriate ABO compatible match for most cases except the O recipients and A and B donors.

Table 1. Clinical and demographic characteristics of patients receiving a blood type ABO-incompatible (ABOi) kidney transplant and matched ABO-compatible (ABOc) controls.

	ABOi	ABOc	<i>p-value</i>
number of patients	50	100	
age recipient (median and range)	54 years (22-75)	55 years (19-77)	n.s.*
age donor (median and range)	50 years (26-75)	55.5 years (23-70)	n.s.
donor male:female ratio	27:23	58:42	
recipient male:female ratio	32:18	63:37	
previous transplantation (%)	18 %	13 %	n.s.
previous pregnancy (%)	30 %	34 %	n.s.
previous blood transfusion (%)	20 %	24 %	n.s.
pre-emptive transplantation	10 (20%)	10 (10%)	n.s.
number HLA MM (median)	4	4	n.s.
ABO blood group recipient (number and % of total)	O 34 (68%) A 8 (16%) B 8 (16%) AB 0 (0%)	O 39 (39%) A 45 (45%) B 13 (13%) AB 3 (3%)	
% of patients with panel reactive antibodies >4 %**	10%	4%	n.s.

*n.s.: not significant (p -value >0.05) **most recent % of panel reactive antibodies positivity obtained before kidney transplantation. A PRA of <5% was considered as negative by the reference laboratory (Leiden, the Netherlands).

Kidney allograft survival of ABOi transplantations

The majority of ABOi patients showed an uncomplicated clinical course and graft survival censored for death at 1 year, 3 years and 5 years was 96%, 90% and 90% compared to respectively 98%, 96% and 96% in the ABOc group (Figure 1). ABOi graft survival was slightly worse but not statistically different from the ABO compatible control group (log rank analysis $p=0.43$). Two patients in the ABOi group died during follow-up; one suicide and one patient because of abdominal sepsis due to an incarcerated hernia cicatricalis. Serum creatinine concentrations at 1 and 3 years follow-up were significantly higher ($p < 0.05$) in the ABOi group (median 137 $\mu\text{mol/L}$ and 154 $\mu\text{mol/L}$) compared to the ABOc group (median 114 $\mu\text{mol/L}$ and 123 $\mu\text{mol/L}$). This difference in graft function was largely attributable to the decreased graft function in ABOi patients after their AMR had resolved. The median serum creatinine concentration in the ABOi patient group without AMR was 135 $\mu\text{mol/L}$ at 1 year and 124 $\mu\text{mol/L}$ at 3 years follow-up. For ABOi patients with early AMR these values were 172 $\mu\text{mol/L}$ and 172 $\mu\text{mol/L}$, respectively.

Two patients had an exceptionally poor renal function at 3 months post-transplantation. One case was caused by a surgical problem leading to severe problems with renal blood supply and subsequent partial renal infarction and the other case was due to an AMR resistant to treatment. Of note is one patient that presented 3 months post-transplantation with an urosepsis with *Serratia* species and a very severe acute AMR necessitating transplantectomy. Anti-A titers had sharply risen (IgM >5000 and IgG 512).

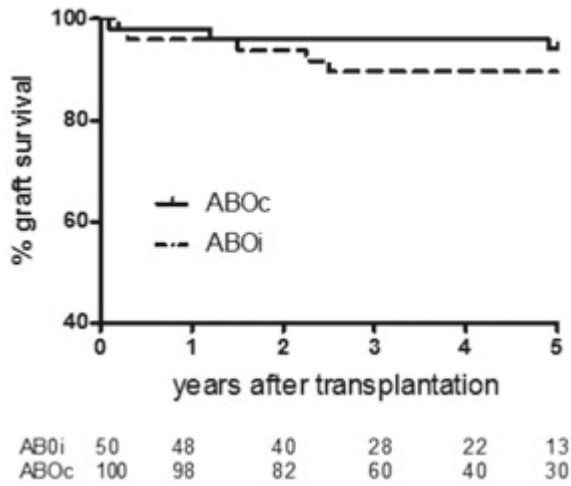


Figure 1. Renal allograft survival censored for death in the blood group ABO incompatible (n=50) and matched ABO compatible (n=100) transplantations.

Numbers of patients in study at every year after transplantation are shown below the x-axis.

Kidney allograft rejection of ABOi transplantations

In eleven patients acute AMR was observed, all within the first week after transplantation except for the case of late acute AMR described above. Donor specific antibodies against HLA were only found in 2 patients, both having anti-HLA-DQ7 antibodies with a MFI of 20.000 in the single antigen beads Luminex assay. The C4d staining was positive in 8 biopsies, weak to focally positive in 2 and negative in 1 kidney biopsy. Five of these early rejections were categorized as AMR at the time of biopsy and responded to treatment with IVIG and steroids. In 5 cases the renal biopsy showed a mixed type rejection with evidence for the co-existence of both cellular and AMR. T-cell depleting therapy was used in case of an inadequate response to IVIG and steroids. In one of these patients graft loss occurred 3 months after transplantation because of treatment resistant AMR. One patient had an acute cellular rejection at 7 months when his tacrolimus through level was inadvertently low.

Routine allograft kidney biopsies at day 7 after kidney transplantation showed C4d positive staining in 8 out of 19 (42%) patients but without other positive criteria to support the diagnosis of AMR.

We could not find any significant relation between antibody titers post-operatively and subsequent AMR, as all titers remained low after transplantation (<1:8, data not shown). Because of this observation, we stopped the post-transplantation

immunoabsorptions after the first 25 patients. This change in protocol did not alter the frequency of AMR and in fact most AMR episodes were observed in the patients that received post-transplantation immunoabsorptions (9 out of 11 patients with AMR). In the group of ABOi patients with an early AMR episode only one patient was diagnosed with transplant glomerulopathy seven years after transplantation.

Adverse events

All adverse events, possibly related to a change in immune suppression, notably the use of rituximab and IVIG, were recorded. The post-transplantation viral infections in the ABOi group were caused by cytomegalovirus (n=2), BK virus (n=3) and herpes zoster virus (n=3). In addition, 1 case of *Pneumocystis carinii* pneumonia was recorded. The frequency of these infections is in the range of our ABO compatible program and cannot be specifically related to the use of rituximab or IVIG.

DISCUSSION

Newly developed desensitization protocols have greatly facilitated the development of ABOi kidney transplantation programs in many countries. Similar to other groups, we have successfully applied the Swedish desensitization protocol for more than 5 years and did not observe any significant side effects. By combining the immunoabsorption procedure with hemodialysis it was also possible to reduce the total number of procedures. This is not only time-effective but also less burdensome for dialysis patients.

In accordance with a number of other studies (8-11), the graft survival of ABOi kidney transplants was not significantly different from matched ABOc kidney transplants. However, it should be noted that the median serum creatinine concentration in the ABOi group was higher at follow-up compared to the ABOc group. This was due to the subgroup of ABOi patients who experienced an AMR. Although the rejection episodes could be treated effectively in most of these patients, the graft function was permanently negatively affected. However, progressive loss of graft function because of ongoing AMR was not observed and overall 5 years graft survival was therefore not significantly lowered compared to the ABOc group. However, the median follow-up of 3 years is relatively short and close monitoring of the ABOi graft results is mandatory. Most cases of AMR occurred within the first 25 patients (9 out of 11 patients with AMR) included in the program and were related to ABO incompatibility, as we could detect DSA only in 2 cases. In spite of a thorough analysis of our procedure we cannot explain the high frequency of AMR at the start of our ABOi program with a subsequent decrease. The frequency of AMR in our study is in contrast to publications by other groups using an identical protocol (8, 9, 12) but a similar or higher rate of rejection has been described by others using different pretransplantation desensitization procedures (12-14). This highly variable incidence of AMR between the published case

series is difficult to explain. Although different definitions of acute rejection may play a role, our definition of a rise in serum creatinine concentration combined with biopsy proven AMR did not leave any uncertainty about the diagnosis. Others have found an increased frequency of AMR in blood group O recipients (15), which may have negatively influenced our results, as the majority of patients in our study carried the blood group O serotype. However, Tyden et al. had a similar high percentage of these patients in his ABOi program but still had a remarkably low incidence of AMR (7). Therefore, the relevance of the O blood group in the recipient remains an open question.

In agreement with the experience of others (16, 17), we used an upper limit for anti-ABO IgG antibody titers of 1:256 to allow patients to enter our program. Notably, the determination of anti-donor blood type antibody titers is susceptible to subjective interpretation of the semi-quantitative test result and the same serum sample may give very different results when independently tested in different laboratories (18). This large variation in determination of titers precludes any sound comparison between the results from different centers and may partly explain the differences in the incidence of AMR. In our protocol we excluded patients with high anti-ABO blood group titers, as it was very difficult to reach a titer of <1:8 in these cases. However, it is not known what constitutes a safe threshold for the pretransplant antibody titer. Again, the lack of a uniform and reliable test for measurement of antibody titers limits analysis of the combined published data.

We did not observe a relation between post-transplantation anti-A/B titers and rejection, and all post-transplantation titers remained low. Therefore, we removed the post-transplantation immunoadsorptions from the protocol. As stated before, the frequency of AMR actually was lower in the 25 patients who did not receive the post-operative immunoadsorptions. Other groups have also reported on the results of ABO-incompatible transplantation without post-transplantation plasmapheresis or immunoadsorption, confirming that such a strategy can indeed be followed without a significant increase in the incidence of AMR (11, 13, 14, 19).

The combined elements of the current ABOi protocol have been chosen on a rationale based on the proposed mechanism of action of each drug. For instance, pretreatment with rituximab depletes the circulating B-cell population. This may be important in the prevention of increased anti-A and anti-B antibody synthesis after ABOi kidney transplantation as rituximab treatment diminishes de novo humoral immune responses. However, a recent report showed that a rituximab-free ABOi protocol yields similar excellent short- and long-term results after kidney transplantation (20). This is of considerable interest as rituximab treatment is not only costly but also has been associated with increased frequencies of viral infections including BK and polyoma viral infections (8, 21, 22).

Given these results, it seems that a simplified protocol with immunoadsorption and immune suppressive drugs in the pretransplantation period is equally effective. The need for IVIG in the ABOi protocol remains to be established as this is not a standard

procedure in some other ABO-incompatible kidney transplantation protocols (8, 14, 19, 23).

After transplantation we routinely stopped prednisone and continued with tacrolimus and MMF. Others found that this policy may lead to an increase in the incidence of acute rejection and is associated with a decreased graft survival (24). However, prednisone could be safely stopped at 3 months after ABOi kidney transplantation in our group of patients.

In conclusion, the use of the “Swedish protocol” with immunoadsorption, IVIG and rituximab added to a standard triple immune suppressive regimen, has been used successfully for more than 5 years in our transplantation center despite an initial high frequency of AMR. It allows for ABOi kidney transplantation with good long-term graft survival comparable to ABO compatible kidney transplantation. Important questions, such as the upper level of anti-A and anti-B titers that can be accepted before kidney transplantation and the optimal preconditioning treatment, remain to be answered. The ABOi treatment protocol is of value for those patients that cannot be matched to a donor kidney because of blood group incompatibility. Although our center is involved in an effective national donor kidney exchange program, the O-positive recipients benefit in particular from the ABOi protocol as they have the lowest chance of success in the kidney exchange program (25, 26).

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Chapter 4

ABO-incompatible kidney transplant patients have a higher bleeding risk after antigen-specific immunoadsorption.

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ABSTRACT

Background

Pre-transplant removal of anti-blood group ABO antibodies is the cornerstone of all current ABO-incompatible (ABOi) transplantation programs. In our protocol, plasmapheresis (PP) is performed with a plasmafilter followed by immunoadsorption (IA) of anti-ABO antibodies. The bleeding complications of this technique are not known.

Methods

We analysed the data of all 65 consecutive ABOi kidney transplantations between March 2006 and October 2013 and compared these with matched 130 ABO-compatible (ABOc) kidney transplantations. Cases differed from controls in the preoperative regimen which included IA-PP and rituximab, tacrolimus, mycophenolate mofetil, prednisone and immunoglobulines. Data on platelet count, blood loss and red blood cell (EC) transfusions during 48 hours postoperatively were collected.

Results

ABOi patients received EC transfusions more frequently than controls (29% vs. 12%, $p=0.005$). Intra-operative blood loss was higher (544 vs. 355 ml, $p < 0.005$) and they experienced more major bleeding (≥ 3 EC within 24 hours, 15% vs. 2%, $p < 0.0005$). Platelet count decreased by 28% after the pre-operative IA-PP. In a multivariate model, only the number of preoperative IA-PPs was associated with the number of ECs given (OR per IA-PP 1.9, $p < 0.05$).

Conclusion

ABOi kidney transplant patients have a high postoperative bleeding risk, correlating with the number of preoperative IA-PP sessions performed.

INTRODUCTION

The cornerstone of ABO incompatible (ABOi) and HLA antibody desensitization protocols is reduction of pre-existing concentrations of antibodies against ABO blood group or HLA antigens within the graft recipient prior to transplantation. In particular, ABO-incompatible kidney transplantation is now accepted as a suitable alternative transplantation program with excellent outcomes for patient and graft survival. This has resulted in increasing numbers of patients receiving an ABOi kidney graft worldwide and a better use of the potential of living kidney donors (1-4). Plasmapheresis (PP) is an essential procedure within all current desensitization protocols and may consist of plasma separation by either a centrifugation technique or by plasmafiltration using a large pore hemofilter. During PP the plasma is exchanged for either fresh frozen plasma (FFP) or a human albumin solution. In the case of ABO desensitization, the need for plasma exchange can be circumvented by the use of immunoadsorption (IA). In this particular procedure, the separated plasma is led over an anti-ABO adsorbing column before returning to the circulation of the patient. In patients undergoing PP an increased risk for hemorrhage has been reported, which can in part be attributed to the removal of coagulation factors when plasma is exchanged for albumin solution instead of FFP. In addition, the anticoagulants used during PP treatment can cause bleeding complications and PP with the centrifugation technique leads to substantial loss of platelets of up to 50% (5). In PP using the filtration technique an occasional case of platelet depletion was described (6), but any association with bleeding tendency has not been reported. Also in ABOi kidney transplantation there are indications that the desensitization procedure may be associated with a higher incidence of bleeding complications postoperatively (7-9). However, there has not been a systematic analysis of bleeding complications associated with the ABOi desensitization procedure in a large group of patients compared to ABO-compatible (ABOc) controls. In our protocol, PP is performed with a plasmafilter followed by IA of anti-ABO antibodies using the Glycorex device. To study the possible bleeding complications in more detail, we analyzed intra-operative blood loss and the need for red blood cell (EC) transfusions in 65 consecutive ABOi kidney transplant patients, compared this to 130 matched ABOc controls and studied the causative mechanisms.

MATERIAL AND METHODS

Patients

We analyzed all consecutive patients of a blood group ABOi kidney transplant between March 2006 and October 2013 in the Erasmus Medical Center in Rotterdam, the Netherlands (n=65). Controls were patients of an ABOc kidney transplant matched for age of donor and recipient in this period (n=130). Cases differed from controls in the preoperative regimen as has been described in detail before (10). In short, the regimen included rituximab 375 mg/m² 4 weeks before transplantation; tacrolimus 0.1 mg/kg

BID, mycophenolate mofetil 1000 mg BID; prednisone 20 mg once daily starting two weeks before transplantation and immunoglobulines 0.5 g/kg one day preoperatively. Furthermore, PP was performed pre-operatively in all but three ABOi patients. PP was performed with a plasmaFlux PSu filter (Fresenius Medical Care, Bad Homburg, Germany) followed by adsorption of anti-ABO antibodies with the Glycosorb® device, coated with synthetically derived blood group A or blood group B antigen (Glycorex Transplantation, Lund, Sweden). In 4 hours 1.5 L plasma/hour was led over the column, using unfractionated heparin infusion 1000 U/hour to prevent clotting. The first 30 patients additionally received also *postoperative* IA as per protocol, performed on days 3, 6 and 9 (or days 2, 5 and 8 depending on the day of surgery). ABOi and ABOc patients received the same immunosuppressive regimen after transplantation: tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID, prednisone 50 mg BID for three days and 20 mg once daily thereafter. All kidney transplant patients in our center receive unfractionated heparin 12.000 IU/24hours from 4 hours after renal artery anastomosis until postoperative day 5. During implantation, the graft is flushed with heparin.

Data collection

The following data were collected from the medical files: sex and age of donor and recipient, ABO blood group, blood urea nitrogen (BUN), dialysis dependency prior to transplantation, platelet count before IA-PP, preoperatively and during two weeks postoperatively; coagulation tests, including the activated partial thromboplastin time (aPTT) and prothrombin time-based International Normalized Ratio (PT-INR); transfusion with EC within 48 hours after surgery and during two postoperative weeks. Major bleeding was defined as ≥ 3 EC within 24 hours. Intra-operative blood loss documented in the anesthesia files was also recorded.

Statistics

For analysis the data obtained within the total ABOi and ABOc patient groups were compared for the first 48 hours after surgery. After this period, the ABOi group was divided into two groups of patients, one that had received postoperative IA-PP and one group that had not. Data were analyzed using GraphPad Prism version 6.0. Mann-Whitney U test, Wilcoxon signed rank test, paired t-test and unpaired t-test were used to determine differences between groups. Fisher's exact test was performed on discrete variables. Multivariate binary regression analysis was performed on variables known for influencing the risk of bleeding, e.g. blood group O (11, 12). For this analysis IBM SPSS Statistics 21 was used. The statistical significance level was determined as $p \leq 0.05$.

RESULTS

Baseline characteristics

Age and sex of donors and patients did not differ between groups. Patients did not differ in age (54 vs. 53 years in controls, $p > 0.1$) with the majority of patients being male (68%

vs. 67% in controls). More ABOi patients were on dialysis than controls (72 vs. 55%, $p < 0.05$) and their BUN was significantly higher than that of ABOc patients, 25 vs. 21 $\mu\text{mol/L}$ (Table 1). As expected, blood group O was overrepresented in the ABOi program (65%), since their anti-ABO antibodies against all non-type O donors limit their donor pool. In ABOc controls the reverse phenomenon was present: 38% of patients were blood group O, which is less than the reported prevalence (47%) of blood group O in blood donors in the Netherlands. A median of 4 IA-PPs was performed pre-operatively, with a range of 0 to 10 sessions depending on the original anti-ABO titer and its decline during treatment with IA-PPs (Table 1).

Immunoadsorption plasmapheresis substantially lowers platelet count but does not result in changes of coagulation tests

The platelet count before the start of the IA-PP was $233 \times 10^9/\text{L}$ on average, comparable to a mean of $230 \times 10^9/\text{L}$ in ABOc controls one day preoperatively ($p > 0.1$). However, after the preoperative IA-PPs platelet count decreased with 28%, leading to $169 \times 10^9/\text{L}$ platelets on average one day preoperatively in ABOi patients ($p < 0.0001$, Figure 1). This decline in platelet count led to a preoperative platelet count of $< 100 \times 10^9/\text{L}$ in 14 patients (22% of ABOi patients), which was different from the control group, where only one patient (1%) had a platelet count $< 100 \times 10^9/\text{L}$ ($p < 0.0001$). One week after transplantation the platelet count was comparable to preoperative levels (mean $178 \times 10^9/\text{L}$ day 7) and returned to pre-IA-PP levels after two weeks in the patients receiving only preoperative IA-PP ($n=35$, mean $223 \times 10^9/\text{L}$ day 14).

Table 1. Baseline characteristics of ABO-incompatible and ABO-compatible kidney transplant patients.

	ABO-incompatible	ABO-compatible	P
number of patients	65	130	-
age recipient (mean, SEM ^a)	53.6 (1.7)	53.5 (1.1)	n.s.
male sex recipient (number, %)	44 (68)	87 (67)	n.s.
BUN ^b day -1 (mmol/l, mean, SD ^c)	25 \pm 9	21 \pm 7	<0.005
dialysis dependency (number, %)	47 (72)	71 (55)	<0.05
recipient blood group (number, %)	O n=42 (65)	O n=50 (38)	<0.005
	A n=11 (17)	A n=57 (44)	-
	B n=12 (18)	B n=18 (14)	-
	AB n=0	AB n=5 (4)	-
number of preoperative plasmapheresis (median, IQR ^d)	4 (2)	-	-
number of postoperative plasmapheresis n=30 (median, IQR)	3 (0.25)	-	-
aPTT (median, IQR)	30 (9.5)	28 (6)	n.s.
PT-INR (median, IQR)	1.0 (0.1)	1.0 (0.1)	n.s.

^a SEM: standard error of the mean ^b BUN: blood urea nitrogen ^c SD: standard deviation ^d IQR: interquartile range

The first 30 patients received additional *postoperative* IA-PP and their platelet count remained non-significantly lower within the first week compared to the group with only preoperative IA-PP (n=21), but their platelet count was restored to significantly higher levels two weeks after transplantation (postoperative IA-PP 291 vs only preoperative IA-PP 223 $\times 10^9/L$; $p < 0.05$). The platelet count on day 14 in the group of patients with postoperative IA-PP exceeded even the numbers recorded before the desensitization procedure started (291 vs. 235 $\times 10^9/L$; $p < 0.05$). Platelet count in the total ABOi group remained significantly lower than in ABOc controls from the day prior to transplantation to day 10 (all $p < 0.0005$).

Both the aPTT and the PT-INR did not differ between groups (aPTT median 30 sec. vs. 28 sec. in controls, $p > 0.1$, laboratory normal range 22-32 seconds; PT-INR median 1.0 IU both groups, $p > 0.1$, Table 1).

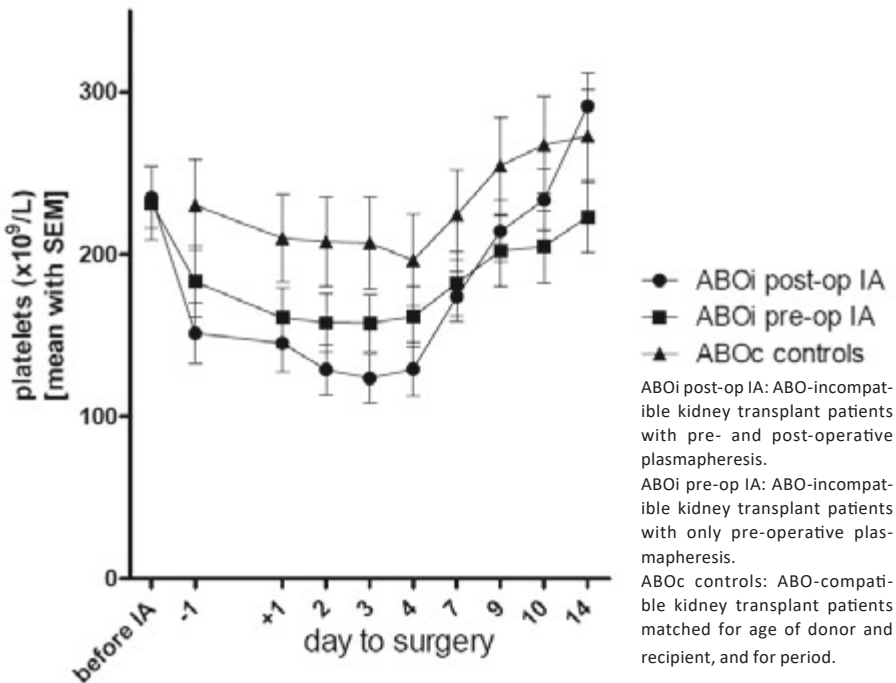


Figure 1. Platelet count in ABO-incompatible patients and their ABO-compatible controls. Platelet count before start of the plasmapheresis (PP) (mean 233 $\times 10^9/L$) was comparable to controls (230 $\times 10^9/L$) one day preoperatively ($p > 0.1$). Platelet count fell by 28% one day pre-operatively in ABO-incompatible patients (169 $\times 10^9/L$, $p < 0.0001$). Platelets in ABO-incompatible patients remained lower up to day 10 compared to controls (all $p \leq 0.001$). In the group of ABOi patients with postoperative PP (first 30 patients) platelet count was higher after two weeks than without postoperative PP (292 vs. 223 $\times 10^9/L$; $p = 0.02$). Platelet count after postoperative PP was even higher at day 14 than before PP (292 vs. 235 $\times 10^9/L$; $p = 0.02$).

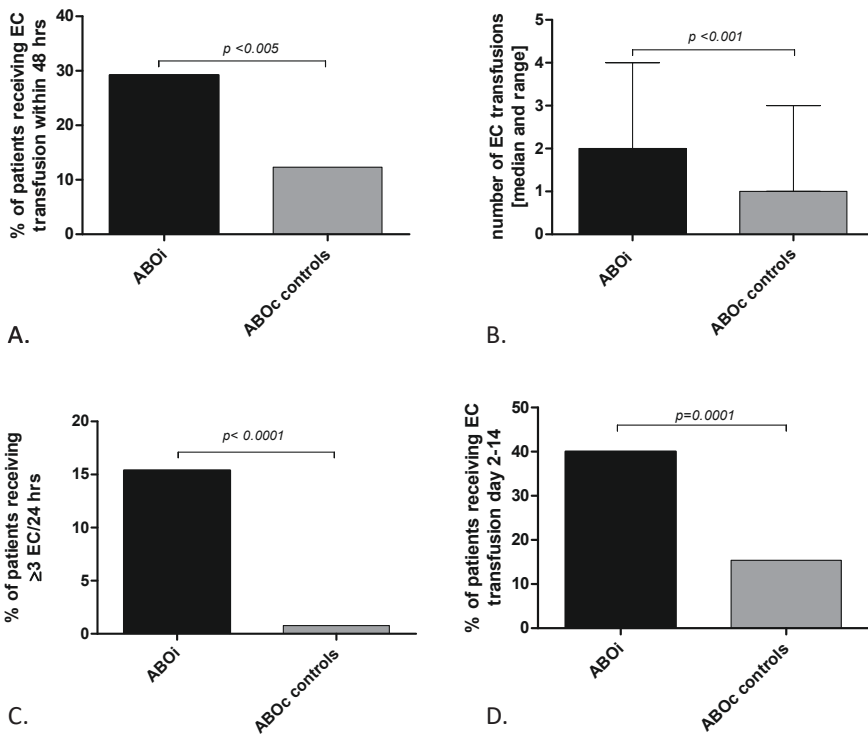
The number of preoperative IA-PPs is strongly associated with the need for red blood cell transfusion 48 hours postoperatively

ABOi patients needed EC transfusion more frequently than ABOc controls within 48 hours postoperatively: 29% of ABOi patients received EC transfusion vs. 12% of controls ($p < 0.005$, Figure 2A). In the group of patients that received EC transfusion, more ECs per patient were given in the ABOi patients than in controls (median 2 EC vs. 1 EC, $p < 0.001$, Figure 2B). The following variables were tested for correlation with the dichotomous dependent EC transfusion within 48 hours postoperatively: sex of recipient, age of recipient, BUN, dialysis requirement prior to transplantation, platelet count before IA-PP, platelet count one day preoperatively both linear and dichotomous $< 100 \times 10^9/L$, delta platelet count (decrease from IA-PP to one day preoperatively), aPTT, PT-INR, ABO blood group, the number of preoperative IA-PPs and intra-operative blood loss. Only the number of preoperative IA-PPs and blood group O correlated with the need for EC transfusion (univariate analysis: OR 1.9, OR 7.1, all $p < 0.05$). In multivariate regression analysis only preoperative IA-PP predicted postoperative EC transfusion within 48 hours: the risk of transfusion increased independently with every preoperative IA-PP session (OR 1.9, $p < 0.05$, Table 2). Only two of the 18 patients with less than 4 preoperative IA-PPs received EC transfusion within the first 48 hours, while 16 of the 44 patients with 4 or more IA-PPs received EC transfusion ($p < 0.05$), irrespective of platelet count (platelet > 100 and PP ≥ 4 : 38% vs. platelet < 100 and PP ≥ 4 : 40% of patients receiving EC in this time period, $p > 0.9$, Figure 3A). The number of preoperative IA-PP sessions did influence preoperative platelet count: the 21 patients with less than 4 preoperative IA-PPs had a higher platelet count than the 44 patients with 4 or more IA-PP sessions (mean $209 \times 10^9/L$ vs. $143 \times 10^9/L$, $p = 0.001$, Figure 3B).

ABOi blood group O patients received EC transfusion more frequently than non-group O patients during 48 hours postoperatively (40% vs. 9%, $p = 0.008$). Blood group O patients underwent more preoperative IA-PP sessions (median 5 vs. 2, $p < 0.0001$). The strong association between blood group O and EC transfusion disappeared after correction in multivariate analysis for the number of preoperative IA-PPs.

Major bleeding is more frequent in the ABOi patient group, especially in women

Ten ABOi patients experienced major bleeding within 48 hours postoperatively compared to only one ABOc patient (15% vs. 1%, $p < 0.0001$, Figure 2C). Three of these ABOi patients underwent relaparotomy, in four patients the anesthesia report described massive blood loss and difficult hemostasis with intra-operative blood loss ranging from 1200 to 2800 ml. One patient had hypotension and acute kidney injury for which hemofiltration was initiated. In the other two patients the major bleeding was contributed to "oozing". Women were overrepresented in major bleeding: 70% of major bleeders were female, while only 35% of ABOi patients were female (Fisher's exact test $p = 0.02$).



ABOi: ABO-incompatible kidney transplant patients EC transfusion: red blood cell transfusion
 ABOc controls: ABO-compatible kidney transplant patients matched for age of donor and recipient, and for period.

Figure 2. ABO-incompatible patients received red blood cell transfusions more frequently than controls. ABO-incompatible patients received blood transfusions more frequently than ABO-compatible controls (29% vs. 12%, $p=0.005$) during 48 hours postoperatively (A). In this time period, more red blood cell (EC) transfusions were given in the ABO-incompatible patients needing transfusion than in controls (median 2 EC vs. 1 EC, $p < 0.001$) (B). ABO-incompatible patients experienced more major bleeding (3 or more EC within 24 hours) in 48 hours postoperatively than ABO-compatible controls (15% vs. 1%, $p < 0.0001$) (C). ABO-incompatible patients received blood transfusion more frequently from 48 hours after transplantation up to day 14 than ABO-compatible controls (40% vs. 15%, $p=0.0001$) (D).

Blood transfusion within two weeks postoperatively

A substantial part of ABOi patients needed EC transfusion after the direct postoperative period, from 48 hours up to day 14: 40% of ABOi patients received EC transfusion vs. 15% of controls in this period (Figure 2D, $p=0.0001$).

Intra-operative blood loss

Of 5 ABOi and 9 ABOc patients data on intra-operative blood loss were missing, all in patients without EC transfusion the day of surgery. ABOi patients lost more blood intra-operatively than controls (543 +/- 65 vs. 355 +/- 34 ml, $p < 0.005$, Figure 4).

Table 2. Multivariate analysis of need for red blood cell transfusion 48 hours postoperatively.

	OR	95% CI	P
male sex recipient	0.46	0.10-2.11	n.s.
number of preoperative PP	1.91	1.16-3.16	0.01
platelet count 1 day pre-operatively	1.01	1.00-1.02	n.s.
blood loss surgery	1.00	1.00-1.00	n.s.
blood group O	3.37	0.36-31.41	n.s.

OR: odds ratio CI: confidence interval PP: plasmapheresis

Hemoglobin level

We studied whether the decision for blood transfusion was made at comparable hemoglobin levels. The hemoglobin level in patients receiving EC transfusion within 48 hours did not differ between ABOi and ABOc controls: the median hemoglobin level the day of EC transfusion was 4.6 vs. 4.8 mmol/L, $p > 0.1$. Hemoglobin levels did not differ between ABOi patients before start of IA-PP and ABOc controls one day preoperatively (7.3 +/- 0.14 vs. 7.5 +/- 0.08 mmol/L respectively, $p > 0.1$). After preoperative IA-PP however, the average hemoglobin level was lower compared to ABOc controls one day preoperatively (6.8 +/- 0.15 vs. 7.5 +/- 0.08 mmol/L, $p < 0.0001$). The average decrease in hemoglobin concentration from the day before surgery till day +2 postoperatively was comparable between ABOi patients and controls (-0.7 vs. -0.9 mmol/L, $p > 0.05$), but the former received significantly more EC transfusions. This finding is in line with the observation that ABOi patients in general have a higher intra-operative blood loss and have more major bleedings postoperatively.

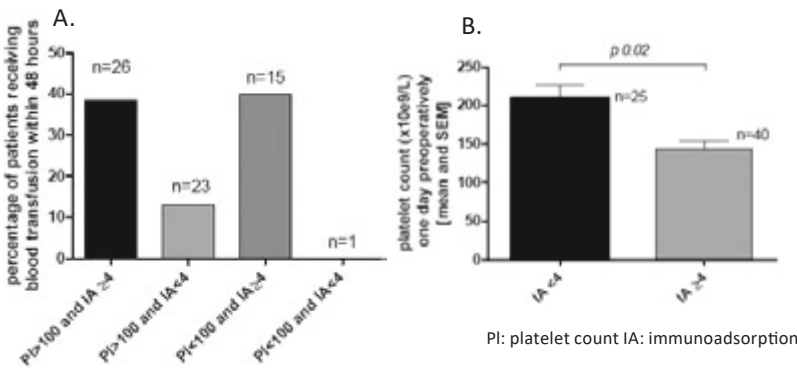


Figure 3. Plasmapheresis correlates with red blood cell transfusion and with platelet count. Only two of the 18 patients with <math>< 4</math> plasmapheresis sessions (PP) received red blood cell (EC) transfusion within 48 hours postoperatively, while in the 44 patients with ≥ 4 PPs 16 of them received EC transfusion, irrespective of platelet count. Platelet > 100 and PP ≥ 4 : 38% vs. platelet < 100 and PP ≥ 4 : 40% of patients receiving EC in this time period, $p > 0.9$ (A). The number of preoperative PPs did influence preoperative platelet count: 21 patients with PP < 4 had an average platelet count of $209 \times 10^9/L$ vs. $143 \times 10^9/L$ in patients with ≥ 4 IA, $p = 0.001$ (B).

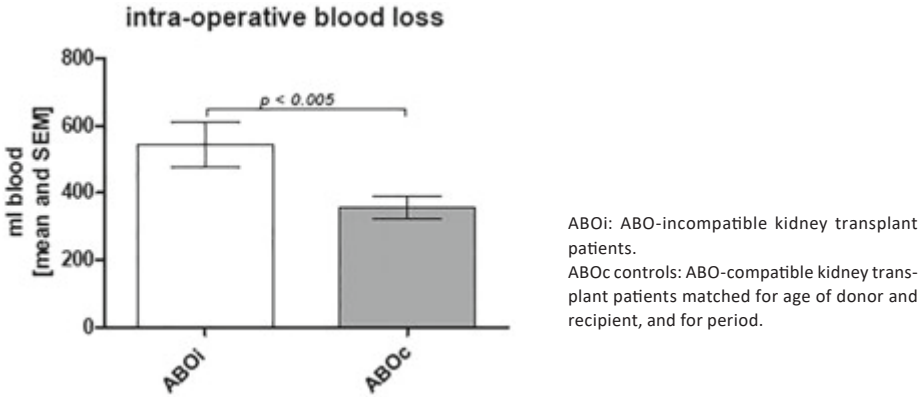


Figure 4. Intra-operative blood loss. ABO-incompatible patients lost more blood intra-operatively than ABO-compatible control patients (536 +/- 66 vs. 364 +/- 34 ml, mean and SEM, $p < 0.005$).

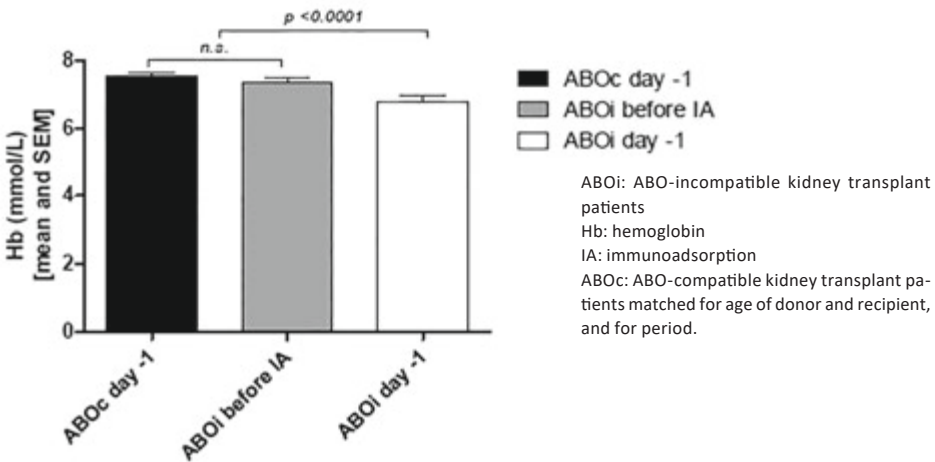


Figure 5. Hemoglobin level. Hemoglobin levels were comparable between ABO-compatible patients 1 day pre-operatively and ABO-incompatible patients before immunoabsorption [7.54 (0.08) vs. 7.34 (0.14), $P = 0.12$], but decreased after pre-operative immunoabsorption [6.79 (0.15), $p < 0.0001$].

DISCUSSION

ABOi kidney transplant patients needed more blood transfusions than their ABOc controls. In this cohort of 65 ABOi patients and 130 ABOc controls, ABOi patients had more blood intra-operative blood loss, received EC transfusions more than twice as frequently as their controls in the first 48 hours postoperatively, and significantly more major bleeding was noted.

The number of IA-PPs appeared to be strongly associated with the need for EC transfusion. It has been known that PP via the centrifugation technique leads to thrombocytopenia but little is known about thrombocytopenia as a result of PP using a plasma filter. In this study, we show that IA by plasma filtration also leads to a remarkable decrease in platelet count. However, despite the marked decrease in platelet count in our ABOi cohort, the platelet count did not correlate with EC transfusion in uni- and multivariate regression analysis. Only the number of preoperative IA-PPs predicted postoperative EC transfusion, irrespective of the platelet count. For instance, the higher transfusion rate in patients receiving 4 or more IA-PPs was not influenced by platelet count. In other words, although frequent IA-PP decreases platelet count, the blood transfusion rate is largely independent of the platelet count. The singular association between IA-PPs and EC transfusion was further strengthened by our analysis of the postoperative period beyond 48 hours. Again in the subgroup of patients receiving postoperative IA-PP, we noticed an increased need for blood transfusion as compared to the other ABOi patients, while their platelet count was not significantly lower within this period.

Our findings are supported by anecdotal data in the literature on a higher bleeding tendency in ABOi kidney transplant patients treated with PP, performed with and without IA (7-9, 13). The correlation between number of PPs and risk of bleeding can be extrapolated from two other studies. ABOi pediatric kidney transplant patients had more bleeding complications when more PP sessions were applied (14). Higher anti-ABO titers necessitating an intensified PP regimen led to more bleeding complications compared to low anti-ABO titers in a cohort of 14 Korean ABOi patients (15).

Frequent contact with the plasma filter membrane thus seems to cause a bleeding tendency, which may be explained by coagulation abnormalities or platelet dysfunction rather than thrombocytopenia. The plasma dialyzer membrane used in our ABOi patients is a full barrier for platelets. However, platelet count and function are influenced during a hemodialysis session using dialyzers made from similar types of synthetic membranes as during PP (16). Daugirdas and Bernardo review this topic extensively, including studies on polysulfone membranes and its inhibitory effect on platelet count and function (16–18). Platelet count falls approximately 10% during the first 30 minutes of dialysis and typically returns to predialysis values thereafter. Both the formation of platelet aggregates and the activation of platelets can lead to this decrease in platelet count (19). The type of membrane (synthetic vs. cellulose), as well as the sterilization method, influence these phenomena (20). The increased blood shear stress or the formation of microbubbles might play a role in platelet activation (21). Activated platelets have a shortened life-span and prolonged bleeding times can be measured directly after hemodialysis (22, 23). Therefore, thrombocytopenia and platelet dysfunction may follow repeated PP as a consequence of the procedure and materials used, similar to hemodialysis.

Besides platelet dysfunction induced by the polysulfone membrane, coagulation abnormalities could be an alternative hypothetical explanation for the higher

transfusion need. The normal PT-INR and aPTT argue against this possibility. This is further supported by a report on 14 ABOi German patients in whom D-dimer, fibrinogen, plasminogen, thromboelastography and antithrombin-III were found to be similar before and after PP with IA (8).

Notably, the hemoglobin level in ABOi patients after the preoperative IA-PP sessions was lower than in ABOc controls before surgery while a similar transfusion policy was used for both groups. This raised the possibility that the EC transfusion rate was increased in the ABOi group postoperatively solely because of a lower hemoglobin level before surgery. However, the higher intra-operative blood loss and the higher rate of major bleeding argue against the lower hemoglobin level pre-operatively as a singular explanation for the higher transfusion rate.

Two remarkable findings need to be discussed. Blood group O patients needed EC more frequently during 48 hours postoperatively. The association between blood group O and EC transfusion could be explained by a higher number of IA-PPs performed in patients with blood group O, as a consequence of higher anti-ABO titers in blood group O patients (24). Another explanation for the higher transfusion rate might be a higher bleeding tendency in blood group O subjects *per se*. Blood group O is also overrepresented in for example women with heavy menstrual bleeding (11) and children with post-tonsillectomy hemorrhage (12). The reciprocal also holds true, with a higher incidence of thrombosis in non-group O individuals (25). This may be related to the 25% lower levels of von Willebrand Factor in individuals with blood group O vs. non-O (26). Another outcome was the higher risk of major bleeding in women. Studies in anticoagulation therapy and management of cardiovascular disease also reveal a higher risk of bleeding among women (27). The reason for this bleeding tendency in women is not clear.

Our results reveal a higher transfusion rate and bleeding tendency in patients undergoing IA-PP. We hypothesize that the causative mechanism is platelet dysfunction induced by contact with the plasma filter membrane, combined with blood loss caused by the filtration technique. We have not excluded so far the possibility that the higher blood transfusion rate was caused by the adsorption column and not the plasma filter membrane alone. Fibrinogen levels can indeed be significantly reduced after IA (28). More bleeding compared to controls, however, was also observed in ABOi patients receiving PP without IA (7, 15). Also the normal coagulation times in our ABOi cohort argue strongly against loss of coagulation factors by adhesion to the column, as hypofibrinogenemia would prolong coagulation tests, which require the production of a fibrinogen clot as an end point, like aPTT and PT-INR.

In conclusion, immunoabsorption plasmapheresis prior to ABOi kidney transplantation exposes patients to a significantly higher bleeding risk than ABOc controls. Blood transfusion leads to HLA sensitization, especially in patients with baseline panel reactive antibodies (29, 30) such as ABOi kidney transplant patients (2, 7). Therefore, further investigations are warranted to clarify the precise mechanisms and to implement anticoagulation protocols for this specific patient category.

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Chapter 5

Post-transplantation immunoadsorption can be withheld in ABO-incompatible kidney transplant recipients.

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ABSTRACT

Background

After ABO-incompatible kidney transplantation, *postoperative* plasma exchange (PE) or immunoadsorption (IA) is performed per protocol or depending on postoperative A/B-titers to prevent acute rejection. However, the need for postoperative PE or IA is not known.

Methods

Since 2006, 30 consecutive patients received three standard *postoperative* IAs. Starting from 2009, the last 46 patients received only preoperative IA. Preoperative desensitization consisted of rituximab, tacrolimus, mycophenolate mofetil, prednisone and intravenous immunoglobulins. Antigen-specific IA was performed pre-operatively with the Glycosorb® device. Biopsy-proven acute rejections either antibody-mediated (AMR) or mixed cellular and antibody-mediated (MAR) within three months were recorded.

Results

The postoperative titer in patients with postoperative IA did not exceed 1:16 (IgG 1:4 [$<2-16$] median and range). The postoperative IgG titer was not significantly different after abandoning postoperative IA, although three patients had titers of 1:32 and one patient even 1:128. Rejections tended to be more frequent in the group with postoperative IA: 6 AMR and 3 MAR were recorded in 30 patients, versus 4 AMR and 1 MAR in the 46 patients without postoperative IA (30 vs. 11%, $p=0.067$). Baseline characteristics differed however: in the group with postoperative IA the vast majority had blood group O (87 vs. 52%, $p=0.003$). Also the IgG titer on the day of transplantation was higher (1:4 [$<2-16$] vs. 1:2 [$<2-32$], $p=0.007$). All 14 patients with AMR and MAR rejections had postoperative IgG titers $\leq 1:16$.

Conclusion

Postoperative removal of A/B antibodies can be safely removed from the ABOi transplantation protocol using strict preoperative criteria for antibody lowering.

INTRODUCTION

Thirty years after the first deliberate ABO-incompatible kidney transplantations were performed, protocols to cross ABO blood group barriers differ substantially across centers worldwide (1). The cornerstone of these different programs is the reduction of A/B antibodies at time of transplantation by plasmapheresis. Different plasma exchange approaches are applied: “classical” plasma exchange is performed in most centers in the United States (2, 3) and Australia (4), while double-filtration plasmapheresis aiming at specifically removing the immunoglobulin fraction is a common procedure in both Japan and Europe (5). Many European centers use antigen-specific immunoadsorption (IA) with synthetically derived blood group A or blood group B columns (6), but experience with non-antigen-specific IA, such as protein A and polyclonal sheep antihuman IgG antibodies is mounting (7, 8).

Originally, a standard number of postoperative plasma exchange sessions were performed to sustain the lowered A/B antibody titers in the first 2 weeks after transplantation, as this was believed to be important for the prevention of antibody-mediated rejection. For example, the Stockholm protocol as described by Tydén provided for three standard postoperative IA sessions, and if the A/B antibody titer rose two dilutions, another postoperative IA session was scheduled (6). However, the need for postoperative antibody removal strategies is a matter of debate. The Freiburg group was the first to abandon pre-emptive postoperative IA: only if the postoperative IgG titer reached a certain threshold, >1:8 in the first postoperative week or >1:16 in the second, then postoperative IA sessions were scheduled (9, 10). This “on-demand” protocol did not seem to result in more rejection (9) and has been adapted by others lowering the postoperative threshold to >1:8 (11-13) and even >1:32 (7) to initiate IA. In our center, we started ABO-incompatible kidney transplantation in 2006, applying the original Stockholm protocol. An interim analysis after 30 transplantations showed that postoperative A/B antibody titers remained low and we did not find an association between titers and rejection (14). Therefore, we decided to stop postoperative IA. In this report we compare the outcomes of the first cohort of patients treated with postoperative IA versus the second cohort of patients treated without postoperative IA.

METHODS

Patients and protocol

We analyzed all consecutive recipients of a blood group ABOi kidney transplant between March 2006 and September 2014 in the Erasmus Medical Center in Rotterdam, the Netherlands (n=76). In short, the regimen included rituximab 375 mg/m² 4 weeks before transplantation; tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID; prednisone 20 mg once daily starting two weeks before transplantation and intravenous

immunoglobulins 0.5 g/kg one day preoperatively. Antigen-specific IA was performed preoperatively in all but three ABOi recipients. Plasmapheresis was performed with a plasmaFlux PSu filter (Fresenius Medical Care, Bad Homburg, Germany) followed by adsorption of A/B antibodies with the Glycosorb® device, coated with synthetically derived blood group A or blood group B antigen (Glycorex Transplantation, Lund, Sweden). In 4 hours, 1.5 L plasma/hour was led over the column, using unfractionated heparin infusion 1000 U/hour to prevent clotting. The first 30 patients additionally received *postoperative* IA as per protocol, performed on days 3, 6 and 9 (or days 2, 5 and 8 depending on the day of surgery). Starting in December 2009, the last 46 patients received only preoperative IA. The postoperative immunosuppressive regimen after transplantation consisted of tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID, prednisone 50 mg BID for three days and 20 mg once daily thereafter in all patients.

Determination of antibody titers

Our procedure to determine hemagglutinin titers has been described in detail before (14). In short, red blood cells of the donor were used to determine IgG and IgM titers against donor ABO-blood group using the fully automated ORTHO BioVue system column agglutination technology. The titer before IA was defined as the highest titer within one month before transplantation. The titer on the day of transplantation was determined on the morning of transplantation, one day after the last preoperative IA (allowing for antibody rebound). The postoperative titer was defined as the highest titer during two weeks postoperatively.

Data collection

Biopsy-proven acute rejections either antibody-mediated (AMR) or mixed cellular and antibody-mediated (MAR) within three months according to revised Banff '09 criteria were recorded (15). Biopsies were taken *for cause* when kidney function deteriorated without an alternative explanation. Renal function at month 12 was documented.

Statistics

For analysis the patients were divided into the postoperative IA group (n=30) and the non-postoperative IA group (n=46). Data were analyzed using GraphPad Prism version 6.0 and the Mann-Whitney U-test was used to determine differences between groups. The statistical significance level was determined as $p \leq 0.05$.

RESULTS

Baseline characteristics

Initial titers of $\leq 1:256$ were accepted for enrollment in the desensitization program, although one exception was made in a patient without postoperative IA with an initial

IgG titer 1:512. The target IgG titer after the last IA was <1:8. Baseline characteristics differed between groups. In the group with postoperative IA the vast majority had blood group O (87 vs. %, $p=0.003$, table I). The titer the day of transplantation was higher in the group with postoperative IA (median IgG 1:4 [$<2-16$] vs. 1:2 [$<2-32$], $p=0.007$, and IgM 1:2 [$<1-16$] vs. 1:1 [$<1-16$], $p=0.005$, Table 1). The number of HLA mismatches on A, B and DR loci, peak PRA and the number of previous kidney transplantations were comparable.

Rejection episodes

Rejections were non-significantly more frequent in the group with postoperative IA: 6 AMR and 3 MAR were recorded in 30 patients, versus 4 AMR and 1 MAR in the 46 patients without postoperative IA (30 vs. 11%, $p=0.067$, Figure 1). Treatment consisted of pulse methylprednisolone and IVIG; extra IA was performed only in the group with postoperative IA. One patient was also treated with rATG because of insufficient renal function recovery. One patient with AMR Banff type 3 underwent transplantectomy (16).

Table 1. Baseline characteristics of ABOi recipients with versus ABOi recipients without postoperative antigen-specific immunoadsorption.

	postoperative IA	only preoperative IA	<i>p-value</i>
recipients (number)	30	46	-
blood group O	26 (87%)	24 (52%)	**
blood group donor > recipient, numbers (%)			
A>O	19 (63)	5 (11)	***
B>O	7 (23)	6 (13)	n.s.
B>A	1 (3)	4 (9)	n.s.
A>B	1 (3)	7 (15)	n.s.
AB>O	-	2 (4)	n.s.
AB>A	-	8 (17)	*
AB>B	2 (7)	3 (7)	n.s.
age recipient (mean)	49	54	n.s.
no. of preop IA	4 (2-7)	3.5 (0-10)	n.s.
no. of postop IA	3 (1-8)	-	-
no. of kidney transplantations	1.17 (0.07)	1.24 (0.08)	n.s.
peak PRA%, median (range)	4 (0-75)	4 (0-85)	n.s.
HLA mismatches on A, B, DR loci, mean (SEM)			
MM on A	1.20 (0.11)	1.32 (0.11)	n.s.
MM on B	1.37 (0.12)	1.40 (0.11)	n.s.
MM on DR	1.23 (0.12)	1.26 (0.11)	n.s.
IgG before IA, median (range)	32 (<2-128)	16 (<2-512)	n.s.
IgM before IA, median (range)	32 (<1-128)	16 (<1-256)	n.s.
IgG the day of transplantation, median (range)	4 (<2-16)	2 (<2-32)	*
IgM the day of transplantation, median (range)	2 (<1-16)	1 (<1-16)	**

IA: immunoadsorption PRA: panel reactive antibodies HLA: human leucocyte antigen

* $p \leq 0.05$ ** $p \leq 0.005$ *** $p \leq 0.0005$

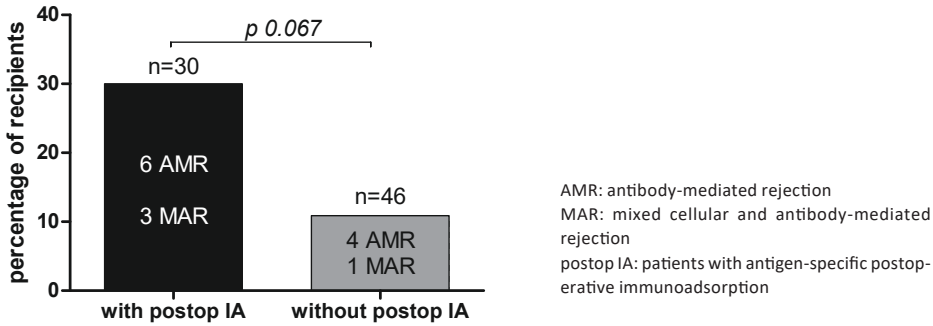
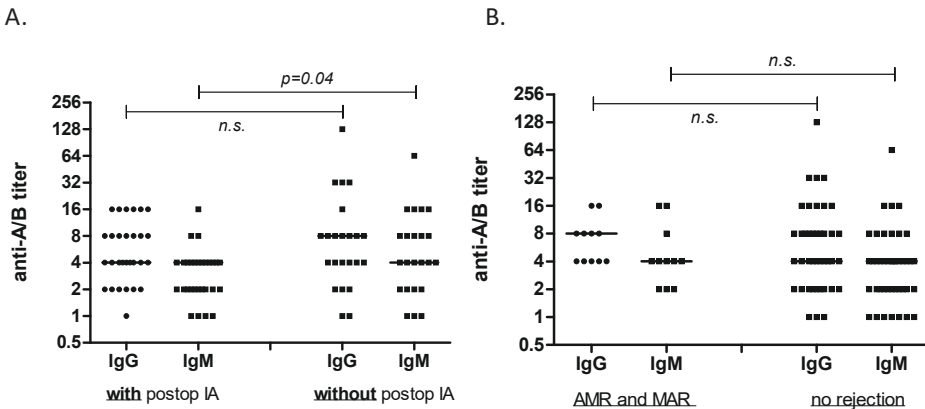


Figure 1. Antibody-mediated and mixed cellular and antibody-mediated rejection in ABO-incompatible kidney transplant recipients treated with and without postoperative immunoadsorption.



with postop IA: ABO-incompatible kidney transplant recipients treated with preoperative and standard 3 postoperative immunoadsorption sessions.
without postop IA: ABO-incompatible kidney transplant recipients treated with preoperative immunoadsorption only.
AMR: antibody-mediated rejection MAR: mixed cellular and antibody mediated rejection

Figure 2. Highest postoperative A/B antibody titers day 1 to day 14. Postoperative IgG titers were comparable between recipients with and without postoperative immunoadsorption, while postoperative IgM titers were slightly higher after abandoning postoperative IA (A). When comparing recipients with rejection and those without rejection, no significant association between postoperative immunoadsorption and rejection could be observed (B).

Anti-A/B titers

The postoperative titer in patients with postoperative IA did not exceed 16 (IgG 1:4 [$<2-16$] and IgM 1:4 [$<1-16$], Figure 2A) and there was no net change in postoperative titers versus titers the day of transplantation (change in dilution step median 0 [minus 2 - plus 3], data not shown). After abandoning postoperative IA, the postoperative titer was available for 23 patients. The median postoperative IgG titer of these 23 patients did not differ compared to the group with postoperative IA. Three patients had titers of 1:32 and one patient even 1:128 (median IgG 1:4 with postoperative IA

vs 1:8. without postoperative IA, $p=0.62$, Figure 2A). Postoperative IgM titers were slightly higher after abandoning postoperative IA (IgM 1:4 [$<1-16$] with postoperative IA vs 1:4 [$<1-64$] without, $p=0.04$). When comparing patients with rejection (AMR and MAR) and those without rejection, no significant association between postoperative IA and rejection could be observed. Of the 14 patients experiencing biopsy-proven acute rejection, 11 postoperative titers were available and none exceeded 16 (Figure 2B).

Renal function

Twelve month follow-up was available for 26 patients in the postoperative IA group: 2 patients had died (suicide and abdominal sepsis) and two grafts failures occurred (month 3 ongoing rejection and month 4 intractable AMR during Gram-negative sepsis (16)). 35 patients without postoperative IA had one-year follow-up. No death or graft loss occurred within this group. Creatinine levels were comparable between groups (145.5 vs. 140.0 $\mu\text{mol/L}$, $p=0.7$, Figure 3).

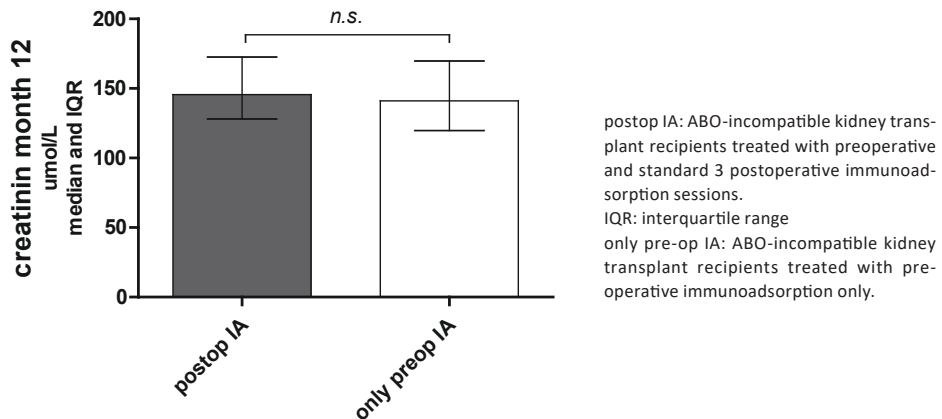


Figure 3. Kidney function one year follow-up. One year after transplantation, kidney function was similar in ABO-incompatible kidney transplant recipients treated with and without postoperative immunoadsorption.

DISCUSSION

Changing the original Stockholm protocol to an ABO-incompatible kidney transplantation program without postoperative IA does not lead to more rejection episodes and does not affect renal graft function at one year follow-up. Postoperative titers did not predict acute rejection episodes: the 14 patients who did experience a biopsy-proven acute rejection within three months had postoperative A/B-titers not exceeding 16. Postoperative IA therefore seems to be an unnecessary procedure, at least in a protocol with initial IgG titers of $\leq 1:256$ and a target titer after the last IA of $< 1:8$.

The safety of abandoning standard postoperative IA has been demonstrated in titers remaining below 16 (9). The uneventful clinical course in our cohort without rejection in 5 patients with postoperative IgG titers of 1:16 and in 4 patients with 1:32 or even higher however, is in contradiction with outcomes in Japanese and American patients treated with preoperative PE or splenectomy, of whom postoperative titers exceeding 1:16 correlated with rejection (17-19). However titers are not one-to-one comparable between centers (20).

The retrospective analysis of our cohort has overt limitations. After abandoning postoperative IA, titer measurements were only performed in 23 out of 46 patients. However, the postoperative titers in the group without postoperative IA were in a similar range as the postoperative titers in the group with postoperative IA. Secondly, the cohort of patients with standard postoperative IA experienced more AMR and MAR than reports of other centers using the Stockholm protocol (21). As described before we could not fully explain this discrepancy (14), although baseline characteristics reveal in this first cohort a higher frequency of blood group O, associated with rejection (22). Third, our protocol has a cut-off entry titer of IgG \leq 1:256 (one exception was made) and whether our findings can be extrapolated to higher initial titers is unclear.

Our data are in agreement with others (7, 9, 10) and indicate that circulating anti-A/B antibodies after kidney transplantation are present and may increase, but their presence does not have to lead to AMR. This condition is termed accommodation but the underlying mechanisms are only partly understood and may include impaired A/B-antigen presentation in the donor organ or a decreased cytotoxic effect of A/B antibodies on renal endothelium (23-25). Finally, critical appraisal of postoperative PE/IA is justified, since PE/IA may lead to a higher rate of infection and bleeding observed after ABOi kidney transplantation (26-29).

In conclusion, postoperative removal of anti-A/B antibodies can be safely removed from the ABOi transplantation protocol using strict preoperative criteria for antibody lowering.

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Chapter 7

Late antibody-mediated rejection after ABO-incompatible kidney transplantation during Gram-negative sepsis.

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ABSTRACT

Background

The major challenge in ABO-incompatible transplantation is to minimize antibody-mediated rejection. Effective reduction of the anti-ABO blood group antibodies at the time of transplantation has made ABO-incompatible kidney transplantation a growing practice in our hospital and in centers worldwide. ABO antibodies result from contact with A- and B-like antigens in the intestines via nutrients and bacteria. We demonstrate a patient with fulminant antibody-mediated rejection late after ABO-incompatible kidney transplantation, whose anti-A antibody titers rose dramatically following *Serratia marcescens* sepsis.

Case presentation

A 58-year-old woman underwent an ABO-incompatible kidney transplantation for end-stage renal disease secondary to autosomal dominant polycystic kidney disease. It concerned a blood group A1 to O donation. Pre-desensitization titers were 64 for anti-blood group A IgM and 32 for anti-blood group A IgG titers. Desensitization treatment consisted of rituximab, tacrolimus, mycophenolate mofetil, corticosteroids, immunoadsorption and intravenous immunoglobulines. She was readmitted to our hospital 11 weeks after transplantation for *S. marcescens* urosepsis. Her anti-A IgM titer rose to >5000 and she developed a fulminant antibody-mediated rejection.

We hypothesized that the (overwhelming) presence in the blood of *S. marcescens* stimulated anti-A antibody formation, as *S. marcescens* might share epitopes with blood group A antigen. Unfortunately we could not demonstrate interaction between blood group A and *S. marcescens* in incubation experiments.

Conclusion

Two features of this post-transplant course are remarkably different from other reports of acute rejection in ABO-incompatible kidney transplantation: first, the late occurrence 12 weeks after kidney transplantation and second, the very high anti-A IgM titers (>5000), suggesting recent boosting of anti-A antibody formation by *S. marcescens*.

BACKGROUND

Both the HLA and the ABO blood group system determine the risk of rejection in clinical organ transplantation. The major challenge in blood group ABO-incompatible (ABOi) transplantation is to minimize antibody-mediated rejection (AMR). In recent years, ABOi kidney transplantation programs have been developed that minimize the risk for AMR and show excellent graft survival. The key to success has been effective reduction of the ABO antibodies prior to transplantation. This is usually achieved by repeated plasmapheresis with or without the use of a specific immunoadsorption procedure. A low concentration of ABO antibodies creates a window of opportunity for graft acceptance by an incompletely understood immunological phenomenon called “accommodation” (1,2). Within the first week after transplantation, AMR may occur but these can usually be effectively reversed by current standard AMR treatment protocols. Anti-ABO titers usually remain low after transplantation. ABO antibodies are traditionally referred to as “natural occurring”, since these antibodies were thought to occur without prior immunization. For over more than half a century, evidence is mounting that ABO antibodies most likely result from contact with A- and B-like antigens in the intestines via nutrients and bacteria, and develop early in childhood (3,4). Therefore, boosting of ABO antibody titers may occur by infections with Gram-negative bacteria (5) and could, at least theoretically, cause AMR of ABOi kidney transplants (6,7). We present a case of a late fulminant AMR of an ABOi kidney transplant which may have been triggered by Gram-negative bacteremia.

CASE PRESENTATION

A 58-year-old woman underwent living unrelated ABOi kidney transplantation. Her medical history revealed hypertension and autosomal dominant polycystic kidney disease, for which she had been on peritoneal dialysis. She had never been pregnant and never received any blood products. The donor kidney came from her 59-year-old husband and the HLA mismatch was 1-2-2 on A, B and DR loci respectively. She had no current or historical panel reactive antibodies. The donor blood group was A1 and the recipient's was O. ABO desensitization treatment consisted of rituximab 375 mg/m² 4 weeks before transplantation; tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID; prednisone 20 mg once daily starting two weeks before transplantation and immunoglobulines 0.5 g/kg one day preoperatively. Five plasmapheresis sessions, followed by adsorption of anti-A antibodies with the Glycosorb® device coated with synthetically derived blood group A antigen, were performed in the week before transplantation. Her anti-A titer was 64 (IgM) and 32 (IgG) before treatment and decreased to 2 at the day before kidney transplantation. The surgical procedure was complicated by peri-transplant hematoma, for which erythrocyte concentrate and platelet transfusions were given (of blood group O donors). Immunosuppressive therapy

after transplantation consisted of tacrolimus 4 mg BID, mycophenolate mofetil 1000 mg BID and prednisone 20 mg once daily. Valgancyclovir for cytomegalovirus (CMV) prophylaxis was started post-transplantation. Direct graft function was noted.

During admission, our patient experienced transient diarrhea and was treated for urinary tract infection with ciprofloxacin. Urine cultured *Pseudomonas aeruginosa* and *Serratia (S.) marcescens* (both $> 10^5$ colony forming units (cfu)). Before discharge, a routine biopsy on day 14 revealed normal renal parenchyma, with no signs of rejection. Staining for C4d on endothelial cells was positive, which is often seen after ABOi kidney transplantation and by itself does not indicate rejection. Anti-A titers remained low: one day postoperative the IgG titer was 2 and the IgM titer 8; at discharge, IgM titers were 1 and IgG titers were < 2 . Renal function improved to a serum creatinine of 113 $\mu\text{mol/l}$ at time of hospital discharge.

Seven weeks post-transplantation, patient was readmitted for fever and loose stools. She had developed new onset diabetes mellitus, for which intravenous insulin was started. Abdominal ultrasound revealed a swollen transplant with signs of pyelonephritis with multiple micro-abscesses. A 10-day course of ceftazidime and ciprofloxacin was started for suspected pyelonephritis as the urine culture identified various uropathogens, not further specified.

Eleven weeks post transplantation, patient returned to our emergency department with fever, tachycardia and pain over the renal allograft. Serum creatinine had risen to 115 $\mu\text{mol/l}$ with a C-reactive protein of 163 mg/l . Ultrasonography of the transplant kidney showed no gross abnormalities with normal renal vascular flow. Cultures of blood, urine and sputum were drawn and imipenem/cilastatin therapy was initiated. Only the blood culture became positive for *S. marcescens* sensitive to imipenem. In the next 5 days, serum creatinine increased further to 275 $\mu\text{mol/l}$ in combination with severe fluid retention. A newly obtained transplant ultrasound disclosed non-measurable diastolic blood flow. On the clinical suspicion of rejection, a three-day course of methylprednisolone 1000 milligram intravenous was initiated and a transplant biopsy was performed. The kidney biopsy revealed AMR type 3 Banff '09, with extended hemorrhagic infarction and positive C4d staining (Figure 1) (8). The anti-A IgM titer was >5000 and anti-A IgG titer 512. Transplantectomy was performed as a renal scintigraphy showed no perfusion. A swollen and hemorrhagic kidney transplant was removed and chronic intermittent hemodialysis was initiated. A repeated anti-A titer one month later was 256 for IgM and 32 for IgG (Figure 2).

EXPERIMENTS

We hypothesized that the (overwhelming) presence in the blood of *S. marcescens* stimulated anti-A antibody formation, as *S. marcescens* might share epitopes with blood group A antigen. We chose to perform a hemagglutination inhibition assay instead of direct (serum) agglutination with bacteria, as the latter could occur because of

possible aspecific clotting. *S. marcescens* obtained from the blood of our patient was frozen and stored until use. The thawed sample was plated on a (blood group free) Trypticase Soy agar (Becton Dickinson, USA) and grown at 37°C overnight. Cultures were suspended in phosphate buffered saline (PBS) and the concentration of bacteria in suspension was assessed using McFarland standards and plate counting the following day. Bacterial suspensions with concentrations ranging from 10⁵ cfu/ml to 10⁸ cfu/ml PBS and a PBS control were then either kept at 4 degrees Celsius, boiled, or sonicated. Subsequently, these different *S. marcescens* suspensions were incubated with anti-A plasma for 30 minutes at 37°C. Next, blood group A erythrocytes 4% (Sanquin blood supply, the Netherlands) were added and subsequently incubated for 15 minutes at room temperature. A PBS control was added to confirm visual agglutination after addition of A erythrocytes.

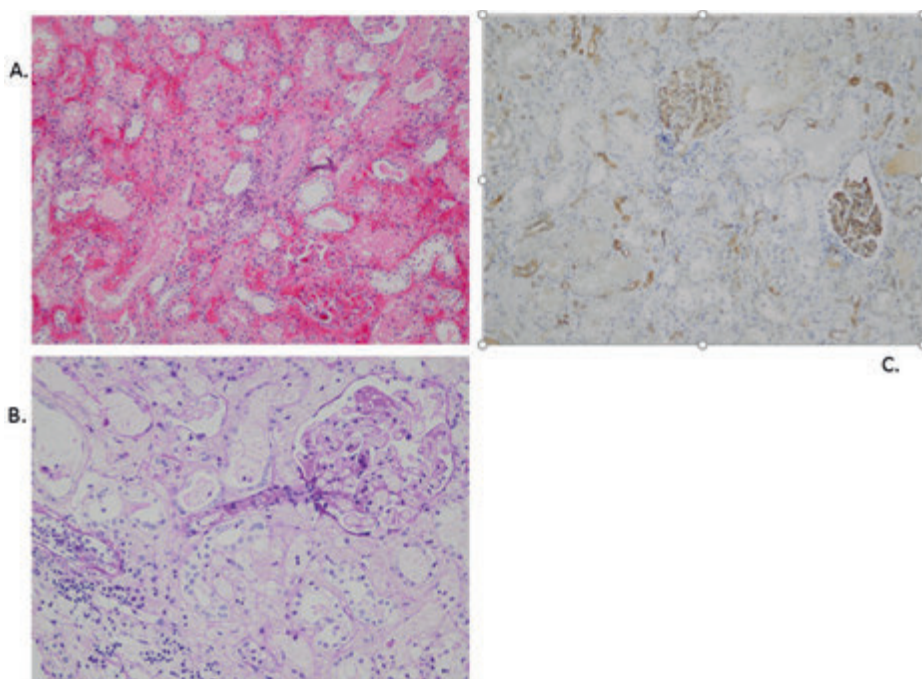


Figure 1. Kidney transplant biopsy 12 weeks after ABOincompatible kidney transplantation.

A. Severe hemorrhage of the cortex and congestion of the glomeruli and tubulointerstitial compartment, with only minimal influx of inflammatory cells. There is a thrombus in the arteriole of the glomerulus. (H&E staining; original magnification 10x).

B. Congestion of the glomerulus with fibrinoid necrosis of the arteriole. There is ischemia of the tubuli. An artery shows a transmurial inflammation, of both mononuclear cells and neutrophils. (Periodic acid-Schiff-Diastase stain; original magnification 20x).

C. Positive staining of more than 50% of the peritubular capillaries and all the glomeruli. (Immunohistochemistry for C4d; original magnification 10x).

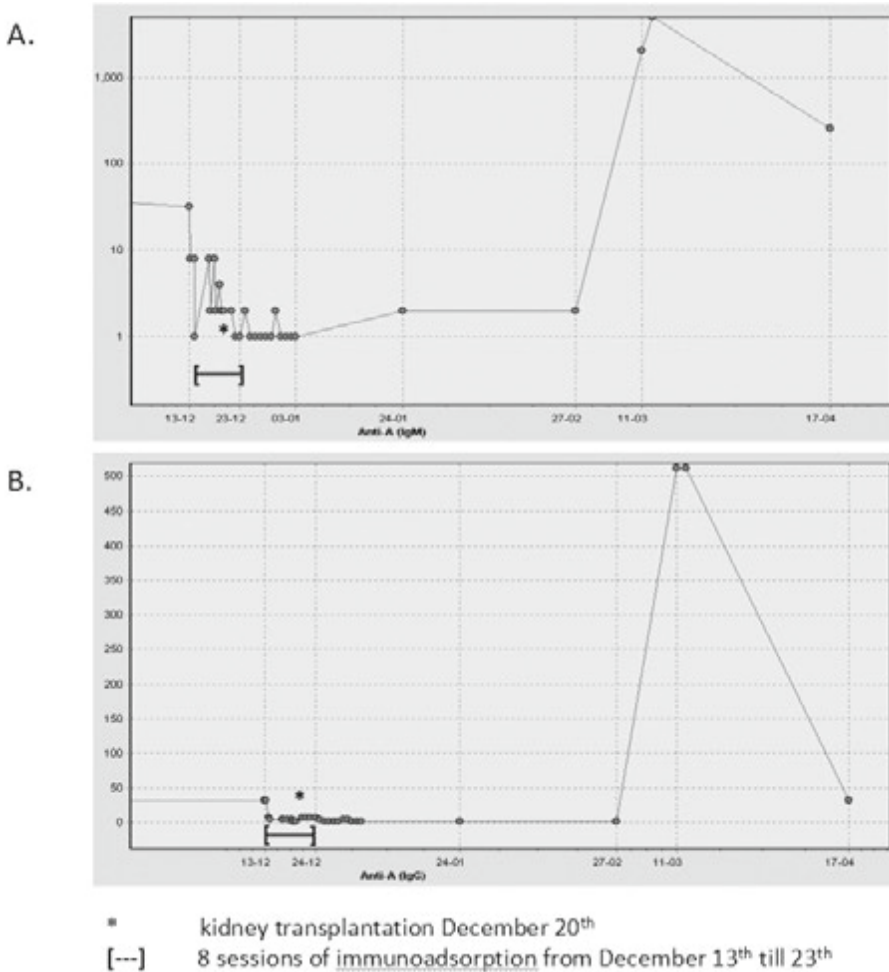


Figure 2. Course of anti-A antibody titers before and after ABO-incompatible kidney transplantation. The anti-A IgM (A.) and IgG (B.) titers were 64 and 32 respectively before pre-operative immunoadsorption (December 13th), decreased to 2/2 pre-operatively (December 20th) and were 1/<2 at discharge. During AMR they increased to >5000/512, decreasing to 256/32 one month later (logarithmic scale).

We hypothesized that preincubation with a certain “threshold” amount of bacteria would prevent hemagglutination. We extrapolated this experimental design from the methods of Springer et al. who demonstrated interaction between Gram-negative bacteria and anti-ABO antibodies in 1961 (9) (see Discussion for more details). Antibody titer changes were investigated as well: after centrifugation the supernatant was stored at 4°C and anti-A titers were measured the following day by adding A erythrocytes in serial plasma dilutions and compared to the original titer. Titers were described as the highest dilution at which hemagglutination was still visible.

We also performed experiments of bacterial incubation with human serum without addition of A erythrocytes, before measuring a possible change in titer the following day. In a parallel experiment, incubation with anti-A plasma took place for 2 hours at room temperature (for IgM binding).

RESULTS

In the first experiment serum was pre-incubated with unboiled bacterial suspensions in increasing concentrations. After addition of blood group A erythrocytes however, agglutination was still observed. As pre-incubation with viable bacteria did not result in inhibition of hemagglutination, i.e. did not absorb antibodies from the serum, we also measured a possible change in antibody titers. The original titer was diluted threefold with bacterial suspension and A erythrocytes in a 1:1:1 ratio. However, compared to the PBS control, pre-incubation with bacterial suspensions did not lower the titer of anti-A plasma when A erythrocytes were added.

Subsequently bacterial suspensions were boiled for 2.5 hours to unmask antigens that theoretically may have been hidden by the bacterial capsule. IgM titers were reduced but only one-fold, which was regarded as non-significant. When a surplus of bacterial suspension (4.8×10^9 cfu/ml) was added, agglutination was still present. In the last set of experiments, bacterial suspensions were boiled and centrifuged at higher speed (14000 rpm) than the previous experiment to prevent loss of light antigens, or sonicated to prevent denaturation of antigens. Similar amounts of bacterial suspension were incubated with or without A erythrocytes. However, both boiling and sonication did not prevent agglutination compared to PBS control.

DISCUSSION

We demonstrate a patient with fulminant antibody-mediated rejection after ABOi kidney transplantation, whose anti-A IgM titers rose dramatically following *S. marcescens* sepsis.

Two features of this post-transplant course are different from other reports of acute rejection in ABOi kidney transplantation: first, the late occurrence of AMR 12 weeks after kidney transplantation and second, the very high anti-A IgM titers (>5000).

Long term patient survival has not been shown to be significantly different between ABO-compatible and incompatible kidney transplant recipients (10). However, a higher risk of AMR exists, occurring mainly in the direct postoperative period. In the 51 patients with detailed time of onset of AMR in the literature, only 4 experienced AMR 12 weeks or later after transplantation (11-19). Of all the 65 recipients of an ABOi kidney allograft in our center so far, 9 experienced AMR and 3 a combined AMR and cellular rejection, all within three months except for the patient presented in this case report (20). The

pathological role of anti-ABO IgG versus IgM on the ABOi renal allograft is a matter of debate (7,12,21). Takahashi hypothesizes on two distinct types of AMR after ABOi kidney transplantation: he states that type I AMR is caused by re-sensitization due to ABO-blood group antigens, occurs early postoperatively and is characterized by an IgG antibody rise. Type II AMR on the contrary is caused by primary sensitization due to ABO-blood group-associated antigens. IgM titer rises more than IgG and it takes longer for this type of AMR to develop (7). He accompanies this hypothesis with two examples: A 34-year-old blood group O recipient receiving a blood group B renal allograft, experienced an AMR on postoperative day 19 during urosepsis with *Klebsiella pneumoniae*. IgM rose from 4 to 64 and IgG from < 2 to 4. The second example is a 68-year-old male blood group B recipient who received a blood group A renal allograft. On postoperative day 9 his graft was removed because of an untreatable AMR in the presence of Methicillin-resistant *Staphylococcus epidermidis* pneumosepsis. His IgM titer rose from 2 to 512 and IgG remained < 2 during the clinical course. We therefore hypothesized that also this AMR had a different etiology than re-sensitization by blood group A antigens: a Gram-negative sepsis.

ABO antibodies are not “naturally occurring” and result from contact with A- and B-like antigens in the intestines via nutrients and bacteria (3,4). ABO antibodies are either absent at birth or present via placental transfer and breastfeeding. Before the age of 3, the infant’s gut becomes colonized with commensal bacteria expressing A- and B-like antigens. The developing immune system produces antibodies against the antigens not present on its own erythrocytes. The continuing influence of gut bacteria on ABO antibody formation is reflected in permanent detectable IgM titers, for example the IgM titers measured in kidney transplant recipients before ABOi kidney transplantation. In 1969 Springer and Horton fed 23 very young infants (35 weeks or younger) and 14 adults killed *Escherichia coli* O86 (4). It concerned blood group A and O individuals, both healthy subjects as well as patients with intestinal disorders: 16 children with diarrhea, 2 adults with ulcerative colitis and 2 adults with colon carcinoma. The majority had a fourfold or greater increase in anti-B antibodies after ingestion, infants more than adults and diarrheic patients more than healthy controls. Moreover, six out of seven infants without a baseline titer had titers of 16 or greater after ingestion. In the same paper, Springer demonstrated that anti-human blood group A and B antibodies in chickens can be neutralized by injecting live *Escherichia coli* O86.

Many Gram-negative bacteria with human blood group activity are identified. For example Yi sequenced the entire *Escherichia coli* O86 gene cluster and identified all the genes responsible for the blood group B-like antigen biosynthesis (22).

There is more evidence that bacterial suspensions are able to reduce anti-ABO titers by binding these ABO antibodies to the bacteria: Springer et al. assessed the blood group activity of 282 Gram-negative bacteria (9). Different bacterial suspensions were incubated with series of human serum with minimal 4 agglutination titers for two hours. Almost 50 percent of these 282 strains exhibited anti-ABO activity. Bacteria with only

one specificity far outnumbered those with two or all three ABO specificities, in which anti-O and anti-B were predominant.

Strong evidence that gut bacteria are able to trigger ABO antibody formation is reported by Daniel-Johnson et al. (23). He describes severe hemolytic transfusion reactions in two blood group B recipients of a blood group A platelet donor. Although platelet transfusion is preferably performed ABO identical or at least blood group compatible, the limited availability of matched platelet donors makes platelet donation across ABO barriers a common practice. This is infrequently followed by hemolysis as only a small amount of (ABOi) donor plasma is present. In contrast, in the blood group A platelet donor described by Johnson et al., the anti-B IgG titer rose to 16384 after taking three tablets of probiotics per day. Furthermore, the solubilized form of this probiotic was found to be able to reduce the measured anti-B in plasma of a randomly chosen blood group A donor threefold, from 64 to 8 after incubation at room temperature *in vitro*.

In ABOi solid organ transplantation the relation between sepsis and AMR is also reported. A pediatric ABOi kidney transplant recipient experienced biopsy-proven AMR during pyelonephritis, with an increase in anti-B titers to 64 and 128 for IgG and IgM, respectively (6). Oya et al. report on an ABOi living donor liver transplantation with an anti-B titer rise during an intra-abdominal hematoma infected with *Serratia marcescens*. Subsequently thrombotic microangiopathy developed. The authors suggest that interaction between the anti-donor ABO antibodies and the endothelial cells of the graft played a causative role in this microangiopathy (24). Unfortunately, in our case, we could not demonstrate an anti-A antibody binding capacity of the *S. marcescens* strain isolated from our patient. There are several possible explanations for this. First, the *Serratia* colonies grew rather mucoid which is an indication of a capsule. The capsule might have been impermeable to ABO antibodies. However, even boiling which has been presumed to remove the capsule, or sonication to unmask the bacterial cell wall expressing other antigenic epitopes, did not change the results. Second, the amount of bacteria might have been insufficient. However, we also performed incubation with a very viscous density without a change in titer. Third, assay temperature might play a role. However, temperature was adjusted for IgM antigen binding to room temperature and for IgG antigen binding to 37°C and this did not result in inhibition of agglutination. Fourth, the time for interaction between anti-A antibodies and bacterial epitopes and subsequently with A erythrocytes was shorter than in the experiments carried out by Springer. In addition, Springer and Horton describe in their methods the possibility of “non-agglutinating-in-saline” ABO antibodies and their detection with anti-human serum after immunizing chickens with ABO antigens. We did not explore this possibility.

Next to *S. marcescens* sharing a comparable epitope with antigen A, another explanation for the development of antibody-mediated rejection during *S. marcescens* sepsis exists. This might be a change in antigenicity of the A antigen. *S. marcescens* was cultured in our patient’s urine several times and subsequently in her blood. *S.*

marcescens is a Gram-negative bacillus and belongs to the family of *Enterobacteriaceae* (25). Mannose-sensitive pili of *S. marcescens* are known to stimulate renal scarring (26). This renal scarring could have hypothetically enhanced changes in ABO antigenicity in the kidney graft.

Conclusion

ABO antibodies result from contact with gut bacteria. A Gram-negative sepsis could theoretically boost anti-ABO antibody formation. We demonstrated a patient whose anti-A titers rose dramatically after Gram-negative sepsis, leading to a type 3 antibody-mediated rejection. Despite comparable incubation experiments in the literature, we could not demonstrate an interaction between *S. marcescens* and anti-A antibodies. Therefore it remains uncertain whether bacteremia can be the cause of antibody-mediated rejection in ABOi kidney transplantation.

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Chapter 8

Summary and conclusions.

SUMMARY

The overall aim of studying ABO-incompatible (ABOi) kidney transplantation is to improve outcomes for recipients of a kidney allograft. The studies presented in this thesis investigated the risk of ABOi kidney transplantation. Specifically, the benefits and risks of specific desensitization elements were described, and the status of ABOi kidney transplantation compared to ABO-compatible (ABOc) donation was investigated.

In **chapters 2 and 6** we compared outcomes of ABOi kidney transplant recipients with ABOc recipients. We performed a systematic review of all single center studies of ABOi kidney transplantation with a control group of ABOc living donor transplantations from the same hospital (**chapter 2**). Our meta-analysis revealed slightly inferior graft and patient survival compared to recipients of an ABOc allograft with similar baseline characteristics: for every 100 transplanted recipients, after one year 96 patients are alive with a functioning ABOi allograft versus 98 alive patients with a functioning ABOc allograft (4 vs 2 graft losses). Half of the reported causes of death in ABOi patients were of infectious origin, versus only 13% in ABOc patients. The observed lower graft survival was irrespective of induction therapy, region, era and filtration technique. Antibody-mediated rejection, severe non-viral infection, and bleeding were also more common after ABOi transplantation. To counsel incompatible donor-recipient pairs on outcomes after ABOi transplantation, information on outcomes after deceased donor transplantation is also needed. Therefore, in **chapter 6** all consecutive Dutch ABOi kidney transplant recipients were compared to matched deceased donor as well as living donor ABOc recipients. Patient survival after ABOi kidney transplantation was superior to deceased-donor controls (HR for death 0.67 [95%CI 0.48-0.92] in ABOi vs ABOc deceased donor recipients). Graft survival in the total ABOi cohort however was no different from that among deceased donor ABOc recipients. Half of this ABOi cohort however was treated with rituximab alone induction and their outcomes trended towards inferior graft survival compared to combined B- and T-cell targeted induction therapy.

Chapters 3, 4 and 5 described analyses of the ABOi program at the Erasmus Medical Center.

In **chapter 3** the first cohort of ABOi kidney transplant recipients was described in detail, with a focus on antibody-mediated rejection. For each of the first 50 ABOi allograft recipients, two ABOc living donor allograft controls were matched for period, recipient age and number of HLA mismatches. Antibody-mediated rejection was observed frequently, in 11 out of 50 ABOi patients. Five of these rejections were diagnosed as mixed cellular and antibody-mediated rejection at time of biopsy. This analysis led to reflections on the desensitization protocol and specifically the induction agent. The observed high frequency of rejection compared to the literature on this “Swedish” protocol (1, 2) led to a protocol change in 2015, in which rituximab was replaced by alemtuzumab (**chapter 6**).

In **chapter 4**, the impression of clinicians treating ABOi transplant recipients that ABOi recipients “ooze easily” was substantiated. We analyzed the data of 65 consecutive ABOi kidney transplant recipients and compared them to 130 control patients matched for age of donor and recipient in the period 2006-2013. Erythrocyte concentrate blood transfusion (EC) was administered more than twice as frequently in ABOi recipients in the first 48 hours after transplantation than in controls (29% vs 12% of patients). Both the number of patients needing blood transfusion and the number of ECs per transfusion were higher in the ABOi group, and more major bleeding was observed (15% vs 2% received ≥ 3 EC). The platelet count dropped significantly in ABOi recipients during desensitization resulting in 22% of ABOi recipients with a preoperative platelet count below $100 \times 10^9/L$. Of note is that in ABOi patients 40% of blood group O recipients received EC versus 9% of non-blood group O recipients. However, only the number of immunoadsorptions (IA) correlated in multivariate analysis with the number of postoperative EC transfusions. An observed drop in hemoglobin level after desensitization likely played a role in the higher blood transfusion rate. On top of that, intraoperative blood loss was also significantly higher in ABOi recipients (544 vs 355 ml). Traditional coagulation tests such as the aPTT and the PT-INR did not differ between ABOi and ABOc recipients. This finding of a higher risk of bleeding was also substantiated in the meta-analysis in **chapter 2**, irrespective of immunoadsorption versus plasmapheresis technique.

In **chapter 5**, we demonstrated that after abandoning postoperative IA the median postoperative anti-blood group titers remained low and similar compared to the group with standard three postoperative IA sessions (median highest two-week postoperative IgG titer of 4 vs 8 with and without postoperative IA). Furthermore, there was no correlation between the occurrence of rejection and blood group titers. Rebound of A/B antibodies was observed in some patients in the group without postoperative IA, resulting in titers of 32 and even 128, although this was in the absence of rejection. All five patients with (mixed cellular) antibody mediated rejection in the 46 patients without postoperative IA had postoperative titers of ≤ 16 .

In **chapter 6** different induction agents were compared in the Dutch national cohort of ABOi kidney transplant recipients. Of the 296 recipients described, the three commonly used regimens were rituximab (n=146), rituximab/basiliximab (n=50) and alemtuzumab (n=92). In this explorative analysis, baseline characteristics, such as transplant center and year of transplantation, differed. Alemtuzumab treated recipients were older, while rituximab treated recipients had longer dialysis vintage and preemptive transplantation was performed less frequently. Rituximab/basiliximab and alemtuzumab induction both trended towards better outcomes compared to rituximab induction alone (HR for graft failure compared to rituximab of 0.32 [95%CI 0.04-2.45] and 0.87 [95%CI 0.36-2.13] respectively). This may be driven by the lower rejection rates: 47% of rituximab vs 22% of rituximab/basiliximab vs 4% of alemtuzumab treated recipients experienced rejection in the first year ($p < 0.001$). Renal function at year one was significantly better for both alemtuzumab and rituximab/basiliximab versus

rituximab treated recipients. Patient survival after ABOi kidney transplantation was similar in the three different regimens. As opposed to the high contribution of infection to death in the meta-analysis in **chapter 2**, only two of the reported 18 deaths (with a truncated follow-up of five years) were of infectious origin. Neither of the two infectious deaths were in the combined T- and B-cell directed regimens.

In **chapter 7** an ABOi recipient was described who experienced a fulminant antibody-mediated rejection during a Gram-negative sepsis. Her anti-A titer rose to IgM >5000 and IgG 512. The onset at three months after transplantation and the exceptionally high titers led to the speculation that bacterial antigens led to boosting of her anti-A antibodies. We described evidence in the literature for this blood group antibody booster hypothesis. A platelet donor taking probiotics led to severe hemolytic transfusion reactions in two platelet recipients (3). The solubilized form of this probiotic could reduce blood group titers after incubation. In the 1960s, Springer fed bacterial suspensions to men and children and demonstrated a rise in their A/B titers (4). In our experiments with suspension of anti-blood group A plasma and A erythrocytes, preincubation with *Serratia marcescens* could not prevent hemagglutination. In the supernatant there were no antibody titer changes, so the booster hypothesis could not be substantiated in our experiments.

CONCLUSIONS

The studies in this thesis have shown that ABOi kidney transplantation using a living donor is superior to ABOc deceased donor transplantation. The inferior outcomes of ABOi compared to ABOc in living donor transplantation continue to be an incentive to invest in adequate donor selection and kidney exchange programs. ABOi kidney transplant recipients experienced more antibody-mediated rejection. This increased immunological risk is also reflected in the trend towards better allograft survival with combined T- and B-cell targeting than rituximab induction alone. Postoperative immunoadsorption is of no benefit in the prevention of antibody-mediated rejection. One of the benefits of limiting the number of immunoadsorption sessions is to avoid excess blood transfusions.

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Chapter 9

Discussion, future perspectives and recommendations.

DISCUSSION

One of the main goals of this thesis was to establish the risks and benefits of ABOi kidney transplantation versus ABOc and deceased donor kidney transplantation. Therefore, we performed a meta-analysis of all published comparative studies and evaluated in more detail the results of the national ABOi program.

Comparison with ABOc living donor and deceased donor transplantation

The meta-analysis in chapter 2 demonstrated slightly inferior patient and death-censored graft survival after ABOi as compared to ABOc living donor transplantation (uncensored graft survival after one year of 96 vs 98% (RR 0.97, 95%CI 0.96-0.98). Scurt et al. published a meta-analysis on the same topic one year later and revealed worse outcomes than those described in our analysis (1): one-year patient survival of 96.9 vs 97.8% in ABOi vs ABOc (OR 2.17 [95%CI 1.63-2.90]) and death-censored graft survival of 95.8 vs 95.0% (OR 2.52 [95%CI 1.8-3.54]). This study however was heavily criticized for using odds ratios instead of relative risks and for including overlapping patient populations leading to an overestimation of their findings (2, 3). As opposed to our meta-analysis, Scurt et al. also included earlier reports before the introduction of rituximab when recipients underwent splenectomy to prevent antibody rebound. Interestingly, the supplemental material of Scurt et al. contained a subgroup analysis of rituximab versus no rituximab and revealed better outcomes for the rituximab group with death-censored graft survival with OR 1.72 [95%CI 1.01-2.94] and remaining inferior patient survival as compared to ABOc with OR 1.97 [95%CI 1.14-3.42]. This underscores that splenectomy for ABOi kidney transplantation is obsolete (4). Despite the fact that ABOi kidney transplantation in the Netherlands has been performed without splenectomy, outcomes as described in chapter 6 were inferior to the outcomes described in the meta-analysis in chapter 2 (uncensored graft survival after one year in ABOi was 91.8% in the Dutch cohort versus 96% in the meta-analysis). The Dutch ABOc control recipients also had inferior outcomes as compared to the meta-analysis (uncensored graft survival after one year 95.7 versus 98%). The much higher patient and donor age of up to 10 years in the Dutch cohort as compared to the meta-analysis is a likely explanation for the observed lower patient survival in both ABOi and ABOc. A possible contribution to the inferior outcomes in the Dutch cohort is the initial induction regimen with rituximab alone, as a trend towards inferior graft survival was observed for rituximab versus rituximab/basiliximab or alemtuzumab. Of the 26 studies in the meta-analysis, only one study, the original Swedish protocol, applied a regimen with rituximab only (5). Except for two studies with either ATG or basiliximab induction (6, 7) all other studies in the meta-analysis combined T-cell directed induction with rituximab. These observations strongly argue for the complementary role of an anti-T-cell agent in the induction regimen as discussed later in this section.

Despite a higher mortality in the first year after ABOi kidney transplantation as compared to propensity matched ABOc deceased donor recipients, patient survival

thereafter was superior (chapter 6). This is in line with a recent analysis of the United States Scientific Registry of Transplant Recipients (SRTR). Massie et al. demonstrated superior patient survival compared to matched waiting list candidates who received a (mostly deceased) donor transplant or remained on the waitlist, from 180 days post-transplant onwards (8). Contrary to this study, we did not account for candidates dying on the waiting list while waiting for a deceased donor kidney transplant in the survival analysis. This immortal time bias in chapter 6 may reflect an underestimation of the beneficial effect of ABOi kidney transplantation over (waiting for) ABOc deceased donor transplantation. We did perform a sensitivity analysis on dialysis vintage, which revealed a non-significant better patient survival after ABOi versus ABOc deceased donor transplantation (HR for death 0.82 [95%CI 0.59-1.14]).

The findings in the meta-analysis and the national cohort substantiate the choice of ABOi living donor kidney transplantation over ABOc deceased donor transplantation. As outcomes stay behind with ABOc living donor transplantation, they call for elaborate living donor transplant programs to increase options for ABOc living donor donation. These outcomes are also an incentive to address the elements of desensitization treatment in order to improve outcomes after ABOi kidney transplantation.

Antibody-mediated rejection

The results of this thesis show that antibody-mediated rejection is an obstacle after ABOi kidney transplantation as it occurred in 11 of the first 50 ABOi kidney transplant recipients at our center (chapter 3). Of note is that most of these rejection episodes (nine out of 11) took place in the first cohort of 25 recipients. Blood group O was overrepresented in this initial cohort (88%). The higher anti-A/B titers in blood group O recipients are a risk factor for ABMR and impact long-term graft survival after ABOi kidney transplantation (9-11). In the Dutch national cohort (chapter 6) a trend towards inferior outcomes for blood group O was also observed. The importance of the recipient blood group and titers limits external validation of findings in ABOi reports without blood group or titer information. The meta-analysis in chapter 2 however could not disclose blood group information as its purpose was to analyze outcomes for recipients of an ABOi kidney allograft as compared to receiving an ABOc allograft from the same center. This meta-analysis did reveal a high risk of ABMR after ABOi kidney transplantation versus ABOc living donor recipients. As PRA, DSA, retransplantation and HLA mismatches were comparable to ABOc control recipients from the same center, it is likely that the risk of ABMR could be attributed to the blood group incompatibility and not to “unchangeable” patient characteristics. Preoperative A/B titers are a risk factor for ABMR and graft loss (10). All ABOi desensitization protocols indeed recognize the importance of low preoperative IgG A/B titers and define target titers preoperatively. The comparison of these targets between centers, however, is limited by the different assays used for A/B titer measurement (12). Baseline and preoperative A/B titers seem to be more important for accommodation to occur than postoperative antibody removal (13, 14). The study in chapter 5 described that if preoperative titers are held

tight, postoperative antibody removal can be withheld in ABOi recipients. Although these findings have been substantiated in other reports (15, 16), Tobian et al. did find a correlation between postoperative titers and ABMR (17).

Avoiding (postoperative) antibody removal is important as antibody removal leads to a higher blood transfusion rate (chapters 2 and 4). Other reports have also demonstrated the contribution of plasmapheresis to bleeding risks (18). At our center we have curtailed the number of IA sessions by increasing the plasma volume from 6 to 12 liters (unpublished data, oral communication Dr. van Agteren). Different from rebound, accommodation could be disrupted by a booster production of antibodies. Chapter 7 described a recipient with an unusually late ABMR with very high IgM/IgG titers and explored the possibility of a booster effect caused by a Gram-negative sepsis. Although data on bacterial induced booster production of A/B antibody exist (19, 20), we could not demonstrate that co-culture with the *Serratia marcescens* strain resulted in anti-A titer changes. Since reports on ABMR during sepsis after ABOi kidney transplantation are scarce, the theoretical breach of accommodation by bacteremia is not likely to be relevant in clinical practice.

So, establishing accommodation and avoiding ABMR is key in ABOi kidney transplantation, as the observed higher ABMR rate likely contributes to the higher mortality as observed in the meta-analysis.

Induction therapy

Another critical appraisal of desensitization is induction therapy. Rituximab has replaced splenectomy and is a key component in the majority of desensitization protocols. The Opelz registry demonstrated a trend towards inferior graft survival when rituximab was omitted (21). The dosage may be a matter of debate but the use of rituximab is widespread (22). The retrospective analysis in chapter 6 described a relatively large cohort of recipients treated with alemtuzumab (n=92). ATG is often administered in the United States instead of rituximab (6), whereas ATG has been combined with rituximab in France (23). The ABOi literature lacks randomized controlled trials comparing rituximab with other induction therapies. Given both the high risk of ABMR and the higher risk of infection (chapter 2), the major challenge in ABOi kidney transplantation is to administer induction therapy that prevents the need for rejection treatment without excessively compromising the infectious burden. The 3C study compared alemtuzumab to basiliximab induction in ABOc kidney transplant recipients and demonstrated that such a reduction in rejection episodes is feasible without an increase in infections (24). In this study, alemtuzumab led to a 58% proportional reduction in biopsy-proven acute rejections as compared to basiliximab, with serious infection rates of 32% in both groups. Alemtuzumab in the national ABOi cohort correlated with a strikingly low risk of rejection: only 4% of alemtuzumab treated recipients vs 47% of rituximab and 22% of rituximab/basiliximab treated recipients were treated for rejection in the first year after transplantation ($p < 0.001$, chapter 6).

To conclude, it is high time for a trial of induction treatment in ABOi kidney transplant recipients, keeping in mind the differences in methods of titer measurement and antibody removal protocols.

FUTURE PERSPECTIVES

Novel desensitization strategies

A promising desensitization agent is imlifidase, an IgG-degrading enzyme derived from *Streptococcus pyogenes*. It has shown promise in HLA-incompatible kidney transplantation through the rapid and complete removal of IgG from the blood (and tissue) within several hours (25). IgG started to recur after seven to 14 days. The question arises as to whether this cleaving agent can enable ABOi (deceased donor) kidney transplantation. A major obstacle is that imlifidase does not cleave IgM. A/B antibody IgM titers have a modest correlation with IgG and their relevance to ABOi kidney transplantation is not fully understood. Tierney et al. described seven consecutive A2 living donor kidney transplantations in non-immunized O recipients (PRA 0%) with an IgG titer of <8 treated with depleting induction but without desensitization treatment (26). Four recipients had discrepantly high IgM titers ranging from 32 to 128 of whom three experienced severe rejection. In the three patients with IgM titers of 4 to 8, one recipient with an IgM titer of 4 experienced rejection. More reports suggest that IgM blood group antibodies are relevant in preventing AMR after ABOi kidney transplantation, in contrast to IgM antibodies against HLA molecules (27). Therefore the role of imlifidase may be limited to recipients with low IgM titers.

Accommodation

A different approach to lower the risk of antibody-mediated rejection after ABOi kidney transplantation is to lower the antigenicity of the incompatible kidney allograft. Tanabe et al. have investigated blood group antigen expression in kidney biopsies by immunohistochemistry in six ABOi kidney transplant recipients (28). Blood group antigen expression decreased to 64% after 10 years as compared to the implantation biopsy. In protocol biopsies of four ABOc control recipients blood group antigen expression remained stable at 99.8% after the same follow-up. Several theoretical options exist to impede the interaction of A/B antibodies and their corresponding antigens: these include reducing antigen expression by infusion of recombinant galactosidase enzyme (29) and neutralization of A/B antibodies (30). Although promising in *in vitro* experiments, so far these ideas have not been further investigated clinically.

Priority programs for ABO-incompatible kidney transplant candidates

For a kidney transplant candidate with an intended ABOi donor, the current policy in the Netherlands is to participate in the national kidney exchange program for two rounds, that is, six months. The success of this program is modest: of the 48 ABOi candidates

in this program in 2018, eight received an ABOc exchange allograft via this exchange program, 11 underwent ABOi kidney transplantation and seven were transplanted with a ABOc living donor outside the program (31). To improve the exchange rate for ABOi in such a program, either the pool can be enlarged or allocation rules can be adapted in favor of ABOi candidates. The first can be achieved by stimulating ABOc couples to voluntarily participate in exchange programs and by non-directed donation (32). The latter was simulated in a computer integrated allocation approach prioritizing immunized and blood group incompatible candidates (33): of 90 actual kidney transplantations 16 were ABOi, whereas the simulated allocation resulted in 95 transplantations of which only five were ABOi. Blood group incompatibility could thus be circumvented in the majority of these recipients.

Allowing A2 incompatible (deceased donor) kidney transplantation

The kidney transplant candidate without a living donor is dependent on allocation programs for deceased donors (34). In the United States, transplantation of deceased donor blood types A2(B) is allowed for blood type B candidates with acceptable low (IgG \leq 4) anti-A titers since 2014 (35). Outcomes after 560 A2 incompatible kidney transplantations appeared similar to ABOc transplantation in a retrospective United Network for Organ Sharing (UNOS) analysis (36). A simulation study in the United Kingdom permitting A2 donation resulted in more HLA 0-0-0 mismatched donor kidneys and shorter waiting times for B recipients (37).

RECOMMENDATIONS

The studies in this thesis have investigated desensitization treatment for ABOi kidney transplantation. Its findings have the following clinical implications:

- Invest in an elaborate living kidney donation program to enable chances of finding an ABO-compatible living donor in due time (chapters 2 and 6).
- In the absence of an ABOc living donor, when an ABOi donor is available, proceed with ABOi kidney transplantation instead of deceased donor transplantation (chapter 6).
- Be prepared for the higher bleeding risk in ABOi recipients (chapters 2 and 4). Desensitization results in an average fall in hemoglobin level of 0.55 mmol/L and more intraoperative blood loss (chapter 4).
- Abandon postoperative immunoadsorption (chapter 5).
- The higher risk of (antibody-mediated) rejection should guide induction therapy to optimize outcomes after ABOi kidney transplantation (chapters 3 and 6). Combined T- and B-cell targeted therapy likely results in better outcomes than rituximab induction alone.

- Outcomes after ABOi kidney transplantation should be interpreted in the context of recipient blood group (O versus non-O) (chapters 3 and 6).

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Chapter 10

NEDERLANDSE SAMENVATTING.

Samenvatting

Discussie

Toekomstperspectieven

Aanbevelingen

SAMENVATTING

Het doel van het bestuderen van bloedgroep ABO-incompatibele (ABOi) niertransplantatie is het verbeteren van uitkomsten voor niertransplantatie patiënten. In de studies van dit proefschrift hebben we de risico's van ABOi niertransplantatie onderzocht. De voor- en nadelen van specifieke onderdelen van de desensibilisatie behandeling werden geanalyseerd, alsmede de status van ABOi niertransplantatie in verhouding tot andere vormen van niertransplantatie.

In **hoofdstuk 2 en 6** hebben we uitkomsten vergeleken van ontvangers van een ABOi niertransplantaat met ontvangers van een ABO-compatibel (ABOc) niertransplantaat. We hebben systematisch alle studies geanalyseerd die zowel de ABOi als de ABOc niertransplantaties uit hetzelfde centrum in dezelfde tijdsperiode bestudeerd hebben (**hoofdstuk 2**). Deze meta-analyse liet zien dat ABOi ontvangers een iets slechtere patiënt- en transplantaatoverleving hebben dan vergelijkbare ABOc ontvangers: voor elke 100 transplantaties bleken na één jaar in deze meta-analyse 96 ABOi ontvangers in leven met een functionerend niertransplantaat, vergeleken met 98 ABOc ontvangers (4 versus 2 transplantaat verliezen). Van de gerapporteerde doodsoorzaken na ABOi niertransplantatie bleek de helft een infectieuze oorzaak te betreffen, versus slechts 13% na ABOc niertransplantatie. De slechtere transplantaatoverleving bleek onafhankelijk van inductietherapie, regio, periode en het gebruik van plasmaferese versus immunoabsorptie. Antistof-gemedieerde afstoting, ernstige (niet-virale) infecties en bloedingen kwamen ook meer voor na ABOi niertransplantatie. Voor adequate informatieverstrekking aan incompatibele koppels zijn deze bevindingen relevant. Het is echter voor deze voorlichting ook belangrijk om naast de vergelijking met ABOc levende donor niertransplantatie, ook de vergelijking met ABOc overleden donor niertransplantatie te maken.

Daartoe werden in **hoofdstuk 6** alle Nederlandse ABOi niertransplantatie ontvangers vergeleken met zowel 'gematchte' ABOc overleden donor als 'gematchte' ABOc levende donor ontvangers. De patiëntoverleving na ABOi niertransplantatie was beter dan na ABOc overleden donor niertransplantatie (hazard ratio (HR) voor overlijden 0.67 [95%CI 0.48-0.92] in ABOi versus ABOc overleden donor ontvangers). De transplantaatoverleving na ABOi niertransplantatie verschilde niet van die na ABOc overleden donor niertransplantatie. De helft van deze ABOi niertransplantatie ontvangers was behandeld met rituximab inductie, en van hen neigde de transplantaatoverleving naar matigere uitkomsten dan van hen die behandeld werden met gecombineerde T- en B-cel inductie.

Hoofdstukken 3, 4 en 5 bevatten studies over het ABOi programma van het Erasmus Medisch Centrum (MC).

De eerste 50 ABOi niertransplantatie ontvangers van het Erasmus MC werden beschreven in **hoofdstuk 3**, met een analyse van het optreden van antistof-gemedieerde afstoting. Voor deze analyse werd elke ABOi ontvanger uit dit eerste cohort van 50 'gematcht' met 2 ABOc levende donor niertransplantatie ontvangers. Deze 'matching'

oftewel koppeling gebeurde op basis van het jaar van transplantatie, de leeftijd van de ontvanger en het aantal humaan leucocytenantigenen (HLA) mismatches. Antistof-gemedieerde afstoting kwam vaak voor, namelijk bij 11 van de 50 ABOi ontvangers. Vijf van deze 11 afstotingen vertoonden in het biopt tekenen van een gemengd cellulaire en antistof-gemedieerde afstoting. Deze bevindingen vormden aanleiding tot reflectie op het gehanteerde inductieprotocol. Het frequent optreden van afstoting, waaronder ook cellulaire afstoting, zeker in vergelijking met andere publicaties over dit Zweedse ABOi protocol (1,2), was aanleiding tot aanpassing van het protocol. In 2015 werd rituximab (anti-CD20, depletie van B-cellen) als inductietherapie vervangen door alemtuzumab (anti-CD52, depletie van T, B en NK cellen) (**hoofdstuk 6**).

Het idee onder klinici dat tijdens ABOi niertransplantatie moeilijker hemostase bereikt kon worden, werd onderzocht in **hoofdstuk 4**. Daartoe hebben we de eerste 65 ABOi ontvangers in het Erasmus MC vergeleken met 130 ABOc ontvangers, 'gematcht' voor leeftijd van de donor en van de ontvanger in de periode 2006-2013. Bloedtransfusie in de eerste 48 uur vond meer dan twee maal zo vaak plaats na ABOi dan na ABOc niertransplantatie (29% vs 12%). Zowel het aantal ontvangers dat een bloedtransfusie nodig had, als het aantal eenheden erythrocyten concentraat (EC) dat per transfusie gegeven werd, was hoger in ABOi ontvangers. Dit gold eveneens voor het optreden van 'majeure bloeding', hier gedefinieerd als ≥ 3 ECs (15% vs 2% in ABOc). De trombocyten (bloedplaatjes) daalden significant tijdens desensibilisatie voorafgaand aan ABOi niertransplantatie, resulterend in 22% van de ABOi ontvangers met een preoperatief trombocyten getal van $<100 \times 10^9/L$. Een opvallende bevinding was dat 40% van de ontvangers met bloedgroep O een bloedtransfusie onderging, in tegenstelling tot slechts 9% van de ontvangers met bloedgroep A of B. Echter, na correctie in een multivariate analyse correleerde alleen nog het aantal sessies immunoabsorptie (IA) met het aantal postoperatieve bloedtransfusies. Het hemoglobinegehalte daalde tijdens desensibilisatie voorafgaand aan ABOi niertransplantatie met gemiddeld 0.55 mmol/L, hetgeen een factor was in de grotere bloedtransfusie behoefte. Daarnaast hadden ABOi ontvangers ook meer bloedverlies intra-operatief dan ABOc ontvangers (544 vs 355 ml). De stollingstesten aPTT en PT-INR verschilden niet tussen ABOi en ABOc ontvangers. Het grotere bloedingsrisico zoals beschreven in **hoofdstuk 4** werd ook gevalideerd in de meta-analyse in **hoofdstuk 2**, waarbij de techniek van immunoabsorptie versus plasmaferese geen factor bleek.

In **hoofdstuk 5** lieten we zien dat bij het weglaten van postoperatieve immunoabsorptie (IA) de bloedgroep titers laag bleven en vergelijkbaar waren met het beleid van standaard drie postoperatieve IAs (de hoogste IgG titer binnen 2 weken na transplantatie was mediaan 4 versus 8 zonder postoperatieve IA ($p=0.6$)). Bovendien was er geen samenhang tussen het optreden van afstoting en de hoogte van de bloedgroep titers. 'Rebound' van A/B antistoffen kwam wel voor in ontvangers zonder postoperatieve IA, resulterend in IgG titers van 32 tot zelfs 128. Bij deze rebound trad er echter geen afstoting op. In de groep van 46 ontvangers zonder postoperatieve IA

hadden vijf een (gemengd cellulaire en) antistof-gemedieerde afstoting, van wie de postoperatieve IgG titers allen ≤ 16 bleven.

In **hoofdstuk 6** werden verschillende inductietherapieën binnen het nationale cohort van ABOi niertransplantatie ontvangers met elkaar vergeleken. Bij de beschreven 296 ABOi ontvangers werden rituximab (n=146), rituximab/basiliximab (n=50) en alemtuzumab (n=92) het meest gebruikt als inductietherapie. Deze inductievergelijking was exploratief, dat wil zeggen niet gecorrigeerd voor mogelijke bias. Karakteristieken zoals transplantatiecentrum en jaar van transplantatie verschilden. De ontvangers behandeld met alemtuzumab waren ouder, terwijl de met rituximab behandelde ontvangers langer gedialyseerd hadden en minder frequent pre-emptief getransplanteerd werden. ABOi ontvangers behandeld met zowel de combinatie rituximab/basiliximab als met alemtuzumab inductie hadden niet-significant betere uitkomsten dan na inductie met alleen rituximab (HR voor transplantaat falen vergeleken met rituximab 0.32 [95%CI 0.04-2.45] respectievelijk 0.87 [95%CI 0.36-2.13]). De trend naar slechtere uitkomsten na (alleen) rituximab zou verklaard kunnen worden door meer afstoting: 47% rejectie in het eerste jaar na rituximab versus 22% na rituximab/basiliximab versus 4% na alemtuzumab inductie (p < 0.001). De nierfunctie na 1 jaar was beter na zowel alemtuzumab als na rituximab/basiliximab vergeleken met rituximab inductie. De patiëntoverleving verschilde niet binnen de drie groepen. In tegenstelling tot de grote bijdrage van infecties aan overlijden in de meta-analyse in **hoofdstuk 2**, hadden binnen het nationale cohort maar 2 van de 18 gerapporteerde sterfgevallen (weliswaar binnen 5 jaar na niertransplantatie) een infectieuze doodsoorzaak. Geen van deze 2 infectieuze sterfgevallen waren bij ontvangers met gecombineerde T- en B-cel inductie.

In **hoofdstuk 7** werd een ABOi niertransplantatie ontvanger beschreven die een fulminante antistof-gemedieerde afstoting doormaakte tijdens een *Gram-negatieve* sepsis. Haar anti-A titer steeg tot IgM >5000 en IgG 512. Het optreden van afstoting drie maanden na niertransplantatie in combinatie met de zeer hoge titers brachten ons tot de hypothese dat herkenning van bacteriële epitopen lijkend op bloedgroep A antigenen, tot 'boostering' van haar anti-A antistoffen geleid heeft. Een aantal publicaties ondersteunt deze booster hypothese: een bloeddonor die trombocyten doneerde had veel probiotica tot zich genomen, waarna ernstige hemolytische transfusiëreacties optraden bij 2 patiënten die zijn trombocyten getransfundeerd hadden gekregen (3). Deze probiotica in opgeloste vorm bleken na incubatie met plasma de bloedgroep titers verlaagd te hebben. In de jaren 60 heeft Springer volwassenen en kinderen opgeloste *E. Coli* bacteriën laten drinken en toonde aan dat hun anti-B titer hierop fors steeg (4). In experimenten beschreven in **hoofdstuk 7** hebben we anti-A plasma geïncubeerd met *Serratia marcescens*. Door vervolgens A erythrocyten toe te voegen kon onderzocht worden of deze pre-incubatie met bacteriën de hemagglutinatie geremd had, wat helaas niet het geval bleek. In het supernatant waren er geen verschillen in titers. De booster hypothese werd dus niet ondersteund door onze experimenten.

DISCUSSIE

Vergelijking ABOi met ABOc levende donor en ABOc overleden donor niertransplantatie

De meta-analyse in hoofdstuk 2 liet een iets slechtere patiënt- en transplantaat-overleving zien na ABOi dan na ABOc levende donor niertransplantatie (1-jaars transplantaatoverleving niet-gecorrigeerd voor overlijden 96% versus 98% (RR 0.97, 95%CI 0.96-0.98). De meta-analyse over hetzelfde onderwerp een jaar later (Scurt et al.) liet slechtere uitkomsten zien na ABOi niertransplantatie dan onze analyse (5): 1-jaars patiëntoverleving van 96.9 versus 97.8% in ABOi versus ABOc (OR 2.17 [95%CI 1.63-2.90] en transplantaatoverleving gecorrigeerd voor overlijden 95.8 versus 95.0% (OR 2.52 [95% CI 1.8-3.54]). Deze studie is echter flink bekritiseerd vanwege het gebruik van odds ratio's in plaats van relatieve risico's en voor het includeren van overlappende patiëntenpopulaties, welke beide tot een overschatting van het verschil geleid hebben (6, 7). In tegenstelling tot onze meta-analyse, betrof Scurt et al. ook studies vòòr de introductie van rituximab, toen splenectomie ingezet werd om antistof 'rebound' te voorkomen. De 'Supplemental Materials' van Scurt et al. bevatte een subgroep analyse van de splenectomie versus de rituximab groep. De rituximab groep had een betere transplantaatoverleving gecorrigeerd voor overlijden met een OR van 1.72 [95%CI 1.01-2.94] maar met nog steeds een slechtere patiëntoverleving vergeleken met ABOc met een OR van 1.97 [95%CI 1.14-3.42] (5). Deze data laten zien dat splenectomie voor ABOi niertransplantatie obsoleet is (8). Ondanks dat in Nederland geen splenectomie toegepast werd, zijn de Nederlandse uitkomsten na ABOi zoals beschreven in hoofdstuk 6 slechter dan de uitkomsten zoals beschreven in de meta-analyse in hoofdstuk 2 (1-jaars transplantaatoverleving niet gecorrigeerd voor overlijden na ABOi in het nationale cohort 91.8% versus 96% in de meta-analyse). De nationale ABOc 'gematchte' controle ontvangers hadden óók slechtere uitkomsten dan in de meta-analyse (1-jaars transplantaatoverleving niet gecorrigeerd voor overlijden was 95.7% versus 98%). De fors hogere leeftijd van zowel donor als ontvanger van elk circa 10 jaar zal zeker een rol gespeeld hebben in de lagere patiëntoverleving in zowel de nationale ABOi als de nationale ABOc populatie. Een mogelijk bijdragende factor van de inferieure uitkomsten in het nationale cohort is het initiële gebruik van (alleen) rituximab inductie, aangezien er een trend onderscheiden kon worden naar een slechtere transplantaatoverleving voor rituximab versus zowel rituximab/basiliximab als alemtuzumab. Van de 26 studies waarop de meta-analyse gestoeld was, had alleen de studie die het 'Zweedse protocol' beschrijft, monotherapie rituximab als inductie (1). Behalve twee studies met ATG of met basiliximab (9, 10) betreffen de andere studies een regime waarbij rituximab gecombineerd werd met een T-cel gerichte inductie. Deze bevindingen pleiten voor de toegevoegde waarde van een (tevens) T-cel gerichte therapie bij ABOi inductie zoals ook verderop in deze discussie beschreven wordt.

Ondanks een hogere mortaliteit in het eerste jaar na ABOi niertransplantatie dan na 'propensity-matched' ABOc overleden donor niertransplantatie, is de patiëntoverleving

daarna beter (hoofdstuk 6). Dit komt overeen met een recente analyse van de Amerikaanse Scientific Registry of Transplant Recipients (SRTR). Massie et al. liet zien dat de overleving na ABOi niertransplantatie superieur was aan 'gematchte' wachtlijst kandidaten die een niertransplantatie ondergingen (merendeels van een overleden donor) dan wel nog op de wachtlijst hiervoor stonden, vanaf 180 dagen na niertransplantatie (11). Anders dan in deze studie, corrigeerden wij in hoofdstuk 6 niet voor de sterfte die kan optreden bij wachtlijst kandidaten in de 'survival analyse'. Deze 'immortal time bias' in hoofdstuk 6 wijst op een onderschatting van het gunstige effect van ABOi niertransplantatie ten opzichte van (wachten op) een overleden donor niertransplantatie. Wel hebben we een sensitiviteitsanalyse uitgevoerd op dialyse duur, welke een niet-significante betere patiëntoverleving liet zien na ABOi versus ABOc overleden donor niertransplantatie (HR voor overlijden 0.82 [95%CI 0.59-1.14]).

De bevindingen van de meta-analyse en het nationale cohort bekrachtigen de keuze voor ABOi niertransplantatie in plaats van ABOc overleden donor niertransplantatie. Omdat de uitkomsten wel matiger zijn dan na ABOc levende donor niertransplantatie, zijn ze aanleiding tot het stimuleren van een uitgebreid levende donor niertransplantatieprogramma om de kans op een ABOc aanbod te vergroten. Tevens zijn deze bevindingen aanleiding tot het kritisch beoordelen van de onderdelen van desensibilisatie behandelingen, om de uitkomsten na ABOi niertransplantatie te verbeteren.

Antistof-gemedieerde afstoting

De resultaten beschreven in dit proefschrift laten zien dat antistof-gemedieerde afstoting een probleem is na ABOi niertransplantatie: in 11 van de 50 eerste ABOi ontvangers in het Erasmus MC trad antistof-gemedieerde afstoting (ABMR) op (hoofdstuk 3). Daarbij moet vermeld worden dat 9 van deze 11 afstotingen optraden in de eerste 25 ontvangers. Bloedgroep O ontvangers vormden de overgrote meerderheid (88%) van dit cohort. De hogere anti-A/B titers in bloedgroep O ontvangers vormen een risicofactor voor ABMR en benadelen de lange termijn transplantaatoverleving na ABO niertransplantatie (12-14). In het Nederlandse nationale cohort (hoofdstuk 6) hadden bloedgroep O ontvangers een niet-significante slechtere transplantaatoverleving. Het belang van de bloedgroep en de antistof titers van ontvangers zorgen ervoor dat als deze informatie ontbreekt, de externe validatie van vergelijkende studies binnen ABOi niertransplantatie kleiner wordt. Bij de meta-analyse in hoofdstuk 2 ontbrak bloedgroepinformatie echter en lag de focus op de vergelijking tussen ABOi en ABOc uit hetzelfde centrum. Wel liet de meta-analyse een hoger risico zien op ABMR na ABOi dan na ABOc levende donor niertransplantatie. Aangezien panel reactieve antistoffen (PRA), donor specifieke antistoffen (DSA), retransplantatie en humaan leucocytenantigenen (HLA) mismatches vergelijkbaar waren met ABOc controle ontvangers uit hetzelfde centrum, leek het verhoogde ABMR risico te komen door de ABO incompatibiliteit en niet door 'vaststaande' patiënteigenschappen. Preoperatieve A/B titers zijn een risicofactor voor het optreden van ABMR en voor transplantaat verlies (13). Alle ABOi

desensibilisatie protocollen erkennen dan ook het belang van lage preoperatieve IgG A/B titers en definiëren streeftiters om te kunnen opereren. Een vergelijking van deze streeftiters tussen verschillende centra wordt echter bemoeilijkt door de verschillende testen die gebruikt worden om A/B titers te meten (15). De starttiter voorafgaand aan desensibilisatie en de direct preoperatieve titer lijken een grotere bijdrage te leveren aan het wel of niet optreden van accommodatie dan postoperatieve titerverlaging (16, 17). De studie in hoofdstuk 5 beschreef dat postoperatieve plasmaferese (immunoadsorptie) achterwege gelaten kan worden in ABOi ontvangers, mits de preoperatieve titers laag gehouden worden. Ook al zijn deze bevindingen overeenkomstig andere publicaties (18, 19), Tobian et al. vond wel een correlatie tussen postoperatieve titers en het optreden van ABMR (20).

Het limiteren van (postoperatieve) plasmaferese (immunoadsorptie) is belangrijk voor het vermijden van bloedtransfusie (hoofdstuk 2 en 4). Andere publicaties hebben ook deze bijdrage van plasmaferese aan bloedingsrisico beschreven (21). In ons centrum hebben we het aantal sessies immunoadsorptie kunnen beperken door het gewisselde plasmavolume te verhogen van 6 naar 12 liter (ongepubliceerde data, mondelinge communicatie dr Van Agteren). Naast 'rebound', kan accommodatie ook teniet gedaan worden door 'boostering' van antistof productie. Hoofdstuk 7 beschreef een ontvanger met een late ABMR met hoge IgM en IgG titers en onderzocht of haar *Gram-negatieve* sepsis tot een 'boostering' van haar anti-A antistoffen geleid zou kunnen hebben. Alhoewel er beschreven is dat bacteriën A/B antistofproductie kunnen 'boosteren' (3, 4), resulteerde het kweken van *Serratia marcescens* met anti-A plasma niet in een anti-A titer verandering van dit plasma. Er zijn weinig publicaties over ABMR tijdens sepsis na ABOi niertransplantatie. De theorie van de 'accommodatiebreuk' door bacteriëmie speelt in de dagelijkse praktijk waarschijnlijk geen grote rol.

Accommodatie en het vermijden van ABMR zijn dus zeer relevant voor ABOi niertransplantatie, waarbij het frequenter optreden van ABMR waarschijnlijk bijdraagt aan de hogere sterfte zoals beschreven in de meta-analyse.

Inductie therapie

Bij desensibilisatie is naast het verwijderen van de aanwezige antistoffen door middel van plasmaferese, ook het remmen van antistof productie middels inductietherapie van belang. Rituximab heeft splenectomie vervangen en is een onderdeel van de inductie in de overgrote meerderheid van de desensibilisatie protocollen. De Opelz registratie liet een niet-significant slechtere transplantaatoverleving zien bij desensibilisatie zonder rituximab (22). Over de dosis wordt gediscussieerd in de literatuur, maar het gebruik op zich van rituximab is alomtegenwoordig (23). De retrospectieve analyse in hoofdstuk 6 beschreef een relatief groot cohort van ABOi ontvangers behandeld met alemtuzumab (n=92). ATG wordt in de Verenigde Staten vaak gebruikt in plaats van rituximab (9), en de combinatie ATG/ rituximab is toegepast in Frankrijk (24). Er bestaan geen gerandomiseerd-gecontroleerde studies die rituximab in het kader van ABOi vergelijken met andere inductiestrategieën. Aangezien zowel ABMR als

infectie vaker optraden (hoofdstuk 2), is de opgave voor ABOi niertransplantatie om inductietherapie toe te passen die voorkomt dat therapie tegen afstoting nodig is, en die op hetzelfde moment de infectiedruk laag houdt. De '3C studie' vergeleek alemtuzumab met basiliximab inductie in ABO-compatibele niertransplantatie ontvangers en liet zien dat het verminderen van rejectie haalbaar is terwijl het aantal infecties gelijk bleef (25). In deze studie gaf alemtuzumab een 58% proportionele reductie in biopsie bewezen acute afstotingen vergeleken met basiliximab, met ernstige infecties die bij 32% van de ontvangers in beide groepen optraden. In onze nationale studie resulteerde alemtuzumab in opvallend weinig afstotingen: slechts 4% van de alemtuzumab versus 47% van de rituximab versus 22% van de rituximab/ basiliximab ontvangers werden behandeld voor afstoting in het eerste jaar ($p < 0.001$, hoofdstuk 6).

Het is dan ook hoog tijd voor een inductiestudie in ABOi niertransplantatie, waarbij wel rekening gehouden dient te worden met verschillen in titer bepalingen en antistof reductie (plasmaferese) protocollen.

TOEKOMSTPERSPECTIEVEN

Nieuwe desensibilisatie strategieën

Imlifidase is een nieuw desensibilisatie medicijn en afkomstig van een enzym van *Streptococcus pyogenes* dat IgG afbreekt. Het lijkt veelbelovend voor HLA-incompatibele niertransplantatie door de snelle en volledige afbraak van IgG in bloed en weefsel binnen enkele uren (26). Nieuw IgG kwam na zeven tot 14 dagen terug. Dit roept de vraag op of dit 'IgG klievende' enzym ook ingezet kan worden voor ABOi niertransplantatie. Een belangrijke hindernis is dat imlifidase geen IgM 'kleeft'. IgM A/B titers hebben enigszins een correlatie met IgG titers, en hoe relevant IgM titers zijn voor ABOi niertransplantatie is eigenlijk nog niet duidelijk. Tierney et al. beschreef zeven A2 levende donor niertransplantatie ontvangers in niet-geïmmuniseerde bloedgroep O ontvangers (PRA 0%) met een IgG titer < 8 die behandeld werden met depletierende inductie maar zonder desensibilisatie/ plasmaferese (27). Vier ontvangers hadden opvallend hoge IgM titers tussen de 32 en 128, van wie drie een ernstige afstoting doormaakten. Andere publicaties wijzen ook op de relevantie van IgM anti-bloedgroep antistoffen voor ABMR na ABOi niertransplantatie, hetgeen anders is dan IgM anti-HLA antistoffen, die niet relevant lijken voor ABMR in (ABOc) niertransplantatie (28). Dit in ogenschouw nemende zou imlifidase in eerste instantie alleen bij lage IgM A/B titers ingezet kunnen worden.

Accommodatie

Een andere aanpak in de preventie van ABMR na ABOi niertransplantatie zou het verminderen van de antigeniciteit van de ABOi donornier kunnen zijn. Tanabe et al. onderzocht bloedgroep antigen expressie in nierbiopsieën door middel van immunohistochemie in zes ABOi niertransplantatie ontvangers (29). Deze expressie nam

af tot een niveau van 64% op jaar 10 ten opzichte van implantatie biopsieën. In protocol biopsieën van ABOc ontvangers bleef de bloedgroep antigeen expressie met 99.8% op hetzelfde niveau na dezelfde follow-up. Er zijn verschillende (theoretische) opties die de interactie tussen A/B antistoffen en A/B antigenen zouden kunnen remmen: het verminderen van A/B antigeen expressie door recombinant galactosidase enzym te infunderen (30) of het neutraliseren van A/B antistoffen (31). Alhoewel veelbelovend in *in vitro* experimenten, zijn deze opties nog niet toegepast in klinische studies.

Prioritering programma's voor ABO-incompatibele niertransplantatie kandidaten

Voor een niertransplantatie kandidaat met een beoogde ABOi donor, is het Nederlandse beleid om dit ABOi koppel te laten participeren in de nationale cross-over; in het algemeen voor 2 rondes (6 maanden). Het succes van dit beleid is echter beperkt: van de 48 ABOi niertransplantatie kandidaten in dit programma in 2018, ontvingen slechts acht een ABOc cross-over donornier, ondergingen 11 (desensibilisatie voor een) ABOi niertransplantatie en werden zeven mensen getransplanteerd met een ABOc donor nier buiten de cross-over (32). Om de cross-over kansen voor ABOi kandidaten te verbeteren, kan ofwel de pool (dus het programma) uitgebreid worden of kunnen de allocatie regels aangepast worden ten faveure van ABOi kandidaten. Het eerste kan bereikt worden door te stimuleren dat ook ABOc koppels vrijwillig meedoen in de nationale cross-over en door anonieme ('non-directed') nierdonatie (33). De tweede optie, aangepaste allocatie, werd gesimuleerd in een computer gestuurde allocatie die prioriteit gaf aan geïmmuniseerde en bloedgroep incompatibele ontvangers (34): van de 90 daadwerkelijk uitgevoerde niertransplantaties waren er 16 ABOi, terwijl computer-allocatie resulteerde in 95 transplantaties, waarvan slechts 5 ABOi. Bloedgroep incompatibiliteit kon dus in de meerderheid van de gesimuleerde transplantaties vermeden worden.

Het toestaan van A2-incompatibele overleden donor niertransplantatie

Een niertransplantatie kandidaat zonder een levende donor is aangewezen op overleden donor programma's (35). In de Verenigde Staten zijn de regels voor allocatie in 2014 zo gewijzigd dat nieren van een overleden donor met A2(B) gealloceerd kunnen worden aan bloedgroep B ontvangers met lage anti-A titers (IgG \leq 4) (36). Een retrospectieve analyse van de United Network for Organ Sharing (UNOS) database liet voor 560 A2-incompatibele niertransplantaties vergelijkbare uitkomsten zien als voor ABOc niertransplantaties (37). Een studie in het Verenigd Koninkrijk simuleerde overleden donor A2-incompatibele nierdonatie hetgeen resulteerde in meer niertransplantaties met 0-0-0 HLA mismatches en een kortere wachttijd voor bloedgroep B ontvangers (38).

AANBEVELINGEN

De studies in dit proefschrift hebben desensibilisatie behandeling voor ABO-incompatibele niertransplantatie onderzocht. De bevindingen hebben de volgende klinische implicaties:

- Investeer in een uitgebreid levende donor niertransplantatieprogramma om de kansen op een ABO-compatibele donornier van een levende donor te vergroten (hoofdstuk 2 en 6).
- Indien er geen ABOc nier van een levende donor beschikbaar is, en wel een ABOi donor, transplanteer dan ABOi met een levende donor in plaats van (te wachten op) een overleden donor niertransplantatie (hoofdstuk 6).
- Wees bedacht op een hoger bloedingsrisico in ABOi ontvangers (hoofdstuk 2 en 4). De desensibilisatie behandeling resulteert in een hemoglobine daling van gemiddeld 0.55 mmol/L en meer intra-operatief bloedverlies (hoofdstuk 4).
- Pas geen postoperatieve immunoabsorptie toe (hoofdstuk 5).
- Het hogere risico op (antistof-gemedieerde) afstoting moet betrokken worden in de keuze van de inductietherapie (hoofdstuk 3 en 6). Gecombineerde T- en B-cel gerichte inductietherapie lijkt betere resultaten te geven dan rituximab alleen.
- Bij vergelijkende studies binnen ABOi niertransplantatie moet de bloedgroep van de ontvangers vermeld worden (O versus non-O) (hoofdstuk 3 en 6).

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Chapter 11

APPENDICES.

List of abbreviations

List of publications

PhD portfolio

Curriculum Vitae

Dankwoord

LIST OF ABBREVIATIONS

ABMR	antibody-mediated rejection
ABOc	ABO-compatible
ABOi	ABO-incompatible
AMR	antibody-mediated rejection
ANOVA	Analysis of Variance
aPTT	activated partial thromboplastin time
ATG	anti-thymoglobulin
ATZ	alemtuzumab
BAS	basiliximab
BUN	blood urea nitrogen
cfu	colony forming units
CI	confidence interval
CMV	cytomegalovirus
DAG	Directed Acyclic Graph
DFPP	double-filtration plasmapheresis
DSA	donor-specific antibodies
EC	erythrocyte concentration
eGFR	estimated glomerular filtration rate
HLA	human leucocyte antigen
HR	hazard ratio
IA	immunoabsorption
Ig	immunoglobulin
IL-2	Interleukin-2
IL-2RAb	Interleukin-2 Receptor Antibody
IQR	interquartile range
IVIG	intravenous immunoglobulin
MAR	mixed cellular and antibody-mediated rejection
MM	mismatch
n.s.	not significant
OR	odds ratio
PBS	phosphate buffered saline
pcr	polymerase chain reaction
PE	plasma exchange
PI	platelet count
post-op	postoperative
PP	plasmapheresis
PRA	panel-reactive antibodies

pre-op	preoperative
PT-INR	prothrombin time-international normalized ratio
RR	relative risk
RTX	rituximab
<i>S. marcescens</i>	<i>Serratia marcescens</i>
SD	standard deviation
SEM	standard error of the mean
SRTR	Scientific Registry of Transplant Recipients
UNOS	United Network for Organ Sharing

LIST OF PUBLICATIONS

Betjes MGH, Sablik K, Litjens N, Otten HG, de Weerd AE. ARHGDI B and AT1R autoantibodies are differentially related to the development and presence of chronic antibody-mediated rejection and fibrosis in kidney allografts. *Human Immunology*, *accepted* December 2020.

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PhD PORTFOLIO

Name	Anna Elisabeth (Annelies) de Weerd
Erasmus MC department	Internal Medicine, section Nephrology and Kidney Transplantation
Promotor	Prof. dr. R. Zietse
Copromotor	dr. M.G.H. Betjes

Date	Activity	EC
24-09-2020	Nephso meeting Transplantation	0.05
04-03-2020	Bootcongres 2020 Wetenschappelijk voorjaarscongres NTV (1 oral, 1 poster)	0.30
13-02-2020	5de Regionale nascholing Nefrologie en Transplantatie anno 2020: op koers! (3 presentations)	0.10
31-01-2020	Transplantatie Symposium 2020	0.30
28-01-2020	Masterclass Critical Care Nephrology	0.20
14-11-2019	Klinisch Review Symposium 2019	0.20
15-09-2019	ESOT 2019: Europeans Society of Organ Transplantation (1 oral, 2 posters)	0.85
29-08-2019	Erasmus MC - Scientific Integrity	0.30
13-03-2019	Bootcongres 2019 Wetenschappelijk voorjaarscongres NTV (4 orals)	0.45
18-01-2019	BROK herregistratie	0.25
15-11-2018	Klinisch Review Symposium 2018	0.15
04-06-2018	ATC 2018: American Transplant Congress (1 oral)	0.65
15-03-2018	Joint NTV-BTS Transplantation Congress 2018 (1 poster)	0.40
08-02-2018	4e Regionale nascholing Nefrologie en Transplantatie anno 2018: de huidige koers! (1 presentation)	0.30
18-01-2018	Transplantatie Symposium 2018	0.20
08-06-2017	Regionale nefro pathologie nascholing	0.05
02-04-2017	ATC 2017: American Transplant Congress	0.85
08-03-2017	Bootcongres 2017 Wetenschappelijk voorjaarscongres NTV (1 oral)	0.35
14-12-2016	Workshop Nefrologie Papendal	0.20
24-11-2016	Masterclass Kidney Transplantation Padua	0.50
06-10-2016	Transmurale Transplantatiezorg	0.15
09-03-2016	Bootcongres 2016 Wetenschappelijk voorjaarscongres	0.35
04-02-2016	3de Regionale nascholing: Niertransplantatie anno 2016: nieuwe inzichten en uitdagingen!	0.25
01-01-2016	Journal Club Internal Medicine	1.00
19-11-2015	Klinisch Review Symposium 2015	0.20

13-09-2015	ESOT 2015: Europeans Society of Organ Transplantation (1 oral)	0.85
25-06-2015	Regionale Nierbiopsie Avond	0.10
11-03-2015	Bootcongres 2015 Joint NTV/ BTS Congres	0.55
13-11-2014	Erasmus MC – BROK (Basic course Rules and Organisation for Clinical researchers)	1.50
17-10-2014	ESOT + AST joint meeting: personalizing transplantation: from new diagnostics to new therapeutics	0.55
11-09-2014	Praktische Nefropathologie	0.45
03-04-2014	Masterclass in Kidney Transplantation Antwerp	0.50
26-03-2014	Bootcongres 2014 wetenschappelijk voorjaarscongres NTV (1 oral)	0.50
20-03-2014	Sixth Rotterdam Course in Electrolyte and Acid-Base disorders	0.20
01-01-2014	Teaching master students, nurses (in training), fellows internal medicine and nephrology	72.0
01-01-2014	Organizer minor: Solid Organ Transplantation	20.00

CURRICULUM VITAE

Annelies de Weerd werd op 8 februari 1979 geboren te Rotterdam. In 1997 behaalde zij haar VWO-diploma aan het Marnix Gymnasium in Rotterdam. Na het behalen van haar propedeuse Rechten aan de Vrije Universiteit Amsterdam in 1998, begon zij datzelfde jaar aan de studie Geneeskunde, eveneens aan de Vrije Universiteit. Met het afstudeerprofiel Immunologie en Ontstekingsprocessen behaalde zij cum laude haar artsexamen in 2004. Zij startte als arts-assistent op de afdeling Inwendige Geneeskunde in het Ikazia Ziekenhuis, alwaar zij in 2005 haar opleiding tot internist aanving (opleiders dr. A. Dees, Prof. dr. H.A.P. Pols en Prof. dr. J.L.C.M. van Saase). In 2008 heeft zij gedurende een jaar haar opleiding onderbroken om onderzoek te kunnen doen op het Transplantatie Laboratorium in het Erasmus MC (supervisors Prof. dr. C.C. Baan en Prof. dr. W. Weimar). In 2011 startte zij met haar differentiatie Nefrologie (opleider Prof. dr. R. Zietse). Vanaf december 2013 is zij werkzaam als internist-nefroloog in het Erasmus MC. Het onderzoek wat zij de afgelopen jaren gedaan heeft naar ABO-incompatibele niertransplantatie (supervisors Prof. dr. R. Zietse en dr. M.G.H. Betjes), heeft uiteindelijk geresulteerd in dit proefschrift.

DANKWOORD

Het leuke van werken in een ziekenhuis is dat je zo ontzettend veel collega's hebt. Dat geldt onverminderd voor het verrichten van klinisch onderzoek. Ik wil dan ook een grote groep mensen bedanken die mij dagelijks helpen en inspireren.

Michiel, mijn copromotor: Het is niet zonder risico om jou één korte vraag te stellen over welk nefrologisch onderwerp dan ook. Na jouw vlammend wetenschappelijk betoog sta ik geïnspireerd, maar ook met drie nieuw uit te voeren plannen weer buiten. Je hebt een scherp oog voor het scheiden van zin en onzin: het door jou tot de essentie brengen heeft het werk altijd beter gemaakt. En niet onbelangrijk, je kan altijd gezellig ouwehoeren over elk niet-nefrologisch onderwerp.

Bob, behalve als mijn promotor, heb ik je ook mogen meemaken als opleider en sectorhoofd. Dit alles doe je met grote kundigheid waarbij je ook graag blijft dokteren: Jij stond vooraan in de rij voor *Tattoo Bob* klompen om de covidzorg te dragen.

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Madelon, jij hebt het ABO-incompatibele niertransplantatieprogramma opgezet in Rotterdam, aangespoord door Willem Weimar. En nog steeds als er gedesensibiliseerd moet worden, zijn de ogen op jou gericht als plasmaferese, immunoadsorptie en hemodialyse in serie geknutseld moeten worden.

Ik wil alle co-auteurs van de verschillende hoofdstukken bedanken.

Marije, Margriet en Jan: Wat is het leuk om met collega's in den lande samen te werken aan nationale data. We zijn inmiddels vier baby's en zes afwijzingen verder maar publicatie komt in zicht!

Naast mij staan twee goede mannen:

Martijn, jij bent in alles mijn tegenpool en je zult je wel groen en geel ergeren aan mijn niet zo clean-desk policy en mijn amodieuze verstandhouding met ICT. Je bent een geweldige, zeer collegiale collega.

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ik ook de transplantatiechirurgen betrekken en in het bijzonder Diederik en Robert en de verpleegkundig specialisten Kelly en Erika.

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De Verpleegkundig Specialisten Louise, Marleen, Mireille en Mirjam: Een feestcommissie is niet compleet zonder jullie, en ook op de werkvloer is het fijn samenwerken: Marleen, met jouw goede humeur en dito mensenkennis doe ik graag samen poli.

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De polidames, Judith, de transplantatiecoördinatoren, Saida, de verpleging van de dialyse en van de afdeling wil ik danken voor hun goede zorgen voor (ABO-incompatibele niertransplantatie) patiënten.

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Lieve Jonah, Elaine en Sanne: Meegroeien met jullie kleine rituelen en grootse avonturen is een enorme rijkdom. De overdracht is steeds vaker van jullie naar ons en dat is maar goed ook! Hoe had ik anders kunnen weten dat 'Wie is de mol?' geen stom programma is, maar een goede aanleiding voor slaapfeestjes met veel cola en nog meer popcorn; dat je Alicia Keys heel goed kunt combineren met de Matthäus-Passion; en dat je met een tandenborstel en rietje prachtige schilderijen kan maken?

Pieter: grote liefde met een kleine bijdrage aan de letter van dit proefschrift, maar de grootste bijdrage aan alles van waarde er omheen: Ik ben je zo dankbaar voor ons leven samen. Ik verheug me enorm op een wandeling over de kliffen van Yorkshire, eindigend in de pub met een *pint* (of bitter, jij, en ik -hoe kansloos- shandy).

Let's have great fun!

