Author's response to reviews

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

Authors:

Annelies E de Weerd (a.deweerd@erasmusmc.nl)
Alieke G Vonk (a.vonk@erasmusmc.nl)
Hans J van der Hoek (h.vanderhoek@erasmusmc.nl)
Marian C van Groningen (m.clahsen-vangroningen@erasmusmc.nl)
Willem Weimar (w.weimar@erasmusmc.nl)
Michiel GH Betjes (m.g.h.betjes@erasmusmc.nl)
Madelon van Agteren (m.vanagteren.1@erasmusmc.nl)

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Author's response to reviews: see over
Point by point reply to the reviewers of BMC Nephrology

Manuscript:

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

Annelies de Weerd¹,², Alieke G. Vonk³, Hans van der Hoek⁴, Marian van Groningen⁵, Willem Weimar¹, Michiel Betjes¹, Madelon van Agteren¹.

We thank the reviewers for these compliments, constructive comments and careful reading of the manuscript. Below we have answered the questions by the individual reviewers in a point by point reply. The revised manuscript is enclosed, in which the changes are highlighted in yellow.

Reviewer: Daniele Focosi

minor essential revisions:

1) “the title should include "... sepsis from Serratia marcescens", given the highly likely link between antigen(s) from this bacterium and anti-A titer rise.”

   We have changed the title to ‘Late antibody-mediated rejection after ABO-incompatible kidney transplantation during Gram-negative sepsis.’ The interaction between bacterial antigen and ABO antibodies has been demonstrated in Gram-negative bacteria and not exclusively in Serratia Marcescens (Springer, Williamson et al. 1961; Yi, Shao et al. 2005).

2) “the titer (colony numbers) of Serratia marcescens in urine during admission should be detailed, as well as reasons for discharging without treatment of
infection/colonization (the likely source of retrograde pyelonephritis seven weeks later).”

During the first admission *Pseudomonas aeruginosa* (> $10^5$ cfu) and *Serratia marcescens* (> $10^5$ cfu) were cultured, for which she was treated with ciprofloxacin until the first outpatient visit. After discontinuing antibiotics *Enterococcus durans* ($10^{3-4}$ cfu) was cultured. In the absence of dysuria this Gram-positive culture was regarded as colonization and antibiotic treatment was not initiated.

3) “the authors should clarify ("various uropathogens") whether Serratia was still present in urine during admission at week 7.”

Seven weeks after transplantation she was readmitted and the urine cultured different uropathogens not further specified (> $10^5$ cfu). Our microbiology laboratory does not routinely specify uropathogens when three or more different bacteria are cultured. A specification of these uropathogens was unfortunately not demanded by the treating clinician at the time.

4) “the authors should explain why they administered nephrotoxic beta-lactam imipenem in absence of a bacterial isolate and 5 days later they administered high-dose steroids to a patient with bacteremia just on the suspicion of rejection and far before the result of graft biopsy.”

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 umol/l with a C-reactive protein of 163 mg/l. The differential diagnosis included pyelonephritis and rejection of the allograft. Awaiting culture results, antibiotic treatment was initiated because our
patient appeared ill, had 39.3 °C fever and pain over the renal allograft. Imipenem is registered for complicated urinary tract and abdominal infections. Estimated renal function was 42 ml/min (MDRD formula), imipenem dosing is adjusted when eGFR is <30 ml/min. As her renal function deteriorated rapidly, including fluid retention, and ultrasound disclosed a non-measurable diastolic blood flow, the clinical decision was to initiate anti-rejection therapy awaiting the pathology results. Corticosteroids were given to a patient in whom a bacteria was cultured in her blood 5 days earlier, but blood cultures had become negative, her blood pressure remained stable and CRP was decreasing from 163 mg/l to 42 mg/l.

5) “the authors should state whether the husband (donor) was A1 or A2 blood type.”

    Donor blood group was A1.

6) “In their response to reviews the authors state ”In the incubation experiments performed commercially available donor plasma and A erythrocytes were used.” I suppose that the sentence ”these different S. marcescens suspensions were incubated with anti-A plasma for 30 minutes at 37 °C” should be interpreted as ”plasma from a group B donor”. If yes, a purified, commercial anti-A (A1 or A2) antiserum would have been a better (more specific and less confounding) choice than whole plasma.”

    Pooled plasma from two blood group B donors was used with known anti-A1 titers (c-negative, E-negative, en K-negative).

7) “the kinetics of anti-B isoagglutinins (whether assayed) should be reported to
corroborate the hypothesis of bacterial trigger.”

Anti-B antibody titers were not investigated.

8) “the role of intra-graft infection (pyelonephritis, as in the pediatric case by Schaefer et al.) vs. systemic infection as the actual proinflammatory trigger should be clarified.”

In the report by Schaefer et al. a patient aged 18 experienced an AMR nine days after kidney transplantation, with a rise in IgG (anti-B) titer to 64 during pyelonephritis (Schaefer, Tonshoff et al. 2013). Culture results were not mentioned. Two subsequent rises in IgG titer were reported in association with pyelonephritis episodes. The authors discuss that this secondary increase of isoagglutinin titers is triggered by a systemic infection and results in an acute antibody-mediated rejection. We can only speculate on whether anti-A formation is boosted because:

- ‘blood group A-like antigens’ are present in the circulation,
- or A antigen itself is upregulated and more easily accessible on the cell surface for antibodies during intragraft infection,
- or neutralizing antibodies to protect the host against infection damage the graft by cross-reacting with blood group A antigen (Takahashi 2007; Takahashi and Saito 2013).

9) “a reference to Banff classification should be added when stating "type 3"”.

Antibody-mediated rejection type 3, Banff ‘09 (Sis, Mengel et al. 2010).

10) “the cause of recipient's death, whether related to sepsis or surgery, should be disclosed.”

After the transplantectomy, the hospital course was very complicated: she was admitted to the ICU after a bradycardia-induced arrest and developed severe motoric and sensible axial neuropathy. Furthermore she experienced a primo CMV infection. She was transferred to a rehabilitation center. I do not have insight in the medical record after this period, but have spoken to her husband: his wife passed away two years later after having been living in a nursing home.
11) “type of immunoabsorption (likely anti-B GlycoSorb columns) should be disclosed.”

Plasmapheresis was performed with a plasmaFlux PSu filter (Fresenius Medical Care, Bad Homburg, Germany) followed by adsorption of anti-A antibodies with the Glycosorb® device, coated with synthetically derived blood group A antigen (Glycorex Transplantation, Lund, Sweden).

12) “the following reference (reporting anti-B titer rise in an ABO-incompatible liver transplant recipient with Serratia sepsis) should be cited:


We thank the reviewer for attending us on this interesting article (Oya, Sato et al. 2008). Eight days after ABO-incompatible living donor liver transplantation, the anti-B titer rose to 64. On day 13, an intra-abdominal hematoma infected with Serratia marcescens was drained surgically. After re-operation thrombotic microangiopathy (TMA) developed (thrombocytopenia, fragmentation of red blood cells in the peripheral blood smear, elevated LDH). The authors suggest that interaction between anti-hemagglutinin and the endothelial cells of the graft contribute to the development of TMA. The authors do not explore on a possible causative role of Serratia marcescens infection on the anti-B titer rise.
Reviewer: Emanuele Cozzi

Reviewer's report:

This interesting paper by de Weerd and colleagues deals with an untreatable AMR episode in a case ABOi (A into O) renal transplant coinciding with a septic event due to Serratia Marcescens. The authors speculate that the Serratia Marcescens may have triggered the production of the very high titers of anti-A antibodies observed and that these are responsible for the graft loss.

This may well be the case although evidence for an unequivocal causative link between the 2 events has not been provided.

On the other hand, it is unquestionable that:

- the titers of isoagglutinins reached in this patient are certainly compatible with AMR.

- A pyelonephritis-associated increase of the isoagglutinin IgG/IgM titers associated with a biopsy-proven AMR in a ABOi renal recipient has been reported,

- the development of infection may increases both breadth and strength of anti-HLA antibodies [reference to this paper should be added].

Still, the authors fail to provide evidence that antibodies directed to other (HLA or non-HLA) specificities may have played a causative(?)/contributory(?) role in this event.

As a consequence the reviewer strongly believes that the following points may be of help to improve the manuscript:
MAJOR COMPULSORY REVISION

1) “A complete patient history (this is a female) should be provided: did she have any pregnancy? What about past transfusions?”

The patient had no children and reported no pregnancies or miscarriages. Before transplantation, she had never received blood transfusions.

2) “The patients received transfusions perioperatively. How many blood donors were used?”

She received three blood transfusions of three different blood group O donors and one platelet transfusion on the day of transplantation. These platelets were harvested in five different blood group O donors. A small amount of anti-A containing plasma could have been transfused during this platelet transfusion. During admission, anti-A IgG remained low however (maximum IgG 2).

3) “What is the anti-HLA antibody profile prior to transplantation and at the various time points where ABO antibodies were studied? Were there any DSA at any time after transplantation (especially at the time of rejection). It would also help if anti-AT1R antibodies were studied.”

We strongly agree with the reviewer that DSA would be helpful in this case, but unfortunately neither DSA nor anti-AT1R antibodies were investigated. The HLA profile prior to transplantation was as follows:

HLA typing in donor:

A2 A3
Mismatch on HLA A, B and DR loci was 1-2-2 respectively. The complement-dependent cytotoxicity-crossmatch two months prior to transplantation was negative. Current and historical panel reactive antibodies were absent.

4) “The treatment is not comprehensible: what do the authors mean by “Rituximab 4 weeks”? Same for MMF and other drugs (including doses of IVIG). Also which column did the authors use?”

Plasmapheresis was performed with a plasmaFlux PSu filter (Fresenius Medical Care, Bad Homburg, Germany) followed by adsorption of anti-A antibodies with the Glycosorb® device, coated with synthetically derived blood group A antigen (Glycorex Transplantation, Lund, Sweden). The immunosuppressive regimen before transplantation 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 mg/kg one day preoperatively. After transplantation patient received tacrolimus 0.1 mg/kg BID, mycophenolate mofetil 1000 mg BID, prednisone 50 mg BID for three days and 20 mg once daily thereafter.
5) “The reviewer disagrees with the statement: “...this patient is remarkably different..., due to the exceptionally late occurrence of AMR12 weeks after kidney transplantation ....”. In fact, in the Tobian paper that the authors cite, 3 of the 7 episodes reported occurred 15 weeks after transplantation or later! This must be amended as needed.”

We have searched the literature for the time of onset of AMR after ABO-incompatible kidney transplantation and summarized the following:

Two out of seven patients with AMR in the article by Tobian et al. experienced AMR beyond 10 weeks after transplantation (15 and 19 weeks) (Tobian, Shirey et al. 2010). A Japanese cohort of 14 ABO-i blood type O recipients experienced early graft loss within six months, of which 11 are due to AMR. All 11 AMRs occurred within 8 weeks after transplantation. (Toki, Ishida et al. 2009). Sivakumaran et al. reported 4 AMR in 10 ABO-i kidney transplant recipients, three of whom had early AMR 6, 7 and 16 days after transplantation. One patient experienced very late AMR on day 363 and lost the renal allograft (Sivakumaran, Vo et al. 2009). Stewart et al. administrated eculizumab to a patient with severe AMR on postoperative day 4 (Stewart, Collins et al. 2012). Of 125 Korean ABO-i kidney transplant recipients, 3 experienced AMR on postoperative day 22, 46 and 207 (Kong, Ahn et al. 2013). In an American cohort of 16 ABO-incompatible kidney recipients, 1 early AMR occurred within 30 days, whereas no late AMR occurred in contrast to positive crossmatch transplantations performed in this center (Padmanabhan, Ratner et al. 2009). Toki et al. described 57 ABO-i kidney transplant recipients and compared the 11 patients in whom AMR develops within 3 months with the 46 patients without early AMR. In the follow-up period no AMR occurs in the latter (Toki, Ishida et al.
The thirteen patients with AMR after ABO-i kidney transplantation reported by Fidler et al. all experienced AMR within 16 days postoperatively (Fidler, Gloor et al. 2004).

In these reports, a total of 51 patients is described with AMR, of which 4 occurred 12 weeks or later after transplantation (8%). Furthermore, of all the 65 recipients of an ABO-i 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 in this case report presented to BMC Nephrology (Agteren Van M. et al., Journal of Transplantation, in press).

We conclude with the reviewer that AMR 12 weeks after transplantation is rare, but not exceptionally late. In accordance with the reviewers critics we have changed ‘exceptionally late’ to ‘late’.

6) “The reviewer disagrees with the statement: “...Anti-ABO titers usually remain low after transplantation and are not boosted by the graft under adequate immune suppression”. What is the evidence behind this statement? What do you mean by “remain low”? In fact, in the Tobian paper 3 out of 8 patients WITHOUT episodes of AMR had HIGH anti-ABO antibody titers (128 or even greater).”

The different isohemagglutinin assays make titer comparisons between centers difficult. We assess hemagglutination with the fully automated ORTHO Bio Vue system, using the red blood cells of the donor. With this method, we have never documented an IgG titer of 562 or greater after transplantation. However, higher IgG titers (1024) have been reported during AMR (Toki, Ishida et al. 2009), (Fidler, Gloor et al. 2004). The striking feature is the very high IgM titer >5000. In for example a patient with IgG 1024 described by Toki et al., maximal IgM titer is 128. The pathological role of anti-ABO IgG versus IgM on the renal allograft is a matter of
Takahashi hypothesizes on two distinct types of AMR after ABO-i kidney transplantation (Takahashi 2007). 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. He accompanies this hypothesis with two examples: A 34-year old blood group O recipient receiving a blood group B renal allograft, who experiences an AMR on postoperative day 19 during urosepsis with *Klebsiella pneumonia*. IgM titers 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 receives 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 would therefore emphasize the very high IgM titer and have adapted the manuscript as such.

7) “The authors seem to have overlooked the fact that the titer of 1/512 in the Tobian paper refers to IgG (gamma chain!) and not IgM. So they cannot compare their >5000 data that refers to IgM (as Tobian only refers to IgG in his paper). Furthermore, the detrimental role of IgM in these patients is possibly debatable.”

The reviewer is correct, please see answer 6.

8) “A1 and A2 donors are not equally immunogenic: Which one did you have
here?’

The donor has blood group A1.

References


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

Annelies de Weerd¹², Alieke G. Vonk³, Hans van der Hoek⁴, Marian van Groningen⁵, Willem Weimar¹, Michiel Betjes¹, Madelon van Agteren¹.

¹ Erasmus Medical Center Rotterdam, department of Nephrology; ² corresponding author;
³ Erasmus Medical Center Rotterdam, department of Microbiology and infectious diseases; ⁴ Erasmus Medical Center Rotterdam, department of Hematology, ⁵ Erasmus Medical Center Rotterdam, department of Pathology.

a.deweerd@erasusmc.nl; a.vonk@erasusmc.nl; h.vanderhoek@erasusmc.nl; m.clahsen-vangroningen@erasusmc.nl; w.weimar@erasusmc.nl; m.g.h.betjes@erasusmc.nl;
m.vanagteren.1@erasusmc.nl

²Address for correspondence

Annelies de Weerd

Erasmus Medical Center Rotterdam,

Department of Nephrology, room D-411

P.O. Box 2040, 3000 CA Rotterdam, The Netherlands.

phone: 31-10-7034607 / 31-6-18834197       fax: 31-10-7044718

email: a.deweerd@erasusmc.nl
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.

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. 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*.

**Keywords**

ABO-incompatible kidney transplantation

*Serratia marcescens*

Antibody-mediated rejection

Bacteremia-induced anti-ABO antibodies
**Background**

Both HLA and ABO blood group system determine the risk of rejection in clinical organ transplantation. The major challenge in blood group ABO-incompatible (ABO-i) transplantation is to minimize antibody-mediated rejection (AMR). In recent years, ABO-i 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 immuno adsorption 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 and are not boosted by the graft under adequate immune suppression.

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 ABO-i kidney transplants [6, 7].

We present for the first time a case of a late fulminant AMR of an ABO-i kidney transplant which may have been triggered by Gram-negative bacteremia.

Case report

A 58-year-old woman underwent living unrelated ABO-i 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
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 mg/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 cytomegolovirus (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 > 10⁵ 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 ABO-i kidney transplantation and by itself does not indicate rejection. Anti-A titers remained low: one day post-operative 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 µmol/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 umol/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 umol/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 $10^5$ cfu/ml to $10^8$ 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. 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 x 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 ABO-i kidney transplantation, whose anti-A IgM titers rose dramatically following *S. marcescens* sepsis.

Two features of this post-transplant course are remarkably different from other reports of acute rejection in ABO-i kidney transplantation: first, the exceptionally 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 ABO-i 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 [Agteren van M. et al., Journal of Transplantation, in press]. The pathological role of anti-ABO IgG versus IgM on the ABO-i renal allograft is a matter of debate [7, 12, 20]. Takahashi hypothesizes on two distinct types of AMR after ABO-i kidney transplantation: he states that type I AMR is caused by re-sensitization due to ABO-blood group antigens, occurs early postoperative 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 pneumonia*. 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 ‘natural 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 ABO-i 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 [21].

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. [22]. 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 (ABO-i) donor plasma is present. In contrast, in the by Johnson described blood group A platelet donor 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 ABO-i solid organ transplantation the relation between sepsis and AMR is also reported. A pediatric ABO-i 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 a ABO-i 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 [23]. 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* [24]. Mannose-sensitive pili of *S. marcescens* are known to stimulate renal scarring [25]. 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 ABO-i kidney transplantation.
Consent

The patient has died.

Written informed consent was obtained from the husband for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

List of abbreviations

ABO-i   blood group ABO-incompatible
AMR    antibody-mediated rejection
cfu    colony forming units
CMV    cytomegalovirus
HLA    human leucocyte antigen
Ig     immunoglobulin
PBS    phosphate buffered saline
PCR    polymerase chain reaction
S. marcescens  Serratia marcescens
Competing interests

The authors declare that they have no competing interests.

Author’s contributions

AdW designed and performed the experiments and wrote the manuscript.

AV designed and supervised the experiments and corrected the manuscript.

HvdH performed the experiments.

MvG supplied the pathology reports and corrected the manuscript.

WW treated the patient and corrected the manuscript.

MB participated in the literature review and corrected the manuscript.

MvA supervises the ABO-i kidney transplant program and corrected the manuscript.

All authors read and approved the final manuscript.
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