SUPPLEMENTAL MATERIALS

ABO-incompatible kidney transplantation in perspective of deceased donor transplantation and induction strategies: a propensity-matched analysis.

FULL METHODS

Patients

Data on all kidney transplantations performed since January 2006, the year of the first ABOi transplantation in the Netherlands, were obtained from the Dutch Organ Transplant Registry (NOTR Nederlandse Orgaan Transplantatie Registratie). Written informed consent was obtained from all patients. We included procedures up to March 2019. Exclusion criteria were age below 16 at time of transplantation, combined liver kidney or kidney pancreas transplantations, and desensitization for HLA-incompatible kidney transplantation. Baseline anti-A/B titers were defined as low (till 1:8), intermediate (1:16-1:64) and high (≥1:128) IgG and IgM titers. This trial is approved by the institutional review board of the Erasmus Medical Center (MEC-2018-1325) and was registered in March 2019 in the Netherlands Trial Register [NTR7587, www.trialregister.nl]. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the ‘Declaration of Istanbul on Organ Trafficking and Transplant Tourism’.

ABOi treatment protocol

ABOi candidates were advised to participate in the national kidney exchange program, in general for 2 rounds. If unsuccessful, and if baseline anti-donor ABO titer was 1:256 or lower, ABOi candidates were deemed eligible for ABOi kidney transplantation. Desensitization consisted of immunoadsorption (IA) with Glycosorb® (Glycorex Sweden), pre-operative initiation of tacrolimus, mycophenolate mofetil and corticosteroids and Intravenous Immunoglobulin (IVIG) 0.5 gr/kg one day preoperatively. The type of induction agent was determined by center and era, not by patient characteristics. Six centers in the Netherlands have an ABOi program, of which one center administered alemtuzumab from start of their program. The remaining five centers started the ABOi program with rituximab induction, and
switched to another regimen after observing a higher incidence of rejection compared to the ABOc program. Two centers switched to alemtuzumab and three centers to the combination of rituximab/basiliximab. This resulted in the following three main regimens (details in table S1 of the Supplementary Materials):

1. Rituximab 375 mg/m² day minus 28.
2. Rituximab 375 mg/m² day minus 28 and basiliximab 20 mg intravenously day 0 intraoperatively and day plus 4.
3. Alemtuzumab 30 mg subcutaneously day minus 30 with or without additional 15 mg subcutaneously day minus 1.

Maintenance immunosuppression consisted of prednisolone, tacrolimus 0.075-0.1 mg/kg daily and mycophenolate mofetil 1000-2000 mg/daily during the first 6 months and was then continued according to local practice. Target tacrolimus trough levels were 10-15 µg/L initially. Targets levels have been changed to 8-12 µg/L in more recent years since the introduction of basiliximab and alemtuzumab.

**ABOc treatment protocol**

Since 2009 basiliximab was introduced as induction therapy in 5 out of 6 centers. Before, no induction was administered. The last center started basiliximab in 2014. T-cell depleting therapy thymoglobulin (rATG) was administered in a small subset of highly sensitized patients. 1 center administered rituximab in a clinical trial for several years (Table) [1]. Maintenance immunosuppression and tacrolimus trough levels were identical to ABOi recipients.

**Outcome definitions**

Graft failure was defined as the definitive initiation of renal replacement therapy, death with a functioning graft was considered a competing event. Rejection was defined as treatment for a rejection episode, whether biopsy-proven or not. Administrative censoring was performed for follow-up after September 2019, ensuring that all patients had at least six months of follow-up. Kidney function was expressed as estimated GFR, calculated with the CKD-EPI formula.

**Covariates**

All baseline covariates were obtained from the Dutch Organ Transplant Registry (NOTR Nederlandse Orgaan Transplantatie Registratie).
Statistical Methods
Distributions of continuous variables were visualized with histograms. We presented means and standard deviations for normally distributed variables, and the medians and the 25th and 75th percentiles for variables with a skewed distribution. We presented frequencies and proportions for categorical variables. Baseline differences between procedures with ABOi, deceased ABOc, and living donor ABOc were tested with one way ANOVA if variables were normally distributed, and the Kruskal-Wallis test if the distribution of the variable was skewed. \( \chi^2 \) tests were used to compare frequencies between the groups.

Missing data
In order to deal with missing data for BMI, cumulative dialysis duration, the number of HLA mismatches, and peak panel reactive antigen level, we performed multiple imputation using chained regression equations [2]. The predictor matrix was set to a minimal correlation of 0.2 between the target variable and potential predictor variables. All values were imputed using predictive mean matching. We created 20 imputed datasets that were used for further analysis. Imputation results were checked using diagnostic plots.

Causal model ad propensity score matching
We created a hypothesized causal model for the possible effect of having an ABOi donor on the risk of adverse outcome after kidney transplantation. The causal model was visualized with a Directed Acyclic Graph (DAG) using the dagitty.net software [3]. A causal DAG encodes assumptions about possible associations (edges) between variables (nodes) and more importantly, the absence of associations (possibly conditional on other variables) in the data [4]. Take the DAG \( X \leftarrow Z \rightarrow Y \) for example: \( X \) and \( Y \) are associated via \( Z \). However, if we were to condition on \( Z \), the association would no longer be present. Thus the DAG implies \( X \) is independent of \( Y \) conditional on \( Z \). Whether this implication holds true can be tested with a regression of \( X \) on \( Y \) and \( Z \). If the association between \( Y \) and \( X \) after conditioning on \( Z \) is not different from 0, we have no evidence for a violation of the assumed independence of \( X \) and \( Y \) conditional on \( Z \) in the data. However, if the association is different from 0, this means that the data does not support the causal model. This procedure is repeated for other conditional independencies implied by the model. Violations of the conditional independencies in the data would mean that the causal model requires
revision. Revisions may include adding and edge $X \rightarrow Y$ or another node (e.g. another confounder) with edges between $X$ and $Y$, such as $X \leftarrow C \rightarrow Y$, for example. The implications from the revised model are then tested, and if violated, the model is revised again. This iterative process continues until a causal model is identified that is sufficiently supported by the data. Subsequently, the DAG can be used to define the adjustment set that will close all backdoor paths between the exposure and the outcome.

The adjustment set that we identified included recipient age, peak panel reactive antigen levels, recipient blood group, sex, and transplant center. In order to adjust for confounding variables, we created propensity scores using logistic regression for ABOi versus ABOc with deceased and living donors respectively, that included the adjustment set. To optimize the propensity score model we evaluated possible Box-Cox transformations for recipient age and peak panel reactive antigen levels. Ultimately we chose to take natural log transformations for both. We performed the matching within each center to ensure balance across centers, as we suspected that residual confounding by center may occur otherwise. We matched the ABOi procedures to ABOc procedures in a 1:4 ratio. We tested duration of dialysis as a mediator and a confounder via both DAGs and found neither to violate the conditional independencies tests. Therefore, we performed the main analysis with duration of dialysis as a mediator that was not included in the adjustment set, and a sensitivity with duration of dialysis as a confounder included in the adjustment set.

**Main outcome analysis**

After matching we estimated the cumulative incidence of graft failure and the survival probability with a function graft as mutually exclusive competing events. Next, a cause-specific Cox proportional hazards model was fitted to estimate the causal effect of having an ABOi donor compared to either a ABOc living or deceased donor. The proportional hazards assumption was checked by plotting Schoenfeld’s residuals by time [5].

**Analysis of outcomes after ABOi by induction therapy and blood group**

We carried out an explorative analysis of the impact of induction therapy on the outcomes after ABOi kidney transplantation. We limited these analyses to the three most commonly used regimens: induction with rituximab alone, a combination of rituximab/basiliximab, and alemtuzumab. Induction with rituximab only was
introduced at the start of the ABOi program and abandoned later. As a consequence the follow-up duration in the rituximab alone group was markedly longer. To ensure comparability of results, administrative censoring at 5 years follow-up was performed in all three groups. Furthermore, as the induction therapies were determined largely by center and era, and not individual patient characteristics, we did not expect that propensity score adjustment would alleviate confounding bias. Residual confounding by center and era would remain. We chose to estimate crude cumulative incidences and hazard ratios instead. Similar procedures were used to determine the difference in outcome by recipient blood group in the ABOi group.

**Software and data**

All analyses were performed on a digital research (DRE) platform, a secure cloud based data analysis platform on Microsoft Azure architecture (www.andrea-consortium.org). We used dagitty.net (version 2.3), R version 3.5.1 with the RStudio shell (version 1.1.463) and the packages tableone (0.9.3), dagitty (0.2-2), mice (3.3.0), car (3.0-2), MatchIt (3.0.2), cmprsk (2.2-7), and survival (2.42-3). Data and analysis scripts for the present manuscripts can be accessed on the DRE platform. Access can be requested via the corresponding author.

**Funding**

None.
### Table S1. Treatment protocols in the six centers performing ABO-incompatible kidney transplantation.

| Center | period   | induction | plasma exchange technique | IVIG                                      | preoperative immunosuppressive drugs |
|--------|----------|-----------|---------------------------|-------------------------------------------|--------------------------------------|
| A.     | 2008-2017 | d -28: rituximab 375 mg/m² | d -7: PE till IgG <8 | d -1: 0.5 g/kg | d -7/- 10: TAC 0.1 mg/kg/d MMF 2000 mg/d prednisone 30 mg/d |
|        | 2018-present | d -28: alemtuzumab 30 mg s.c. |                           |                                          |                                      |
| B.     | 2009-2016¹ | d -28: rituximab 375 mg/m² | d -7: IA                  | d -1: 0.5 g/kg | d -14: TAC 0.15 mg/kg/d MMF 2000 mg/d prednisone 20 mg/d |
|        | 2016¹²-present | d -28: rituximab 375 mg/m²; d 0, +2: basiliximab 20 mg |                           |                                          |                                      |
| C.     | 2006-2015³ | d -28: rituximab 375 mg/m² | d -7: IA if titers are >8 standard 3 postop IA abandoned in 2009 | d -1: 0.5 g/kg | d -14: TAC 10 mg/d MMF 2000 mg/d prednisone 20 mg/d |
|        | 2015⁴-present | d -21:alemtuzumab 30 mg s.c. |                           |                                          |                                      |
| D.     | 2008-2015³ | d -30: rituximab 375 mg/m² | d -7: IA if titers are >8 | d -1: 0.5 g/kg if baseline titer >8 | d -13: MMF 2000 mg/d |
|        | 2015⁵-present | d -30: rituximab 375 mg/m²; d 0, +2: basiliximab 20 mg |                           |                                          |                                      |
| E.     | 2009-2014 | d -28: rituximab 375 mg/m² | d -6: IA                  | d -1: 0.5 g/kg | d -14: TAC 0.15 mg/kg/d MMF 1500 mg/d prednisone 20 mg/d |
|        | 2015-present | d -28: rituximab 375 mg/m²; d 0, +2: basiliximab 20 mg |                           |                                          |                                      |
| F.     | 2010-present | d -30: alemtuzumab 30 mg s.c.; d -1: alemtuzumab 15 mg s.c. if baseline IgG >256: bortezomib 1.3 mg/m² (d -44, d -40, d -36 and d -33) | d -7: IA if titer increase >2 dilutions: repeat IA | d -1: 0.5 g/kg | d -14: TAC 6 mg/d MMF 1000 mg/d prednisone 30 mg/d |

IA immunoadsorption  MMF mycophenolate mofetil  PE plasmapheresis  s.c. subcutaneous  TAC tacrolimus
Table S2. Baseline characteristics of the unmatched cohort.

|                                | ABO incompatible (n = 296) | ABO compatible living donor (n = 4272) | ABO compatible deceased donor (n = 4086) | p-value |
|--------------------------------|---------------------------|----------------------------------------|----------------------------------------|---------|
| **Recipient age (yrs)** (median [IQR]) | 54.00 [44.75, 64.00]     | 52.00 [40.00, 61.00]                   | 58.00 [47.00, 66.00]                   | <0.001  |
| **Recipient sex: Male (%)**   | 199 (67.2)                | 2589 (60.6)                            | 2494 (61.0)                            | 0.08    |
| **Recipient BMI (kg/m^2)** (mean (sd)) | 25.61 (4.14)             | 25.55 (4.37)                           | 26.06 (4.40)                           | <0.001  |
| **Primary kidney disease (%)** |                           |                                        |                                        | <0.001  |
| diabetic nephropathy           | 19 (6.4)                  | 301 (7.0)                              | 442 (10.8)                             |         |
| glomerulonephritis             | 68 (23.0)                 | 819 (19.2)                             | 720 (17.6)                             |         |
| urologic                       | 6 (2.0)                   | 77 (1.8)                               | 86 (2.1)                               |         |
| polycystic kidney disease      | 58 (19.6)                 | 489 (11.4)                             | 403 (9.9)                              |         |
| vascular                       | 69 (23.3)                 | 964 (22.6)                             | 1270 (31.1)                            |         |
| benign/malignant tumor         | 2 (0.7)                   | 21 (0.5)                               | 14 (0.3)                               |         |
| other/ not reported            | 57 (19.3)                 | 1435 (33.6)                            | 1050 (25.7)                            |         |
| hereditary nephropathies       | 17 (5.7)                  | 166 (3.9)                              | 101 (2.5)                              |         |
| preemptive (%)                 | 116 (39.6%)               | 1925 (45.3%)                           | 231 (5.7%)                             | <0.001  |
| time on dialysis (days) (median [IQR]) | 225 [0.551]            | 105 [0.533]                            | 1191 [703, 1754]                       | <0.001  |
| Previous transplantation (n) (mean (sd)) | 1.16 (0.50)            | 1.11 (0.36)                            | 1.17 (0.48)                            | <0.001  |
| **Recipient blood group (%)**  |                           |                                        |                                        | <0.001  |
| A                              | 57 (19.3)                 | 1942 (45.5)                            | 1564 (38.3)                            |         |
| AB                             | 0 (0.0)                   | 189 (4.4)                              | 191 (4.7)                              |         |
| B                              | 44 (14.9)                 | 474 (11.1)                             | 474 (11.6)                             |         |
| O                              | 195 (65.9)                | 1667 (39.0)                            | 1857 (45.4)                            |         |
| Donor Age (yrs) (median [IQR]) | 55.00 [45.00, 63.00]      | 54.00 [45.00, 61.00]                   | 55.00 [45.00, 63.00]                   | 0.11    |
| Donor sex: Male (%)            | 126 (42.6)                | 1884 (44.1)                            | 2228 (54.5)                            | <0.001  |
| Total HLA mismatches (n) (mean (sd)) | 3.47 (1.39)            | 3.45 (1.55)                            | 2.74 (1.42)                            | <0.001  |
| Peak PRA                       |                           |                                        |                                        | <0.001  |
| 1% - 4%                        | 259 (87.5)                | 3716 (87.2)                            | 3291 (80.5)                            |         |
| 5% - 84%                       | 32 (10.8)                 | 483 (11.3)                             | 577 (14.1)                             |         |
| 85% - 100%                     | 5 (1.7)                   | 62 (1.5)                               | 218 (5.3)                              |         |
| Transplant center (%)          |                           |                                        |                                        | <0.001  |
|    | A                | B                | C                | D                | E                | F                |
|----|------------------|------------------|------------------|------------------|------------------|------------------|
|    | 11 (3.7)         | 323 (7.6)        | 407 (10.0)       | 50 (16.9)        | 31 (10.5)        | 74 (25.0)        |
|    | 516 (12.1)       | 1209 (28.3)      | 757 (18.5)       | 823 (19.3)       | 757 (17.7)       | 644 (15.1)       |
|    | 838 (20.5)       | 834 (20.4)       |                  |                  | 564 (13.8)       | 686 (16.8)       |
| Year of transplantation (median [IQR]) | Year of transplantation (median [IQR]) | Year of transplantation (median [IQR]) | <0.001 |
| 2014 | [2011-2016] | 2013 | [2010-2016] | 2012 | [2009-2016] | <0.001 |
| Induction therapy (%) | <0.001 |
| alemtuzumab | 92 (31.1) | 1 (0.0) | 6 (0.1) |
| alemtuzumab + bortezomib | 5 (1.7) | 0 (0.0) | 0 (0.0) |
| basiliximab | 1 (0.3) | 2659 (62.2) | 2368 (58.0) |
| basiliximab + alemtuzumab | 0 (0.0) | 1 (0.0) | 4 (0.1) |
| rATG | 1 (0.3) | 68 (1.6) | 132 (3.2) |
| none | 0 (0.0) | 1465 (34.3) | 1518 (37.2) |
| rituximab | 146 (49.3) | 78 (1.8) | 57 (1.4) |
| rituximab + basiliximab | 50 (16.9) | 0 (0.0) | 1 (0.0) |
| rituximab + basiliximab + eculizumab | 1 (0.3) | 0 (0.0) | 0 (0.0) |
| Maintenance therapy (%) | <0.001 |
| tacrolimus-antiproliferatives-steroids | 286 (96.6) | 3852 (90.2) | 3493 (85.5) |
| CsA-antiproliferatives-steroids | 6 (2.0) | 420 (9.8) | 593 (14.5) |
| tacrolimus-antiproliferatives-no steroids | 1 (0.3) | 0 (0.0) | 0 (0.0) |
| CNI - no antiproliferatives-steroids | 3 (1.0) | 0 (0.0) | 0 (0.0) |

BMI body mass index  CNI calcineurin inhibitor  CsA ciclosporin  HLA human leucocyte antigen  IQR interquartile range  PRA panel reactive antibodies  rATG rabbit anti-thymocyte globulin  SD standard deviation
Table S3. Conditional Independencies.

| Implied conditional independency | estimate | 2.5%  | 97.5% | p-value |
|---------------------------------|----------|-------|-------|---------|
| aboi || donor_age | age | -0.003 | | | |
| age || dialysisdaysrenine | number_transplantations | 0.001 | 0.001 | 0.001 | 0.000 |
| age || kidney_disease_dn | 6.876 | 5.841 | 7.911 | 0.000 |
| age || peak_pra | number_transplantations | -0.001 | -0.016 | 0.013 | 0.851 |
| age || recipientbloodgroup | 0.233 | | | 0.758 |
| age || recipientsex | 0.863 | | | 0.005 |
| center || dialysisdaysrenine | number_transplantations | 0.000 | | | |
| center || dialysisdaysrenine | age | 0.000 | | | |
| center || donor_age | age | 0.000 | | | |
| center || kidney_disease_dn | -0.390 | | | |
| center || number_transplantations | age | -0.101 | | | |
| center || peak_pra | number_transplantations | 0.004 | | | |
| center || peak_pra | age | 0.002 | | | |
| center || recipientbloodgroup | -0.057 | | | |
| center || recipientsex | -0.003 | | | |
| dialysisdaysrenine || donor_age | age | -7.528 | -9.012 | -6.044 | 0.000 |
| dialysisdaysrenine || donor_age | number_transplantations | -5.637 | -7.062 | -4.213 | 0.000 |
| dialysisdaysrenine || kidney_disease_dn | 151.053 | 84.290 | 217.816 | 0.000 |
| dialysisdaysrenine || recipientbloodgroup | 33.241 | | | 0.089 |
| dialysisdaysrenine || recipientsex | | | | |
| donor_age || kidney_disease_dn | 0.491 | -0.488 | 1.471 | 0.326 |
| donor_age || number_transplantations | age | -1.453 | -2.089 | -0.818 | 0.000 |
| donor_age || peak_pra | number_transplantations | -0.028 | -0.042 | -0.015 | 0.000 |
| donor_age || peak_pra | age | -0.034 | -0.046 | -0.023 | 0.000 |
| donor_age || recipientbloodgroup | -1.064 | | | 0.133 |
| donor_age || recipientsex | 0.332 | | | 0.252 |
| kidney_disease_dn || number_transplantations | -0.046 | -0.060 | -0.033 | 0.000 |
| kidney_disease_dn || peak_pra | 0.000 | -0.001 | 0.000 | 0.000 |
| kidney_disease_dn || recipientbloodgroup | 0.018 | | | 0.233 |
| kidney_disease_dn || recipientsex | 0.015 | | | 0.014 |
| number_transplantations || recipientbloodgroup | 0.007 | | | 0.773 |
| number_transplantations || recipientsex | -0.024 | | | 0.010 |
| peak_pra || recipientbloodgroup | -2.362 | | | 0.057 |
| recipientbloodgroup || recipientsex | -0.012 | | | |
Table S4. Correlation of baseline anti-A/B titers and the occurrence of rejection in ABO-incompatible kidney transplant recipients.

| IgG  | No rejection number of recipients, (%) | Rejection number of recipients, (%) | IgM  | No rejection number of recipients, (%) | Rejection number of recipients, (%) |
|------|--------------------------------------|-------------------------------------|------|--------------------------------------|-------------------------------------|
| <1   | 24 (11.4)                            | 3 (3.5)                             | <2   | 7 (3.3)                              | 1 (1.2)                             |
| 1:1  | 1 (0.5)                              | 0 (0)                               | 1:1  | 17 (8.1)                             | 1 (1.2)                             |
| 1:2  | 27 (12.8)                            | 11 (12.9)                           | 1:2  | 15 (7.1)                             | 4 (4.7)                             |
| 1:4  | 25 (11.8)                            | 5 (5.9)                             | 1:4  | 40 (19.0)                            | 11 (12.9)                           |
| 1:8  | 24 (11.4)                            | 14 (16.5)                           | 1:8  | 31 (14.7)                            | 4 (4.7)                             |
| 1:16 | 28 (13.3)                            | 5 (5.9)                             | 1:16 | 45 (21.3)                            | 17 (20.0)                           |
| 1:32 | 17 (8.1)                             | 13 (15.3)                           | 1:32 | 26 (12.3)                            | 20 (23.5)                           |
| 1:64 | 30 (14.2)                            | 13 (15.3)                           | 1:64 | 12 (5.7)                             | 11 (12.9)                           |
| 1:128| 22 (10.4)                            | 13 (15.3)                           | 1:128| 9 (4.3)                              | 12 (14.1)                           |
| 1:256| 6 (2.8)                              | 5 (5.9)                             | 1:256| 9 (4.3)                              | 4 (4.7)                             |
| 1:512| 7 (3.3)                              | 3 (3.5)                             |      |                                      |                                      |

\[X^2\text{-test}\ p=0.081 \quad X^2\text{-test}\ p=0.0004\]
Table S5. Baseline characteristics of the ABOi group by recipient blood group.

|                        | Blood group A (n = 57) | Blood group B (n = 44) | Blood group O (n = 195) | p-value |
|------------------------|------------------------|------------------------|-------------------------|---------|
| **Recipient age (yrs)** (median [IQR]) | 57.00 [42.00, 65.00]   | 48.50 [44.00, 63.25]   | 55.00 [45.00, 63.00]   | 0.83    |
| **Recipient sex: Male (%)** | 42 (73.7)             | 24 (54.5)              | 133 (68.2)              | 0.11    |
| **Recipient BMI (kg/m^2)** (mean (sd)) | 26.34 (5.07)           | 25.22 (4.45)           | 25.50 (3.71)            | 0.31    |
| **Primary kidney disease (%)** |                        |                        |                         | 0.15    |
| diabetic nephropathy    | 7 (12.3)               | 3 (6.8)                | 9 (4.6)                 |         |
| glomerulonephritis      | 13 (22.8)              | 9 (20.5)               | 46 (23.6)               |         |
| urologic                | 0 (0.0)                | 2 (4.5)                | 4 (2.1)                 |         |
| polycystic kidney disease | 7 (12.3)              | 9 (20.5)               | 42 (21.5)               |         |
| vascular                | 8 (14.0)               | 11 (25.0)              | 42 (21.5)               |         |
| other/ not reported     | 18 (31.6)              | 9 (20.5)               | 30 (15.4)               |         |
| hereditary nephropathies | 4 (7.0)                | 1 (2.3)                | 12 (6.2)                |         |
| **Preemptive**          | 23 (40.4%)             | 24 (54.5%)             | 71 (36.4%)              | 0.09    |
| **Time on dialysis (days)** (median [IQR]) | 217.00 [0.00, 545.00]  | 0.00 [0.00, 369.00]    | 244.00 [0.00, 565.50]   | 0.08    |
| **Previous transplantation (n) (mean (sd))** | 1.28 (0.82)            | 1.23 (0.52)            | 1.10 (0.35)             | 0.04    |
| **Donor Age (yrs)** (median [IQR]) | 52.00 [39.00, 61.00]   | 55.50 [48.75, 65.00]   | 55.00 [46.00, 63.00]    | 0.19    |
| **Donor sex: Male (%)** | 25 (43.9)              | 20 (45.5)              | 81 (41.5)               | 0.87    |
| **Total HLA mismatches (n)** (mean (sd)) | 3.42 (1.27)            | 3.18 (1.51)            | 3.38 (1.49)             | 0.67    |
| **Peak PRA (%)** |                        |                        |                         | 0.04    |
| 1-4%                   | 44 (77.2)              | 38 (86.4)              | 177 (90.8)              |         |
| 5% - 84%               | 10 (17.5)              | 6 (13.6)               | 16 (8.2)                |         |
| 85% - 100%             | 3 (5.3)                | 0 (0.0)                | 2 (1.0)                 |         |
| **ABO IgG titer (%)** |                        |                        |                         | <0.001  |
| <1                     | 13 (28.2)              | 6 (16.7)               | 4 (2.5)                 |         |
| 1:1                    | 0 (0.0)                | 1 (2.8)                | 0 (0.0)                 |         |
| 1:2                    | 13 (28.3)              | 8 (22.2)               | 6 (3.8)                 |         |
| 1:4                    | 10 (21.7)              | 8 (22.2)               | 9 (5.6)                 |         |
| 1:8                    | 5 (10.9)               | 4 (11.1)               | 25 (15.6)               |         |
| ABO IgM titer (%) | <0.001 |
|-------------------|--------|
| <2                | 3 (6.7)| 0 (0.0) | 3 (2.2)|
| 1:1               | 6 (13.3)| 3 (9.4)| 4 (3.0)|
| 1:2               | 7 (15.6)| 3 (9.4)| 3 (2.2)|
| 1:4               | 12 (26.7)| 6 (18.8)| 16 (11.9)|
| 1:8               | 6 (13.3)| 5 (15.6)| 16 (11.9)|
| 1:16              | 8 (17.8)| 9 (28.1)| 30 (22.2)|
| 1:32              | 2 (4.4)| 4 (12.5)| 26 (19.3)|
| 1:64              | 0 (0.0)| 2 (6.2)| 14 (10.4)|
| 1:128             | 0 (0.0)| 0 (0.0)| 17 (12.6)|
| 1:256             | 1 (2.2)| 0 (0.0)| 6 (4.4)|

| Transplant center (%) | 0.98 |
|-----------------------|------|
| A                     | 1 (1.8)| 2 (4.5)| 8 (4.1)|
| B                     | 3 (5.3)| 3 (6.8)| 13 (6.7)|
| C                     | 21 (36.8)| 17 (38.6)| 73 (37.4)|
| D                     | 10 (17.5)| 7 (15.9)| 33 (16.9)|
| E                     | 4 (7.0)| 5 (11.4)| 22 (11.3)|
| F                     | 18 (31.6)| 10 (22.7)| 46 (23.6)|

| Year of transplantation (mean (sd)) | 0.30 |
|-------------------------------------|------|
| 2013.79 (2.94)                      | 2013.57 (3.08)| 2013.08 (3.46)|

| Induction therapy (%) | 0.66 |
|-----------------------|------|
| alemtuzumab           | 26 (45.6)| 11 (25.0)| 55 (28.2)|
| alemtuzumab + bortezomib| 0 (0.0)| 1 (2.3)| 4 (2.1)|
| basiliximab           | 0 (0.0)| 0 (0.0)| 1 (0.5)|
| rATG                  | 0 (0.0)| 0 (0.0)| 1 (0.5)|
| rituximab             | 22 (38.6)| 24 (54.5)| 100 (51.3)|
| rituximab + basiliximab| 9 (15.8)| 8 (18.2)| 33 (16.9)|
| rituximab + basiliximab + eculizumab | 0 (0.0)| 0 (0.0)| 1 (0.5)|

| Maintenance therapy (%) | 0.40 |
|-------------------------|------|
| tacro-antiproliferatives-steroids | 54 (94.7)| 44 (100.0)| 188 (96.4)|
|                     | BMI | CNI | HLA |
|---------------------|-----|-----|-----|
| CsA-antiproliferatives-steroids | 1 (1.8) | 0 (0.0) | 5 (2.6) |
| tacrolimus-antiproliferatives-no steroids | 0 (0.0) | 0 (0.0) | 1 (0.5) |
| CNI - no antiproliferatives-steroids | 2 (3.5) | 0 (0.0) | 1 (0.5) |

BMI body mass index  CNI calcineurin inhibitor  CsA ciclosporin  HLA human leucocyte antigen  IQR interquartile range  PRA panel reactive antibodies  rATG rabbit anti-thymocyte globulin  SD standard deviation
Figure S1. Directed Acyclic Graph of the causal model. Directed Acyclic Graph showing the causal model for the relation between ABO incompatible transplantation and survival with a functioning graft. The circles denote nodes, baseline covariates, the arrows are edges. An edge denotes the assumed causal relation between two variables. For example the level of peak_pra ‘listens’ to number_transplantations. Importantly, the absence of an edge encodes the assumption that two variables are unrelated. For example, peak_pra and recipient_blood_group are assumed to be unrelated, this is mathematically notated as peak_pre _||_ recipient_blood_type. This assumption, called the independence assumption can be tested by regressing peak_pra on recipient_blood_type. If the association between the variables is not different from 0, the assumption holds. The independence assumptions have been tested and the results are shown in Supplemental Table S3.
Figure S2. Baseline characteristics of the matched cohort represented with histograms. Density plots for baseline covariate distribution after propensity score matching. ABO incompatible procedures are shown in black, ABO-compatible deceased donor in light blue, ABO-compatible living donors in red.
Figure S3 A. Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients according to baseline anti-A/B IgG titers. Outcomes for ABOi kidney transplant recipients (n=296) were divided according to low titers ≤1:8 in red, intermediate titers 1:16-1:64 in light blue and high titers ≥1:128 in black. The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure with patient death considered as a competing event.
Figure S3 B. Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients according to baseline anti-A/B IgM titers. Outcomes for ABOi kidney transplant recipients (n=296) were divided according to low titers ≤1:8 in red, intermediate titers 1:16-1:64 in light blue and high titers ≥1:128 in black. The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure with patient death considered as a competing event.

| years | 0  | 2  | 4  | 6  | 8  | 10 |
|-------|----|----|----|----|----|----|
| IgM ≤ 1:8 | 93 | 86 | 84 | 76 | 72 | 69 |
| IgM 1:16-1:64 | 95 | 87 | 83 | 77 | 71 | 69 |
| IgM ≥1:128 | 24 | 24 | 22 | 20 | 20 | 18 |
Sensitivity analysis of patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared to matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients; *dialysis duration prior to transplantation added to the propensity-score matching*. Outcomes for ABOi kidney transplant recipients (n=296) were compared to propensity matched ABOc recipients (n=1184) from the same centers, with living and with deceased donors. The matching variables included: recipient age, peak panel reactive antibody levels, recipient blood type, recipient sex, and *dialysis duration prior to transplantation*. ABOi recipients are marked in black, ABOc living donor recipients are marked in light blue, and ABOc deceased donor recipients are marked in red. The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure with patient death considered as a competing event.
Figure S5. Sensitivity analysis of patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared to matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients; diabetic nephropathy as primary kidney disease added to the propensity-score matching. Outcomes for ABOi kidney transplant recipients (n=296) were compared to propensity matched ABOc recipients (n=296) from the same centers, with living and with deceased donors. The matching variables included: recipient age, peak panel reactive antibody levels, recipient blood type, recipient sex, diabetic nephropathy as primary kidney disease. ABOi recipients are marked in black, ABOc living donor recipients are marked in light blue, and ABOc deceased donor recipients are marked in red. The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure with patient death considered as a competing event.
Sensitivity analysis of patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients compared to matched blood group compatible (ABOc) living and deceased donor kidney transplant recipients; *rituximab induction was excluded from the analysis*. Outcomes for ABOi kidney transplant recipients (n=142) were compared to propensity matched ABOc recipients (n=568) from the same centers, with living and with deceased donors. The matching variables included: recipient age, peak panel reactive antibody levels, recipient blood type, recipient sex, *with exclusion of recipients treated with rituximab induction*. ABOi recipients are marked in dark blue, ABOc living donor recipients are marked in light green, and ABOc deceased donor recipients are marked in red. The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure with patient death considered as a competing event.
Figure S7. Patient survival and cumulative incidence of graft failure in blood group incompatible (ABOi) kidney transplant recipients by recipient blood group. Outcomes for ABOi kidney transplant recipients were compared according to recipient blood group, that is A (n=57), B (n=44) and O (n=195). The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure with patient death considered as a competing event. Blood group A recipients are marked in black, blood group B recipients are marked in red and blood group O recipients are marked in light blue. The dashed lines represent patient survival with a functioning graft, the solid lines represent kidney graft failure.
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