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Early Thrombotic Microangiopathy After ABO-Incompatible Living Donor Kidney Transplantation

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Introduction: Although long-term graft survival is comparable with that of ABO-compatible (ABOc) renal transplantation, the risk of antibody-mediated rejection (ABMR) following ABO-incompatible (ABOi) transplantation is higher and can occur as an early thrombotic microangiopathy (TMA).

Methods: We designed a retrospective multicenter study, including all patients who presented with a TMA (histological and/or biological) after an ABOi transplantation ($<$ 1 month) and compared with matched controls who had a favorable initial course with a normal biopsy.

Results: Between 2013 and 2022, 375 ABOi kidney transplants were performed and 23 patients (6.1%) developed TMA (median: 1 day, interquartile range [IQR]: 0–3 days). Twenty-one patients (91.3%) had biological TMA. Among 23 early graft biopsies, histological evidence of active TMA was found in 17 cases (80.9%). All patients received treatment: 20 of 23 received at least 1 session of plasmapheresis and 19 of 23 received at least 1 injection of eculizumab. Eight early graft losses (30.4%) occurred (median: 7 days, IQR: 3–16 days). IgG and IgM anti–blood group antibody (ABGA) levels (peak and last pregraft assay) were significantly higher in the TMA group (peak: $P = 0.01$ for IgG and $P = 0.0006$ for IgM; last assay before kidney transplantation [KT]: $P < 0.0001$ for IgG and $P = 0.0003$ for IgM). A level $\geq$ 1/8 for IgG and $\geq$ 1/4 or IgM before transplantation were significantly and independently predictive of the occurrence of TMA. No other predictive factors were found.

Conclusion: TMA after ABOi transplantation is not a rare phenomenon and is associated with a poor prognosis in nonresponders-to-treatment patients. ABGA titer performed by hemagglutination is an imperfect marker of the occurrence of such a phenomenon.

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Despite excellent long-term results for renal graft and patient survival,[1-4] living donor ABOi KT is associated with an increased risk of mortality, graft loss, and ABMR[2,3] in the first years posttransplantation compared with living ABOc KT. These differences are probably related to the higher rate of early graft loss in ABOi KT[5] and the increased risk of infectious disease related to the immunosuppression and desensitization protocols for ABOi KT.[6] The modern strategy for this kind of KT includes the infusion of anti-CD20 monoclonal antibodies and plasmapheresis to achieve the lowest ABGA titer.[7] Nevertheless, ABGA seems to be an imperfect prognostic marker and ABMR can occur after ABOi KT with low titer of ABGAs.[8] Risk stratification based on ABGA remains a major issue in ABOi KT.[9]

Early TMA (systemic or localized) is a recognized phenomenon after ABOc KT,[10] being an integral part of the diagnosis of anti–human leukocyte antigen (HLA) ABMR.[11,12] The definition and diagnostic criteria of ABMR after ABOi KT are less well-reported and are not considered as a distinct entity in the Banff International Classification. Renal TMA lesions are associated with the diagnosis of ABMR after ABOi KT[13] and the occurrence of early TMA is more frequent than in ABOc KT.[14] Nevertheless, these studies are monocentric and the frequency of TMA after ABOi KT, the long-term evolution of this complication and its consequences on graft survival, depending on the treatment proposed are not well-determined.

Here, we report on a French multicentric retrospective study to determine the frequency of early TMA after living donor ABOi KT, factors associated with its occurrence. and its long-term impact on graft survival.

METHODS

Study Design: Flow Charts and Patients

We conducted a multicentric retrospective study involving 11 French KT centers (flow charts in Figure 1), in which all the kidney transplant recipients (KTRs) who fulfilled the following eligibility criteria were included (TMA group):

1. ABOi KT recipient between 2013 and 2022.
2. Delayed graft function (at least 1 session of hemodialysis after KT) or slow graft function (serum creatinine $>$ 250 µmol/l on day 5 after KT).
3. Presence of biological signs of TMA and/or histological signs of TMA in kidney graft biopsy during the 4 weeks after KT. Biological TMA was diagnosed clinically based on the presence of thrombocytopenia with microangiopathic hemolytic anemia in the absence of any other apparent clinical cause. The presence of thrombocytopenia was defined as the platelet count $<$ 50 000/l or $>$ 50% lower from the platelet count on the day of transplantation. Microangiopathic hemolytic anemia was indicated by markedly increased serum lactate dehydrogenase levels and the additional presence of fractionated erythrocytes in blood smears. A pathological diagnosis of TMA was made based on the presence of 1 or more of the following conditions: fibrin thrombi in the glomeruli and/or small arteries and arterioles; intracapillary or arteriolar thrombosis; and vascular fibrinoid necrosis.
4. No preformed or *de novo* anti-HLA donor-specific antibody (DSA), measured with Luminex single antigen assay. *De novo* DSAs were tested at the time of the TMA diagnosis as well at 3 months post-KT.

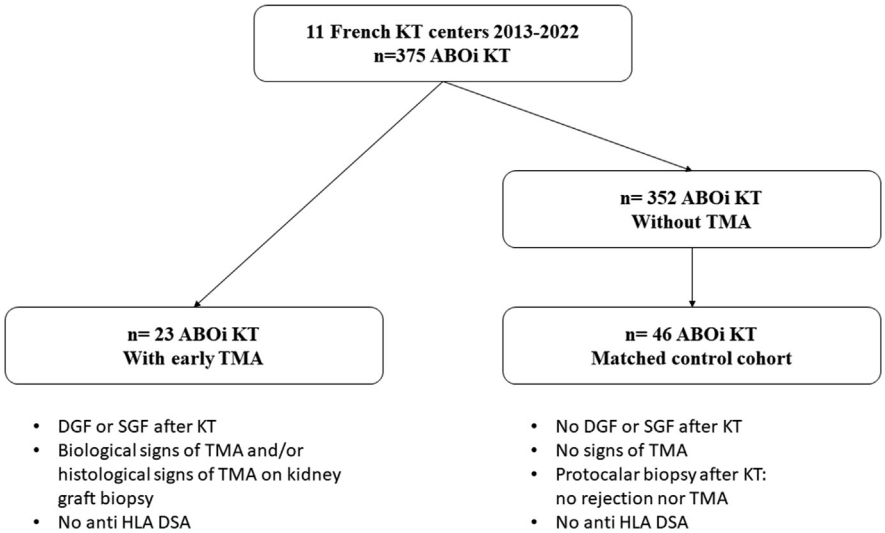

Figure 1. Flow chart. ABOi, ABO-incompatible, DGF, delayed graft function; DSA, donor-specific antibodies; HLA, human leukocyte antigen; KT, kidney transplantation; SGF, slow graft function TMA, thrombotic microangiopathy.

We built a matched control cohort (2:1) in which all the KTRs fulfilled the following eligibility criteria (control group):

1. ABOi KT recipient in the same period, between 2013 and 2022.
2. No delayed graft function or slow graft function after transplantation.
3. Absence of biological signs of TMA after KT.
4. Month-3 protocol biopsy after KT showing no sign of rejection or TMA.
5. No preformed or *de novo* anti-HLA DSA. DSA were tested at 3 months post-KT in all patients of the control group.

Clinical and laboratory information were extracted from electronic databases and the patients' medical records. Transplant biopsies were "for cause" biopsies in the TMA group (delayed graft function or slow graft function) and were month-3 protocol biopsy in the control group. Biopsies were scored according to the Banff classification and reviewed in each center.[15] C4d positivity was defined by a C4d score > 1 on immunofluorescence, or > 0 on immunohistochemistry. We evaluated kidney graft function by estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease formula.[16]

According to French law (loi Jardé), because this was an anonymous retrospective study, institutional review board approval was not required. 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.

Immunosuppression Protocol for ABOi KT

All the centers included in the study used the same immunosuppressive protocol for ABOi KT. All patients received 1 course of rituximab (1 infusion, 375 mg/m^2) 1 month before transplantation. Depending on the ABGA titer and local practice, sessions of plasmapheresis (plasma exchange, specific-immunoadsorption, or double filtration plasmapheresis) were initiated 2 weeks before KT. Induction therapy was based on antithymocyte globulins or basiliximab according to HLA sensitization and local practice. All patients received a triple-immunosuppression therapy consisting of tacrolimus, mycophenolate mofetil, and steroids.

ABGA Isohemagglutinin Titers

Titration of anti-A and anti-B antibodies were performed using the gel filtration method in each center. Recipient plasma was serially diluted with saline solution, and 0.8% of the red blood cell (ID-DiaCell ABO, DiaMed GmbH, Cressier, Switzerland) suspension (50 μl) was mixed with the recipient plasma (25 μl) in gel cards. The reaction mixture was distributed on neutral gel cards (NaCl, Enzyme Test and Cold Agglutinins, DiaMed GmbH, Cressier, Switzerland) and allowed to stand for 15 minutes at room temperature before measuring the IgM antibody titer. To measure the IgG antibody titer, the same distribution was performed with specific IgG gel cards (Coombs Anti-IgG [rabbit], DiaMed GmbH, Cressier, Switzerland) and recipient plasma were allowed to react at 37°C for 15 minutes. The gel cards were centrifuged at 900 to 1000 rpm for 10 minutes using DIAMED centrifuge 24S immediately before determining the result. The titer endpoint was the reciprocal of the highest dilution demonstrating macroscopic agglutination.

Biopsy-Based Transcriptomics

Biopsy-based transcriptomics for the diagnosis of ABMR was available in one of the KT centers.[17] Briefly, a reverse transcriptase multiplex ligation-dependent probe amplification assay was used. This enables the simultaneous evaluation of a restricted gene panel using formalin-fixed paraffin-embedded blocks. Gene expressions were interpreted using an open-access classifier (https://kidney-transplant-classifier.herokuapp.com/) which classifies each case between "molecular signal of ABMR," "molecular signal of TCMR," or "molecular signal of non-rejection". According to the 2019 Banff classification,[15] the expression of previously validated gene transcripts or classifier associated with ABMR can substitute for microvascular inflammation above threshold (defined by glomerulitis + peritubular capillaritis score ≥ 2), C4d, or DSA criteria.

Statistical Methods

Quantitative data were presented as mean (standard deviation), or median (IQR) when data were not normally distributed. Qualitative data were presented as percentages. The nonparametric Wilcoxon (quantitative data) and Mann-Whitney tests (qualitative data) were used to compare characteristics between the 2 groups. The Kaplan–Meier method was used to assess patient and graft survival and log rank test to compare the groups ($P < 0.05$ was considered statistically significant). A receiver operating characteristic curve was used to calculate the area under the curve to identify the optimal cut-off value for ABGA titer (IgG and IgM) for TMA prediction in KTRs after ABOi KT. For logistic regression, predictors with a $P < 0.10$ were included in the model. A 2-sided $P < 0.05$ was considered statistically significant. Results were presented as odds ratios with 95% confidence intervals. All analyses were performed using STATVIEW version 5.0 (SAS

Institute, Cary, NC) and GraphPad Prism version 8.0 software (GraphPad Software, San Diego, CA).

RESULTS

Early TMA: General Characteristics

During the study period, 375 living donor ABOi KT were performed in 11 KT centers and 23 KTRs experienced early TMA, that is, 6.1%. Among them, 11 patients (47.8%) developed delayed graft function and required hemodialysis after KT, whereas 12 had a slow graft function (serum creatinine $>$ 250 μmol/l on day 5). Twenty-one KTRs had biological signs of TMA (21/23:91.3%) after a median time of 1 day post-KT (IQR: 0–3): median serum lactate dehydrogenase level was 830 U/L (IQR: 561–1044), fractionated erythrocytes in blood were present in 19 of 21 cases (90.5%), haptoglobin was collapsed in all cases, and median platelet count was 55×10^9/L (IQR: 21–78). Median eGFR was 15.2 ml/min per 1.73m^2 (IQR: 10.1–23.3) at time of diagnosis. Graft imaging (doppler ultrasound or computed tomography scan) was normal in 14 of 23 cases, but showed cortical necrosis in 7 of 23 cases and a suspicion of venous thrombosis in 2 of 23 cases.

An early kidney graft biopsy was performed in all the patients after a median time of 8 days post-KT (IQR: 5–14.2). Histological findings consisted of active arteriolar and glomerular TMA in 17 of 23 cases (73.9%) coupled with microvascular inflammation (glomerulitis + peritubular capillaritis) $\geq$ 2 in 10 of 23 cases (43.5%). C4d was positive along the peritubular capillaries in 17 of 23 cases (73.9%). All biopsies presented with acute tubular necrosis lesions. Only 1 patient presented with a grade 1A T cell–mediated associated with microvascular inflammation.

In one center, biopsy-based transcriptomics for the diagnosis of ABMR were available. Consequently, these were performed on all biopsies from this center, that is, 5 of 23 cases (21.7%) and 13 of 46 controls (28.3%). These evidenced a molecular signal of ABMR in all cases of biological and/or pathological TMA. All biopsies presented C4d deposits. Two biopsies (2/5, 40%) presented isolated acute tubular necrosis. One biopsy presented with acute tubular necrosis and microvascular inflammation $\geq$ 2. One biopsy presented with TMA with isolated peritubular capillaritis = 1. One biopsy presented with TMA with isolated glomerulitis = 2. Using histological and molecular criteria, all these biopsies met the 2019 Banff criteria for ABMR. In the control group, showing no sign of rejection or TMA, molecular assessment failed in 3 cases because of insufficient material. In all the other biopsies a "non-rejection" molecular signal was evident.

Table 1. General characteristics of recipients and donors and strategy for desensitization in the 2 groups of patients

Characteristics	TMA group n = 23	Control group n = 46	P value
Mean age of recipient ± SD, yrs	46.9 ± 14.8	48.4 ± 14.1	0.71
Sex of recipient M/F: ratio	17/6: 2.8	32/14: 2.3	0.71
BMI of recipient, mean ± SD	24.1 ± 4.0	23.6 ± 4.0	0.72
Dialysis before KT, n (%)	14 (60.8)	25 (54.3)	0.61
Cause of ESRD n (%)			0.65
IgA nephropathy	5 (21.7)	12 (26.1)	
CGN (non-IgA)	7 (30.4)	10 (21.7)	
CTIN	6 (26.1)	9 (19.6)	
ADPKD	3 (13.1)	5 (10.9)	
Other	2 (8.7)	10 (21.7)	
Diabetes before KT, n (%)	2 (8.7)	6 (13.4)	0.58
HLA sensitization, n (%)	11 (47.8)	15 (32.6)	0.22
Mean age of donor ± SD, yrs	51.9 ± 10.1	50.2 ± 16.4	0.79
Sex of donor M/F: n	8/15: 0.5	19/27: 0.7	0.60
BMI of donor, mean ± SD	25.5 ± 3.5	24.7 ± 3.1	0.21
Blood group D/R – n (%)	A/O: 14 (60.9)	A/O: 27 (58.7)	0.54
	B/O: 5 (21.7)	B/O: 2 (4.3)	
	AB/O: 0	AB/O: 2 (4.3)	
	B/A: 2 (8.7)	B/A: 5 (10.9)	
	AB/A: 2 (8.7)	AB/A: 1 (2.2)	
	A/B: 0	A/B: 9 (19.6)	
	AB/B: 0	AB/B: 0	
Plasmapheresis, n (%)	21 (91.3)	41 (89.1)	0.78
IA	16 (69.6)	22 (47.8)	0.08
PE	6 (26.1)	18 (39.1)	0.28
DFPP	5 (21.7)	8 (18.4)	0.67
Specific IA	3 (13.1)	5 (10.9)	0.79
n session – median	6	5	0.08
Induction, n (%)			
Antithymocytes globulins	13 (56.5)	21 (45.7)	0.39
Basiliximab	10 (43.5)	25 (54.3)	0.39

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CGN, chronic glomerulonephritis; CTIN, Chronic tubulointerstitial nephritis; D, donor; DFPP, double filtration plasmapheresis; ESRD, end-stage renal disease; F, female; HLA, human leukocyte antigen; IA, immunoadsorption; KT, kidney transplantation; M, male; PE, plasma exchange; PP, plasmapheresis; R, recipient; TMA, thrombotic microangiopathy.

Early TMA: Outcomes

Twenty patients (86.9%) received plasmapheresis: 19 received plasma exchanges, 6 received immunoadsorption, and 5 received both plasma exchanges and immunoadsorption, with a median number of sessions of 5 (IQR: 3–6). Twelve KTRs received high dose of steroids (3 infusions of 500 mg methylprednisolone), 4 received anti-thymocytes globulins (1.5 mg/kg for 3 days) and 1 i.v. Igs (2g/kg). Eculizumab (900 mg) was introduced in 19 of 23 KTRs after a median time of 5 days post-KT (IQR: 1–13), with a median number of 5 infusions (IQR: 2.7–6.7). All the treatments received by each of the 23 KTRs after TMA diagnosis are summarized in Supplementary Table S1.

After treatment, biological signs of TMA disappeared in all cases without recurrence. We reported 7 primary nonfunctioning grafts (30.4%) that required kidney transplant graftectomy in 6 of 7 cases. We recorded 2 later graft losses at 5.5 and 17 months after KT. One year death-censored graft survival was 63.7%. Only 1 KTR in the

TMA group died 91 months after KT because of severe COVID-19; no death was reported in the control group.

Early TMA: Comparison to the Control Cohort

There was no significant difference in the recipients' and the donors' characteristics between the 2 groups (Table 1). Regarding strategies for desensitization and immunosuppressive therapy, there were no statistical differences between the 2 groups (Table 1). Results of ABGA (both IgG and IgM) before desensitization (before plasmapheresis), at time of KT (before surgery) are presented in Figure 2. IgG and IgM levels before desensitization and at the time of transplantation were statistically significantly higher in the TMA group than in the control group.

ABGAs after KT were not statistically different between the 2 groups. At the time of TMA diagnosis, median IgG titer was 1:4 (IQR = 1:1–1:8) in the TMA group and 1:2 (IQR = 1:1–1:4) in the control group ($P = 0.06$). Median IgM titter was 1:1 (IQR = 1:1–1:4) in the TMA group and 1:1 (IQR = 1:1–1:2) in the control group ($P = 0.55$). Two patients presented a rebound of ABGAs in the TMA group and 1 in the control group ($P = 0.64$).

One-year death-censored graft survival was significantly higher in the control group (Figure 3): 100% versus 63.7% in the TMA group ($P < 0.001$). Mean eGFR in 1-year functioning grafts was lower in the TMA group than in the control group: 33.6 ± 15.8 versus 60.8 ± 16.8 ml/min per 1.73 m^2 ($P < 0.001$) at 1 month after transplantation and 41.3 ± 20.5 versus 56.4 ± 15.7 ml/min per 1.73 m^2 ($P = 0.005$) at 1 year after transplantation, respectively.

Cut-Off Value of ABGA Titer for Predicting Early TMA

The receiver operating characteristic curve determined that the optimal cut-off value of ABGA titer for predicting early TMA after ABOi KT was 1:64 for IgG and 1:16 for IgM before desensitization, and 1:8 for IgG and 1:4 for IgM at time of KT. The respective area under the curve, sensitivity, specificity, and positive and negative predictive values are reported in Table 2.

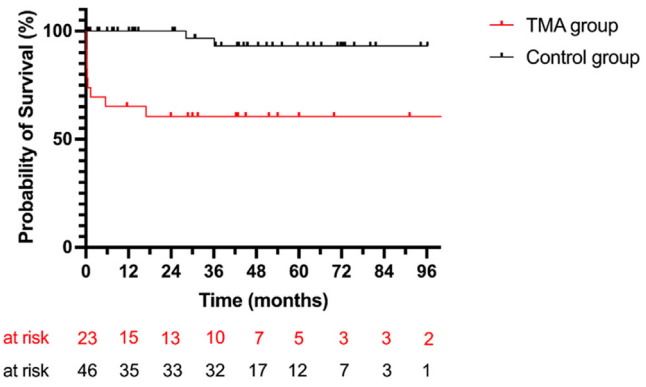

Figure 2. Anti–blood group antibodies titter (IgG and IgM) (a) before desensitization and (b) at time of KT. KT, kidney transplantation; TMA, thrombotic microangiopathy.

Factors Associated With the Occurrence of Early TMA After ABOi KT

Univariate and multivariate logistic regression analyses to determine factors associated with the occurrence of early TMA after ABOi KT are reported in Tables 3 and 4. In multivariate analysis, IgG ≥ 1:8 (odds ratio = 10.4; 95% confidence interval:1.9–57.7; $P = 0.007$) and IgM ≥ 1:4 (odds ratio = 4.9; 95% confidence interval: 1.2–21.1; $P = 0.03$) before transplantation but not before desensitization were associated with the occurrence of TMA.

DISCUSSION

Here, we report on the first multicentric study and the largest cohort investigating TMA after ABOi living donor KT. Data published on this complication are very scarce and derived solely from monocentric studies. To date, the largest study was from Tasaki *et al*. who reported 15 cases of TMA during the first 4 weeks post-KT in a cohort of 87 ABOi KTRs (17.2%).[14] The second cohort was from Kishida *et al*. in the setting of ABOi living donor liver transplantation; they reported 11 cases of early TMA over 29 ABOi liver transplantations (11/29: 37.9%).[18] In these studies, TMA was diagnosed by sudden and severe thrombocytopenia, followed by hemolytic anemia with fractionated erythrocytes in the blood smear following transplantation, but not necessarily confirmed by a graft biopsy because obtaining a graft biopsy at that time might be judged too risky. In our study, the incidence of this complication was lower, but remains significant (6.1%). Tasaki *et al*. reported 4 graft losses in their 15 KTRs with early TMA (26.7%).[14] We report the prognosis of such a phenomenon compared with a normal course of ABOi KT. We recorded 7 primary nonfunctioning grafts (30.4%) and 2 later graft losses in our cohort of KTRs. Death-censored graft survival was significantly higher in the control group: 100% at 1 year post-KT versus 63.7% in the TMA group. Nevertheless, in KTRs who responded to treatment, kidney graft survival was excellent, despite a lower eGFR at 3 months improving at 12 months post-KT.

Early TMA after ABOi KT is probably linked to an ABMR. We confirm this hypothesis because biopsy-based transcriptomics evidenced a molecular signal of ABMR in all cases of biological and/or pathological TMA. Histological lesions of ABMR in the context of HLA DSAs have been largely reported[10,11] and are well-described in the Banff classification.[15] TMA lesions are an integral part of the definition of humoral

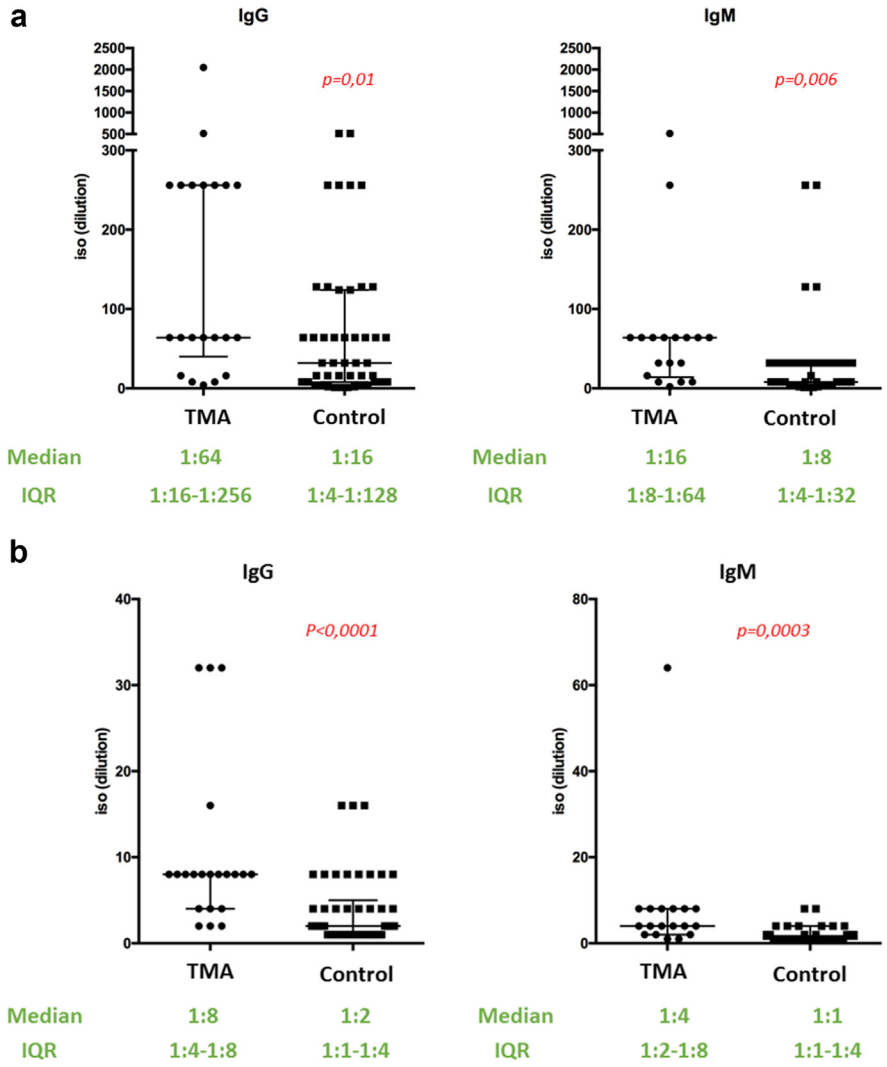

Figure 3. Death-censored graft survival in the TMA group and control. TMA, thrombotic microangiopathy.

rejection.[15] The definition of ABMR in the setting of ABO incompatibility is not depicted as a separate entity in the Banff classification and its histologic appearance is less well-defined. Fidler *et al*. report in 32 ABOi KT the histological findings of ABMR particularly linked to a process of TMA.[13] Nine of 32 (28%) developed clinical ABMR. Biopsy revealed glomerular thrombi (78%), mesangiolysis (78%), peritubular capillary C4d staining (56%) and neutrophil infiltration (67%), interstitial hemorrhage and necrosis (56%), and arteriolar thrombi (33%). Subclinical ABMR was diagnosed by protocol biopsies in 4 patients. Findings

Table 2. Predictive values of anti–blood group antibody titers (IgG and IgM) for thrombotic microangiopathy after ABOi KT

Parameter	AUC	*P*	Cut-off	Se (%)	Sp (%)	PPV (%)	NPV (%)
IgG Des	0.679	0.02	1:64	54.3	76.2	43.2	83.3
IgM Des	0.730	0.006	1:16	52.6	77.8	56.2	83.3
IgG KT	0.799	< 0.0001	1:8	76.1	71.4	57.7	85.4
IgM KT	0.799	0.0003	1:4	73.7	72.2	56.5	84.8

ABOi, ABO-incompatible; AUC, area under the curve; Des, before desensitization; KT, before KT; NPV, negative predictive value; PPV: positive predictive value; Se: sensitivity; Sp: specificity.

Table 3. Univariate logistic regression analysis to determine factors associated with the occurrence of early TMA after ABOi KT

Parameter	OR	95% CI	*P value*
Age of recipient	1.01	0.97–1.04	0.68
Sex, M of recipient	0.81	0.26–2.48	0.71
BMI of recipient	0.97	0.86–1.09	0.65
Age of donor	0.99	0.96–1.02	0.63
Sex M, of donor	1.31	0.47–3.73	0.61
BMI of donor	0.89	0.75–1.05	0.16
IA	0.40	0.14–1.16	0.09
PP, number of sessions	0.86	0.73–1.01	0.07
Induction: basiliximab	1.55	0.56–4.24	0.39
IgG before Des ≥ 1:64	3.81	1.19–12.1	0.02
IgM before Des ≥ 1:16	3.89	1.08–13.99	0.04
IgG before KT ≥ 1:8	7.96	2.48–25.48	0.0005
IgM before KT ≥ 1:4	7.28	2.01–25.64	0.002

ABOi, ABO-incompatible; BMI, body mass index; CI, confidence interval; Des, desensitization; IA, immunoadsorption; KT, kidney transplantation; M, male; OR, odds ratio; PP, plasmapheresis; TMA, thrombotic microangiopathy.

Table 4. Multivariate logistic regression analysis to determine factors associated with the occurrence of early TMA after ABOi KT

Model 1			
Parameter	**OR**	**95% CI**	***P***
IA	0.49	0.11–2.10	0.34
PP – number of sessions	1.00	0.81–1.30	0.96
IgG before Des ≥ 1:64	1.04	0.14–7.6	0.97
IgG before KT ≥ 1:8	10.41	1.88–57.7	0.007
Model 2			
Parameter	**OR**	**CI 95%**	***P***
IA	1.07	0.85–1.34	0.56
PP – number of sessions	0.94	0.11–7.64	0.17
IgM before Des ≥ 1:16	2.51	0.42–15.06	0.31
IgM before KT ≥ 1:4	4.95	1.16–21.08	0.03

ABOi, ABO-incompatible; CI, confidence interval; Des, desensitization; IA, immunoadsorption; KT, kidney transplantation; OR, odds ratio; PP, plasmapheresis; TMA, thrombotic microangiopathy.
Model 1 with anti-blood group antibodies IgG and model 2 with IgM.

consisted of glomerular thrombi (100%), mesangiolysis (25%), and C4d staining (100%). An early kidney graft biopsy was performed in the patients of our study. Histological findings were active arteriolar and glomerular TMA in 17 of 23 cases coupled with microvascular inflammation, glomerulitis and peritubular capillaritis in 10 of 23 cases (43.5%). In their study Tasaki *et al*. performed 1-hour post-KT biopsies in 82 out of 87 patients who received ABOi KT[14]: the glomerulitis, vasculitis, peritubular capillaritis, and C4d deposition on peritubular capillaritis scores were significantly higher in the TMA group than in the non-TMA group. In our study, biological signs of TMA were present in 91.3% of the TMA group as depicted in the study by Tasaki *et al*.[14] Nevertheless, 2 KTRs in our study exhibited TMA histological lesions but no biological signs of TMA. Early systemic TMA after ABOi KT could be the highest grade of ABMR after KT; and ABMR could have a heavy impact on the long-term outcome of ABOi KT.[19]

The pathophysiological process of ABMR in this setting is not well-understood and is reported to be connected to the level of ABGAs, IgG and IgM. Patient-conditioning protocols, including plasmapheresis or immunoadsorption to remove the potentially injurious anti–donor blood group antibodies together with the use of rituximab appear to be effective and well-tolerated.[1] Studies investigating the correlation of ABGA and the occurrence of ABMR after ABOi KT are discordant. In their study, Toki *et al*. found in multivariate analysis that anti–blood group IgG antibody titers of 1:32 at the time of transplantation (odds ratio = 9.52; $P = 0.041$) were independent risk factors for ABMR regardless of baseline anti–blood group IgG antibody titers.[19] Tobian *et al*. found correlation between the posttransplant ABGAs and the occurrence of ABMR with a cut of off of 1/64 with a good negative predictive value but a low sensitivity and specificity of 57.1% and 79.5%, respectively.[20] Shimura *et al*. did not find any correlation between ABGA (IgG and IgM) and the occurrence of ABMR.[8] In the same way, Ishihara *et al*. did not report a significant association between ABGA titer (IgG and IgM) and microvascular inflammation in the graft, sign of humoral rejection.[21] However, Tasaki *et al*. reported in a multivariate logistic regression analysis that pretreatment IgG antibody titer ≥ 64-fold and pretransplant IgM antibody titer ≥ 16-fold were significant risk factors for systemic *de novo* TMA in ABOi KT.[14] In our study, the only factor associated with the occurrence of an early TMA after ABOi KT was the ABGA titer: the cut-off with the best area under the curve was 1/64 for IgG and 1/16 for IgM before desensitization, and 1/8 for IgG and 1/4 for IgM after desensitization. Even if there is a strong correlation, this biomarker is not perfect and positive predictive values were very low (some patients with high antibody titers did not develop TMA) and efforts should be made to better predict hyperacute and acute humoral rejection after ABOi KT. Antibody titers were routinely measured by isohemagglutinin assays using red blood cells. ABO blood group antigens expressed on red blood cells are not identical to those of the kidney: for KTRs with blood group A, red blood cells expressed A-II, A-III, and A-IV antigen subtypes, whereas endothelial cells expressed A-II subtype in the kidney and epithelial cells expressed A-III and A-IV subtypes.[22] In blood group B KTRs, B antigen is limited to subtype II.[22] All ABGAs measured using red blood cells cannot bind to kidney endothelial cells and trigger TMA. Furthermore, interpretation of isohemagglutinin assays could be difficult, and the results may vary from one laboratory to another depending on the temperature and the test used (tube or gel). It should be noted that the Diamed gel card method used to measure ABGA titers tends to yield lower values than the tube method, as reported by Masterson *et al*.[23] This may explain the lower thresholds observed in our study (IgG ≥ 1:8, IgM ≥ 1:4 at transplantation). Despite this, these thresholds were statistically significant predictors of early TMA, highlighting their clinical relevance within the context of this method. Accurate methods must be developed to measure ABGA more precisely, because the Luminex technology has revolutionized the detection of anti-HLA antibodies. Flow cytometric techniques are beginning to address these questions,[24] but are not yet routinely used. Recent work by Tasaki *et al*.[25] identified CD31 as a key carrier of ABO antigens on KECs, distinct from the Band 3 protein found on red blood cells. Their development of the CD31-ABO microarray demonstrated that antibody binding to

endothelial-specific ABO antigens could more accurately predict ABMR than conventional isohemagglutinin assays. These findings underscore the complexity of endothelial-immune interactions and suggest that further studies are needed to evaluate the predictive value of anti-ABO antibodies measured directly against endothelial antigens in the context of TMA risk.

In addition, we report on a large experience in the treatment of early TMA post-ABOi KT and the use of eculizumab in this setting. Nineteen of 23 patients (82.6%) received at least 1 dose of anticomplement therapy. In all patients, biological signs of TMA resolved; however, we noted 6 graft losses in patients treated with eculizumab (6/19: 31.6%). Although ABGAs bind to ABO antigens on endothelial cells, the upregulation of the complement inhibitor might contribute to circumventing TMA or ABMR in the acute phase after ABOi KT. Moreover, in all patients presenting with an episode of TMA, we did not report an increase of ABGAs after KT, except in 2 patients, as if the antibodies were trapped in the graft as suggested by Tasaki *et al*.[14] and Nakamura *et al*.[26] in their study, in which they detected intragraft ABGAs. As per this hypothesis, plasmapheresis may probably not be the best therapeutic option in this case. Blocking the complement with eculizumab could be a better alternative to treat TMA in this context. Because of its mechanism of action to target the complement pathway, it has been also used in desensitization protocols to prevent ABMR.[27-29] Although the incidence of ABMR was decreased in patients given eculizumab-based desensitization protocols compared with those having not received eculizumab, at 1 year, chronic ABMR lesions were similar in both groups.[30] Eculizumab has been also used to treat ABMR in ABOc[31] and ABOi kidneytransplant patients.[32-34] In addition, *in vitro* and *in vivo*, eculizumab was found to inhibit hemolytic reaction after ABOi red blood cell transfusion.[35]

There are some limitations in our work. First, this is a retrospective matched cohort study that could have induced some declarative bias. Second, small sample size is a limitation while assessing various outcomes in this study. The major limitation is the measurement of ABGAs: all the evaluations with agglutination assays were performed in different laboratories without a central confirmation for the study and we therefore, emphasize the variability of the test between laboratories because of possible technical issues. Consequently, caution should be exercised when interpreting the cut off for ABAGs, IgG, and IgM. Lastly, TMA after transplantation might involve genetic complement variants or mutations involving the KTR, and/or the graft, which may have precipitated the onset of the TMA. Unfortunately, such analyses were not systematically performed. Despite these limitations, our cohort is homogenous in terms of the desensitization protocol, well-phenotyped, and thanks to the multicentric nature of the study, this might have more impact for clinicians involved in KT.

To conclude, we report here the largest series of early TMA after ABOi KT. This phenomenon related to an ABMR process, is not rare and insignificant, but is associated with a poor prognosis in nonresponder-to-treatment KT. In responders, graft survival seems to be excellent despite a lower eGFR in the follow-up compared with controls. Treatment with plasmapheresis and/or eculizumab seems to be the best therapeutic option in this case. The isoagglutinin titer performed by hemagglutination is an imperfect marker of the occurrence of such a phenomenon, with a low positive predictive value. Further analysis is required to elucidate the specificity of ABGAs and the circumstances contributing to the incidence of TMA in ABOi KT.

DISCLOSURE

All the authors declared no conflicting interests.

DATA AVAILABILITY STATEMENT

All relevant data are within the paper.

AUTHOR CONTRIBUTIONS

DB designed the study, participated in the performance of the research, collected and analyzed the data, and wrote the paper. TdN participated in the performance of the research, collected the data, provided feedback and critical review of the paper. All the authors collected the data, provided feedback and critical review of the paper.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Characteristics and treatment of the 23 patients with early thrombotic microangiopathy.

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