ORIGINAL ARTICLE

Factors Associated with Antibody-Mediated Rejection and Graft Outcomes in Pediatric Kidney Transplantation: A 35-Year Single-Center Study

Facteurs associés au rejet médié par les anticorps et à la survie du greffon en transplantation rénale pédiatrique: Etude monocentrique sur 35 ans

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Abstract

Introduction: Antibody-mediated rejection (ABMR) is a major complication that compromises allograft survival after kidney transplantation (KT). This study aimed to identify factors associated with biopsy-proven ABMR in pediatric kidney recipients and to evaluate its impact on graft and patient survival.

Methods: We conducted a retrospective cohort study including pediatric kidney transplant recipients (≤20 years) at a single center over a 35-year period (1989–2024). Multivariate logistic regression identified factors associated with ABMR. Graft survival was analyzed using Kaplan–Meier and Cox regression models. A competing risk analysis (Fine and Gray model) was performed to account for death with a functioning graft.

Results: Ninety-seven transplant recipients were included. ABMR occurred in 22 patients (22.7%). On multivariate analysis, post-transplant anti-HLA-DQ donor-specific antibodies (OR = 18.5; 95% CI: 3.2-105; p = 0.001), hyperuricemia (OR = 3.9; 95% CI: 1.3-12.5; p = 0.021), hypertension requiring ≥2 antihypertensive agents (OR = 9.6; 95% CI: 2.1–39; p = 0.004), and cyclosporine use as first-line calcineurin inhibitor (OR = 3.8; 95% CI: 1.1-13.2; p = 0.039) were independently associated with ABMR. ABMR was an independent predictor of graft loss (HR = 5.2; 95% CI: 2.1-9.1; p = 0.004), confirmed in competing risk analysis (sHR = 4.8; 95% CI: 1.9-8.7; p = 0.006). No significant association was found with patient survival.

Conclusion: ABMR significantly compromises graft survival in pediatric KT. Identification of modifiable risk factors, including metabolic and immunological parameters, may help optimize post-transplant management and improve outcomes.

Key words: kidney transplantation, Pediatric, Allograft rejection, Antibody-mediated rejection, Cellular rejection

Résumé

Introduction : Le rejet médié par les anticorps (ABMR) constitue une complication majeure compromettant la survie du greffon après transplantation rénale (TR). Cette étude avait pour objectif d'identifier les facteurs associés à un ABMR prouvé par biopsie chez les receveurs pédiatriques de greffe rénale et d'évaluer son impact sur la survie du greffon et du patient.

Méthodes : Nous avons mené une étude de cohorte rétrospective incluant des receveurs pédiatriques de greffe rénale (≤ 20 ans) dans un centre unique sur une période de 35 ans (1989–2024). Une régression logistique multivariée a été utilisée pour identifier les facteurs associés à l'ABMR. La survie du greffon a été analysée à l'aide des courbes de Kaplan–Meier et du modèle de régression de Cox. Une analyse en risques compétitifs (modèle de Fine et Gray) a été réalisée afin de prendre en compte le décès avec greffon fonctionnel.

Résultats : Quatre-vingt-dix-sept receveurs de greffe ont été inclus. Un ABMR est survenu chez 22 patients (22,7 %). En analyse multivariée, les anticorps spécifiques du donneur anti-HLA-DQ post-transplantation (OR = 18,5 ; IC 95 % : 3,2-105 ; p = 0,001), l'hyperuricémie (OR = 3,9 ; IC 95 % : 1,3-12,5 ; p = 0,021), l'hypertension nécessitant au moins deux antihypertenseurs (OR = 9,6 ; IC 95 % : 2,1–39 ; p = 0,004), et l'utilisation de la ciclosporine comme inhibiteur de la calcineurine de première ligne (OR = 3,8 ; IC 95 % : 1,1-13,2 ; p = 0,039) étaient indépendamment associés à l'ABMR. L'ABMR était un facteur prédictif indépendant de perte du greffon (HR = 5,2 ; IC 95 % : 2,1-9,1 ; p = 0,004), résultat confirmé par l'analyse en risques compétitifs (sHR = 4,8 ; IC 95 % : 1,9-8,7 ; p = 0,006). Aucune association significative n'a été retrouvée avec la survie des patients.

Conclusion : L'ABMR compromet significativement la survie du greffon en transplantation rénale pédiatrique. L'identification de facteurs de risque modifiables, notamment métaboliques et immunologiques, pourrait permettre d'optimiser la prise en charge post-transplantation et d'améliorer les résultats.

Mots clés: Transplantation rénale, rejet, rejet humoral, rejet cellulaire

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Introduction

Kidney transplantation (KT) is the gold standard treatment for children with end-stage renal disease (ESRD), improving both survival and quality of life [1]. Advances in healthcare infrastructure, surgical techniques, and immunomodulatory therapies have significantly improved short and medium-term graft and patient survival. Nonetheless, long-term survival remains problematic [2]. Indeed, 50% of children who undergo KT will require a second transplant before the age of 25 [3].
Antibody-mediated rejection (ABMR) is one of the main complications of KT. Its occurrence represents a critical turning point, necessitating early and tailored management. Advances in immunology and immunohistochemistry have enhanced our understanding of the phenomena underlying rejection. However, the exact pathophysiological mechanisms remain uncertain.
ABMR is the primary factor compromising long-term graft survival [4]. Therefore, identifying factors associated with its occurrence is key to optimizing the management of transplanted children, enabling personalized care.
The aims of our study were to identify the factors associated with ABMR following KT and to evaluate the impact of ABMR on graft and patient survival.

Methods

Study Type

This was a retrospective and descriptive study that included pediatric kidney transplant recipients followed up in the Pediatric departments at Charles Nicolle Hospital (CNH) in Tunis over a 35-year period (from January 1, 1989, to December 31, 2024).

Patients

Patients were included if they met the following criteria: underwent KT at an age ≤ 20 years. For patients who had multiple transplants, each one was considered as an independent KT.
The study excluded the following KTs: those complicated by hyperacute rejection, those with vascular thrombosis leading to graft loss in the immediate post-transplant period, those with less than 3 months of follow-up, those in patients whose diagnosis of rejection was based solely on clinical and biological criteria without histological confirmation, and those in patients with incomplete medical records that prevented proper data analysis. Among the 123 kidney transplant episodes initially screened, 9 transplant episodes complicated by isolated biopsy-proven cellular rejection without associated histological evidence of antibody-mediated rejection were excluded from the study.

Patient Classification Criteria
Evaluation of Graft Function

Graft function was evaluated by calculating the glomerular filtration rate (GFR), which is expressed in mL/min/1.73m² of body surface area (BSA) [5]. For patients under 18 years of age, GFR was determined using the modified Schwartz formula (2009), while for patients over 18 years, the MDRD (Modification of Diet in Renal Disease) formula was used.

Chronic Kidney Disease (CKD)

CKD was defined in 2012 by the "kidney disease: Improving Global Outcomes" (KDIGO) group as the presence of renal impairment markers and/or a decrease in glomerular filtration rate (GFR) persisting for more than 3 months. CKD is classified into stages of increasing severity based on GFR levels [6].

Renal Allograft Rejection
Clinical and Biological Aspects

Renal graft biopsy (RGB) was not routinely performed in our department after KT. It was indicated based on the presence of clinical and/or biological signs suggestive of rejection, including increased serum creatinine levels, hypertension, and a significant reduction in 24-hour urine output.

Graft Ultrasound

Graft ultrasound was performed on all patients suspected of rejection. The examination used a low frequency curved abdominal probe.

Types of Rejection

Rejection was classified based on the timing of occurrence relative to KT:
- Hyperacute Rejection: occurs immediately after the establishment of vascular anastomoses or within 72 hours of transplantation. It results from preformed cytotoxic antibodies (ACC) in the recipient directed against donor antigens present on the graft [7]. Patients who experienced hyperacute rejection were excluded from this study.
- Acute Early or Late Rejection: This type of rejection corresponds to acute graft dysfunction:
  - Early Acute Rejection: Occurs within the first year after KT [8].
  - Late Acute Rejection: Occurs after the first-year post-transplantation [8].

Both types are characterized by a sudden decline in graft function, often requiring prompt diagnosis and management to preserve graft viability. Two types of acute rejection can be intertwined:
- ABMR
- Cellular-mediated Acute Rejection.

- Chronic Rejection refers to a more gradual deterioration of graft function over time. This type of rejection can be either humoral or cellular in nature [7].

Graft Biopsy

RGB is a crucial step in diagnosing the cause of renal graft dysfunction. It can be performed via percutaneous, transvenous, or surgical routes. According to the Banff Foundation for Allograft Pathology [9]. The data from the RGB were initially classified according to different versions of the Banff classification. Since the elementary lesions were the same across these versions, we reclassified the different RGBs according to the same diagnostic criteria to standardize our results. We used the diagnostic criteria outlined in the Banff 2017 classification.

Immunological Aspects of Kidney Transplantation

The detection of cytotoxic antibodies relies on cellular methods (microlymphotoxicity test (LCT) and flow cytometry) as well as acellular methods (ELISA and Luminex method) [10].

In our study, the crossmatch was performed at the Immunology Laboratory of CNH for all transplant recipients. The crossmatch was done using donor cells from peripheral blood in the case of a living donor (LD), or from lymph nodes, or, if unavailable, from the spleen in the case of a deceased donor (DD). For the recipients, the sera used were the serum from the day of the graft call and any historically positive sera stored in the Immunology Laboratory's serum bank.

Before 2014, the technique used for the crossmatch was the LCT. From 2014 onwards, the crossmatch combined LCT with flow cytometry. Donor-specific anti-HLA antibodies (DSA) were assessed using Luminex single antigen bead assays when available. DSA positivity was defined by a mean fluorescence intensity (MFI) ≥1000. DSAs were further categorized according to HLA class and specificity, with a particular focus on anti-HLA-DQ DSAs given their known clinical relevance.

All kidney transplants performed in our study had a negative crossmatch. The search for anti-HLA antibodies before and after KT was conducted at the Immunology Laboratory of our hospital for all our transplant recipients. Before the introduction of the Luminex method, the LCT was the first-line test used for detecting these antibodies. If the LCT was positive, or if there was suspicion of rejection despite a negative LCT for cytotoxic antibodies, an additional search for these antibodies using ELISA was performed. The Luminex technique was later introduced at the Immunology Laboratory of CNH and became the first-line test for detecting cytotoxic antibodies starting in 2019.

Given the small number of events and quasi-complete separation for DSA positivity, estimates should be interpreted with caution.

Post-Transplantation Period

• Immunosuppressive Treatment (IS)

The goal of immunosuppressive (IS) treatment is to prevent alloimmunization against foreign HLA and non-HLA antigens present on the renal graft. This treatment is based on a combination of molecules with different mechanisms of action to prevent rejection.

• Immunosuppression Protocol

Induction Treatment: the induction treatment combined corticosteroids (Methylprednisolone) with either a polyclonal T lymphocyte-depleting antibody (ATG® or Thymoglobulin®) or a monoclonal anti-CD25 antibody (Basiliximab). The initial corticosteroid dose was administered intravenously, ranging from 1 to 2 mg/kg/day. The dosage was then gradually reduced and switched to an oral form until reaching a maintenance dose of 10 mg/day

Maintenance Treatment: the maintenance treatment included oral corticosteroids (Prednisone or Prednisolone), an antimetabolite (Azathioprine (AZA) or Mycophenolate Mofetil (MMF)), a calcineurin inhibitor (Cyclosporine or Tacrolimus), and Sirolimus.

• Pharmacological Therapeutic Monitoring of Immunosuppressants

The dosage of IS was adjusted based on plasma levels and therapeutic goals. The pharmacological therapeutic monitoring was based on:

- Tacrolimus: Residual concentration (C0) and the area under the curve (AUC).
- Cyclosporine: C0, concentration at 2 hours (C2), and AUC.
- Mycophenolate Mofetil (MMF): C0 and AUC.

Intra-Individual Variability of IS: the evaluation of intra-individual variability of IS was based on calculating the coefficient of variation (CV) for each immunosuppressor. This coefficient was calculated using the formula: CV=σMCV = \frac{\sigma}{M}CV=Mσ where: σ\sigmaσ = standard deviation and MMM = mean value of the drug concentration.

Data Collection

The data collection was done using a form containing information about the pre-, peri-, and post-transplantation periods.

The Period Before Transplantation

For each transplant recipient, we collected the following data:

• Donor Information: Age and gender, type of donor, relationship for living donors, immunological characteristics: (blood group, HLA typing)

• Recipient Information: Epidemiological data, viral serologies, and medical history, time to transplantation, immunological characteristics: (Blood group, HLA typing, Presence of cytotoxic antibodies before transplantation). For patients with positive cytotoxic antibodies post-transplantation, the following details were included: (Nature of the cytotoxic antibodies found: Anti-HLA class I (HLA I), Anti-HLA class II (HLA II), Anti-Major Histocompatibility Complex class I chain-related antigens A (MICA), the technique used for detecting cytotoxic antibodies, the specificity of the antibodies. MICA antibody testing was not systematically performed across the study period, precluding reliable statistical analysis.

• Data Collection: The data collection process

involves comprehensive information gathering pre-, peri-, and post-transplantation, including donor and recipient characteristics, immunological factors, and transplantation specifics such as graft function, complications, and immunosuppressive therapy. Hyperuricemia was defined as a serum uric acid level >360 µmol/L (6 mg/dL) in females and >420 µmol/L (7 mg/dL) in males, or the use of urate-lowering therapy.

• Rejection Classification: Rejection is classified based on clinical, biological, and histological findings, specifically according to the Banff 2017 criteria. This includes classifying rejection as early or late and distinguishing between humoral and cellular types of rejection.

• Outcome definition: Graft loss was defined as the need for permanent return to dialysis. Death with a functioning graft was considered separately and not included as graft loss. Given the potential competing risk of death with a functioning graft, a sensitivity analysis using a competing risk model (Fine and Gray method) was performed.

• Sensitivity analyses: To address potential temporal heterogeneity, we conducted a sensitivity analysis stratified by transplantation era (pre-2010 vs post-2010), corresponding to the introduction of Luminex-based immunological assessment and changes in immunosuppressive protocols. Within each era, the associations between predefined risk factors and ABMR were re-analyzed using the same regression models as in the primary analysis.

• Statistical Analysis: Statistical analyses were performed using IBM SPSS software. Continuous variables were expressed as mean ± standard deviation or median [interquartile range], and categorical variables as frequencies and percentages. Multivariate logistic regression was used to identify factors independently associated with antibody-mediated rejection. Variables with $p < 0.1$ in univariate analysis and clinically relevant covariates were included in the multivariate model. Graft survival was estimated using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazards regression was performed to identify independent predictors of graft loss. Given the potential competing risk of death with a functioning graft, a sensitivity analysis was performed using a competing risk model based on the Fine and Gray method. Subdistribution hazard ratios (sHR) were estimated. To account for temporal changes in clinical practice, a sensitivity analysis stratified by transplantation era (before and after 2010) was performed. Missing data were handled using a complete-case analysis approach

• Survival Analysis: The survival analysis aims to evaluate graft and patient survival in relation to rejection, with the use of Kaplan-Meier curves, Logrank tests, and Cox regression to adjust for potential confounding factors.

Results

A total of 97 patients were included, consisting of 56 boys and 41 girls. The baseline demographic, clinical and biological characteristics are summarized in Table 1.

In our series, we observed three different IS induction treatment protocols. Anti-lymphocyte serum (ALS) was administered in 93 cases (95.9%), of which 21 (22.6%) subsequently experienced rejection. Corticosteroid therapy based on MP was initiated in all patients, with an initial dose of 2 mg/kg/day in 44 cases (45.4%). The distribution of IS induction treatment protocols in our study was as follows: MP + ALS: Administered in 95.9% of cases (n=93), MP + Basiliximab: Administered in 3.1% of cases (n=3), MP alone: Administered in 1% of cases (n=1). The maintenance IS treatment consisted of triple immunosuppressive therapy in all cases: Azathioprine was prescribed in 19 patients (19.6%), all transplanted before the year 2000, with 4 of them (21%) subsequently experiencing rejection. Cyclosporine was used as the first-line CNI in 46 cases (47.4%), of which 8 (16%) later experienced rejection.

The therapeutic combinations observed in our study were distributed as follows:

- Corticosteroid + MMF + Tacrolimus: 51.5% of cases (n=50).
- Corticosteroid + MMF + Cyclosporine: 27.8% of cases (n=27).
- Corticosteroid + Azathioprine + Cyclosporine: 19.6% of cases (n=19).
- Corticosteroid + MMF + Sirolimus: 1% of cases (n=1).

The median coefficient of variation (CV) for Tacrolimus was 20.4% [17.5-29.2]. Nineteen patients (33.3%) receiving Tacrolimus had a CV greater than or equal to 27%, among whom 10 (52.6%) subsequently experienced rejection. The median CV for Cyclosporine was 52% [25-63.5] (Rejection (+): 52% [34-66] vs. Rejection (-): 51% [31-63]). The prevalence of patients with anti-class I antibodies (ACAs) before KT was 7.2% (n=7). Among these, 28.6% subsequently experienced rejection. Post-transplant, ACAs were detected in 31 patients (32%), with 12 (38.7%) of them later experiencing rejection. The median time to the appearance of these antibodies post-transplant was 56 months [1.4-106.4].

Post-transplant DSAs were detected in 7 patients (7.2%), all of whom developed ABMR, suggesting a very high positive predictive value in our cohort; however, this observation should be interpreted with caution due to the limited number of events.

Table 2 illustrates the clinical, biological, and outcome characteristics of our patients after renal transplantation. In our series, 22 patients (22.7%) experienced at least one histologically confirmed ABMR. The median time from transplantation to ABMR diagnosis was 62 months [23-102]. The rejection was classified as early in 18.2% (n=4) of cases. It was acute in 63.7% (n=14) and chronic in 36.3% (n=8) of cases, corresponding to an overall prevalence of 14.4% for acute ABMR and 8.2% for chronic ABMR. Half of the cases (50%) involved active antibody-mediated rejection. Associated cellular rejection lesions were observed in 13.6% of cases.

Table 1. Baseline characteristics of patients prior to the transplantation

	n(%)/Mean ± SD	Humoral graft rejection	
		Yes	No
Total	97	22	75
Sex (male/female)	56/41 (sex ratio:1.4)	sex-ratio 0.8	sex-ratio: 1.6
Age at Kt	15.4 ± 3.2 ans	15.4 ± 2.4	15.4 ± 3.5
Underlying kidney disease			
• CTIN	53(54,6%)	12(22.6%)	41(77.35%)
• CKDu	23(23.7%)	5(21.7%)	18(78.26%)
• CGN	20(20.6%)	5(25%)	15(75%)
• CVN	1(1%)	-	1(1%)
Pre-transplant dialysis modalities			
• HD	64(65%)	12(18.8%)	52(81.2%)
• PD	33(34%)	-	-
Residual urine output			
• < 100 ml/d	35(36,1%)	9(25,7%)	26(74.3%)
• 100 ml et 500 ml/d	27(27,8%)	-	-
• > 500 ml/d	35(36,1%)	-	-
Time from initiation of RRT to Kt(months)	23[15 – 43.6]	19.5[12.5 – 41.7]	23.8[15.4 – 44]
Past history of HTN	62(63.9%)	14 (22.6%)	48(77.4%)
Growth retardation	42(43.3%)	10(23.8%)	32(76.2%)
Pre-transplant transfusion history	35(36.1%)	7(20%)	28(80%)
Donor type			
• LRD	58(59.8%)	16(27,6%)	42(72.4%)
• DD	39(40.2%)	6(15.4%)	33(84.6%)
Donor characteristics			
• Median age (Years) Mean ± SD	38.3±10.3	40.5±11	37.8±9
• Sex (Male/Female)	49/48(sex-ratio:1.02)		
Donor–recipient matching			
• Matched	5(52.6%)	8(15.7%)	43(74.4%)
ABO compatibility			
• ABO compatible	76(82.6%)	17(22.4%)	59(77.63%)
HLA matching			
• Number of HLA mismatches	3[2 – 3]	3[2 – 3]	3[2-3]
0	7(7.2%)	3(3.1%)	4(4.1%)
1-2	20(20.6%)	4(4.1%)	16(16.5%)
3-5	70(72.2%)	15(15.5%)	55(56.7%)
Number of HLA mismatches per locus			
• HLA-A mismatches			
0	16(16.5%)	6(6.2%)	10(10.3%)
1	63(64.9%)	13(13,4%)	50(51.5%)
2	18(18.6%)	3(3.1%)	15(15.5%)
• HLA-B mismatches			
0	11(11.3%)	3(3.1%)	8(8.2%)
1	67(69.1%)	16(16.5%)	51(52.6%)
2	19(19.6%)	3(3.1%)	16(16.5%)
• HLA-DR mismatches			
0	25(25.8%)	5(5.2%)	20(20.6%)
1	70(72.2%)	17(17.5%)	53(54.7%)
2	2(2%)	-	2(2%)
Left kidney harvested	69 (71.1%)	19 (27.5%)	50(72.5)
Warm ischemia time (minutes)	37 ± 9.5	37.5± 8.3	37.3 ± 10.1

Kt: Kidney transplantation; **CTIN:** Chronic Tubulointerstitial Nephropathy; **CKDu:** Chronic Kidney Disease of Unknown/Undetermined origin; **CGN:** Chronic Glomerular Nephropathy; **CVN:** Chronic Vascular Nephropathy; **HD:** Hemodialysis; **PD:** Peritoneal dialysis; **RRT:** renal replacement therapy; **HTN**: Hypertension; **LRD:** Living related donor, **DD:** deceased donor; **HLA:** Human Leukocyte Antigen

Table 2. Clinical, biological, and post-transplant outcomes

Baseline characteristics	n(%)/Mean ± SD	Humoral graft rejection	
		Yes	**No**
DGF	13(13.4%)	1(7.7)	12(92.3%)
Median time to serum creatinine nadir(d)	15.5d [5 – 27.3]	16d [3 – 29]	13d[7 – 30
Duration of first hospitalization (days)	30[24 – 43]	31[25 – 47]	30[24 – 41]
Serum creatinine clearance at discharge (mL/min/1.73 m²BSA)	73.1 ± 28.3	71.3 ± 23	74.5 ± 20.3
Vascular complications			
• Graft renal artery stenosis	5(5.2%)	2(40%)	3(4%)
• Lymphocele	7(7.2%)	2(28.6%)	5(6.6%)
Urological complication			
• VUR	13(13.4%)	3(3.1%)	10(10.3%)
• Urinary fistla	3(3.1%)	-	3(3.1%)
• Transplant kidney stones	1(1%)	1(1%)	-
• Uro-peritoneum	1(1%)	-	1(1%)
Infectious complication			
• UTIs	43 (44.3%)	9 (20.9%)	34(79%)
• Pneumonia	17(17.5%)	4(23.5%)	13(76.4%)
• Gastrointestinal infection	21(21.6%)	14(14.4%)	7(33.3%)
• Sepsis	14(14.4%)	5(35.7%)	9(64.2%)
• CMV infection	32(33%)	10(31.3%)	22(68.7%)
• HSV infection	15(15.6%)	6(40%)	9(60%)
• VZV infection	13(13.4%)	2(15.4%)	11(84.6%)
• BK-Virus infection	11(11.3%)	5(45.5%)	6(54.5%)
• SARS-COV2	7(7.2%)	2(28.6%)	5(71.4%)
• Fungal and parasitic infections	7(7.2%)	1(14.2%)	6(85.8%)
Post-transplant malignancy	4(4.1%)	-	4(4.1%)
Post-transplant HTN	63(64.9%)	17 (27%)	
Metabolic complications			
• PTDM	9(9.3%)	1(11.1%)	8(88.9%)
• Hypertriglyceridemia	41(42.3%)	13(31.7%)	28(68.3%)
• Hyperuricemia	29(29.9%)	13 (44.8%)	16(55.1%)
Anemia and transfusion			
• Post-transplant anemia	39(40.2%)	16 (41%)	23(59%)
• Post-transplant transfusion	22(22.7%)	12(54.5%)	10(45.5%)

DFG: Delayed graft function; **BSA:** Body surface area; **VUR:** Vesicoureteral reflux; **UTI:** Urinary tract infection; **CMV:** Cytomegalovirus; **HSV:** Herpes simplex virus; **VZV:** Varicella-Zoster Virus; **SARS- COV2:** Severe Acute Respiratory Syndrome Coronavirus 2; **HTN:** Hypertension; **PTDM:** Post-transplant diabetes mellitus

The median follow-up duration was 6.5 years [2.5-13]. In multivariate Cox regression analysis, antibody-mediated rejection was independently associated with reduced graft survival (HR = 5.2; 95% CI: 2.1-9.1; p = 0.004). Factors associated with ABMR after multivariate analysis are presented in Table 3. In our study, the cumulative graft survival was 86% at 5 years, 75% at 10 years (Figure 1). Univariate survival analysis showed a statistically significant association between rejection and graft survival (Table 4). We did not find any statistically significant association between rejection and transplant recipient survival.

Table 3. Multivariate analysis of factors associated with antibody-mediated rejection

Factors associated with rejection	Adjusted OR	95% CI	p
Post-transplant hyperuricemia	3.9	1.3-12.5	0.021
First-line CNIs: Cyclosporine	3.8	1.1-13.2	0.039
Post-transplant HLA-DQ DSA	18.5	3.2-105	0.001
Hypertension ≥2 drugs	9.6	2.1-39	0.004

CNI: Calcineurin inhibitor; **HLA:** Human Leukocyte Antigen; **DSA:** Donor-Specific Antibody; **OR:** Odds ratio; **CI:** Confidence interval

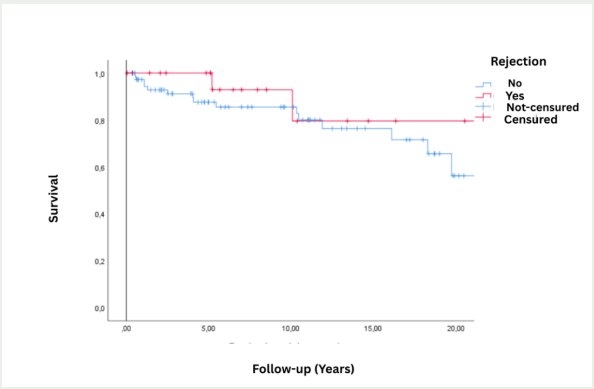


Figure 1. Kaplan–Meier survival curves according to antibody-mediated rejection status

Table 4. Competing risk analysis for graft loss (Fine and Gray model)

Variable	sHR	95% CI	p-value
ABMR	4.8	2.1 – 9.1	0.004
Post-transplant sepsis	3.1	1.3 – 7.5	0.014
Primary glomerular nephropathy	3.0	1.2 – 8.2	0.021
Lymphocele	3.2	1.2 – 8.8	0.018

ABMR: Antibody-mediated rejection

In sensitivity analyses stratified by transplantation era (pre-2010 vs post-2010), the main associations identified in the overall cohort, particularly post-transplant anti-HLA-DQ antibodies, hyperuricemia, and cyclosporine use remained consistent across both periods. Although effect sizes were attenuated in the earlier era, the direction and statistical significance of these associations were preserved.

In competing risk analysis accounting for death with a functioning graft, antibody-mediated rejection remained significantly associated with graft loss (sHR = 4.8; 95% CI: 1.9-8.7; p = 0.006) (Table 5).

Table 5. Multivariate analysis of graft survival

Variable	HR	95% CI	P
Post-transplant sepsis	3.3	1.2–8	0.016
ABMR	5.2	1.9-8.7	0.006
Primary glomerular chronic nephropathy	3.3	1.2–9	0.023
Lymphocele	3.5	1.3–10	0.02

HR : Hazard ratio ; **CI :** Confidence interval; **ABMR:** Antibody-mediated rejection

Discussion

In our cohort, 22.7% of pediatric kidney transplant recipients developed biopsy-proven ABMR. Our ABMR rate is consistent with previous pediatric studies reporting rates between 15-30% [11-20]. Although the exclusion of isolated cellular rejection may limit external generalizability, its impact is likely minimal given the small number of excluded cases and the inclusion of mixed rejection phenotypes when humoral lesions were present, thereby preserving internal validity.

ABMR occurred predominantly as a late event, with early rejection observed in only 18.2% of cases [21]. This distribution likely reflects favorable immunological conditions in our cohort, including a high proportion of living donors, systematic negative crossmatch, and widespread use of induction therapy. These findings are consistent with contemporary studies reporting a predominance of late ABMR [21,22]. Clinically, ABMR was mainly characterized by graft dysfunction, often associated with hypertension, which should raise suspicion when newly occurring or worsening after transplantation.

Our findings align with current understanding that ABMR is primarily driven by donor-specific antibodies (DSAs) targeting endothelial HLA antigens, leading to complement activation and microvascular injury [23]. The presence of C4d-negative ABMR cases further supports the role of complement-independent pathways.

We identified several independent factors associated with ABMR, including post-transplant anti-HLA-DQ donor-specific antibodies, hyperuricemia, HTN requiring multiple antihypertensive agents, and the use of cyclosporine as the primary CNI [24-27]. Notably, only anti-HLA-DQ DSAs remained independently associated with ABMR, suggesting a predominant role of this specific subtype rather than DSAs as a whole. The wide confidence interval suggests limited precision and potential overestimation due to the small number of events and quasi-complete separation. This finding is consistent with growing evidence highlighting the central role of anti-HLA-DQ DSAs in chronic alloimmune injury and graft loss. These findings support systematic monitoring of anti-HLA-DQ DSAs and aggressive control of modifiable risk factors such as hypertension and hyperuricemia [27].

Hyperuricemia also emerged as an independent risk factor, supporting the hypothesis that metabolic disturbances may contribute to endothelial dysfunction and alloimmune activation [28-31]. In contrast, traditional factors such as donor type and HLA mismatch burden were not significantly associated with ABMR in our study, possibly reflecting the relatively homogeneous immunological risk profile and limited sample size.

The role of anti-MICA antibodies could not be adequately assessed due to non-systematic testing, particularly in earlier transplantation eras, and therefore remains inconclusive in our cohort. [27]

ABMR is not only immunological but also influenced by modifiable metabolic and hemodynamic factors [31].

Importantly, ABMR was a strong independent predictor of graft loss. This association remained significant in

competing risk analysis accounting for death with a functioning graft, confirming the robustness of this finding. No significant association was observed between ABMR and patient survival, likely due to the limited number of events. From a clinical perspective, these findings underscore the importance of identifying high-risk patients and optimizing modifiable factors, including immunosuppressive exposure, metabolic control, and blood pressure management.

This study has several limitations, including its retrospective design, relatively small sample size, and the long study period encompassing major changes in immunological assessment and clinical practice. This long study period encompasses major shifts in immunological assessment (introduction of Luminex) and immunosuppressive strategies, which may have influenced ABMR detection and management. However, sensitivity analyses stratified by transplantation era confirmed the consistency of our results, supporting their robustness.

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