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TOCILIZUMAB IN KIDNEY TRANSPLANT RECIPIENTS WITH caAMR

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1. **INTRODUCTION**

Chronic active antibody-mediated rejection (caAMR) is one of the leading causes of graft loss in kidney transplant recipients [1-3]. It is estimated that in the USA about 5,000 kidney transplants / year undergo functional failure mainly due to cAMR[4]. The diagnosis of caAMR is histological, based on the Banff 2022 classification criteria (Table 1), and relies on evidence of recent interaction between donor-specific antibodies (DSA) and vascular endothelium, indicated by signs of microvascular inflammation (MVI), including glomerulitis (g) and peritubular capillaritis (ptc), along with chronic histological lesions such as transplant glomerulopathy (cg > 0) and/or severe multilayering of the peritubular capillary basement membrane [5,6]. The pathogenic mechanism is primarily mediated by the presence of DSA (mainly anti-HLA donor-specific antibodies), which activate complement and cause direct endothelial damage, recruiting innate immune cells such as neutrophils, macrophages, and NK cells [7–9]. Recent evidence has highlighted the complexity and variability of caAMR, with some patients showing MVI in the absence of DSA and C4d staining [10]. The 16th Banff meeting in 2022 introduced new phenotypes, including MVI-positive with DSA and C4d-negative (MVI+ DSA–C4d–) and probable AMR in DSA-positive patients with subthreshold MVI (g + ptc < 2) [11]. A study by Sablik et al. demonstrated that these emerging phenotypes are associated with graft survival of less than 5 years compared to patients not classified as AMR [10]. The pathophysiology of MVI+ DSA–C4d– lesions may be mediated by NK and T cells rather than antibodies [12–14].

Diagnostic Criteria for caAMR According to Banff 2022 Classification: all three criteria must be met for diagnosis

1. Chronic AMR (antibody-mediated rejection) lesions* present, including at least one of the following:
    - Transplant glomerulopathy (cg > 0) in the absence of evidence of chronic thrombotic microangiopathy (TMA) or recurrent/de novo glomerulonephritis; includes changes visible only by electron microscopy (cg1a).
    - Severe multilayering of the peritubular capillary basement membrane (requires electron microscopy).
2. At least one of the following:
    - Linear C4d staining in peritubular capillaries or medullary vasa recta (C4d2 or C4d3 by immunofluorescence on frozen sections, or C4d > 0 by immunohistochemistry on paraffin sections)
    - Moderate or greater microvascular inflammation ([g + ptc] ≥ 2), in the absence of recurrent or de novo glomerulonephritis. In the presence of acute TCMR (T-cell-mediated rejection), borderline infiltrate or infection, ptc ≥ 2 alone is insufficient; g must be ≥ 1.
    - Transcriptomic diagnostics on biopsy for AMR/MVI above a defined threshold, if rigorously validated and available as a substitute for MVI.
3. Serological evidence of circulating donor-specific antibodies (anti-HLA DSA or against other antigenic specificities):
    - If a complete DSA test has not yet been performed, it must be conducted according to STAR guidelines. Detection of non-HLA antibodies (including ABO antibodies in ABO-incompatible transplants) may be used as a Banff serological criterion for AMR diagnosis, if testing protocols are sufficiently standardized and clinically validated for the appropriate clinical context.
    - C4d staining may substitute for DSA. DSA testing is strongly recommended whenever criteria 1 and 2 are met.

* Other lesions may support the diagnosis of AMR but are not diagnostic alone:

- De novo arterial intimal fibrosis (cv), excluding other causes
- Presence of leukocytes within sclerotic intima, supporting chronic AMR diagnosis in the absence of prior TCMR history.

Table 1. Diagnostic Criteria for caAMR According to Banff 2022 Classification

Currently, no approved treatment exists for caAMR, representing an unmet clinical need [15]. Although a phase II pilot study showed the efficacy of an anti-CD38 antibody in improving renal function and histological remission in patients with active AMR and caAMR, no therapeutic consensus has been established [16].

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in B and T cell activation and proliferation, acute inflammatory response, and modulation of regulatory T cells and Th17 cells [17]. Blocking the interaction between IL-6 and IL-6 receptor (IL-6R), already validated in autoimmune disease treatment, represents a promising option to counter MVI progression by acting on antibody secretion, regulatory-effector T cell balance, and DSA-mediated endothelial activation [18–19].

Preclinical studies in murine skin transplant models have shown that IL-6 inhibition reduces antibody rebound, pro-inflammatory cytokines, and promotes regulatory T cell expansion [20].

Clazakizumab (CLZ), an anti-IL-6 antibody, demonstrated significant reduction in DSA MFI in a phase 2 study, but without evident benefits on molecular and histological rejection scores. Moreover, CLZ was associated with a high risk of gastrointestinal perforation, leading to early termination of the phase 3 IMAGINE study [21–22].

Tocilizumab (TCZ), another humanized monoclonal anti-IL-6 receptor antibody, has shown efficacy in reducing DSA, MVI, and stabilizing transplant glomerulopathy in retrospective studies [23–25]. Tocilizumab is currently approved by regulatory agencies (FDA, EMA, and AIFA) for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis. In 2017, Choi et al. reported the benefits of intravenous tocilizumab as rescue therapy in 36 patients

with caAMR unresponsive to intravenous immunoglobulin (IVIg), Rituximab, and Plasma Exchange—therapies approved for acute antibody-mediated rejection; observed outcomes included reduced DSA levels, stabilized renal function at 24 months post-treatment, and reduced microvascular inflammation (MVI) with concurrent stabilization of transplant glomerulopathy (TG) in 12-month biopsies [23].

However, this study has raised concerns regarding the immunosuppressive burden on patients. A recent Italian study evaluated the efficacy of intravenous Tocilizumab as first-line therapy for caAMR, achieving similar results in terms of laboratory findings (renal function stabilization and DSA reduction) and histological outcomes (reduced microvascular inflammation and C4d deposition, stabilization of transplant glomerulopathy and interstitial inflammation/tubular atrophy—IFTA) [26].

Nevertheless, these studies are limited by monocentric and non-randomized cohorts [25,27–29]. The ongoing INTERCEPT study is a multicenter, open-label, randomized controlled clinical trial aimed at evaluating the efficacy of TCZ in caAMR [30].

2. STUDY OBJECTIVES

This study, encompassing both retrospective and prospective components, was conducted at the Kidney Transplant Center of the Nephrology, Dialysis, and Transplantation Unit of the Sant'Orsola-Malpighi University Hospital, under the supervision of Professor G. La Manna. To increase the sample size, an international collaboration was established with the Transplant Center in Grenoble.

The primary objective of the study was to evaluate the efficacy of tocilizumab (TCZ) as a first-line treatment for chronic active antibody-mediated rejection (caAMR) in kidney transplant recipients followed at our center. To assess the therapeutic efficacy of TCZ, the following parameters were evaluated:

- Laboratory parameters: delta serum creatinine (sCreat.), delta eGFR (estimated glomerular filtration rate) and delta proteinuria between the treatment start visit and the visit after 24 months, along with DSA title;
- Histological parameters: stabilization of chronic lesions (Transplant glomerulopathy-TG, multilayering basement membrane/BM and arteriolar fibrosis) and regression of active lesions (e.g. peritubular capillaritis-PTC, reaction with vascular endothelium) according to the Banff Classification.

Secondary objectives were the monitoring of adverse effects associated with the specific therapy and the potential need for temporary or permanent discontinuation of treatment.

3. MATERIALS AND METHODS

3.1. Study Design

This is a non-sponsored observational study conducted for scientific purposes and health protection.

Although the study was originally designed as a single-center investigation, a section of the analysis was carried out in collaboration with the Grenoble Transplant Center, thereby conferring a multicenter dimension to specific segments of the research. This approach aimed to increase statistical power and enhance the generalizability of the findings.

The study consists of two temporally consecutive arms:

Prospective arm: Enrollment of patients with a new diagnosis of chronic antibody-mediated rejection (cAMR) attending the Kidney Transplant Follow-Up Clinic of the Nephrology, Dialysis, and Transplant Unit at Sant'Orsola-Malpighi University Hospital, directed by Prof. G. La Manna, from 2018 to the present.

Retrospective arm: Inclusion of the same patients enrolled in the prospective arm, with the objective of analyzing clinical and laboratory parameters during the 12–24 months preceding the initiation of tocilizumab (TCZ) therapy. This design allows for a homogeneous cohort to evaluate longitudinal trends in renal function (serum creatinine [sCreat], estimated glomerular filtration rate [eGFR]), proteinuria, and donor-specific antibody (DSA) titers, ensuring greater robustness in data interpretation.

3.2. Study Population

Inclusion Criteria:

Adults with a diagnosis of cAMR based on the Banff Classification [5].

Provision of written informed consent.

Availability of data on renal function (sCreat, eGFR), proteinuria, and Luminex-based DSA quantification during the 12–24 months prior to baseline (T0) (required only for the retrospective arm).

Exclusion Criteria:

Recipients of combined organ transplants (heart-kidney, liver-kidney, or kidney-pancreas).

Treatment with immunosuppressive therapies such as rituximab, plasma exchange, intravenous immunoglobulin (IVIg), or eculizumab within 12 months prior to enrollment.

Histological evidence of concomitant pathologies (e.g., recurrence of primary nephropathy, diagnosis of de novo nephropathy, acute cellular rejection, BK polyomavirus nephropathy).

3.3. Treatments

In patients diagnosed with caAMR according to Banff criteria, standard maintenance immunosuppressive therapy was combined with subcutaneous administration of TCZ at a dose of 162 mg every 14 days.

3.4. Visits and Assessments – Prospective Component

Schedule of visits and evaluations:

At baseline (T0), clinical, laboratory, and histological data were collected. Inclusion and exclusion criteria were verified, and informed consent was obtained.

Patients meeting eligibility criteria and providing consent were enrolled. At

semiannual visits, the following were assessed: general physical examination, concomitant therapies, and laboratory tests (Table 2). A tolerance window of ±15 days was allowed for these visits.

Every 6 months, anti-HLA antibody screening was performed, and treatment satisfaction was evaluated. Additional blood samples were collected for biohumoral and cellular analyses, which are still under investigation.

PARAMETER	T0 (Baseline)	T1 (6 months)	T2 (12 months)	T3 (18 months)	T4 (24 months)
Medical History	X				
Inclusion/Exclusion Criteria	X				
Concomitant Therapies	X	X	X	X	X
Informed Consent	X				
General Physical Examination	X	X	X	X	X
Laboratory Tests	X	X	X	X	X
Anti-HLA Antibody Screening	X	X	X	X	X
Cytokine Panel and Lymphocyte Subpopulation Analysis + Biological Material Storage (serum and urine)	X	X	X	X	X
Adverse Events		X	X	X	X
Treatment Satisfaction Assessment		X	X	X	X

Table 2: Schedule of visits and evaluations of enrolled patients.

Laboratory Assessments

At each scheduled visit, the following laboratory parameters were evaluated:

Hematology and Biochemistry: Complete blood count; serum creatinine (mg/dL); estimated glomerular filtration rate (eGFR, mL/min); electrolytes (Na/K, mmol/L); calcium/phosphate (mg/dL); uric acid (mg/dL); liver enzymes (AST, ALT, GGT, U/L); fasting glucose (mg/dL); glycated hemoglobin (HbA1c, mmol/mol); C-reactive protein (CRP).

Urinary Analysis: Complete urinalysis; 24-hour proteinuria (mg/24h); urinary albumin-to-creatinine ratio (Alb-U/Creat-U).

Immunological Profile: Lymphocyte subpopulations (immunophenotyping); anti-HLA antibody screening; cytokine panel.

Therapeutic Drug Monitoring: Blood levels of concomitant immunosuppressive agents (tacrolimus, cyclosporine, everolimus), using reference ranges applied in routine clinical practice.

Virological Screening: Quantitative PCR for cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and BK Polyomavirus (BKV) DNA, according to standard clinical protocols.

3.5 Statistical Methods

Demographic, clinical, and laboratory characteristics of the patients were summarized using descriptive statistics. Qualitative variables were expressed as absolute frequencies and percentages, whereas quantitative variables were reported as median and interquartile range (IQR: first and third quartile), according to their observed distribution.

Group comparisons (patients with effective vs. non-effective treatment response) were performed using non-parametric tests: the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. Temporal trends in renal function (eGFR) and proteinuria (UACR) were analyzed using mixed-effects regression models, which estimate mean trajectories over time while accounting for intra-patient variability, outliers, and loss to follow-up. Kaplan–Meier curves were used to describe the time from transplantation to rejection onset.

Statistical significance was set at $p < 0.05$. All analyses were conducted using R software, version 4.5.0.

3.6 Laboratory Methods

Peripheral Blood Mononuclear Cell (PBMC) Collection

PBMCs were obtained from study participants after informed consent at baseline (T0) and subsequently at T1, T2, T3, and T4.

PBMCs were isolated from whole blood by density-gradient centrifugation using Ficoll (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), counted, and cryopreserved for subsequent flow cytometric and gene characterization analyses.

The isolation protocol for a 9 mL EDTA blood sample was as follows:

- Dilute peripheral blood in PBS at a 1:2 ratio.
- Carefully layer 10 mL of diluted blood over 4.5 mL of Ficoll in a 15 mL Falcon tube.

- Centrifuge at 1500 rpm for 30 minutes at 21°C.
- Aspirate the plasma layer, leaving the mononuclear cell ring at the plasma–Ficoll interface.
- Transfer the mononuclear cell layer to a 50 mL Falcon tube and bring to volume with PBS.
- Mix and centrifuge at 1500 rpm for 5 minutes at 21°C; remove the supernatant completely.
- For cell counting, use a Bürker chamber and stain cells with Trypan Blue. Prepare 190 µL of Trypan Blue and 10 µL of cell suspension, mix, and load 10 µL into the counting chamber. Count cells in squares delimited by the triple line.

The total cell number will be calculated using the formula:
cells/mL= mean counted cells × chamber dilution factor (10,000) × Trypan Blue dilution factor (20)
Cells will be centrifuged and resuspended in FBS + 10% DMSO (minimum 20 million PBMCs per vial). Vials will be stored at –80°C overnight and then transferred to liquid nitrogen.
Cryopreserved PBMCs will be used for flow cytometric analysis of intracellular and surface markers or for cell culture assays.

Urine Collection
Urinalysis is an essential tool for evaluating patients with impaired renal function, proteinuria, hematuria, urinary tract infection, or nephrolithiasis, providing critical information on primary kidney diseases and systemic conditions. Moreover, urine represents a valuable source of material for identifying biomarkers of renal injury and toxicity.

Urine samples were collected from study participants after informed consent. The following protocol describes the collection and storage procedure for a 30 mL urine sample:

- Collect urine in a container equipped with an integrated sampling probe.
- Maintain the sample at 4°C and transfer urine into 15 mL tubes using the probe.
- Centrifuge at 1500 rpm for 15 minutes at controlled temperature.
- Aliquot the supernatant into cryopreservation tubes (500 µL per aliquot) to prevent repeated freeze–thaw cycles.
- Store all aliquots in freezing boxes at –20°C until analysis.

Urine samples will be subsequently used for quantitative assays of proteins, cytokines, chemokines, antibodies, and renal injury or rejection biomarkers using Luminex technology.

4. **RESULTS**

4.1 Descriptive Analysis of the Study Population

The study population consisted of 28 consecutive patients followed at the Kidney Transplant Center of the Nephrology, Dialysis, and Transplant Unit at Sant'Orsola-Malpighi University Hospital, Bologna (Table 3). Females accounted for 32% of the cohort, with a median age of 37.8 years (interquartile range [IQR]: 31.2–46). Patients were categorized according to underlying nephropathy, with the following distribution: polycystic kidney disease in 25% of cases, glomerulonephritis in 28.6%, vascular nephropathy and tubulointerstitial disorders each in 3.6%, while 39.3% presented other forms of nephropathy or unknown etiology.

The population was further stratified by pre-transplant immunization status, assessed through Panel Reactive Antibody (PRA) values (last and peak). Immunosuppressive regimens were also recorded, distinguishing between induction therapy (basiliximab or thymoglobulin) and maintenance therapy (corticosteroids, tacrolimus, cyclosporine, mycophenolic acid, and everolimus). Renal function at discharge post-transplant was documented, with median serum creatinine of 1.50 mg/dL (IQR: 1.39–1.90) and median eGFR of 53 mL/min/1.73 m² (IQR: 40–66).

Baseline Characteristic	N	N = 28[1]
Female Gender -N (%)	28	9 (32%)
Age	28	37.8 [31.2, 46]
Nephropathy -N (%)	28	
PKD		7 (25%)
Glomerulonephritis		8 (28.6%)
Vascular disease		1 (3.6%)
Tubulo-interstitial disease		1 (3.6%)
Other/ Unknown		11 (39.3%)
Pre-emptive Transplantation- N (%)	27	2 (7.4%)
Last PRA	22	0 [0, 6]
Max PRA	22	6 [0, 68]
Living Donor- N (%)	28	3 (11%)
Induction Therapy	18	
Basiliximab		11 (61%)
Antithymoglobulin		7 (39%)
Maintenance Therapy	28	
Steroids		27 (96.3%)
Tacrolimus (Fk)		17 (61%)
CyA		11 (39%)
MMF		21 (75%)
Everolimus		2 (7.1%)
eGFR at the discharge of transplant (mL/min/1.73 m2)	24	53 [40, 66]
Serum Creatinine at the discharge of transplant (mg/dL)	25	1.50 [1.39, 1.90]

[1]n (%); Median [Q1, Q3]

Table 3: Descriptive Analysis of the Study Population

Regarding histological characteristics, all enrolled patients underwent a kidney transplant biopsy at baseline (T0), which was required for the diagnosis of chronic antibody-mediated rejection (cAMR). Histological evaluation was performed according to the Banff criteria [5].

4.2 Descriptive Analysis at the Time of cAMR Diagnosis (T0)

At the time of chronic antibody-mediated rejection (cAMR) diagnosis, the mean onset time from transplantation was 10.6 years, while the median was 8.75 years.

Survival analysis was performed using Kaplan–Meier curves to assess the time interval between kidney transplantation and the onset of chronic antibody-

mediated rejection. The curve illustrates the cumulative probability of remaining free from rejection over time, accounting for censored patients and those who experienced the event (Figure 1) . As reported above, the mean onset time was 10.6 years, and the median was 8.75 years, indicating substantial variability in event latency among patients. This approach enabled visualization of the temporal distribution of cAMR within the cohort and identification of potential long-term risk patterns.

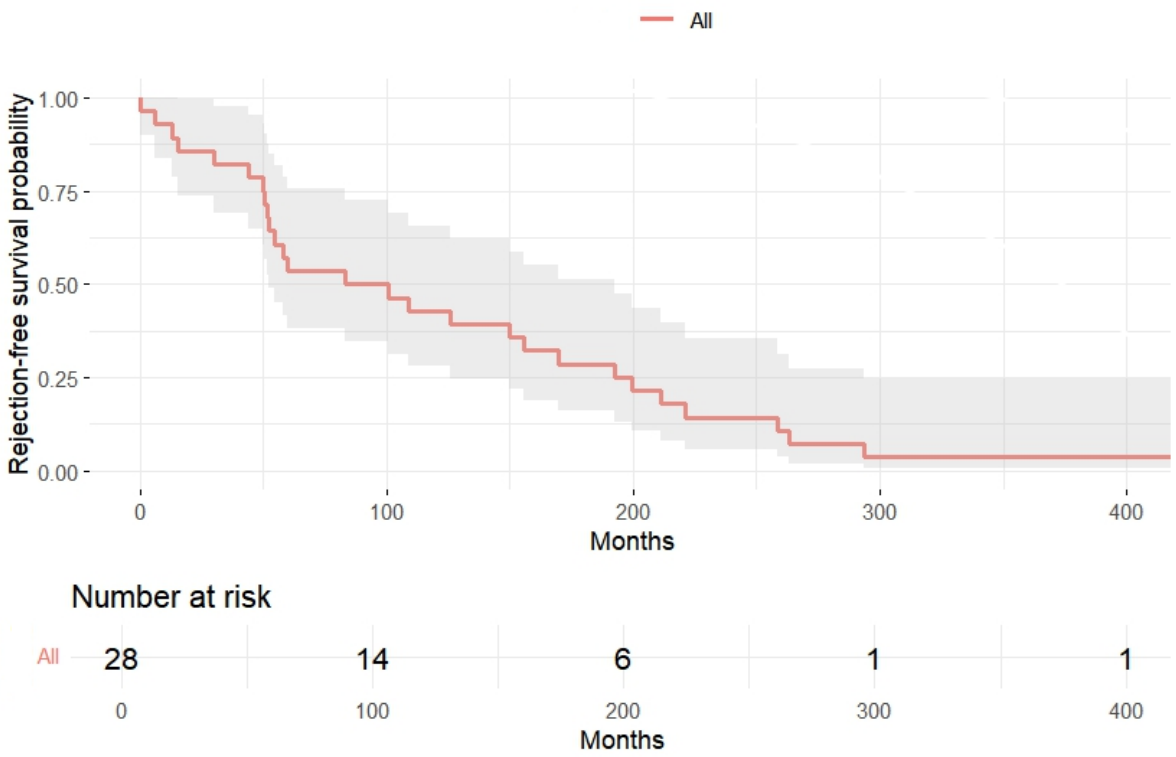


Figure 1. Kaplan–Meier curve illustrating the time interval between kidney transplantation and the diagnosis of active chronic antibody-mediated rejection (cAMR). The graph shows the cumulative probability of remaining free from rejection over time, accounting for censored patients and those who experienced the event. The mean onset time was 10.6 years, while the median onset time was 8.75 years.

The clinical and laboratory characteristics of patients at baseline (T0), corresponding to the diagnosis of cAMR, are summarized in the corresponding table. Specifically, renal function was represented by a median serum creatinine of 1.97 mg/dL (interquartile range [IQR]: 1.72–2.45), a median estimated glomerular filtration rate (eGFR) of 37 mL/min/1.73 m² (IQR: 30–43), and proteinuria assessed by urinary albumin-to-creatinine ratio (UACR) with a median value of 262 mg/g (IQR: 124–1,098).

Characteristic	**N**	**N = 28**[1]
eGFR	28	37 [30, 43]
sCreat	28	1.97 [1.72, 2.45]
UACR	26	262 [124, 1,098]
steroids	28	26 (93%)
CNI	28	28 (100%)
MMF	28	21 (75%)
mTORi	28	3 (11%)
Azathioprine	28	0 (0%)

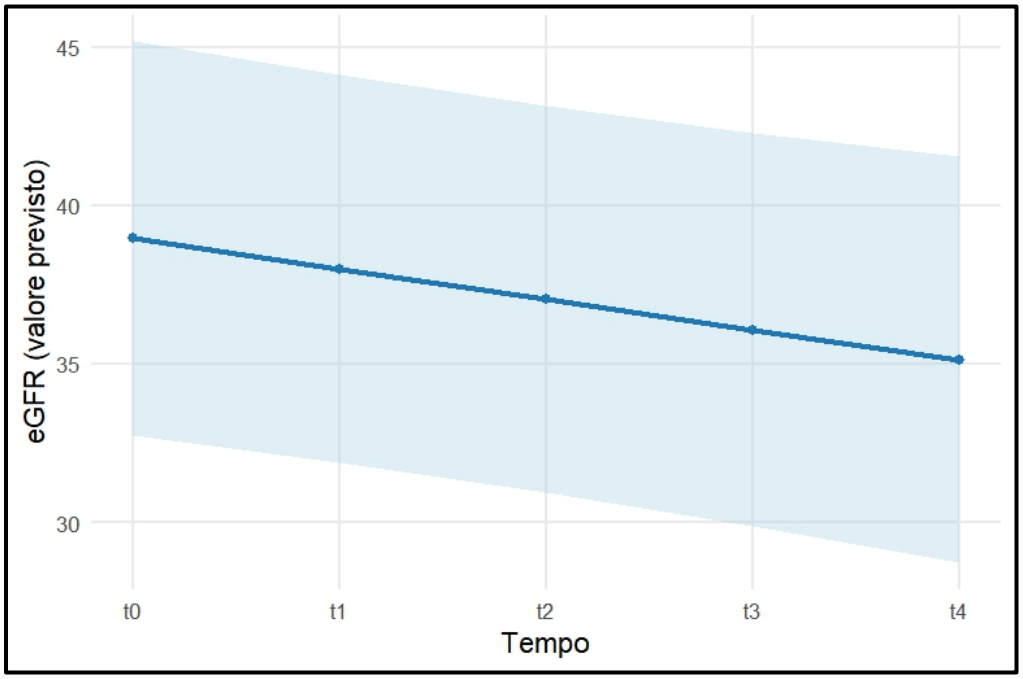
[1]Median [Q1, Q3]; n (%)

At the time of diagnosis, 19 out of 28 patients tested positive for Donor-Specific Antibodies (DSA), including 5 with class I DSA (mean MFI: 7,040) and 16 with class II DSA (mean MFI: 15,431.25).

4.3 Main Study Results

Serum creatinine, eGFR, proteinuria (UACR), and DSA titers were analyzed longitudinally at T1 (6 months after treatment initiation), T2 (12 months), T3 (18 months), and T4 (24 months) to assess the evolution of these parameters during follow-up.

Regarding eGFR, analyses—although conducted on a sample of 28 patients—revealed a trend toward progressive decline, with an estimated decrease of approximately 1 mL/min/1.73 m² per timepoint. The applied model does not merely compute point-by-point means but estimates an overall longitudinal trend, accounting for complex factors such as intra-patient variability and outliers. Although the model does not directly provide a statistical significance value (*p*-value), the robustness of the result is supported by technical parameters in the output (Figure 2).



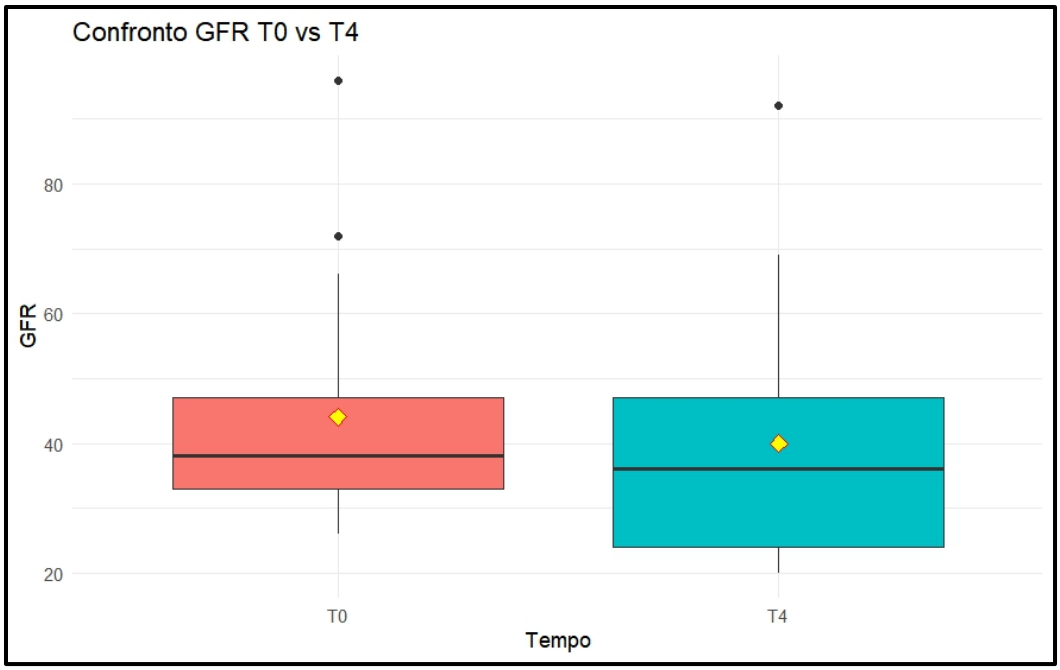


Figure 2. The eGFR analysis revealed a trend toward progressive decline, with an estimated decrease of approximately 1 mL/min/1.73 m² per timepoint.

Regarding proteinuria, assessed as UACR, despite marked inter-individual variability (95% confidence interval), a significant reduction was observed throughout the follow-up period (Figure 3).

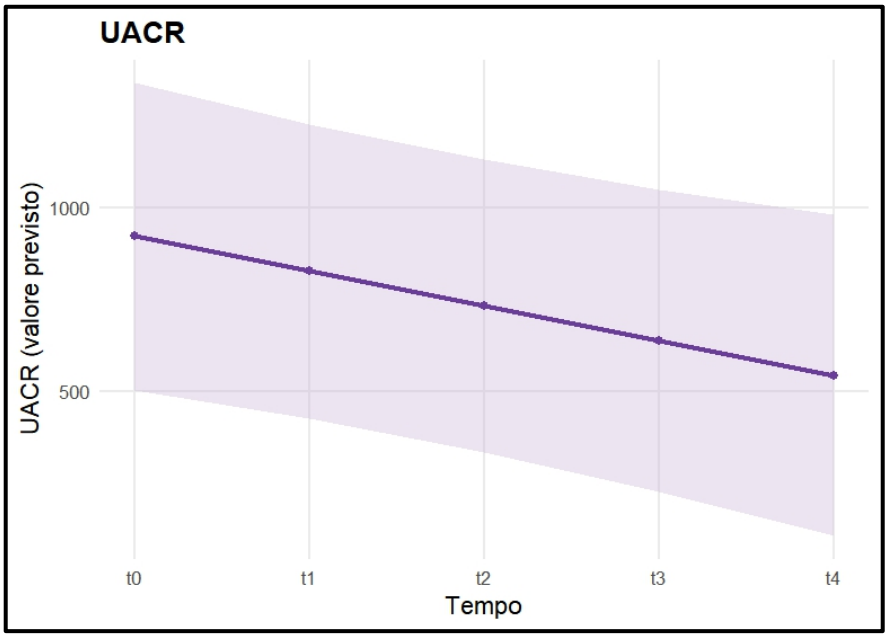


Figure 3. Proteinuria analysis, assessed as UACR, showed a significant reduction throughout the follow-up period.

The analysis of DSA, both class I and class II, revealed a downward trend in Mean Fluorescence Intensity (MFI) levels between baseline (T0) and the final timepoint (T4). Although the *p*-values obtained for both classes did not reach the threshold for statistical significance, this result cannot be interpreted as evidence of no effect. It is plausible that the lack of significance is attributable to the limited sample size, which compromises the statistical power of the analysis and increases the risk of a type II error—namely, failure to detect a true effect. Therefore, the observed findings should be interpreted with caution and may gain greater relevance in larger populations (Figure 4).

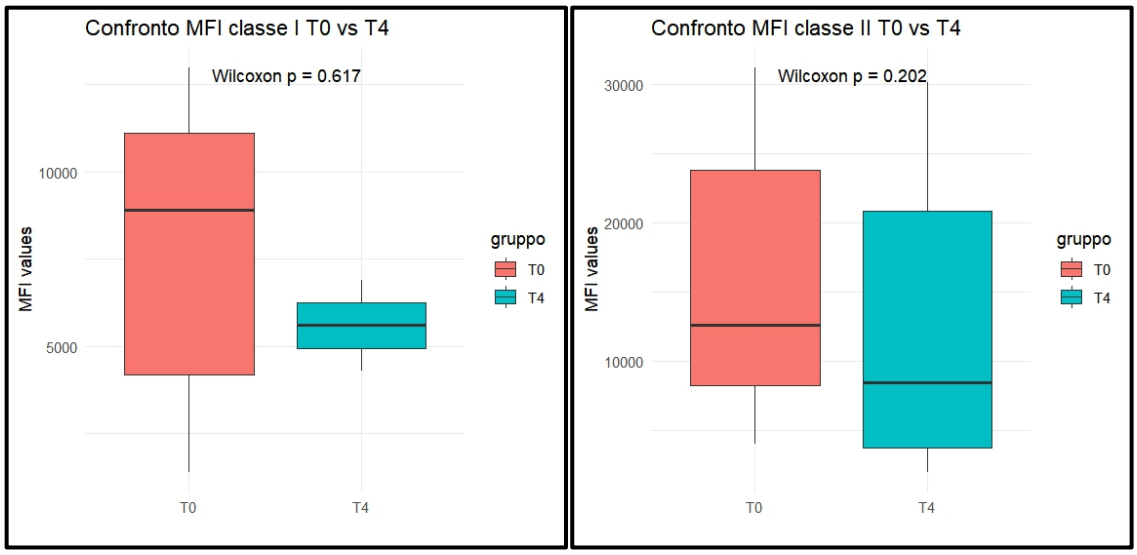


Figure 4. The DSA analysis, both class I and class II, revealed a downward trend in Mean Fluorescence Intensity (MFI) levels between baseline (T0) and the final timepoint (T4).

The retrospective analysis was performed by collecting eGFR data for the 12 months preceding the initiation of TCZ therapy. The linear mixed-effects model of eGFR trajectory demonstrated a significant difference between the pre-TCZ and post-TCZ periods (p = 0.0316) (Figure 5).

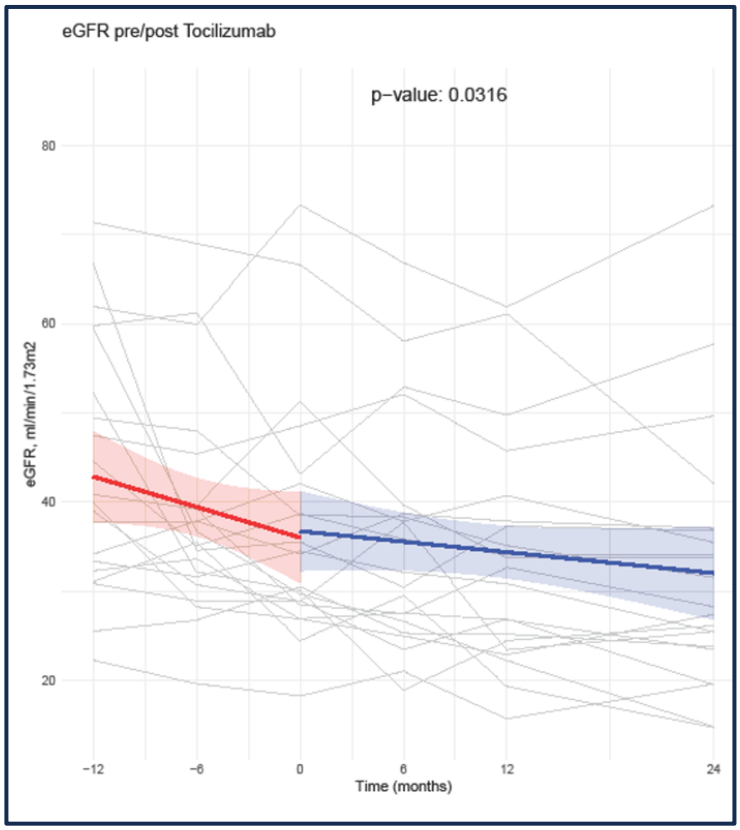


Figure 5. The retrospective analysis of eGFR demonstrated a significant difference between the pre-TCZ and post-TCZ periods.

4.4 Multicenter Study Results

Collaboration with the Kidney Transplant Center in Grenoble (France) allowed expansion of the study cohort to a total of 64 patients. All consecutive adult kidney transplant recipients between July 2018 and July 2022 from the nephrology and transplant units in Grenoble (France) and Bologna (Italy) were included. The same inclusion and exclusion criteria previously described were applied to the entire cohort.

Patients were monitored longitudinally for renal function (eGFR, calculated using the CKD-EPI formula), urinary albumin-to-creatinine ratio (UACR, g/g), adverse events and serious adverse events related to treatment, patient and graft survival, and DSA levels.

Analysis of eGFR trajectory using a linear mixed-effects model revealed a significant difference between the pre- and post-treatment periods with tocilizumab (TCZ): −1.2 ± 0.2 vs. +0.03 ± 0.2 mL/min/1.73 m²/month, respectively ($p = 0.001$; Figure 6A). When considering only patients with at least two years of follow-up, eGFR trajectory decreased from −1.2 ± 0.2 mL/min/1.73 m²/month in the pre-TCZ period to −0.15 ± 0.1 mL/min/1.73 m²/month after two years of treatment ($p < 0.001$; Figure 6B).

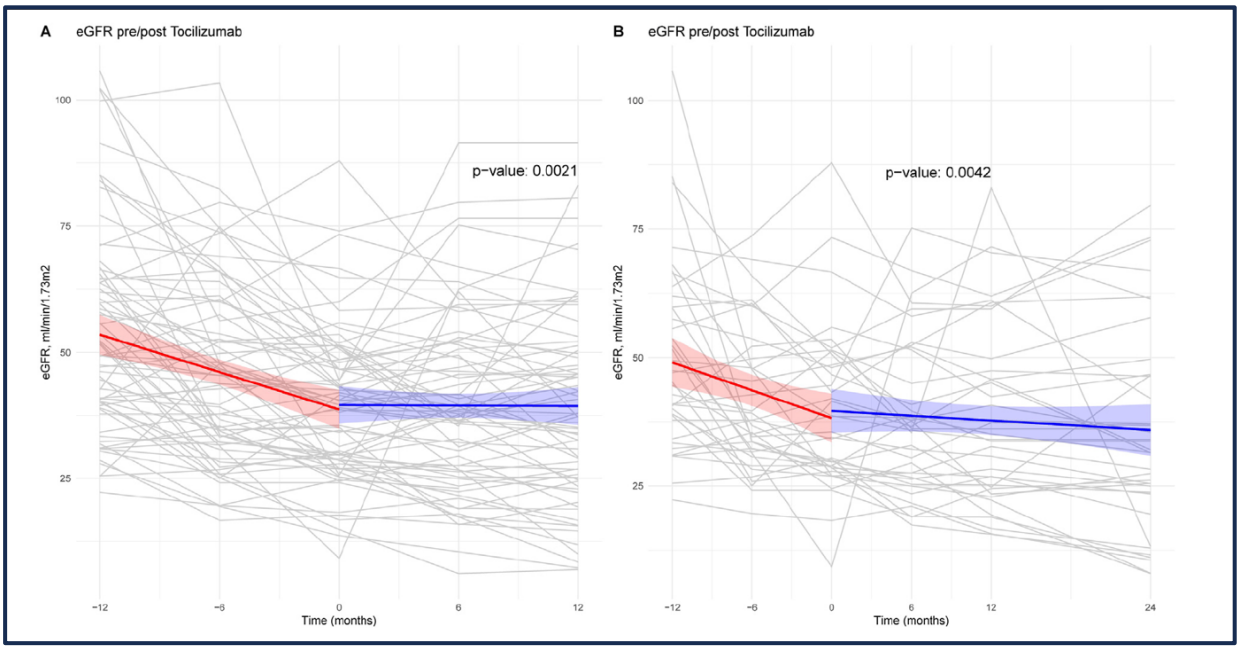


Figure 6. Estimated Glomerular Filtration Rate (eGFR) Trajectories Before and After Tocilizumab Therapy. Panel (A) presents a mixed-effects linear regression model analyzing eGFR changes from 12 months prior to 12 months following the initiation of Tocilizumab. Panel (B) extends the post-treatment observation period to 24 months. Individual patient trajectories are depicted by grey lines, illustrating eGFR evolution over time. The time point "0" marks the commencement of Tocilizumab administration for the management of antibody-mediated rejection. The reported p-*value reflects the statistical significance of the difference between the pre- and post-treatment periods, based on a comparison of the two regression models.*

Moreover, in the overall cohort, the urinary albumin-to-creatinine ratio (UACR) showed a significant reduction at one year after TCZ treatment, decreasing from 1.0 ± 1.3 g/g to 0.6 ± 0.7 g/g ($p = 0.033$) (Figure 7).

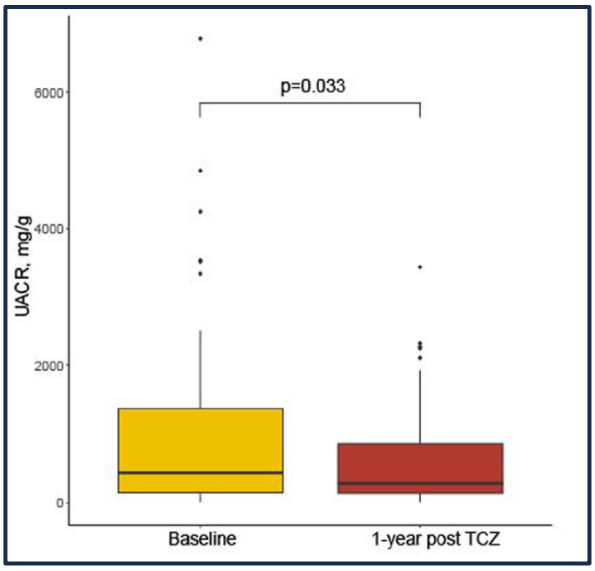


Figure 7. The UACR analysis of the overall cohort, showed a significant reduction at one year after TCZ treatment.

At Tocilizumab initiation, the median MFI of DSA was 9,537 [1,426–15,075], decreasing to 7,250 [3,100–14,975] at 1-year post-treatment (p = 0.001) (Figure 8)

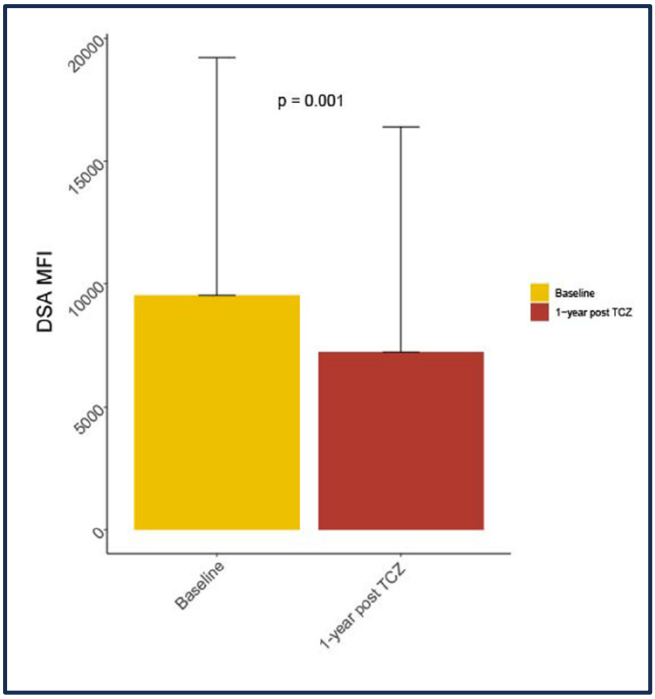


*Figure 8. The MFI analysis of DSA at the time of diagnosis (baseline) and after 1-year post TCZ showed a decline from a median MFI of 9,537 [1,426–15,075] to 7,250 [3,100–14,975] (*p *= 0.001).*

5. **Histological Evaluation**

All patients from the Grenoble cohort underwent protocol follow-up kidney biopsies. Figure 9 depicts the temporal changes in glomerulitis (g) scores, peritubular capillaritis (ptc) scores, combined g + ptc scores, C4d staining, and chronic glomerulopathy (cg) lesions between the initial biopsy confirming microvascular inflammation (MVI) and the assessment performed one year after Tocilizumab (TCZ) therapy. At 12 months post-treatment, a statistically significant reduction was observed in the g score ($p = 0.014$), whereas ptc, g + ptc, C4d, and cg scores remained unchanged. Notably, within the g-score category, the proportion of patients with a score of 3 decreased from 29.3% prior to TCZ initiation to 22% at one-year post-treatment ($p = 0.032$).

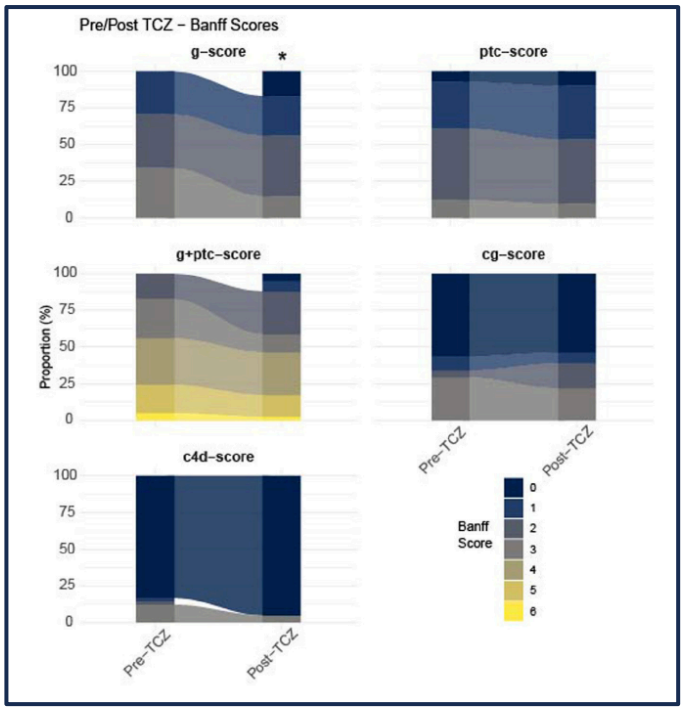


*Figure 9. Plot of percentage of Banff Scores in biopsies before Tocilizumab (pre-TCZ) and 1-year after TCZ treatment (post-TCZ). Banff scores are g: glomerulitis, Ptc: Peritubular capilaritis, g + ptc: addition of g and ptc scores, c4d: complement deposition, cg: chronic glomerulopathy. *p-value = 0.014.*

6. Discussion

Kidney transplantation represents the gold-standard therapy for patients with end-stage chronic kidney disease. Therefore, improving long-term graft survival is currently one of the main goals of the transplant scientific community. To date, the leading cause of long-term graft loss is chronic active antibody-mediated rejection (caAMR), making the identification of an effective treatment a primary priority, as this remains an unmet clinical need.

The pathophysiology of antibody-mediated rejection involves a central role of antibodies, B lymphocytes, and plasma cells, which explains why current therapy focuses on the depletion of these components through immunoglobulins (IVIg), Rituximab, and/or plasma exchange [31–33]. Although these treatments have proven effective in acute forms of antibody-mediated rejection, outcomes in caAMR have been disappointing [34].

Given these premises, it is clear that the efforts of the scientific-transplant community have turned to identifying new therapeutic strategies effective against cAMR. Interleukin 6 (IL-6) is a cytokine that plays a key role in regulating inflammation and in the development, maturation and activation of B and T lymphocytes and plasma cells [35]. Overproduction of IL-6 has been associated with numerous pathologies characterized by autoimmunity and uncontrolled production of antibodies [17].
It has also been shown that the interaction between IL-6 and its receptor (IL-6R) represents a fundamental step in the production of allo-antibodies in immunized animal models; the interruption of the interaction between IL-6 and IL-6R led to a significant reduction of alloantibodies, a reduction in the splenic and medullary production of antibodies, the direct inhibition of the production of anti-HLA antibodies by the plasma cells and the production of T cells with regulatory activity (Tregs) [20].

Tocilizumab (Actemra / RoActemra, Roche / Genentech, San Francisco, CA) is a humanized
monoclonal antibody directed against the IL-6 receptor, binding both the soluble and membrane-bound forms. In several studies, TCZ, used as rescue therapy or as first-line treatment, has been associated with reduction in DSA levels, stabilization of renal function at 24 months from the start of treatment along with reduction in microvascular inflammation and stabilization of transplant glomerulopathy (TG) in biopsies at 12 months [25–29].

In our study, we sought to evaluate the efficacy of Tocilizumab (TCZ) as a first-line therapeutic option for chronic active antibody-mediated rejection (caAMR). This decision was driven by the persistent challenge posed by caAMR, which remains a major contributor to late graft loss and for which effective treatment strategies are still lacking. We prospectively analyzed patients with newly diagnosed caAMR who initiated TCZ as first-line therapy, while retrospectively collecting data on renal function—expressed as estimated glomerular filtration rate (eGFR)—for the 12 months preceding treatment. This approach allowed us to characterize both pre-treatment and post-treatment trajectories, providing insight into the potential impact of TCZ on graft function over time.

Although the limited sample size precluded statistical significance, the observed trends were clinically relevant. Specifically, we noted an improvement in renal function following TCZ initiation, as reflected by eGFR dynamics both when comparing pre- and post-treatment periods and within the post-treatment phase (from baseline [T0] to month 4 [T4]). In addition to eGFR, reductions in proteinuria—assessed via urine albumin-to-creatinine ratio (UACR)—and attenuation of donor-specific antibody (DSA) strength, measured by mean fluorescence intensity (MFI), were observed. These findings suggest that TCZ may exert a beneficial effect not only on renal function but also on immunological activity, which is consistent with its mechanism of action targeting the IL-6 pathway.

To enhance the robustness of our analysis and mitigate the limitations imposed by small sample size, we established a collaborative effort with the Grenoble Transplant Center, resulting in a combined cohort of 64 patients. Our study demonstrated an overall stabilization of estimated glomerular filtration rate (eGFR) as early as one year following initiation of Tocilizumab (TCZ), with this effect reaching statistical significance at two years post-treatment. This finding suggests that IL-6 receptor blockade may exert a sustained protective effect on renal function in the context of chronic active antibody-mediated rejection (caAMR). The beneficial impact of TCZ was further supported by a reduction in proteinuria and a significant decrease in glomerulitis (g) score at the one-year follow-up, indicating an improvement in microvascular inflammation.

In addition to histological and functional improvements, we observed a marked reduction in both the number of donor-specific antibodies (DSA) and their mean fluorescence intensity (MFI) at one year post-treatment. This observation aligns with previous evidence implicating interleukin-6 (IL-6) as a key driver of B-cell activation and HLA-DSA production [36]. By attenuating IL-6 signaling, TCZ may reduce antibody titers and thereby mitigate ongoing alloimmune injury, ultimately contributing to improved graft survival.

When analyzing predictors of therapeutic response, patients with fewer chronic glomerulopathy (cg) lesions exhibited a more pronounced benefit from TCZ, suggesting that the degree of chronicity may influence treatment efficacy. These findings underscore the importance of early intervention: initiating TCZ in the initial stages of caAMR and microvascular inflammation (MVI) may maximize its therapeutic potential before irreversible chronic damage occurs.

7. Conclusion

Our findings strongly support the potential of Tocilizumab (TCZ) as a first-line therapeutic strategy for kidney transplant recipients exhibiting histological evidence of microvascular inflammation (MVI). The observed improvement in eGFR trajectories, coupled with reductions in donor-specific antibody (DSA) number and mean fluorescence intensity (MFI), underscores the capacity of IL-6 receptor blockade to mitigate alloimmune injury and preserve graft function. Importantly, the data suggest that early initiation of TCZ during the evolution of MVI may amplify these benefits and sustain renal function over time, positioning TCZ as a compelling candidate for timely intervention in chronic active antibody-mediated rejection (caAMR).

While these results are promising, limitations such as the small sample size, single-center design, and heterogeneity of inclusion criteria must be acknowledged. The inclusion of patients with caAMR alongside those with MVI without detectable DSA or C4d deposition introduces variability that could influence treatment response. Nevertheless, the multicenter approach implemented at a later stage of this study, enhances external validity and reflects the growing interest in IL-6 blockade as a targeted immunomodulatory strategy.

Future research should prioritize large, multicenter, prospective trials with stratification by histological and immunological profiles to refine patient selection and optimize therapeutic outcomes. Establishing TCZ as an early intervention in graft inflammation could represent a paradigm shift in the management of caAMR, addressing a critical unmet need in transplantation medicine.

8. **Bibliography**

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Receptor Antibody Attenuates Donor-Specific Antibody Responses in a Mouse Model of Allosensitization. Transpl Immunol (2013) 28:138–43. doi:10. 1016/j.trim.2013.03.003

9. **Abstract**

Kidney transplantation (KT) is the treatment of choice for patients with end-stage renal disease (ESRD), offering significant improvements in survival and quality of life. Despite advances in short-term graft survival, long-term outcomes remain suboptimal, with chronic active antibody-mediated rejection (cAMR) representing a major cause of late graft loss, primarily driven by donor-specific anti-HLA antibodies (DSA). Diagnosis of cAMR relies on both laboratory and histological criteria, including the presence of DSA and microvascular lesions with or without C4d deposition. Current therapies effective in acute AMR have shown limited benefit in cAMR, highlighting the need for novel strategies. Interleukin-6 (IL-6) plays a pivotal role in inflammation and in the activation of B and T lymphocytes and plasma cells, promoting antibody production. Tocilizumab (TCZ), a humanized monoclonal antibody targeting both soluble and membrane-bound IL-6 receptors, is approved for autoimmune diseases and may offer a promising approach for alloimmune modulation in transplantation. This observational study, combining prospective and retrospective components, was primarily conducted at a single center with a multicenter extension through collaboration with the Grenoble Transplant Center. The primary objective was to assess renal function in KT recipients with cAMR following TCZ therapy, while secondary endpoints included histomorphological, biohumoral, and cytological evaluations. By inhibiting IL-6 signaling, TCZ may reduce DSA titers, attenuate microvascular inflammation, and improve long-term graft survival, addressing a critical unmet need in transplantation medicine.