Evidences of histologic Thrombotic Microangiopathy and the impact in renal outcomes of patients with IgA nephropathy

Short Title: IgA Nephropathy and Thrombotic Microangiopathy

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ABSTRACT

Introduction: IgA nephropathy (IgAN) is the most common primary glomerulopathy worldwide. According to the Oxford Classification, changes in the kidney vascular compartment are not related with worse outcomes. This paper aims to assess the impact of thrombotic microangiopathy (TMA) in the outcomes of Brazilian patients with IgAN.

Materials and Methods: Analysis of clinical data and kidney biopsy findings from patients with IgAN to assess the impact of TMA on renal outcomes.

Results: The majority of the 118 patients included were females (54.3%); mean age of 33 years (25;43); hypertension and hematuria were observed in 67.8% and 89.8%, respectively. Median creatinine: 1.45mg/dL; eGFR: 48.8ml/min/1.73m^2; 24-hour proteinuria: 2.01g; low serum C3: 12.5%. Regarding to Oxford Classification: M1: 76.3%; E1: 35.6%; S1: 70.3%; T1/T2: 38.3%; C1/C2: 28.8%. Average follow-up: 65 months. Histologic evidence of TMA were detected in 21 (17.8%) patients and those ones presented more frequently hypertension (100% *vs.* 61%, p <0.0001), hematuria (100% *vs* 87.6%, p=0.0001), worse creatinine levels (3.8 *vs*. 1.38 mg/dL, p=0.0001), eGFR (18 *vs.* 60 ml/min/1.73m^2), p =0.0001), low serum C3 (28.5% *vs*. 10.4%, p =0.003), lower hemoglobin levels (10.6 *vs.* 12.7g/dL, p<0.001) and platelet counts (207,000 *vs*. 267,000, p=0.001). Biopsy findings of individuals with TMA revealed only greater proportions of E1 (68% *vs.* 32%, p = 0.002). Individuals with TMA were followed for less time (7 *vs*. 65 months, p<0.0001) since they progressed more frequently to chronic kidney disease (CKD) requiring renal replacement therapy (RRT) (71.4% *vs*. 21,6%, p<0.0001). Male sex, T1/T2, and TMA were independently associated with progression to CKD-RRT.

Conclusions: In this study patients with TMA had worse clinical manifestations and outcomes. In terms of histologic evidence, E1 distinguished patients with TMA from other patients. These findings indicate that vascular compartment may also be a prognostic marker in IgAN patients.

Keywords: IgA Nephropathy; kidney biopsy; pathology; prognostic factors; thrombotic microangiopathy; complement protein

INTRODUCTION

IgA nephropathy (IgAN) is a highly prevalent condition worldwide and ranks as the most common primary glomerulopathy in some countries.[1-3] Given the high prevalence of the disease and the fact that about 30% of the patients with IgAN progress to chronic kidney disease (CKD) requiring kidney replacement therapy (KRT),[1,4] it is imperative to identify clinical and histology markers associated with worse renal outcomes.

The most widely accepted explanation for the IgAN pathogenesis is the 4-hit hypothesis, in which Hit 1 involves the production of hypoglycosylated IgA1; Hit 2 starts with the production of IgG antibodies that recognize hypoglycosylated IgA1; Hit 3 regards the formation of potentially nephritogenic IgG/IgA1 immune complexes; and in the Hit 4 there is deposition of formed complexes in the glomerular mesangium and capillaries, thereby activating the immune system and leading to the recruitment of inflammatory cells, cytokines, and the activation of the complement system.[1,2,5]

The Oxford Classification (OC)[6,7] was first published in 2009 as an attempt to identify kidney biopsy alterations possibly associated with worse outcomes in patients with IgAN. Mesangial hypercellularity, segmental glomerulosclerosis, and interstitial fibrosis/tubular atrophy have been associated with progression to CKD-KRT, while endocapillary hypercellularity was first correlated with function decline in patients on immunosuppressant therapy and later with worse renal outcomes. An updated version of the Oxford Classification was published in 2017,[8] and cellular crescents were added as markers of worse renal outcomes. It should be mentioned that vascular alterations were not included in the Oxford Classification, since they were not associated with worse outcomes in patients with IgAN. However, recent studies[9-11] have looked into the role of vascular alterations and their ties with the outcomes of patients with IgAN, shedding light on a matter yet unresolved in the literature.

Thrombotic microangiopathy (TMA) is a histology finding of vascular involvement associated with some renal conditions – atypical and typical hemolytic-uremic syndrome,

eclampsia, accelerated hypertension, thrombotic thrombocytopenic purpura – that may also be induced by certain drugs.[12,13] Histology and serum findings of TMA have been associated with other primary and secondary glomerulopathies – lupus nephritis, ANCA-associated vasculitis, focal segmental glomerulosclerosis, and IgA nephropathy – and correlated with worse renal outcomes in individuals with IgAN.[9,10,14,15]

This study aimed to assess the impact of histologic findings of TMA on the renal outcomes of individuals with IgAN seen at a healthcare center in Brazil.

MATERIALS AND METHODS

Study Design and Population

This retrospective single-center study included patients diagnosed with IgA nephropathy based on kidney biopsy findings. Patients with IgAN secondary to systemic conditions (Henoch-Schönlein purpura, liver disease, autoimmune disease, HIV infection) and individuals with insufficient follow-up or outcome data were excluded, along with patients with fewer than eight glomeruli for analysis via the Oxford Classification.

The following clinical data were considered at the time of kidney biopsy: age; sex; serum creatinine (SCr); estimated glomerular filtration rate (e-GFR); 24-hour proteinuria and/or urine protein/creatinine (UPC) ratio; hematuria; hypertension; serum C3 level; serum IgA; hemoglobin; platelet count; lactate dehydrogenase (LDH); and indirect bilirubin. The glomerular filtration rate was estimated based on the CKD-EPI[16] equation. Hematuria was defined as ≥3 red blood cells/high-power field in a sample of urine. Hypertension was defined as a blood pressure ≥140 and/or 90mmHg[17]. The reference ranges laboratory tests were as follows: C3 (90-180mg/dL); IgA (69-382mg/dL); LDH (135-214U/L); indirect bilirubin (0.2 ; 0.8mg/dL); haptoglobin (30-200mg/dL). Anemia was defined as hemoglobin <12g/dL for

101 females and 13g/dL for males.[18] Thrombocytopenia was defined as having a platelet count
102 <150,000/mm^3.[19] Presence of schistocytes was verified via peripheral blood smear tests.

103 Patients were also analyzed for prescription of angiotensin-converting-enzyme (ACE) inhibitors
104 or angiotensin II receptor blockers (ARBs), corticosteroids, and other immunosuppressants. The
105 end of follow-up was defined either by the last visit of the patient to the healthcare unit or by
106 referral to dialysis or kidney transplantation.

107

108 *Histopathology*

109 Kidney biopsy specimens analyzed by light microscopy were stained by hematoxylin and eosin
110 (H&E), Masson's trichrome, periodic acid–Schiff (PAS), and periodic acid silver methenamine
111 stain (PAMS). Analysis by immunofluorescence microscopy included serum anti-IgA, IgG,
112 IgM, C3, C1q, kappa and lambda light chains, and fibrinogen, with positive deposition defined
113 when intensity ≥1. The biopsies were reviewed by a renal pathologist and classified based on
114 the latest version of the Oxford Classification.[8]

115 As previously described in the literature,[20] acute TMA histologic findings were categorized
116 based on renal compartments. In glomeruli: presence of thrombi, edema, or endothelial
117 denudation, fragmented red blood cells, mesangiolysis, microaneurysms; in arterioles: thrombi,
118 edema, or endothelial denudation, intramural fibrin, fragmented red blood cells, edema of the
119 intima, myocyte necrosis; and in arteries: thrombi, myxoid intimal change, intramural fibrin,
120 fragmented red blood cells. Chronic findings included in glomeruli: double contour in
121 capillaries with mesangial interposition; in arterioles hyaline deposits; in arteries fibrous intimal
122 thickening and concentric lamination resembling the bulb of an onion. Histologic findings of
123 TMA were assessed only based on light microscopy examination.

124

125 *CD68 Immunohistochemistry*

Formalin-fixed, paraffin-embedded tissue was sectioned at 2μm and stained with rabbit monoclonal anti-CD68 (Santa Cruz Biotechnology) as the primary antibody. All cases were stained by hand using routine protocols, including deparaffinization, followed by antigen retrieval (tissue section was boiled in 1mM EDTA, pH 8.0 for 10 min followed by cooling at room temperature for 20 min), protein blocking (DPB-125S; Spring, Pleasanton, CA, USA), incubation for primary antibody at room temperature for 30 min (1:40) and for secondary goat anti-rabbit IgG (DHRR-999; Spring, Pleasanton, CA, USA) at 1:360. Detection was performed with streptavidin/horseradish peroxidase (SPB-125; Spring) and developed with Stable DAB (Spring). CD68-positive cells in the glomeruli and tubulointerstitium were quantified and the final count was expressed as number of cells/glomerulus and cells/field, respectively.

Endpoint Analysis

Progression to CKD-KRT was the primary endpoint assessed. Secondary endpoint included achieving an e-GFR ≤60ml/min/1.73m^2.

Statistical Analysis

The distribution of variables was assessed with the Shapiro-Wilk test. Qualitative variables were expressed as proportions and compared against each other via the chi-squared test or Fisher's exact test. Variables following a parametric distribution were expressed as mean values ± standard error and compared against each other with Student's t-test. Variables with non-parametric distributions were expressed as median values (p25 and p75) and compared against each other with the Mann-Whitney U test. Logistic regression was used in multivariate analysis. Statistical significance was attributed to p-values <0.05.

RESULTS

Exclusion criteria accounted for the removal of 50 patients from the original population of 168 individuals. Of the remaining 118 patients, 65 were females (55%), 86 were whites (73%), 80 (67.8%) presented with hypertension and 106 (89.8%) hematuria (89.8%). Low serum C3 was detected in 15 (12.5%) patients. Table 1 shows patient data and laboratory tests at the time of kidney biopsy. Histologic findings based on the Oxford Classification were as follows: M1 (76.3%), E1 (35.6%), S1 (70.3%), T1/T2 (38.3%), and C1/C2 (28.8%). Histologic evidence of TMA was seen in 21 (17.8%) patients. The median follow-up time was 65 months, and 36 individuals progressed to CKD-KRT.

Table 2 describes the presence of serum findings consistent with TMA in patients with and without histologic evidence of TMA. When compared to individuals without signs of TMA in kidney biopsy, patients with histologic evidence of TMA had more anemia (81.3% *vs*. 24.7%, p=0.001), more schistocytes in peripheral blood (44.5% *vs*. 3.1%, p=0.0007), and were more prone to developing thrombocytopenia, albeit not significantly (14.3% *vs*. 4.1%, p=0.07).

The analysis of patients with and without TMA on kidney biopsy (Table 3) revealed that the first group had a greater proportion of hypertensive individuals (100 *vs*. 61, p<0.0001) and with hematuria (100 *vs*. 87.6, p=0.0001). Patients with TMA had worse median serum creatinine levels (3.8 *vs*. 1.38 mg/dL, p=0.0001), eGFR (18 *vs*. 60.2 ml/min/1.73m^2, p=0.0001), and more frequent low serum C3 (28.5 *vs*. 10.4, p=0.003). There was no difference in the treatment prescribed to the two groups of patients. In regard to the histology parameters of the Oxford Classification, solely a higher proportion of individuals with E1 was observed (68% vs. 32%, p<0.002). Patients with histologic evidence TMA were followed for less time (7 *vs*. 65 months, p<0.0001), since a greater portion of them progressed to CKD-KRT (71.4% *vs*. 21.6%, p<0.0001) and in less time (3 *vs*. 16 months, p=0.003) on account of the quicker eGFR decrease they experienced (-6.8 *vs*. -0.65 ml/min/1.73m^2/year, p=0.01).

The comparison of patients that progressed or not to CKD-KRT (Table 4) revealed that most of the individuals in the first group were males (63.8% *vs*. 36.5%, p=0.01), younger (30 *vs*.

177 34 years of age, p=0.04), had hypertension (86.1% *vs*. 56.1%, p=0.0016), worse creatinine
178 levels at the biopsy time (3 *vs*. 1.2mg/dL, p<0.0001), lower eGFR (22.5 *vs*. 64.8ml/min/1.73m^2,
179 p<0.0001), and more frequent low serum C3 (25.7% *vs* 7,3%, p=0.01), without difference in
180 proteinuria. Kidney biopsy findings pointed to a greater proportion of patients with T1/T2
181 (54.2% *vs*. 23,2%, p<0.0002) and a greater proportion of individuals with TMA (41.7% *vs*.
182 7,32%, p<0.0001). No difference was seen in the treatment prescribed to both patient groups.
183 Patients progressing to CKD-KRT were followed for less time (7 *vs*. 69 months, p<0.0001) on
184 account of more pronounced eGFR decreases (-8.17 *vs*. -0.21 ml/min/1.73m^2/ano, p<0.0001).

185 Immunohistochemistry for CD68 (Figure 1) was performed to look into macrophage-
186 mediated tissue inflammation in 76 patients, nine from the group of 21 patients with TMA
187 (43%) and 67 without TMA (69.1%). Figure 1 shows immunohistochemistry images derived
188 from renal biopsy specimens. No statistically significant difference was found when patients
189 with and without TMA were compared for number of CD68 positive glomerular (4.3 *vs*. 2.25,
190 p=0.12) or interstitial (25.4 *vs*. 18.3, p=0.25) cells. The comparison between patients
191 progressing or not to CKD-KRT did not yield significant differences for glomerular cells
192 labeled positive for CD68 (3.35 *vs*. 2.24, p=0.38), but significantly more CD68 interstitial cells
193 were seen in individuals progressing to CKD-KRT (32.5 *vs*. 15.6, p<0.0001) (Table 4).

194 Logistic regression performed to assess risk factors independently related to the primary
195 end point found that female sex was protective against the condition (Hazard Ratio - HR 0.26;
196 95% CI 0.09-0.72, *p*=0.01), while T1/T2 scores (HR: 11.9; 95% CI 4.1-35.3, p<0.001) and
197 histologic signs of TMA (HR: 11.5; 95% CI 3.2; 41.7, p<0.001) were associated with
198 progression to CKD-KRT (Table 5). Figure 2 shows the differences in renal survival for patients
199 with and without evidences of TMA in kidney biopsy.

200

201 **DISCUSSION**

Some clinical findings associated with worse outcomes in individuals with IgAN – male sex, older age, persistent microscopic hematuria, hypertension, proteinuria, and creatinine levels on kidney biopsy[21-24] – have been described as a factor in the progression to CKD-KRT within five years seen in approximately 30% of the individuals with the condition.[4,22,25] In light of this fact and the variety of histologic findings to consider, the need to standardize the analysis of kidney biopsy of IgAN patients and the search for histology-related prognostic factors led to the creation of histologic classifications,[6-8,26] with the Oxford Classification standing as the most widely accepted among pathologists and nephrologists. However, the OC does not consider TMA despite its role in disease progression and although it has been described to affect 2.2% to 53% of the individuals with IgAN.[11,26] Discrepancies in the frequency of involvement by TMA may be ascribed to differences among populations, although the adoption of diverse criteria to diagnose the condition – some including electron microscopy examination and immunohistochemistry staining protocols – is a relevant matter to consider.

El Karoui *et al*[11] studied a multi-center population of patients with IgAN and TMA. The author found that 68 (53.15%) of 128 patients had histologic evidence of TMA, a proportion higher than the reported in other studies. Diagnosis of TMA was based on light microscopy and immunohistochemistry staining for CD61. Patients with TMA progressed more frequently to CKD-KRT. A multicenter study carried out in Spain[27] included patients with IgAN and malignant hypertension. In the study, 13/186 patients (7%) were diagnosed with malignant hypertension. Patients with the condition were predominantly men in the fourth decade of life. Ten patients were on KRT at the end of the follow-up period. Three of the six individuals submitted to kidney transplantation returned to dialysis due to chronic allograft nephropathy associated with recurrent IgAN, although they did not have signs of malignant hypertension. Cai *et al*[10] studied 944 patients with IgAN from a single center in China, in which 194 patients with histologic evidence of TMA were found based on light and electron microscopy examination. Patients with TMA were older than their counterparts. Most were males with worse renal function, more proteinuria, and, interestingly enough, no differences in serum TMA markers. As

seen in previous literature reports, patients with TMA reached the target endpoint more frequently and quickly. Haas *et al*[26] looked into 2290 patients with IgAN and found 49 (2.2%) with TMA based on light and electron microscopy examination. The patients in this group were younger and had worse renal function and proteinuria on admission. They were not analyzed for progression to CKD-KRT. Other studies support these findings.[10,29-31] Our study found histologic evidence of TMA in 17.8% of the included patients, confirming some of the clinical and workup findings described previously in the literature (more individuals with hypertension, worse renal function, and worse outcomes).[9-11] As reported in other studies,[9,10] patients often do not present with clinical or workup findings indicative of TMA. Therefore, in our study only anemia and increased schistocyte counts stood out in the comparison of individuals with and without histologic evidence of TMA, since the groups were not different in terms of platelet counts, serum LDH, or haptoglobin levels.

Cases of IgAN associated with complement factor mutation have been described,[31-34] some of which effectively treated with Eculizumab.[32] What is not known is whether patients with IgAN and TMA actually present with a combination of the two diseases or if immunoglobulin deposition associated with the onset of the disease might produce local manifestations of TMA. The early pathogenesis of TMA has been linked to endothelial lesion.[35,36] Studies have shown that in addition to tropism by immunoglobulin degradation and complement factors in the mesangium, patients with IgAN also produce anti-endothelial cell antibodies,[37-38] thereby causing endothelial lesion and dissociation of the endothelium and the glomerular basement membrane, activation of the immune system (cytokines, inflammatory cells, complement system) and the coagulation cascade, producing local glomerular thrombosis.[35,39,40] On the other hand, the complement system may be activated via the lectin pathway as hypoglycosylated IgA1 – linked to the pathogenesis of IgAN – binds to endothelial cells.[41-43] This finding may be demonstrated by the overwhelming positivity for C4d in patients with IgAN and TMA without co-deposition of C1q.[10,35] Both theories are plausible, since it is not rare to find patients with only histologic and no clinical signs of TMA. We did not observe

differences in the profile or intensity of immunoglobulin deposition (IgG, IgM) or complement proteins (C3, C1q) in our series between patients with and without TMA or individuals that progressed versus patients that did not progress to CKD-KRT. However, none of the patients with TMA had deposition of IgG or C1q, while 10.3% of the patients without TMA had deposition of IgG and 7.3% of C1q. Chua *et al*[9] employed markers not used in routine clinical practice to assess the complement system and analyze depositions of C4d, C5b-9, mannose-binding lectin and Factor B in the renal tissue of patients with IgAN with and without TMA. The authors found an association between C4d and TMA and the presence of the two conditions with worse renal survival, thus stressing the role of the complement system in the pathogenesis of TMA in patients with IgAN.

By approaching the complement system from a systemic standpoint, we found, in our series, more patients with low C3 levels in the TMA group when compared with individuals without TMA and with the group of patients that progressed to CKD requiring KRT. This finding is not reflected in renal tissue, in which C3 deposition did not occur differently in patients with or without TMA (85.7 *vs*. 77.3, p=0.57) or in individuals progressing or not to CKD-KRT (80.5 *vs*. 78.0, p=0.95). In regard to MEST-C and TMA parameters, we found associations only with parameter E, which identifies active glomerular endothelial lesions in association with findings consistent with TMA. Since parameter E is modifiable via treatment, it is important to notice that no significant difference was seen in the treatment prescribed to the two groups of patients.

Recent studies[2,43] pointed to the relevance of glomerular tissue expression of CD68 as an additional marker that allows pathologists to more accurately define parameter E for endocapillary hypercellularity, as described by Soares *et* al.[43] In our series, this marker did not elicit differences in the number of cells labeled for CD68 in the glomeruli or tubulointerstitium of patients with and without TMA. However, expression was increased in the tubulointerstitium of patients progressing to CKD requiring KRT on account of the histologic evidence of chronic disease described previously by Soares et al[43].

The limitations of this study derive from the fact that is was carried out in a single center and that diagnostic examination for TMA was based only on light microscopy, without the aid of immunohistochemistry staining or electron microscopy. However, studies performed in a single center are known for more consistent compliance with procedure, follow-up, and treatment processes.

In conclusion, our study found that patients with IgAN and histologic evidences of TMA had clinically worse kidney function, more hypertension and hematuria, greater proportions of low serum C3 at kidney biopsy, with a larger amount of individuals with endocapillary hypercellularity (E1). Histologic evidence of TMA were not concurrent with clinical/laboratory markers of the condition. Patients with histologic evidence of TMA progressed more frequently and quickly to CKD-KRT when compared to individuals without TMA. Having signs of TMA in kidney biopsy specimens was an independent marker for progression to CKD-KRT when compared to other histologic predictors in the Oxford Classification. Additional studies are required to investigate the role of the complement system in TMA and IgAN and support the development of new therapeutic targets.

DISCLOSURE

The authors declare no competing interests

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TABLES

Table 1. Baseline clinical characteristics and kidney biopsy findings of patients with IgA Nephropathy.

	N=118
Age (years)	33 (25;43)
Female sex (n/%)	65 / 55
Serum creatinine (mg/dL)	1.45 (0.99;2.6)
e-GFR by CKD-EPI (ml/min/1.73m^2)[b]	48.8 (27.5;78.5)
24h-proteinuria (g)	2.01 (1.1;3.7)
Serum albumin (g/dl)	3.4 (2.9;3.8)
Hematuria (n/%)	106 / 89.8
Hypertension (n/%)	80 / 67.8
Low serum C3 levels (n/%)	15 / 12.5
Follow-up (months)	65 (27;115)
ΔeGFR (ml/min/1.73m^2/year)	-1.25 (-7.11 ; 0.91)
CKD-KRT (n/%)	36 / 30.5
Time to CKD-KRT (months)	9 (3;38)
Kidney Histology - Oxford Classification (n/%)	
M1	90 / 76.3
E1	42 / 35.6
S1	83 / 70.3
T1/T2	45 / 38.3
C1/C2	34 / 28.8
Thrombotic Microangiopathy (n/%)	21 / 17.8

eGFR: estimated glomerular filtration rate, *CKD requiring RRT:* Chronic Kidney Disease requiring Renal Replacement Therapy, *M1*: mesangial hypercellularity, *E1:* endocapillary hypercellularity, *S1:* segmental glomerulosclerosis, *T1/T2*: tubular atrophy or interstitial fibrosis, *C1/C2*: cellular crescent

Table 2. Laboratory findings of thrombotic microangiopathy in patients with and without histologic evidence of TMA.

	TMA (n=21)	**No-TMA (n=97)**	***p***
Anemia* (n/%)	13 / 61.9	24 / 24.7	0.002
Low Platelet count (n/%)	3 / 14.3	4 / 4.1	0.07
High serum LDH level (n/%)	13 / 61.9	45 / 46.4	0.29
Low serum haptoglobin level (n/%)	2 / 9.5	2 / 2.6	0.29
High serum indirect bilirubin level (n/%)	0 / 0	4 / 4.12	0.77
Schistocytes on peripheral blood smear (n/%)	4 / 19	3 / 3.1	0.02

LDH: Lactate Dehydrogenase.
** Defined as hemoglobin <12g/l for females and <13g/l for males*[20]

Table 3. Analysis of clinical parameters and laboratory findings in IgAN patients with and without histologic evidence of TMA.

	TMA (n=21)	**No-TMA (n=97)**	***p***
Male (n/%)	10 / 47.6	43 / 44.3	0.62
Age (years)	32 (27;41)	33 (24;44)	0.83
Hypertension (n/%)	21 / 100	59 / 61	<0.0001
Hematuria (n/%)	21 / 100	85 / 87.6	0.0001
Serum creatinine (mg/dL)	3.8 (2.2;5.8)	1.38 (0.91;1.9)	0.0001
e-GFR by CKD-EPI (ml/min/1,73m^2)[a]	18.3 (9.2;30.5)	60.2 (35.1;87.5)	0.0001
24h-proteinuria (g)	1.9 (0.9;3.96)	2 (1.3;3.6)	0.86
Serum albumin (g/dL)	3.2 (2.55;3.9)	3.5 (3.1;3.8)	0.26
Low serum C3 levels (n/%)	6 / 28.5	9 / 10.4	0.003
Treatment			
ACE inhibitor or ARB alone (n/%)	19 / 89.4	78 / 80.4	0.37
Corticosteroids (n/%)	11 / 52.6	63 / 64.7	0.35
Other immunosuppressants (n/%)	8 / 36.8	34 / 35.2	0.9
Kidney Histology – Oxford Classification (n/%)			
M1	19 / 89.7	75 / 77.3	0.23
E1	14 / 68	31 / 32	0.002
S1	16 / 78.9	70 / 72.1	0.54
T1/T2	12 / 57.9	35 / 36.1	0.07
C1/C2	8 / 32.1	26 / 26.8	0.57
Immunofluorescence positivity (n/%)			
IgM	6 / 28.5	23 / 23.7	0.84
IgG	0 / 0	10 / 10.3	0.26
C3	18 / 85.7	75 / 77.3	0.57
C1q	0 / 0	7 / 7.2	0.44
CD68 Immunohistochemistry (cells/field)			
Glomeruli	4.3 (3.02;6.0)	2.25 (1.56;5)	0.12
Tubulointerstitium	25.4 (14;34.7)	18.3 (9.8;28.3)	0.25
Follow-up (months)	7 (3;21)	65 (27;115)	<0.0001
ΔeGFR (ml/min/1,73m^2/year)	-6.8 (-24;0)	-0.65 (-4.48;2.3)	0.01
CKD-KRT (n/%)	15 / 71.4	21 / 21.6	<0.0001
Time to CKD-KRT (months)	3 (3;7)	16 (4;64)	0.003

e-GFR estimated glomerular filtration rate, *M1:* mesangial hypercellularity, *E1:* endocapillary hypercellularity, *S1:* segmental glomerulosclerosis, *T1/T2:* tubular atrophy or interstitial fibrosis, *C1/C2:* cellular crescent, *CKD requiring RRT:* Chronic Kidney Disease requiring Renal Replacement Therapy

[a]As determined by the Chronic Kidney Disease–Epidemiology Collaboration equation

494 **Table 4.** Clinical parameters and laboratory findings of IgAN patients progressing or not to CKD-KRT

	CKD-KRT (n=36)	No CKD-KRT (n=82)	*p*
Age (years)	30 (24;40)	34 (26;50)	0.04
Male sex (n/%)	23 (63,8)	30 (36,5)	0.01
Creatinine (mg/dL)	3 (2.3;5.6)	1.2 (0.9;1.7)	<0.0001
eGFR (ml/min/1.73m^2)	22.5 (9.6;36)	64.8 (40;91.7)	<0.0001
Proteinuria (g/day)	2.4 (1.3;4.1)	1.58 (1;2.98)	0.07
Albumin (g/dL)	3.4 (2.7;3.8)	3.5 (3;3.9)	0.61
Hematuria (n/%)	32 / 88.9	70 / 85.3	0.77
Hypertension (n/%)	31 / 86.1	46 / 56.1	0.0016
Consumption of C3 (n/%)	9 / 25.7	6 / 7.3	0.01
Follow-up (months)	7 (3;39)	69 (35;122)	<0.0001
ΔGFR (ml/min/1.73m^2/year)	-8.17 (-31;2.46)	-0.21 (-2.1;2.7)	<0.0001
Kidney Histology – Oxford Classification (n/%)			
M1	21 / 58.33	60 / 73.1	0.13
E1	16 / 44.4	24 / 29.2	0.13
S1	24 / 66.6	51 / 62.2	0.68
T1/T2	20 / 54.2	19 / 23.2	0.002
C1/C2	13 / 36.1	18 / 21.9	0.11
Immunofluorescence positivity (n/%)			
IgM	11 / 30.5	18 / 21.9	0.44
IgG	3 / 8.3	7 / 8.53	0.74
C3	29 / 80.5	64 / 78	0.95
C1q	2 / 5.5	5 / 6.1	0.75
CD68 Immunohistochemistry (cells/field)			
Glomeruli	3.35 (1.87;5.82)	2.24 (1.47;5.04)	0.38
Tubulointerstitium	32.5 (24.6;51.9)	15.6 (8.99;24.4)	<0.0001
TMA (n/%)	15 / 41.7	6 / 7.32	<0.0001
Treatment			
ACE inhibitor or ARB (n/%)	17 / 47.2	54 / 65.8	0.06
Corticosteroids (n/%)	13 / 36.1	40 / 48.8	0.23
Other immunosuppressants (n/%)	8 / 22.2	16 / 19.6	0.81

495 *eGFR* estimated glomerular filtration rate. *SD* standard deviation. *M1:* mesangial hypercellularity. *E1:* endocapillary
496 hypercellularity. *S1:* segmental glomerulosclerosis. *T1/T2:* tubular atrophy or interstitial fibrosis. *C1/C2 cellular*
497 crescent. *ACEi* angiotensin-converting enzyme inhibitor. *ARB* angiotensin II receptor blocker *CKD-RRT:* Chronic
498 Kidney Disease requiring Kidney Replacement Therapy
499 [a]As determined by the Chronic Kidney Disease–Epidemiology Collaboration equation
500

501 [
502 **Table 5.** Logistic regression analysis for the primary outcome[a] adjusted for sex, hypertension,
503 creatinine, eGFR, TMA, and the Oxford classification.

		95% Confidence Interval for HR		
Variable	**HR**	**Lower**	**Upper**	**p**
Female	0.26	0.09	0.72	0.01
T1/T2	11.9	4.1	35.3	<0.0001
TMA	11.5	3.2	41.7	<0.0001

504 *HR* hazard ratio. *CI* confidence interval. *T1/T2* mild to severe tubular atrophy or
505 interstitial fibrosis. *TMA:* Thrombotic Microangiopathy
506 [a]CKD requiring RRT: Chronic Kidney Disease requiring Renal Replacement
507 Therapy

FIGURE LEGENDS

Figure 1. CD68 immunohistochemistry of kidney biopsies of IgAN patients labeled positive in the A) glomerulus (x400) and B) tubulointerstitium (x200).

Figure 2. Survival free of dialysis of patients with IgAN with and without evidence of TMA in kidney biopsy specimens.

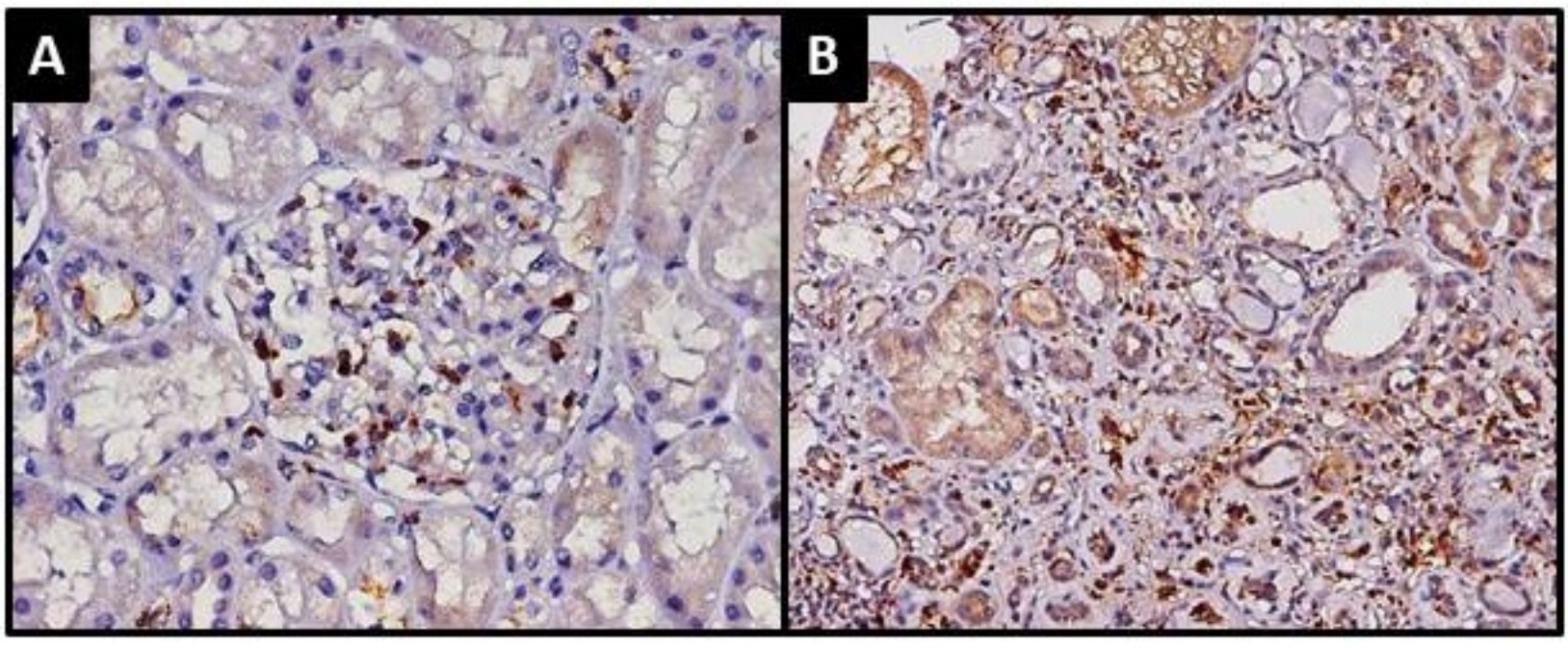
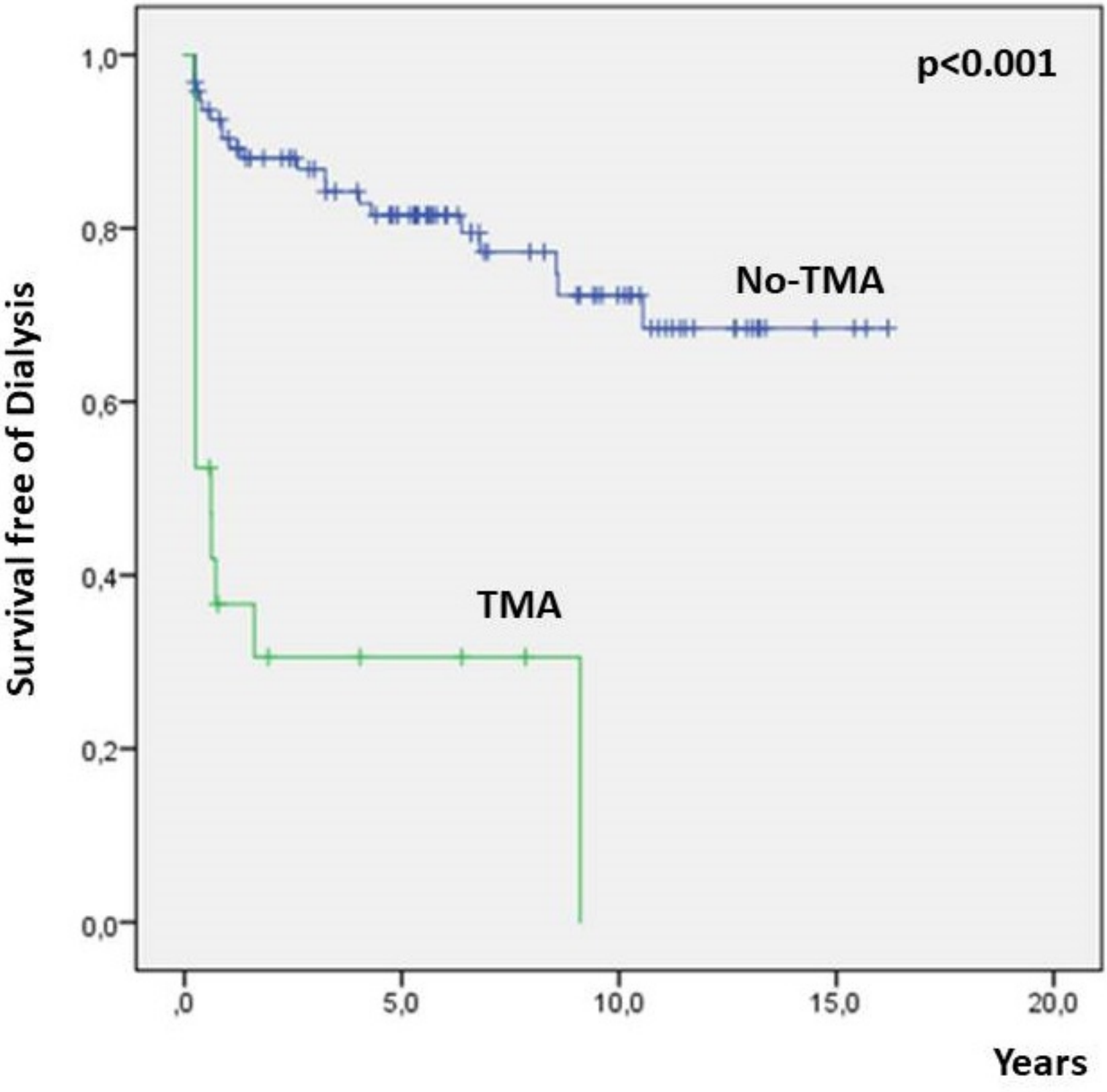


Figure 1


p<0.001
No-TMA
TMA
Survival free of Dialysis
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Years

Figure