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Kidney Complement Immunohistochemistry in Thrombotic Microangiopathy Subtypes

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INTRODUCTION

Thrombotic microangiopathy (TMA) is the term used for conditions characterized by nonimmune thrombocytopenia, microangiopathic hemolytic anemia, and organ dysfunction.[1] Kidney involvement is a common feature of TMA because of the vulnerability of glomerular vessels to endothelial damage and occlusion.[2] Determining TMA etiology can be difficult but is important for successful treatment.[3] At present, there is no diagnostic test that reliably identifies complement-mediated TMA.[1] Genetic sequencing is important; however, genetic abnormalities are only identified in approximately 50% of patients, and the absence of a detectable variant in complement genes does not exclude complement-mediated disease.[2,4] Although the cause of TMA cannot be established from kidney histopathologic morphology alone, the availability of kidney tissue presents the opportunity for immunohistology analysis to provide key diagnostic information.[2]

Characteristic kidney biopsy features of TMA reflect endothelial injury. Pathological complement activity results in complement cleavage product deposition that can be detected by immunohistology. Given the centrality of complement to TMA pathogenesis, we hypothesized that detailed immunohistology examination of complement activation within kidney biopsies could distinguish complement-mediated TMA from other causes and identify cases likely to benefit from complement inhibition. We assessed clinicopathological correlations of TMA and looked for associations with immunohistochemistry for a wide range of complement proteins. We specifically questioned whether differences in histological features and complement immunohistochemistry could differentiate TMA of different etiologies that have similar clinical features at time of diagnosis.

RESULTS

From our institution's pathology database, we identified 23 patients with atypical hemolytic uremic syndrome (aHUS) defined according to published criteria,[5] confirmed to have TMA on kidney biopsy between April 25, 1997 to December 31, 2020, and with surplus tissue available for complement staining (Supplementary Methods and Supplementary References). We also identified control patients with secondary TMA, including 9 with hypertension-associated TMA (hTMA) and 4 with pregnancy-associated TMA (pTMA) (Table 1).

All patients with aHUS were tested for complement gene variants. Six patients were found to have known pathological variants, 5 to have variants of unknown significance, and 12 to have no variants detected. Patients with aHUS with complement gene variants were older than patients with aHUS with variants of unknown significance. There were no other significant differences in clinical presentation or outcome. Consequently, the aHUS cohort were analyzed together (Table S1).

Table 1. Comparison of demographics, clinical features and clinical outcomes

Characteristics	aHUS (n = 23)	Hypertension-associated TMA (n = 9)	Pregnancy- associated TMA (n = 4)	P-value
Male sex, n (%)	20 (87)	5 (56)	0 (0)	0.0013[a,b]
Age, yrs, mean ± SD	42.6 ± 15.9	41.7 ± 15.2	31.3 ± 5.1	0.25[c]
Ethnicity, n (%)				0.16[d]
Black	5 (22)	2 (22)	2 (50)	
White	13 (57)	5 (56)		
Asian	1 (4)	1 (11)	1 (25)	
Other/not stated	4 (17)	1 (11)	1 (25)	
Systolic blood pressure, mm Hg, mean ± SD	202 ±44.6	206 ±37.2	126.0± 8.5	0.029[c,e]
Serum creatinine, μmol/l, median (IQR)	657 (393–867)	422 (200–544)	88 (49–272)	0.0067[d,e]
Protein-to-creatinine ratio, mg/mmol, median (IQR)	223 (75–439)	208 (64–506)	162 (5–308)	0.77[d]
Serum C3, g/l, mean ± SD (0.70–1.70 g/l)	1.22 ±0.19	1.22± 0.20	1.12 ±0.20	0.65[c]
Serum C4, g/l, mean ± SD (0.16–0.54 g/l)	0.38 ±0.12	0.40 ±0.13	0.17 ±0.049	0.011[c,e]
Platelet count, x 10^9/l, Mean ± SD (130–370 x 10^9/l)	86.5 ±72.8	174 ±98.8	174 ±160	0.27[c]
Development of CKD G5 and/or ESKD, n (%)	14 (61)	5 (56)	0 (0)	0.078[a]
Death, n (%)	3 (13)	1 (11)	0 (0)	0.59[a]
Length of follow-up, yrs, median (IQR)	4.69 (0.54–6.13)	8.20 (2.39–9.41)	5.92 (3.20–8.93)	0.25[d]

CKD, chronic kidney disease; ESKD, end-stage kidney disease; IQR, interquartile range.
[a]Chi-square.
[b]P <0.005.
[c]Brown-Forsythe 1-way analysis of variance.
[d]Kruskal-Wallis test.
[e]P <0.05.

Demographics

Of the aHUS patients, 87% were male compared with 56% of patients with hTMA (Table 1). Patients with pTMA presented at a younger age (mean: 31.3 ± 5.1 years) than both patients with aHUS and those with hTMA (mean: 42.6 ± 15.9 years and 41.7 ± 15.2 years, respectively).

Clinical Features

There were no significant differences between clinical features of aHUS and hTMA at the time of biopsy (Table 1). Mean systolic blood pressure was 202 ± 45 mm Hg in aHUS and 206 ± 37 mm Hg in hTMA, but lower in pTMA (mean: 126 ± 8 mm Hg). Serum creatinine was increased at 767 ± 555 μmol/l in aHUS, compared with 518 ± 552 μmol/l in hTMA and 136 ± 27 μmol/l in pTMA. Serum C4 was lower in pTMA than aHUS and hTMA but within the normal range (0.17 g/l vs. 0.38 g/l and 0.40 g/l, $P = 0.01$).

Treatment

Four patients with aHUS received eculizumab (3 with concurrent plasma exchange) and 3 had plasma exchange alone. No other participants received eculizumab or plasma exchange. The remaining patients with aHUS did not receive eculizumab because they presented before eculizumab was widely available ($n = 4$, 17%), achieved remission spontaneously ($n = 3$, 13%), or presented with advanced kidney disease ($n = 9$, 39%). Three of the 4 patients receiving eculizumab had mesangial or capillary C5b9 staining and 2 of 4 had positive FHR5 staining. Both patients with FHR5 staining and 2 of 3 patients with mesangial C5b9 staining, treated with eculizumab, recovered renal function.

Clinical Outcomes

The renal end points of doubling creatinine and end-stage kidney disease occurred frequently in aHUS (61%) and hTMA (56%) but were not observed in pTMA. Four patients with aHUS (17%) and 1 with hTMA (11%) died.

Histopathological Features

Light microscopy of the kidney biopsy revealed changes typical of TMA in all cases (Figure S1). Arteriolar endothelial swelling was a feature of most patients with aHUS and hTMA, and arteriolar thrombus formation was seen in both conditions. Features of chronic damage, including glomerulosclerosis and interstitial fibrosis were also seen in both aHUS and hTMA. Patients with pTMA showed milder changes, lacking chronic damage. Electron microscopy demonstrated endothelial swelling with flocculent material and denuded areas of basement membrane to a similar degree in both aHUS and hTMA (Figure S2). Kidney biopsy and clinical features did not distinguish aHUS from hTMA.

Immunostaining

All samples obtained from controls (healthy tissue and thin basement membrane disease) were negative for complement staining. C5b9 deposition was observed on the peritubular capillary wall and in the mesangium in

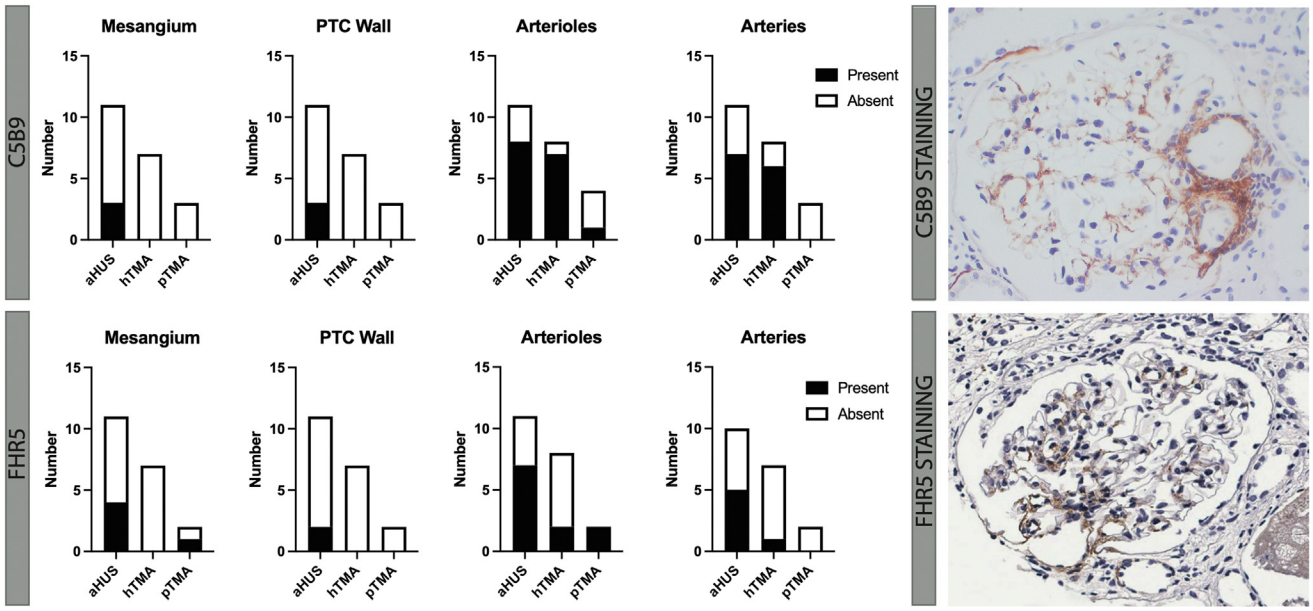


Figure 1. Complement component staining in TMA. C5b9 deposition was observed on the capillary wall and in the mesangium in a proportion of patients with aHUS, but not in the other conditions. C5b9 was observed in the arterioles in most cases of aHUS and less intensely in some patients with hTMA. FHR5 deposition was observed in the mesangium and on the capillary wall of some patients with aHUS, but not in hTMA, and was more commonly seen in the arterioles in aHUS. Representative images of C5b9 and FHR5 staining are shown. aHUS, atypical hemolytic uremic syndrome; hTMA, hypertension-associated TMA; pTMA, pregnancy-associated TMA; PTC, peritubular capillaries; TMA, thrombotic microangiopathy.

3 of 11 patients with aHUS (27%), but not in the other conditions (Figure 1, Table S2). FHR5 deposition was observed in the mesangium (4/11, 36%) and on the peritubular capillary wall (2/11, 18%) of patients with aHUS, but not hTMA, and was more commonly seen in the arterioles in aHUS (7/11 [64%] vs. 2/8 [25%]). C4d deposits were more prevalent in aHUS (14/44 samples, 32%) than hTMA (4/30 samples, 13%), and no glomerular C4d deposition was seen in hTMA. There was no difference in the number of glomerular CD68 positive cells between the groups ($P = 0.53$).

Correlation of Clinical Features

Correlations between mesangial light microscopy features, immunostaining, and serum creatinine are shown in Figure S3. C5b9 and FHR5 staining negatively correlated with signs of chronic damage (percentage global sclerosis, interstitial fibrosis, and tubular atrophy). There was no significant difference in time from disease onset to biopsy in those with positive and negative C5b9 and FHR5 immunostaining.

DISCUSSION

We have confirmed the observations of Chua *et al.*[6] that patients with TMA have considerably more complement deposition in the kidney than healthy or disease controls. Our findings support a pathological role for complement activation in aHUS regardless of genetic testing. Glomerular depositions of C5b9 and FHR5 appear to be more common and more intense in patients with TMA because of aHUS and may help to distinguish aHUS from hTMA.

Our study suggests that it is not possible to distinguish aHUS from hTMA based on clinical or pathological features alone. Furthermore, within the aHUS TMA cohort, apart from age, there were no major differences in the clinical features or kidney pathology in the subcohort with complement genetic variants, validating previously reported data.[7] The findings of C5b9 and FHR5 deposition in aHUS are of particular clinical interest, although this will need confirming in larger cohorts. Limitations of this study are that this was a retrospective kidney-biopsy based series; thus, full clinical details were not available, and nephritic factor and other complement autoantibody data are lacking.

Complement assays on kidney biopsy tissue could be used to identify cases of complement-dependent TMA, or aHUS, at diagnosis. This is clinically relevant because aHUS would be expected to improve with therapeutic complement inhibition with agents such as eculizumab. Whereas complement inhibition is not recommended for hypertensive emergency, some cases of secondary TMA may have complement deposition and respond to complement inhibition.[6,8] Complement inhibitors are costly and associated with adverse effects, so identification of patients unlikely to improve with complement inhibition would protect patients from needless exposure. We propose that C5b9 and FHR5 staining could aid this process.

In summary, the current identification of patients with TMA who are likely to benefit from therapeutic complement inhibition is unreliable. The presence of complement genetic variants can identify cases caused by aberrant complement activity, and complement gene testing should be performed in all patients with diagnostic features suggestive of aHUS, including those with hTMA. Complement immunohistochemistry of kidney biopsy tissue can assist identification of aHUS or TMA likely to improve with complement inhibition.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Figure S1. Light microscopy of TMA.

Figure S2. Electron microscopy of TMA.

Figure S3. Correlogram to demonstrate correlation between mesangial light microscopy features, immunostaining and serum creatinine.

Table S1. Comparison of patients with aHUS with complement gene variants to those without.

Table S2. Immunohistochemical staining results of thrombotic microangiopathy (TMA) kidney sections.

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