Patients with hypertension-associated thrombotic microangiopathy may present with complement abnormalities

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Thrombotic microangiopathy (TMA) is a pattern of endothelial damage that can be found in association with diverse clinical conditions such as malignant hypertension. Although the pathophysiological mechanisms differ, accumulating evidence links complement dysregulation to various TMA syndromes and in particular the atypical hemolytic uremic syndrome. Here, we evaluated the role of complement in nine consecutive patients with biopsy-proven renal TMA attributed to severe hypertension. Profound hematologic symptoms of TMA were uncommon. In six out of nine patients, we found mutations *C3* in three, *CFI* in one, *CD46* in one, and/or *CFH* in two patients either with or without the risk *CFH*-H3 haplotype in four patients. Elevated levels of the soluble C5b-9 and renal deposits of C3c and C5b-9 along the vasculature and/or glomerular capillary wall, confirmed complement activation *in vivo*. In contrast to patients without genetic defects, patients with complement defects invariably progressed to end-stage renal disease, and disease recurrence after kidney transplantation seems common. Thus, a subset of patients with hypertension-associated TMA falls within the spectrum of complement-mediated TMA, the prognosis of which is poor. Hence, testing for genetic complement abnormalities is warranted in patients with severe hypertension and TMA on renal biopsy to adopt suitable treatment options and prophylactic measures.

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Severe hypertension may induce thrombotic microangiopathy (TMA) within the renal vasculature associated with fibrinoid necrosis of arterioles and the glomerular capillary tufts. The exact mechanism remains to be established, but TMA may occur when autoregulation fails to counteract the hypertension-induced shear stress. In those patients with hypertension as the primary pathologic process, aggressive management of blood pressure is effective in resolving acute features of TMA and at least partially restoring renal function.[1] However, numerous other processes may be relevant, particularly in those patients who are not responding to standard treatment and are becoming dialysis-dependent.

During the last decade, the alternative pathway (AP) of complement activation has been linked to TMA and in particular to the atypical hemolytic uremic syndrome (aHUS): a rare syndrome of microangiopathic hemolytic anemia, thrombocytopenia, and renal insufficiency. AP is a continuously active immune surveillance and effector system operating in circulation and on the cell surface, which is tightly regulated to prevent damage to the self. In aHUS, AP dysregulation can occur at the endothelial surface, leading to the formation of the terminal complement complex (i.e., C5b-9) and subsequent endothelial cell damage.[2,3] AP dysregulation can be due to mutations in genes that either regulate or activate AP and/or autoantibodies that inhibit complement-regulatory proteins.[4,5] The penetrance of aHUS is incomplete, indicating that a second hit such as hypertension is required for disease manifestations.[6] The prognosis is extremely poor,[4,7] but blockade of the terminal complement pathway has dramatically improved the clinical outcome.[8–10]

In clinical practice, it is often a diagnostic challenge to differentiate hypertension-associated TMA from complement-mediated disease. This is particularly the case in patients presenting without profound hemolysis and/or thrombocytopenia. If one refrains from a complete diagnostic work-up, including a comprehensive search for complement abnormalities, a subset of patients may progress to end-stage renal disease without receiving optimal treatment. Moreover, the correct diagnosis is of utmost importance to adopt suitable prophylactic measures prior to kidney transplantation. Here,

we hypothesized that AP dysregulation is an often unrecognized, but treatable cause of hypertension-associated TMA. To test this hypothesis, we in retrospect thoroughly analyzed AP in 9 patients with severe hypertension diagnosed with TMA on renal biopsy. The study included 8 cases without profound hematologic signs of TMA. Furthermore, to explore whether indeed the prognosis of these patients is poor, we evaluated the long-term renal outcome, including the disease course after transplantation.

RESULTS

Baseline characteristics

Fourteen consecutive patients who fulfilled the inclusion criteria of hypertension-associated TMA were included from January 2005 onward; 5 patients were excluded because of secondary TMA (antiphospholipid syndrome, $n = 1$; scleroderma renal crisis, $n = 1$), immune-complex glomerulonephritis ($n = 2$), or the lack of DNA material ($n = 1$). Hence, 9 patients were included.

All 9 patients were evaluated at the Maastricht University Medical Centre, and 6 of them were referred from an outside institution. The baseline characteristics have been depicted in Table 1. At the time of presentation, a clinical diagnosis of malignant nephrosclerosis was clinically inferred. In all patients, mild-to-moderate hypertensive retinopathy was found, and papilledema was observed in 1 case (no. 8). Indeed, 7 patients had a known medical history of hypertension, including 2 patients with documented episodes of preeclampsia (nos. 1, 7) and/or malignant hypertension (no. 7). Proteinuria and hematuria were found in patients not presenting with anuria. Renal biopsies revealed characteristic lesions of TMA including endothelial cell swelling, reduplication of the glomerular basement membrane, wrinkling of the glomerular capillary wall, and/or mesangiolysis. Fibrin thrombi were localized in the glomeruli of 6 and in the vasculature of 5 tissue samples. Prominent intimal fibrosis, myxoid intimal alterations, and/or fibrinoid necrosis of the renal arteries were also found, reflecting preexisting severe hypertension. Assays of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, or ADAMTS13, at presentation were normal in 5 patients tested (nos. 1–5) and immunological tests were uniformly negative. Furthermore, iatrogenic causes, infections, and pregnancy were ruled out. However, aHUS was not suspected because profound hematologic signs of TMA were lacking in all but 1 patient (no. 3); moreover, none of the patients had a family history consistent with familial aHUS. Thus, a diagnosis of hypertension-associated TMA was established.

Complement abnormalities

DNA samples were tested; genetic AP abnormalities were identified in 6 (67%) of 9 patients, most of which were found in heterozygosity (Table 2). The mutated genes included *C3*, *CFI*, *CFH*, and *CD46*. The *C3* and *CFI* mutations included the missense variants c.481C>T (R161W) and c.452A>G (N151S), respectively. The *CFH* mutations included c.2558G>A (C853R) and c.2850G>T (Q950H), the latter of which is a rare variant of unknown significance present also in the normal population. The *CD46* mutation included the 6 base-pair deletion c.811_816delGAGACT (ΔD237/S238). Four patients carried the -332C>T and c.2808G>T single nucleotide polymorphisms that tag the *CFH*-H3 haplotype.[11,12] Also, the homozygous genomic deletion of *CFHR1* and *CFHR3* was identified in 1 patient, and circulating factor H autoantibodies were not found.

Complement ELISAs

At the time of renal biopsy, plasma sC5b-9 levels were measured by enzyme-linked immunosorbent assays (ELISAs) and although functional studies of AP and classical pathway were unremarkable, increased sC5b-9 levels were found in all patients. The upper limit of normal was set at 337 ng/ml ($n = 20$ healthy control subjects, mean ± 2 SD), whereas sC5b-9 levels ranged from 440 to 4,200 ng/ml in our cohort (Table 2).

Complement activation *in vivo*

Renal biopsies were stained for complement components. Tissue specimens of 8 patients were available. Deposits of C3c and C5b-9 were found in patients with complement abnormalities along the vasculature and/or glomerular capillary wall (Figure 1, Supplementary Table S1), confirming complement activation.[2] Also, deposits of C4d, a biomarker for

Table 1 | Baseline clinical features and laboratory evaluation

Patient No.	Age (yr)	Sex	BP (mm Hg)	SCr (μmol/l)	uProt (g/d)	uRBC	ESRD	Hb (mmol/l)	LDH (U/l)	MAHA[a]	Platelets (×10^9/l)[b]
1	38.4	F	184/140	1730	NA	NA	Y	5.1	1800	Y	224
2	40.3	M	205/114	1195	2.3	Y	Y	5.7	1104	Y	158
3	37.7	M	200/120	586	3.9	Y	Y	5.3	2125	Y	100
4	32.0	F	180/120	1138	NA	NA	Y	5.9	1486	Y	142
5	65.0	M	195/105	162	1.5	Y	N	7.9	271	N	98
6	41.1	F	180/120	334	0.7	Y	Y	7.5	291	N	285
7	28.5	F	224/122	1065	1.6	Y	Y	5.1	298	N	228
8	27.9	M	240/150	673	1.6	Y	Y	7.9	165	N	133
9	44.0	F	220/120	649	0.4	Y	Y	8.2	339	N	340

BP, blood pressure; ESRD, end-stage renal disease; F, female; Hb, hemoglobin; LDH, lactate dehydrogenase; M, male; MAHA, microangiopathic hemolytic anemia; N, no; NA, not applicable; SCr, serum creatinine; uProt, proteinuria; uRBC, hematuria; Y, yes.
[a]Cases with hemolytic anemia and schistocytes on peripheral blood smear were defined as MAHA.
[b]Lower limit of normal = 130 ×10^9/l.