The syndromes of thrombotic microangiopathy

10

Recent travels (summary)

The kidney donor recipient with a diagnosis of “hypertensive” end–stage kidney disease (ESKD) who presented with graft failure in the kidney from his mother, as presented in **Chapter 1**, intrigued me because morphologic features of chronic thrombotic microangiopathy (TMA), identical to those found on native kidney biopsy, developed in the graft. Blood pressure, however, was well–controlled after kidney transplantation, suggesting a mechanism independent of hypertension. The genetic studies, requested a decade after the patient had progressed to ESKD, indicated hereditary complement–mediated (C–)TMA rather than secondary atypical hemolytic uremic syndrome (HUS) related to hypertension. During the patient’s disease course, none of the attending physicians considered C–TMA because systemic hemolysis, as seen in primary atypical HUS (i.e., prototypic C–TMA), was not present. Thus, if one assumes that coexisting conditions reflect the etiology of disease, C–TMA can be missed, having impact on treatment and prognosis in the era of complement–specific drugs. We therefore studied the role of complement dysregulation along the spectrum of so–called “secondary” atypical HUS.

This thesis illuminates that complement dysregulation is common in a subset of patients with TMA, coexisting conditions, and severe kidney disease not responding to standard of care and/or relapsing disease, resembling primary atypical HUS. This, in particular, is the case in patients with coexisting hypertensive emergency, pregnancy, and *de novo* TMA after kidney transplantation. Our studies highlight the need for an updated and true etiology–based nomenclature that makes so much more sense. The term C–TMA was introduced to define patients with TMA on the background of complement dysregulation, either with coexisting conditions or not. The presence of hereditary and/or acquired factors can inform the risk of TMA recurrence in patients with C–TMA. We developed and validated a specific serum–based *ex vivo* test that enables us to recognize complement dysregulation on the endothelium. This *ex vivo* test facilitates the identification of C–TMA and may guide treatment decisions and monitoring during follow–up. Thus, our data are a first step to pursue precision medicine.

HYPERTENSIVE EMERGENCY AND C–TMA

The incidence of hypertensive emergency, defined as impending or progressive target organ dysfunction secondary to severe hypertension, has declined over the last decades. Also, the prognosis has improved from a “malignant” disease, with mortality rates up to 80% within 2 years from diagnosis[211] to a 10–year survival of >95%.[74] Kidney disease, both acute and chronic, however, has been linked to morbidity and mortality.[212] Most patients with acute kidney injury (i.e., ~75%) respond to rapid blood pressure control, whereas ~20% patients progress to ESKD.[74] Patients with microangiopathic hemolytic anemia had highest levels of

serum creatinine (median of 690 versus 120 µmol/L),[79] pointing to TMA as a potential factor associated with ESKD.

The hypothesis that complement dysregulation, pathognomonic for C–TMA, is key for poor outcomes and, in particular, ESKD, was tested in a pilot cohort, including 9 patients with TMA and coexisting hypertensive emergency (**Chapter 2**). Patients invariably presented with severe kidney disease; all but 1 patient required dialysis despite rapid blood pressure control. Most patients presented without profound hematologic abnormalities and thus, a diagnosis of so–called malignant nephrosclerosis was inferred. Notably, genetic studies demonstrated a high prevalence (i.e., *n*/*N*=6/9, 67%) of pathogenic variants in complement genes linked to C–TMA.

Patients with C–TMA who start eculizumab early have the best possible chance to recover kidney function.[13,14] Genetic studies, however, are time–consuming and lack sensitivity; thus, the decision to start treatment should not await genetic test results.[11] Routine complement measures, such as C3, soluble C5b9, and functional assays, also lack sensitivity and specificity.[51,67,86] Therefore, a specific serum–based *ex vivo* test using endothelial cells was developed to recognize patients with complement dysregulation in the earliest possible stage of disease (**Chapter 3**). Patients with TMA, coexisting hypertensive emergency, and severe kidney disease often presented with massive *ex vivo* C5b9 formation, whereas normal *ex vivo* C5b9 formation was found in patients with dense deposit disease (i.e., complement dysregulation in the fluid phase)[213] and patients with biopsy–proven arterionephro–sclerosis not presenting with hypertensive emergency. Thus, *ex vivo* C5b9 formation reflects the dynamics of complement activation on the endothelium (i.e., solid phase). At the time of quiescent disease, *ex vivo* C5b9 formation normalized on the resting endothelium; pre–incubation with adenosine diphosphate that causes endothelial perturbation[51] resulted in massive *ex vivo* C5b9 formation, indicating that a precipitating factor is needed for unrestrained complement activation to occur. Serum samples from patients treated with eculizumab attenuated C5b9 to form. *Ex vivo* C5b9 formation on the endothelium can therefore aid the recognition of C–TMA and may guide treatment during follow–up.

Next, additional patients were recruited from the Cliniques universitaires Saint–Luc, Brussels, Belgium, to study diagnostic and risk factors for complement dys–regulation in patients with TMA, coexisting hypertensive emergency, and severe kidney disease (**Chapter 4**). Again, profound hematologic abnormalities appeared uncommon, underscoring the need for a kidney biopsy to detect the TMA. Neither morphologic nor immunologic features on kidney biopsy, however, can be used to define etiology. Massive *ex vivo* C5b9 formation was found in 68% tested patients and associated with rare variants in complement genes. Also, patients with massive *ex vivo* C5b9 formation seem to benefit from eculizumab, with a renal response in

most of the treated patients. It is important to stress that *ex vivo* C5b9 formation reflects the dynamic process of complement on the endothelium, whereas rare variants in complement genes indicate the predilection for disease. Pathogenic variants in complement genes, indeed, were associated with relapsing disease. Thus, assessment of *ex vivo* C5b9 formation and screening for rare variants in complement genes can better categorize the TMA into different groups with therapeutic and prognostic implications.

Two independent cohort studies of patients with TMA and coexisting hypertensive emergency showed a high prevalence of rare variants in complement genes (i.e., ~55%) and moreover, eculizumab induced a renal response in >70% treated patients,[97,119] validating our observations.

THE RECOGNITION OF C–TMA

C–TMA has been linked to poor outcomes, with high rates of ESKD and TMA recurrence,[8,9] whereas most patients with secondary atypical HUS respond to treatment directed towards the coexisting condition.[139] Based on our clinical experience in patients with TMA and coexisting hypertensive emergency, the hypothesis that complement dysregulation is key for poor (kidney) outcomes in patients with “secondary” atypical HUS was tested in a well–defined cohort of 65 patients with TMA (**Chapter 5**). At baseline, massive *ex vivo* C5b9 formation on the endothelium was associated with severe kidney disease, rare variants in complement genes, and a favorable response to eculizumab, validating that the *ex vivo* test can aid the recognition of C–TMA in patients with coexisting conditions. Massive *ex vivo* C5b9 formation was common in the setting of hypertensive emergency, pregnancy, and, to a lesser extent, *de novo* TMA after kidney transplantation. Most of these patients, indeed, did not respond to standard of care and rapidly progressed to ESKD, whereas eculizumab appeared effective and prevented ESKD in 86% patients. Prolongation of eculizumab’s interdose interval appeared to block *ex vivo* C5b9 formation on the perturbed endothelium and prevented TMA recurrence despite a functional activity of the classical pathway >10% (recommended activity for complement inhibition, <10%),[11] corroborating data from Giuseppe Remuzzi’s group.[92] Thus, the *ex vivo* test, when performed in a specialized laboratory, facilitates the identification of C–TMA in patients with coexisting conditions, guides treatment, and helps to monitor patients during follow–up. As expected, TMA recurrence was associated with rare variants in complement genes. This study provides a rationale for an updated nomenclature on TMAs, as is discussed later.

Patients with TMA and coexisting autoimmunity were not studied in **Chapter 5**. Patients with the antiphospholipid syndrome (APS), characterized by thrombotic and/or obstetric complications with persistent antiphospholipid autoantibodies, may

present with TMA. Murine data suggested that complement, at least partly, is involved in the development of APS–related TMA.[128] Preliminary data showed that patients' serum induced C5b9 formation on hybrid endothelial cells that lack glycosylphosphatidylinositol–anchored complement regulatory proteins (i.e., CD55 and CD59) and corresponding complement–depending cell killing.[129] Variants in complement genes (classified as benign or of unknown significance) were found in some patients. From a kidney point of view, however, ESKD is uncommon in patients with APS–related TMA[107] as compared to those with C–TMA not treated with therapeutic complement inhibition,[8,9] suggesting a pathogenic mechanism not linked to complement dysregulation. We therefore studied the role of complement dysregulation in APS–related TMA (**Chapter 6**). *Ex vivo* C5b9 formation on the perturbed endothelium did not differ from controls, kidney tissue sections did not show complement deposits, and the disease course differed from C–TMA (i.e., 12 [92%] out of 13 patients with APS–related TMA stabilized and/or improved kidney function without therapeutic complement inhibition), excluding complement dysregulation. The non–complement fixing IgG2, a "thrombotic" subclass of anti–β2 glycoprotein–1 and anti–cardiolipin autoantibodies,[132,133] was found on the endothelium after serum incubation; neither IgG1 nor IgG3 were found. Therefore, anti–phospholipid autoantibodies may exert direct effects on the endothelium[134] and/or cause so–called annexin A5 resistance,[135] leading to thrombophilia. Standard of care, that is, anticoagulation either with immunosuppressive drugs or not, should therefore be started instead of therapeutic complement inhibition. The life–threatening catastrophic APS (~1% patients with APS), defined as (microvascular) thrombosis in at least 3 organs that develop in less than 1 week, with mortality >40%[214] may be an exception as case reports suggest that add–on therapeutic complement inhibition offer survival benefit.[215-218] Prospective (un)controlled studies, however, are needed to test this premise.

Patients with C–TMA typically present with acute features of TMA on kidney biopsy, whereas a small subset of patients may present with chronic features of TMA, that is, double contour formation of the glomerular basement membrane. In general, the clinical phenotype of patients with chronic TMA is poorly understood.[11] Murine data showed that a C3 gain–of–function protein (i.e., p.Asp1115Asn) drives chronic rather than acute TMA with heavy proteinuria.[32] The C3 gain–of–function protein (p.Arg161Trp) is prevalent in the Limburg Renal Registry's C–TMA cohort and has been associated with nephrotic–range proteinuria in more than half the patients,[66] suggesting chronic damage. The genotype–phenotype correlation was studied to better understand the etiology and disease course of patients with chronic TMA (**Chapter 7**). C3 p.Arg161Trp and probably other C3 gain–of–function proteins commonly present with morphologic features of chronic TMA and heavy proteinuria, often with normal platelets (11 [73%] out of 15 events). This is particular the case in

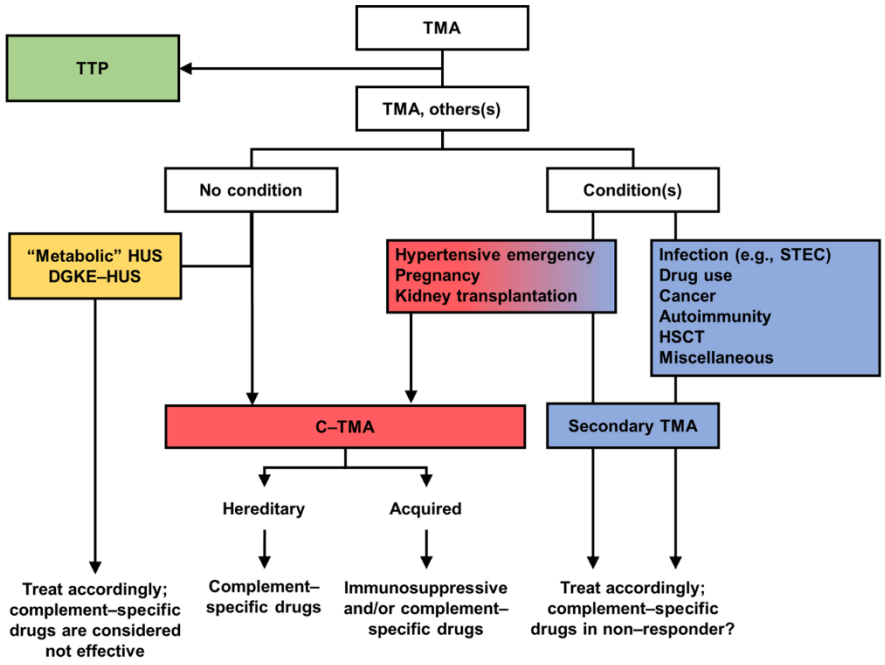
"late" TMA recurrence after kidney transplantation; all patients progressed to graft loss. Morphologic features on kidney biopsy and the clinical course of disease resembled the so–called C3KI mice. C5 inhibition rescued affected C3KI mice with chronic TMA.[32] Prospective controlled studies are needed to test whether patients with chronic C–TMA in isolation benefit from therapeutic complement inhibition or not. To state the obvious, early recognition is of utmost clinical importance. Proteinuria >1 g/day, although aspecific, may be a marker of TMA recurrence and should prompt a diagnostic work–up.

Taken together, our studies provide a rationale to update HUS International's nomenclature on the TMAs,[10,11] focusing on the correct recognition of complement dysregulation in patients presenting with coexisting conditions (**Chapter 8**). Moreover, HUS indicates hemolysis and uremia, whereas profound hematologic abnormalities can be lacking in patients with "organ–limited" TMA and coexisting conditions. Thus, primary atypical HUS, indicating a diagnosis of exclusion, should be replaced by C–TMA to improve the recognition of complement dysregulation in patients with TMA and coexisting conditions. C–TMA should be considered in patients with TMA and severe kidney disease not responding to standard of care and/or relapsing disease. Provocative studies, including our observations,[101,105,140] demonstrated that C–TMA is prevalent in patients with coexisting hypertensive emergency,[97,119] pregnancy,[120,145] and *de novo* TMA after kidney transplantation.[39] In contrast, C–TMA is uncommon in patients with other coexisting conditions.[100] Patients with TMA, excluding thrombotic thrombocytopenic purpura, at higher risk for C–TMA should be screened for unrestrained endothelium–restricted complement activation, rare variants in complement genes, and autoantibodies that inhibit complement regulation to better categorize the TMA (Figure 1). (Of note, patients with thrombotic thrombocytopenic purpura and severe kidney disease may present with complement dysregulation.[219]) Rare variants in complement genes and, to a lesser extent, factor H autoantibodies inform the long–term prognosis. This information should be added to the classification, that is, hereditary and acquired C–TMA. Our retrospective analyses suggest that patients with severe kidney disease not responding to standard of care and, in particular, those with massive *ex vivo* C5b9 formation, should be selected for therapeutic complement inhibition, either with eculizumab or other complement–specific drugs under development. We therefore initiated the "*Complement Prospective Evaluation of TMA on the Endothelium*" (COMPETE; NCT04745195) study to test this premise in patients with TMA and coexisting conditions.

IMPROVING PATIENT CARE BEYOND THE TMA

In women (~20%), pregnancy is an important precipitating factor for C–TMA to manifest.[47] C–TMA often develops late in pregnancy or postpartum,[120] whereas

Figure 1. Pragmatic approach to diagnosis and treatment of TMA.



Patients with TMA should be tested for the enzymatic activity of ADAMTS13 (i.e., >10% excludes thrombotic thrombocytopenic purpura [TTP]). Patients with a normal activity of ADAMTS13 should be screened for coexisting conditions. Of note, patients with coexisting hypertensive emergency, pregnancy, or *de novo* TMA after kidney transplantation may have C–TMA rather than secondary TMA. Most patients with no coexisting conditions have C–TMA, although primary TMA related to recessive variants in *DGKE* and metabolic causes should be considered in children.

DGKE, diacylglycerol kinase epsilon. HSCT, hematopoietic stem cell transplantation. STEC, Shiga toxin–producing E. *coli*.

thrombotic thrombocytopenic purpura is more common in the second and third trimester[147] due to a rise in von Willebrand factor multimers.[220] The counseling of women predisposed to complement dysregulation, both patients and asymptomatic carriers of rare variants in complement genes, who wish to start pregnancy is difficult as data are scarce. Numerous women at–risk therefore decided not to become pregnant. Maternal and fetal outcomes were studied to better understand the natural course of pregnancy in women predisposed to complement dysregulation (**Chapter 9**). The risk in such women appeared to be too pessimistic as C–TMA occurred in <20% pregnancies, often in the setting of additional precipitants. Of note, all but 1 patient had normal kidney function prior to pregnancy. Eculizumab recovered kidney function in all but 1 treated patient, corroborating previous studies.[120,145] Rare variants in complement genes per se cannot predict the risk of C–TMA in a given pregnancy.

Also, the burden of preeclampsia and HELLP (hemolysis, elevated liver enzymes, low platelets) appeared lower than anticipated. Previous studies linked HELLP[114,151] and, to a lesser extent, preeclampsia[150] to (rare) variants in complement genes. Four patients with HELLP from the Limburg Renal Registry presented with mild–to–moderate acute kidney injury (serum creatinine ranged from 54 to 227 μmol/L) and normal *ex vivo* C5b9 formation on the perturbed endothelium, 1 of whom had a variant of unknown significance identified in *CFHR2* (data not shown); after delivery, kidney function recovered in all without sequalae. In contrast to C–TMA, ESKD–related to preeclampsia and HELLP is uncommon,[208,221-223] most variants in complement genes are of unknown significance, and morphologic features reflect defects in vascular endothelial growth factor's function, that is, endotheliosis,[207] rather than thrombi. Moreover, preeclampsia and HELLP have been reported in pregnant women treated with eculizumab.[116,206] Thus, whether preeclampsia and HELLP fall within the spectrum of complementopathies remains debatable.

Pregnancy should be considered individually and carefully planned in women predisposed to complement dysregulation. Monitoring for at least 3 months after delivery is warranted in centers of expertise. In patients with active disease, eculizumab should be immediately available. Future studies need to assess the risk of pregnancy in women with a history of C–TMA and sequalae, such as hypertension and chronic kidney disease.