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Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies☆

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ABSTRACT

Background: The secondary forms of hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (secondary TMA) emerge as complications of coexisting diseases.
Objectives: We hypothesized that secondary TMA could be characterized by the presence of relative ADAMTS13 deficiency and complement activation, and this relationship may have a prognostic value for outcome.
Patients and methods: Fifty-three patients with thrombotic microangiopathy (TMA) and coexisting disease (such as malignancies, sepsis, heart surgery with extracorporeal circulation, solid organ transplantation, systemic autoimmune disorders), 41 patient controls, and 34 healthy controls were enrolled in our case-control study with 30 days follow-up. Complement profile (from serum) and activation products, von Willebrand factor (VWF, from EDTA plasma), and ADAMTS13 activity were determined.
Results: ADAMTS13 activity was reduced, while VWF level was elevated in secondary TMA patients. The activity of the classical, lectin and alternative pathways, as well as the levels of C3, C4, and Factor H were significantly lower in secondary TMA patients, and were accompanied by high activation product levels (C3a and sC5b-9). Factor H concentration correlated to relative ADAMTS13 deficiency (i.e. VWF/ADAMTS13 ratio ($r = -0.368$, $p = 0.019$)). 28/53 patients (53%) died during the follow-up period. Increased sC5b-9, C3a, and C reactive protein levels were all associated with a poor patient outcome.
Conclusions: Our results indicate that the secondary TMA syndrome and its poor outcome is characterized by relative ADAMTS13 deficiency, inflammation, and complement activation with consumption via the classical and alternative pathways. It is yet to be determined whether complement inhibition could be a possible therapeutic option for patients with secondary TMA.

1. Introduction

Thrombotic microangiopathy (TMA) is a pathology diagnosis established by the presence of thrombosis in small blood vessels, detachment and swelling of endothelial cells, and widening of subendothelial space (George and Nester, 2014). The common denominator in various TMA forms is activation and dysfunction of the endothelium (Goldberg et al., 2010). The clinical manifestations of TMA typically consist of microangiopathic hemolytic anemia, and thrombocytopenia with various degree of kidney damage. Multiple etiologies may lead to development of TMA, including ADAMTS13 (A Disintegrin and Metalloprotease with TromboSpondin motif repeats 13) deficiency resulting in thrombotic thrombocytopenic purpura (TTP); dysregulation of the complement alternative pathway, which predisposes to atypical hemolytic uremic syndrome (aHUS); or infection with Shiga-like toxin-producing bacteria caus-

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ing typical HUS (STEC-HUS). However, TMA may also develop as a complication of various coexisting diseases or their treatments (George et al., 2012; George and Charania, 2013), such as of malignant hypertension, systemic autoimmune disease, cancer, drug treatment, hematopoietic stem cell transplantation, solid organ transplantation, open heart surgery, glomerulopathies, protein-losing conditions, infections (including human immunodeficiency virus infection), and sepsis.

A decrease in plasma ADAMTS13 activity is specific for patients with acquired or hereditary TTP on one hand. On the other hand, there is increasing evidence that a substantial proportion of patients with secondary forms of TMA may have slightly or greatly reduced plasma ADAMTS13 activity. Moderate or severe decrease in plasma ADAMTS13 activity was reported in patients with metastatic cancer in multiple case-series, and in systematic reviews of case reports (Lechner and Obermeier, 2012; Francis et al., 2007; Mannucci et al., 2003a). Similarly, reduced or severely deficient ADAMTS13 activity was found in adult (Merayo-Chalico et al., 2014; Jiang et al., 2014; Mannucci et al., 2003b) and in pediatric (Muscal et al., 2011) patients with systemic lupus erythematosus and systemic sclerosis. In addition, a decrease in ADAMTS13 activity was described in secondary TMA in response to inflammatory (Claus et al., 2009; Bockmeyer et al., 2008), infective (Karim et al., 2013), or extracorporeal stimuli including solid organ transplantation (Verbiest et al., 2014; Mannucci et al., 2005). Decreased ADAMTS13 activity relative to increased von Willebrand factor (VWF) levels, appears to be a universal, clinically useful biomarker of such processes (Schwameis et al., 2015). Nevertheless, its relationship with other biomarkers of microangiopathy and with the short-term outcome of TMA has not yet been explored in depth (Claus et al., 2009).

Von Willebrand factor multimers may provide a surface for Factor H binding, for the modulation of complement activation and regulation, as well as for the cleavage of VWF by ADAMTS13, as reported by a series of recent, *in vitro* (Feng et al., 2015, 2013a; Rayes et al., 2014; Turner and Moake, 2013) and animal (Tati et al., 2013) studies. Accordingly, both complement activation and dysregulation have been shown to play important roles in the pathogenesis of aHUS, and in some cases of TTP. Hence, our group found elevated complement activation product C3a, and sC5b-9 levels in a substantial proportion of TTP patients (Reti et al., 2012); this observation was confirmed by an independent study (Westwood et al., 2014). Furthermore, in their comprehensive review of this topic, Riedl et al. summarized the observational evidence currently available on the role of complement, together with that of inflammation, coagulation, and endothelial activation in the spectrum of various thrombotic microangiopathies (Riedl et al., 2014). While the relationship between complement and aHUS, TTP, or STEC-HUS appears to be supported by observational data (Cataland et al., 2014; Feng et al., 2013b; Noris et al., 2012; Volokhina et al., 2015), the association between complement activation and secondary TMA has not yet been investigated formally in large-scale, case-control studies. Moreover, in spite of multiple *in vitro* evidence for the potential of von Willebrand factor to facilitate complement activation, *in vivo* observations on complement activation related to ADAMTS13 activity and VWF antigen (VWF:Ag) levels are still lacking in secondary TMA patients.

Based on earlier clinical studies into ADAMTS13 activity and VWF:Ag levels, as well as on observations on the contributory role of ULVWF to *in vitro* complement activation, we hypothesized that relative ADAMTS13 deficiency with the formation of ULVWF is a distinctive feature of the different forms of secondary thrombotic microangiopathies. Furthermore, we also hypothesized that the presence of ULVWF may be associated with *in vivo* complement activation, and a worse clinical outcome in patients with secondary TMA. To test these hypotheses, we conducted a case-control study in TMA patients. TMA was associated with cancer, systemic autoimmune disease, or open heart surgery (including solid organ transplantation cases). We measured ADAMTS13 activity, VWF:Ag and complement protein-, regulator-, and activation product levels, as well as explored the associations between these biomarkers of secondary TMA and in-hospital mortality.

2. Materials and methods

2.1. Patients and samples

Fifty-three patients with secondary TMA, 41 patient controls, and 34 healthy controls were enrolled into this single-site, laboratory-based investigation. The laboratory has been providing diagnostic services in Hungary since August 2007 for patients suspected of having various forms of thrombotic microangiopathies. The study was conducted in conformity with the Helsinki Declaration. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee on human clinical research. The recruitment of patients was closed in February 2015.

By definition, secondary TMA was present when all of the following criteria were met: (1) thrombocytopenia (<150 G/L), (2) anemia with signs of direct Coombs negative hemolysis and with fragmented erythrocytes in the peripheral blood smear, (3) evidence of coexisting disease or treatment, such as any of the following: malignancy, autoimmune disease, sepsis, solid organ transplantation, and open heart surgery. The exclusion criteria were the following: TMA after hematopoetic stem cell transplantation, drug-induced TMA, and TMA secondary to malignant hypertension. (Only very few patients belonging to these groups were investigated during the study period. Therefore, these patients were excluded in order to keep the study groups homogenous). Patients fulfilling only one, i.e. (1) or (2) of the above criteria, but with coexisting disease according to (3), were included into the 'patient control' group. Thirty-four healthy (age- and sex-matched) Hungarian controls were recruited by an outpatient department of occupational health. Subjects without known disease or regular medical treatment (except for adequately controlled hypertension), were enrolled into this group. Table 1 shows the baseline clinical and laboratory data of the patients and of the controls enrolled in this study.

Samples (serum, EDTA-anticoagulated plasma, and sodium-citrate-anticoagulated plasma) were drawn from the antecubital vein, or from a central venous catheter. The cells and the supernatant were immediately separated by centrifugation, and transferred to our laboratory in a coolbox. Separated samples were taken in aliquots and then, stored at −70 °C until analysis.

2.2. Determination of laboratory parameters

The fluorogenic substrate FRETS-VWF73 was applied to determine ADAMTS13 enzyme activity and inhibitors in citrated plasma or serum samples (Reti et al., 2012). VWF:Ag level was measured in EDTA plasma with an in-house ELISA based on Cejka (1982), using primary and horseradish peroxidase-labeled polyclonal rabbit anti-human VWF antibodies (Dako Cytomation, Glostrup, Denmark), as described earlier (Gombos et al., 2009). VWF:Ag determination was calibrated against pooled normal plasma (defined as 100%), and was performed in a subgroup of the study population (in n = 41 patients, 35 patient controls, and 25 healthy controls) due to the limited number of samples available. Functional assessment of the lectin- and alternative pathways was done with a commercially available test (Wieslab MBL and AP ELISA KITs, EuroDiagnostica, Malmö, Sweden) (Seelen et al., 2005) according to the manufacturer's instructions, whereas total classical pathway activity was