Pathophysiology of thrombotic thrombocytopenic purpura : the "two-hit" paradigm

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Pathophysiology of Thrombotic Thrombocytopenic Purpura: the “Two-Hit” paradigm

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INTRODUCTION

Thrombotic microangiopathies

Thrombosis is the pathologic formation of a blood clot within the vascular lumen. Intravascular clotting should always be regarded as pathologic, because blood clots physiologically form only at the sites of vascular wall injury. Thrombotic diseases constitute the main primary cause of death and disability worldwide and are the second most frequent secondary cause of death in patients with cancer.[1] Thrombosis may occur in virtually any vessel of the blood circulation and therefore thrombotic diseases are classified according to the type and location of the vessel that is occluded (Figure 1). Thrombosis in the vessels of microscopic caliber (terminal arterioles and capillaries) is the defining characteristic of thrombotic microangiopathies. Thrombotic microangiopathies are a group of diseases or clinical syndromes with overlapping clinical characteristics and heterogeneous etiology, highlighting the multicausal nature of microvascular thrombotic disease.[2] Classifying thrombotic microanciopathies is difficult. There are many possible classification criteria (by clinical symptoms, by associated clinical condition, by pathophysiology, by any other characteristic), and we propose a reasonable classification in Table 1. Idiopathic thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS) are the two most important forms of idiopathic thrombotic microangiopathy. From a clinical standpoint, the two diseases are characterized by acute episodes of thrombocytopenia and microangipathic hemolytic anemia (Coombs negative hemolytic anemia with signs of red blood cell fragmentation).

Atypical HUS has prominent renal involvement with acute renal failure (which is a required criterion for the diagnosis of HUS), whereas TTP frequently (but not invariably) displays neurological manifestations. From a pathophysiological standpoint, aHUS is characterized by hyperactivation of the alternative complement pathway.[3] Hyperactivation of the alternative pathway is usually caused by rare nonsynonymous mutations in complement factor or complement regulation genes.[3] The patterns of inheritance of aHUS are articulated and characterized by variable penetrance, considerable locus heterogeneity and epistasis. Idiopathic TTP is characterized by a severe deficiency of the von Willebrand factor (VWF) cleaving protease, ADAMTS13. This form of microvascular thrombosis is the main focus of this thesis.

Thrombotic thrombocytopenic purpura and severe ADAMTS13 deficiency

Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy characterized by acute episodes of widespread microvascular thrombosis causing severe ischemic organ damage.[4] In the late 1990s it was discovered that the plasmatic activity of the von Willebrand factor (VWF) cleaving protease, ADAMTS13, is severely deficient in individuals with TTP. This discovery represented a turning point in the understanding of the pathophysiology of the disease.[5] ADAMTS13 (**a** **d**isintegrin **a**nd **m**etalloproteinase with a **t**hrombo**s**pondin type 1 motif, member **13**) is the thirteenth member of the ADAMTS family of metalloproteases. Severe ADAMTS13 deficiency (i.e., a plasmatic activity of the protease below 10% of normal) is either due to circulating anti-ADAMTS13 autoantibodies (i.e. acquired deficiency)[6] or, less frequently, to recessively inherited mutations of *ADAMTS13*

(i.e. congenital deficiency).[7] Although not all of the patients with a clinically defined TTP present with severely reduced ADAMTS13, the finding of severe ADAMTS13 deficiency has been shown by several studies to define a distinct etiologic subgroup of the disease.[5] In TTP patients with severe ADAMTS13 deficiency, the pathologic presence in the circulation of uncleaved ultralarge VWF multimers is considered as the mechanism responsible for VWF-mediated platelet aggregation and thrombosis.[4] The clinical severity of TTP is heterogeneous both in terms of short and long term prognosis. The studies presented in this thesis investigate the pathophysiology of TTP with ADAMTS13 deficiency, in an effort to better understand the clinical heterogeneity that characterizes TTP patients.

We studied both congenital (Section I, Chapters 1-4) and acquired (Section II, Chapters 5-9) forms of TTP in order to identify factors influencing clinical heterogeneity.

In **Chapter 1**, we sought to compile a catalog of all genetic variants in the *ADAMTS13* gene that are associated with congenital TTP. We systematically studied the type and location of these variants, the results of functional studies and their association with disease phenotype. The aim was to discover whether genotype-phenotype relationships existed. We found that *ADAMTS13* genotype is a determinant of clinical heterogeneity.

In **Chapter 2**, we expanded these observations and conducted a study measuring residual ADAMTS13 activity in 29 patients with congenital TTP. The goal was to find whether a residual ADAMTS13 activity exists in congenital TTP patients and if that activity is associated with phenotype severity. We found that residual

ADAMTS13 activity is a determinant of clinical heterogeneity and we described genotype-phenotype relationships.

In **Chapter 3**, we described a case of congenital TTP. By measuring residual ADAMTS13 we sought to determine if ADAMTS13 activity is abolished during acute TTP in patients with congenital TTP who have measurable activity in remission.

In **Chapter 4**, we summarized all recent studies on residual ADAMTS13 activity in TTP, discussing their pathophysiologic implications.

In **Chapter 5**, we introduced acquired TTP, summarizing knowledge on the role of anti-ADAMTS13 autoantibodies in the disease.

In **Chapter 6**, we compared the clinical severity of acquired TTP in first episodes and recurrences, in order to determine whether clinical episode number is associated with disease severity. We found that episode number is a determinant of clinical heterogeneity in TTP.

In **Chapter 7**, we studied VWF-related measurements during acute disease and remission of TTP. The goal was to see if changes of VWF properties (e.g. its conformation) were associated with the onset of thrombosis in patients with TTP and if these properties were associated with the disease severity. We found that multimeric VWF pattern is associated with acute severity of TTP.

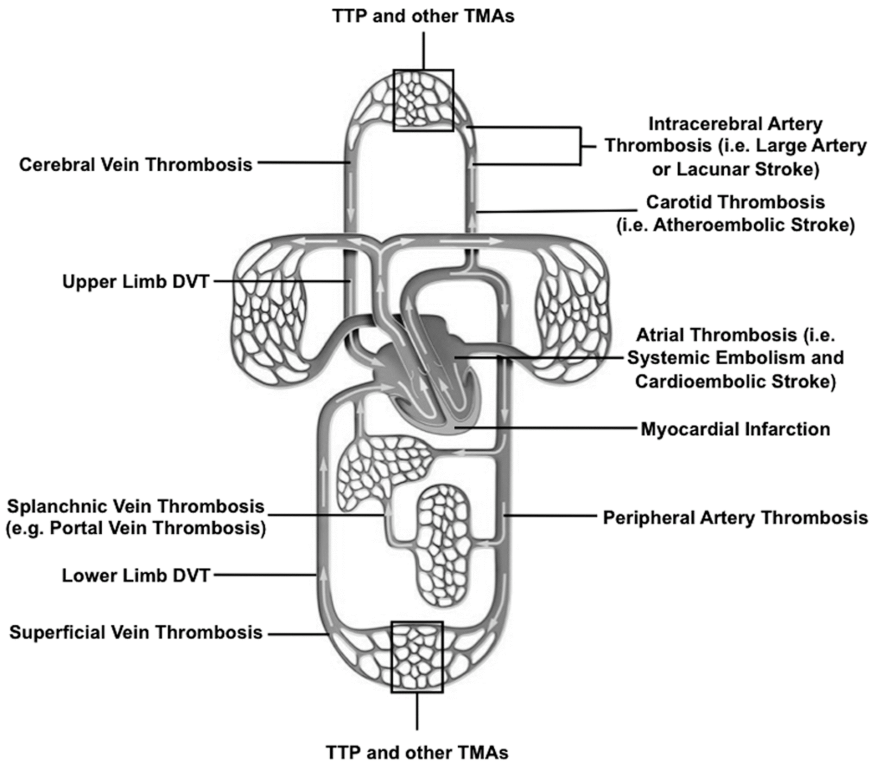
In **Chapter 8**, we studied several ADAMTS13- and anti-ADAMTS13 autoantibody-related measurements in acquired TTP patients, in order to determine their association with acute disease severity and with the occurrence of disease recurrence. We found several associations between study measurements

and clinically relevant endpoints, such as number of plasma exchange procedures needed to achieve remission or the development of recurrence.

In **Chapter 9**, we reported the case of a patient with a history of acquired TTP who was safely treated with thienopyridines (i.e. drugs associated with the development of drug-induced-TTP). The report demonstrates that these drugs do not necessarily induce TTP in patients with a history of the disease.

Figures

Figure 1. Localization of thrombotic diseases.
Adapted from *http://www.humanbody.dke-explore.com/clipart/human/image_human056.htm*.



TTP, thrombotic thrombocytopenic purpura; TMA, thrombotic microangiopathy; DVT, deep vein thrombosis.

Tables

Table 1. Classification of thrombotic microangiopathies.

Name	Localization of thrombosis	Pathophysiology	Clinical notes
Idiopathic			
Thrombotic thrombocytopenic purpura	Widespread Brain, heart, bowel typically involved	Severe ADAMTS13 deficiency (*ADAMTS13* mutations or anti-ADAMTS13 autoantibodies) Ultralarge VWF mediated thrombosis	Fever, neurological and cardiac involvement are frequent Purpura is the most common manifestation
Atypical hemolytic uremic syndrome	Widespread Renal circulation prominently involved	Hyperactivation of the alternative complement activation pathway (mutations in complement factor or regulator genes or auto-antibodies against complement regulators)	Prominent renal involvement with acute renal failure
Catastrophic antiphospholipid antibody syndrome	Widespread (including large vessels)	Anti-phospholipid autoantibodies	Positive test for anti-phospholipid antibodies Thrombosis may occur also in large vessels
Secondary			
Secondary thrombotic thrombocytopenic purpura			
Cancer	Widespread	Cancer-related endothelial damage and activation Metastatic cell embolization Cancer-related hypercoagulability	Poor response to PEX
Bone marrow transplantation	Widespread	Endothelial damage and activation	Poor prognosis
Drug induced (dose independent)	Widespread	Development of acute disease after use of the drug (quinine, ticlopidine, clopidogrel) Anti-ADAMTS13 autoantibodies may be present	Acute disease days/weeks after the use of the drug
Drug induced (dose dependent)	Widespread	Dose- and duration-dependent toxic effect of chemotherapy (e.g. mitomycine, gemcitabine, etc.)	Insidious onset of symptoms
HIV infection	Widespread	HIV-mediated endothelial damage and activation	May be sensitive to anti-retroviral therapy

Typical hemolytic uremic syndrome	Widespread Renal circulation prominently involved	Infection by enterohemorrhagic *E. Coli* O157:H7	Bloody-diarrhoea prodrome. Prominent renal involvement with acute renal failure
Hemolysis elevated liver enzymes low platelet (HELLP) syndrome[a]	Widespread Liver is affected	Unknown The disease is considered a severe form of pre-eclampsia	May have mild symptoms Typical manifestations are epigastric pain and malaise
Disseminated intravascular coagulation[b]	Widespread	Hyperactivation of the coagulation cascade in response to a variety of diseases	Prominent hemorrhagic symptoms
Autoimmune-disease associated thrombotic microangiopathy	Widespread	Manifestation of the primary autoimmune disorder	Insidious onset Frequent renal involvement

a HELLP is classified as secondary TMA because it develops in pregnant women with preeclampsia

b Disseminated intravascular coagulation is secondary to many different disease/conditions

VWF, von Willebrand factor; PEX, plasma exchange.

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