Thrombotic microangiopathies: thrombotic thrombocytopenic purpura / hemolytic uremic syndrome

**ABSTRACT**
Thrombotic microangiopathies (TMAs) are pathological conditions characterized by generalized microvascular occlusion by platelet thrombi, thrombocytopenia, and microangiopathic hemolytic anemia. Two typical phenotypes of TMAs are hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Other disorders occasionally present with similar manifestations. Depending on whether renal or brain lesions prevail, two pathologically indistinguishable but somehow clinically different disorders have been described: HUS and TTP. Injury to the endothelial cell is the central and likely inciting factor in the sequence of events leading to TMA. Loss of physiological thrombore sistance, leukocyte adhesion to damaged endothelium, complement consumption, abnormal von Willebrand factor release and fragmentation, and increased vascular shear stress may then sustain and amplify the microangiopathic process. Intrinsic abnormalities of the complement system and of the von Willebrand factor pathway may account for a genetic predisposition to the disease that may play a paramount role in particular in familial and recurrent forms. In the case of diarrhea-associated HUS (D+HUS), renal endothelial damage is mediated (at least in large part) by the bacterial agent Shigatoxin (Stx), which is actually a family of toxins elaborated by certain strains of Escherichia coli and Shigella dysenteriae. Outcome is usually good in childhood, Shiga toxin-associated HUS, whereas renal and neurological sequelae are more frequently reported in adult, atypical, and familial forms of HUS and in TTP. Recent studies have demonstrated that deficiency in the von Willebrand factor cleaving protease ADAMTS13, due to deficiency of ADAMTS13 can be genetic or more common, acquired, resulting from autoimmune production of inhibitory anti-ADAMTS13 antibodies, that causes TTP. During the last decade, atypical HUS (aHUS) has been demonstrated to be a disorder of the complement alternative pathway dysregulation, as there is a growing list of mutations and polymorphisms in the genes encoding the complement regulatory proteins that alone or in combination may lead to aHUS. Approximately 60% of aHUS patients have so-called ‘loss-of-function’ mutations in the genes encoding the complement regulatory proteins, which normally protect host cells from complement activation: complement factor H (CFH), factor I (CFI) and membrane cofactor protein (MCP or CD46), or have ‘gain-of-function’ mutations in the genes encoding the complement factor B or C3. In addition, approximately 10% of aHUS patients have a functional CFH deficiency due to anti-CFH antibodies. Although TMAs are highly heterogeneous pathological conditions, one-third of TMA patients have severe deficiency of ADAMTS13. Platelet transfusions are contraindicated. Plasma infusion or exchange (PE) is the only treatment of proven efficacy.

Keywords: thrombotic microangiopathies, thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, kidney failure, von Willebrand factor.

INTRODUCTION

Thrombotic microangiopathies (TMAs) are pathological conditions characterized by the presence of microangiopathic hemolytic anemia (due to shear stress in the microcirculation), generalized microvascular occlusion caused by platelet-rich thrombi (renal involvement is common), and thrombocytopenia (platelet consumption).\(^2\) The two classical phenotypic manifestations of TMAs are thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS), serious conditions that put the patient's life at risk. Hemolytic-uremic syndrome is characterized by three already cited clinical signs (classical “triplet”), while TTP is characterized by a set of five manifestations: the “triplet” associated with fever and neurological signs. However, the two diseases cannot be clinically distinguished. Those TMAs should be differentiated from disseminated intravascular coagulation (DIC) and from consumptive thrombo-hemorrhagic disorders.\(^2\) In fact, the spectrum of clinical manifestations of such disorders is very similar, the neurological abnormalities being commonly considered a characteristic of TTP, while acute kidney failure is a characteristic of HUS. Patients with those syndromes can have both, or none, abnormalities. This distinction has been valued to a certain extent because of the suggestion that treatment with plasma exchange (PE) would be appropriate to TTP, but not to HUS. Even considering that both are significantly clinically and histologically related, most recent investigations have shown that TTP and HUS have independent and non-related outcomes. Currently, investigations related to TTP have focused on the regulation of the blood coagulation protein, the von Willebrand factor (vWF), while studies on HUS have focused on understanding the mechanisms of renal endothelial injury.

It is worth emphasizing that the PE treatment is the combination of plasmapheresis (which can remove unusually large von Willebrand factor multimers and autoantibodies against ADAMTS13) and infusion of frozen fresh or cryosupernatant plasma (containing additional metalloprotease).\(^2\)\(^,\)\(^20\)

DIAGNOSTIC CRITERIA FOR TMAS (TTP AND HUS)

According to previous studies,\(^2\)\(^,\)\(^4\)\(^,\)\(^5\) TMAs have been defined as having all the following alterations: (1) microangiopathic hemolytic anemia (hemoglobin ≤ 12 g/dL), negative Coombs test, undetectable serum haptoglobin (< 10 mg/dL), more than two fragmented red blood cells (schizocytes) in a 100-x microscopic field (Figure 1), and an increase in lactic dehydrogenase (LDH) above the institutional baseline; (2) thrombocytopenia (platelet count ≤ 100x10^9/L); and (3) organic dysfunction (renal and neurological involvement) of variable intensity with no signs of DIC.\(^6\)

The differential diagnosis between HUS and TTP based on routine laboratory findings is usually very difficult.\(^7\)

Approximately 90% of children with HUS have diarrhea [HUS associated with diarrhea (D+ HUS)] as a prodrome, usually with blood, caused by Shiga toxin (Stx), typically produced by *Escherichia coli* of the serotype O157:H7.\(^8\) With support therapy, its mortality is 3%,\(^9\) and, thus, treatment with PE is rarely necessary. Because that procedure is not used in children with HUS, the same diagnosis in adults can indicate that PE treatment is not necessary. Therefore, some authors avoid using the term HUS for adults, even in the presence of kidney failure. It is worth emphasizing that acute kidney failure manifests in 55% to 70% of the cases;\(^10\)\(^,\)\(^12\) however, kidney function recovers in most cases (more than 70% in several case series).\(^12\)\(^,\)\(^15\)

Leukocytosis can be extreme, in the form of a leukemoid reaction, being a positive predictor of acute mortality and residual nephropathy.\(^16\)\(^,\)\(^19\) Children with diarrhea (D+ HUS) present a significantly higher leukocyte count than children with atypical HUS (D- HUS). This finding suggests that the intestinal disorder is an important factor implicated in the generation of leukocytosis.\(^17\) In the Osaka outbreak in 1996, leukocyte count and C reactive protein (CRP) levels were higher in the group of children with diarrhea who developed HUS, when compared with infected children without that complication.\(^18\)

Atypical HUS involves a heterogeneous group of patients not infected by the Stx-producing bacterium (5% of the HUS cases), which should be excluded as a cause of the disease. Atypical HUS can be sporadic or familial (that is, more than one family member affected by the disorder, once exposure to Stx-producing *E. coli* is excluded). The forms of D- HUS have a poor prognosis. More than 50% progress to chronic kidney failure or irreversible cerebral damage, and 25% evolve to death during the acute phase of the disease.\(^16\) Recently, genetic studies have documented that the familial form is associated with genetic abnormalities of the complement regulatory protein, and there is evidence that similar genetic alterations can also predispose to sporadic cases of D- HUS.\(^21\)

Unlike TTP, HUS is rarely induced by genetic mutations of complement regulation factors (factors B, H, and I, and CD46) and by auto-antibodies against factor H.\(^21\)
In the era preceding its effective treatment, TTP was defined by the following “pentad” of clinical manifestations: thrombocytopenia; microangiopathic hemolytic anemia; neurological abnormalities; kidney failure; and sudden fever. Efficacy of the treatment with PE requires immediate diagnosis and a reduction in the number of clinical criteria necessary for diagnosing the disease. The diagnostic criteria that have been used more recently include only thrombocytopenia and microangiopathic hemolytic anemia, with no apparent alternative etiology. The use of fewer criteria is supported by the clinical findings at presentation of patients whose diagnosis of TTP had been confirmed by the presence of severe deficiency of ADAMTS13: neurological and renal abnormalities were uncommon, fever was rare, and no patient had the complete “pentad” of clinical manifestations. The feasibility of effective treatment and the reduction in number of the diagnostic criteria have resulted in an eight- to ten-fold increase in the number of patients treated with PE in cases of TTP. Because the diagnosis of TTP requires that treatment to be considered, neither the deficiency of ADAMTS13, nor the deficiency of complement regulators are sufficient for the development of TTP or atypical HUS, respectively.

**Acute inflammation in the pathogenesis of HUS**

Initially described in 1925 as a syndrome comprising kidney failure, hemolytic anemia, and thrombocytopenia, HUS is usually considered a kidney disease with systemic complications. While in TTP, the focus is on von Willebrand factor and ADAMTS13, in HUS, the focus is on the mechanism of renal endothelium injury mediated by Stx (at least, mostly), a family of toxins produced by certain strains of *Escherichia coli* and *Shigella dysenteriae*.

Unlike TTP, patients with D+ HUS have plenty of evidence of an acute inflammatory response, whose magnitude predicts clinical outcome. There is evidence that inflammatory cells and their byproducts play a major role in: (1) loss of the intestinal barrier function, promoting the movement of endotoxin and Stx into the circulation; (2) delivery of Stx to target organs; (3) sensitization of target organs to Stx by increasing the intracellular glycolipid receptor expression; and (4) direct injury of target organ endothelium.

The gastrointestinal disorder in patients with Stx-producing *E. coli* varies from an aqueous diarrhea to a severe form of hemorrhagic colitis. Colonic inflammation could play a role in the local intestinal microangiopathy, in the Stx transportation from the intestinal lumen through lamina propria into the circulation, and in the generation of severe systemic inflammatory response. Recent evidence shows that Stx induces marked chemotactic response of human intestinal epithelial cells. By using a colonic epithelial cell line, Stx led to superinduction of interleukin-8 (IL-8) with increase in both IL-8 protein and mRNA, despite the known inhibitory effects of Stx on protein elongation. Secretion of IL-8 into the lamina propria might create a chemokine gradient sufficient to recruit polymorphonuclear leukocytes (PMNs). Both IL-8 and tumoral necrosis factor alpha (TNF-alpha) are very elevated in stools of patients infected with Stx-producing *S. flexneri*. Several potential consequences of the inflammatory response in lamina propria exist. Transepithelial migration of PMNs can lead to transient loss of colonic barrier function and cause the passage of luminal contents into circulation. A recent study has shown the movement of PMNs from the basolateral membrane to the apical side of intestinal epithelial cells and the increase in translocation of both Stx1 and Stx2 in the opposite direction. Loss of

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**Figure 1. Peripheral blood smear of a patient with TTP.**

and because almost all adults who do not meet the diagnostic criteria for PTT can benefit from PE, some authors use the term TTP for almost all adults.

It is worth noting that, although a certain degree of kidney impairment with proteinuria and/or hematuria is common, arterial hypertension and acute kidney failure requiring dialysis are rare in TTP. The distinction between TTP and HUS is extremely important, because of the cases of atypical HUS, since D+ HUS affects mainly children, who typically have diarrhea before the episode of kidney failure. It is worth emphasizing the recent evidence that
of barrier function could contribute to direct entry of Stx into the circulation, and endotoxemia could promote a systemic cytokine response. Increasing evidence, both in vitro and in vivo, suggests that Stx binds to PMNs, which is the trigger for their activation, and promotes adherence of PMNs to the target endothelium. Recently, Te Loo et al. have found that Stx binds to PMNs (not via Gb3) and that these cells are direct carriers of the toxin from the intestine to target organs. Shiga toxin circulates in the body, but can be preferentially located in the kidney, as a result from the high concentration of its receptor, globotriaosylceramide (Gb3), in the endothelium.

The D+ HUS (Table 1) is caused by Stx1 and Stx2, a component of the subunit A of 33 kDa and five subunits B of 7 kDa each, produced by Escherichia coli O157: H7. In the circulation, it binds to the Gb3 receptor, which is widely expressed on glomerular endothelial cells. After receptor-mediated endocytosis, the subunit A of Stx is internalized and activated, leading to the depuration of specific adenosines in 28S ribosomal RNA, resulting in irreversible inhibition of protein elongation and apoptosis of endothelial cells. This releases significant levels of Willebrand factor multimers (UL-VWFMS) into the circulation, resulting in platelet thrombi in the microcirculation. Glomerular endothelial cells and renal tubular epithelial cells express the Gb3 receptor, contributing to the development of kidney failure in infected patients. Thus, E. coli O157:H7 associated with TMA seems to be induced independently from the plasma levels of ADAMTS13:AC7 (normal activity of the vWF-cleaving protease), and efficacy of the treatment with PE is much less evident in such case.

By definition, the kidney is the target organ in HUS caused by Stx-producing E. coli. That vulnerability is mostly due to the high density of Gb3 receptors in renal tubular epithelial and microvascular endothelial cells. In fact, renal tubular epithelial cells are maybe the major source of Gb3, in a quantity equal to or possibly greater than that of Vero cells. The pending question is how much of that vulnerability is due to the cytotoxic environment. The studies by Hughes et al. and King et al. have shown that those cells are extremely sensitive to the ribotoxic effects of Stx. Those studies have increased the possibility that acute tubular necrosis plays a role in kidney failure. Human glomerular microvascular endothelial cells express less Gb3 and are less sensitive to the ribotoxic effects of Stx. Even if the subsequent events are less well known, it is clear that the thrombotic state induced by Stx in HUS involves more than direct toxic effect. Pretreatment of the glomerular microvascular endothelial cells with TNF-alpha substantially increased the density of the receptors and their sensitivity to Stx. Those data suggest that inflammatory cytokines contribute to glomerular injury, because of an increase in Gb3 expression on glomerular microvascular endothelial cells, thus increasing the sensitivity of those cells to the ribotoxic effects of Stx and their vulnerability to undergo apoptosis. If the finding that Stx increases the expression of adhesion molecules is true for glomerular microvascular endothelial cells, such mechanism could serve as an accelerator of the glomerular injury due to an increase in Stx delivery.

Approximately 50% of the patients with atypical HUS (Table 2) carry a heterogeneous mutation in one of the four genes that encode the complement factors H (CFH), I (CFI), B (CFB), membrane cofactor protein (MCP) or CD46. Those proteins control the complement activation on cell surface and limit cell injury mediated by complement in the host tissue. However, because of only partially known reasons, the glomerular endothelium is especially sensitive to loss of complement regulation, and the microvascular damage in atypical HUS is mainly limited to the renal circulation.

Since 1974, reduced serum levels of complement component C3 and normal serum levels of C4 have been shown in patients with atypical HUS (Table 2). Patients with HUS and low levels of C3 have high activated complement (C) component levels, including C3b, C3c, and C3d. Granular deposits of C3 in glomeruli and arterioles during acute disease are consistent with complement activation and local C3 consumption. Positive staining for C9 in glomeruli and small arteries with intimal proliferation and thrombosis indicates activation through the final lytic pathway (membrane attack complex C5b-9).

It is worth noting that the complement system consists of several plasma proteins and membrane-bound proteins that protect against the invasion of microorganisms. There are three activation pathways (classical, lectin, and alternate), which produce proteases denominated C3 and C5 convertases, which cleave C3 and C5, respectively, and bind to the membrane attack complex. Hydrolysis of C3 in the plasma initiates the alternate pathway, leading to C3b deposition on almost all surfaces exposed to plasma. In host cells, complement activation is controlled by both receptors, fluid-phase and membrane-anchored receptors, favoring C3b cleavage to inactive C3b (iC3b) by CFI (cofactor activity) and dissociating the multicomponents of C3 and C5-convertases (decay
Thrombotic microangiopathies

### Table 1  Classification and Treatment of the Different Forms of HUS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causes</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+ HUS</td>
<td>Stx-producing Escherichia coli</td>
<td>Support</td>
</tr>
<tr>
<td></td>
<td>Shigella dysenteriae type 1</td>
<td>Support and antibiotics</td>
</tr>
<tr>
<td>Non-Stx HUS (sporadic)</td>
<td>Bacterium (Streptococcus pneumoniae)</td>
<td>Antibiotics and plasma</td>
</tr>
<tr>
<td></td>
<td>Virus (HIV)</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>Drugs (antineoplastic, antiplatelet,</td>
<td>Drug interruption and plasma</td>
</tr>
<tr>
<td></td>
<td>immunosuppressive drugs)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pregnancy</td>
<td>Delivery, plasma</td>
</tr>
<tr>
<td></td>
<td>Post-partum</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>Systemic diseases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lupus</td>
<td>Steroids and plasma</td>
</tr>
<tr>
<td></td>
<td>Scleroderma</td>
<td>Blood pressure control</td>
</tr>
<tr>
<td></td>
<td>Antiphospholipid syndrome</td>
<td>Oral anticoagulant agents</td>
</tr>
<tr>
<td></td>
<td>Idiopathic</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>Genetic (CFH, MCP, CFI)</td>
<td>Plasma</td>
</tr>
<tr>
<td>Familial</td>
<td>Genetic (CFH, MCP, CFI), plasma</td>
<td>Plasma</td>
</tr>
</tbody>
</table>

Complement component C3b also participates in the formation of C5-convertase and initiates the attack complex that causes cell lysis. That thin regulation is based on a certain number of membrane regulators (CR1, DAF, MCP, and Cd59) and fluid-phase regulators (Factor H) that protect the host cells.

This results in the formation of the membrane attack complex and recruitment of inflammatory cells, events that cause injury and retraction of endothelial cells, platelet adhesion and aggregation, an increase in

### Table 2  Genetic Abnormalities and Clinical Evolution of Patients with Atypical HUS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Major effect</th>
<th>Principal efeito</th>
<th>Frequency (%)</th>
<th>Short-term response with plasma therapy</th>
<th>Long-term evolution</th>
<th>Outcome of kidney transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>Factor H</td>
<td>Does not bind to endothelium</td>
<td>20-30</td>
<td>Remission rate: 60% (dose- and time-dependent)</td>
<td>Death rate or CKF: 70%-80%</td>
<td>Recurrence rate: 80%-90%</td>
</tr>
<tr>
<td>CFHR1/3</td>
<td>HR1/R3 factor</td>
<td>Antibody anti-CFH</td>
<td>6</td>
<td>Remission rate: 70%-80% (PE and immunosuppressive agents)</td>
<td>CKF rate: 30%-40%</td>
<td>Recurrence rate: 20%</td>
</tr>
<tr>
<td>MCP</td>
<td>MCP</td>
<td>Lack of surface expression</td>
<td>10-15</td>
<td>Non definitive indication for therapy</td>
<td>Death rate or CKF: &lt;20%</td>
<td>Recurrence rate: 15%-20%</td>
</tr>
<tr>
<td>CFI</td>
<td>Factor I</td>
<td>Low cofactor level or activity</td>
<td>4-10</td>
<td>Remission rate: 30%-40%</td>
<td>Death rate or CKF: 60%-70%</td>
<td>Recurrence rate: 70%-90%</td>
</tr>
<tr>
<td>CFB</td>
<td>Factor B</td>
<td>Stabilization of C3b convertase</td>
<td>1-2</td>
<td>Remission rate: 30%</td>
<td>Death rate and CKF: 79%</td>
<td>Remission in one case</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>Resistance to C3b inactivation</td>
<td>5-10</td>
<td>Remission rate: 40%-50%</td>
<td>Death rate and CKF: 60%</td>
<td>Recurrence rate: 40%-50%</td>
</tr>
<tr>
<td>TM</td>
<td>TM</td>
<td>Reduction in C3b inactivation</td>
<td>5</td>
<td>Remission rate: 60%</td>
<td>Death rate and CKF: 60%</td>
<td>Remission in one case</td>
</tr>
</tbody>
</table>
the tissue factor with binding and activation of Factor VII, and formation of thrombin and fibrin polymers. That scenario applies particularly to the glomerular capillary bed, which is a fenestrated endothelium, whose surface of the basal membrane is rich in polymers prone to Factor H1 binding, which could explain the localization of the HUS vascular injury.49,50

Membrane cofactor protein is also widely expressed in the kidneys and could be found in glomerular endothelial cells by use of immunohistochemistry analysis.48-51 It plays an important role in the protection of glomerular endothelial cells against C3 activation, because the cofactor activity in the extract of those cells was completely blocked by antibodies anti-MCP.51 Complement factor H and MCP act in conjunction to control the complement activation in host cells. Mutation in CFH and in MCP results in complement activation and HUS, indicating that the functions of those complement regulators do not overlap, being both required for complement control and activation.

The lesion of D+ HUS cannot be distinguished from that of the atypical form based on histological analysis. It is characterized by thickening of the capillaries and arterioles, endothelial edema and detachment, and accumulation of proteins and cell debris in the subendothelial space. Platelet thrombi occlude the capillary lumen. Hemolysis occurs and distorted and fragmented erythrocytes are evident on peripheral blood smears. The lesions typically affect the kidneys (mainly glomeruli and arterioles), but other organs can be involved, such as brain, heart, lungs, gastrointestinal tract, and pancreas.

**Thrombotic Thrombocytopenic Purpura (TTP)**

Immediate recognition of TTP is important, because the disease responds well to treatment with PE,20 but TTP is associated with a high mortality rate when not treated. In the era preceding the effective treatment with PE, 90% of the patients with TTP died from systemic microvascular thrombosis that caused myocardial and cerebral infarction and kidney failure.19 However, recognizing TTP may be difficult because of the variety of presentations and lack of specific criteria. The only consistent abnormalities are microangiopathic hemolytic anemia, characterized by fragmentation of red blood cells, and thrombocytopenia,7 manifestations that can also occur in other conditions.

Prior to effective therapy, the diagnosis of TTP was based on the progressive appearance of the “pentad” of clinical manifestations: microangiopathic hemolytic anemia, thrombocytopenia, renal and neurological abnormalities, and fever. However, the recognition of the efficacy of the therapy with PE resulted in the adoption of more comprehensive diagnostic criteria so that treatment could be initiated faster. A randomized study has shown the efficacy of the therapy with PE only for microangiopathic hemolytic anemia and thrombocytopenia with no apparent alternative cause; the frequency of neurological and renal abnormalities and fever was smaller than that in previous studies.20 Thrombotic thrombocytopenic purpura occurs primarily in adults. Children with microangiopathic hemolytic anemia, thrombocytopenia, and kidney failure are considered to have HUS.52,53 That is typically preceded by abdominal pain and diarrhea, and has been recognized since 1983 as a complication of infection caused by the Stx-producing bacterium, *E. coli* O157: H7. Currently, 91% of the children with typical HUS survive with support care, without PE treatment.

Those observations have suggested that TTP and HUS are two distinct syndromes,1 an interpretation supported by studies that found a severe deficiency (<5% of activity) in the vWF-cleaving protease, the ADAMTS13, in patients diagnosed with TTP, but not in patients with HUS. ADAMTS13 cleaves the large vWF multimers, which are synthesized and secreted by endothelial cells. When ADAMTS13 is not present, the result is an abnormal formation of large vWF multimers in the plasma and an increased ability to react with platelets and cause disseminated platelet thrombi, characteristic of TTP.1

However, in fact TTP and HUS are not distinct syndromes, since their essential diagnostic criteria – microangiopathic hemolytic anemia and thrombocytopenia – are the same. Neurological abnormalities are commonly considered as characteristic of TTP, and kidney failure as characteristic of HUS. Sometimes, patients with those syndromes have none of those abnormalities or have both.59 In addition, the name of the syndrome – TTP or HUS – has acquired clinical importance, due to the suggestion that treatment with PE can be appropriate to TTP, but not to HUS.53 Thus, the term “TTP” is used to describe microangiopathic hemolytic anemia and thrombocytopenia occurring in adults, with no apparent alternative cause, and with or without associated abnormalities, conditions or causes.

Thrombotic thrombocytopenic purpura is rare in children; in adults, it affects mainly women, black individuals, and obeses.31

The role of measuring the activity of ADAMTS13 and inhibitors remains uncertain.31
The vWF multimers are produced inside megakaryocytes and endothelial cells and stored in granules inside the platelets and in the Weibel-Palade bodies of endothelial cells. When compared to plasma ones, such large multimers bind more efficiently to the glycoprotein Iba component of the platelet glycoprotein Ib/IX/V receptor for vWF. This may be due to the fact that the binding site for the Iba glycoprotein in the vWF monomeric subunit is more exposed in large multimers than in small ones, which are usually circulating. The initial attack of a small amount of large vWF multimers against Iba glycoprotein, and subsequent formation of the IIb/IIIa glycoprotein complex of activated platelet by ADP, induces in vitro platelet aggregation in the presence of increased shear stress.22,54

Plasma vWF metalloproteinase usually prevents the entry of large vWF multimers in the circulation (or persistence). That enzyme breaks down the multimers by cleaving the peptide bindings in the vWF monomeric subunit in position 842–843 (between tyrosine and methionine). The metalloproteinase is called ADAMTS13 (disintegrin and metalloproteinase with thrombospondin-1-like domain), a member of the family of the calcium and zinc-dependent proteases. ADAMTS13 has a sequence arginine-glycine-aspartate, is mainly produced in hepatocytes, and its gene is located in chromosome 9q34.55

The large vWF multimers are probably cleaved by ADAMTS13 directly on the surface of endothelial cells. The thrombospondin-1-like domain of ADAMTS13 can bind to the enzyme in the thrombospondin receptor on the surface of endothelial cells.56

Severe deficiency of ADAMTS13 can be caused by genetic mutations (ADAMTS13: AC) or by autoantibodies (ADAMTS13: INH) acquired against that enzyme. In 1994, Tandon et al.57 reported that approximately 80% of the patients with acquired TTP had autoantibodies against CD36. In 2009, Davis et al.58 showed that ADAMTS13 binds especially to CD36 in vitro. CD36 is expressed in endothelial cells, platelets, and monocytes, and binds to thrombospondin 1. It has not been clarified whether the autoantibodies block the ADAMTS13 binding to endothelial cells, but this is the only way of interfering with efficient cleavage of vWF by ADAMTS13, resulting in TTP.

**Diseases that can mimic the clinical findings of TTP**

Because the diagnostic criteria of TTP are not specific, multiple systemic diseases can mimic TTP, resulting in misdiagnosis. The systemic diseases include disseminated malignant neoplasias,69,70 systemic infections,61-63 malignant hypertension,64,69 systemic lupus erythematosus, and other kidney diseases.69 In addition, physicians should maintain continued vigilance regarding the existence of possible alternative diseases, even after establishing the diagnosis of TTP, and starting treatment with PE.

**Treatment of D+ HUS**

Correction with isotonic saline or ringer lactate to prevent oliguria on the first four days, as well as the correction of electrolytic abnormalities by use of dialysis seem to play a relevant role in survival in cases of D+ HUS in the short run.65 The use of analgesics, anti-inflammatory and antimotility agents should be avoided; morphine and acetaminophen have good effects. Approximately 80% of the patients need blood transfusion because of symptomatic anemia. Platelet transfusion is indicated for important hemorrhages or procedures.

The use of antibiotics that interact with bacterial DNA, such as gyrase inhibitors (fluoroquinolones), alkylating agents (mytomycin C, TMP/SMX), and β-lactam antibiotics induce lysogenesis of the bacteriophage, with an increase in Stx expression. Ikeda et al.66 have shown that fosfomycin administered on the second day of diarrhea protects against the development of HUS caused by *E. coli* O157:H7.

Oral administration of the first generation of the Gb3 analogue - Synsorb-Pk67 - when initiated after the diagnosis of HUS failed to improve the course of the disease in a randomized study.

**Treatment for atypical HUS**

Despite the poor prognosis of atypical HUS, after the introduction of the therapy with plasma, the mortality rate dropped from 50% to 25%.13,68-70 However, the effectiveness of the use of plasma in the treatment of acute episodes is still controversial.71 Some studies have shown that a consistent percentage of patients with atypical HUS respond to treatment with plasma. Plasma exchange has been proposed to be relatively more effective than plasma infusion, because it should remove potentially toxic substances from the circulation of patients.

Treatment with plasma should be initiated within 24 hours from presentation, since its delay can increase the frequency of therapeutic failure.23

Other treatments, such as antiplatelet agents, prostacyclins, heparin or fibrinolytic agents, steroids, and immunoglobulins, have been tested, and the results have been inconsistent.13
Diagnostic and Prognostic Role of Measuring ADAMTS13

Measuring ADAMTS13 may not guarantee initial diagnosis and therapeutic decision, but it is important for the prognosis. Even if most patients with severe ADAMTS13 deficiency do not have kidney failure, measuring its activity can distinguish TTP from HUS. Some patients with severe ADAMTS13 deficiency can have acute kidney failure. An ADAMTS13 activity < 5% seems to be specific for TTP, but it does not identify all patients who can relapse; ADAMTS13 activity < 10% essentially identifies all patients at risk for relapsing, but it is not specific for that purpose; patients with sepsis and liver cirrhosis can also have ADAMTS13 activity < 10%.

Treatment with “Plasma Exchange”

Plasma exchange is the essential treatment for all patients diagnosed as having TTP, with or without kidney failure, but the number of PE sessions required for remission is extremely variable. Before effective treatment, most survivors were children, which can reflect their inherent resistance to thrombosis, as suggested by observations that venous and arterial thromboses are rare in children.

The hypothesis that tries to explain the efficacy of PE treatment considers that ADAMTS13 deficiency is corrected by the infusion of plasma and inhibitor; autoantibodies are removed by use of apheresis, resulting in recovery of ADAMTS13 activity. However, most adult patients diagnosed with TTP do not have severe ADAMTS13 deficiency, and many seem to also respond to PE, like those presenting with bloody diarrhea or who have TTP induced by quinine. The mechanism of the possible efficiency of the PE treatment in those patients is unknown.

Even if several cases have suggested that plasma cryosupernatant, which is deficient in vWF, can be better than fresh plasma as a reposition product in PE, a small randomized clinical trial failed in the attempt to confirm that finding. Based on those observations, PE should be performed every day and maintained until platelet count returns to normal. Levels of LDH, which reflect tissue ischemia and hemolysis, are also markers of response to treatment.

Complications of the Treatment with “Plasma Exchange”

When deciding about initiating PE treatment, the potential complications and confidence in TTP diagnosis should be taken into account. Of 206 consecutive patients assessed over a period of nine years in the registry of Oklahoma, 57 (28%) had complications and the death of five (2.4%) was attributed to that therapeutic approach. The deaths were caused by hemorrhage or pneumothorax, complicating the insertion of central venous catheter (two patients), or sepsis attributed to central venous catheter (three patients). Two other patients had cardiac arrest with pulse electrical activity: one caused by anaphylactic reaction to plasma and the other caused by cardiac tamponade related to catheter insertion.

Adjunctive Treatment

There is evidence that the PE treatment would have only a transient effect on the presumably autoimmune basis of the disease, and that additional immunosuppressive treatment could lead to a longer-lasting response.

Treatment with immunosuppressive agents is reserved for patients suspected of having ADAMTS13 autoimmune deficiency. Corticoids are the immunosuppressive agents initially administered. Others agents, such as rituximab and cyclosporine, are used for more critically-ill patients. Aspirin is not used as adjuvant treatment, but is appropriate for patients with standard cardiologic or neurological indication and without severe thrombocytopenia.

Mortality

Despite the institution of treatment considered optimal, mortality among patients with TTP remains approximately 15%. However, 50% of the deaths can be attributed to complications from the treatment with PE or hospitalization, such as sepsis, hemorrhage, and thrombosis.

Treatment of the Patients Who Achieved Remission

In patients with severe ADAMTS13 deficiency, the risk of relapse is approximately 40%, but it is rare in patients with less severe deficiency; 50% of these patients can relapse, most within one year. The value of maintaining the immunosuppressive treatment or of measuring ADAMTS13 activity during remission is unknown. Patients can have severe ADAMTS13 deficiency for many years with no clinical evidence of TTP. In continuing with treatment, it is paramount to insist with the patient to immediately have a platelet count, when any acute symptom occurs.

Because many young women develop TTP associated with pregnancy, the risk of relapse with a future pregnancy becomes a concern. Follow-up study of 30 pregnancies in 19 women who had TTP revealed that most subsequent pregnancies were not affected.
LONG-TERM OUTCOME
Even if relapse is a major problem, it is not the only one. After recognizing the disease, patients have a significantly altered quality of life, with progressive memory loss and fatigue.92

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