

Controversies in Differentiating Thrombotic Thrombocytopenic Purpura and Hemolytic Uremic Syndrome

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Abstract

The diagnoses of thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) often remain questionable, forcing the clinician to make the difficult decision of initiating therapy based on symptomatology and clinical judgment and, sometimes, instinct. An increased awareness of characteristic symptoms and early diagnoses of TTP and HUS are of utmost importance, given the excellent results obtained with prompt plasma exchange therapy. Tremendous progress has been made in understanding TTP and HUS since TTP was first described more than 75 years ago at Mount Sinai. However, several questions are still not definitively answered. In this article, we will review background on both entities, and then describe the controversy in differentiating between them.

Key Words: Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome.

IN 1924, DR. ELI MOSCHCOWITZ, of The Mount Sinai Hospital, published the first case study of a young woman with “an acute febrile pleomorphic anemia with hyaline thrombosis of the terminal arterioles and capillaries”; she died after two weeks (1). Her illness was characterized by fever, bleeding, neurologic and renal abnormalities and a microangiopathic hemolytic anemia. This syndrome subsequently became known as Moschcowitz’s disease, or “thrombotic thrombocytopenic purpura (TTP).” Thirty years later, a similar illness was described in children and named hemolytic uremic syndrome (HUS) (2).

The clinical and pathophysiologic relationship between these two entities has been discussed and debated for more than 40 years. In

this article, we will review some background on both entities, and then describe the controversy in differentiating between them.

Epidemiology

In the United States, approximately 1,000 new cases of TTP are diagnosed each year, with women affected twice as often as men. Most patients range in age from 20–60; the disease is uncommon in the elderly and in infants. Although the majority of patients with TTP have no known clearly associated risk factor, it may occur in the third trimester of pregnancy or as an adverse reaction to some medications, and has been associated with autoimmune disorders (3).

There are two primary types of TTP: the single acute episode and chronic relapsing TTP. The acute episode is much more common and severe. Two-thirds of patients experience a single episode which does not recur after recovery. For the remaining one-third, the disease recurs intermittently. Chronic relapsing TTP is a much rarer entity, with approximately 25 patients in all of North America in 1996. It occurs with frequent episodes at regular intervals, every 3–4 weeks, and usually occurs in children (4).

Two types of HUS have been described (5). Diarrhea-associated HUS occurs most com-

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monly in children epidemically in the first two years of life, and it is usually self-limited. The majority of these cases have been associated with a bloody diarrhea prodrome secondary to *Escherichia coli* 0157:H7. Sporadic, nondiarrhea-associated HUS is seen more commonly in adults and is sometimes associated with a mild viral prodrome. We will focus more on this nondiarrhea type of HUS, as this is the entity which is difficult to distinguish from TTP.

TTP is characterized by the typical findings of severe thrombocytopenia (usually less than 10,000 platelets/mm³ in acute episode), hemolytic anemia, schistocytosis, and polychromasia; central nervous system ischemia and neuropathy, may range in severity from transient bizarre mentation and behavior to sensory-motor deficits, aphasia, seizures, or coma; fever, which occurs in only a minority of patients; and renal abnormalities, with hematuria and/or proteinuria. Renal failure is rare. Symptoms of ischemia in the gastrointestinal circulation, especially abdominal pain, have been recognized with increasing frequency (3).

The nondiarrhea form of HUS is defined by the triad of acute renal failure, intravascular hemolysis, and thrombocytopenia. Severe renal dysfunction is a prominent feature and often requires dialysis. Thrombocytopenia, hemolytic anemia, and LDH elevations are present but are usually less profound in HUS than in TTP. In addition, HUS is frequently associated with hypertension. Mortality for HUS is much lower than for TTP (5).

Because TTP can sometimes involve severe renal disease and HUS can involve extrarenal disease, the two disorders can be especially difficult to distinguish. Frequently, the same patient will be described as having HUS by nephrologists and TTP by hematologists.

Pathophysiology

The basic pathologic processes of TTP and HUS have been proposed to involve endothelial cell injury and the subsequent release of platelet-aggregating substances which result in the formation of thrombotic lesions in terminal arterioles and capillaries. The initial endothelial cell damage may involve apoptosis. Mitra et al. have shown that plasma from patients with TTP and nondiarrhea HUS induced apoptosis in human microvascular endothelial cells (6). Another group of investigators also provided support for this mechanism in demonstrating apoptosis of endothelial cells in spleens removed from patients with TTP (7).

The specific underlying cause of these thrombi has not been established. Various platelet-aggregating substances have been proposed and will be reviewed in this paper. These microvascular thrombi, which Moschowitz described in his 16-year-old patient as "hyaline," were subsequently described by Asada and his group by immunohistochemical studies to be composed of aggregated platelets with an abundance of von Willebrand factor (vWF), but containing little fibrin (8).

The lesions of TTP are seen most commonly in the microvasculature of the brain, kidney, pancreas, heart, spleen, and adrenal glands, but can be found throughout the body, though less commonly in the lung or liver. The partial and complete occlusions in many organs, leading to organ failure, along with the consumptive thrombocytopenia, result in the clinical manifestations of TTP.

Although it is generally accepted that the thrombotic lesions give rise to the characteristic manifestations of TTP, the cause of the lesions remains unclear. They are believed to be the result of intrusion into the circulation of one or more platelet-aggregating substances. It is thought that platelet-aggregating factor p37, reported to induce clumping by binding to platelet glycoprotein IV (9), is derived from an infectious agent, and that normal patients and patients with TTP in remission develop antibodies to this infectious agent (10).

Calpain, a calcium-dependent cysteine protease (11), and cathepsin, a non-calcium-dependent cysteine protease (12), are thought to be released from either injured tissue or endothelial cells. Calpain is reported to proteolyze vWF multimers into fragments that can agglutinate platelets (11). This would, however, be unusual, given that proteolysis of vWF multimers into small fragments is commonly associated with loss of vWF-regulated platelet aggregation. vWF multimer abnormalities have recently been a focus of intense investigation in this disorder.

von Willebrand Factor

vWF monomers (270 kDa) form multimers of varying sizes via disulfide bridges (13). vWF multimers are produced within endothelial cells and megakaryocytes and are stored within the Weibel-Palade bodies of endothelial cells, which are the predominant source of vWF multimers, and the alpha granules of platelets.

Unusually Large vWF Multimers

In 1982, Moake and colleagues described unusually large multimers of vWF (ULvWF) in the plasma of patients with chronic relapsing TTP. They proposed that endothelial cells secrete these large multimers, which are even larger than the largest vWF multimers found in normal plasma. These ULvWFs were secreted retrograde into the subendothelium, and promoted platelet adhesion after vascular injury (14). A vWF-cleaving protease responsible for cleaving the large vWF multimers into the sizes seen in normal plasma has been identified in the platelet-poor plasma cryosupernatant portion of normal plasma. Deficiency or absence of this protease has been reported in TTP patients, but not in HUS patients (15). Fluid shear stress also appears to play a role in inducing platelet aggregation (16) (Table 1).

In 1989, Frangos et al. found a substance in cryosupernatant capable *in vitro* of reducing reversibly the size of ULvWF multimeric forms released by endothelial cells in culture into smaller vWF multimers ordinarily found in the circulation (17). This activity had characteristics of a limited disulfide bond reductase.

The mechanism was further clarified by Tsai in 1996 (18) and Furlan in 1997 (19), when they independently described a metal-containing proteolytic enzyme (metalloproteinase) in normal plasma, which could cleave the peptide bond between tyrosine at position 842 and methionine at position 843 in the monomeric vWF subunits, thereby degrading large multimers. This metalloproteinase can cleave vWF multimers *in vitro* if the forms are partially unfolded mechanically (as by shear stress) (20) or chemically (as by guanidine HCl) (18), or due to other chaotropic conditions such as low ionic strength or urea in the presence of divalent cations (19).

Hence, it is proposed that there is tandem action of the ULvWF reductase followed by a vWF metalloproteinase *in vivo*. It was proposed that the proteolysis prevented the reformation of ULvWF forms, which may occur *in vivo* in the absence of vWF metalloproteinase activity.

TABLE 1

Abnormalities of vWF Metabolism Proposed for TTP

Unusually large vWF multimers
Defective processing of multimers
Fluid shear stress

There is biochemical precedent for analogous tandem sequences of reductase-protease activities in human biology. In 1977, Stathakis et al. in Australia described the production of angiotensin by a disulfide bond reductase. Plasmin autoproteolysis follows and results in angiotensin fragments (21).

An important finding suggesting that the metalloproteinase has a role in normal physiology is that cleavage *in vitro* of vWF and ULvWF multimers results in the generation of 176 kD and 140 kD vWF fragments identical to those found in normal plasma (18).

Presumably in a patient with TTP, the high levels of shear stress caused by platelet thrombi in the circulation cause vWF unfolding and hence proteolysis. Therefore, the persistence of the ULvWF multimers in patients with acute TTP remains to be explained. A reasonable explanation to be offered is a defect in the proteolysis of the ULvWF which is the focus of the next series of investigations.

Defective Processing of Multimers

A potentially important finding was made in 1997, when Furlan et al. described 4 patients with chronic relapsing TTP who had deficiency of vWF-cleaving protease activity in plasma (22). This supported the hypothesis that the ULvWF multimers are responsible for the formation of platelet aggregates in the circulating blood. Because no inhibitor of the enzyme was found, the deficiency was thought to be congenital.

In 1998, two different groups, one led by Furlan (15) and another led by Tsai (23), published data on the pathogenesis of the more common type of TTP, characterized by a single acute episode. Tsai's evidence showed von Willebrand factor-cleaving protease deficiency in acute TTP. Inhibitors to the protease were found in 67% of plasma samples from the acute phase of disease. Tsai provided evidence, using affinity chromatography, that the inhibitor is an immunoglobulin G (IgG) antibody.

In their 1998 work, Furlan and associates presented a key finding regarding the inhibitor (15). They show the activity of vWF-cleaving protease and the level of its inhibitor in patients with nonfamilial TTP and HUS, and familial TTP and HUS. Plasma samples were collected during the acute event and in remission.

Most of the patients with acute nonfamilial TTP have some protease inhibitor preventing the breakdown of the multimers into smaller

fragments, while in remission, they have some protease activity. In contrast, in the group of patients with nonfamilial HUS, there is no detectable inhibitor, consistent with breakdown of multimers. Patients with familial TTP were all found to have a protease deficiency of less than 5% of normal activity, but no inhibitor. In familial HUS, again, normal protease levels were found, without evidence of an inhibitor. Data from these two studies warrant further investigation into the role of protease level measurement in differentiating TTP from HUS. Recently, the von Willebrand factor-cleaving protease has been identified to be a marker of the ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 motif) zinc metalloprotease family, and is more specifically called ADAMTS13 (24). Several mutations of ADAMTS13 have been reported and found to have clinical correlation. Homozygous or doubly heterozygous ADAMTS13 mutations were found in patients clinically affected with TTP (25).

A link between the TTP and altered immune reactivity has been proposed, but as of yet, the reasons for the transient autoantibody production and the selective antigenic targeting of the protease are not known (26–28).

Ticlopidine, a platelet aggregation inhibitor, has also been associated with TTP, and it has recently been found that patients with ticlopidine-associated TTP have also acquired antibodies to the metalloproteinase (29).

Despite the progress made in understanding the pathophysiology of TTP, the complexity of this mechanism is attested to by the work of Moore et al. (30). In their study, they find that abnormalities in vWF-cleaving protease activity occur not only in a significant portion of patients with TTP, but also in patients with other thrombocytopenic disorders such as idiopathic thrombocytopenic purpura, disseminated intravascular coagulation, and systemic lupus erythematosus. They concluded that abnormalities of vWF protease activity do not correlate completely with the presence of TTP.

Shear Stress

How do the ULvWF multimers aggregate platelets? Fluid shear stress (i.e., frictional forces generated by the relative parallel motion between fluid planes during flow) in the microcirculation may be important during episodes of TTP in promoting attachment of ULvWF multimers to shear-altered vWF receptors on

platelets, which results in platelet aggregation (16).

Increased shear stress has been shown to promote platelet aggregation *in vitro* by stimulating ULvWF multimer binding to shear-altered glycoprotein Ib (GPIb) component of platelet GPIb-IX-V receptors. These multimers subsequently bind to GPIIb/IIIa on adenosine diphosphate (ADP)-activated platelets (31). Platelet aggregation requires binding of the multimers to both types of platelet receptors. Presumably, after small thrombi are formed in the microcirculation of TTP patients, there is further increased shear stress on the platelets in flowing blood and hence increased platelet aggregation (16, 31).

Chow et al. (32) performed flow cytometry on platelet aggregates from samples obtained from healthy and TTP patients. It was shown that after applying 90 dynes/cm² shear stress for 1 minute, there was an increase in both size and complexity, presumably representing the platelet aggregates. A study of a TTP patient showed that the platelet aggregation during the acute episode disappeared when the patient recovered. As mentioned earlier, Asada and his group performed immunohistochemical studies on these platelet aggregates which occluded microvessels and found them to contain vWF (8).

In summary, the pathophysiology of TTP appears to involve the ULvWF polymers synthesized by endothelial cells (33). Normally, these large vWF multimers undergo proteolysis in the high shear environment of the arterial circulation by the enzyme vWF metalloproteinase (top). Proteolysis reduces the size of the polymers and consequently inhibits their binding to platelet glycoprotein (GP) Ib/IX/V and GP IIb/IIIa receptors. In both forms of TTP, ULvWF polymers accumulate in the circulation, bind to both types of platelet vWF receptors under high-flow conditions, and cause the systemic platelet aggregation responsible for the arterial ischemic symptoms characteristic of the disorder.

Hemolytic-Uremic Syndrome

The great majority of cases of HUS are associated with a bloody diarrheal prodrome. The nondiarrhea-associated HUS, occurring predominantly in adults, can be idiopathic, but in some instances has been found to be associated with HIV, bone marrow transplant, cancer and autoimmune diseases, or can be associated with cyclosporine, mitomycin, and other chemotherapeutic agents.

Pathophysiology

In the diarrhea-associated HUS, the causative agent has been identified as an *E. coli* which produces shiga-like toxins (verotoxins 1 and 2), the most common serotype being 0157:H7. *Shigella dysenteriae* is the other significant diarrhea-producing organism associated with HUS which produces shiga toxin.

Endothelial cells are the primary targets for shiga toxins (5, 34, 35). The verotoxins bind through their B subunits to the terminal trisaccharide linkage of globotriosyl ceramide (Gb3), the predominant membrane receptor for shiga toxin on the endothelial cell (5). The A subunit is then internalized by endocytosis and following proteolysis yields an enzyme capable of cleaving an N-glycoside bond resulting in removal of an adenosine nucleotide from the 28S ribosomal RNA that makes up 60S ribosomal subunits (5, 36, 37). This renders the ribosome inactive, suppressing protein synthesis leading to endothelial cell death. Gb3 expression is much greater in renal cells than in other cells, which probably accounts for the extensive renal disease occurring in HUS.

In both forms of HUS, it is thought, bacterial toxins cause endothelial cell toxicity and lead to organ damage. The endothelial cells become separated from the basement membrane and platelets are activated by contact with exposed collagen. Platelet aggregation occurs, aided by release of ULvWF multimers, which are also involved in HUS (38), reduced prostacyclin levels, which have anti-aggregating properties, and increased production of thromboxane, a platelet-aggregating agent produced by activated platelets (39, 40). Capillary and arteriolar lumina are narrowed by swollen endothelial cells, thrombi, and other vasoactive substances released by platelet and endothelial cells. These effects result in subsequent reduced kidney function (35).

Morigi et al. recently provided evidence supporting the role of verotoxin 1 (VT-1) in directly inducing platelet adhesion and thrombus formation on endothelial cells under high shear stress and showed that microvascular endothelial cells are significantly more sensitive to this thrombogenic effect of VT-1 than large vessel endothelial cells (41). In addition, they demonstrated the vital role of endothelial adhesion molecules in thrombus formation.

Treatment

Before the introduction of exchange plasmapheresis and infusion, TTP was fatal in 80% of cases. It is thought that plasma infusion replaces the metalloproteinase, while the exchange removes the autoantibody and possibly also the vWF polymers. Restoration of processing of ULvWF multimers was demonstrated in chronic relapsing TTP by infusion of fresh-frozen plasma (FFP) (42); cryosupernatant, which is the cryoprecipitate-depleted fraction of FFP that contains the metalloproteinase without the largest vWF polymers (42); or solvent/detergent-treated plasma which renders a potentially less infectious preparation (16). It has now been shown that these plasma preparations contain the vWF-cleaving protease (15, 23). If plasmapheresis is not immediately available, infusion of normal FFP can be used until plasmapheresis is arranged. Steroids have been shown to be effective (43), presumably in suppressing autoantibody production in acute TTP or TTP associated with ticlopidine.

Splenectomy was described by Cuttner in 1974 and has shown some success, especially in patients with frequent relapses, presumably because the spleen is a major site of autoantibody production (44). However, splenectomy can be very dangerous in these patients, given their profound thrombocytopenia and risk for severe hemorrhage.

Platelet transfusion should be avoided except for a life-threatening bleeding episode, since such treatment has been associated with worsening renal and neurologic status (45).

Suppressing autoantibody production with azathioprine or cyclophosphamide in patients with refractory, single episodes of TTP has shown some success (4, 46). There is some evidence that vincristine can also be helpful, as it depolymerizes platelet microtubules and may produce unfavorable vWF binding conditions by altering the GPIb-IX-V and/or GPIIb-IIIa receptors on platelet surfaces (47). Treatment options for TTP and HUS are summarized in Table 2.

Although the diarrhea-associated HUS is self-limited, patients with HUS require early and careful management of acute renal failure with fluid and electrolyte balance. Blood transfusions and hemodialysis are frequently required. In the adult form of HUS, FFP is recommended in addition, especially if the patient complains of neurologic symptoms. The use of FFP has been tried with variable success for HUS patients, although it remains unclear what its mechanism of action is.

TABLE 2
Treatment Options

TTP
Exchange plasmapheresis
Cryosupernatant
Glucocorticoids
Splenectomy
Immunosuppression
Vincristine
HUS
Management of acute renal failure
Blood transfusion
Plasmapheresis

TABLE 3
Differentiating TTP and HUS

Similarities
· Clinical symptoms
· Labs (thrombocytopenia/hemolytic anemia/renal dysfunction)
· Endothelial cell damage/vWF multimer release/platelet thrombi
Differences
· Protease deficiency/inhibitor in TTP vs normal protease levels in HUS

Differentiating Between TTP and HUS

TTP and HUS are two entities with similarities in clinical symptoms and laboratory results, and in the fundamental mechanism of endothelial cell damage/loss of antithrombotic properties/resulting platelet thrombi formation, making it difficult to differentiate between them.

Although the precise mechanism by which each entity results in these thrombi remains somewhat elusive, evidence points toward involvement of ULvWF multimers in the pathogenesis of both TTP and HUS. TTP appears to involve defective von Willebrand factor-cleaving protease activity secondary to either an autoimmunity or deficiency, while HUS has been shown to have normal protease activity. Thus, further investigation of the predictive value of protease level measurement, which may enable physicians to distinguish between TTP and HUS, is warranted.

The finding of the protease difference may explain why plasma exchange therapy is more effective in TTP than in HUS. However, plasma exchange is not always effective in patients with an initial acute episode of TTP, possibly because more inhibitor is produced for a longer time, in which case more efficient techniques to remove autoantibody may increase survival. The important question is: Are TTP and nondiarrhea-associated HUS manifestations of one disease, representing different points along the same clinical spectrum, or are they indeed two distinct entities? (See Table 3.)

Future Therapies

It has been suggested that blockade of platelet receptors GPIb and GPIIb/IIIa (48–50) may be part of the future therapy of these enti-

ties, as binding to both receptors is required for initiation of the platelet-aggregating process. At present, two monoclonal antibodies which can block vWF binding to GPIIb/IIIa are abciximab, which is used in angioplasty, and integrilin, which is a polypeptide derivative. These blockers may offer a therapeutic alternative for those patients with acute TTP who do not respond to FFP. However, these antibodies pose a potentially serious danger in that the patients who do not respond to plasma or steroids are generally extremely thrombocytopenic. Thus, using an agent which reduces the function of the few remaining platelets may cause fatal hemorrhage. These agents have thus far not been used in the treatment of TTP or HUS.

Currently, studies to develop an agent that can block the binding of large vWF multimers to platelet GPIb receptor are underway (48, 51). Hemorrhage is a complication of these compounds in severely thrombocytopenic patients and therefore initial clinical trials will have to be limited to patients with refractory TTP unresponsive to standard therapy.

Synthesis of the metalloproteinase for replacement may be another therapeutic option for patients with recurrent TTP. And finally, inhibitors of apoptosis may also play a therapeutic role in the treatment of TTP and HUS.

Summary

In summary, an increased awareness of characteristic symptoms and early diagnosis of TTP and HUS are of utmost importance, given the excellent results obtained with prompt plasma exchange therapy. Before the introduction of plasma exchange, the prognosis for these disorders was extremely poor, whereas now survival reaches upwards of 90%. However, the diagnoses of TTP and HUS often remain questionable, forcing the clinician to

make the difficult decision of initiating plasma exchange therapy based on symptomatology and clinical judgment, and sometimes instinct. Frequently, it is possible to confirm the diagnosis only after initiating plasma exchange and seeing improvement in the patient's status. The quality of supportive measures has also contributed to the great difference in clinical outcome. However, since the acceptance of plasma therapy as the main treatment modality, there have been no further significant advances in the clinical management of TTP and HUS.

Because of the low incidence of these diseases, it has been difficult to perform prospective randomized trials of different treatment regimens. Thus, recommendations are based on reports of a limited number of patients, and anecdotal reports. It remains unclear how long plasma exchange therapy should continue. There are also no clear data on supportive measures most likely to be beneficial.

Tremendous progress has been made toward understanding TTP and HUS since TTP was first described at Mount Sinai more than 75 years ago. However, several questions remain. Are TTP and nondiarrhea-associated HUS actually the same disease entity? Can vWF-cleaving protease activity measurement predict which patients will benefit most from plasma exchange? What initiates apoptosis of endothelial cells in TTP, and are there other mechanisms of endothelial cell damage, for example, microbial infections? What is the precise sequence of events leading from endothelial cell damage to thrombotic microvessel obstruction in both TTP and HUS? What is the long-term outcome of patients after recovery from an episode of TTP or HUS, and what is the risk of relapse? Further efforts in understanding the pathophysiology, and investigating novel therapeutic options by way of future clinical trials will help shed more light on these two elusive disease entities.

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