

The Role of ADAMTS13 in the Pathogenesis of Thrombotic Thrombocytopenic Purpura–Hemolytic Uremic Syndrome

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Abstract: The identification, characterization, and clinical observation of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin-1–like domains) have provided important insights into the pathogenesis of thrombotic thrombocytopenic purpura (TTP). ADAMTS13 is a plasma enzyme essential for postsecretion proteolytic processing of von Willebrand factor (VWF). Absence of ADAMTS13 is associated with the occurrence of abnormally large multimers of VWF and is also associated with the occurrence of TTP. Initial assumptions that absent ADAMTS13 was itself the etiology of TTP have been tempered by subsequent observations that ADAMTS13 activity can be severely deficient without clinical abnormalities and that patients can have characteristic clinical features of TTP without severe ADAMTS13 deficiency. A current interpretation of these observations is that ADAMTS13 deficiency is a major risk factor for the development of TTP, but it is neither always necessary nor sufficient to cause TTP. This interpretation is consistent with other vascular and thrombotic disorders in which multiple risk factors and associated conditions contribute to the etiology of acute events.

ADAMTS13, von Willebrand Factor, and Platelet Adhesion

The identification, characterization, and clinical observation of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin-1–like domains) in the past 8 years^{1–8} have provided important insights into the pathogenesis of thrombotic thrombocytopenic purpura (TTP).⁹ ADAMTS13 is a plasma enzyme that cleaves von Willebrand factor (VWF) following VWF secretion by endothelial cells. Endothelial cells are the site of VWF synthesis and most VWF is secreted into the subendothelial matrix, where it combines with collagen and provides the surface for platelet adhesion following blood vessel damage. VWF secreted into plasma does not interact with platelets unless there is platelet activation. In plasma, VWF circulates as multimers of various sizes, with larger multimers being more reactive with platelets when vessel damage and platelet activation occur. Even with normal proteolytic processing by ADAMTS13, VWF is the largest plasma protein, with strings of multimers achieving a molecular weight of up to 20,000,000 daltons. VWF multimers with linear dimensions of over 2 μm , the diameter of a normal platelet, have been visualized by electron microscopy.¹⁰ In the absence of

Keywords

ADAMTS13, von Willebrand factor, VWF, thrombotic thrombocytopenic purpura, TTP, hemolytic uremic syndrome, HUS

normal proteolytic processing of VWF by ADAMTS13, even larger VWF molecules occur that may adhere to circulating platelets and cause spontaneous aggregation.⁹

ADAMTS13 Deficiency and the Etiology of TTP

The recent observations regarding ADAMTS13 and TTP were anticipated 23 years ago by Moake and others when they observed abnormally large multimers of VWF in patients with chronic relapsing TTP.¹¹ Some of the patients in this original report¹¹ had acquired TTP; at least 1 woman had congenital TTP, a syndrome first described by Schulman and colleagues in 1960¹² and Upshaw in 1978.¹³ Moake and others recognized the relevance of these observations, since the abnormally large plasma multimers of VWF had a greater ability to interact with circulating platelets than normal sized VWF multimers and could contribute to the formation of intravascular platelet thrombi. Although these abnormal VWF multimers were larger than normal plasma VWF, they were similar in size to the VWF multimers synthesized by endothelial cells and stored in their secretory granules. Therefore Moake and others hypothesized that in TTP a plasma enzyme responsible for proteolytic modification of secreted VWF was absent.¹¹ Although evidence supporting the hypothesis of postsecretion proteolytic modification of VWF was later suggested in 1990 by Dent and others,¹⁴ it was not until 1996 that Furlan et al¹ and Tsai² isolated a VWF-cleaving protease from plasma. Subsequent developments in the ADAMTS13-VWF-TTP story occurred rapidly. The VWF-cleaving protease activity was found to be absent in patients with both acquired and congenital TTP^{3,4} and patients with acquired TTP were found to have activity in their sera that neutralized the protease activity.^{5,6} When the protease was isolated, it was found to be a member of a metalloprotease family of enzymes and characterized as ADAMTS13.^{7,8,15} Patients with congenital TTP were found to have mutations in the *ADAMTS13* gene.^{8,16-19} This rapid development of new molecular and clinical knowledge led to the compelling hypothesis that absent ADAMTS13 activity was the etiology of TTP.

This hypothesis gained further support because it provided an explanation for the effectiveness of plasma therapy in treating TTP. Prior to the systematic use of plasma therapy, the mortality rate for patients with TTP was 90%.²⁰ Initial reports in the 1970s described remissions from TTP with whole blood-exchange transfusions,²¹ plasma infusions,²² and plasma exchange.²³ Then, in 1991, Rock and the Canadian Apheresis Study Group published a landmark randomized clinical trial documenting the efficacy of plasma exchange treatment compared to plasma infusion.²⁴ In this study, 40 (78%) of

the 51 patients randomized to plasma exchange therapy survived.²⁴ These observations suggested that patients with acquired TTP, presumed to be caused by autoantibody inhibition of ADAMTS13 activity, require apheresis to remove the autoantibody and a large volume of plasma infused during plasma exchange to provide ADAMTS13 activity in excess of in vivo autoantibody neutralization.⁹ In contrast, patients with congenital TTP could be managed simply by intermittent plasma infusions to replace missing ADAMTS13 activity.^{13,25} With these observations and inferences it appeared that we now understood the cause of TTP and the mechanism of effective treatment.

Limitations of the ADAMTS13 Deficiency Hypothesis

The limitations of this hypothesis soon became apparent. Several patients with congenital absence of ADAMTS13 but no evidence of TTP have been described.²⁶ Other patients with congenital absence of ADAMTS13 do not manifest overt TTP until adulthood.^{11,26,27} Many patients with congenital absence of ADAMTS13 have long intervals between acute episodes of TTP, which may be triggered by pregnancy or acute inflammatory conditions.¹³ Patients have been described who have recovered from acquired TTP and had stable hematologic remissions for months or years in spite of undetectable ADAMTS13 activity.²⁸⁻³⁰

Initial reports suggested that ADAMTS13 deficiency distinguished TTP from hemolytic uremic syndrome (HUS), since patients described as having HUS did not have severe ADAMTS13 deficiency.^{5,31} However, interpretation of these studies is uncertain because they do not present quantitative, reproducible clinical criteria to define patients as having either TTP or HUS. Distinguishing of TTP from HUS is difficult because these syndromes have similar pathologic features (thrombotic microangiopathy^{32,33}) and identical defining clinical characteristics (thrombocytopenia and microangiopathic hemolytic anemia). Although it is commonly stated that TTP is characterized by predominant neurologic abnormalities and HUS is characterized by predominant renal failure, in clinical practice they are not distinct. Children with typical HUS, preceded by bloody diarrhea caused by enterohemorrhagic species of *Escherichia coli* such as *E. coli* 0157:H7, do have predominant renal failure, but 15–25% of children with typical HUS also have severe neurologic abnormalities.³⁴⁻³⁶ TTP and HUS are not distinct syndromes in adult patients; many have both severe neurologic abnormalities and acute renal failure, or neither.³⁷ Furthermore, many patients with severe ADAMTS13 deficiency have no neurologic abnormalities and some patients with severe ADAMTS13 deficiency have acute

renal failure.^{37,38} Although acute renal failure only rarely occurs in patients with severe ADAMTS13 deficiency, patients without severe ADAMTS13 deficiency have the same frequency of severe neurologic abnormalities and the same severity of thrombocytopenia and hemolytic anemia as those with severe ADAMTS13 deficiency.³⁷ Thus, the inability to distinguish the clinical syndromes described as TTP and HUS in adults and the similar clinical features of adult patients with or without severe ADAMTS13 deficiency support the use of the comprehensive term TTP-HUS to describe the adult syndromes.^{37,39,40}

Additional observations that the correlation between severe ADAMTS13 deficiency and the occurrence of TTP is not perfect is that the frequency of severe ADAMTS13 deficiency is extremely variable in case series of adults with TTP (or, as described in some case series, TTP-HUS) (Table 1).^{28,31,37,41,42} In these case series, the percentage of patients with TTP who had severe ADAMTS13 deficiency ranged from 12% to 80%. Most patients with severe ADAMTS13 deficiency were described as having idiopathic TTP. In 2 of these case series, patients with idiopathic TTP were distinguished from patients who had a prior stem cell transplant procedure, in whom TTP was associated with pregnancy, in whom there was a potential drug-associated etiology, in whom there was a prodrome of bloody diarrhea, or who had an additional diagnosis (such as systemic lupus erythematosus) or were subsequently discovered to have an alternative etiology (such as sepsis or disseminated malignancy). But even in this restricted category of patients, the frequency of severe ADAMTS13 deficiency ranged from 30%³⁷ to 80%.²⁸ These observations emphasize the diversity of syndromes described as TTP or TTP-HUS.

Only undetectable or severely deficient ADAMTS13 activity (<5% of normal) is considered to be specific for TTP.⁴³ But many patients in whom the diagnosis of TTP is considered have moderate deficiencies, 5–25% of normal. In some patients with TTP, a severe deficiency of ADAMTS13 may be masked by prior transfusions, since the half-life of ADAMTS13 in plasma is 2–3 days.^{25,44} In addition, patients with acute multisystem disorders that may mimic TTP-HUS can have reductions in deficiencies of ADAMTS13 activity (Table 2). In patients with severe sepsis or acute flares of systemic lupus erythematosus, levels of ADAMTS13 activity may be perilously close to the levels described as specific for TTP (ie, <5%).⁴³ It may be that any acute illness resulting in increased levels of plasma VWF will be associated with a correspondingly decreased levels of ADAMTS13 activity.^{45,46}

The hypothesis that severe ADAMTS13 deficiency is the etiology of TTP has been tested in an experimental mouse model. ADAMTS13-deficient mice were generated by gene targeting to remove exons 1–6, which encode

Table 1. Frequency of Severe ADAMTS13 Deficiency Among Patients with TTP

| Case Series | Total Patients | Patients with Severe ADAMTS13 Deficiency* |
|-------------------------------|----------------|---|
| Veyradier et al ³¹ | 63 | 44 (70%) |
| Mori et al ⁴¹ | 18 | 12 (67%) |
| Raife et al ⁴² | 107 | 50 (47%) |
| Zheng et al ²⁸ | | |
| All patients | 37 | 16 (43%) |
| Patients with idiopathic TTP | 20 | 16 (80%) |
| Vesely et al ³⁷ | | |
| All patients | 185 | 22 (12%) |
| Patients with idiopathic TTP | 167 | 20 (30%) |

* Severe ADAMTS13 deficiency is usually defined as <5% of normal activity.

TTP = thrombotic thrombocytopenic purpura.

Table 2. ADAMTS13 Activity in Patients with Acute Multisystem Disorders that May Mimic TTP

| Disorder | ADAMTS13 Activity |
|---|-------------------|
| Sepsis ^{37,40} | 6–100% |
| Systemic lupus erythematosus ^{37,40} | 7–100% |
| HELLP syndrome ^{67*} | 12–48% |

* An acronym for an obstetric complication manifested by hemolysis, elevated liver function tests, and low platelet counts.

TTP = thrombotic thrombocytopenic purpura.

most of the protease domain of *ADAMTS13*.⁴⁷ Although the resulting homozygous ADAMTS13-null mice had no specific VWF-cleaving protease activity, hematologic parameters were normal and there was no evidence for thrombocytopenia or microangiopathic hemolytic anemia. However, the ADAMTS13-deficient mice had an increased thrombotic tendency and were more susceptible to death from infusion of verotoxin-2, a bacterial toxin involved in the pathogenesis of typical childhood HUS. Verotoxin-2 infusion caused more frequent thrombocytopenia and greater mortality in the ADAMTS13-null mice than in the control mice. Peripheral blood from the ADAMTS13-null mice demonstrated microangiopathic changes following verotoxin-2 infusion. These observations support the concept that severe ADAMTS13 deficiency may not always be sufficient to cause overt TTP.

Table 3. Risk Factors and Conditions Associated with Occurrence of Acute Episodes of TTP–HUS

| |
|---|
| Major Risk Factor |
| ADAMTS13 deficiency |
| Minor Risk Factors |
| Age 20–60 years |
| Black race |
| Female sex |
| Pregnancy |
| Obesity |
| Acute illness (such as cardiac surgery, pancreatitis) |

TTP–HUS = thrombotic thrombocytopenic purpura–hemolytic uremic syndrome.

Risk Factors for the Development of TTP–HUS

Although severe ADAMTS13 deficiency may not always be sufficient to cause TTP, clinical observations and experimental data suggest that severe ADAMTS13 deficiency is the major risk factor for development of TTP–HUS (Table 3). In addition to severe ADAMTS13 deficiency, multiple other risk factors are associated with TTP. In all reported case series of TTP–HUS among adults, there are more women than men.²⁷ This is distinct from the equal occurrence of girls and boys among children with typical diarrhea-positive HUS.^{34,36} Moreover, pregnancy is clearly an additional risk factor for the development of TTP. There are reports of 15 women from 9 families with congenital ADAMTS13 deficiency who had their first episode of TTP coincident with their first pregnancy.^{11,26,27} Pregnancy as a trigger for TTP in women with congenital ADAMTS13 deficiency was emphasized in the report by Furlan and Lämmle of 2 families, each with 3 siblings who had undetectable ADAMTS13 activity.²⁶ In each of these 2 families, 2 sisters had their first episodes of TTP during their first pregnancies while a brother continued to remain asymptomatic.²⁶ Acute episodes of TTP may occur in all pregnancies in women with congenital TTP, unless intensive plasma infusion prophylaxis is provided.⁴⁸ In all case series that describe pregnancy, 79 (13%) of 601 women were diagnosed during pregnancy.²⁷ However pregnancy should not be overemphasized as a risk factor in women who have recovered from acquired TTP as typically subsequent pregnancies are not complicated by recurrent TTP.⁴⁸

Black race is another risk factor for the development of TTP. An increased relative frequency of blacks among patients with TTP has been suggested in multiple case series^{28,37,49-51} and confirmed by data from the Oklahoma TTP–HUS Registry, which documented an incidence rate ratio of black patients versus non-black patients,

standardized for age and sex, of 3.07 (95% confidence interval, 2.16–4.37).⁵² When this analysis was restricted to patients with severe ADAMTS13 deficiency, the standardized incidence rate ratio of black patients to non-black patients was even greater: 9.29 (95% confidence interval, 4.33–19.93).⁵²

Other risk factors for TTP–HUS include obesity,³⁷ bacterial infections,⁵³ cardiac surgery,⁵⁴ and acute inflammatory conditions such as pancreatitis.⁵⁵

Relation of ADAMTS13 Deficiency to Other Risk Factors for TTP–HUS

Although current observations suggest that severe ADAMTS13 deficiency is not the sole etiology for TTP–HUS, it may be related to other apparent risk factors and help provide an understanding for interactions among these risk factors.

An increased risk for acute episodes of TTP during pregnancy may be related to the increased plasma concentration of VWF that occurs during pregnancy,⁵⁶ which is associated with a corresponding decrease of ADAMTS13.⁵⁷ The higher frequency of TTP–HUS among women, especially during pregnancy, could also be related to the ability of estrogen to potentiate platelet activation and aggregation.⁵⁸ Any condition causing an increased plasma concentration of VWF, an acute-phase reacting plasma protein,⁵⁹ can potentially cause a corresponding decrease of ADAMTS13.^{45,46} Although this inverse relation of substrate and enzyme may be an *in vitro* artifact of the ADAMTS13 assay, it may also reflect *in vivo* consumption of ADAMTS13. Therefore, increased plasma concentrations of VWF and correspondingly decreased plasma concentrations of ADAMTS13 may also be involved in the acute inflammatory conditions that appear to be associated with the development of TTP–HUS.⁵³⁻⁵⁵ These acute inflammatory conditions may be similar to conditions in cell culture studies that have demonstrated the secretion of abnormally large multimers of VWF stimulated in a dose-dependent manner by interleukin-8 and tumor necrosis factor- α .⁶⁰ These experimental data suggest that inflammatory cytokines may cause the accumulation of abnormally large VWF multimers in plasma and on the surface of endothelial cells and provide a potential link between inflammation and thrombosis in the etiology of acute episodes of TTP–HUS.^{60,61}

The risk factors of female sex and black race are similar to risk factors for other autoimmune disorders, such as systemic lupus erythematosus.⁶² In addition, the increased incidence among blacks may be related to their higher levels of VWF,⁶³ which may increase their risk for VWF-mediated platelet thrombus formation in the absence of ADAMTS13.

The Clinical Course of TTP-HUS Related to Severe ADAMTS13 Deficiency

The confirmation that acquired TTP can be caused by autoantibodies has provided important information for patient management. Relapses of TTP occur almost exclusively among patients with severe ADAMTS13 deficiency,³⁸ in whom an autoimmune etiology is assumed. Not only is the risk for relapse associated with severe ADAMTS13 deficiency, the demonstration of severe ADAMTS13 deficiency associated with a high titer of inhibitory activity may predict a more prolonged and complicated course with multiple exacerbations when plasma exchange treatments are diminished.²⁸ In these patients, immunosuppressive treatment in addition to plasma exchange may be required. Although early case series reporting on patients treated with steroids⁶⁴ showed no better outcomes than case series in which none of the patients were treated with steroids,²⁴ the patients have been too heterogeneous to accurately interpret the results. In patients who have relapsed TTP, or who have a prolonged clinical course with multiple exacerbations when plasma exchange is diminished, glucocorticoid therapy is appropriate, and even more intensive immunosuppressive treatment with rituximab, cyclophosphamide, or cyclosporine may be necessary.^{28,65,66}

Conclusions

The discovery of ADAMTS13, the documentation of its role in the physiologic processing of VWF, and the demonstration of its involvement in the pathogenesis of TTP have been important advances. However, severe ADAMTS13 deficiency does not detect all patients who may be appropriately diagnosed with TTP and who may respond to plasma exchange treatment. Further clinical observations will provide better insight into the interactions of the multiple risk factors and associated conditions that can combine to cause the explosive and potentially fatal syndromes of TTP and HUS.

References

- Furlan M, Robles R, Lammle B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. *Blood*. 1996;87:4223-4234.
- Tsai H-M. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. *Blood*. 1996;87:4235-4244.
- Furlan M, Robles R, Solenthaler M, et al. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood*. 1997;89:3097-3103.
- Furlan M, Robles R, Solenthaler M, Lammle B. Acquired deficiency of von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood*. 1998;91:2839-2846.
- Furlan M, Robles R, Galbusera M, et al. Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*. 1998;339:1578-1584.
- Tsai H-M, Lian ECY. Antibodies to von-Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998;339:1585-1594.
- Zheng X, Chung D, Takayama TK, et al. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem*. 2001;276:41059-41063.
- Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*. 2001;413:488-494.
- Moake JL. Thrombotic microangiopathies. *N Engl J Med*. 2002;347:589-600.
- Fowler WE, Fretto LI, Hamilton KK, Erickson HP, McKee PA. Substructure of human von Willebrand factor. *J Clin Invest*. 1985;76:1491-1500.
- Moake JL, Rudy CK, Troll JH, et al. Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *N Engl J Med*. 1982;307:1432-1435.
- Schulman I, Pierce M, Lukens A, Currimbhoy Z. Studies on thrombopoiesis: I. A factor in normal plasma required for platelet production: chronic thrombocytopenia due to its deficiency. *Blood*. 1960;16:943-957.
- Upshaw JD Jr. Congenital deficiency of a factor in normal plasma that reverses microangiopathic hemolysis and thrombocytopenia. *N Engl J Med*. 1978;298:1350-1352.
- Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM. Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor. *Proc Natl Acad Sci U S A*. 1990;87:6306-6310.
- Plaimauer B, Zimmermann K, Volkel D, et al. Cloning, expression, and functional characterization of the von Willebrand factor-cleaving protease (ADAMTS13). *Blood*. 2002;100:3626-3632.
- Kokame K, Matsumoto M, Soejima K, et al. Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. *Proc Natl Acad Sci U S A*. 2002;99:11902-11907.
- Matsumoto M, Kokame K, Soejima K, et al. Molecular characterization of ADAMTS13 gene mutations in Japanese patients with Upshaw-Schulman syndrome. *Blood*. 2004;103:1305-1310.
- Pimanda JE, Maekawa A, Wind T, et al. Congenital thrombotic thrombocytopenic purpura in association with a mutation in the second CUB domain of ADAMTS13. *Blood*. 2004;103:627-629.
- Veyradier A, Lavergne J-M, Ribba A, et al. Ten candidate ADAMTS13 mutations in six French families with congenital thrombotic thrombocytopenic purpura (Upshaw-Schulman syndrome). *J Thromb Haemost*. 2004;2:424-429.
- Amorosi EL, Ulmann JE. Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature. *Medicine*. 1966;45:139-159.
- Bukowski RM, Hewlett JS, Harris JW, et al. Exchange transfusions in the treatment of thrombotic thrombocytopenic purpura. *Semin Hematol*. 1976;13:219-232.
- Byrnes JJ, Khurana M. Treatment of thrombotic thrombocytopenic purpura with plasma. *N Engl J Med*. 1977;297:1386-1389.
- Bukowski RM, King JW, Hewlett JS. Plasmapheresis in the treatment of thrombotic thrombocytopenic purpura. *Blood*. 1977;50:413-417.
- Rock GA, Shumak KH, Buskard NA, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. *N Engl J Med*. 1991;325:393-397.
- Barbot J, Costa E, Guerra M, et al. Ten years of prophylactic treatment with fresh-frozen plasma in a child with chronic relapsing thrombotic thrombocytopenic purpura as a result of a congenital deficiency of von Willebrand factor-cleaving protease. *Br J Haematol*. 2001;113:649-651.
- Furlan M, Lammle B. Aetiology and pathogenesis of thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome: the role of von Willebrand factor-cleaving protease. *Best Pract Res Clin Haematol*. 2001;14:437-454.
- George JN. The association of pregnancy with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Curr Opin Hematol*. 2003;10:339-344.
- Zheng XL, Kaufman RM, Goodnough LT, Sadler JE. Effect of plasma exchange on plasma ADAMTS13 metalloprotease activity, inhibitor level, and clinical outcome in patients with idiopathic and non-idiopathic thrombotic thrombocytopenic purpura. *Blood*. 2004;103:4043-4049.
- Stude J-D, Hovinga JK, Radonic R, et al. Familial acquired thrombotic thrombocytopenic purpura: ADAMTS13 inhibitory autoantibodies in identical twins. *Blood*. 2004;103:4195-4197.
- Ashida A, Nakamura H, Yoden A, et al. Successful treatment of a young infant who developed high-titer inhibitors against VWF-cleaving protease (ADAMTS-13): important discrimination from Upshaw-Schulman syndrome. *Am J Hematol*.

- 2002;71:318-322.
31. Veyradier A, Obert B, Houllier A, Meyer D, Girma JP. Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. *Blood*. 2001;98:1765-1772.
32. Laszik Z, Silva F. Hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura, and systemic sclerosis (systemic scleroderma). In: Jennett JC, Olson JL, Schwartz MM, Silva FG, eds. *Heptinstall's Pathology of the Kidney*. Philadelphia: Lippincott-Raven; 1998:1003-1057.
33. Hosler GA, Cusumano AM, Hutchins GM. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome are distinct pathologic entities: a review of 56 autopsy cases. *Arch Pathol Lab Med*. 2003;127:834-839.
34. Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med*. 1990;323:1161-1167.
35. Elliott EJ, Robins-Browne RM, O'Loughlin EV, et al. Nationwide study of haemolytic uremic syndrome: clinical, microbiological, and epidemiological features. *Arch Dis Child*. 2001;85:125-131.
36. Gerber A, Karch H, Allerberger F, Verweyen HM, Zimmerhackl LB. Clinical course and the role of Shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997-2000, in Germany and Austria: a prospective study. *J Infect Dis*. 2002;186:493-500.
37. Vesely SK, George JN, Lammle B, et al. ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. *Blood*. 2003;101:60-68.
38. Sadler JE, Moake JL, Miyata T, George JN. Recent advances in thrombotic thrombocytopenic purpura. In: Broudy VC, Berliner N, Larson RA, Leung LLK, eds. *Hematology 2004*. Washington, DC: American Society of Hematology; 2004:407-423.
39. George JN. How I treat patients with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Blood*. 2000;96:1223-1229.
40. George JN, Vesely SK, Terrell DR. The Oklahoma thrombotic thrombocytopenic purpura-hemolytic uremic syndrome (TTP-HUS) registry: a community perspective of patients with clinically diagnosed TTP-HUS. *Semin Hematol*. 2004;41:60-67.
41. Mori Y, Wada H, Gabazza EC, et al. Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of vWF-cleaving protease activity. *Transfusion*. 2002;42:572-580.
42. Raife TJ, Atkinson B, Montgomery RR, Vesely SK, Friedman K. Severe deficiency of VWF-cleaving protease (ADAMTS13) activity defines a distinct population of thrombotic microangiopathy patients. *Transfusion*. 2004;44:146-150.
43. Bianchi V, Robles R, Alberio L, Furlan M, Lammle B. Von Willebrand factor-cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. *Blood*. 2002;100:710-713.
44. Furlan M, Robles R, Morselli B, Sandoz P, Lammle B. Recovery and half-life of von Willebrand factor-cleaving protease after plasma therapy in patients with thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 1999;81:8-13.
45. Reiter RA, Knobl P, Varadi K, Turecek PL. Changes in von Willebrand factor-cleaving protease (ADAMTS13) activity after infusion of desmopressin. *Blood*. 2003;101:946-948.
46. Mannucci PM, Parolari A, Canciani MT, Alemanni F, Camera M. Opposite changes of ADAMTS-13 and von Willebrand factor after cardiac surgery. *J Thromb Haemost*. 2005;3:397-399.
47. Motto D, Zhang W, Zhu G, et al. Additional environmental and/or genetic factors are required to trigger TTP in ADAMTS13-deficient mice. *Blood*. 2004;104:77a.
48. Vesely SK, Li X, McMinn JR, Terrell DR, George JN. Pregnancy outcomes after recovery from thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Transfusion*. 2004;44:1149-1158.
49. Török TJ, Holman RC, Chorba TL. Increasing mortality from thrombotic thrombocytopenic purpura in the United States: analysis of national mortality data, 1968-1991. *Am J Hematol*. 1995;50:84-90.
50. Conlon PJ, Howell DN, Macik G, Kovalik EC, Smith SR. The renal manifestations and outcome of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome in adults. *Nephrol Dial Transplantation*. 1995;10:1189-1193.
51. Elkins SL, Wilson PP, Files JC, Morrison FS. Thrombotic thrombocytopenic purpura: evolution across 15 years. *J Clin Apheresis*. 1996;11:173-175.
52. Williams LA, Terrell DR, Lammle B, et al. The incidence of TTP-HUS: racial disparity among patients with severe ADAMTS13 deficiency. *Blood*. 2004;104:244a-245a.
53. Creager AJ, Brecher ME, Bandarenko N. Thrombotic thrombocytopenic purpura that is refractory to therapeutic plasma exchange in two patients with occult infection. *Transfusion*. 1998;38:419-423.
54. Naqvi TA, Baumann MA, Chang JC. Post-operative thrombotic thrombocytopenic purpura: a review. *Int J Clin Pract*. 2004;58:169-172.
55. Boyer A, Chadda K, Salah A, Bonmarchand G. Thrombotic microangiopathy: an atypical cause of acute renal failure in patients with acute pancreatitis. *Intensive Care Med*. 2004;30:1235-1239.
56. Stirling Y, Woolf L, North WRS, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. *Thromb Haemost*. 1984;52:176-182.
57. Mannucci PM, Canciani MT, Forza I, et al. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. *Blood*. 2001;98:2730-2735.
58. Moro L, Reineri S, Piranda D, et al. Nongenomic effects of 17beta-estradiol in human platelets: potentiation of thrombin-induced aggregation through estrogen receptor-beta and Src kinase. *Blood*. 2005;105:115-121.
59. Collet JP, Montalescot G, Vicaut E, et al. Acute release of plasminogen activator inhibitor-1 in ST-segment elevation myocardial infarction predicts mortality. *Circulation*. 2003;108:391-394.
60. Bernardo A, Ball C, Nolasco L, Moake J, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104:100-106.
61. Dong JF, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;100:4033-4039.
62. McCarty DJ, Manzi S, Medsger TA Jr, et al. Incidence of systemic lupus erythematosus: race and gender differences. *Arthritis Rheum*. 1995;38:1260-1276.
63. Miller CH, Haff E, Platt SJ, et al. Measurement of von Willebrand factor activity: relative effects of ABO blood type and race. *J Thromb Haemost*. 2003;1:2191-2197.
64. Bell WR, Braine HG, Ness PM, Kickler TS. Improved survival in thrombotic thrombocytopenic purpura - hemolytic uremic syndrome. *N Engl J Med*. 1991;325:398-403.
65. Zheng XL, Pallera AM, Goodnough LT, Sadler JE, Blinder MA. Remission of chronic thrombotic thrombocytopenic purpura treated with cyclophosphamide and rituximab. *Ann Int Med*. 2003;138:105-108.
66. Cataland SR, George JN, Kraut EH, et al. Improved clinical outcome and ADAMTS13 activity following cyclosporine therapy in patients with TTP. *Blood*. 2004;104:243a.
67. Lattuada A, Rossi E, Calzarossa C, Candolfi R, Mannucci PM. Mild to moderate reduction of a von Willebrand factor cleaving protease (ADAMTS-13) in pregnant women with HELLP microangiopathic syndrome. *Haematologia*. 2003;88:1029-1034.