the Ministry of Health, Labour and Welfare of Japan for Blood Coagulation Abnormalities (to Y. Fujimura).

References

[1] L. N. Roberts, R. K. Patel, and R. Arya, "Haemostasis and thrombosis in liver disease," *British Journal of Haematology*, vol. 148, no. 4, pp. 507–521, 2010.

[2] A. Tripodi, M. Primignani, and P. M. Mannucci, "Abnormalities of hemostasis and bleeding in chronic liver disease: the paradigm is challenged," *Internal and Emergency Medicine*, vol. 5, no. 1, pp. 7–12, 2010.

[3] T. Lisman and R. J. Porte, "Rebalanced hemostasis in patients with liver disease: evidence and clinical consequences," *Blood*, vol. 116, no. 6, pp. 878–885, 2010.

[4] P. G. Northup, V. Sundaram, M. B. Fallon et al., "Hypercoagulation and thrombophilia in liver disease," *Journal of Thrombosis and Haemostasis*, vol. 6, pp. 2–9, 2008.

[5] J. L. Moake, "Thrombotic microangiopathies," *New England Journal of Medicine*, vol. 347, no. 8, pp. 589–600, 2002.

[6] Y. Fujimura, M. Matsumoto, H. Yagi, A. Yoshioka, T. Matsui, and K. Titani, "Von Willebrand factor-cleaving protease and Upshaw-Schulman syndrome," *International Journal of Hematology*, vol. 75, no. 1, pp. 25–34, 2002.

[7] G. G. Levy, W. C. Nichols, E. C. Lian et al., "Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura," *Nature*, vol. 413, pp. 488–494, 2001.

[8] K. Kokame, M. Matsumoto, K. Soejima et al., "Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11902–11907, 2002.

[9] M. Furlan, R. Robles, M. Galbusera et al., "Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome," *New England Journal of Medicine*, vol. 339, no. 22, pp. 1578–1584, 1998.

[10] H. M. Tsai and E. C. Lian, "Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura," *New England Journal of Medicine*, vol. 339, no. 22, pp. 1585–1594, 1998.

[11] E. Moschcowitz, "Hyaline thrombosis of the terminal arterioles and capillaries: a hitherto undescribed disease," *Proceeding New York Pathological Society*, vol. 24, pp. 21–24, 1924.

[12] E. L. Amorosi and J. E. Ultmann, "Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature," *Medicine*, vol. 45, pp. 139–159, 1966.

[13] M. Matsumoto, H. Chisuwa, Y. Nakazawa et al., "Living-related liver transplantation rescues reduced vWF-cleaving protease activity in patients with cirrhotic biliary atresia," *Blood*, vol. 96, p. 636a, 2000.

[14] X. Zheng, D. Chung, T. K. Takayama, E. M. Majerus, J. E. Sadler, and K. Fujikawa, "Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura," *Journal of Biological Chemistry*, vol. 276, no. 44, pp. 41059–41063, 2001.

[15] K. Soejima, N. Mimura, M. Hirashima et al., "A novel human metalloprotease synthesized in the liver and secreted into the blood: possibly, the von Willebrand factor-cleaving protease?" *Journal of Biochemistry*, vol. 130, no. 4, pp. 475–480, 2001.

[16] M. Uemura, K. Tatsumi, M. Matsumoto et al., "Localization of ADAMTS13 to the stellate cells of human liver," *Blood*, vol. 106, no. 3, pp. 922–924, 2005.

[17] M. Suzuki, M. Murata, Y. Matsubara et al., "von Willebrand factor-cleaving protease (ADAMTS-13) in human platelets," *Biochemical and Biophysical Research Communications*, vol. 313, no. 1, pp. 212–216, 2004.

[18] N. Turner, L. Nolasco, Z. Tao, J. F. Dong, and J. Moake, "Human endothelial cells synthesize and release ADAMTS-13," *Journal of Thrombosis and Haemostasis*, vol. 4, no. 6, pp. 1396–1404, 2006.

[19] M. Manea, A. Kristoffersson, R. Schneppenheim et al., "Podocytes express ADAMTS13 in normal renal cortex and in patients with thrombotic thrombocytopenic purpura," *British Journal of Haematology*, vol. 138, no. 5, pp. 651–662, 2007.

[20] P. M. Mannucci, M. T. Canciani, I. Forza, F. Lussana, A. Lattuada, and E. Rossi, "Changes in health and disease of the metalloprotease that cleaves von Willebrand factor," *Blood*, vol. 98, no. 9, pp. 2730–2735, 2001.

[21] M. Uemura, Y. Fujimura, S. Ko, M. Matsumoto, Y. Nakajima, and H. Fukui, "Pivotal role of ADAMTS13 function in liver diseases," *International Journal of Hematology*, vol. 91, no. 1, pp. 20–29, 2010.

[22] Y. D. Park, A. Yoshioka, K. Kawa et al., "Impaired activity of plasma von Willebrand factor-cleaving protease may predict the occurrence of hepatic veno-occlusive disease after stem cell transplantation," *Bone Marrow Transplantation*, vol. 29, no. 9, pp. 789–794, 2002.

[23] M. Matsumoto, K. Kawa, M. Uemura et al., "Prophylactic fresh frozen plasma may prevent development of hepatic VOD after stem cell transplantation via ADAMTS13-mediated restoration of von Willebrand factor plasma levels," *Bone Marrow Transplantation*, vol. 40, pp. 251–259, 2007.

[24] M. Uemura, Y. Fujimura, T. Matsuyama et al., "Potential role of ADAMTS13 in the progression of alcoholic hepatitis," *Current Drug Abuse Reviews*, vol. 1, no. 2, pp. 188–196, 2008.

[25] M. Ishikawa, M. Uemura, T. Matsuyama et al., "Potential role of enhanced cytokinemia and plasma inhibitor on the decreased activity of plasma ADAMTS13 in patients with alcoholic hepatitis: relationship to endotoxemia," *Alcoholism: Clinical and Experimental Research*, vol. 34, supplement 1, pp. S25–S33, 2010.

[26] T. Matsuyama, M. Uemura, M. Ishikawa et al., "Increased von Willebrand factor over decreased ADAMTS13 activity may contribute to the development of liver disturbance and multiorgan failure in patients with alcoholic hepatitis," *Alcoholism: Clinical and Experimental Research*, vol. 31, supplement 1, pp. S27–S35, 2007.

[27] M. Uemura, T. Matsuyama, M. Ishikawa et al., "Decreased activity of plasma ADAMTS13 may contribute to the development of liver disturbance and multiorgan failure in patients with alcoholic hepatitis," *Alcoholism: Clinical and Experimental Research*, vol. 29, no. 12, supplement 3, pp. 264S–271S, 2005.

[28] T. Lisman, T. N. Bongers, J. Adelmeijer et al., "Elevated levels of von Willebrand factor in cirrhosis support platelet adhesion despite reduced functional capacity," *Hepatology*, vol. 44, no. 1, pp. 53–61, 2006.

[29] H. B. Feys, M. T. Canciani, F. Peyvandi, H. Deckmyn, K. Vanhoorelbeke, and P. M. Mannucci, "ADAMTS13 activity to antigen ratio in physiological and pathological conditions associated with an increased risk of thrombosis," *British Journal of Haematology*, vol. 138, no. 4, pp. 534–540, 2007.

[30] M. Uemura, Y. Fujimura, M. Matsumoto et al., "Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis," *Thrombosis and Haemostasis*, vol. 99, no. 6, pp. 1019–1029, 2008.

[31] S. Ko, E. Okano, H. Kanehiro et al., "Plasma ADAMTS13 activity may predict early adverse events in living donor liver transplantation: observations in 3 cases," *Liver Transplantation*, vol. 12, no. 5, pp. 859–869, 2006.

[32] T. Kobayashi, H. Wada, M. Usui et al., "Decreased ADAMTS13 levels in patients after living donor liver transplantation," *Thrombosis Research*, vol. 124, no. 5, pp. 541–545, 2009.

[33] I. T. A. Pereboom, J. Adelmeijer, Y. Van Leeuwen, H. G. D. Hendriks, R. J. Porte, and T. Lisman, "Development of a severe von Willebrand factor/ADAMTS13 dysbalance during orthotopic liver transplantation," *American Journal of Transplantation*, vol. 9, no. 5, pp. 1189–1196, 2009.

[34] E. Okano, S. Ko, H. Kanehiro, M. Matsumoto, Y. Fujimura, and Y. Nakajima, "ADAMTS13 activity decreases after hepatectomy, reflecting a postoperative liver dysfunction," *Hepato-Gastroenterology*, vol. 57, no. 98, pp. 316–320, 2010.

[35] M. Yagita, M. Uemura, T. Nakamura, A. Kunitomi, M. Matsumoto, and Y. Fujimura, "Development of ADAMTS13 inhibitor in a patient with hepatitis C virus-related liver cirrhosis causes thrombotic thrombocytopenic purpura," *Journal of Hepatology*, vol. 42, no. 3, pp. 420–421, 2005.

[36] F. Durand and D. Valla, "Assessment of prognosis of cirrhosis," *Seminars in Liver Disease*, vol. 28, no. 1, pp. 110–122, 2008.

[37] Z. Kmieć, "Cooperation of liver cells in health and disease," *Advances in Anatomy, Embryology, and Cell Biology*, vol. 161, pp. 1–151, 2001.

[38] D. C. Rockey, "Hepatic blood flow regulation by stellate cells in normal and injured liver," *Seminars in Liver Disease*, vol. 21, no. 3, pp. 337–350, 2001.

[39] F. Schaffner and H. Popper, "Capillarization of hepatic sinusoids in man," *Gastroenterology*, vol. 44, pp. 239–242, 1963.

[40] M. Hattori, Y. Fukuda, M. Imoto, Y. Koyama, I. Nakano, and F. Urano, "Histochemical properties of vascular and sinusoidal endothelial cells in liver diseases," *Gastroenterologia Japonica*, vol. 26, no. 3, pp. 336–343, 1991.

[41] L. Albornoz, D. Alvarez, J. C. Otaso et al., "Von Willebrand factor could be an index of endothelial dysfunction in patients with cirrhosis: relationship to degree of liver failure and nitric oxide levels," *Journal of Hepatology*, vol. 30, no. 3, pp. 451–455, 1999.

[42] D. Ferro, C. Quintarelli, A. Lattuada et al., "High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia," *Hepatology*, vol. 23, no. 6, pp. 1377–1383, 1996.

[43] T. Knittel, K. Neubauer, T. Armbrust, and G. Ramadori, "Expression of von Willebrand factor in normal and diseased rat livers and in cultivated liver cells," *Hepatology*, vol. 21, no. 2, pp. 470–476, 1995.

[44] S. Urashima, M. Tsutsumi, K. Nakase, J. S. Wang, and A. Takada, "Studies on capillarization of the hepatic sinusoids in alcoholic liver disease," *Alcohol and Alcoholism*, vol. 1B, pp. 77–84, 1993.

[45] L. Amitrano, M. A. Guardascione, V. Brancaccio et al., "Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis," *Journal of Hepatology*, vol. 40, no. 5, pp. 736–741, 2004.

[46] I. R. Wanless, F. Wong, L. M. Blendis, P. Greig, E. J. Heathcote, and G. Levy, "Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension," *Hepatology*, vol. 21, no. 5, pp. 1238–1247, 1995.

[47] K. Oka and K. Tanaka, "Intravascular coagulation in autopsy cases with liver diseases," *Thrombosis and Haemostasis*, vol. 42, no. 2, pp. 564–570, 1979.

[48] A. Pluta, K. Gutkowski, and M. Hartleb, "Coagulopathy in liver diseases," *Advanced Medical Science*, vol. 55, pp. 16–21, 2010.

[49] Y. Fujimura, M. Matsumoto, and H. Yagi, *Thrombotic Microangiopathy*, Springer, Tokyo, Japan, 2008.

[50] A. Padilla, J. L. Moake, A. Bernardo et al., "P-selectin anchors newly released ultralarge von Willebrand factor multimers to the endothelial cell surface," *Blood*, vol. 103, no. 6, pp. 2150–2156, 2004.

[51] C. A. Siedlecki, B. J. Lestini, K. K. Kottke-Marchant, S. J. Eppell, D. L. Wilson, and R. E. Marchant, "Shear-dependent changes in the three-dimensional structure of human von Willebrand factor," *Blood*, vol. 88, no. 8, pp. 2939–2950, 1996.

[52] J. A. Dent, S. D. Berkowitz, J. Ware, C. K. Kasper, and Z. M. Ruggeri, "Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 16, pp. 6306–6310, 1990.

[53] M. Akiyama, S. Takeda, K. Kokame, J. Takagi, and T. Miyata, "Crystal structures of the noncatalytic domains of ADAMTS13 reveal multiple discontinuous exosites for von Willebrand factor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 46, pp. 19274–19279, 2009.

[54] A. K. Davis, R. S. Makar, C. P. Stowell, D. J. Kuter, and W. H. Dzik, "ADAMTS13 binds to CD36: a potential mechanism for platelet and endothelial localization of ADAMTS13," *Transfusion*, vol. 49, no. 2, pp. 206–213, 2009.

[55] M. Furlan, R. Robles, and B. Lämmle, "Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis," *Blood*, vol. 87, no. 10, pp. 4223–4234, 1996.

[56] S. Kinoshita, A. Yoshioka, Y. D. Park et al., "Upshaw-Schulman syndrome revisited: a concept of congenital thrombotic thrombocytopenic purpura," *International Journal of Hematology*, vol. 74, pp. 101–108, 2001.

[57] S. Kato, M. Matsumoto, T. Matsuyama, A. Isonishi, H. Hiura, and Y. Fujimura, "Novel monoclonal antibody-based enzyme immunoassay for determining plasma levels of ADAMTS13 activity," *Transfusion*, vol. 46, no. 8, pp. 1444–1452, 2006.

[58] S. C. Meyer, I. Sulzer, B. Lämmle, and J. A. Kremer Hovinga, "Hyperbilirubinemia interferes with ADAMTS-13 activity measurement by FRETS-VWF73 assay: diagnostic relevance in patients suffering from acute thrombotic microangiopathies," *Journal of Thrombosis and Haemostasis*, vol. 5, no. 4, pp. 866–867, 2007.

[59] C. K. Kasper, L. Aledort, D. Aronson et al., "A more uniform measurement of factor VIII inhibitors," *Thrombosis et Diathesis Haemorrhagica*, vol. 34, no. 2, pp. 869–872, 1975.

[60] N. Afdhal, J. McHutchison, R. Brown et al., "Thrombocytopenia associated with chronic liver disease," *Journal of Hepatology*, vol. 48, no. 6, pp. 1000–1007, 2008.

[61] R. H. Aster, "Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia," *Journal of Clinical Investigation*, vol. 45, no. 5, pp. 645–657, 1966.

[62] M. Peck-Radosavljevic, M. Wichlas, J. Zacherl et al., "Thrombopoietin induces rapid resolution of thrombocytopenia after orthotopic liver transplantation through increased platelet production," *Blood*, vol. 95, no. 3, pp. 795–801, 2000.

[63] J. García-Suárez, C. Burgaleta, N. Hernanz, F. Albarran, P. Tobaruela, and M. Alvarez-Mon, "HCV-associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha2b-interferon therapy," *British Journal of Haematology*, vol. 110, no. 1, pp. 98–103, 2000.

[64] H. S. Ballard, "Hematological complications of alcoholism," *Alcoholism: Clinical and Experimental Research*, vol. 13, no. 5, pp. 706–720, 1989.

[65] H. E. Gerritsen, P. L. Turecek, H. P. Schwarz, B. Lämmle, and M. Furlan, "Assay of von Willebrand factor (vWF)-cleaving protease based on decreased collagen binding affinity of degraded vWF. A tool for the diagnosis of thrombotic thrombocytopenic purpura (TTP)," *Thrombosis and Haemostasis*, vol. 82, no. 5, pp. 1386–1389, 1999.

[66] A. E. Schorer, C. F. Moldow, and M. E. Rick, "Interleukin 1 or endotoxin increases the release of von Willebrand factor from human endothelial cells," *British Journal of Haematology*, vol. 67, no. 2, pp. 193–197, 1987.

[67] I. Tornai, J. Hársfalvi, Z. Boda, M. Udvardy, G. Pfliegler, and K. Rak, "Endothelium releases more von Willebrand factor and tissue-type plasminogen activator upon venous occlusion in patients with liver cirrhosis than in normals," *Haemostasis*, vol. 23, no. 1, pp. 58–64, 1993.

[68] A. Bernardo, C. Ball, L. Nolasco, J. F. Moake, and J. F. Dong, "Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand-factor multimers under flow," *Blood*, vol. 104, no. 1, pp. 100–106, 2004.

[69] W. J. Cao, M. Niiya, X. W. Zheng, D. Z. Shang, and X. L. Zheng, "Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells," *Journal of Thrombosis and Haemostasis*, vol. 6, no. 7, pp. 1233–1235, 2008.

[70] Y. Kume, H. Ikeda, M. Inoue et al., "Hepatic stellate cell damage may lead to decreased plasma ADAMTS13 activity in rats," *FEBS Letters*, vol. 581, no. 8, pp. 1631–1634, 2007.

[71] M. Niiya, M. Uemura, X. W. Zheng et al., "Increased ADAMTS-13 proteolytic activity in rat hepatic stellate cells upon activation in vitro and in vivo," *Journal of Thrombosis and Haemostasis*, vol. 4, no. 5, pp. 1063–1070, 2006.

[72] Y. Fujimura and M. Matsumoto, "Registry of 919 patients with thrombotic microangiopathies across Japan: database of Nara Medical University during 1998–2008," *Internal Medicine*, vol. 49, no. 1, pp. 7–15, 2010.

[73] H. Fukui, B. Brauner, J. C. Bode, and C. Bode, "Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay," *Journal of Hepatology*, vol. 12, no. 2, pp. 162–169, 1991.

[74] P. Tandon and G. Garcia-Tsao, "Bacterial infections, sepsis, and multiorgan failure in cirrhosis," *Seminars in Liver Disease*, vol. 28, no. 1, pp. 26–42, 2008.

[75] M. Sala, A. Forner, M. Varela, and J. Bruix, "Prognostic prediction in patients with hepatocellular carcinoma," *Seminars in Liver Disease*, vol. 25, no. 2, pp. 171–180, 2005.

[76] R. A. Claus, C. L. Bockmeyer, M. Sossdorf, and W. Lösche, "The balance between von-Willebrand factor and its cleaving protease ADAMTS13: biomarker in systemic inflammation and development of organ failure?" *Current Molecular Medicine*, vol. 10, no. 2, pp. 236–248, 2010.

[77] R. A. Reiter, K. Varadi, P. L. Turecek, B. Jilma, and P. Knöbl, "Changes in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation," *Thrombosis and Haemostasis*, vol. 93, no. 3, pp. 554–558, 2005.

[78] C. L. Bockmeyer, R. A. Claus, U. Budde et al., "Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor," *Haematologica*, vol. 93, no. 1, pp. 137–140, 2008.

[79] K. Iyoda, M. Kato, T. Nakagawa et al., "Thrombotic thrombocytopenic purpura developed suddenly during interferon treatment for chronic hepatitis C," *Journal of Gastroenterology*, vol. 33, no. 4, pp. 588–592, 1998.

[80] A. Hori, M. Kami, T. Hamaki et al., "Thrombotic thrombocytopenic purpura without any evidence of thrombotic lesions at autopsy," *Annals of Hematology*, vol. 80, no. 8, pp. 496–498, 2001.

[81] A. Shibuya, A. Satomichi, S. Nagaba, H. Endoh, S. Kuwao, and K. Saigenji, "Thrombotic thrombocytopenic purpura in autoimmune hepatitis," *Journal of Gastroenterology*, vol. 36, no. 8, pp. 569–573, 2001.

[82] J. V. Nally and E. N. Metz, "Acute thrombotic thrombocytopenic purpura. Another cause for hemolytic anemia and thrombocytopenia in cirrhosis," *Archives of Internal Medicine*, vol. 139, no. 6, pp. 711–712, 1979.

[83] S. Killick, S. Jeffery, M. Otter, C. Rist, and D. Bevan, "Thrombotic thrombocytopenic purpura in a patient with genetic haemochromatosis, liver cirrhosis and an iron-free focus," *British Journal of Haematology*, vol. 99, no. 4, pp. 839–841, 1997.

[84] K. Kitano, Y. Gibo, A. Kamijo et al., "Thrombotic thrombocytopenic purpura associated with pegylated-interferon alpha-2a by an ADAMTS13 inhibitor in a patient with chronic hepatitis C," *Haematologica*, vol. 91, article ECR34, supplement 8, 2006.

[85] F. Lambot, B. Hanson, and B. Sztern, "Thrombotic thrombocytopenic purpura mediated by an ADAMTS13-inhibitor related to a treatment with pegylated-interferon alpha-2a and ribavirine in a patient with chronic hepatitis C," *Presse Medicale*, vol. 39, pp. 1207–1210, 2010.

[86] M. Deutsch, E. K. Manesis, E. Hadziyannis, D. Vassilopoulos, and A. J. Archimandritis, "Thrombotic thrombocytopenic purpura with fatal outcome in a patient with chronic hepatitis C treated with pegylated interferon-a/2b," *Scandinavian Journal of Gastroenterology*, vol. 42, no. 3, pp. 408–409, 2007.

[87] M. Sallée, E. Crétel, R. Jean et al., "Thrombotic thrombocytopenic purpura complicating interferon therapy in chronic C hepatitis," *Gastroenterologie Clinique et Biologique*, vol. 32, no. 2, pp. 145–146, 2008.

[88] M. Shimizu, S. Nomura, K. Ishii et al., "The significance of ADAMTSI3 in a patient with thrombotic thrombocytopenic purpura complicated autoimmune hepatitis," *Thrombosis and Haemostasis*, vol. 101, no. 3, pp. 599–600, 2009.

[89] K. Sonomoto, T. Miyamura, H. Watanabe et al., "A case of systemic lupus erythematosus complicated with autoimmune hepatitis and thrombotic thrombocytic purpura," *Japanese Journal of Clinical Immunology*, vol. 32, no. 2, pp. 110–115, 2009.

[90] Y. Usta, F. Gurakan, Z. Akcoren, and S. Ozen, "An overlap syndrome involving autoimmune hepatitis and systemic lupus erythematosus in childhood," *World Journal of Gastroenterology*, vol. 13, no. 19, pp. 2764–2767, 2007.

[91] M. Lenzi, S. Bellentani, G. Saccoccio et al., "Prevalence of non-organ-specific autoantibodies and chronic liver disease in the general population: a nested case-control study of the Dionysos cohort," *Gut*, vol. 45, no. 3, pp. 435–441, 1999.

[92] M. Y. Hsieh, C. Y. Dai, L. P. Lee et al., "Antinuclear antibody is associated with a more advanced fibrosis and lower RNA levels of hepatitis C virus in patients with chronic hepatitis C," *Journal of Clinical Pathology*, vol. 61, no. 3, pp. 333–337, 2008.

[93] S. Tamura, Y. Sugawara, Y. Matsui et al., "Thrombotic microangiopathy in living-donor liver transplantation," *Transplantation*, vol. 80, no. 2, pp. 169–175, 2005.

[94] B. D. Clifford, D. Donahue, L. Smith et al., "High prevalence of serological markers of autoimmunity in patients with chronic hepatitis C," *Hepatology*, vol. 21, no. 3, pp. 613–619, 1995.

[95] A. Iorio, M. Basileo, E. Marchesini et al., "The good use of plasma. A critical analysis of five international guidelines," *Blood Transfusion*, vol. 6, no. 1, pp. 18–24, 2008.

[96] W. I. Youssef, F. Salazar, S. Dasarathy, T. Beddow, and K. D. Mullen, "Role of fresh frozen plasma infusion in correction of coagulopathy of chronic liver disease: a dual phase study," *American Journal of Gastroenterology*, vol. 98, no. 6, pp. 1391–1394, 2003.

[97] H. Yagi, M. Konno, S. Kinoshita et al., "Plasma of patients with Upshaw-Schulman syndrome, a congenital deficiency of von Willebrand factor-cleaving protease activity, enhances the aggregation of normal platelets under high shear stress," *British Journal of Haematology*, vol. 115, no. 4, pp. 991–997, 2001.

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Paradigm shift of childhood thrombotic thrombocytopenic purpura with severe ADAMTS13 deficiency

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In this issue

Summary

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disease with pathological conditions termed thrombotic microangiopathy (TMA). TTP is thought to predominantly affect adults and to rarely occur in children. Currently, TTP is defined by a severe deficiency in the activity of ADAMTS13, a metalloprotease that specifically cleaves unusually large von Willebrand factor multimers under high shear stress. Genetic mutations in and acquired autoantibodies to ADAMTS13 cause congenital TTP (termed Upshaw-Schulman syndrome [USS]) and acquired TTP, respectively. Because of very few overt clinical signs for TTP, USS is often misdiagnosed as chronic idiopathic thrombocytopenic purpura or overlooked during childhood. However, in women with USS, pregnancy can induce thrombocytopenia followed by the development of TTP. Furthermore, early childhood cases of acquired idiopathic TTP have not been characterized. From 1998 to 2008, our institution at Nara Medical University functioned as a TMA referral center in Japan and collected a large dataset on 919 TMA patients (Intern Med 2010;49:7–15). This registry contains 324 patients with a severe deficiency in ADAMTS13 activity, including 41 patients with USS and 283 patients with acquired TTP. Of note, the latter population contains 17 patients who were enrolled as children (≤ 15 years old), including 14 children with idiopathic TTP and three with connective tissue disease-associated TTP. Of the 14 patients with idiopathic TTP, five were very young children (under 2 years old). This study focused on these 58 patients (41 USS and 17 acquired TTP) who were diagnosed with a severe deficiency in ADAMTS13 activity during childhood, causing a paradigm shift in our concept of TTP.

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Thrombotic microangiopathies (TMAs) are pathological conditions that are characterized by organ dysfunction due to platelet thrombi in the microvasculature, consumptive thrombocytopenia, and microangiopathic hemolytic anemia (MAHA). Two of the typical TMA phenotypes are life-threatening generalized diseases, termed thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) [1–4].

A disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 (ADAMTS13) is a metalloprotease that specifically cleaves the Tyr1605–Met1606 bond in the von Willebrand factor (VWF)-A2 domain [5]. In the absence of ADAMTS13 activity (ADAMTS13:AC), unusually large VWF multimers (UL-VWFMs) are released from vascular endothelial cells (ECs) and improperly cleaved, causing them to accumulate in the circulation and induce the formation of platelet thrombi in the microvasculature under conditions of high shear stress. Currently, a severe deficiency in ADAMTS13:AC, which results either from genetic mutations in the *ADAMTS13* gene or acquired autoantibodies to ADAMTS13, is thought to be a specific feature of TTP but not HUS [6,7].

TTP was first described in 1924 by Moschcowitz [8], who documented a 16-year-old female who died of multiorgan failure after a clinical disease course of 1 week. An autopsy revealed hyaline membrane thrombi in the small arteries of multiple organs, except for the lung. In 1966, Amorosi and Ultmann [9] examined 16 new patients and reviewed 255 previously documented patients in order to establish a clinical 'pentad', consisting of MAHA, thrombocytopenia, renal failure, fluctuating neurological signs, and fever. Since then, TTP has been considered a life-threatening but rare disease that occurs mainly in adults and presents with predominant neurotropic clinical signs. Because of this classification, the estimated frequency of TTP was low (3.7 per million) [10] before the discovery of ADAMTS13.

On the other hand, in 1955 Gasser et al. [11] described five children who died of acute renal insufficiency, and their autopsies showed prominent necrosis of the renal cortex. This study established the clinical 'triad' for HUS, which consisted of MAHA, thrombocytopenia, and renal insufficiency. In addition, after it was determined that there was a close relationship between HUS and enterohemorrhagic *Escherichia coli* infection, particularly strain O157:H7 that produces a Shiga-like toxin, studies showed that HUS typically affects children with prominent nephrotropic clinical signs [12].

From 1998 to 2008, our institution at Nara Medical University has functioned as a TMA referral center in Japan and collected a large dataset of 919 patients who have TMA but not disseminated intravascular coagulation (DIC) [13]. This registry contains 324 patients with a severe ADAMTS13:AC deficiency (less than 3% of normal), including 41 patients with congenital TTP (Upshaw-Schulman syndrome [USS]) with variable clinical symptoms and 283 patients with acquired TTP. Notably, the latter population includes 17 patients who were diagnosed with TTP as children ($\leq$ 15 years old), including 14 with idiopathic TTP and three with connective tissue disease (CTD)-associated TTP. Surprisingly, the 14 patients with idiopathic TTP included five patients who were very young infants (under 2 years old), which significantly differed from the previous concept of TTP. Therefore, the aim of this study was to characterize these 58 patients (41 USS and 17 childhood TTP) in order to examine the paradigm shift in our understanding of TTP.

Glossary

ADAMTS13	**a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13**
ADAMTS13:AC	**ADAMTS13 activity**
ADAMTS13:INH	**ADAMTS13 inhibitor**
ai-TTP	**acquired idiopathic TTP**
BU	**Bethesda unit**
CR-TTP	**chronic relapsing TTP**
CTD	**connective tissue disease**
DIC	**disseminated intravascular coagulation**
EC	**endothelial cell**
FFP	**fresh frozen plasma**
HPS	**hemophagocytic syndrome**
HUS	**hemolytic uremic syndrome**
ITP	**idiopathic thrombocytopenic purpura**
MAHA	**microangiopathic hemolytic anemia**
PE	**plasma exchange**
PNH	**paroxysmal nocturnal hemoglobinuria**
SNP	**single nucleotide polymorphism**
TMA	**thrombotic microangiopathy**
TTP	**thrombotic thrombocytopenic purpura**
UL-VWFM	**unusually large VWF multimer**
USS	**Upshaw-Schulman syndrome**
VWF	**von Willebrand factor**
VWF-CP	**VWF-cleaving protease**
WPBs	**Weibel-Palade bodies**

Diagnostic criteria for thrombotic microangiopathy and thrombotic thrombocytopenic purpura

As previously described [13], patients were considered to have TMA if they met all of the following criteria:

- MAHA (hemoglobin [Hb] $\leq$ 12 g/dL), Coombs test negative, undetectable serum haptoglobin (< 10 mg/dL), more than two fragmented red blood cells (RBC) (schistocytes) in a microscopic field with a magnification of 100, and concurrent increased serum lactate dehydrogenase (LDH) above the institutional baseline;
- thrombocytopenia (platelet count $\leq 100 \times 10^9$/L);
- a variable degree of organ dysfunction (renal or neurological involvement) without DIC [14,15].

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It is difficult to differentially diagnose HUS and TTP based on routine laboratory data. Therefore, as a rule, the plasma levels of ADAMTS13:AC were determined for all patients who were suspected to have TMA, and patients with a severe ADAMTS13:AC deficiency were classified as having TTP regardless of the clinical signs. This protocol was important because our registry included patients with congenital TTP or an ADAMTS13:AC deficiency (USS), which generally have fewer clinical signs, often isolated thrombocytopenia, than patients with acquired TTP.

Within the large dataset of 324 patients with a severe ADAMTS13:AC deficiency who were enrolled in our registry between 1998–2008 [10], 58 patients were diagnosed with a severe ADAMTS13:AC deficiency during childhood, of which 41 had congenital TTP (USS) and 17 were diagnosed with acquired TTP, including 14 with idiopathic TTP and three with CTD-associated TTP.

Assays for plasma ADMTS13:AC and ADAMTS13:INH

Until March 2005, ADAMTS13:AC was determined with a classic VWFM assay in the presence of 1.5 mol/L urea using purified plasma-derived VWF as a substrate according to the method described by Furlan et al. [16]. In our laboratory, the detection limit of this assay was 3% of the normal control [17].

In 2005, our laboratory developed a novel chromogenic ADAMTS13-act-ELISA using both an N- and C-terminal tagged recombinant VWF substrate (termed GST-VWF73-His). This assay was highly sensitive, and the detection limit was 0.5% of the normal control [18]. Since 2005, the classic VWFM assay was completely replaced with this novel chromogenic act-ELISA. Both assays show a high correlation between the plasma ADAMTS13:AC levels ($R^2 = 0.72$, $P < 0.01$) with similar means ± SD in healthy individuals (102.4 ± 23.0% vs. 99.1 ± 21.5%), as was shown previously [18]. Thus, the results obtained using the chromogenic act-ELISA were used in this study. In addition, we have categorized plasma ADAMTS13:AC levels of < 3%, 3~ < 25%, and 25~50% of the normal control as a severe, moderate, and mild deficiency, respectively.

Since 2005, ADAMTS13:INH has also been evaluated with the chromogenic act-ELISA by means of the Bethesda method [19]. Prior to this inhibition assay, the tested samples were heat-treated at 56 °C for 60 min to eliminate endogenous enzymatic activity. The ADAMTS13:INH assay consists of two steps. In the 1st step, the test or control plasma is heat-inactivated, mixed with an equal volume of intact normal pooled plasma, and incubated for 2 hours at 37 °C. After the incubation, the residual enzyme activity is measured. One Bethesda unit is defined as the amount of inhibitor that reduces the enzymatic activity by 50% of the control value, and values greater than 0.5 U/mL are considered significant.

Pathogenesis of thrombotic thrombocytopenic purpura

ADAMTS13-producing cells

ADAMTS13 is a metalloproteinase that consists of 1427 amino acids and a multi-domain structure, including a signal peptide, short propeptide, metalloproteinase domain, disintegrin-like domain, thrombospondin-1 (TSP1) domain, cysteine-rich domain, spacer domain, seven additional TSP1 repeats, and two CUB domains [20]. The *ADAMTS13* gene is located on chromosome 9q34, and initial northern blotting studies indicated that ADAMTS13 mRNA is exclusively expressed in the liver [20]. Subsequent immunological studies with *in situ* hybridization analyses indicated that ADAMTS13 is unambiguously produced in hepatic stellate cells (Itoh cells) [21]. However, ADAMTS13 was also identified in platelets [22], vascular ECs [23], and kidney podocytes [24]. Therefore, an outstanding and important question is which organ is most responsible for maintaining the plasma levels of ADAMTS13:AC. In this regard, we have two observations that suggest that the liver is the major ADAMTS13-producing organ. Childhood patients with advanced billiary cirrhosis due to bile duct atresia often showed pathological features of TMA with low plasma levels of ADAMTS13:AC (20–30%), but these clinical signs disappeared and plasma ADAMTS13:AC rapidly recovered to normal levels after a successful liver transplantation [25]. Adulthood patients with cirrhosis that was largely related to hepatitis C infection tended to have lower plasma ADAMTS13:AC levels that correlated with their clinical severity, and the lowest values were approximately 20–30% of the normal levels [26].

Cleavage of unusually large von Willebrand factor multimer

Although the mechanism by which TTP develops in the absence of ADAMTS13:AC has not been fully elucidated, accumulating evidence has provided a hypothesis as illustrated in *figure 1* [27]. In this proposed model, UL-VWFMs are produced exclusively in vascular ECs and stored in an intracellular organelle termed Weibel-Palade bodies (WPBs) and then released into the circulation upon stimulation. Under physiological conditions, epinephrine acts as an endogenous stimulus but other stimuli are largely unknown. In contrast, under non-physiological conditions, DDAVP (1-deamino-8-D-arginine vasopressin), hypoxia, and several cytokines such as interleukin (IL)-2, IL-6, IL-8, and tissue necrotizing factor (TNF)-α act as stimuli that up-regulate VWF release. Once ECs are stimulated, UL-VWFMs and P-selectin, both stored in WPBs, move to the membrane surface of ECs, where P-selectin anchors UL-VWFMs on the EC surface [28]. Under these circumstances, high shear stress generated in the microvasculature induces a change in the UL-VWFM molecule that alters its conformation from a

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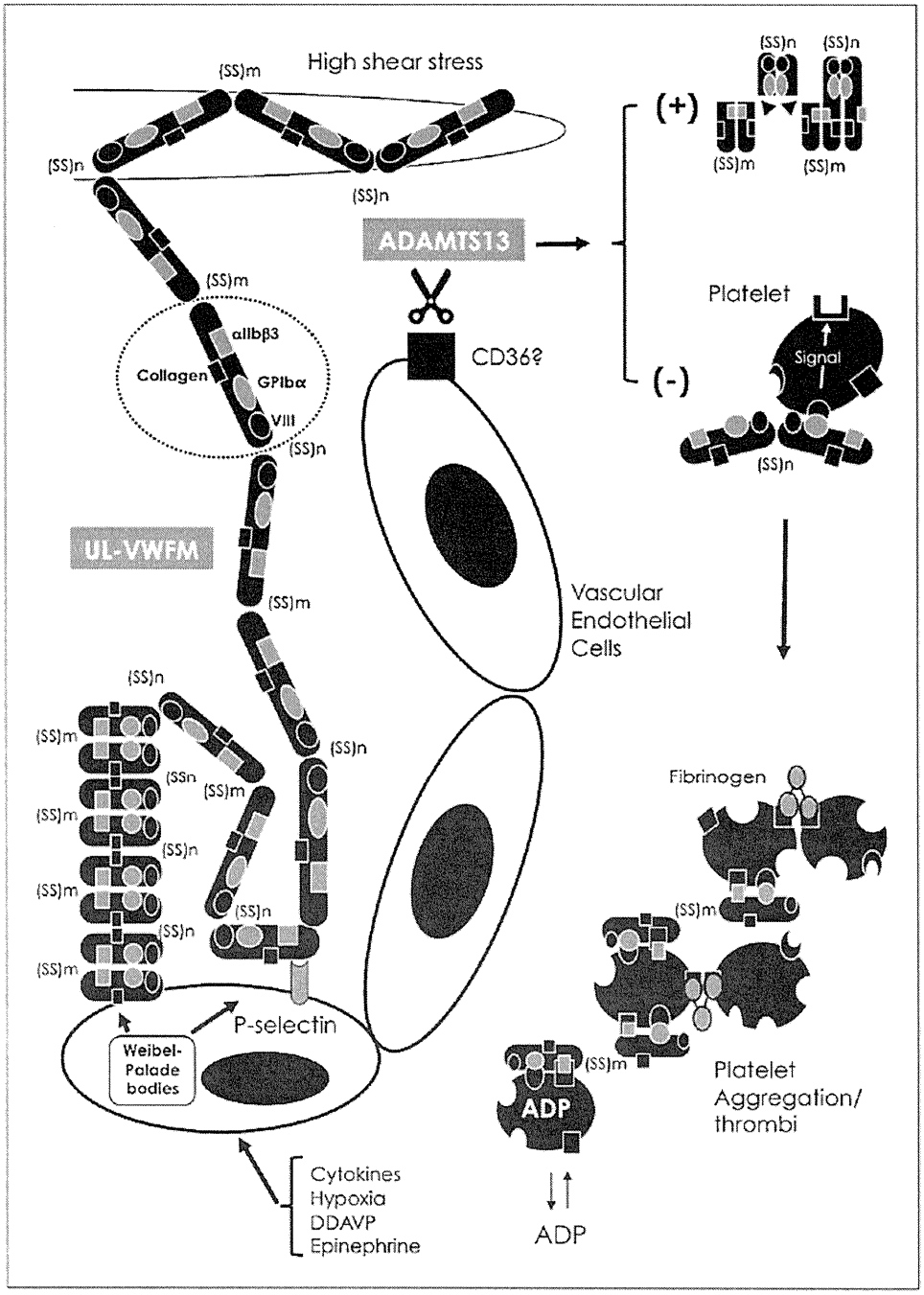

FIGURE 1

Proposed mechanism of platelet thrombi under high shear stress in the absence of ADAMTS13:AC

Unusually large von Willebrand factor multimers (UL-VWFMs) are produced in vascular endothelial cells (ECs) and stored in Weibel-Palade bodies (WPBs). UL-VWFMs are released from WPBs into the circulation upon stimulation by cytokines, hypoxia, DDAVP, and epinephrine. P-selectin that co-migrates from WPBs anchors UL-VWFMs on the vascular EC surface. Under these circumstances, high shear stress changes the molecular conformation of UL-VWFM from a globular to an extended form, allowing ADAMTS13 to access this molecule. In the absence of ADAMTS13:AC, UL-VWFMs are left uncleaved, allowing them to excessively interact with platelet glycoprotein (GP) Ibα and activate platelets via intraplatelet signaling, which results in the formation of platelet thrombi (dotted circle indicates a VWF subunit, which contains a set of binding domains with factor VIII, subendothelial collagen, platelet GPIbα, and integrin αIIbβ3).

globular to an extended form, allowing ADAMTS13 to cleave UL-VWFM. In this context, it has been postulated that multiple exocites within the disintegrin-like/TSP1/cysteine-rich/spacer (DTCS) domains of ADAMTS13 play an important role in interacting with the unfolded VWF-A2 domain [29]. Furthermore, although a direct link to TTP pathogenesis had not been shown, in 1994 Tandon et al. [30] reported that approximately 80% of patients with acquired TTP had autoantibodies to CD36. Recently, Davis et al. [31] showed that recombinant (r) ADAMTS13 specifically binds to rCD36 *in vitro*. Thus, it is possible that ADAMTS13 more efficiently cleaves newly released UL-VWFMs that exist as solid-phase enzymes

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anchored to the vascular EC surface by binding to CD36 because CD36 is a receptor for TSP1, which is a repeated domain within the ADAMTS13 molecule.

In 2001, we clearly showed that pre-existing UL-VWFMs in the plasma of USS patients began to disappear within 1 hour and completely disappeared 24 hours after ADAMTS13 was replenished with infusions of fresh frozen plasma (FFP) as shown in *figure 2* [32]. Retrospectively, these results indicated that exogenous ADAMTS13 could efficiently cleave both UL-VWFMs that pre-existed in the circulation and the newly produced molecules at the EC surface. Related to this phenomenon, Zhang et al. [33] recently analyzed the crystal structure of the VWF-A2 domain and found that the ADAMTS13 cleavage site within this domain is not exposed to the outer surface of the molecule, indicating that the enzyme cannot readily access this site. More recently, Zanardelli et al. [34] proposed that the '2-site initial interaction mechanism between VWF and ADAMTS13', in which a binding site in the VWF C-terminal domains (D4CK) is constitutively exposed, allows this domain to interact with the ADAMTS13 C-terminal domains [TSP1(5-8)/CUB]. Under high shear stress, the '2-site initial interaction' may help expose this binding site within the VWF-A2 domain and favor the correct positioning of the ADAMTS13 spacer domain. Once the higher-affinity interaction between the spacer domain and the VWF-A2 domain is achieved, the metalloproteinase domain of ADAMTS13 can access and cleave the Tyr1605–Met1606 bond within the VWF-A2 domain.

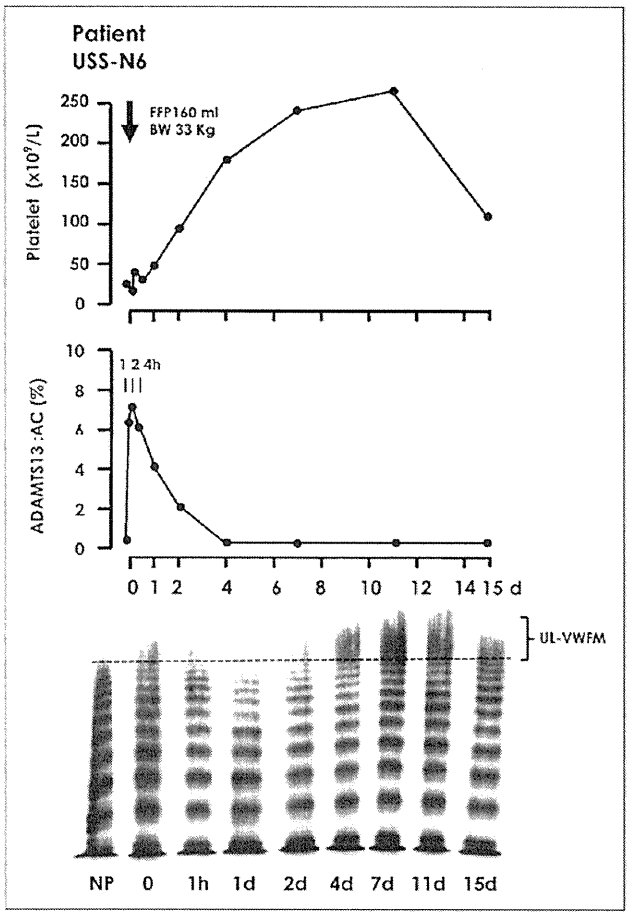

FIGURE 2

Effect of fresh frozen plasma (FFP) infusion on platelet counts, ADAMTS13:AC, and VWFM patterns in patient USS-N6

A total of 160 mL of FFP was transfused into female patient USS-N6 (BW 33 kg). As shown in the top panel, her platelet counts increased from 23 × 10^9/L before the FFP infusion to 251 × 10^9/L at 11 days after the infusion. The middle panel shows the plasma levels of ADAMTS13:AC that were re-examined by the chromogenic act-ELISA using deep-frozen plasma samples. Note that 4 days after the infusion, the plasma ADAMTS13:AC decreased to the pre-infusion level (< 0.5%). In the lower panel, the pre-existing UL-VWFM levels before the FFP infusion rapidly disappeared 24 hours after infusion, and 4 days later, UL-VWFMs re-appeared in the plasma. It should be noted that the platelet count began to decrease concomitantly with the re-appearance of UL-VWFMs (cited from [32] with a slight modification).

Anti-ADAMTS13 autoantibodies

Soejima et al. [35] were the first to report that the cysteine-rich and spacer domains of ADAMTS13 are a major binding site for ADAMTS13 autoantibodies in acquired TTP. Subsequently, Klaus et al. [36] showed that there are multiple antibody binding sites within the ADAMTS13 molecule. Now it is accepted that anti-ADAMTS13 neutralizing autoantibodies target epitopes within the spacer domain [37]. More recently, Pos et al. [38] identified three amino acids, Arg660, Tyr661, and Tyr665, within the spacer domain of ADAMTS13 that are critical for the binding of both the VWF-A2 domain and anti-ADAMTS13 autoantibodies.

Upshaw-Schulman syndrome (congenital TTP/deficiency in ADAMTS13:AC)

Background

The classic hallmarks of USS are repeated childhood episodes of chronic thrombocytopenia and MAHA that are reversed by infusing fresh frozen plasma (FFP). The most striking clinical feature is severe neonatal jaundice with a negative Coombs test that requires exchange blood transfusion therapy. Although USS is now defined as a congenital ADAMTS13:AC deficiency due to genetic mutations, there was a lengthy history that led to this conclusion, as has been described in detail in previous publications [39]. In fact, the term USS had almost been embedded in 1997, when the assay for VWF-cleaving protease (VWF-CP) activity (now ADAMTS13;AC) was established. This is because the pathogenic features that were initially postulated for the disease, such as a defect in 'platelet-stimulating factor', 'decreased plasma fibronectin

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level', or 'lack of thrombopoietin', have been entirely excluded by subsequent investigations. Instead, the practical diagnostic term 'chronic relapsing TTP' (CR-TTP) has long been used. This term was coined by Moake et al. [40], who found that UL-VWFMs were present in the plasma of 4 CR-TTP patients during the remission phase, but disappeared during the acute phase. In 1997, Furlan et al. [41] showed that four CR-TTP patients, different from those of Moake et al. [40], lacked VWF-CP activity, but did not address ADAMTS13:INH. Retrospectively, however, each two CR-TTP patients, reported by Moake et al. [40] and Furlan et al. [41], were congenital TTP, and the remaining two each were acquired TTP. Under these circumstances, we re-visited the term USS [17], which included analyzing three Japanese patients with USS, and found that they uniformly had a severe deficiency in VWF-CP activity (determined by VWFM assay in the presence of 1.5 mol/L urea) in the absence of its inhibitors. The parents of these patients were asymptomatic with a moderately decreased activity (17–60% of normal), except for one carrier who had very low VWF-CP activity (5.6% of normal). Later, this carrier was shown to have a unique single nucleotide polymorphism (SNP), a P475S mutation in the *ADAMTS13* gene in one allele, which is very common in Japanese people (9.6% of normal individuals are heterozygous for the P475S mutation) [42]. However, Levy et al. [43] provided solid evidence that linked congenital TTP or USS and *ADAMTS13* gene mutations. Since this discovery, approximately 100 patients have been identified worldwide [44], but the precise incidence is completely unknown because USS is an extremely rare disease.

ADAMTS13 gene knock-out humans and mice

Although USS patients consistently lack ADAMTS13:AC, they do not always have acute symptoms, and symptoms often become evident only when the patients have infections or become pregnant. In both instances, vascular EC injuries might be involved, and these cases have been indirectly shown to have elevated plasma levels of cytokines or soluble thrombomodulin [45]. However, studies on *ADAMTS13* gene knock-out mice [46,47] showed that UL-VWFMs were detectable in the blood, although the mice did not have acute symptoms. Considering these results, investigators have assumed that an ADAMTS13:AC deficiency is prothrombotic but alone is insufficient to provoke acute symptoms. Therefore, second hits or triggers must exist. However, the lack of symptoms in knock-out mice sharply contrasts the clinical symptoms of USS. For example, USS patients, but not mice, were reported to have acute clinical aggravation soon after receiving infusions of DDAVP [48,49]. However, it is still controversial whether mice have a receptor to DDAVP. Furthermore, there are striking differences between humans and mice during pregnancy. In our studies, nine USS females had a history of pregnancy and all had thrombocytopenia during the 2nd–3rd trimesters. When this thrombocytopenia was not well managed, they developed clinical signs of TTP and the fetus died in many cases [50]. However, this disease course was not found in knock-out mice.

Natural history of 41 Upshaw-Schulman syndrome patients in Japan

USS is inherited in an autosomal recessive fashion, indicating that the female-to-male ratio in the patient population should be one-to-one. However, in our registry of 41 USS patients from 36 families (Table I), the female-to-male ratio was 25-to-16 with an apparent female predominance. Furthermore, all patients had a severe ADAMTS13:AC deficiency (under 3% of normal), except for one USS-GG2 patient (ADAMTS13:AC 2.4–3.4%).

Although severe neonatal jaundice is a typical sign of early-onset bouts of USS, our analysis indicates that such cases represent a relatively small number (16/41, 39%) of patients. Thirty-two patients (32/41, 78%) had repeated episodes of thrombocytopenia during childhood, but many USS patients were primarily misdiagnosed with idiopathic thrombocytopenic purpura (ITP) or Evans syndrome. Therefore, the age at which these patients were diagnosed with CR-TTP or USS was widely distributed from 1 month to 63 years. Sixteen patients (16/41, 39%) were diagnosed with TTP beyond childhood.

Of particular interest, pregnant women with USS inevitably have thrombocytopenia during the 2nd–3rd trimester when the plasma VWF levels rapidly increase with the appearance of UL-VWFM. *Figure 3* presents data for two female patients who were siblings in an USS-L family and were diagnosed based on their precise natural history around pregnancy followed by an examination of ADAMTS13:AC and ADAMTS13:INH [50]. Furthermore, an *ADAMTS13* gene analysis gave a solid diagnosis of USS. As illustrated in these two cases, USS is thought to have two clinical phenotypes, the early-onset and late-onset types. However, generally we cannot find any clear differences in the plasma ADAMTS13:AC levels between these two phenotypes, even when examined by the sensitive act-ELISA.

Regarding severe renal complications in USS patients, we know that two patients thus far have received hemodialysis for chronic renal insufficiency. One patient, USS-C3 (male, born in 1972), was diagnosed with USS at 8 years of age, and then given prophylactic FFP infusions that were continued for the rest of his life. However, his renal function deteriorated yearly and he began to receive hemodialysis when he was 24 years old. During the clinical course of his disease, he experienced chronic heart failure and died of a sudden cardiac attack at 38 years of age. The other patient, USS-JJ3 (male, born in 1980), was diagnosed with USS at 16 years of age, after which he received prophylactic FFP infusions. However, his renal function deteriorated and he has been receiving hemodialysis since he was 26 years old.

La Presse Médicale

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TABLE I

Registration of 41 Japanese patients with Upshaw-Schulman syndrome (USS)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
											From when		
1	A4	1999	M	+	+	< 0.5	C-Hetero	p.R268P/ p.C508Y	4 m	+	4 m		[53]
2	B3	1986	F	+	+	< 0.5	Homo	p.Q449X	2 m	+	11 m		[53]
3	C3	1972	M	–	+	< 0.5	Homo	c.414+1G>A	8 y	+	8 y	Dead (chronic heart failure at the age of 36)	[54]
4	D4	1978	F	+	+	< 0.5	C-Hetero	c.414+1G>A/ p.I673F	4 y	+	4 y		[54]
5	E4	1985	M	+	+	< 0.5	C-Hetero	p.I673F/ p.C908Y	5 y	–	–		[54]
6	F3	1993	M	+	+	0.6	C-Hetero	p.R193W/ p.1244+2 T>G	2.5 y	–	–		[54]
7	G3	1987	F	+	+	< 0.5	C-Hetero	c.686+1G>A/ p.R1123C	14 y	–	–		[54]
8	H3	1951	M	–	–	0.6	C-Hetero	p.A250V/ c.330+1G>A	51 y	+	50 y	Dead (renal failure at the age of 51)	[51]
9	I4	1972	M	–	+	< 0.5	C-Hetero	p.H234Q/ p.R1206X	2 y	+	2 y		[55]
10	J3	1977	F	–	+	< 0.5–0.8	C-Hetero	p.R312C/ c.3198del CT	3 y	+	22 y		[56]
11	J4	1979	M	–	+	< 0.5	C-Hetero	p.R312C/ c.3198del CT	5 y	–	–		[56]
12	K3	1976	F	–	+	< 0.5–0.7	C-Hetero	p.Y304C/ p.G525D	27 y	+	27 y		[50]
13	K4	1978	F	+	+	< 0.5	C-Hetero	p.Y304C/ p.G525D	25 y	+	25 y		[50]
14	L2	1967	F	–	–	< 0.5	C-Hetero	p.R125VfsX6/ p.Q1302X	25 y	–	–		[50]
15	L3	1972	F	–	+	< 0.5	C-Hetero	p.R125VfsX6/ p.Q1302X	25 y	–	–		[50]

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TABLE I (*Continued*)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion		Remarks	Ref.
											From when		
16	M3	1969	F	–	–	< 0.5	C-Hetero	p.R193W/ p.R349C	33 y	–	–		[50]
17	M4	1971	F	–	–	< 0.5	C-Hetero	p.R193W/ p.R349C	30 y	–	–		[50]
18	N6	1986	F	+	+	< 0.5	C-Hetero	p.H234R/ c.3220delTACC	4 y	+	4 y		[17]
19	O4	1958	F	–	–	< 0.5	C-Hetero	p.I178T/ p.Q929X	26 y	+	26 y		[50]
20	P3	1971	M	-	+	< 0.5	C-Hetero	p.C908Y/ p.C322G, p.T323R, p.F324L	3 y	+	21 y		[42]
21	Q1	1983	M	+	+	< 0.5–0.7	C-Hetero	p.G227R/ p.C908Y	6 y	+	11 y		[56]
22	Q2	1988	M	+	+	< 0.5	C-Hetero	p.G227R/ p.C908Y	2 y	+	7 y		[56]
23	R5	1982	F	–	+	< 0.5	C-Hetero	p.R193W/ p.A606P	23 y	+	23 y		[50]
24	S3	1982	F	–	+	0.9	Not determined		4 y	+	*		
25	T4	1981	F	+	+	< 0.5	Homo	c.3220delTACC	1 m	+	*		[56]
26	U3	1990	F	+	+	< 0.5	Homo	c.2259delA	4 m	+	*		[56]
27	V3	1983	F	+	+	< 0.5	C-Hetero	p.W1081X/ p.R193W	6 y	+	6 y		[56]
28	W4	1990	F	–	+	< 0.5	C-Hetero	p.G550R/Not determined	15 y	+	15 y		[56]
29	X5	1963	F	–	–	< 0.5	Not determined		40 y	–	–		
30	Y3	1960	F	–	+	< 0.5	C-Hetero	p.G385E/ p.R1206X	45 y	+	45 y		[56]
31	Z3	1971	F	–	+	< 0.5	Homo	p.R193W	25 y	–	–		[50]
32	AA3	1987	F	–	–	< 0.5	Not determined		19 y	–	–		
33	BB3	1947	M	–	–	< 0.5	Homo	p.R193W	55 y	–	–		[56]
34	CC5	2004	M	+	+	< 0.5	C-Hetero	p.Q723K/ p.R398C	2 y	+	2 y		[56]

TABLE I (*Continued*)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childhood	ADAMTS13:AC (%)	Disease-causing ADAMTS13 gene mutations		Age of TTP diagnosis	Prophylactic FFP infusion	From when	Remarks	Ref.
35	DD5	2007	F	—	+	< 0.5	C-Hetero	p.R268P/ p.Y304C	1 m	—	—		[56]
36	EE4	2003	M	+	+	< 0.5	Homo	c.2259delA	4 y	—	—		[56]
37	FF3	1991	F	—	+	< 0.5	Homo	p.Q449X	6 y	+	6 y		[56]
38	GG2	1931	M	—	—	2.4–3.4	Homo	p.C1024R	63 y	+	63 y	Dead (stroke at the age of 79)	[56]
39	HH4	2003	F	+	+	< 0.5	C-Hetero	p.Q449X/ c.4119delG	1 y	—	—		[56]
40	II3	1977	F	+	+	< 0.5	Not determined		9 m	+	10 y		
41	JJ3	1980	M	—	+	< 0.5	C-Hetero	c.1885delT/ p.C908Y	12 y	+	25 y	Hemodialysis	[56]

C-Hetero: compound heterozygotes; Homo: homozygotes.

There have been two fatal USS cases, one is the above-mentioned USS-C3 and the other is patient USS-I4 (male, born in 1972), whose natural history was previously described in detail [51]. Briefly, patient USS-I4 was diagnosed with late-onset USS when he was 50 years old. The next year he received a cholecystectomy and then experienced a bout of TTP, which led to renal insufficiency. He received extensive treatment, including PE and hemodialysis but did not improve, and he died of renal insufficiency at the age of 51 years.

ADAMTS13 gene analysis

The parents of USS patients are usually asymptomatic carriers, and a major population of patients from unrelated parents is a compound heterozygote, while a minor population of patients from related parents is a homozygote [42,43,51–56].

We performed *ADAMTS13* gene analyses in 38 out of 41 USS patients and disease-causing mutations were identified in 37 patients: nine with homozygous and 28 with compound heterozygous *ADAMTS13* gene mutations. Furthermore, five of these 37 patients were siblings. Therefore, within 64 [2 × (37 − 5)] allelic numbers (n) for *ADAMTS13* gene mutations, the three most frequently found mutations were in the following order: p.R193W (n = 8), p.Q449X (n = 5), and p.C908Y (n = 4). All these mutations were unique to Japanese individuals, perhaps to East-Asians, and were totally different from Europeans and white and black Americans. In addition, to date, we have not found an apparent association between specific *ADAMTS13* mutations and clinical phenotypes. However, Camilleri et al. [57] reported that some single nucleotide polymorphisms in the *ADAMTS13* gene could modulate ADAMTS13:AC and its secretion, indicating that further investigations are required.

Patient USS-GG2 (male, born in 1931) suddenly developed a bout of TTP when he was 63 years old. After this incident, he had repeated TTP bouts and required prophylactic FFP infusions under a clinical diagnosis of CR-TTP. Even under these circumstances, he developed a cerebellar infarction at 76 years of age. During the infusion intervals, ADAMTS13:AC was often measured and determined to be 2.4–3.4% of the normal levels but ADAMTS13:INH was not detected. Most recently, the patient was diagnosed with USS with the homozygous missense mutation C1024R based on an *ADAMTS13* gene analysis (unpublished).

Treatment

Except for exchange blood transfusions to treat jaundice in newborns, USS patients usually respond well to small FFP infusions. Therefore, the question arises; what is the best marker for deciding this indication? As suggested above, mild thrombocytopenia seems to occasionally occur in USS patients during childhood, but this condition might be overlooked

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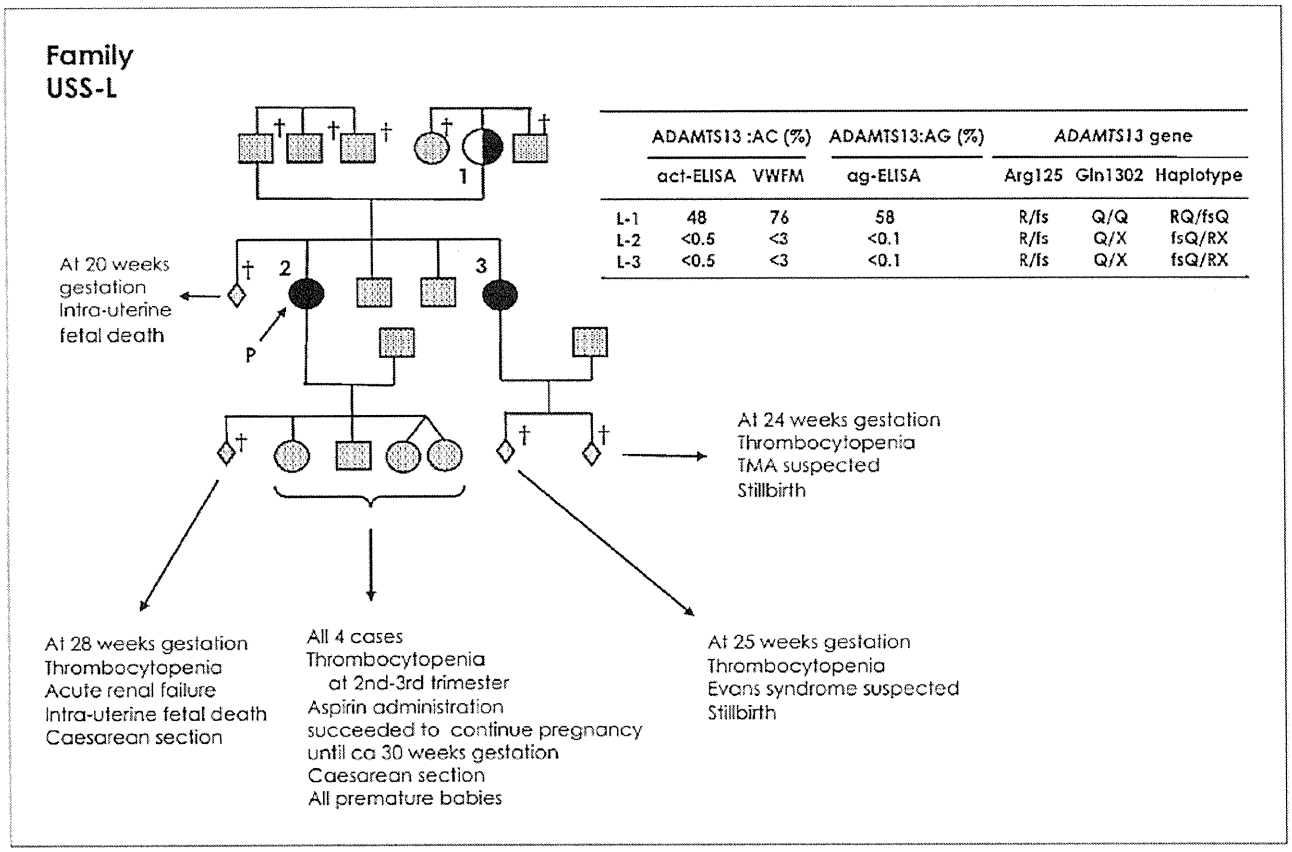

FIGURE 3

Family pedigree and ADAMTS13 analyses in family USS-L

The propositus is L2, and L3 is her younger sister. Both siblings had an abortion along with thrombocytopenia of an unknown etiology. When propositus L2 became pregnant the second time, she had mild thrombocytopenia and her physician recommended low-dose aspirin, which enabled her to maintain the pregnancy until 30 weeks of gestation. However, it was uncertain why and how aspirin worked in this occasion. She delivered a live but premature baby by caesarean section. Then, she successfully bore three more children with the same treatment. All of the babies were premature and alive. During childhood, L2 had no episodes of thrombocytopenia, but L3 was diagnosed with ITP at 3 years of age. At 25 years of age, propositus L2 was diagnosed with USS based on an analysis of ADAMTS13:AC and ADAMTS13:INH. In addition, an *ADAMTS13* gene analysis provided solid evidence that the two siblings are compound heterozygotes for *ADAMTS13* gene mutations (p.R125fsx6/p.Q1302X). Squares and circles indicate males and females, respectively, and an arrow with P indicates the proposita. Closed circles and closed squares represent USS patients. The half-closed circles and squares represent asymptomatic carriers. The cross indicates deceased (cited from [50] with a slight modification).

because of the paucity of clinical signs. Thus, mild thrombocytopenia alone may not be a good marker. However, in clinical practice, some USS patients receive prophylactic FFP infusions (5–10 mL/kg BW) every 2–3 weeks because the half-life of ADAMTS13:AC in the plasma is thought to be 2–3 days, while other patients receive FFP infusions only when acute TTP bouts develop. In our registry, 26 of 41 (63%) USS patients received prophylactic FFP infusions. Currently, USS patients receive FFP infusions based on the physician's observations and the frequency of TTP bouts. However, the efficacy of prophylactic FFP infusions needs to be more precisely evaluated over a long observation period because our two patients who developed renal insufficiency had been receiving FFP infusions since they were clinically diagnosed with CR-TTP.

One serious adverse effect of repeated plasma infusions is that nine out of 41 (22%) USS patients were infected with hepatitis C virus. In this regard, virus-free rADAMTS13 preparations would be a promising product for USS patients.

Acquired thrombotic thrombocytopenic purpura/ADAMTS13:AC deficiency

Figure 4 shows age and gender distribution of acquired TTP. Of 195 patients with acquired TTP and a severe ADAMTS13:AC deficiency due to ADAMTS13:INH, 17 (6%) were childhood patients, including 14 with acquired idiopathic TTP (ai-TTP)

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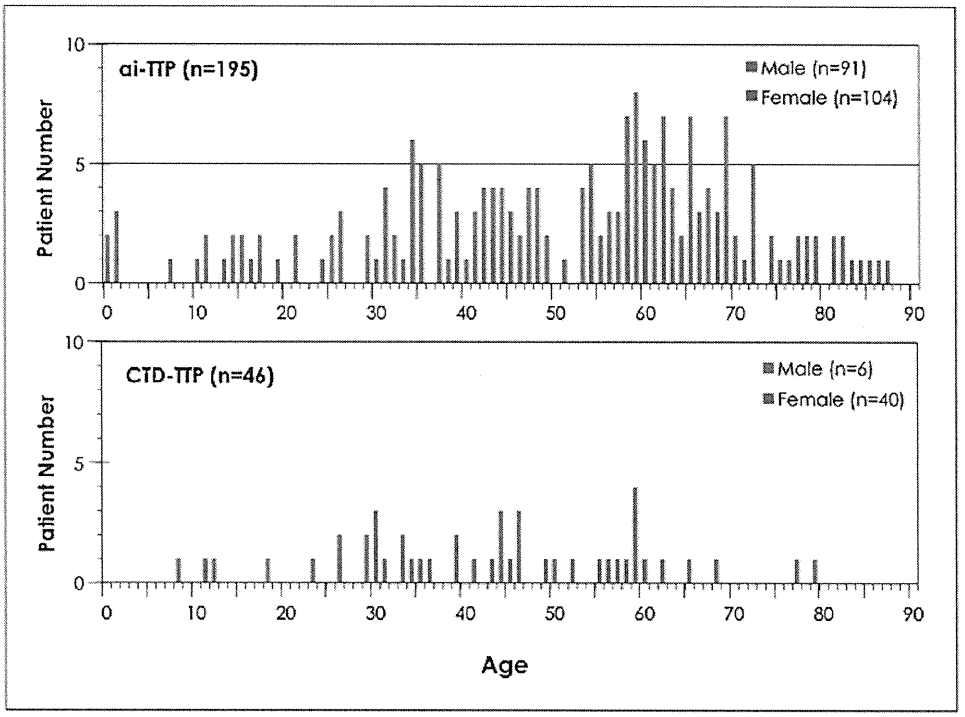

FIGURE 4

Age and gender distribution of patients with acquired idiopathic (ai)-thrombotic thrombocytopenic purpura (TTP) and connective tissue disease (CTD)-associated TTP

The upper panel shows the age and gender distribution of 195 ai-TTP patients who were registered at Nara Medical University during 1998–2008 and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. The largest population peak is found at approximately 60 years old, and we identified 14 patients (14/195, 7.2%) aged less than 15 years. Of note, five were very young children below 2 years of age.

The lower panel shows the age and gender distribution of 46 CTD-TTP patients who were registered at our institution during the same time period and were determined to have a severe ADAMTS13:AC deficiency and be positive for ADAMTS13:INH. This CTD-TTP patient population is widely distributed across ages, but is more common in patients between 30–60 years old. There is an apparent female predominance (41/46, 89%) in CTD-TTP patients in this registry, and three childhood patients were identified.

and three with connective tissue disease (CTD)-associated TTP. Of note, the former group included five patients who were less than 2 years of age and were initially misdiagnosed with other thrombocytopenic disorders, such as ITP, HUS, and hemophagocytic syndrome (HPS). It is important to examine and compare the detailed clinical features of these TTP patients to adulthood patients in order for physicians to ascertain that TTP is not a rare childhood disease. Therefore, we herein describe the clinico-laboratory features of these five young infants with ai-TTP and three childhood patients with CTD-associated TTP.

Idiopathic (ai-) thrombotic thrombocytopenic purpura

Table II summarizes the features of the 14 childhood patients with ai-TTP, including five infantile patients. Case 1 was previously reported [58] and case 5 was more recently described [59]. Interestingly, in contrast to the adulthood ai-TTP patients with a severe ADAMTS13:AC deficiency, the childhood patients had a slightly male predominance (female:male = 5:9). Five patients (5/14, 33%) had apparent prodromal illnesses, such as an upper respiratory tract infection ($n = 3$), Rotavirus infection ($n = 1$), or urinary tract infection ($n = 1$). Ten patients (10/14, 67%) had neurological findings, including headache ($n = 5$), altered mental status ($n = 4$), hemiparesis ($n = 1$), seizures ($n = 1$), and vision disturbance ($n = 1$). These patients exclusively presented with renal involvement (11/14, 73%) and fever (13/14, 93%). All the patients had hemolytic anemia (Hb, 4.5–11.3 g/dL) and thrombocytopenia (platelet, 7–38 $\times$ 10^9/L), but their serum creatinine levels (Cr, 0.19–1.0 mg/dL) remained within the normal range. Most of the childhood patients had five clinical signs that are characteristic of classic TTP ('pentad'), but six patients, including five young infants aged below 2 years, were initially misdiagnosed with other thrombocytopenic disorders, such as ITP ($n = 2$), HUS ($n = 2$), HPS ($n = 1$), and paroxysmal

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TABLE II

Clinical features in childhood patients with acquired TTP with severe ADAMTS13 deficiency

Case	Age	Sex	Prodromal illness	Initial diagnosis	Clinical findings on admission					
					Neurological symptom	Renal involvement	Cr (mg/dL)	Fever	Hb (g/dL)	Platelet (×109/L)
1	9 m	F	Rotavirus	HUS	Altered mental status	Yes	0.3	Yes	4.5	2
2	19 m	F	URI	ITP	No	Yes	0.19	Yes	11.3	38
3	19 m	M	URI	HUS	Altered mental status	Yes	1	Yes	5.4	9
4	12 m	M	No	HPS	Hemiparesis	Yes	0.31	Yes	8.5	38
5	8 m	M	No	ITP	No	No	0.19	Yes	8.7	25
6	7 y	M	No	TTP	Altered mental status	No	0.32	Yes	5.4	7
7	10 y	M	UTI	TTP	No	Yes	0.4	No	10.5	19
8	11 y	M	No	TTP	Headache	Yes	0.7	Yes	8.8	3
9	11 y	F	URI	TTP	No	Yes	0.6	Yes	5.5	6
10	13 y	F	No	TTP	Headache	No	0.58	Yes	5.7	12
11	14 y	F	No	TTP	Altered mental status, convulsion	No	0.4	Yes	7.8	6
12	15 y	M	No	PNH	Headache	Yes	1	Yes	10.5	17
13	15 y	M	No	TTP	Headache	Yes	0.91	Yes	8.1	11
14	14 y	M	No	TTP	Headache, visual disturbance	Yes	1.05	Yes	7	28
15	8 y	F	SLE	DIC	Altered mental status, headache	Yes	0.44	Yes	8.8	38
16	11 y	F	MCTD	TTP	Headache	Yes	0.6	Yes	11.9	43
17	12 y	F	SLE	TTP	No	No	0.61	Yes	5.6	1

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
1	< 0.5	> 100	19	No	SP	Yes	No	Alive	Cerebral infarction	[58]
2	< 0.5	4.3	3	Yes	SP, PSL	Yes	Yes	Dead		
3	< 0.5	2.3	3	Yes	PSL	Yes	No	Alive		
4	< 0.5	1.7	2	No	PSL	Yes	No	Alive	Cerebral infarction	
5	< 0.5	4.8	6	No	SP, PSL	Yes	No	Alive		[59]
6	< 0.5	2.1	3	No	Rituximab	No	No	Alive		
7	< 0.5	0.5	No	No	SP, PSL, MZR	Yes	No	Alive		
8	< 0.5	1.3	No	Yes	SP	No	No	Alive		
9	< 0.5	5.6	5	No	PSL	Yes	No	Alive		
10	< 0.5	2	9	No	SP, PSL	No	No	Alive		

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TABLE II (*Continued*)

Case	ADAMTS13: AC (%)	ADAMTS13: INH (BU/ml)	Treatments				Outcome		Clinical course remarks	Ref.
			PE (times)	FFP infusions	Immunosupressive agents	Platelet transfusion	Relapse	Prognosis		
11	< 0.5	3.2	17	No	SP, PSL, VCR	No	No	Alive		
12	< 0.5	34	39	No	PSL, VCR, CSA	No	Yes	Alive		
13	< 0.5	2.3	3	No	SP, PSL, AZT	No	No	Alive		
14	< 0.5	6.8	30	Yes	PSL	No	No	Alive		
15	< 0.5	1.2	5	Yes	SP, PSL, CY	No	No	Alive		
16	< 0.5	1.8	3	No	SP, PSL	No	No	Alive		
17	< 0.5	0.7	9	Yes	SP, PSL	No	No	Alive		

Cr: creatinine; Hb: hemoglobin; ADAMTS13:AC: ADAMTS13 activity; ADAMTS13:INH: ADAMTS13 inhibitor; PE: plasma exchange; FFP: fresh frozen plasma; URI: upper respiratory infection; UTI: urinary tract infection; SLE: systemic lupus erythematosus; MCTD: mixed connective tissue disease; TTP: thombotic thrombocytopenic purpura; ITP: idiopathic thrombocytopenic purpura; HUS: hemolytic uremic syndrome; HPS: hemophagocytic syndrome; PNH: paroxysmal noctural hemoglobinuria; SP: steroid pulse; PSL: predonosolone; MZR: mizoribine; VCR: vincristine; CSA: cyclosporine A; AZT: azathioprine; CY: cyclophosphamide.

nocturnal hemoglobinuria (PNH, $n = 1$). After analyzing ADAMTS13, they were all correctly diagnosed with ai-TTP.

Of these 14 childhood patients with ai-TTP, 13 received plasma exchange (PE, 2–39 times, median 5 times), including four patients who subsequently received a FFP infusion. They also received immunosupressive therapy, including steroid pulse ($n = 7$), predonisolone ($n = 12$), vincristine ($n = 2$), cyclosporin ($n = 1$), azathioprine ($n = 1$), mizoribine ($n = 1$), and rituximab ($n = 1$). As an adjunctive therapy, the patients were given intravenous immune globulin ($n = 3$) or an antiplatelet agent ($n = 2$). Of note, seven patients received platelet transfusions before or after they were diagnosed with acquired TTP. In five of the seven patients who received platelet transfusions, there were no apparent serious complications. However, case 1 developed general convulsions soon after the platelet transfusion, and case 2 died from bleeding without appreciable hemostatic effects from the platelet transfusion. As consequence, 13 out of 14 childhood patients with ai-TTP achieved one clinical remission, but two patients relapsed, including one who died. We think that clinicians should be aware of the existence of ai-TTP during very early childhood, and herein we present a short summary for each of these five infants with ai-TTP.

Case 1

In March 2000, a 9-month-old girl presented with a fever. She subsequently showed loss of appetite, a drop in physical activity, a pale complexion, and vomiting followed by diarrhea related to a Rotavirus infection. On the following day, these symptoms continued and generalized petechiae appeared. She was taken to a family doctor who determined that she had severe anemia and thrombocytopenia. As a result, she was admitted to a nearby hospital for treatment. Upon admission, she was drowsy and her laboratory findings showed severe anemia (Hb, 4.5 g/dL), thrombocytopenia (platelet, 2.0×10^9/L), hyperbilirubinemia (total bilirubin, 2.6 mg/dL), and an elevated LDH level (2,925 IU/L). Both direct and indirect Coombs' tests were negative, and fragmented RBCs were detected in the blood film. The hemostatic tests showed the following: prothrombin time (PT, 14.0 sec), activated PTT (35.9 sec), fibrinogen (268 mg/dL), thrombin-antithrombin complex (TAT, 31.7 µg/L), D-dimer (7.14 µg/mL), and fibrin degradation product (FDP, 82.3 µg/mL). The levels of blood urea nitrogen (BUN) and creatinine were within the normal ranges (25 mg/dL and 0.3 mg/dL, respectively). Neither Shiga-like toxin nor *E. coli* O157:H7 was detected in her stool. However, she had proteinuria, hematuria, and marked petechiae on her body due to thrombocytopenia. She was tentatively diagnosed with HUS, and she received five units of platelet transfusion. Soon after completing the platelet transfusion, she developed generalized convulsions followed by right hemiplegia, and therefore, PE therapy was immediately instituted. On the following day, both CT and MRI examinations of her brain revealed a diffuse hemorrhagic infarction in the left posterior region. The PE therapy was continued for the next 37 days on a total of 19 occasions, along with steroid pulse therapy and high-dose intravenous immunogloburin (IVIG) infusions until clinical improvements were noted. The ADAMTS13:AC and ADAMTS13:INH titers measured by the VWFM assay were less than 3% and greater than 100 BU/mL, respectively (later, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasmas, and the respective data were

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less than 0.5% and greater than 100 BU/mL [58]). Now, almost 10 years have passed, and the patient is apparently healthy with a minimal sequela of the right hemiplegia.

Case 2

In July 2000, a 19-month-old girl presented with a fever and cough. The next day, she was taken to a family doctor and then transferred to a nearby hospital because of thrombocytopenia (platelet, 21×10^9/L). Upon admission, her laboratory data revealed slight anemia (Hb, 11.3 g/dL), thrombocytopenia (platelet, 38×10^9/L), and an elevated LDH level (994 IU/L). An analysis of a bone marrow aspiration showed no abnormalities, and therefore she was suspected to have ITP. She was administered high-dose IVIG with steroid therapy but her platelet count did not increase. Her platelet count slightly increased soon after the platelet transfusions, while the number of schistocytes in the blood films gradually increased. This patient never had renal dysfunction or neurological signs. Thus, her physician suspected that the patient had USS but did not measure ADAMTS13:AC. The patient was given an infusion of 80 mL of FFP, but her platelet count did not increase. During this period, she was alert and no clinical deterioration was noted. Three months after admission, plasma samples from this patient were sent to our laboratory for ADAMTS13:AC and ADAMTS13:INH testing. Based on the results of the VWFM assays, the patient was diagnosed with a severe ADAMTS13:AC deficiency (< 3%) with ADAMTS13:INH (4.0 BU/mL). However, in those days we were unable to clearly determine whether this patient had USS and developed alloantibodies or acquired TTP with autoantibodies to ADAMTS13. The patient was not given PE therapy because she did not show any clinical deterioration during the subsequent 3 months. Therefore, she was discharged and then carefully observed at the outpatient clinic. However, 1 month after discharge, she was re-admitted to the hospital and received PE therapy because of exacerbated anemia and thrombocytopenia. However, her clinical signs did not improve, even after whole blood exchange therapy. Thus, she was treated with RBC and platelet transfusions, but 2 weeks later she fell into coma and died of tracheal bleeding, which was 8 months after her first hospital admission (plasma ADAMTS13:AC and ADMTS13:INH in this patient were measured only on one occasion. In recent years, both values were re-evaluated by the chromogenic act-ELISA using deep-frozen plasma samples, and the data were less than 0.5% and 4.3 BU/mL, respectively).

Case 3

In July 2002, a 19-month-old boy developed a low-grade fever and cough followed by petechiae. He was taken to a family doctor because his nasal bleeding did not stop. The doctor noted thrombocytopenia and anemia and suspected HUS, and the patient was transferred to a local hospital. He had mild thrombocytopenia (platelet, 70×10^9/L) soon after birth, which spontaneously improved. Upon admission, he was drowsy and his laboratory data showed anemia (Hb, 5.4 g/dL), thrombocytopenia (platelet, 9×10^9/L), elevated LDH (1991 IU/L), and proteinuria. He was administered FFP infusions, steroid therapy, and IVIG. A platelet transfusion was performed but his platelet counts did not significantly increase. Since he was negative for DIC markers, the patient was clinically diagnosed with TTP and then administered PE therapy. After three consecutive PE therapies, he became alert, recovered, and his laboratory markers returned to normal levels. The ADAMTS13:AC and ADAMTS13:INH titers were measured by classic VWFM assays using frozen plasma that was obtained before the PE therapy was administered, and the results were less than 3% and 2.3 BU/mL, respectively (later, chromogenic act-ELISA gave values of less than 0.5% and 2.3 BU/mL, respectively). His plasma ADAMTS13:AC deficiency with ADAMTS13:INH continued for more than 6 months but without appreciable clinical manifestations. After 4 years, ADAMTS13:AC (77%) had normalized and ADAMTS13:INH (< 0.5 BU/ml) was absent.

Case 4

In June 2002, a 13-month-old boy developed a fever followed by dark urine and diarrhea. He was taken to a nearby clinic, where he was determined to have leukocytosis (WBC, 12,000/μL), anemia (Hb, 7.2 g/dL), and thrombocytopenia (platelet, 46×10^9/L). In addition, a peripheral blood film showed phagocytosis and therefore the patient was diagnosed with suspected HPS. He was transferred to a university hospital where platelet transfusions were performed twice for two consecutive days, but his platelet counts only transiently increased. Soon after the second platelet transfusion, a bone marrow aspiration was performed, but the HPS diagnosis was not confirmed. On the other hand, there was a transient increase in his platelet count (platelet, 40×10^9/L) after he was infused with a small amount of FFP, and therefore the physician suspected a diagnosis of USS. Therefore, the patient received a daily plasma infusion therapy for the next 5 days. However, hematuria developed followed by right hemiplegia. An MRI revealed a hemorrhagic infarction (3 × 4 cm) in the left parieto-occipital region. Based on the clinical course, he was eventually diagnosed with ai-TTP. After he received PE therapy for two consecutive days with orally administered prednisolone, his clinical conditions rapidly improved and his laboratory findings recovered. After his recovery, the ADAMTS13:AC and ADAMTS13:INH levels were tested using the classic VWFM assay and deep-frozen plasma samples that were obtained before the PE therapy, and the results were less than 3% and 1.9 BU/mL, respectively (later, these values were re-examined with the chromogenic act-ELISA, and the results were less than 0.5% and 1.9 BU/mL, respectively). He subsequently improved and was discharged. Three years later, his plasma

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TABLE III

Comparison of clinical features and outcomes between childhood and adulthood patients with acquired idiopathic (ai)-TTP

Ai-TTP (n = 195)	Childhood patients (n = 14)	Adulthood patients (n = 181)
Age at the onset of TTP bouts (years old), Median (25, 75 percentile)	11 (1.6, 14)	57 (41, 65)
Female (%)	35.7	55.2
"Pentad"		
(1) Platelet count ($\times 10^9$/L), Median (25, 75 percentile)	15 (8, 24)	10 (7, 17)
(2) Hemoglobin (g/dL), Median (25, 75 pecentile)	8.0 (5.6, 8.8)	7.5 (6.3, 8.8)
(3) Renal involvement (%)	71.4	75.7
Serum creatinine (mg/dL), Median (25, 75 percntile)	0.5 (0.3, 0.9)	1.0 (0.7, 1.3)
(4) Central nervous system involvements (%)	71.4	79.6
(5) Fever ($\geq$ 37.0 °C) (%)	92.9	69.6
Times of plasma exchange	5.5 (3.0-17.5)	ND
Mortality in the current episode of TTP bouts (%)	7.1	15.5

ND: not determined.

ADAMTS13:AC had normalized. At present, he has fully recovered and has no residual right hemiplegia.

Case 5

In January 2005, a 9-month-old boy with generalized petechiae and a fever was referred to a local hospital, where he was determined to have thrombocytopenia (platelet, 9×10^9/L). He was admitted to a university hospital and diagnosed with acquired TTP based on ADAMTS13:AC (< 3%) and ADAMTS13:INH titers (2.8 BU/mL) that were determined using the classic VWFM assays. (Later, these values were re-examined by the chromogenic act-ELISA, and the results were less than 0.5% and 4.8 BU/mL, respectively). After he was diagnosed with ai-TTP, he was administered PE therapy for six consecutive days at a different hospital. His clinical symptoms rapidly improved, but the increase in platelet counts was only transient and his platelet count was consistently lower than 10×10^9/L. To prevent serious bleeding complications, the physician administered oral prednisolone, together with continuous low-dose platelet transfusions. Two months later, he was discharged, despite having an ADAMTS13:AC deficiency with ADAMTS13:INH that lasted for at least 8 months. Two years later, we were able to examine the plasma ADAMTS13:AC and ADAMTS13:INH in this patient, and determine that ADAMTS13 had fully normalized [59].

Table III compares the clinical features and outcomes of the childhood patients (n = 14) and adulthood patients (n = 181) with ai-TTP in our registry [13].

Connective tissue disease-associated thrombotic thrombocytopenic purpura

In 1939, Gitlow and Goldmark [60] first reported a close relationship between 'classic' TTP and systemic lupus erythematosus (SLE). In 1999, Brenner et al. [61] described five patients with childhood-onset 'classic' TTP and reviewed 30 other patients who were previously described in the literature. According to their analysis, nine (9/35, 26%) fulfilled four or more ACR criteria for SLE and eight (8/35, 23%) had incipient SLE. Interestingly, of the five patients who were initially diagnosed with 'classic' idiopathic TTP in their laboratory, three were diagnosed with SLE within 3 years, and the remaining two patients fulfilled the ACR classification criteria for SLE within 4 years of disease onset. However, at that time, ADAMTS13:AC assays were not generally available, and therefore no data on ADAMTS13 was provided in their report.

In our registry of 919 patients with TMAs, 221 had CTD-associated TMA, of which 92 had SLE-associated TMA [13]. For the 221 CTD-TMA and/or 92 SLE-TMA patients, the number of patients with a severe ADAMTS13:AC deficiency with ADAMTS13:INH was 46 and 24, respectively. Furthermore, within the 221 patients with CTD-TMA, 11 developed the disease in childhood (less than 15 years of age), including eight patients with SLE, 1 with RA (rheumatoid arthritis), and two with MCTD (mixed connective tissue disease), in whom three had a severe ADAMTS13:AC deficiency. These three patients included two SLE- and one MCTD-associated TTP patients, and they uniformly had relatively low titers of ADAMTS13:INH (0.7–1.8 BU/mL) at

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the onset of TTP, which slightly differed from those with ai-TTP (table II). Here we briefly describe these three childhood patients with CTD-associated TTP (cases 15–17) due to their relevance in clinical practice.

Case 15

In April 2005, a 7-year-old girl was determined to have proteinuria and occult blood in her urine based on a school health examination. The patient was admitted to a nearby university hospital for further examination in June 2005, where she was diagnosed with SLE (Lupus nephritis) based on her clinical manifestations and the following laboratory findings: proteinuria, positive for anti-nuclear antibodies and anti-double stranded DNA antibodies, and low complementemia. She was treated with prednisolone, mizoribine, and azathioprine. Because her clinical signs significantly improved with these treatments, she was discharged in September 2005.

In April 2006 (8 years of age), this patient noticed proteinuria and hematuria based on a self-examination at home. The next day, she was admitted to the same hospital. Her laboratory data at the second admission were as follows: Hb (8.8 g/dL), LDH (1608 IU/L), platelet (2×10^9/L), PT (11.0 sec), PTT (31.1 sec), fibrinogen (355 mg/dL), antithrombin (140%), TAT (9.06 µg/L), D-dimer (5.25 ng/mL), FDP (7.9 µg/mL), and schistocytes in a peripheral blood smear. She had a DIC score of 6 according to the DIC diagnostic criteria from the International Society of Thrombosis and Haemostasis [15]. As a result, she was initially treated with nafamostat, but there were no clinical improvements. ADAMTS13:AC and ADAMTS13:INH assays were performed for a differential diagnosis of TTP, and the results were less than 0.5% and 1.2 BU/mL, respectively. Thus, she was diagnosed with SLE-associated TTP and treated with PE (five times), steroid pulse therapy, and cyclophosphamide. These treatments saved her life, and to date, she has not had a relapse of TTP.

Case 16

In July 2006, an 11-year-old girl developed general fatigue, headache, and vomiting. Two days later, she was admitted to a local hospital where laboratory tests indicated the following: Hb (11.9 g/dL), LDH (1636 IU/L), total bilirubin (9.3 mg/dL), platelet (33×10^9/L), PT (12.7 sec), PTT (34.0 sec), fibrinogen (290 mg/dL), antithrombin (> 75%), D-dimer (< 2 ng/mL), FDP (< 5 µg/mL), BUN (18.0 mg/dL), and schistocytes in a peripheral blood smear. For a differential diagnosis, her plasma ADAMTS13:AC and ADAMTS13:INH levels were determined to be less than 0.5% and 1.8 BU/mL, respectively. In addition, upon admission she simultaneously had Raynaud's phenomenon and was positive for anti-nuclear antibodies and anti-RNP antibodies. Thus, she was diagnosed with MCTD-associated TTP, and was administered PE with steroid pulse therapy starting on the third day of hospitalization. During the third PE, she had anaphylactic shock, perhaps related to the infused plasma. Thus, she stopped the PE therapy and continued the steroid pulse therapy alone. As a result of these treatments, she recovered and on the hospital day 14 her ADAMTS13:AC increased to 67% of normal and ADAMTS13:INH became negative. To date, she has had no episodes of TTP.

Case 17

In October 2007, a 12-year-old girl suddenly developed jaundice with a fever. She was admitted to a nearby university hospital, and her routine laboratory data provided the following: Hb (5.6 g/dL), platelet (1×10^9/L), PT (14.0 sec), PTT (40.1 sec), fibrinogen (333 mg/dL), FDP (20.5 µg/mL), total bilirubin (5.5 mg/dL), and schistocytes in a peripheral blood smear. Upon admission, she had low levels of complement, and was positive for anti-nuclear antibodies, anti-double stranded DNA antibodies, and anti-SS-A antibodies. Plasma ADAMTS13:AC and ADAMTS13:INH were simultaneously measured by the act-ELISA and were less than 0.5% and 0.7 BU/mL, respectively. Based on these results, she was diagnosed with SLE-associated TTP and PE therapy was initiated. After three consecutive PE treatments with steroid pulse therapy, her platelet count increased. However, on hospital day 8, her platelet count decreased again, and her ADAMTS13:INH titer increased to 2.2 BU/mL. The PE therapy was re-initiated with steroid pulse therapy. A total of nine rounds of PE therapy and two courses of steroid pulse therapy resulted in remission on hospital day 23. At this time, ADAMTS13:AC and ADAMTS13:INH were 86% and less than 0.5 BU/mL, respectively. To date, she has had no TTP relapses.

Treatment of acquired thrombotic thrombocytopenic purpura

Plasma exchange (PE) is the first line therapy that was demonstrated to be effective in randomized clinical trials for acquired TTP [62]. PE removes ADAMTS13:INH, UL-VWFM, and hazardous cytokines from the circulation in TTP patients, and replenishes ADAMTS13 without circulatory overload. Corticosteroids are often used as an adjunctive treatment. In relapsing or refractory cases, other immunosuppressive drugs such as cyclosporine, cyclophosphamide, vincristine, and rituximab are empirically used. PE therapy should be initiated as soon as possible after TTP is diagnosed, but the onset of therapy tends to be delayed in childhood patients with acquired TTP because of difficult differential diagnoses, especially with HUS, unless ADAMTS13:AC is measured.

In regard to platelet transfusions in TTP patients with a severe ADAMTS13:AC deficiency, these transfusions have consistently been viewed as a contraindication because they may enhance thrombotic complications due to platelet aggregation and thrombus formation under high shear stress generated in the microvasculature. Our experience also partially supports this concept, but such adverse effects happened only on very few occasions. We believe that prophylactic platelet