**Disorders of Platelets**

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1. A 1-year-old boy diagnosed with Glanzmann thrombasthenia is referred to you. Remembering that this disorder is caused by a lack of the glycoprotein complex IIb-IIIa, you perform platelet aggregation studies.

What do you expect to see from the platelet aggregation studies?

A. Increased aggregation to low-dose ristocetin

B. Absent aggregation to all agonists

C. Absent aggregation to all agonists except ristocetin

D. Absent aggregation only to ristocetin

E. Absent second-wave aggregation to ADP and epinephrine

**Explanation**

Patients with Glanzmann thrombasthenia lack the glycoprotein IIb-IIIa complex that is responsible for platelet binding to fibrinogen. Therefore, patients with this condition lack a platelet aggregation response to all agonists except ristocetin. Aggregation to ristocetin is preserved because this tests the interaction of factor glycoprotein complex Ib-IX with von Willebrand, so answer B is not correct.

Increased aggregation to low-dose ristocetin occurs in platelet-type von Willebrand disease (vWD) and type 2B vWD. Both conditions result in increased binding of platelets to von Willebrand factor. In platelet-type vWD the defect is glycoprotein Ib on the platelets, whereas in type 2B vWD the mutation is in the von Willebrand factor molecule. Patients with Bernard-Soulier syndrome lack the glycoprotein complex Ib-IX that is essential for adhesion of platelets to the vascular endothelium mediated by von Willebrand factor. Therefore, patients with this condition lack a platelet aggregation response to ristocetin. Lack of second-wave aggregation to ADP and epinephrine is seen in dense granule storage disease.

2. A 3-day-old Caucasian infant has petechiae on his face and trunk. The infant is well-appearing, and physical examination is otherwise normal. The infant’s CBC is unremarkable except for a platelet count of 6,000/mm3. Platelet morphology is normal with the exception of a few large platelets. There is no maternal history of idiopathic thrombocytopenic purpura or lupus, and maternal platelet count is 187,000/mm3.

From which of the following does the infant’s thrombocytopenia probably result?

A. Alloantibodies reactive against human platelet antigen 1a (HPA-1a) on the platelet surface

B. Autoantibodies reactive against common antigens on the platelet surface

C. Absent platelet alpha granules

D. Autoantibodies reactive against platelet factor 4 and heparin complexes

E. Alloantibodies reactive against glycoprotein Ib-IX

**Explanation**

The most likely explanation for severe thrombocytopenia in an otherwise healthy infant with no concerning maternal history of thrombocytopenia is neonatal alloimmune thrombocytopenia (NAIT) resulting from maternal antibodies directed against paternally derived antigen expressed by the infant’s platelets. The most common antigen involved is HPA-1a, accounting for approximately 80% of such cases in Caucasian infants. In the Asian population the most common antigen is HPA-4a (80%). NAIT can result in severe bleeding and requires prompt treatment. It is recommended that the platelet count be kept above 30,000/mm3. This can be accomplished by giving maternal platelets, if available, or a combination of random donor platelets and intravenous immunoglobulin (IVIg).

If the mother’s platelet count is low or history is concerning for autoimmunity, then maternal immune thrombocytopenia due to IgG autoantibodies reactive against common antigens on the infant’s platelets would be most likely. In this setting, severe hemorrhage is much less common, and treatment with IVIg can be reserved for children with active bleeding.

Autoantibodies against the complex of heparin and platelet factor 4 are the cause of heparin-induced thrombocytopenia. Alloantibodies to glycoprotein Ib-IX can occur in patients with Bernard-Soulier syndrome after platelet transfusions. Absence of alpha granules can be seen in gray platelet syndrome associated with findings on light microscopy.

3. A 15-year-old adolescent presents with acute onset of bruising. On physical examination the patient is lethargic. CBC shows a hemoglobin concentration of 8.7 g/dL, WBC 5,600/mm3 with a normal differential, and platelet count of 6,000/mm3. Creatinine is 0.8 mg/dL. Reticulocyte count is 10%. Peripheral blood smear shows red cell fragmentation and a few large platelets.

What is the appropriate management at this time?

A. Platelet transfusion

B. Intravenous immunoglobulin

C. Hemodialysis

D. Plasmapheresis

E. Rituximab

**Explanation**

This patient has thrombotic thrombocytopenic purpura (TTP). The classic clinical pentad of TTP is microangiopathic hemolytic anemia, thrombocytopenia, decreased renal function, depressed neurological function, and fever. TTP results when the metalloprotease ADAMTS13, responsible for cleaving ultralarge multimers of von Willebrand factor (vWF), is either absent (congenital) or inhibited by antibodies (acquired). Without this protease, platelets bind to large vWF multimers and form microthrombi, resulting in thrombocytopenia and microangiopathic hemolytic anemia. TTP is a medical emergency, so prompt recognition and initiation of plasmapheresis are essential.

High dose-steroids and rituximab can be added for patients who are not responding to plasmapheresis alone. Hemodialysis is first-line treatment for hemolytic uremic syndrome. Platelet transfusions are contraindicated in TTP, and intravenous immunoglobulin does not have a significant role.

4. You are examining a 7-month-old boy who is admitted to the hospital with pneumonia. You notice that he has eczema on his face and scattered petechiae on his trunk. CBC is normal with the exception of a platelet count of 17,000/mm3 with small platelets on the peripheral blood smear.

Which test is most likely to yield a diagnosis?

A. Flow cytometric evaluation of platelet glycoproteins

B. Electron microscopic evaluation of platelets

C. Evaluation of von Willebrand factor multimers

D. Gene mutation or gene product testing

E. Immunohistochemical staining

**Explanation**

This child most likely has Wiskott-Aldrich syndrome (WAS). WAS is an X-linked condition associated with thrombocytopenia, eczema, and immunodeficiency. It is caused by a mutation in the WASP gene and should be considered in any male patient with thrombocytopenia and small platelets. The diagnosis can be confirmed by showing an abnormality in the WASP gene or its protein product. X-linked thrombocytopenia is also caused by a mutation involving WASP but results only in thrombocytopenia without the additional complications of WAS.

Electron microscopic evaluation is used to determine the absence of dense granules (normal platelet size). Flow cytometric evaluation of platelet glycoproteins can diagnose Glanzmann thrombasthenia (normal platelet size) and Bernard-Soulier syndrome (macrothrombocytopenia). Von Willebrand factor levels and multimers can establish a diagnosis of platelet-type von Willebrand disease (vWD) or type 2B vWD (normal size platelets). Immunohistochemical staining can help identify MYH9 protein aggregates in the neutrophils of patients with MYH9-related disorders (macrothrombocytopenia).

5. A 5-year-old girl is referred to you because of recurrent epistaxis. The parents are first cousins. Past medical history is significant for strabismus surgery at 3 years of age. On physical examination you notice oculocutaneous albinism. Platelet count and peripheral blood smear are normal.

Which of the following is most likely to be absent in her platelets?

A. ADAMTS13

B. ADP

C. Fibrinogen

D. Platelet factor 4

E. Thrombin

**Explanation**

Hermansky-Pudlak syndrome, an autosomal recessive disorder, causes a bleeding diathesis due to a lack of dense granules in the platelets, which is demonstrated on electron microscopy. The disease is associated with oculocutaneous albinism, pulmonary fibrosis, strabismus, and nystagmus. Dense granules contain small molecules such as ATP, ADP, serotonin, and calcium. Platelet aggregation studies will therefore show absent second-wave aggregation in response to ADP and epinephrine.

Alpha granules contain proteins such as fibrinogen, platelet factor 4, von Willebrand factor, and thrombospondin. Thrombin is a potent platelet agonist but is not contained in platelet granules. ADAMTS13 is a metalloprotease responsible for cleaving ultralarge von Willebrand multimers and not contained within the platelet.

6. A 4-year-old girl presents with acute onset of bruising, petechiae, and epistaxis. History and physical examination are otherwise unremarkable. CBC reveals a platelet count of 8,000/mm3 but is otherwise normal. Blood smear shows a few large platelets, and you diagnose idiopathic thrombocytopenic purpura (ITP). You decide to treat her with intravenous immunoglobulin (IVIg), given her epistaxis.

Which side effect should you monitor for after IVIg administration?

A. Red cell hemolysis

B. Aseptic meningitis

C. Serum sickness

D. Hypertension

E. Disseminated intravascular coagulopathy

**Explanation**

Treatment for children with newly diagnosed ITP is associated with side effects. Aseptic meningitis is a side effect of IVIg, usually resulting in a severe headache. The majority of children will recover over the course of several days. Often patients with aseptic meningitis are treated with corticosteroids; however, nothing has been shown to decrease the duration of symptoms, and symptoms may recur with reinfusion.

Anti-D immunoglobulin causes antibody-coated red cells to undergo intravascular and extravascular hemolysis and an expected decline in hemoglobin. Fatal reports of disseminated intravascular hemolysis have also been reported with anti-D. Anti-D immunoglobulin is therefore not recommended for children who have significant bleeding or anemia or who are direct antiglobulin test positive. It is also not effective after splenectomy. Serum sickness can occur with rituximab, and hypertension is associated with high-dose corticosteroid administration.

7.A 7-month-old girl is evaluated for gastrointestinal bleeding and easy bruising. Physical examination shows shortened forearms, bruising, and petechiae. Radiographs of her forearms show bilateral absent radii. Her CBC is normal with the exception of a platelet count of 13,000/mm3.

What management do you offer to the family?

A. Gene testing to confirm the diagnosis

B. Chromosome breakage analysis

C. Referral for bone marrow transplant

D. Splenectomy

E. Supportive care with platelet transfusions

**Explanation**

This child has thrombocytopenia absent radii (TAR) syndrome. The genetic defect causing TAR is unknown. Children with TAR usually begin to have resolution of their thrombocytopenia by the second year of life. Therefore, treatment consists of supportive care, including platelet transfusions for episodes of bleeding.

Gene testing and bone marrow transplant should be considered for children with congenital amegakaryocytic thrombocytopenia, which is not associated with skeletal findings. Chromosome breakage analysis and bone marrow transplant are indicated for children with Fanconi anemia (FA). Children with TAR syndrome have normal bilateral thumbs, distinguishing it from FA. Splenectomy can raise the platelet count in X-linked thrombocytopenia or Wiskott-Aldrich syndrome. Intravenous immunoglobulin is used for immune-mediated thrombocytopenia but does not have a role in congenital thrombocytopenias.

8.A 23-year-old woman who had idiopathic thrombocytopenic purpura (ITP) 9 years ago and is now in remission after splenectomy delivers a healthy male infant. During your physical examination you notice he has scattered petechiae. CBC is normal with the exception of a platelet count of 35,000/mm3. The infant is vigorous and alert.

How do you manage this case?

A. Oral corticosteroids

B. Transfusion with maternal platelets

C. Transfusion with random donor platelets

D. Observation

E. Intravenous immunoglobulin

**Explanation**

The maternal history of ITP in this scenario makes a diagnosis of neonatal autoimmune thrombocytopenia more likely. Even though the infant’s mother is in remission after a splenectomy, the mechanism of remission after splenectomy does not eliminate her ability to make antiplatelet antibodies. Neonatal autoimmune thrombocytopenia can occur even in women who had ITP years before pregnancy and are in remission. In autoimmune thrombocytopenia, unlike alloimmune, the antibodies are against common antigens on the platelet surface. Neonatal autoimmune thrombocytopenia is less likely to be associated with severe thrombocytopenia, rarely leads to intracranial hemorrhage, and usually can be managed with conservative measures. Infants do not need treatment but should be watched closely because the platelet count reaches a nadir at 3 to 4 days of life. If treatment is necessary, either intravenous immunoglobulin (IVIg) or high-dose corticosteroids can be used.

Neonatal alloimmune thrombocytopenia is a more severe condition. Such infants need treatment to keep the platelet count above 30,000/mm3. This can be accomplished by giving maternal platelets, if available, or a combination of random donor platelets and IVIg.

9.You are consulted because a child has a platelet count of 21,000/mm3 and requires elective surgery. He is otherwise well and has no evidence of bleeding, including no bruising or petechiae. He has no history of bleeding in the past with tonsillectomy and adenoidectomy. The peripheral blood smear shows platelet clumps.

What do you recommend?

A. Transfuse platelets before surgery.

B. Cancel surgery until the platelet count rises.

C. Repeat the CBC using a tube containing acid citrate dextrose (ACD) or citrate.

D. No further evaluation is necessary before surgery.

E. Repeat the CBC using a tube containing ethylenediaminetetraacetic acid (EDTA).

**Explanation**

This is a case of pseudothrombocytopenia. It is due to antibodies that bind to an antigen exposed only in the presence of EDTA anticoagulant. The antibody causes clumping in vitro only. The best way to confirm the diagnosis is to obtain a CBC in a tube containing citrate, ACD, or heparin. Usually a different anticoagulant will correct the artificially low platelet count.

Platelet transfusion is not necessary because the patient is not truly thrombocytopenic. Surgery should not be canceled until the platelet count recovers, because this might not occur if an EDTA-containing tube is again used for the platelet count. However, it is always best to confirm the diagnosis and exclude other causes of thrombocytopenia before proceeding to surgery.

10. A 24-month-old boy, whose parents are first cousins, is referred to you because of a significant episode of epistaxis. The parents report that the child had bleeding after circumcision and some gum oozing when his primary teeth erupted. Evaluation with a PT/PTT, factor VIII and IX levels, and von Willebrand factor levels were all normal. You suspect a platelet disorder, and platelet aggregation studies reveal absent aggregation to ristocetin but are normal otherwise.

This is due to an absence of which of the following?

A. Glycoprotein VI

B. Glycoprotein IIb-III

C. Platelet HLA antibodies

D. Glycoprotein Ib-IX

E. Dense granules

**Explanation**

This child has a history concerning for a bleeding diathesis. Given the normal coagulation profile and bleeding that is primarily mucosal, consideration should be given to platelet disorders. The platelet aggregation study results are due to an absence of glycoprotein Ib-IX, which results in macrothrombocytopenia and poor platelet function seen in the autosomal recessive disorder Bernard-Soulier syndrome. Glycoprotein Ib-IX causes platelet adhesion to the vascular endothelium via von Willebrand factor.

Glycoprotein VI binds collagen. Glycoprotein IIb-IIIa binds to fibrinogen and is absent in Glanzmann thrombasthenia. HLA antibodies are present on the platelet surface. No condition has been identified that is associated with an absence of HLA antibodies; however, antibodies against HLA are the most common cause of platelet refractoriness after platelet transfusions. Absence of dense granules would result in a lack of second-wave aggregation to ADP and epinephrine.

11. A 15-year-old girl is admitted to the general pediatrics service with intermittent abdominal pain and diarrhea. She is subsequently diagnosed with Crohn disease. You are consulted because her initial evaluation revealed a platelet count of 958,000/mm3 but was otherwise normal.

What testing and treatment should you recommend at this time?

A. Treatment targeted at her Crohn disease

B. Bone marrow evaluation

C. JAK2 kinase mutation testing

D. Alpha-fetoprotein levels

E. Antiplatelet drug therapy

**Explanation**

This child most likely has a reactive thrombocytosis, and treatment should be targeted at the management of the underlying disease. Bone marrow biopsy and JAK2 mutation testing are part of the evaluation for essential (or primary) thrombocytosis, caused by overproduction of platelets by the bone marrow. This condition is very rare in a child of this age with another explanation for thrombocytosis. Thrombocytosis is associated with hepatoblastoma, which is usually seen in younger children and is a speculated result of increased thrombopoietin production from the liver. Checking alpha-fetoprotein levels would therefore be appropriate for a child for whom you have a clinical suspicion of hepatoblastoma based on additional findings such as abdominal mass, distension, and hepatomegaly. Reactive thrombocytosis is not associated with thrombosis, and therefore antiplatelet drug therapy is not indicated.

12. You receive a consult from the neonatal intensive care unit (NICU) for an infant with purpura. The infant was born via uncomplicated vaginal delivery to a 32-year-old woman. Hospital course has been complicated by failure of the hearing screen and a patent ductus arteriosus. On examination you note scattered small, firm, nonblanching blue-red lesions. The infant’s CBC reveals isolated thrombocytopenia with a platelet count of 40,000/mm3. Peripheral blood smear reveals normal red and white cell morphology and platelets that are normal in size.

Which test is most likely to reveal the cause of thrombocytopenia in this neonate?

A. Blood culture

B. Maternal and paternal platelet genotyping

C. Genetic testing for mutations affecting the thrombopoietin receptor

D. Viral PCR

E. Bone marrow biopsy

**Explanation**

The most common cause of thrombocytopenia in the NICU is bacterial sepsis, but careful consideration of additional history and physical examination findings may indicate additional causes. This infant has additional findings consistent with congenital rubella infection, such as a patent ductus arteriosus, hearing loss, and a blueberry muffin rash. Therefore, testing with rubella PCR would be most likely to reveal the source of thrombocytopenia. Maternal and paternal genotyping can be undertaken in suspected cases of neonatal alloimmune thrombocytopenia, which usually is seen in an otherwise healthy infant. Genetic mutations affecting the thrombopoietin receptor agonist are seen in infants with congenital amegakaryocytic thrombocytopenia. A bone marrow biopsy would be necessary only after additional causes of thrombocytopenia were excluded and in the setting of prolonged thrombocytopenia.

13. A 10-month-old girl is found to have thrombocytopenia on a CBC obtained because of blood in her stool. Her platelet count is 24,000/mm3. On the peripheral blood smear the platelets are normal in size. The child has an elevated thrombopoietin level and lack of megakaryocytes on bone marrow biopsy evaluation.

In addition to bleeding, what else do you consider the child to be at risk for?

A. Pancytopenia

B. Sensorineural hearing loss

C. Recurrent infections

D. Liver failure

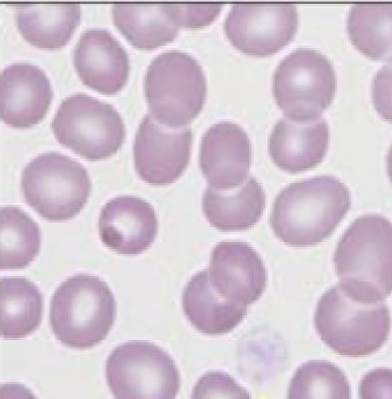
E. T-cell immune deficiency

**Explanation**

This child has the features of congenital amegakaryocytic thrombocytopenia (CAMT). CAMT is caused by mutations affecting the thrombopoietin receptor. Patients with CAMT progress to pancytopenia within the first few years of life, and treatment is with hematopoietic stem cell transplant. Infants with CAMT encounter high mortality from bleeding (30%).

MYH9-related disorders are a group of disorders caused by mutations involving myosin heavy chain-A. The patients can have sky-blue inclusions in their neutrophils called Döhle bodies, nephritis, cataracts, and sensorineural hearing loss in addition to bleeding. In addition, they will have macrothrombocytopenia on the peripheral blood smear. Recurrent infections are part of the clinical picture of Wiskott-Aldrich syndrome. T-cell immunodeficiency is part of DiGeorge syndrome, which also can have associated thrombocytopenia. Liver disease is not associated with any congenital platelet disorders.

14. A 4-year-old boy presents with recurrent epistaxis and numerous bruises. His CBC, PT, and PTT are normal. Testing for von Willebrand disease is also normal. He undergoes platelet aggregation testing and is found to have mild aggregation defects with epinephrine and ADP. His peripheral blood smear is below.



What is this patient’s diagnosis?

A. Bernard-Soulier syndrome

B. Aspirin overdose

C. Hermansky-Pudlak syndrome

D. Glanzmann thrombasthenia

E. Gray platelet syndrome

**Explanation**

Gray platelet syndrome can result in mild aggregation defects with epinephrine and ADP and is diagnosed based on the ghost-like (gray) platelets on the peripheral smear. It also can be diagnosed by genetic testing. This is an autosomal recessive condition characterized by macrothrombocytopenia, splenomegaly, and a mild bleeding diathesis. It can be associated with myelofibrosis. The typical finding of faint platelets on light microscopy comes from an absence of alpha granules with the platelets. Alpha granules contain proteins such as fibrinogen, platelet factor 4, von Willebrand factor, and thrombospondin.

Glanzmann thrombasthenia would show an absence or dysfunction of glycoprotein IIb/IIIa on the surface of platelets. This glycoprotein is the receptor for fibrinogen and results in an inability of the platelets to bind the formed fibrinogen on their surface during the clotting process. Patients with Glanzmann thrombasthenia do have glycoprotein Ib/IX on their surface, which is the receptor for von Willebrand factor, and ristocetin serves as a bridge between glycoprotein Ib/IX and platelets, thus allowing for normal aggregation with this agonist and only this agonist. In contrast, Bernard-Soulier syndrome is an absence of glycoprotein Ib/IX and thus has the exact opposite aggregation pattern to Glanzmann thrombasthenia: absent aggregation with ristocetin but, due to the normal fibrinogen surface receptor, normal aggregation with all other agonists. Patients on aspirin generally have abnormal aggregation only with epinephrine, collagen, and arachidonic acid. Patients with Hermansky-Pudlak syndrome have no dense bodies where ADP is primarily stored and so have absent aggregation or poor aggregation with ADP but usually normal aggregation with other agonists. The dense granules contain small molecules such as ATP, ADP, serotonin, and calcium.

15. You are consulted on a 6-year-old boy with end-stage renal disease because he has oozing from his lines and easy bruising. His PT and PTT are normal, and CBC shows a normal platelet count. He has no family history of bleeding and underwent a renal biopsy 6 months ago without any bleeding. You suspect bleeding secondary to chronic uremia.

What treatment do you recommend at this time?

A. Recombinant factor VIIa

B. Prothrombin complex concentrate

C. Fresh frozen plasma

D. Desmopressin

E. Whole blood

**Explanation**

Bleeding associated with uremia is likely to be a multifactorial condition. Treatment also is therefore aimed at several different aspects that can contribute. Because dysfunctional von Willebrand factor contributes to uremic bleeding, desmopressin is considered appropriate treatment. Additional treatments include dialysis, erythropoietin, cryoprecipitate, and estrogens. Dialysis will improve the underlying uremia, erythropoietin may increase red cell volume and assist with hemostasis, and cryoprecipitate is rich in von Willebrand factor and fibrinogen. The exact mechanism of action of estrogens is unclear, but they have been shown to be effective in reducing the bleeding time and clinical bleeding in patients with uremic bleeding. The remaining options are not indicated for treatment in uremic bleeding.

16. You are consulted because a child newly admitted to the hospital needs frequent platelet transfusions. After platelet transfusion for a platelet count of 13,000/mm3, the child has a platelet count of 20,000/mm3 at 1 hour, 18,000/mm3 at 4 hours, and 19,000/mm3 at 24 hours.

Which of the following is most consistent with these platelet kinetics?

A. Fever

B. Splenomegaly

C. Aplastic anemia

D. Immune thrombocytopenia

E. Normal platelet kinetics

**Explanation**

The patient’s platelet kinetics are most consistent with splenomegaly. In this setting, the transfused platelets rapidly leave circulation and pool in the spleen, resulting in very little incremental increase. The increase observed may hold at first and then will begin to decline again over the next few days. With aplastic anemia, platelet count should respond sufficiently to transfused platelets at 1 hour and 4 hours but may start to show decline at 24 hours. Patients with immune thrombocytopenia can actually have an increase in the platelet count immediately after platelet transfusion, but it will return to baseline within several hours. Fever can increase platelet turnover and transfusion needs but should not result in frank platelet refractoriness.

17. A 3-year-old girl presents to your emergency department with new-onset bruising. Her labs show a platelet count of 17,000/mm3 and a hemoglobin of 7.6 mg/dL. Her reticulocyte count is 3%, and her LDH is elevated at 560 U/L. Her PT and PTT are normal. Her blood smear reveals a predominance of schistocytes. When taking the history, you learn that she had one previous episode of documented thrombocytopenia and often has recurring bruising with infections. Her ADAMTS-13 antibody is negative.

What should be your next course of action?

A. Plasmapheresis

B. Fresh frozen plasma

C. High-dose corticosteroid

D. Platelet transfusion

E. Humate-P

**Explanation**

The child in the clinical vignette has findings of thrombotic thrombocytopenia purpura (TTP). This includes evidence of thrombocytopenia and microangiopathic anemia without another cause. Although her ADAMTS-13 antibodies are negative, it is important to also check the actual ADAMTS-13 level because this can be low in cases of congenital TTP in the absence of an antibody. This diagnosis should be considered in children with cyclic thrombocytopenia, especially in the setting of infections. Management is with fresh frozen plasma replacement, usually every 2 to 3 weeks, which maintains an ADAMTS-13 level of more than 5%. Investigations into recombinant ADAMTS-13 are under way.

Both plasmapheresis and high-dose corticosteroids are part of the management of acquired TTP, which is antibody mediated. A platelet transfusion would not be indicated in either congenital or acquired TTP. Humate-P is useful in von Willebrand disease (vWD). Humate-P may be used in cases of type 2B vWD, which can present with variable thrombocytopenia but is not associated with microangiopathic anemia. Type 2B vWD is an autosomal dominant condition that results in increased binding of von Willebrand factor (with platelets). This leads to rapid clearance of both the platelets and von Willebrand factor multimers.