Bleeding Associated with Thrombocytopenia

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Introduction

Platelets are a critical component of coagulation, most importantly in the formation of the hemostatic platelet plug, and are actively involved in secretion, aggregation, and adhesion [1–3]. Platelets also have an important role as immune cells, aiding in wound healing and vascular integrity [4, 5]. A normal platelet count is between 150,000 and 450,000/mm^3 regardless of age [6], and a decrease in circulating platelets can increase the tendency for bleeding. Low platelet count, or thrombocytopenia, can be caused by numerous abnormalities, broadly categorized by platelet production abnormalities in the bone marrow, loss of platelets after formation, or a combination of the two processes. There are also disorders of platelet function, characterized by platelet-type bleeding symptoms despite normal or slightly low platelet number, which will be discussed in this chapter in more detail. Peripheral loss of platelets can be caused by the immune system inappropriately targeting platelets and resulting in platelet destruction. There are several disorders that result from predominantly antibody-mediated platelet destruction and/or consumption. Three of these disorders, immune thrombocytopenia (ITP), heparin-induced thrombocytopenia (HIT)/thrombosis, and thrombotic thrombocytopenic purpura (TTP), will be discussed here. Immune-mediated platelet refractoriness will also be briefly reviewed.

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Immune Thrombocytopenia

Immune thrombocytopenia (ITP) is a common cause of often-severe thrombocytopenia with variable bleeding symptoms occurring in both adults and children. ITP is an acquired autoimmune disorder with multifactorial etiology including generation of antiplatelet autoantibodies by an immune trigger (most commonly infection), direct T-cell cytotoxicity, and abnormal platelet production in the bone marrow [7–9]. There is no diagnostic test that is confirmatory; therefore, ITP is a diagnosis of exclusion. There are multiple disorders that should be ruled out with clinical history, blood count and smear review, and other laboratory testing as indicated [10]. See Table 13.1 for a list of diagnoses that should be considered in the differential diagnosis of ITP. The management of bleeding in ITP is different from other causes of thrombocytopenia, and therefore, accurate diagnosis is essential.

Primary and Secondary ITP

ITP can be primary (not triggered by another disorder), which is the case in up to 75% of children [14] but much less frequent in adults, or secondary to another disorder, including autoimmune diseases such as systemic lupus erythematosus (SLE), inflammatory bowel diseases (IBD), immunodeficiencies such as common variable immunodeficiency (CVID) or DiGeorge syndrome, thyroid disease, infection (human immunodeficiency virus (HIV), hepatitis C, hepatitis B, *Helicobacter pylori*), chronic lymphocytic leukemia (CLL), or pregnancy [14–16]. Secondary ITP is most successfully treated by optimal management of the underlying condition [17–19].

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Table 13.1 Differential diagnosis of immune thrombocytopenia

Category	Diseases
Macrothrombocytopenia	MYH9 group, familial thrombocytopenias, GATA-1 Group, Bernard-Soulier syndrome, gray platelet syndrome, Montreal platelet syndrome
Congenital thrombocytopenia	TAR, X-linked thrombocytopenias, Wiskott-Aldrich syndrome, Fanconi anemia, Bernard-Soulier syndrome, congenital amegakaryocytic thrombocytopenia, GATA-1-associated X-linked dyserythropoietic anemia, and thrombocytopenia; RUNX1-associated thrombocytopenia
Neonatal thrombocytopenia	Sepsis, congenital infections, perinatal insults, maternal ITP, neonatal alloimmune thrombocytopenia, trisomies [11–13], thrombosis, placental insufficiency
Acquired thrombocytopenia	Infections, drugs, toxins, splenomegaly
Thrombotic microangiopathies	Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, atypical hemolytic uremic syndrome
Marrow infiltration	Leukemia/oncologic process, marrow fibrosis, myelodysplastic syndrome
Other	Rheumatologic, autoimmune lymphoproliferative syndrome, paroxysmal nocturnal hemoglobinuria, common variable immunodeficiency syndrome, von Willebrand disease type 2B, Evans syndrome, pseudothrombocytopenia

TAR thrombocytopenia-absent radius, *ITP* immune thrombocytopenia

Categorization

The International Working Group (IWG) on ITP Consensus Report recommends the following categories to stratify ITP cases according to the duration of disease [15]:

- Newly diagnosed: 0–3 months from diagnosis
- Persistent: 3–12 months from diagnosis
- Chronic: >12 months from diagnosis

These categories guide therapeutic decisions and predict the likelihood of disease resolution.

Symptoms

Platelet-type bleeding, including petechiae, purpura, and bruising, is the most common manifestation. Moderate bleeding that may warrant treatment can include wet purpura, epistaxis, menorrhagia, and oral bleeding. Life-threatening bleeding occurs very rarely [11, 20–23]; examples include gastrointestinal bleeding, hematuria, and central nervous system (CNS) bleeding (including intracranial hemorrhage (ICH)). More significant bleeding is generally thought to occur at the lowest platelet counts (i.e., generally less than 20,000/mm^3), but platelet count correlates poorly with bleeding risk [21].

Natural History of ITP

Childhood ITP is generally self-limited, with about 80% of affected children experiencing spontaneous resolution without recurrence, regardless of interventions and treatments that may be necessary during the course of the disease [14]. Adult ITP is generally chronic, with about 80% of adults having a course that persists indefinitely [12, 24].

Diagnosis

Routine evaluation should include a complete history including documentation of historically normal platelet count (if available) and physical exam. Physical exam should be normal with the exception of any relevant bleeding manifestations. Significant lymphadenopathy or organomegaly is not expected in ITP, and the presence of these abnormalities should prompt consideration of an alternative diagnosis.

Laboratory Testing

The cornerstones of the laboratory evaluation of suspected ITP are the complete blood count (CBC) and peripheral blood smear. The CBC should be normal except for thrombocytopenia, and platelet size is generally variable to large. Significant white blood cell (WBC) or red blood cell (RBC) abnormalities warrant further evaluation. Reticulocytosis should not be present. The peripheral blood smear should show relatively normal erythrocyte and leukocyte populations, and platelets should appear normally granulated and variable in size with the presence of scattered large/giant platelets.

Direct antiglobulin test (DAT; formerly known as direct Coombs test) and serum immunoglobulins are recommended in all newly diagnosed ITP patients, as these markers may be associated with underlying tendency toward autoimmunity [13, 25]. Hepatitis C and HIV testing is recommended for all adults, and *Helicobacter pylori* testing is recommended for high-risk or symptomatic adult patients [13]. ANA (antinuclear antibody) testing can be obtained for patients with high suspicion of autoimmunity [25–28], but is rarely helpful in the absence of symptoms in adults with uncomplicated newly diagnosed ITP [19]. The role of bone marrow examination is controversial and generally recommended only in circumstances where the diagnosis is not clear due to the

presence of atypical features. Guidelines from the IWG recommend bone marrow examinations in patients >60 years old with newly diagnosed ITP [13]. However, the American Society for Hematology (ASH) guidelines do not advocate for routine bone marrow studies prior to initiating steroid treatment or in the case of a patient who fails intravenous immune globulin (IVIG) therapy, based on population studies [14, 29]. Antiplatelet antibody testing has not been shown to have adequate sensitivity and specificity for use in the diagnosis or management of ITP. There are many reports of the presence of positive antibodies in alternative diagnoses, and antibodies are not always present and identifiable in all cases of ITP because of the multifactorial etiology of the disease. Platelet-associated IgG or IgM (known as PA IgG or PA IgM) has a very low sensitivity (reported as low as 40–60%), which significantly limits the diagnostic utility in ITP [30–33]. Glycoprotein-specific antibody testing (direct antibody testing) has a high specificity (~75–95%), but the sensitivity is low [34, 35]. For these reasons, ASH and IWG guidelines recommend against routine platelet antibody testing for the diagnosis of childhood or adult ITP [13, 14].

Management of ITP in Children

Given the typically mild clinical symptoms and expectation of resolution in childhood ITP, most experts recommend observation without treatment regardless of the platelet count in children with no symptoms or with only mild cutaneous findings [14]. However, for children with "wet" bleeding symptoms, including wet purpura, epistaxis, or menorrhagia, treatment is recommended to elevate platelet count to a hemostatic range, facilitating cessation of bleeding and decreasing the risk of ICH and other forms of life-threatening bleeding [14].

Management of ITP in Adults

It is estimated that less than 30% of adults can be successfully managed with observation alone [14]. Current recommendations for management of ITP in adults advise treatment for those patients with a platelet count <30,000/mm^3 or those with bleeding symptoms or need for a procedure.

Management of ITP in Pregnancy

ITP in pregnancy is generally mild in women with no history of ITP prior to pregnancy. It can typically be followed with close observation if the platelet count is >30,000/mm^3 without bleeding symptoms [36]. Affected patients may require treatment for severe thrombocytopenia and bleeding or in anticipation of a procedure, including amniocentesis or delivery. Splenectomy is generally avoided as the ITP usually spontaneously resolves after pregnancy, but is considered a safe second-line intervention in the second trimester for severely affected patients [14, 36]. The optimal platelet count to achieve hemostasis during delivery has not been established. A count of 75,000–80,000/mm^3 is generally recommended [37], especially in the case of epidurals or spinal anesthesia [38], but for uncomplicated deliveries, a platelet count of 50,000/mm^3 is considered safe, even for cesarean section [39]. The ITP diagnosis itself is not an accepted indication for cesarean delivery, as the risk of ICH with vaginal delivery is low (<1%) [19]. The newborn should be monitored for at least 7 days post-delivery for thrombocytopenia secondary to passively acquired maternal antiplatelet antibody [37]. Maternal therapy and platelet count are poor predictors for the neonate's platelet count, with the only reliable predictor being the platelet count and course of thrombocytopenia of an older sibling [40]. In severely thrombocytopenic newborns, imaging to evaluate for ICH should be obtained regardless of symptoms, as it can be clinically silent. If treatment is required in the neonate, intravenous immune globulin (IVIG) can be considered. The affected neonate should have spontaneous resolution as the passively acquired antibodies clear the body, usually by 12 weeks of age [36].

Frontline Therapies

There are several treatment options for the temporary improvement in bleeding symptoms and thrombocytopenia in ITP. These therapies function by interfering with immune destruction of antibody-coated platelets and include corticosteroids, IVIG, or anti-D immune globulin. Steroid dosing and duration recommendations are varied, but the standard first-line treatment for many years has consisted of a course of 1–4 mg/kg/day of oral prednisone for 2–4 weeks (including a taper) [10, 13–15]. Other acceptable steroid regimens include high-dose IV methylprednisolone 30 mg/kg/day (maximum of 1 g/day) and oral prednisone 4–8 mg/kg/day for 3–7 days, followed by a prolonged taper to day 21 [36]. Recent work has investigated whether intensification of treatment using high-dose dexamethasone results in increased remission rates in adults with ITP. One randomized trial found that a complete response rate was higher in the high-dose dexamethasone arm (six 3-week cycles of 0.6 mg/kg/d of dexamethasone pulsed on days 1–4) compared to the standard prednisone arm (1–2 mg/kg/d × 2–4 weeks with taper) [41].

IVIG is typically administered at 1 g/kg IV × 1–2 doses and anti-D immune globulin at 50–75 μg/kg IV × 1 dose. These therapeutic modalities typically increase circulating

platelet count within 24–48 h, whereas corticosteroids usually take 3–5 days, but can take up to 2 weeks, for an effect to be seen [15]. Of note, Rh-negative patients have not shown benefit from anti-D immune globulin.

The choice of therapy depends on a variety of factors including the side effect profile of each agent and the indication for treatment, as well as patient-related factors. Platelet transfusion is generally avoided due to the expected rapid antibody-mediated clearance of transfused platelets and theoretical risk of increased antibody development. IVIG and corticosteroids are both considered acceptable frontline treatment options in pregnancy [14]. Medications that affect platelet number or function should be avoided (aspirin, NSAIDs), and activity restrictions may be required depending on the platelet count and bleeding symptoms. Antifibrinolytic agents (aminocaproic acid and tranexamic acid) can be helpful adjuncts to therapy for mucosal bleeding symptoms [13, 36].

Two small studies using thrombopoietin receptor agonists (TPO-RA) as up-front therapy in ITP have shown promising results [42, 43]. However, additional studies with greater patient numbers are needed in order to evaluate the long-term outcomes with these newer agents before official recommendations on their use as first-line agents can be made.

Second-Line Therapies

For patients who fail frontline therapy or have persistent/severe disease, a second-line therapy may be considered to achieve a more durable platelet response. The choice of agent/treatment is dependent on a variety of factors and should be made on a case-by-case basis. Examples of second-line treatments include splenectomy, rituximab, TPO-RA, and alternative immunosuppressive agents [10, 13, 14]. Encouraging data are becoming available on TPO-RA as second-line therapy for both adult and childhood ITP [44–49]. With the increasing use of TPO-RA, as well as risk for infection, thrombosis, and postoperative complications with splenectomy, fewer patients with ITP are undergoing splenectomy than before [50].

Management of Life-Threatening Bleeding

A patient experiencing life-threatening bleeding in the setting of ITP requires an aggressive treatment approach using a combination of therapies. IVIG and high-dose IV steroids should be given, with consideration of platelet transfusion/drip to facilitate hemostasis acutely [10, 13]. If the patient has signs and symptoms worrisome for ICH, expeditious imaging should be obtained with surgical and neurosurgical consultations as needed. Urgent/emergent splenectomy can be life-saving in the setting of uncontrolled bleeding or neurologic compromise [14].

Heparin-Induced Thrombocytopenia/Thrombosis

Heparin-induced thrombocytopenia (HIT) is a potentially life-threatening clinical syndrome caused by immune reaction and antibody formation to platelets upon exposure to heparin. HIT most commonly occurs after exposure to unfractionated heparin and, very rarely, to low molecular weight heparin products [51, 52]. Although the incidence of antibody development to heparin exposure is higher, actual development of HIT occurs in only about 1–3% of adults receiving treatment doses of unfractionated heparin [53–55]. The incidence is much lower in children, those receiving prophylactic dosing or with heparin used as line flush, and those receiving low molecular weight heparin products [53]. The highest risk appears to be in the cardiac surgery setting [56]. Thrombocytopenia, typically not severe, is the most common clinical manifestation of HIT [53]. Platelet counts rarely fall below 20×10^9/L [57], with median counts ranging from 40,000/mm^3 to 60,000/mm^3 [58]. HIT is not typically associated with bleeding symptoms and, in fact, is associated more commonly with thrombosis [53]. Venous thrombi are more common (occurring in ~30–60% of diagnosed patients) [53, 56, 59, 60] than arterial thrombi, which occur in ~3–10% of patients [53, 60, 61]. Patients with heparin-induced thrombocytopenia and thrombosis (HITT) have high morbidity and mortality rates [59, 60].

Pathophysiology

In susceptible individuals, heparin exposure results in the generation of IgG antibodies that react with heparin and platelet factor 4 (PF4) released from platelet alpha granules, resulting in the formation of heparin-PF4-IgG immune complexes. These complexes bind to Fc gamma receptors on the surface of platelets, subsequently resulting in mild-to-moderate thrombocytopenia, platelet activation, aggregation, and risk of thrombosis due in large part to thrombin generation [52, 53, 62–65]. The onset of thrombocytopenia is typically 5–10 days after first heparin exposure, but is much more rapid (usually within 24–72 h) if there has been previous heparin exposure within months [66, 67]. As in other immune disorders, the incidence of antibody development with heparin exposure is significantly higher than the incidence of clinical HIT [53, 56], and it is not clear why some patients are more susceptible to developing the clinical syndrome than others.

Diagnosis

HIT is a clinical-pathologic syndrome: diagnosis is based on the presence of one or more HIT-associated clinical symptoms and the detection of heparin-PF4-IgG immune complexes [53, 68, 69]. A precipitous drop in platelet count to <100,000/mm^3, or >50% reduction in platelet count in an individual exposed to heparin, should raise suspicion for this diagnosis [58]. Laboratory testing, often with long turnaround times, should only be pursued for patients with a high clinical suspicion. The 4T scoring system is a clinical prediction tool developed to assist clinicians in determining appropriate candidates for laboratory testing [53]. The 4Ts are *T*hrombocytopenia, *T*iming of platelet count fall, *T*hrombosis, and other causes for *T*hrombocytopenia. See Table 13.2 for calculation of the 4T score. Patients with a 4T score of 0–3 points have a low probability of HIT and likely do not require testing for PF4 antibodies. This tool does have limitations, as patients with a high 4T score may not have a diagnosis of HIT [70–72]. Laboratory diagnosis of HIT first involves the ELISA measurement of heparin-dependent IgG antibodies targeted to heparin-PF4 complexes; a negative ELISA test ensures that HIT can be excluded with high probability and heparin can be continued if clinically indicated [73]. However, when positive, confirmatory testing must be obtained because this test is associated with a high false positive rate [74, 75]. The platelet [^{14}C] serotonin release assay (SRA) is considered the gold-standard test for the confirmation of HIT and should be obtained when there is positive ELISA testing [76, 77]. Unfortunately, the SRA test is technically challenging and not widely available, limiting its usefulness when making treatment decisions [53, 74]. Other functional assays for confirmation of a HIT diagnosis have been developed, some of which allow for rapidly available results [73]. However, more data are needed before these tests can replace the current standard for diagnosis.

Table 13.2 The 4T scoring system for diagnosis of HIT

4Ts	Condition	Points
Thrombocytopenia	Platelet count fall >50% and nadir ≤20,000/mm^3	2
	Platelet count fall 30–50% or nadir 10–19,000/mm^3	1
	Platelet count fall <30% or nadir <10,000/mm^3	0
Timing of platelet count fall	Between days 5 and 10 or </= 1 day if prior heparin exposure within the last 30 days	2
	Consistent with fall between 5 and 10 days but unclear, onset after day 10, or fall ≤1 day with prior heparin exposure within 30–100 days	1
	Platelet count fall <4 days without recent heparin exposure	0
Thrombosis or other sequelae	Confirmed new thrombosis, skin necrosis, or acute systemic reaction after IV unfractionated heparin bolus	2
	Progressive or recurrent thrombosis, non-necrotizing skin lesions, or suspected thrombosis, not proven	1
	None	0
Other causes of thrombocytopenia	None apparent	2
	Possible	1
	Definite	0

Interpretation
0–3 points, low probability
4–5 points, intermediate probability
6–8 points, high probability

Treatment

Upon suspicion of a HIT diagnosis, the most important intervention is immediate discontinuation of heparin from all sources, including line flushes, regardless of laboratory confirmation [54, 78]. Given the ongoing risk of thrombosis (as high as 25–50%) despite discontinuation of heparin, these patients require alternative anticoagulation [54, 79]. Generally, low molecular weight heparin should not be used due to the likely presence of cross-reacting antibodies [57, 80]. Warfarin is not a good substitution for heparin secondary to the risk of venous limb gangrene or skin necrosis on initiation [81–83]. Acceptable non-heparin anticoagulant alternatives include the direct thrombin inhibitors lepirudin, argatroban, and bivalirudin, as well as the factor Xa inhibitors danaparoid and fondaparinux [54]. In patients with renal insufficiency, argatroban should be considered, as it is not renally cleared [84]. Whereas there is an abundance of evidence supporting the use of lepirudin, argatroban, and danaparoid in the treatment of HIT, the evidence supporting the use of bivalirudin and fondaparinux is limited to case series and systemic reviews [53, 85], and therefore, these drugs are considered "off-label" in the treatment of HIT. The new direct oral anticoagulants (DOACs) are being used more frequently in patients with HIT and have shown safety and efficacy in case reports, case series, and retrospective reviews [86–88]; however, more data are needed before these agents become standard HIT therapy.

Thrombotic Thrombocytopenic Purpura

Thrombotic thrombocytopenic purpura (TTP) is a rare but potentially life-threatening condition with an incidence of approximately 1–4 per million person years [89, 90]. The disorder is secondary to a deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) [91], responsible for cleaving high molecular weight von Willebrand factor multimers [92, 93]. TTP can be congenital or, more commonly, acquired,

secondary to the development of inhibitory antibodies to ADAMTS13. Acquired TTP is more common in adults than in children, and within the adult population, women and African Americans have the highest incidence [90]. Acquired TTP is very rare in the pediatric population, with an incidence of 0.1 per million; within this group, adolescents are the most commonly affected [94]. TTP is a life-threatening condition with high morbidity and mortality. The mortality rate in untreated TTP approaches 90% [95], but with treatment is approximately 10–20% [96]. Therefore, timely recognition of symptoms is important in decreasing fatality from the disease.

Pathophysiology

The pathophysiologic abnormalities in TTP are secondary to the decreased concentration of the von Willebrand factor (VWF) cleaving protease, ADAMTS13, released by endothelial cells and megakaryocytes [92, 93, 97]. Physiologically, periods of shear stress result in a partial unfolding of ultra-large VWF multimers, allowing ADAMTS13 cleavage at the Tyr-Met bond in the second A-domain of VWF [92, 93, 98]. Without the metalloprotease, the high molecular weight VWF multimers are not cleaved from the surface of the endothelium, passing platelets adhere and aggregate to the long multimers, and platelet thrombi form within the microvasculature, leading to end-organ damage (especially of the kidneys, brain, and heart) and development of microangiopathic hemolytic anemia [99].

In acquired TTP, the ADAMTS13 protease is inhibited by IgG-type antibodies generated by a dysfunctional immune system [91, 100, 101]. Drugs, infection, underlying autoimmunity, pregnancy, and pancreatitis can all lead to the development of acquired TTP [102–109]. Medications implicated in drug-induced TTP include clopidogrel, tacrolimus, sirolimus, mitomycin, alpha interferon, gemcitabine, quinine, and cyclosporine [102, 109, 110]. The ultimate reason for ADAMTS13 inhibitor development is unknown, although the phenomenon likely arises in those patients otherwise predisposed to autoantibody development.

Presentation

The onset of acquired TTP is typically brisk due to overwhelming antibody formation. The clinical presentation varies and may include skin and mucosal bleeding; neurological symptoms that can range in severity from mild (headache) to severe (confusion, seizures, altered mental status); weakness; fever; nausea, vomiting, diarrhea, or abdominal pain; and renal insufficiency [109, 111–113]. Classically, the presentation of TTP has been described by a "pentad" of symptoms: thrombocytopenia, microangiopathic hemolytic anemia, neurologic abnormalities, renal failure, and fever [114]; however, the pentad is variably present with some features found more commonly than others [112]. Laboratory findings in TTP include anemia, thrombocytopenia (often <20,000/mm^3), and reticulocytosis on CBC, the presence of schistocytes and erythrocyte fragments on the peripheral blood smear, and elevated LDH from a combination of hemolysis and tissue damage/ischemia [99, 109, 115].

Diagnosis

The combination of thrombocytopenia, schistocytosis, and LDH is often sufficient to suggest a diagnosis of TTP [99, 112]. Measurement of low (<10%), or absent, ADAMTS13 activity is confirmatory of the disease; to further distinguish congenital vs. acquired types, plasma inhibitory and/or non-inhibitory anti-ADAMTS13 antibodies are measured [95, 113]. Both of these tests can be useful when followed over time to determine response to therapeutic interventions. The degree of ADAMTS13 reduction at presentation has been shown in some studies to be prognostic of risk of relapse [109, 116–118]. Additionally, the presence of detectable antibodies at initial diagnosis has been associated with higher mortality risk and worse clinical outcome [119–121]. ADAMTS13 antibody levels are performed at specialty hematology labs that can require up to a 7-day turnaround. Given the life-threatening and rapidly progressive nature of this disorder, the diagnosis should be made clinically and therapy instituted rapidly, rather than waiting for the results of this testing [120].

Differential Diagnosis

Microangiopathic hemolytic anemia and thrombocytopenia are present in other disorders, and therefore, the clinician must be alert to other possible diagnoses. The most common disease overlap is with atypical hemolytic uremic syndrome (aHUS) and diarrhea-associated hemolytic uremic syndrome (D + HUS). Historically, patients with neurologic symptoms were labeled as having TTP, and patients with more overt renal injury were labeled as having HUS; however, this distinction does not always hold true, leaving patients with an uncertain diagnosis and labeled with the spectrum disorder "TTP-HUS" [95]. Laboratory investigations now make it possible to make a distinction between the two disorders, which becomes crucial in treatment and overall prognosis. Other disorders on the differential include preeclampsia or HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome in pregnancy; autoimmune disorders, including ITP, SLE, antiphospholipid antibody syndrome, and

scleroderma; sepsis; malignancy; disseminated intravascular coagulation (DIC), malignant hypertension, and others [109, 122, 123].

Screening for Other Disorders

A high percentage of patients with acquired TTP eventually develop other underlying autoimmune diseases, including SLE and antiphospholipid antibody syndrome [105, 122, 124]. Testing for autoimmunity, at least by a thorough screening for signs and symptoms specific to autoimmune disorders, should be performed, and development of symptoms should be monitored over time [122]. Additionally, these patients are at risk for thrombosis [107] and should avoid thrombotic risk factors, including estrogen-containing oral contraceptives and medications, long plane flights, smoking, and obesity.

Treatment

The treatment of acquired TTP is dependent on removal of the ADAMTS13 antibody, as well as the high molecular weight VWF multimers, by plasma exchange [99, 125]. Simultaneously, high-dose steroids should be initiated to inhibit antibody production for a more long-term, sustained response [99, 112, 126]. It is also important to remove/treat the offending cause (i.e., drugs, infection) as soon as possible, if known. TTP is a life-threatening disorder that can be rapidly progressive and fatal if plasma exchange is not instituted rapidly upon clinical suspicion. The mortality rate of TTP was over 90% prior to the institution of plasma exchange for treatment, but has now improved to 10–20% [96, 127]. If plasma exchange is not readily available, plasma transfusion can be initiated until exchange is available [128]. Plasma exchange requires a large-caliber catheter for removal of the patient's plasma in exchange for donor fresh frozen plasma (FFP) containing normal ADAMTS13 [99, 112]. Although the standard in exchange is FFP, an alternative is cryoprecipitate-poor plasma, which is deficient in VWF [129]. Plasma exchange should occur daily until the platelet count has normalized (>150,000/mm^3 for at least 1 day), LDH has decreased to normal or near normal, and hemoglobin has begun to rise [111, 118, 130]. More recent studies have demonstrated that ADAMTS13 levels should be >10% prior to discontinuing TPE [120]. LDH levels can also be followed as a marker for disease exacerbation [115].

Non-focal neurologic symptoms have been observed to resolve rapidly and radically upon the institution of plasma exchange, likely because of the removal of the high molecular weight VWF multimers [95, 130]. Platelet count begins to increase within the first few days and usually normalizes by day 7, whereas anemia may worsen initially, requiring continued red cell transfusion support [122, 130]. Renal damage usually requires a lengthier recovery—as long as several months—and full recovery is often uncertain [122]. Platelet count has proven an important predictor of response to plasma exchange; failure of response requires an increase in the number or volume of plasma exchange or addition of other treatment modalities [130]. Similarly, a decrease in platelet count after initial recovery should alert the clinician that control of the disease has not been achieved [130]. Measurement of ADAMTS13 at regular intervals during treatment and remission may provide information on the risk of relapse or persistence of disease [123, 131]. The duration of plasma exchange varies greatly, with most studies reporting a duration ranging from 7 to 20 days [96, 125, 132, 133].

Patients with a more severe course, those who have multiple exacerbations after cessation of plasma exchange, or those with relapses despite plasma exchange and glucocorticoid treatment may benefit from stronger immunosuppression [117]. Rituximab, an anti-CD20 monoclonal antibody, is an immunosuppressive agent now considered a standard second-line therapy for adults with antibody-mediated TTP [134, 135]. There have been recent studies evaluating the use of rituximab as a frontline agent in combination with plasma exchange in high-risk patients, as well as using rituximab as prophylaxis against relapse, with promising results [126, 134, 136, 137]. The drug is especially efficacious in patients with evidence of TTP and underlying systemic autoimmunity [138]. Rituximab depletes CD20-positive B-lymphocytes, preventing antibody formation that can last up to 6–9 months [136, 139]. The drug should be administered in four weekly doses at 375 mg/m^2 [116, 138, 140–142]. There have been concerns about the effectiveness of rituximab administration when given concurrently with plasma exchange because of drug clearance. Although there is evidence that 65% of rituximab is cleared by plasma exchange [139], studies have demonstrated improved outcomes and fewer relapses in patients receiving the combination therapy [135, 137]. Therefore, it is recommended to proceed with rituximab concurrent with plasma exchange, if medically indicated. It is, however, important that the drug be given immediately after plasma exchange to maximize the time in circulation prior to the next exchange. Additional doses of rituximab can be given if ADAMTS13 activity decreases or inhibitor levels increase or if there has not been total B-cell depletion after the standard course [142]. While rituximab is becoming standard of care for treatment of adult TTP, there is less evidence to date for the use of rituximab in pediatric TTP [113, 143].

A variety of other immunosuppressive agents have been trialed in the treatment of TTP, including vincristine, cyclophosphamide, azathioprine, cyclosporine A, IVIG, and others, and results of these case reports and retrospective reviews

are varied [95, 112, 122, 134]. Splenectomy has been performed in TTP patients in an effort to prevent additional relapse, with some success [128, 133, 144]; however, this treatment modality is not routinely recommended because of the complications and risks associated with it [128, 145].

Recurrence

Relapses occur in 20–65% of TTP patients [116, 118, 127, 146–148]. Relapse rates are high in patients presenting with severe ADAMTS13 deficiency (<10%) and can be common in patients with an underlying autoimmune disorder [116–118, 122]. Relapses are most common in the year following the TTP episode [116]. Resumption of plasma exchange is the first treatment choice for relapse, and often rituximab is initiated, if not previously administered. If remission cannot be achieved, other immunosuppressive agents may be trialed [128].

Congenital TTP

Congenital deficiency of ADAMTS13, also known as Upshaw-Schulman syndrome, is less common than acquired TTP and results in a relapsing/remitting TTP syndrome [149]. Approximately 75% of children have their first TTP episode in the neonatal period, and 25% present between 2 months and 4 years of age [113]. Patients with more mild mutations may present in adulthood, often during pregnancy [126, 150]. Neonates present with icterus, hyperbilirubinemia, severe hemolytic anemia that often leads to hemoglobinuria and acute renal insufficiency, and severe thrombocytopenia (<20,000/mm^3) [113]. Older children and adults often present after a triggering event, such as infection, stress, or hormonal changes, with thrombocytopenia and hemolytic anemia [113]. The disorder is inherited in an autosomal recessive pattern, leading to compound heterozygous or homozygous mutations of the ADAMTS13 gene [151, 152]. ADAMTS13 gene sequencing should be obtained (performed only by specialty laboratories) in patients without detectable ADAMTS13 antibodies to confirm the diagnosis of congenital TTP [113, 123, 153].

The approach to treatment of congenital ADAMTS13 deficiency is replacement of the ADAMTS13 protein, typically through transfusion of FFP [99]. This may be done periodically at the time of TTP exacerbation, but after several exacerbations, a scheduled regular transfusion of FFP is typically prescribed to prevent potentially life-threatening complications, usually at 2–3-week intervals [113, 154]. The schedule of transfusion should be based on the individual patient presentation, but increasing the interval between transfusions beyond 4 weeks can increase the risk of relapse [113]. Side effects of FFP, as well as donor exposure, cannot be taken lightly. Importantly, treatment of neonates with congenital TTP often requires plasma exchange, rather than FFP transfusion alone, secondary to the accompanying severe hyperbilirubinemia [113]. An emerging therapy for congenital TTP is a recombinant ADAMTS13 replacement product [145, 155]. Clinical trials with this agent are underway, showing initial safety and efficacy [156]. Therefore, recombinant ADAMTS13 may be a viable therapeutic option in the near future, eliminating the disadvantages of FFP transfusion [153].

Platelet Refractoriness

Platelet transfusions are required for a variety of causes of thrombocytopenia. In most cases, transfusion of platelets is a lifesaving intervention. Approximately 20% of hematology and oncology patients, however, do not achieve the expected response to platelet transfusions and are considered platelet refractory [157]. Platelet refractoriness is associated with an increased risk of morbidity and mortality and is related to significant bleeding events [158]. The definition of "optimal response" to platelet transfusion historically has been defined as an increase in platelet count of at least 5000–10,000/mm^3 1 h after transfusion [159]. More accurately, one can measure the 1-h corrected count increment (CCI), an objective measure of whether a patient is refractory to platelet transfusion [160, 161]. The formula requires the posttransfusion platelet increment, the body surface area of the patient, and the number of platelets transfused: CCI = (platelet increment/μL × BSA in m^2)/number of platelets transfused × 10^{11}. An acceptable CCI value is considered >5000 [162].

Pathophysiology

There are immune and non-immune causes for platelet refractoriness. Non-immune causes are far more common than immune causes and are secondary to acute events that lead to platelet consumption. Non-immune causes of platelet refractoriness include splenomegaly, veno-occlusive disease, DIC, febrile illnesses, sepsis, graft-vs.-host disease, bleeding, and medications, among others [157, 161, 163–166]. A percentage of frequently transfused patients become refractory due to immune destruction of transfused platelets. Immune platelet refractoriness is caused by alloimmunization to human leukocyte antigens (HLA) or, less commonly, human platelet antigens (HPA), after prior exposure (i.e., prior transfusions, maternal-fetal incompatibility during pregnancy, transplantation) [161, 167–169].

Diagnosis

A patient is confirmed to have platelet refractoriness if the 1-h posttransfusion CCI value is <5000 on at least two occasions [162]. To distinguish the type of refractoriness, it is helpful to additionally measure the platelet count at 18–24 h after transfusion to gain information about platelet survival. In non-immune cases of platelet refractoriness, patients typically have a normal 1-h CCI, but platelet survival is decreased (platelet count will return to pre-transfusion levels within 24 h), whereas in immune cases of platelet refractoriness, patients typically have a low 1-h CCI [160, 165, 170–172]. If further testing is desired in patients with suspected alloimmunization, testing for the presence of HLA antibodies should occur first, followed by HPA antibody testing if negative, as HLA antibody development is more common [167]. Some consider a less-than-expected 1-h CCI level diagnostic for alloimmunization and proceed to treatment without further testing.

Management and Prevention

For patients with non-immune platelet refractoriness, the most important intervention is treating the underlying illness or suspected cause [161]. Patients with alloimmunization require HLA-compatible platelet transfusions to raise platelet count increments [173]. This can be achieved by transfusing HLA-antigen-negative platelets (corresponding to specificity of anti-HLA antibodies), HLA-matched platelets, or crossmatch-compatible platelets [166]. Most commonly, patients are transfused with HLA-antigen-negative platelets, as this method does not require obtaining the HLA typing of the patient and provides a larger donor pool [174]. The donor pool for HLA-matched platelets is smaller because they are obtained from donors that are a match for the HLA-A or HLA-B loci [161]. Importantly, these platelet units should be irradiated prior to transfusion to decrease the risk of transfusion-associated graft-vs.-host disease [175]. Crossmatching involves identifying compatible platelet units by crossmatching with the patient's plasma. This method is quick (a few hours) and allows for a larger donor pool than HLA matching [161, 176]. After the introduction of leukoreduction procedures (removal of white blood cells from platelet products by filtration), a near 50% reduction in HLA alloimmunization was observed [177]. Therefore, platelets should be leukoreduced prior to transfusion in frequently transfused patients (i.e., hematology/oncology, parous patients) to prevent alloimmunization. However, it is important to note that leukoreduction does not reduce the incidence of platelet refractoriness if the patient develops HPA antibodies [167, 178, 179].

Platelet Function Disorders

Platelet function disorders are a group of hereditary or acquired disorders characterized by defective platelet function. The platelet count in patients with these disorders may be normal, reduced, or even elevated. Hereditary platelet function disorders are uncommon and often difficult to diagnosis [180]. There are many different types of hereditary platelet defects, which can be categorized broadly based on platelet size. Acquired platelet function disorders are more common than hereditary platelet function disorders, although still rare, and are caused by underlying diseases, infection, drugs, autoimmunity, or trauma [159, 181]. Here we will focus on hereditary platelet function defects.

Platelet Function Disorders with Giant Platelets

Giant platelet disorders include Bernard-Soulier syndrome (BSS), the MYH9 group of platelet disorders, gray platelet syndrome (GPS), and platelet-type von Willebrand disease (VWD). BSS is an autosomal recessive disorder characterized by thrombocytopenia, large platelets, and prolonged bleeding time [182]. The syndrome is secondary to a deficiency of the platelet glycoprotein complex GP1b/IX/V [183, 184], which normally functions to facilitate platelet adhesion to VWF on damaged endothelium [185]. The disorder can be diagnosed with an abnormal platelet function analyzer-100 (PFA-100) assay, by flow cytometry, or by platelet aggregation studies showing normal aggregation responses with ADP, arachidonic acid, collagen, and epinephrine, but absence of aggregation to ristocetin [186]. Presentation varies among patients and can include spontaneous epistaxis, mucocutaneous bleeding, ecchymosis, and gastrointestinal bleeding, all of which can be more severe than the degree of thrombocytopenia; severe bleeding can occur with trauma [180, 185].

The MYH9-related disorder is a syndrome caused by a mutation within the *MYH9* gene that is inherited in an autosomal dominant fashion [187, 188]. The syndrome includes the previously classified disorders May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome [180, 189]. It is characterized by macrothrombocytopenia and mucocutaneous bleeding in early life, with the development of hearing loss, glomerulonephritis, and cataracts with aging [190, 191]. Life-threatening bleeding is rare, but can occur with trauma [180]. Diagnosis should be strongly considered in a patient with macrothrombocytopenia in addition to glomerulonephritis, sensorineural hearing loss, and cataracts [180]. Platelet count is variable from patient to patient, but peripheral smear showing large or

giant platelets and/or neutrophils with Döhle-body inclusions is highly suggestive of MYH9 [190]. Definitive diagnosis is achieved with demonstration of a mutation in the *MYH9* gene.

GPS is a very rare hereditary platelet function disorder secondary to decreased alpha granule content within the platelet [192]. There are case reports of autosomal dominant and autosomal recessive inheritance, but it can also be sporadic [180, 190]. The syndrome is characterized by normal or low platelet count, large/giant platelets, and mucocutaneous bleeding of variable severity [190]. Platelets appear agranular and thus gray on peripheral smear, and platelet aggregation studies may be abnormal to one or more agonists [180].

Platelet-type VWD is an autosomal dominant macrothrombocytopenia secondary to gain-of-function mutations that increase the affinity of GP1b/IX/V for VWF, resulting in shortened platelet survival [190, 193]. Patients present with mucocutaneous bleeding that is usually mild-to-moderate in severity [190]. Diagnosis is confirmed with increased ristocetin-induced platelet aggregation, in addition to a mild reduction of plasma VWF levels and absence of large molecular weight VWF multimers [190]. Platelet-type VWD is similar to type 2B VWD, but is different in that the genetic defect affects the platelet rather than VWF [194].

Treatments of the giant platelet function disorders include antifibrinolytic agents for mild-to-moderate bleeding and platelet transfusions and/or recombinant activated factor VII (rFVIIa) for severe bleeding [180]. Desmopressin may also be used for mild-to-moderate bleeding in patients with BSS, GPS, and MYH9-related disorder. Although desmopressin has no direct effect on platelets, the ultra-large VWF released by desmopressin-stimulated endothelial cells may facilitate platelet adhesion and decrease the bleeding times in these patients [186]. Desmopressin should not be used in platelet-type VWD as it can worsen thrombocytopenia; in these patients, treatment of severe bleeding includes infusion of VWF-factor VIII concentrates and platelets [195].

Platelet Function Disorders with Small Platelets

Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are X-linked platelet function disorders characterized by thrombocytopenia and small platelets [190]. XLT presents with isolated thrombocytopenia, whereas WAS presents with a severe immunodeficiency leading to recurrent infections, allergies, eczema, autoimmunity, and lymphoid malignancy [190, 196]. The disorders are caused by mutations in the gene encoding the Wiskott-Aldrich syndrome protein (WASp) [197–199].

The incidence of WAS is 1/250,000 and usually occurs in patients of European decent [190]. Patients present at birth with bleeding and frequent illness related to the immune dysregulation, which worsens with age [199]. Bleeding may range from mild mucocutaneous bleeding to severe intracranial or gastrointestinal hemorrhage [190]. Immune dysfunction may present with frequent infections or other signs of dysregulation, including eczema, concurrent autoimmune disorders, IBD, vasculitis, arthritis, or lymphoproliferative disorders [190, 199]. Laboratory findings include thrombocytopenia and small platelet volume on CBC, prolonged bleeding time, a decrease in the number and function of T-lymphocytes, low IgM levels, and high IgA and IgE levels [190, 197]. Treatment for WAS includes prophylactic antibiotics against *Pneumocystis jirovecii* pneumonia in infants and children and platelet transfusions to treat severe bleeding [199]. IVIG is indicated for patients with antibody deficiency [199]. Splenectomy has been successful at correcting thrombocytopenia, but the risks of severe infection often outweigh the benefits [199]. The only curative treatment is hematopoietic stem cell transplantation [200, 201].

Platelet Function Disorders with Normal Platelet Size

Disorders of platelet function in which platelets are of normal size include Glanzmann thrombasthenia (GT), congenital amegakaryocytic thrombocytopenia (CAMT), and thrombocytopenia with absent radii (TAR).

GT is an autosomal recessive disorder resulting from homozygous or compound heterozygous mutations of either the *ITGA2B* or *ITGB3* genes [202], leading to a defective platelet integrin $\alpha_{IIb}\beta_3$ receptor (also known as the glycoprotein complex GPIIb/IIIa) [203–205]. This integrin is present in high concentrations on platelets and, when functionally intact, allows strong bonds to form between the platelet and fibrinogen and/or VWF [180, 186]. Absence results in inefficient platelet aggregation [180]. Patients with severe $\alpha_{IIb}\beta_3$ deficiency (<5% expression) are classified as having type I GT, patients with moderate deficiency (10–20% expression of $\alpha_{IIb}\beta_3$) are classified as having type II GT, and patients with a dysfunction $\alpha_{IIb}\beta_3$ receptor are classified as having the "variant" form of GT [186]. Although the degree of bleeding is variable among patients [202, 206], it has been observed that the greater the deficiency of the $\alpha_{IIb}\beta_3$ receptor, the more severe the bleeding symptomatology [204]. Patients typically present with mucocutaneous bleeding beginning in childhood, often before 5 years of age [186, 204]. The most common clinical features are purpura, epistaxis, gingival bleeding, and menorrhagia [204]. GT can be diagnosed with prolonged closure time of the PFA-100; with the absence of aggregation in response to collagen, ADP, epinephrine, or

arachidonic acid in platelet aggregation assays; and/or by absence/decreased levels of CD41 and CD61 and normal levels of CD42 by flow cytometry [180, 186, 193, 207]. Treatment of GT involves antifibrinolytics (tranexamic acid) for mild-to-moderate mucocutaneous bleeding and platelet transfusion and/or rFVIIa for severe bleeding [180, 208]. It is important to note that GT patients may develop antibodies to platelet membrane surface αIIbβ3 antigens on transfused platelets, resulting in inhibition of platelet function or rapid clearance of transfused platelets [208]. In these cases, platelet transfusion should be avoided and rVIIa treatment administered for severe bleeding. Menorrhagia can be treated with oral tranexamic acid and/or hormone therapy [180]. In cases of a severe bleeding phenotype, hematopoietic stem cell transplantation can be considered [208, 209].

CAMT, characterized by severe thrombocytopenia, is an autosomal recessive disorder affecting the *MPL* gene [210]. Mutations in this gene result in altered expression or function of the thrombopoietin receptor [210]. Patients typically present in the neonatal period with bleeding symptoms secondary to severe thrombocytopenia and often progress to pancytopenia and/or severe aplastic anemia within 5–10 years [180, 211]. Thrombocytopenia diagnosed in infancy, along with reduced or absent megakaryocytes in the bone marrow, is suggestive of the disease; definitive diagnosis is obtained with confirmation of mutations in the *MPL* gene [180]. Bleeding symptoms are treated with platelet transfusions, but progression of aplasia requires hematopoietic stem cell transplantation [180, 212–214].

TAR is an autosomal recessive disorder with an uncertain genetic basis that remains the subject of active investigation [195, 215]. Patients present in the neonatal period with severe thrombocytopenia and bilateral absent radii; other clinical features may be present, including cow's milk intolerance, skeletal defects, renal abnormalities, cardiac anomalies, and facial capillary hemangiomas [215, 216]. Unlike in CAMT, the severe thrombocytopenia generally improves throughout childhood [180, 190]. Diagnosis is suggested by congenital thrombocytopenia and the associated clinical abnormalities [180]. Bleeding in infancy may be severe and require platelet transfusions, but with aging, treatment is rarely needed [180].

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