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REVIEW ARTICLE

The role of peripheral blood smear examination in the evaluation of suspected platelet-related disorders in children: A practical approach and an illustrated review

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Abstract

Platelets, along with coagulation factors and vasculature, represent the three main compartments of hemostasis. Upon investigation of a suspected hemostasis disorder, platelet count, size and morphology often offer important clues to the diagnosis or help narrow the differential diagnosis. In this review, we describe a general approach to diagnosing platelet disorders, starting with easily obtained data such as findings of complete blood count (CBC) and microscopic review of a stained peripheral blood smear. We discuss general findings that help separate consumptive from underproduction thrombocytopenia. We further touch on inherited thrombocytopenia disorders after classifying them into those associated with small, normal sized or large platelets. Illustrative microscopic images are provided where contributory. We conclude with a suggested algorithmic step-by-step approach to investigating a suspected platelet disorder in children.

Keywords: platelets, peripheral blood smear examination, inherited thrombocytopenia, algorithmic approach to thrombocytopenia

INTRODUCTION

The practice of modern medicine is most optimal when the clinical context (medical history, family history, and physical examination) is used to guide a targeted, algorithmic focus on additional objective data (e.g., laboratory tests), thus allowing the "big picture" to direct the "small picture". The astounding medical advancement achieved in recent years resulted in revolutionary molecular and genetics discoveries that provide further diagnostic insight on an even smaller scale. These advancements may make it appealing to circumvent certain conventional tests once considered cornerstones of targeted "small picture" diagnostics, such as light microscopy. However, even with more advanced testing available, light microscopy continues to retain its value in guiding further work up. For peripheral blood components, including platelets, microscopic examination of a well-stained peripheral blood smear, when interpreted within the larger clinical context, can narrow the differential diagnoses. This, in turn, can focus the list of molecular / genetic tests needed to establish a final diagnosis, thus supporting cost-efficient and time-efficient practice.

Of the three main cellular components of peripheral blood, platelets have the distinction of not being "real" cells as well as the unfortunate reputation of being the least interesting morphologically. Many physicians only review the platelet number and average size (mean platelet volume or MPV) obtained via complete blood count (CBC) before casting wide nets of specialized tests to evaluate potential platelet disorders. This costly and often unnecessary practice needs to change. In this article, we review the role of peripheral blood smear morphologic findings in guiding the work up of suspected platelet related disorders.

Complete Blood Count and Peripheral Blood Smear Examination

The CBC is the most commonly ordered lab test

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worldwide.[1] It is most informative when data is supplemented by a microscopic examination of a well-stained peripheral blood smear (PBS). Though PBS review tends to focus on white and red blood cells, evaluation of platelet morphology via PBS review can at times reveal important clues that save time and resources by guiding further diagnostic evaluation.

The ideal zone for the purpose of evaluating platelet morphology on a well-prepared PBS slide is called the "reading area". The reading area is located where red blood cells (RBCs) are well spaced. It can be found between the thick area of the smear where RBCs overlap significantly, and the thin area, where RBCs are separated by large gaps of space (Figure 1).

Normal Platelet Number and Size

Platelets are fragments of tissue that originate from cytoplasmic budding of megakaryocytes in the bone marrow. The normal platelet range for term infants, children, and adults is 150,000 to 450,000 platelets/mcL.[2] This equates to at least 10 platelets per high-powered field.[3] Platelets vary slightly in size and measure 2-3 micrometers on average (Figure 2A). By convention, the term large platelet is used for platelets larger than the average platelet but smaller than a normal red blood cell, between 3 and 7 micrometers in size (Figure 2B). Giant platelets are those larger than a normal red blood cell, or larger than 7 micrometers in size (Figure 2C and D). By light microscopy, the only structures of significance that can be seen within platelets are small red or purple granules that correspond to alpha granules.

Using a Peripheral Blood Smear Review to Answer Common Platelet-Related Questions

It is helpful to follow a systematic approach when reviewing a PBS in a patient with a suspected platelet disorder. The following sequential questions can assist in that process.

1. Does the instrument measured platelet count appear accurate (i.e., correlates with PBS estimate?)?
2. If the platelet count is low, is it likely caused by overconsumption or underproduction?
3. Are there any morphological abnormalities visible on the PBS that can provide clues to a diagnosis?

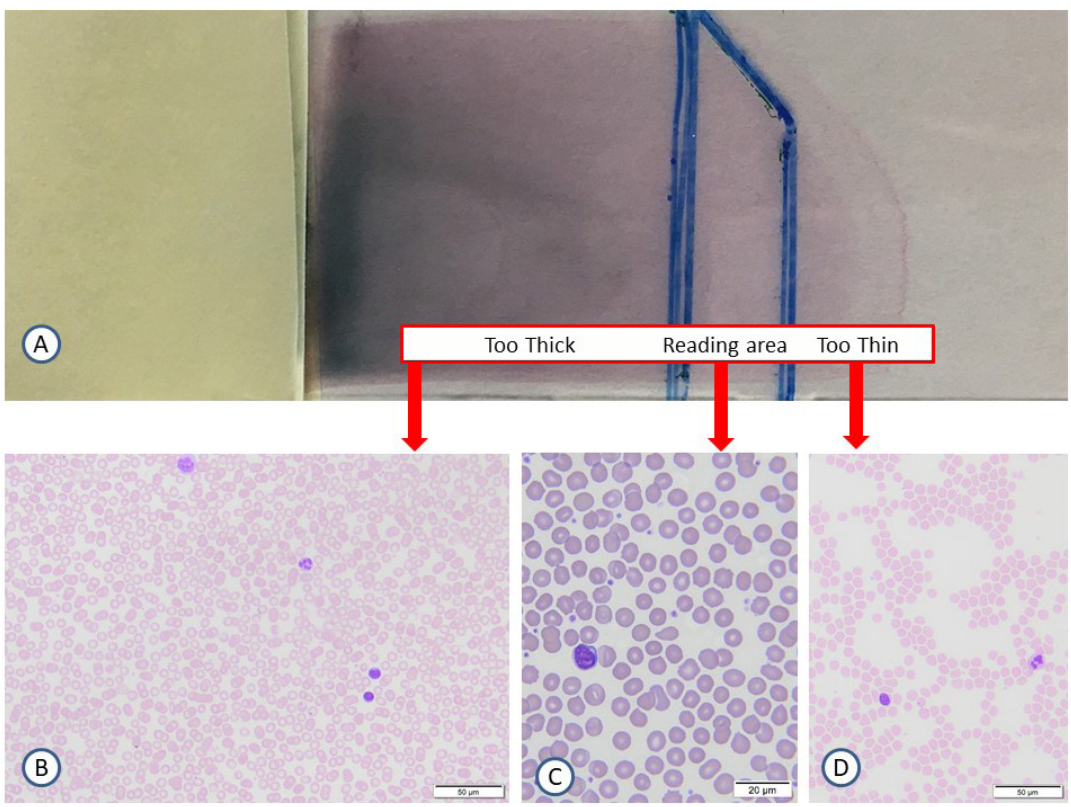


FIG. 1: A stained peripheral blood smear (A) showing an optimal platelet morphology reading area (C) located between a thick (B) and thin (D) areas respectively.

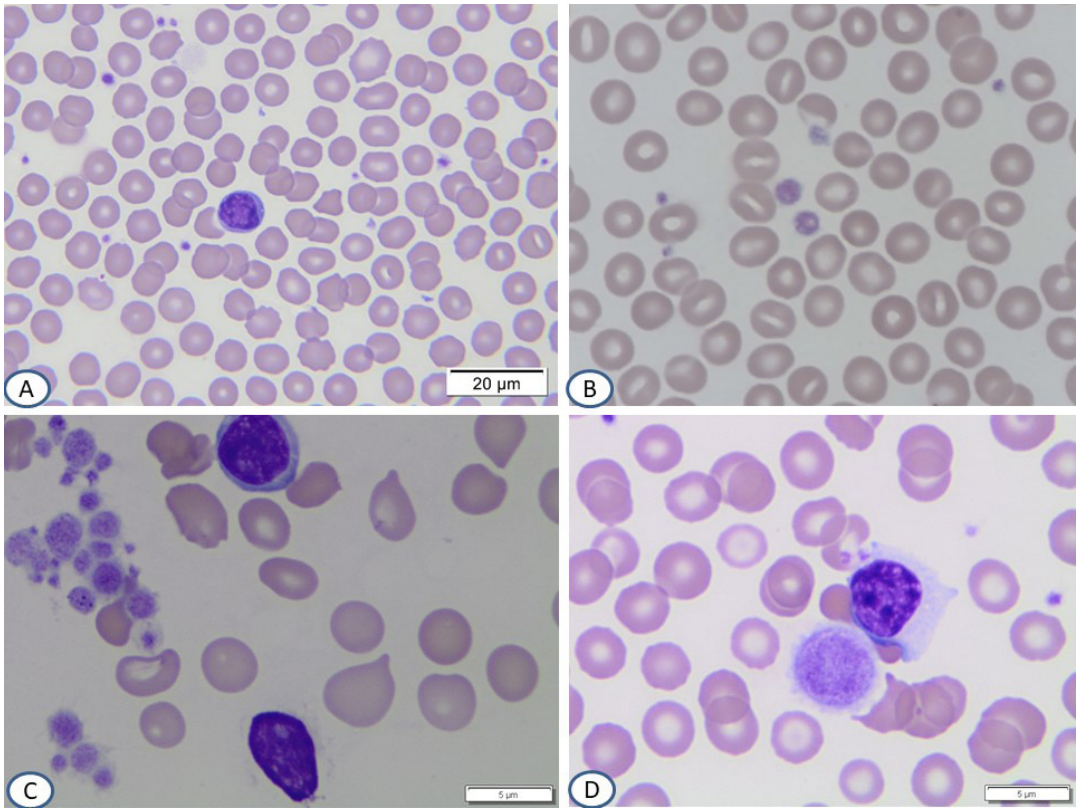


FIG. 2: Normal sized platelets (A), large platelets (B and C) and giant platelets (D).

Question 1: Does the automated platelet count appear accurate?

When evaluating the cause of a patient's abnormal platelet count, the peripheral blood smear is an invaluable source of information. The differential diagnosis of thrombocytopenia, especially in the absence of any mucosal bleeding symptoms, should always include the possibility of an inaccurate automated platelet count. Correlating the instrument-produced platelet count with the estimate made by PBC review can answer the simple yet important question about whether the reported platelet count is accurate. The platelet count can be estimated after reviewing at least 10 high-powered fields on the PBS and using the following equation: Platelet count/μL = average number of platelets present per high-powered x 15,000.[4]

Falsely low automated platelet count (pseudothrombocytopenia) can occur when platelets stick to each other in vitro, forming platelet clumps. Automated cell counters cannot distinguish these clumps from individual cells, so the platelets within the clumps are not counted and excluded from the reported platelet count.[5,6] This artifactual clumping can result from improper collection / storage technique, venipuncture-induced platelet activation, or anticoagulant-induced antibodies.[5,7] Platelet clumps are easily identified on PBS review (Figure 3C-D). It is worth noting that when pseudothrombocytopenia due to platelet clumping occurs, the true platelet count is at least as high as the reported automated count.[6] A stepwise approach to deal with platelet clumps and obtain an accurate platelet count reviewed elsewhere.[6]

Similarly, platelets can adhere to white blood cells and thus be excluded from the reported platelet count. The rare phenomenon of platelets rosetting around the surface of a WBC is called platelet satellitism (Figure 3A-B) and it occurs in a small percentage of patients when ethylenediaminetetraacetic acid (EDTA) is used as the anticoagulant in the collection tube.[8] It does not occur with other anticoagulants, such as citrate. Platelet satellitism has been reported in association with various inflammatory states, including autoimmune diseases, acute trauma, thermal burns, and infection, though has been seen in healthy individuals as well.[9,10] The exact mechanism behind this rare phenomenon is not known.

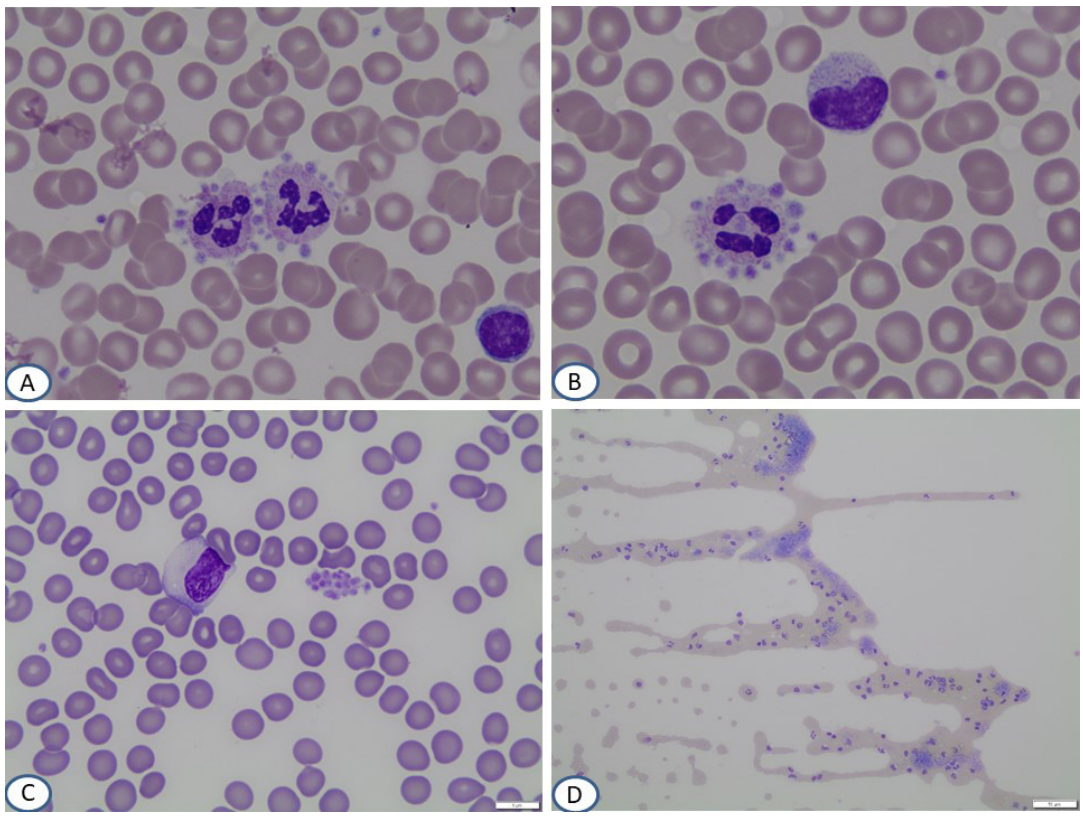


FIG. 3: Platelet satellitism (A and B), platelet clumps in reading area (C), and platelet clumps at feathered edge (D).

Falsely low automated platelet counts can also occur when the platelet size falls significantly outside of the normal range because large / giant platelets or extremely small platelets may not be recognised by the automated cell counter.[11] Some automated cell counters may even mistake large platelets for red blood cells. The presence of many large or giant platelets should prompt evaluation for other haematologic diagnoses.[12]

Pseudo-thrombocytosis, or falsely high platelet count, occurs less frequently than pseudothrombocytopenia.[13] Rarely, red blood cell fragments, microspherocytes, protein aggregates, cytoplasmic fragments of white blood cells, or lipid droplets may be miscounted as platelets by some automated analyzers.[10,13,14] Microspherocytosis and RBC fragmentation, and subsequent pseudo-thrombocytosis, has been well-described in severe burn patients.[10,13]

Question 2: Is the thrombocytopenia caused by overconsumption or underproduction of platelets?

Once pseudothrombocytopenia is excluded as a possible cause, one must then focus on identifying the general pathophysiology of the thrombocytopenia. Thrombocytopenia can be caused by underproduction in the bone marrow (hypoproliferative thrombocytopenia) or by peripheral consumption / destruction / sequestration (hyperproliferative thrombocytopenia).[12,15] Certain features noticeable via PBS review, along with clinical context, can help one identify the aetiology of the thrombocytopenia.

The presence of large, immature platelets in addition to normal sized platelets on a PBS suggests that the marrow is responding to peripheral destruction or consumption of platelets.[12,15] However, homogeneously large platelets in a thrombocytopenic patient should prompt evaluation for a congenital macrothrombocytopenia. Note: Most immature platelets are large, but not all large platelets are immature. Immaturity refers to the amount of RNA content within the platelet, not the size.[12] Thrombocytopenia with small to normally sized platelets and no visible immature platelets may indicate an issue with production.[15,16]

Concurrent morphological abnormalities in red blood cells or white blood cells can also provide clues to the nature of the

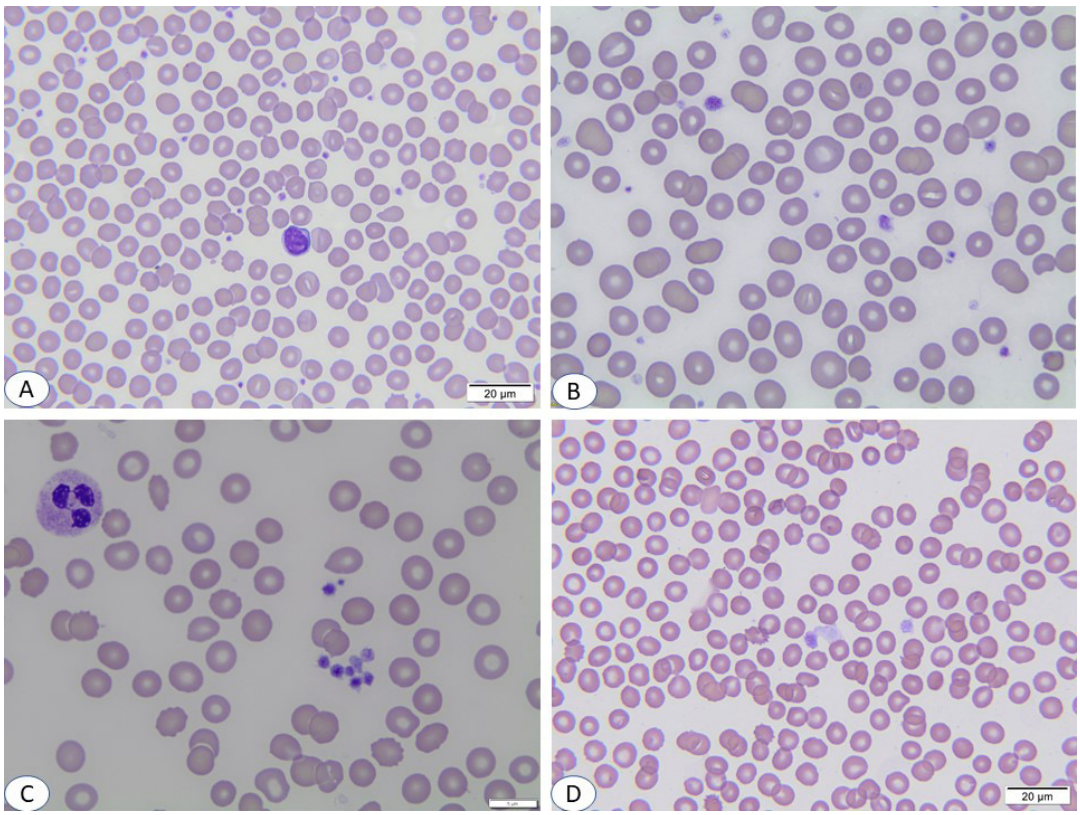


FIG. 4: Size consistency of platelets in normal sample (A) compared to variability in left shifted immature platelets caused by increased consumption in Immune Thrombocytopenic Purpura (B), Von Willebrand Type IIb (C) and irregularly shaped, hypo-granular platelets in this example from a child with Haemophagocytic Lymphohistiocytosis or HLH (D).

thrombocytopenia. These will be discussed further in other sections of this guide, but notable examples include the following: The presence of blasts or left shifted myeloid and/or erythroid precursors may point to a neoplastic process. If schistocytes are evident, microangiopathic processes such as disseminated intravascular coagulation (DIC), haemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) should be urgently considered.[16] Small spherocytes (microspherocytes) may indicate an underlying autoimmune cytopenia process, such as Evan's Syndrome. Toxic granulation or increased number of band cells indicates an infectious or inflammatory process.[16]

Clinical context is an important tool for distinguishing hypo from hyperproliferative thrombocytopenia, but PBS examination can help confirm clinical suspicions or alert to aetiologies that have not been otherwise considered.

Question 3: Are there morphologic abnormalities present in the platelets?

Morphologic alteration in the size, shape, granulation pattern, and size variability of platelets can provide important diagnostic clues. When platelet abnormalities are suspected, there may be concurrent morphologic abnormalities apparent in other blood cells that can aid in diagnostic evaluation as well. Obviously, normal platelet morphology by light microscopy does not rule out a platelet disorder as many platelet function disorders have normal platelet morphology. However, if present, abnormal morphology can provide invaluable insight. Specific morphological abnormalities will be discussed throughout the rest of this manuscript. Platelet size-being the most readily recognizable feature-will be used to stratify these abnormalities.

Platelet Count and Morphology in Certain Inherited Conditions Evident in Childhood

The number of identified hereditary platelet disorders continues to grow as genetic and molecular diagnostics continue to advance.

The following highlights some of the more well-known hereditary platelet disorders but is certainly not an exhaustive list.

Inherited Thrombocytopenia with Small Platelets

Wiskott-Aldrich Syndrome and X-Linked Thrombocytopenia

Wiskott-Aldrich syndrome (WAS) is an X-linked disease which classically presents as a triad of eczema, thrombocytopenia, and susceptibility to infection in a male child.[17] Platelet counts are usually less than 70,000 platelets/μL and the platelets are typically small sized with an MPV less than 5fl, though this can vary.[18] The primary underlying aetiology stems from mutations in the WAS protein resulting in a cascade of immune dysregulation, which can lead to a variety of clinical manifestation such as atopy, immunodeficiency, autoimmunity, lymphoproliferative disorders, and increased risk of leukaemia and lymphoma.[17,18] X-linked thrombocytopenia is a less severe WAS variant that may present with intermittent microthrombocytopenia.[18]

Inherited Thrombocytopenia with Normal Platelet Size

Thrombocytopenia-Absent Radius (TAR) Syndrome

TAR syndrome is a rare congenital disorder caused by an autosomal recessive deletion or mutation in the RBM8A gene. It manifests as hypoproliferative thrombocytopenia and bilateral absence of radii, with preservation of thumbs.[19] The platelet count is typically less than 50,000 platelets/μL in infancy and increases with age.[19] Bleeding symptoms are most severe in the first several months of life.[20] Approximately 90% of patients with TAR syndrome experience symptoms related to thrombocytopenia during the first two years of life, after which there is typically spontaneous resolution.[19] Thrombocytopenia can be intermittent, and can be triggered by physiologic stress, surgeries, or viral illnesses.[19] Patients with TAR can also present with other abnormalities, including congenital heart defects, renal defects, and ulnar hypoplasia.[19]

Congenital Amegakaryocytic Thrombocytopenia (CAMT)

CAMT is a rare autosomal recessive bone marrow failure syndrome that presents in the neonatal period with severe thrombocytopenia and eventually evolves into aplastic anaemia.[21] It is not associated with congenital anomalies.[22] There are different subtypes: type I is an early-onset severe disease with a platelet count typically 21,000 platelets/μL or less. Type II is milder, with platelet counts of 35,000 platelets/μL or less and symptoms presenting between 3-6 years of age. Patients with CAMT are at increased risk of leukaemia.[22]

GATA1-Related Thrombocytopenia

GATA1-related thrombocytopenia occurs in different disorders caused by various mutations in the GATA1 gene on the X-chromosome. Germline GATA1 mutations are responsible for several X-linked forms of congenital macrothrombocytopenia and dyserythropoietic anemia.[23,24] Certain GATA1 mutations result in reduced expression of beta-globin genes, causing a disorder known as "X-linked thrombocytopenia with thalassaemia".[25] Other GATA1 mutations are responsible for a small proportion of Diamond Blackfan anaemia and congenital erythropoietic porphyria cases.[23,24,26] Acquired (somatic) mutations in GATA1 are frequently seen in patients with trisomy 21 associated transient myeloproliferative disorder or acute megakaryoblastic leukaemia.[24] The platelets in GATA1-related thrombocytopenias are large in size and may be dysplastic and/or hypogranular.[26] Platelet aggregation defects also occur in some cases.[27]

SRC-Related Thrombocytopenia

This is a very rare inherited disorder that results from a gain of function mutation in universal tyrosine kinase SRC that causes decreased proplatelet formation.[23,28] This disorder is morphologically interesting because the platelets are misshapen, variable in size, and lack alpha granules.[28] Vacuoles within platelets may also be present. Patients have been noted to have early myelofibrosis with hypercellular bone marrow and trilineage dysplasia, as well as osteoporosis, abnormal dentition, behavioral abnormalities, and mild facial dysmorphism.[23] Patients typically have moderate to severe bleeding symptoms with platelet counts of less than 100,000 platelets/μL.[23]

Inherited Thrombocytopenias with Large Platelets

Type 2B von Willebrand Disease (VWD 2B)

VWD 2B is an inherited platelet disorder caused by a mutation in the GPIb binding domain of von Willebrand factor (VWF) that results in increased

platelet-binding affinity.[29] Thrombocytopenia is a common feature because the enhanced binding of VWF to the platelets creates platelet aggregates which are cleared from circulation via lysis by ADAMTS13.[29] Young large platelets are released early to circulation to replace those clumped and cleared. This can be exacerbated in certain conditions, such as pregnancy, surgery, or after DDAVP administration, which may result in severe mucocutaneous bleeding.[30] VWF multimer analysis and ristocetin-induced platelet binding assays are useful in diagnosis.[11]

Grey Platelet Syndrome

Grey platelet syndrome (GPS) is a rare inherited platelet disorder characterised by progressive macro-thrombocytopenia, myelofibrosis, and splenomegaly. The platelets are uniformly large, but not giant, and are pale or gray due to the absence of α-granules. Platelet counts range between 30,000 to 100,000 platelets/μL, with most patients typically on the higher end of that range.[31] The quantitative decrease in platelets along with the decreased numbers of functional α-granules results in a mild to moderate bleeding tendency in most patients.[32,33] Rarely, severe bleeding has been described in women with menorrhagia.[32,33] Patients with gray platelet syndrome are at risk of developing myelofibrosis, so the presence of teardrop cells or nucleated red blood cells on the peripheral blood smear of a patient with GPS warrants further investigation.[20]

Bernard-Soulier Syndrome

Bernard-Soulier syndrome (BSS) is a hereditary platelet disorder associated with macrothrombocytopenia and mild mucocutaneous bleeding diathesis. It can be inherited in either an autosomal recessive or autosomal dominant pattern.[20] Typical clinical findings include epistaxis, gingival bleeding, and rarely, significant trauma-induced haemorrhage.[20,34] A gene mutation causes deficiency of the glycoprotein (GP)Ib-IX complex, a protein complex necessary for platelet adhesion.[20] Platelet counts may range from moderately low

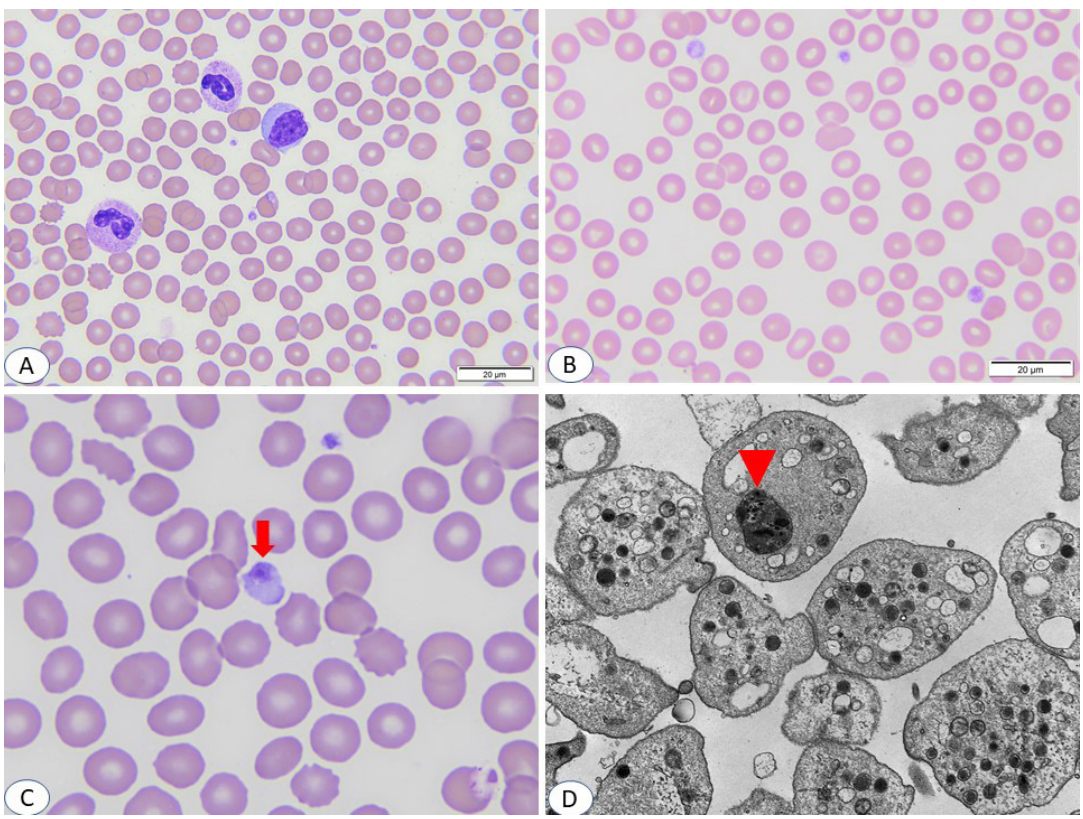


FIG. 5: Small and decreased platelets in Wiskott- Aldrich syndrome (A), large and decreased platelets in Bernard-Soulier syndrome (B) fused alpha granules can be seen on light microscopy (C) and Electron microscopy (D) from a patient with Paris-Jacobsen-Trousseau. Note the large fused alpha granules (arrowhead).

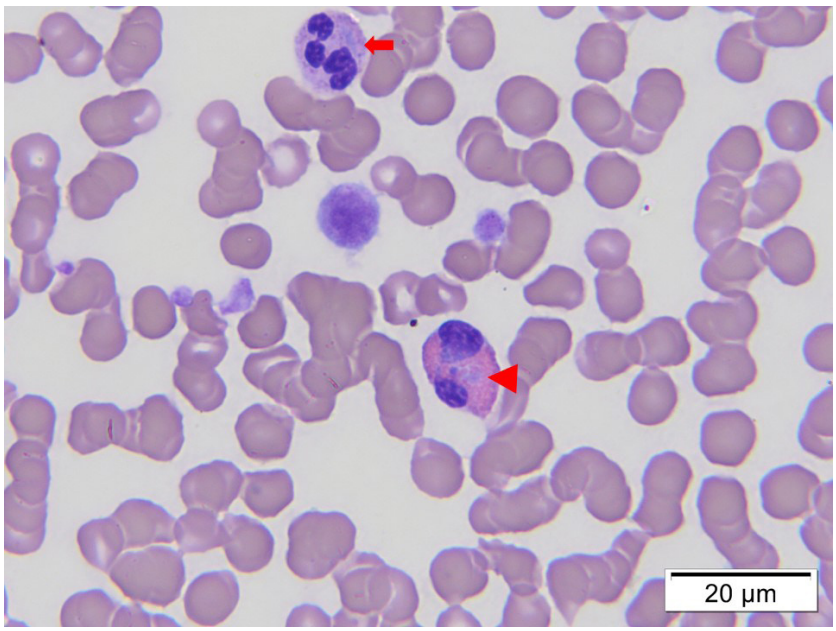


FIG. 6: Large platelets from a case of May-Hegglin anomaly. Note the presence of Döhle bodies in a neutrophil (arrow) and an eosinophil (arrowhead).

(30,000 platelets/μL) to the lower end of normal, though the severity of bleeding phenotype is not strongly correlated with the degree of thrombocytopenia.[34] Of note, automated counters frequently underestimate the true platelet count in BSS because the large, heavily granulated platelets may be counted other blood cells by instruments.[20] Platelets are large to giant in size when examined via light microscropy.[20,34] Platelet aggregation studies will demonstrate reduced response to Ristocetin only.[20]

Paris-Trousseau Syndrome / Jacobson Syndrome

Paris-Trousseau syndrome is caused by a mutation in the FLI-1 gene on chromosome 11q.[35] It is characterised by neonatal thrombocytopenia, platelet dysfunction, and mild mucocutaneous bleeding symptoms.[36] Significant clinical overlap exists with Jacobsen Syndrome, a rare disorder characterised by congenital anomalies including facial dysmorphism, trigonocephaly, skeletal abnormalities, and cardiac defects.[20] Approximately 90% of patients with Jacobsen syndrome have Paris-Trousseau syndrome.[37]

Large fused alpha granules will be evident in approximately 15% of platelets in patients with Paris-Trousseau syndrome.[37] Additional findings include varying degrees of thrombocytopenia and overall larger mean platelet volume, though not all platelets will be large or giant and MPV may be normal in some patients.[20,37,38]

MYH9-Related Disorders

MYH9-related disorders are caused by various mutations in the non-muscle myosin heavy chain gene (MYH9). The spectrum of MYH-9-related disorders include the disorders once known as May-Hegglin anomaly, Fechtner syndrome, Epstein syndrome, and Sebastian syndrome.[39] MYH9 mutations result in varying degrees of thrombocytopenia with large to giant platelets. Platelet counts are typically > 30,000 platelets/μL.[20] Large blue inclusions called Döhle bodies can be seen within granulocytes, as shown in Figure 6.[39] Clinically, patients typically have mild to moderate mucocutaneous bleeding symptoms.[40] MYH9-related disorders can also manifest with sensorineural hearing loss, cataracts, and nephropathy, depending on the specific MYH9 mutation.[20,41,42]

Inherited Platelet Disorders with Normal Platelet Count

Platelet number and morphology, as seen via light microscopy, are normal in certain notable hereditary qualitative platelet disorders. Some of these disorders, such as Chediak-Higashi Syndrome, have visible morphological abnormalities of other blood cell types. Others, like Glanzmann Thrombasthenia, do not.

Glanzmann Thrombasthenia

Glanzmann thrombasthenia is an autosomal recessive disorder caused by an abnormality in the glycoprotein IIb or glycoprotein IIIa genes.[43] Platelets are normal in number, size, and granularity.[20] Mucocutaneous bleeding is often present from infancy and is variable in severity; severe bleeding may occur. Gastrointestinal bleeding and heavy menstrual bleeding may also occur.[43] Platelet aggregation studies show poor response to all agents except Ristocetin and reduced clot retraction may be noted.[12,20] A definitive diagnosis can be made by identifying deficiency of the GPIIb/GPIIIa receptor via monoclonal antibody tests or flow cytometry.

Chediak-Higashi Syndrome

Chediak-Higashi Syndrome is a rare autosomal recessive disorder caused by a defective lysosomal trafficking protein. The disorder is characterised by oculocutaneous albinism, recurrent infections, mild mucocutaneous bleeding, and predisposition to infantile hemophagocytic lymphohistiocytosis (HLH).[44] Allogenic stem cell transplant is the only curative treatment option.[45] Many patients who survive early childhood eventually develop progressive neurological symptoms, regardless of stem cell transplant. Platelet size and count are typically normal but platelet electron microscopy demonstrates decreased numbers of dense granules.[20] Lysosomal inclusions called ceroid bodies are present in granulocytes and platelets and are visible via light microscopy.[12,46] These inclusions are pathognomonic for Chediak-Higashi Syndrome.

Hermansky-Pudlak Syndrome (HPS)

HPS is another rare autosomal recessive disorder caused by mutations involving lysosome trafficking. Similar to Chediak-Higashi Syndrome, it can result in oculocutaneous albinism and dense granule deficiency within platelets, which typically manifests as mild mucocutaneous bleeding.[47] Platelet count and size are normal. Lysosomal inclusions within bone marrow macrophages are seen, but there are typically no abnormal inclusions or morphologic abnormalities evident on peripheral blood smear.[12] Patients with certain types of HPS are prone to pulmonary fibrosis and granulomatous colitis.[47,48]

Platelet Count and Morphology in Reactive Conditions of Childhood

Quantitative platelet abnormalities can result from either intrinsic (primary) defects in the production of platelets or from reactive (secondary) processes affecting the production or survival of platelets. Secondary thrombocytosis and thrombocytopenia are more common than primary etiologies in both children and adults.[49] Secondary thrombocytopenia can be caused by decreased platelet production (hypoproliferative) or increased platelet consumption / destruction (hyperproliferative). In some instances, both processes happen simultaneously and the distinction is less clear.[50] Secondary thrombocytosis is nearly always associated with an infectious or inflammatory process in children.

A thorough history and physical exam in addition to a review of the peripheral blood smear is often sufficient to identify the most probable aetiology of a patient's abnormal platelet count. Careful examination of all blood cells on the peripheral smear is particularly important in this context, as concurrent changes in RBCs and WBCs yield important diagnostic information. Even if PBS review is not sufficient to conclusively identify the underlying cause of a patient's abnormal platelet count, it can provide clues to guide further diagnostic testing.

Secondary Hypoproliferative Thrombocytopenia in Non-Malignant Conditions

Infections

Infections are a common cause of secondary thrombocytopenia, particularly in children. While any infectious or inflammatory process can cause an inflammatory response and subsequent increased platelet production, there are infections that are more commonly associated with suppression of platelet production. Gram negative rod bacteremia[51], disseminated fungal infections[51], necrotising enterocolitis (NEC)[51,52], Dengue fever[53], tick-borne diseases[54], malaria[50], leptospirosis, CMV[50], EBV[50], parvo, untreated HIV[20], and HHV6[55] are all notorious for causing hypoproliferative thrombocytopenia. There may also be a component of superimposed platelet destruction or consumption in many infectious processes, especially in rickettsia and EBV.[50,54] It is prudent to examine the PBS for other signs of infection, such as reactive or atypical lymphocytes, toxic granulation in neutrophils, or a left shift in neutrophil production. Lymphocyte nuclei with cytoplasmic pseudopods and/or a clover shaped nuclei should prompt testing for EBV. PBS from patients with unexplained thrombocytopenia in areas where malaria

and babesia are endemic should be examined carefully for RBC inclusions characteristic of those infections.

Neonates with thrombocytopenia can present a diagnostic challenge because of difficulty distinguishing secondary thrombocytopenia from the various hereditary thrombocytopenia syndromes that often present in infancy. However, infection is by far the most common cause of thrombocytopenia in this patient population and should be the first aetiology considered since untreated infection can be rapidly fatal in neonates. Thrombocytopenia is often an early sign of necrotising enterocolitis and/or impending sepsis in neonates.[52] Of note, congenital CMV can be associated with severe microthrombocytopenia, with unusually small platelets evident on PBS review.[20] Careful review of the PBS in children with unexplained thrombocytopenia can guide testing for specific infections.

Nutritional Deficiencies

Mild to moderate thrombocytopenia may be seen in cases of severe prolonged iron deficiency anaemia (IDA). Evidence of IDA will be apparent on PBS review, with microcytic, hypochromic RBCs, including hypochromic elliptocytes (pencil cells) commonly seen in IDA.[56] Folate and/or vitamin B12 deficiency may also cause decreased platelet production.[20] Concurrent macrocytosis and/or more than five lobes within the neutrophils would support this diagnosis.[56]

Metabolic Disorders

Space limiting or space occupying bone marrow infiltration and subsequent hypoproliferative thrombocytopenia can occur in certain metabolic disorders. An example of the former is osteopetrosis, and of the latter is Gaucher disease. Osteopetrosis often causes pancytopenia, which will be evident on PBS review. In Gaucher disease, this decreased production of platelets coupled with hypersplenism can lead to severe thrombocytopenia in some cases and may be accompanied by anaemia.[57]

Other metabolic disorders can be associated with hypoproliferative thrombocytopenia in the absence of marrow infiltration. Examples include methylmalonic acidemia, isovaleric acidemia, and holocarboxylase synthetase deficiencies. Intermittent neutropenia may also be present in these disorders. Hypothyroidism, particularly in infants, is also sometimes associated with thrombocytopenia, as is neonatal Graves' Disease.[58]

Drug-Associated Hypoproliferative Thrombocytopenia

Cytotoxic drugs such as anti-neoplastic agents and certain immunosuppressants that target rapidly dividing cells may cause bone marrow suppression and subsequent thrombocytopenia. Other drugs may also cause decreased platelet production, though the mechanisms are not always entirely understood. Common drugs associated with decreased platelet production include linezolid, valproic acid, daptomycin, thiazide diuretics, ethanol, tolbutamide, carbamazepine, and gold compounds.[59,60,61] Note: this is not the same process as drug-induced immune-mediated thrombocytopenia, which is discussed in greater detail in the next section, though some drugs are implicated in both immune and non-immune mediated drug-induced thrombocytopenia.

Secondary Hyperproliferative Thrombocytopenia

The MPV and the distribution of platelet size tends to be greater in consumptive or destructive processes, as the bone marrow attempts to compensate by increasing platelet production and release larger platelets.[62] Higher number of large, giant, or immature platelets can be seen on peripheral blood smear review. Immune-mediated thrombocytopenias and thrombosis-driven processes are common examples of secondary hyperproliferative thrombocytopenias.

Immune-Mediated Thrombocytopenia

Immune-mediated processes are a common cause of secondary thrombocytopenia in children. There are several immune-mediated causes, including neonatal alloimmune thrombocytopenia (NAIT), immune thrombocytopenic purpura (ITP), and drug-induced thrombocytopenia.

NAIT is a transient condition that affects up to 1 in 1,200 live births.[63] It occurs when maternally-derived antibodies attack paternally-derived antigens on the infant's platelets. It manifests as moderate to severe congenital thrombocytopenia with varying degrees of mucocutaneous bleeding. Severe bleeding, including intracranial hemorrhage, can occur.[63] Treatment includes platelet transfusions and intravenous immunoglobulin if the infant has severe thrombocytopenia and/or severe bleeding complications.

Immune thrombocytopenic purpura (ITP) is one of the most common bleeding disorders in the paediatric population, estimated to occur in 5-10 children per 100,000 children per year.[64]

The pathophysiology of most cases of ITP is thought to be related to autoantibodies that attack the patient's platelets, though alternative mechanisms may also be involved.[64] In children, a recent or concurrent viral infection is a commonly-identified precipitating event and it is not uncommon to see reactive lymphocytes on the PBS of these patients. ITP typically causes severe thrombocytopenia, with platelet counts often in less than 20,000 platelets/μL.[20] In addition to severe thrombocytopenia, PBS review will often reveal a high proportion of large to giant platelets as well as increased numbers of immature platelets. Most cases are acute and self-limiting, though some patients develop chronic ITP that can last months or years. Patients without bleeding symptoms can be observed, while those with bleeding symptoms are treated with steroids and/or intravenous immunoglobulin. Peripheral smear review to evaluate for leukemic blasts can provide clinicians with some degree of reassurance that it is safe to treat the patient with steroids.

Immune-mediated thrombocytopenia can also be associated with the use of certain medications. Antibiotics are a common culprit and include trimethoprim-sulfamethoxazole, rifampin beta-lactams, and vancomycin.[65] Other offending agents include Quinine, Heparin, thiazides, and antiepileptics such as Dilantin and Valproic acid. Typically the thrombocytopenia is detected 5-10 days after the initiation of the medication and improves days to weeks after the medication is stopped, depending on its half-life.[65] Patients with drug-induced immune thrombocytopenia are at high risk of bleeding complications.[65]

Thrombotic Microangiopathy

Microangiopathic haemolytic anaemia (MAHA) is descriptive term for the non-immune mediated intravascular haemolysis caused by microvascular stenosis.[66] Schistocytes are commonly seen on PBS.[67] Thrombotic microangiopathy (TMA) is a diagnostic term used for a group of disorders characterised by MAHA, thrombocytopenia, and tissue damage in the correct clinical context.[68] These include thrombotic thrombocytopenic purpura (TTP), haemolytic uremic syndrome (HUS), and disseminated intravascular coagulation.[67] In TMA, multiple microvascular thrombi consume platelets and shear red blood cells, leading to severe thrombocytopenia and red blood cell fragmentation.[67] Many conditions that cause TMA require urgent treatment. Thus, the presence of schistocytes and thrombocytopenia on a peripheral blood smear should immediately be brought to the clinician's attention.

Vascular Anomalies

Large vascular anomalies, particularly kaposiform haemangioendotheliomas (KHEs), can trap and destroy platelets and RBCs. This localised form of microangiopathic haemolytic anaemia, termed Kasabach-Merrit phenomenon (KMP), occurs in approximately 70% of KHE cases. KMP may not always manifest immediately, as the likelihood of KMP correlated with the size of the vascular anomaly and the vascular anomaly can continue to grow throughout infancy.[20]

Thrombosis

Because thrombi consist of platelets and fibrin, extensive thrombosis alone can cause consumptive thrombocytopenia. A classic example of this is renal vein thrombosis in neonates, which classically presents with haematuria, a flank mass, and thrombocytopenia.[69] Extensive thrombosis, such as that which occurs in May-Thurner syndrome or in catastrophic antiphospholipid antibody syndrome which can also cause a consumptive thrombocytopenia.[70]

Splenic/Hepatic Sequestration

In patients with hypersplenism, the spleen can sequester up to 90% of the total platelet mass, leaving very few in circulation.[71] Hypersplenism can occur with splenomegaly or increased splenic demand caused by various conditions such as infection, inflammation, autoimmune disorders, or haemoglobinopathies. Other cytopenias can occur as well, including anaemia and neutropenia and are usually more severe than the thrombocytopenia.[20] Less commonly, platelets can be sequestered within the liver as well, as seen in post-transplant patients and in certain haemoglobinopathies. Morphological clues regarding the underlying aetiology of the splenic or hepatic sequestration may be apparent in the red or white blood cells seen on the peripheral blood smear.[56]

Mechanical Destruction

There are multiple aetiologies of mechanical destruction of platelets. Mechanical heart valves and extracorporeal therapies, including extracorporeal oxygenation, cardiopulmonary bypass, haemodialysis, and apheresis can cause destruction of platelets.[72] In all of these, shearing forces created by prosthetic hardware destroys

platelets and red blood cells. A peripheral blood smear will show schistocytes and red blood fragments in addition to mild-moderate thrombocytopenia.[72]

Hypothermia
Hypothermia, whether environmental or related to a disease process / treatment, is known to have an adverse effect on platelets. Hypothermia can cause an increase in mean platelet volume, shape changes, and platelet apoptosis.[73] Hypothermia also causes platelet margination through increasing haematocrit, which affects the platelet shape, decreases blood flow rate, and increases adhesion molecules.[73]

Secondary or Reactive Thrombocytosis

Thrombocytosis is generally defined as a platelet count greater than 450,000 platelets/ μL in children and adults or greater than 650,000 platelets/ μL in neonates.[74] In this context, thrombocytosis is usually a transient reactive phenomenon that resolves once the underlying condition abates. Though acquired and inherited primary thrombocytosis disorders such as essential thrombocythemia and hereditary thrombocytosis do exist, they are rare.[20] In both children and adults, thrombocytosis is commonly secondary to another underlying process.

Infection
Reactive thrombocytosis in children is most commonly the result of infection.[75-77] Many inflammatory cytokines, such as interleukin-6 (IL-6), upregulate thrombopoietin activity, resulting in increased platelet production.[49,78,79] Viral, bacterial, and fungal infections have all been associated with a transient reactive thrombocytosis.[49,80] Infants and young children are particularly prone to dramatic rise in their platelet counts during infections.[75] Other indicators of infection may be present on the peripheral blood smear, such as reactive or atypical lymphocytes, toxic granulation in neutrophils, or a left shift in neutrophil production.

Anaemia
Iron deficiency anaemia (IDA) is another common cause of reactive thrombocytosis in children because iron deficiency induces megakaryocyte proliferation.[49,76,81,82] Of note, unlike other types of reactive thrombocytosis, IDA with thrombocytosis is a risk factor for venous thromboembolism.[82] Iron deficiency even without significant anaemia can still result in reactive thrombocytosis, so it is important to look for signs of iron deficiency on the PBS of patients with thrombocytosis regardless of their haemoglobin level. Acute blood loss or acute haemolytic anaemia can also trigger a reactive thrombocytosis as the marrow tries to abruptly upregulate production. Evidence of acute haemolysis may be visible on PBS in these cases, with visible RBC fragments, polychromasia, or immature RBCs. Vitamin E deficiency, which can also cause a mild haemolytic anaemia, is also associated with thrombocytosis.[20]

Inflammation
As mentioned before, inflammatory cytokines can drive increased platelet production. Thrombocytosis can be considered a non-specific inflammatory marker, though its absence does not exclude the presence of inflammation. Marked thrombocytosis is commonly seen in association with severe tissue damage, such as acute pancreatitis, severe trauma, or thermal burns. Both acute and chronic inflammatory disorders, such as inflammatory bowel disease and Kawasaki disease, can also cause thrombocytosis.[83,84]

Drug-Induced Thrombocytosis
Except for medications designed to stimulate platelet production, such as TPO-agonists, drug-induced thrombocytosis is a rare phenomenon. There have been reports of thrombocytosis associated with miconazole, low molecular weight heparin, epinephrine, vinca alkaloids, corticosteroids, all-trans retinoic acid, gemcitabine, and clozapine.[85] The evidence supporting miconazole and enoxaparin-induced thrombocytosis is stronger relative to the other medications listed. Certain hormones like testosterone, whether exogenous or endogenous, can cause increased platelet production as well as increased red blood cell mass and hemoglobin concentration. Patients with hyperadrenalism may also have elevated platelet counts.[20] Exercise-induced catecholamine release can also cause thrombocytosis, albeit transiently.[86]

Asplenia
Transient extreme thrombocytosis can be seen following surgical splenectomy.[20] Patients with congenital asplenia or hyposplenia may have mild persistent thrombocytosis, though most will have normal platelet counts.[87] Other signs of asplenia, such as Howell-Jolly bodies, may be apparent on PBS.

Platelet Count and Morphology in Neoplastic Conditions

Abnormal platelet count is a common feature of many malignancies. When reviewing the PBS for a patient with an abnormal platelet count, it is important to look for any dysplastic cells, non-artifactual teardrop cells, blasts, or nucleated red blood cells that could indicate an underlying neoplastic process. Neoplastic conditions can be associated with primary thrombocytosis, secondary (reactive) thrombocytosis, or thrombocytopenia. Thrombocytosis can be driven by a primary myeloproliferative disorders, though this by far a more common aetiology in adults (particularly elderly adults) than in children.[49] Certain somatic mutations, such as t(9;22) in BCR-ABL1-positive chronic myeloid leukaemia (CML) and JAK2 mutations in myeloproliferative neoplasms like essential thrombocythemia, can be primary drivers of thrombocytosis in people with these conditions.[49,88]

Many cancers are also associated with secondary thrombocytosis because of the systemic inflammatory response involved.[79] This phenomenon of paraneoplastic thrombocytosis is more prevalent in adult cancers, such as ovarian cancer, breast cancer, lung cancer, and Kaposi sarcoma.[79] However, paraneoplastic reactive thrombocytosis is also common in osteosarcoma and has been reported in other childhood malignancies, such as hepatoblastoma, lymphoma.[89,90]

Thrombocytopenia in the context of a new malignancy is most often secondary to suppressed platelet production that results from malignant infiltration into the bone marrow. In malignancies associated with splenomegaly, like some cases of leukaemia and lymphoma, there can also be a consumptive component to the thrombocytopenia. Rarely, lymphoproliferative malignancies can induce a secondary immune-mediated thrombocytopenia as well.[91] Abnormal platelet morphology, such as abnormal granulation patterns, irregular shapes, and blebbing, can also be seen in various hematologic malignancies and myelodysplastic conditions.[92]

Although marrow suppression is a more common cause of thrombocytopenia in malignancies, there are a few rare inherited thrombocytopenia syndromes that predispose people to developing malignancies. Examples include Familial Thrombocytopenia type-2, germline RUNX1 mutations, and ETV6-related thrombocytopenia.[20] Thrombocytopenia in the context of malignancy in these patients may be a direct result of their inherited condition rather than solely a secondary effect of their malignancy.

Common Artifacts

Aside from clumping (discussed above), degranulation due to aging or excessive shaking of blood specimen is the main artifact encountered. Degranulation may render platelets pale, gray and devoid of granules (Figure 7).

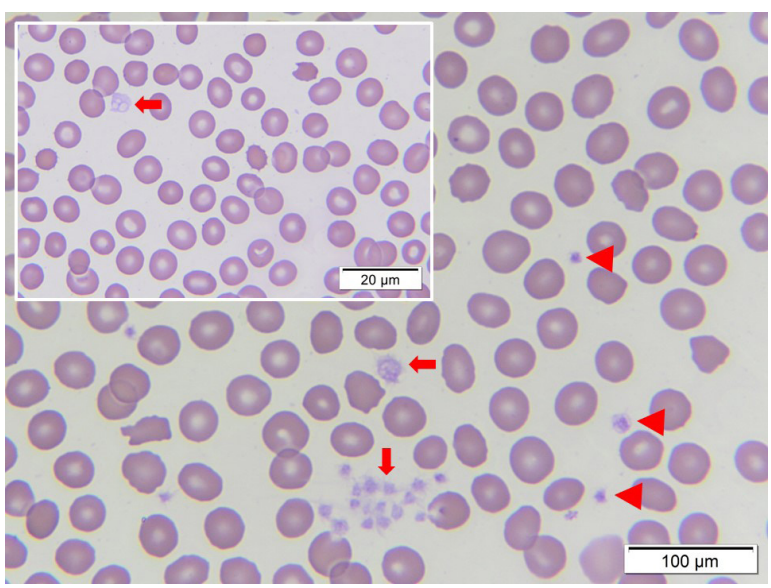
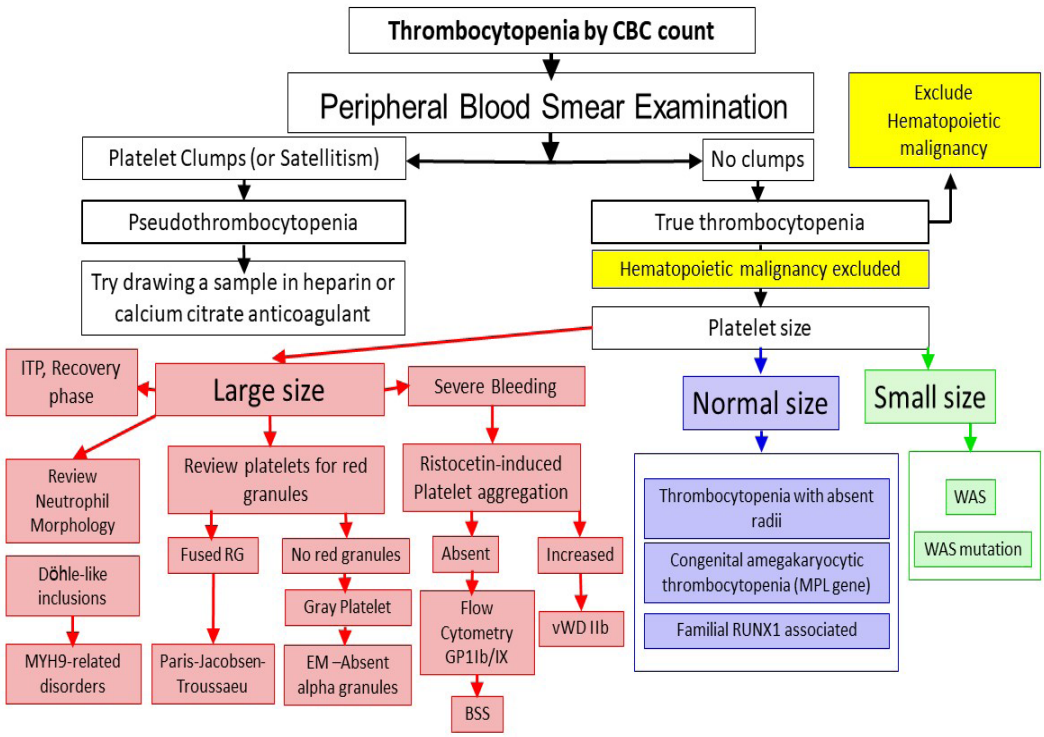


FIG. 7: Degranulated platelets. There is decrease or loss of red granules and vacuolar changes. Compare degranulated platelets (Arrows) to intact platelets (Arrow heads).

An Algorithmic Approach to Thrombocytopenia in Children



BSS= Bernard-Soulier Syndrome.
WAS= Wiskott-Aldrich Syndrome
EM= Electron Microscopy

CONCLUSIONS

A careful review of a well-stained peripheral blood smear can contribute valuable information and guide further workup of platelet related disorders. The following chart outlines a suggested algorithmic approach to investigating thrombocytopenia in children.

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