

# Disorders of platelets and white blood cells:

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## Disorders of platelets

Platelets are among the first responders in the wound healing process. They are second in abundance only to red blood cells with normal counts roughly ranging between 150,000 to 450,000 platelets per cubic millimeters. Normal wound healing requires both normal platelet count and function. Nowadays, platelet counting is mostly automated to save time and manpower. However, these automated methods can't provide accurate results at lower counts, can't detect platelet clumping and can't assess for morphologic abnormalities as large platelets. Add to this, they can completely miss thrombocytopenia in situations as acute leukemia with a very high white count as white blood cell fragments may be counted by machines as platelets preventing a potentially lifesaving platelet transfusion. So, whenever indicated or when in doubt, always order a peripheral blood smear.

The primary regulator of platelet production is thrombopoietin produced by the liver. Receptors for the protein are present on both megakaryocytes and platelets. Levels are upregulated in marrow failure states such as aplastic anemia and can be depressed in liver failure states explaining liver failure induced thrombocytopenia. The average platelet life span is about 7-9 days. Aging platelets are removed from the circulation by the reticuloendothelial system in the liver and spleen. Remember that platelets don't have nuclei as they don't need them. But they have the structures that they need to perform their function, and these include: mitochondria, lysosomes, peroxisomes, alpha granules (contain VWF, fibrinogen, PF4, PDGF) and dense granules (contain ADP, ATP, serotonin, calcium).

Once recruited to a wound site, the following cascade of events occur:

- 1) Platelet adhesion: platelets bind to the defect site by attaching to subendothelial vWF/ collagen via platelet GP1b-IX-V and GPIIb/IIIa receptors.
- 2) Activation: platelet shape changes to expose GPIIb/IIIa receptors.
- 3) Aggregation: this happens via cross linking of platelet activated GPIIb/IIIa by fibrinogen/ vWF
- 4) Propagation of coagulation: coagulation factor complexes/ enzymes attach to the activated platelet surface.

The simplest way to assess for platelet function is through measuring bleeding time. Normally, it should be under 10 minutes. This test is not widely used anymore because it is widely operator dependent and can't be used with concurrent thrombocytopenia. It has been widely replaced by automated tests such as PFA-100 and platelet aggregation studies. Detailed description of these won't be included here as it's not within the scope of this discussion. Some clinical features may help differentiate bleeding due to platelet disorders from coagulation factor related disorders. With platelet related bleed, the bleeding tends to happen immediately after the trauma. Petechiae are more indicative of platelet problems than are bigger bleeds and hemarthroses are rather very unusual in this context. Family history can be very helpful as many of the defects may be genetic.

### A) Quantitative platelet disorders:

We start by describing the phenomenon of pseudothrombocytopenia, where automated testing shows thrombocytopenia in an otherwise healthy individual with no symptoms. This is caused by in vitro platelet clumping in the test tube due to the anticoagulant, EDTA, in the test tube. EDTA activates EDTA antiplatelet antibodies that are present in the patient's blood stream once in the test tube causing platelet clumping. This causes a lot of unexplained cases of thrombocytopenia and repeated count testing. This can usually be resolved by sending blood samples in non EDTA tubes such as sodium citrate and obtaining a blood smear to look for platelet clumping.

Thrombocytopenia can occur due to decreased production by the bone marrow either because of a bone marrow primary disorder (leukemia, bone marrow failure) or loss of external drive such as low thrombopoietin levels seen in end stage liver failure. Thrombocytopenia can also be consumptive as seen in massive bleeds and significant

trauma and can be due to pooling such as spleen sequestration crises seen in young sickle cell anemia patients. The clinical circumstances usually help delineate the cause in these circumstances. Another main mechanism of thrombocytopenia is shortened platelet life span. These can be either of immune or non-immune nature. Non-immune causes of decreased platelet life span include DIC, TTP, HUS and Kasabach-Merritt syndrome. In the following lines, we summarize the most famous immune causes:

- 1) ITP: historically used to be called idiopathic thrombocytopenia purpura, and since the mechanisms became clearer in the past few decades, it's called now immune thrombocytopenia purpura. As the immune system tries to clear a viral infection, it starts by making IgM antibodies. Later class switching occurs and IgG titers rise in a matter of a few weeks. Occasionally, these IgG antibodies may cross react with some of the platelet common antigens if they share similarities with viral antigens. Once the platelets are coated by these antibodies, they'll be phagocytosed by macrophages in the reticuloendothelial system as they pass through it causing thrombocytopenia. The presentation is usually skin and mucosal bleeding in the form of petechiae and purpura in an otherwise healthy child. CBC shows thrombocytopenia with normal hemoglobin; white blood count and differential and blood smear may show large platelets. The process is usually self-limiting in a few weeks to a few months and many cases can be managed by observation, but intervention may be warranted in patients with active bleeding, severe thrombocytopenia (less than 20,000 platelets per mm<sup>3</sup>) or when adequate follow up can't be guaranteed. The first line of active treatment is usually either IVIG or anti-D infusion. IVIG works by flooding the macrophage receptors for antibodies in the spleen so platelets can pass through safely and unharmed. Anti-D will work only on Rh+ patients and it acts through inducing an iatrogenic hemolytic anemia in which the splenic macrophages will be flooded and saturated trying to clear red blood cells from the circulation. The effectiveness of this method depends on the fact that normally, there are roughly 10-30 times more red blood cells in the circulation than platelets. Hemoglobin concentration is expected to drop by 1-3 gm/dL after administration of anti-D, so it's contraindicated if the patient has a concurrent anemia regardless of the cause. Steroids can also be very effective but should not be administered before ensuring the cause of thrombocytopenia is not leukemia. If none of the above works, further lines of therapy include measures as splenectomy, administration of cytotoxic agents such as cyclophosphamide or monoclonal antibodies such as Rituximab (anti CD20). Although spontaneous intracranial bleeding is rare in ITP it can still happen. If it occurs, emergent platelet transfusion and splenectomy is usually indicated. Platelet transfusions are not routinely recommended as antibodies are usually against common human platelet antigens rendering the rise in platelet count transient thus not helpful except in acute bleeds. Most cases are acute, especially in younger children, but some may become chronic especially in older patients and if it's secondary to causes like malignancy.
- 2) Heparin-induced thrombocytopenia: occurs due to anti-heparin antibodies reacting with heparin-platelet factor 4 complex on platelet surface causing platelet activation and consumption. Thrombocytopenia usually occurs 5-10 days after exposure to heparin but may be sooner. It may sometimes present with reduction of platelet count rather than absolute thrombocytopenia and it may be triggered by low molecular weight heparin although less commonly. It's managed by discontinuation of heparin and using an alternative way of anticoagulation. This is a form of drug induced thrombocytopenia. Other drugs can also induce thrombocytopenia such as Quinine, sulfa compounds and valproic acid.
- 3) Immune thrombocytopenia in neonates: the more common form of this is neonatal alloimmune thrombocytopenia. Where the mother is sensitized to a fetal platelet antigen that she doesn't have herself (inherited from father). This condition carries a high risk of intracranial bleeding (over 10%) in the neonate thus daily platelet count monitoring is warranted with platelet transfusions to keep the count above 30,000 per mm<sup>3</sup>. IVIG infusions and steroids may be considered. A head US to evaluate for intracranial bleeding is also indicated. Maternal platelet count is typically normal and subsequent pregnancies tend to be more severely affected. The less common and less serious condition is when the mother has ITP herself and

antiplatelet antibodies pass through the placenta to the fetus causing thrombocytopenia. The antibodies are against both the maternal and fetal antigens. This condition has a much lower risk of bleeding and maternal platelet count is usually low but neonatal thrombocytopenia can still occur if maternal ITP is in remission. IVIG may be considered if clinical bleeding occurs. Nadir platelet count occurs at around 3 days of life and most infants recover within a week or 2.

Rarely, thrombocytopenia can be congenital. Examples of this include:

- 1) Wiskott-Aldrich syndrome: thrombocytopenia, eczema and immunodeficiency. It has X linked recessive inheritance of a mutation in the WASP gene. Platelets are small and have poor function. It's diagnosed via gene testing and flowcytometry for the WASP protein (CD43). It's also associated with autoimmune disorders. And malignancies (usually ALL or lymphomas). The only current cure is stem cell transplantation. Some patients may benefit from splenectomy.
  - 2) X-linked thrombocytopenia: also caused by a mutation to the WASP gene but causes only thrombocytopenia without immunodeficiency or eczema.
  - 3) Thrombocytopenia Absent Radii (TAR) syndrome: abnormal radial bone and thrombocytopenia. Platelet counts usually improve by the age of 2. Platelet transfusions may be needed for symptomatic management.
  - 4) Congenital Amegakaryocytic thrombocytopenia. Autosomal recessive inheritance in the thrombopoietin receptor. Platelet count is typically less than 20,000/mm<sup>3</sup>. It may be associated with other congenital anomalies and most patients progress to aplastic anemia by the age of 5 years. Stem cell transplantation is the only current cure.
- B) Qualitative platelet defects:
- Can be due to a platelet membrane receptor abnormality, storage granule defect or a signal transduction problem.
- 1) Glanzmann's Thrombasthenia: abnormality in the platelet membrane fibrinogen receptor (GPIIb/IIIa). It's inherited in an autosomal recessive pattern and presents with severe bleeding starting in infancy.
  - 2) Bernard-Soulier syndrome: Also inherited as an autosomal recessive disorder. The disease-causing mutation results in absent or abnormal platelet surface receptor for vWF (GP Ib/IX). It presents with mucocutaneous bleeding starting in infancy. It's also characterized by mild to moderate thrombocytopenia.
  - 3) Gray platelet syndrome: usually autosomal recessive. Characterized by macrothrombocytopenia and absent alpha granules on electron microscopy. It's usually a mild disorder and can be associated with myelofibrosis and splenomegaly.
  - 4) Hermansky-Pudlak syndrome: pulmonary fibrosis, oculocutaneous albinism and absent dense granules on electron microscopy. Bleeding is typically mild. Autosomal recessive.

## Disorders of white blood cells:

Here, we will mainly discuss the most important neutrophil disorders covering the basic facts.

- 1) Neutropenia secondary to infection: is the most common form of neutropenia. It can be driven by many mechanisms such as demargination, consumption, and bone marrow suppression. It's self-limiting.
- 2) Drug induced neutropenia: long list, includes many chemotherapeutic agents, sulfa drugs, penicillins, phenothiazine, phenobarbital, and others.
- 3) Autoimmune neutropenia: as the name indicates, it's antibody mediated. It can be associated with other immune cytopenias, immunodeficiency or autoimmune disorders. Examples of possible associations are: ITP, warm autoimmune hemolytic anemia, SLE, Grave's disease, etc. ANC can be variable. Bone marrow biopsy shows normal cellularity with maturation arrest. Natural history is to self-limit unless its secondary to other conditions and duration of this illness is usually shorter than Chronic benign neutropenia of childhood.
- 4) Chronic benign neutropenia of childhood: due to an anti-neutrophil antibody. Presents most of the time before 3 years of age. Rarely results in serious infections. Can last from 6-60 months but the natural history is to self-limit. Managed by supportive care and antibiotic therapy as needed to treat infections.

- 5) Alloimmune neutropenia: caused by maternal alloimmunization to fetal specific neutrophil antibodies. Usual duration is several weeks, and neutropenia can occasionally be severe. If infections occur, they are typically in the form of sepsis, meningitis, pneumonia or skin abscess. Antibiotics and supportive care for infections and GCSF may be considered in severe infections.
- 6) Kostmann's syndrome: or severe congenital neutropenia. Multiple modes of inheritance due to multiple different possible mutations. Most of these mutations affect GCSF receptor resulting in severe neutropenia and absence of myeloid precursors resulting in severe recurrent bacterial and fungal infections. Neutropenia is most of the time severe and starts in early infancy. Bone marrow studies show myeloid hypoplasia with severe maturation arrest. Severe recurrent purulent infections start in early life. Typical organisms include *S. aureus*, *E. coli* and *Pseudomonas*. These patients are at high risk for transformation to MDS/AML. They may be responsive to high doses of GCSF. Bone marrow transplant is curative.
- 7) Cyclic neutropenia: these patients present with periods of neutropenia alternating with periods of normal neutrophil counts. Cycles range from 14 to 28 days but are usually around 3 weeks. Most are inherited in an autosomal dominant fashion. Infections and mouth ulcers occur during the neutropenic phase. The severity of this condition tends to improve with age.
- 8) Schwachman-Diamond syndrome: presents with the usual triad of neutropenia, exocrine pancreatic insufficiency and skeletal abnormalities. Most are autosomal recessive. About 25% eventually develop MDS/AML. Stem cell transplant is indicated in severe cases.
- 9) Chronic idiopathic neutropenia: usually sporadic and not inherited. Moderate to severe neutropenia with recurrent sinopulmonary infections and skin infections. No anti-neutrophil antibodies detected.
- 10) Leukocyte adhesion deficiency: 2 types I and II. Both are autosomal recessive. They are characterized by defects in neutrophil membrane proteins needed for adherence to and rolling on endothelial surfaces (neutrophil movement defect). They both present with severe recurrent soft tissue infections and neutrophilia. Type I is associated with delayed umbilical cord separation and poor wound healing. Type II is characterized by mental retardation, short stature and craniofacial abnormalities.
- 11) Chediak-Higashi syndrome: is a complex syndrome characterized by oculocutaneous albinism, nystagmus, photophobia and recurrent soft tissue and respiratory infections. These patients have neutropenia with giant granules in leukocytes and splenomegaly. The main defect is decreased degranulation of neutrophils resulting in decreased bacterial killing. The usual cause of death in these patients is EBV associated hemophagocytic lymphohistiocytosis.
- 12) Chronic granulomatous disease: presents with severe recurrent skin and sinopulmonary infections with catalase positive bacteria and fungi. Infections can also affect organs like the spleen and bone. These patients are also neutrophilic. The defect is in the oxidase enzyme where no free oxygen radicals can be produced by the neutrophils. Resulting in reduced ability to kill catalase positive organisms.