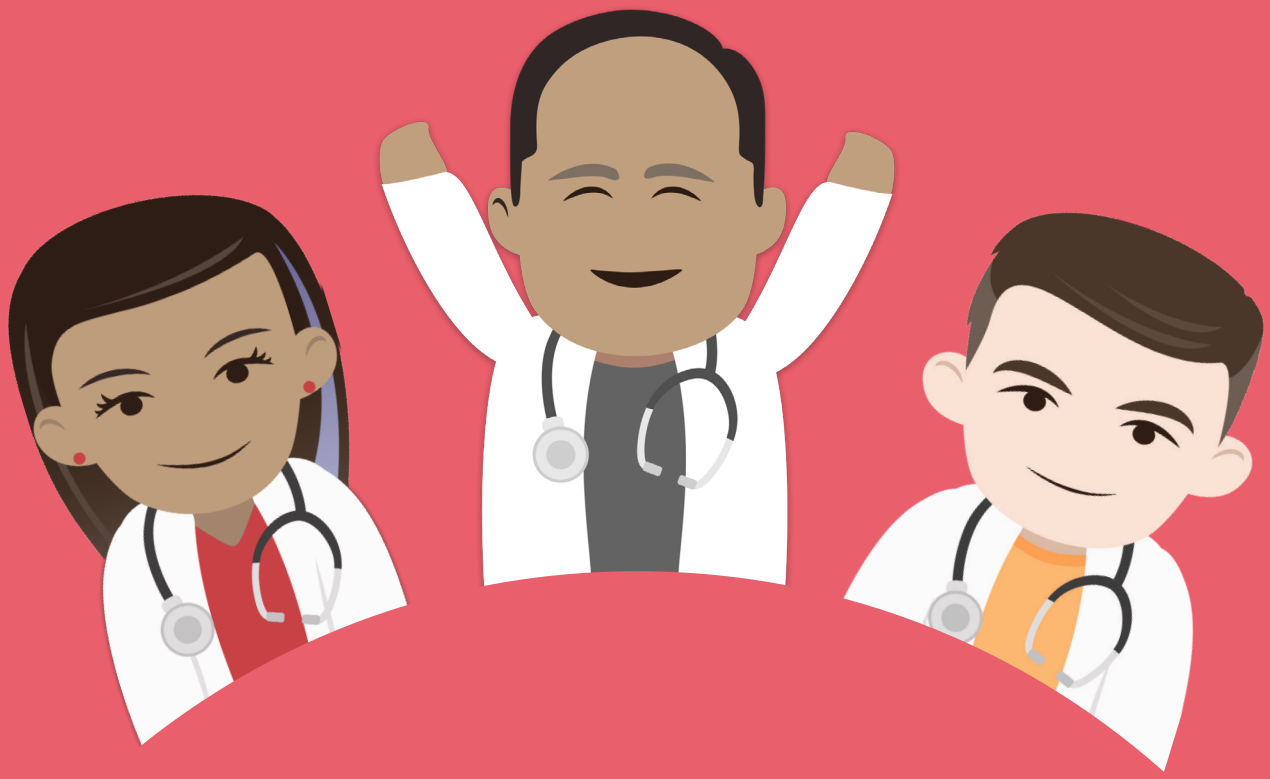
Hematology and Coagulation Essentials
Chapter 4

DIAGNOSING BENIGN WHITE BLOOD CELL (WBC) AND PLATELET DISORDERS



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Diagnosing benign white blood cell (WBC) and platelet disorders

IDENTIFYING LEUKOCYTOSIS AND LEUKOPENIAS

In a patient with leukocytosis, it is important to determine whether the condition is secondary to a reactive condition, such as infection or inflammation, or whether there is an underlying hematologic malignancy. Examples of common hematologic malignancies are acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and chronic lymphocytic leukemia (CLL).

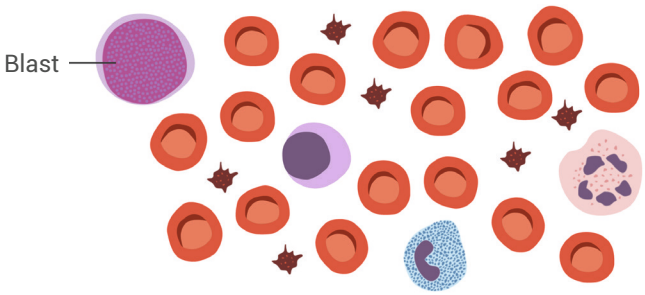
Acute leukemias

In acute leukemias, the white cell count is usually elevated (although it can sometimes remain within normal limits)

Blasts—immature white cells that are overproduced in the bone marrow and spill over into the peripheral blood. It is also very common to see anemia (in addition to the thrombocytopenia) so the RBC and platelet levels should be measured.

If a patient shows a high white cell count, with the presence of blasts, accompanied by anemia and thrombocytopenia, we are potentially dealing with a case of acute leukemia.

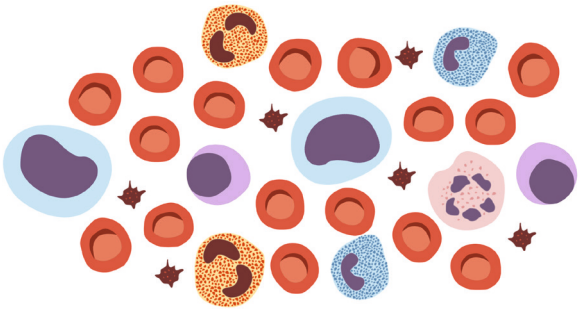
The next appropriate tests to order are a peripheral smear, flow cytometry from the peripheral blood, and a bone marrow exam. Flow cytometry is a method of immunophenotyping which can help identify blasts based on the presence of unique antigens on the cell surface.



Elevated white cell count

Chronic leukemias

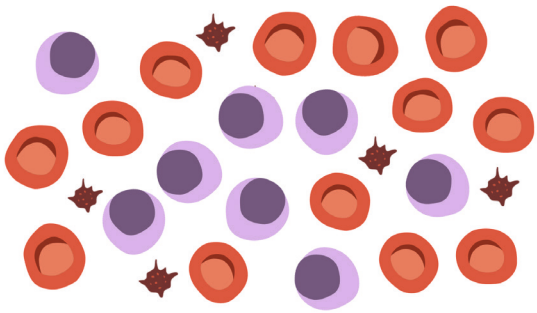
In chronic leukemias the white cell count is typically very high and may reach in excess of 100,000. If we are dealing with a potential case of chronic lymphocytic leukemia (CLL), then the absolute lymphocyte count should be greater than 5,000. The lymphocytes have typical morphology which would be picked up on a peripheral smear exam. Flow cytometry can confirm a case of CLL and a bone marrow examination may actually not be necessary.



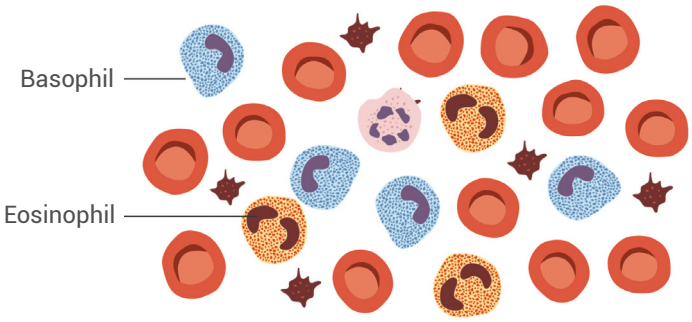
Very high white cell count

In chronic myelogenous leukemia (CML), the high white cell count is typically accompanied by basophilia and eosinophilia. Examination of peripheral blood will show the presence of immature granulocytes. CML requires molecular confirmation, which can be performed from peripheral blood. Unfortunately, flow cytometry is not useful for CML as there are no unique antigens on the tumor cells.

In summary, for both CLL and CML, a peripheral smear exam is warranted. For CLL, flow cytometry of peripheral blood is appropriate. For CML, molecular studies from peripheral blood and a bone marrow exam are indicated. Unlike acute leukemias, anemia and thrombocytopenia are not always seen in chronic leukemia.



CLL—absolute lymphocyte count > 5,000



CML—basophilia and eosinphilia

Leukopenia

Leukopenia may be isolated or part of pancytopenia.

It is important to remember that pancytopenia may be present in acute leukemia or myelodysplastic syndrome (MDS). Patients with MDS may have unicytopenia, bicytopenia, or pancytopenia. If MDS is suspected, a peripheral smear exam and a bone marrow exam are indicated. Cytogenetic and molecular studies are required to confirm MDS.

Causes of leukopenia include

- Bone marrow suppression or failure
- Drugs
- Autoimmune mechanisms
- Severe infections
- Hypersplenism
- Congenital causes

Diagnosing benign white blood cell (WBC) and platelet disorders

DIAGNOSING THROMBOCYTOPENIAS

Thrombocytopenia may be pseudo or true.

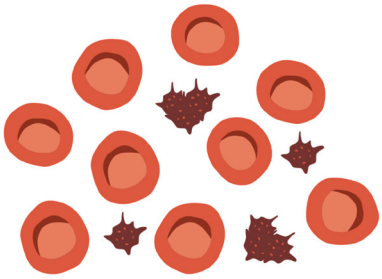
Pseudothrombocytopenia

Pseudothrombocytopenia occurs when reported platelet counts are falsely low.

Common causes of pseudothrombocytopenia include

Platelet clumps

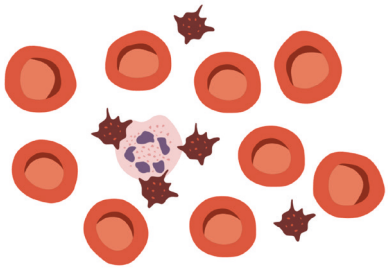
- Commonly occur when blood in collected in tubes containing ethylenediaminetetraacetic acid (EDTA).
- If blood is recollected in blue top tube, clumping should disappear.



Platelet clumps

Platelet satellitism

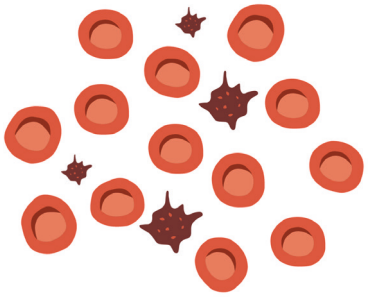
- A rare cause of pseudothrombocytopenia.
- Platelets attach onto neutrophils.
- Usually occurs when blood is collected in tubes containing EDTA.



Platelet satellitism

Numerous large platelets

- Large platelets are categorized by the analyzer as RBCs, resulting in reduced platelet numbers.



Large platelets

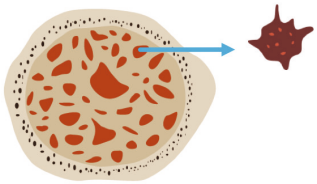
Traumatic venipuncture

Traumatic venipuncture

True thrombocytopenia

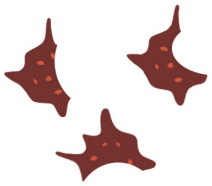
Common causes of true thrombocytopenia include

- Decreased platelet production by the bone marrow
- Increased platelet consumption
- Increased platelet destruction
- Platelet sequestration



Decreased platelet production

Increased platelet consumption



Increased platelet destruction

Decreased platelet production by the bone marrow

Decreased platelet production may be congenital or acquired.

Congenital thrombocytopenia may be divided according to the size of platelets. Wiskott Aldrich syndrome is an X-linked recessive condition characterized by thrombocytopenia, eczema, and immunodeficiency. The platelets are small in size. Thrombocytopenia with absent radii (TAR) syndrome is another congenital thrombocytopenia, in which the platelets are of normal size. Examples of congenital thrombocytopenias with large platelets are Bernard-Soulier syndrome and May-Hegglin anomaly. In May-Hegglin anomaly, the neutrophils also contain blue bodies in their cytoplasm.

Acquired causes of decreased bone marrow production actually can be any cause of bone marrow failure or suppression.

Increased platelet consumption

Microangiopathic hemolysis causes thrombocytopenia due to increased platelet consumption. Examples are disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and hemolytic-uremic syndrome (HUS). All are associated with the presence of schistocytes in the peripheral smear. DIC is associated with abnormal coagulation (abnormal PT and PTT). Coagulation is normal in TTP and HUS (i.e., PT and PTT are normal).

Increased platelet destruction

Idiopathic thrombocytopenic purpura (ITP) and mechanical valves are good examples here.

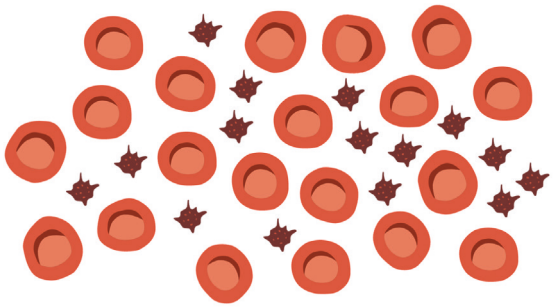
Thrombocytopenia due to sequestration

Platelets may be sequestered in hemangiomas. This is known as Kasabach-Merritt syndrome.

Diagnosing benign white blood cell (WBC) and platelet disorders

INTERPRETING TESTS FOR THROMBOCYTOSIS

Thrombocytosis may be due to reactive or neoplastic causes.



Reactive thrombocytosis

Reactive thrombocytosis is usually related to infection or inflammation. In these cases, the WBC count may be high and markers of inflammation, such as the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), will be high.

Neoplastic thrombocytosis

Thrombocytosis due to a neoplastic causes is typically persistent. Platelet counts are also very high, usually above one million.

Myeloproliferative neoplasms are the most common cause of neoplastic thrombocytosis. These include chronic myelogenous leukemia (CML), polycythemia rubra vera (PRV), and essential thrombocythemia (ET).

Chronic myelogenous leukemia (CML)
- Characterized by high WBC count with basophilia and eosinophilia

Polycythemia rubra vera (PVR)
- Characterized by high Hb and Hct levels

Essential thrombocythemia (ET)
- Characterized by very high platelet count

Myeoproliferative neoplasms require molecular diagnosis. Thus, peripheral blood or bone marrow aspirate should be tested for the expression of the following genes:

- BCR-ABL
- JAK2 mutations
- CALR
- MPL

Other causes of thrombocytosis

A special subtype of myelodysplastic syndrome (MDS) known as 5q-syndrome may cause thrombocytosis.

Patients with acute leukemia typically show thrombocytopenia. However, in very rare cases, acute leukemia may result in thrombocytosis.

Diagnosing benign white blood cell (WBC) and platelet disorders

RECOGNIZING THROMBOCYTOPATHIAS

Thrombocytopathia refers to platelet dysfunction; it may be congenital or acquired.

Congenital thrombocytopathia may be caused by

Disorders of platelet adhesion
- von Willebrand disease, Bernard-Soulier syndrome

Disorders of platelet activation
- Storage pool disorders, Chediak-Higashi syndrome, or Hermansky-Pudlak syndrome

Disorders of platelet aggregation
- Glanzmann disorder

Acquired thrombocytopathia may be caused by

- Drugs (e.g., aspirin, NSAIDs)
- Uremia
- Acquired von Willebrand disease (VWD)
- Myeloproliferative diseases
- Antiplatelet antibodies

Investigation for platelet dysfunction

If thrombocytopathia is suspected, CBC and a peripheral blood smear should be performed.

Bleeding time is the time taken for bleeding to stop, after a defined incision is made into the skin, introduced by Duke in 1910. Ivy made the method more reliable by introducing a blood pressure cuff on the upper arm, which was inflated to 40 mmHg and placing the incision into the anterior surface of the forearm. This test is commonly used to detect qualitative defects of platelets, vascular defects, or von Willebrand disease; however, the test has poor clinical correlation.

The **PFA-100 system** is a platelet function analyzer designed to measure platelet related primary hemostasis. The instrument uses two disposable cartridges which are coated with platelet agonist.

For analysis, whole blood is collected from the patient in a citrate tube and testing should be performed within four hours of collection. Blood is transferred into a sample cup, aspirated by the analyzer, and passed through an aperture in a membrane which is coated with platelet agonists. When platelet aggregation takes place, the aperture closes and the blood flow stops. This is the closure time. One membrane is coated with collagen / epinephrine (CEPI) and the other membrane is coated with collagen / adenosine diphosphate (CADP).

If the CEPI closure time is prolonged but CADP closure time is normal, the thrombocytopathia is most likely due to the presence of aspirin. If both CEPI and CADP closure time are prolonged or CEPI closure time is normal and CADP closure time is abnormal, this denotes platelet dysfunction or von Willebrand disease.

VerifyNow is a rapid, turbidimetric whole blood assay capable of evaluating platelet aggregation. This assay is based on the ability of activated platelets to bind with fibrinogen. This assay can be used to assess the effect of aspirin, clopidogrel (and related drugs), and glycoprotein IIb / IIIa antagonists.

Plateletworks is a test that assesses platelet function using whole blood. The test is available as a kit and uses the impedance cell counter (ICHOR II analyzer), which is commercially available from the Helena Laboratory; therefore, this test can be used as a point-of-care test. This test assesses platelet function by comparing the platelet count before and after exposure to a specific platelet agonist.

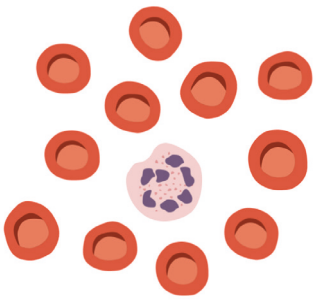
For this test, blood is collected in tubes containing EDTA or platelet agonists such as adenosine diphosphate (ADP), arachidonic acid, or collagen. In the agonist tube, functional platelets should aggregate and the nonfunctional platelets should not aggregate. A hematology analyzer, such as ICHOR II, is then used to count the number of platelets in the EDTA tube and the number of unaggregated platelets in the agonist tube. The unaggregated platelets are considered to be dysfunctional.

In order to calculate the number of functional platelets, the platelet count in the presence of a platelet agonist, should be subtracted from platelet count obtained in the presence of EDTA.

Thromboelastograph (TEG) is a test for primary and secondary hemostasis. It can be used to detect thrombocytopenia and thrombocytopathia. The specifics of this test will be described later in the course.

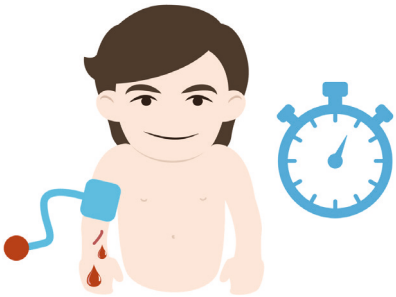
Platelet aggregometry measures the increase in light transmission through platelet rich plasma that occurs when platelets are aggregated, due to the addition of an agonist.

For this test, blood should be collected in citrate tubes and the test should be performed within four hours of blood collection. If the original platelet count of the patient is less than 100,000 then the test might be invalid.

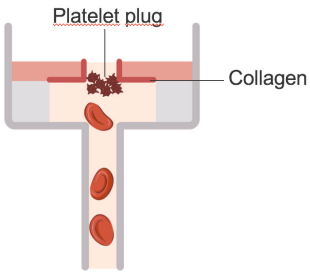


Peripheral smear

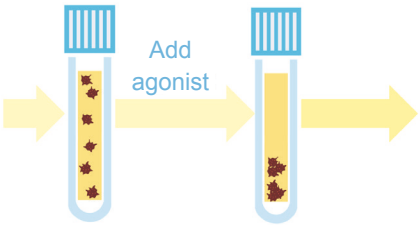
CBC



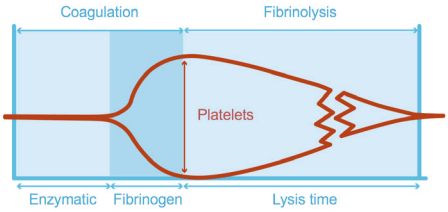
Bleeding time



PFA-100



Platelet aggregometry



TEG