

2. Guidelines for Investigating Cytopenia and Cytosis

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Cytopenia**2.1 Definition of cytopenia****Leucopenia**

Total leukocyte count less than 4,000/mm³

Neutropenia

Absolute neutrophil count less than 2,500/mm³ in an adult. In a neonate less than 2,000/mm³ is worth monitoring.

Anaemia

Please refer guideline to the investigation of anaemia.

Thrombocytopenia

Platelet count less than 150,000/mm³, please refer guidelines on bleeding disorders.

2.2 Causes of Neutropenia**2.2.1 Congenital**

- Diagnosed by morphology
 - Kostmann's syndrome
 - Chediak-Higashi syndrome

- Accompanied by other symptoms
 - Congenital aleukocytosis
 - Shwachman-Diamond syndrome
 - X linked agammaglobulinaemia
 - Diamond Blackfan syndrome
 - Dyskeratosis congenita

- Accompanied by functional disorders
 - Familial severe neutropenia
 - Lazy leukocyte syndrome
 - Congenital dysgranulopoiesis

2.2.2 Acquired

- Drug induced - from history
- Accompanied by infections- bacterial,viral, fungal
- Nutritional – Vit B12 levels, folate levels, serum iron studies
- Bone marrow pathology – HAMs test for PNH
- Toxins – from history
- Immune mediated – positive DS –DNA, ANA ,Coomb's
- Cyclic Neutropaenia

An unexpected apparent neutropenia on an automated counter should always be confirmed on a blood film since it may be factitious. In many clinical circumstances the likely cause of neutropenia will be readily apparent from the patient's medical history. When the history and examination of the blood film do not reveal the cause bone marrow investigation is usually necessary.

Screening for SLE is indicated in adults presenting with neutropenia.

For functional neutrophil disorders the Nitro Blue Tetrazolium test is suggested.

2.3 Pancytopenia

Reduction of all cell lines below the normal range for that age, sex and ethnic origin

2.3.1 Inherited causes of pancytopenia

- Accompanied by metabolic disorders –Gaucher's Disease, Neimann-Pick Disease
- Accompanied by chromosomal disorders- Chromosome fragility
- Due to stem cell disorders - PNH
- Due to bone disorders - Osteopetrosis

2.3.2 Acquired causes of pancytopenia

- Infections –from history, eg-Hepatitis, HIV, Parvo virus
- Drugs – antibiotics, anti cancer
- Chemicals -benzene
- Irradiation – from history
- Malignancies –from BM biopsy-Leukaemia, lymphoma, secondaries
- Immune mediated –ANA, DS-DNA
- Clonal disorders of haemopoiesis – MDS, PNH-flow cytometer
- Alcohol- LFT, history
- Nutritional – B₁₂, Iron

To investigate a patient with pancytopenia, blood picture, bone marrow aspiration and trephine biopsy are essential.

2.4 Aplastic anaemia

Aplastic anaemia is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate with no increase in reticulin.

To diagnose aplastic anaemia two of the following are required in addition to a hypocellular marrow

- Hb less than 10 g/dl
- Platelets less than 100,000/mm³
- Neutrophils less than 1,500/mm³

2.4.1 Severe aplastic anaemia

- Marrow cellularity <25%
 - Or 25 – 50% cellularity with < 30% residual haemopoetic tissue
- With two out of the following
 - Neutrophils less than $0.5 \times 10^9/l$
 - Platelets < 20,000/mm³
 - Reticulocytes < $20 \times 10^9 / l$

2.4.2 Very severe aplastic anaemia

As for severe aplastic anaemia but neutrophils < $0.2 \times 10^9/L$.

2.4.3 Non severe aplastic anaemia

Patients not fulfilling the criteria for severe or not severe aplastic anaemia with a hypocellular marrow with two out of 3 of the following:

- Neutrophils < $1.5 \times 10^9/l$
- Platelets < 100,000/mm³
- Hb < 10 g/dl

2.5 Polycythaemia

True polycythaemia refers to an absolute increase in red cell volume (mass) manifesting as a high haemoglobin level, or packed cell volume

This is further divided as

- Primary – clonal disorder (polycythaemia vera). Over 90% of patients with primary proliferative polycythaemia are positive for the JAK2 mutation.
- Secondary – results from an increased erythropoietin drive either in the presence or absence of hypoxia

Apparent polycythaemia - due to reduction in plasma volume

2.5.1 Criteria for Diagnosis of Polycythaemia Vera

A. Major

- A1 : Raised red cell mass(>25% above predicted or PCV >0.60 in men or >0.56 in women)
 A2 : Absence of cause for secondary erythrocytosis (consider possibility of dual pathology)
 A3 : Palpable splenomegaly
 A4 : Clonality marker, i.e. acquired abnormal bone marrow karyotype

B. Minor

- B1 : Thrombocytosis (platelets >400,000/mm³)
 B2 : Neutrophil leucocytosis (neutrophils > 10x10⁹/l)
 B3 : Splenomegaly
 B4 : Characteristic BFU – E growth or reduced serum erythropoietin

A1 + A2 + A3 or A4 established PV

A1 + A2 + any two of B criteria establishes PV

Splenomegaly in the absence of secondary cause such as portal hypertension

2.5.2 Causes of Secondary polycythaemia

A. With systemic hypoxia

- Hypoxic Lung diseases
- High altitude
- Congenital cyanotic heart disease with shunts
- High affinity haemoglobins
- Red cell metabolic defects
- Heavy smoking
- Local renal hypoxia – renal artery stenosis, end stage renal disease, hydronephrosis, renal cysts

B. Polycythaemia in the absence of systemic hypoxia

- Inherited / congenital polycythaemia
- Abnormal erythropoietin secretion
- Endocrine disorders

2.6 Idiopathic erythrocytosis

Patients not falling into the category of PV. These patients may have germ line mutations and go on to develop overt PV.

Investigations in patients with an absolute erythrocytosis. All these tests fall into the Grade X unless specified.

A. Stage I

- Full blood count and film – non tourniquet
- Arterial oxygen saturation
- Ferritin level
- Renal and liver function tests
- Abdominal ultrasound
- Serum erythropoietin level
- Chest X-ray
- NAP score
- JAK2 mutation analysis (Grade Y)

B. Stage II

- Bone marrow aspirate / trephine
- Cytogenetics (Grade Y)
- BFU – E culture (Grade Y)
- Oxygen dissociation curve (p50) (Grade Y)
- Sleep study (Grade Y)
- Lung function tests (Grade Y)
- Erythropoietin receptor gene analysis (Grade Y)
- VHL analysis (Grade Y)

2.7 Criteria for diagnosis of Essential Thrombocythaemia

2.7.1 Definition

Persistent elevation of platelet count to above $600 \times 10^9/l$ in association with megakaryocytic hyperplasia and tendency for venous thromboses and haemorrhage.

2.7.2 Diagnostic criteria

A. Positive criteria

- Sustained platelet count more than or equal to $600 \times 10^9/l$ for at least 2 months
- Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged mature megakaryocytes.

B. Criteria of exclusion

- No evidence of idiopathic MF
- No evidence of polycythaemia vera
- No evidence of iron deficiency: stainable iron in the marrow or normal red cell mean corpuscular volume
- No evidence of CML
- No evidence of MDS
- No evidence of reactive thrombocytosis

Full blood count, examination of blood picture, bone marrow aspiration with iron stain and trephine biopsy are essential for the diagnosis of ET.

2.8 Causes of reactive thrombocytosis

- Iron deficiency
- blood loss (acute/chronic)
- Hyposplenism/splenectomy
- Tissue damage- post surgery, delivery
- Acute and chronic inflammation
- Malignancy
- Rebound thrombocytosis(following ITP treatment, recovering from infections, chemotherapy)
- Drugs-vincristine, steroids

2.9 Neutrophil leucocytosis

It is the elevation of the absolute neutrophil count above which would be expected in a healthy subject of the same age, sex, race and the physiological status.

2.9.1 Causes of neutrophil leucocytosis

- c. Inherited
Very rare.
- d. Acquired

- Infections – mostly bacterial, some viral -eg IMN
- Tissue damage – Inflammation, infarction
- Acute haemorrhage, haemolysis
- Acute hypoxia
- Heat stress
- Metabolic and endocrine disorders
- Malignant diseases – cancer, leukaemia, MPD
- Drugs and administration of cytokines, chemicals
- Poisoning by chemicals and drugs, snake venom
- vigorous exercise
- Acute pain, epileptic convulsions, electric shock, paroxysmal tachycardia
- Physiological-pregnancy, neonates

Full blood count with differential count, examination of blood picture is essential.

Bone marrow aspiration and trephine biopsy in appropriate conditions.

2.10 References

These guidelines are based on the guidelines published by the British Committee for Standards in Haematology.

1. Guidelines for the Diagnosis and Management of Acquired Aplastic Anaemia
British Journal of Haematology 2003; 123:782-801
2. Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy
British Journal of Haematology 2003; 120:574-596