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**Abstract**

Hemolytic anemias are an important type of anemia in which destruction of RBCs occurs. Manifestations occur in the form of development of anemia along with sign and symptoms of hemolysis. Usually the diagnosis is made by good clinical history relating the cause of anemia and evidence of hemolysis in laboratory evaluation. Therefore, a general approach for making etiological diagnosis of the hemolytic anemia is always needed for classification and management of anemia. In this chapter an effort has been made to provide a relevant approach for the evaluation of hemolytic anemia.

**Introduction**

Anemia is defined as decrease in hemoglobin (Hb) level, according to reference ranges specific for age, gender, and race. For men Hb <13 g/dL and for women Hb <12 g/dL is considered as anemia as per World Health Organization (WHO).

The mean life span of Red Blood Cells (RBCs) is 120 days. Taking a good history followed by proper clinical examination along with laboratory investigations is still considered as the best approach for any case of anemia.

In hemolytic anemias, there is premature destruction of RBC, that is, in less than 120 days (which defines the hemolytic disorder) which in turn to compensate, there is increase in the production capacity of RBCs by bone marrow. When the rate of destruction of red blood cell exceeds the capacity of producing more red cells by bone marrow, the hemolytic disorder will manifest as hemolytic anemias.

Usually the diagnoses of hemolytic anemia are made through the laboratory investigations but a clinical history along with physical examination is also helpful for providing useful information about presence of hemolysis and its probable etiology.

Causes of anemia that can be treated (anemia due to nutritional deficiencies, gastrointestinal bleed, anemia of renal origin, and hemolysis) should be looked for carefully, so that it is not missed.

**Classification**

- On the basis of etiology, hemolytic anemia can be classified as:
  - Inherited or
  - Acquired.
- According to the site of hemolysis (**Table 1**), it is divided into:
  - Intravascular, where RBCs destruction is in the circulation or
  - Extravascular, where destruction occurs in the spleen or liver (within macrophages).
- From the clinical perspective, hemolytic anemia can be
  - acute or
  - chronic.
- According to mechanism (location of the abnormality) (**Table 2**), there may be
  - intrinsic (intracorpuscular) defect or
  - extrinsic (extracorpuscular) defects.
Mechanisms involved in anemia include:

- **Inadequate production**: Stem cell damage or defective red cell maturation.
- **Excessive destruction (hemolysis)**: Intrinsic defect in red cell leading to shortened lifespan or external factors in blood or blood vessels destroy red cells.
- **Blood loss (bleeding)**

**Causes of hemolytic anemia include:**

- **Inherited red blood cell membrane abnormalities**: There is change in shape of cell due to membrane defects and this change in shape is identified as abnormal by spleen and destroyed it.
- **Inherited enzyme deficiencies in red blood cells**: Abnormalities in enzyme levels makes the red blood cell fragile, which makes red blood cell prone to get destroyed easily.

- **Hemoglobin disorders**: Abnormal hemoglobin is because of inherited gene in some people. Abnormal hemoglobin can lead to easy destruction of red blood cells. Disorder includes sickle cell anemia and the thalassemias.

- **Physical damage to RBCs**:
  - During heart-lung surgery
  - In patients with artificial heart valves (as blood flows near devices)
  - In patients with severe burn (exposure to extreme heat)

- **Physical damage to RBCs**:
  - During heart-lung surgery
  - In patients of artificial heart valves
  - In patients with severe burn due to exposure to extreme heat

- **Autoimmune hemolytic anemia**: It can occur in autoimmune conditions like lupus, certain types
of infections, and use of some drugs. This happens when the red blood cells of the body gets destroyed by immune mechanism of its own body.

- Hypersplenism: The spleen is enlarged and becomes overactive. It traps the circulating red blood cells and destroys it.

**General Features of Hemolytic Disorders**

Hemolytic anemias can be differentiated from other anemias by presence of signs and symptoms arising because of hemolysis.

**General examination:** Jaundice, Pallor, Bossing of Skull

**Physical findings:**
- Enlarged spleen (as a preferential site of hemolysis) leading to neutropenia and/or thrombocytopenia
- Enlarged liver (in some cases)
- Skeletal changes, because of bone marrow over activity (seen in severe congenital forms)
- Hemoglobin—From normal to severely reduced
- MCV—Usually increased
- Reticulocytes—Increased (sign of the erythropoietic response by the bone marrow)
- Bilirubin—Increased (mostly unconjugated)
- LDH—Increased
- Haptoglobin—Reduced to absent

**Two main principles for diagnosis:**
- First is to confirm the diagnosis of hemolysis
- Second is to determine its etiology
  The etiology of hemolytic anemia can be determined by:
  - A good clinical history
  - The peripheral blood film

**Clinical Presentation**

- New onset pallor or anemia
- Hemolytic faces—Chipmunk facies
- Jaundice
- Splenomegaly, bossing of skull (in severe congenital cases)
- Gall stones
- Dark colored urine
- Leg ulcers

**Features of Hemolysis**

- Hemoglobin—Normal to severely reduced
- MCV, MCH—Usually increased
- Bilirubin is raised (mostly unconjugated)
- LDH is raised (up to 10 times of normal with intravascular hemolysis)
- Reticulocytes, n-RBC—Raised
- Haptoglobins are reduced to absent
- Urinary hemosiderin, Urobilinogen are +ve
  - Blood Smear
    - Spherocytes—
      - DCT +ve (Autoimmune hemolysis)
      - DCT -ve (H. Sherocytosis, malaria, clostridium)
    - No spherocytes—Hereditary spherocytosis
    - Fragmentation—Microangiopathic, Traumatic

**Laboratory Evaluation of Hemolysis**

See Table 3.

**Metabolic Manifestations of Increased Red Cell Turnover**

There is significant iron loss (requiring treatment), due to persistent hemoglobinuria because of chronic intravascular hemolysis. If frequent blood transfusions are needed or if erythropoiesis is massively increased then iron overload is very common. This iron overload leads to hemochromatosis affecting liver and heart muscles eventually leading to cirrhosis and heart failure respectively.

**Morphological Abnormalities of RBCs in Hemolytic Anemias**

- Schistocytes—thrombotic microangiopathies or cardiac prosthetic valves
- Spherocytes—hereditary spherocytosis, immune hem. anemia, burns, chemical injury to RBC
- Sickled cells—sickle cell disease
- Elliptocytes—hereditary elliptocytosis
- Echinocytes—pyruvate kinase deficiency
- Heinz bodies—G6PD deficiency
- Basophilic stipplings—thalassemia, Wilson’s disease, and lead poisoning
### TABLE 3: Laboratory evaluation of hemolysis

<table>
<thead>
<tr>
<th>Extravascular</th>
<th>Intravascular</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic</strong></td>
<td><strong>Hematologic</strong></td>
</tr>
<tr>
<td>• Peripheral blood film</td>
<td>• Peripheral blood film</td>
</tr>
<tr>
<td>• Reticulocyte count</td>
<td>• Reticulocyte count</td>
</tr>
<tr>
<td>• Bone marrow examination</td>
<td>• Bone marrow examination</td>
</tr>
<tr>
<td>• Polychromatophilia</td>
<td>• Polychromatophilia</td>
</tr>
<tr>
<td>• Raised</td>
<td>• Raised</td>
</tr>
<tr>
<td>• Erythroid hyperplasia</td>
<td>• Erythroid hyperplasia</td>
</tr>
<tr>
<td><strong>Plasma or serum</strong></td>
<td><strong>Plasma or serum</strong></td>
</tr>
<tr>
<td>• Bilirubin</td>
<td>• Bilirubin</td>
</tr>
<tr>
<td>• Haptoglobin</td>
<td>• Haptoglobin</td>
</tr>
<tr>
<td>• Free hemoglobin</td>
<td>• Free hemoglobin</td>
</tr>
<tr>
<td>• Lactate dehydrogenase</td>
<td>• Lactate dehydrogenase</td>
</tr>
<tr>
<td>• Unconjugated decreased</td>
<td>• Unconjugated decreased</td>
</tr>
<tr>
<td>• Absent</td>
<td>• Absent</td>
</tr>
<tr>
<td>• N/increased (Variable)</td>
<td>• N/increased (Variable)</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td><strong>Urine</strong></td>
</tr>
<tr>
<td>• Bilirubin</td>
<td>• Bilirubin</td>
</tr>
<tr>
<td>• Hemosiderin</td>
<td>• Hemosiderin</td>
</tr>
<tr>
<td>• Hemoglobin</td>
<td>• Hemoglobin</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>→ severe cases</td>
<td>→ severe cases</td>
</tr>
</tbody>
</table>

**Flowchart 1: Approach to identify and classify hemolytic anemias**

- Patient with anemia and characteristic laboratory features of hemolysis
  - DAT
  - Nonimmune-mediated HA
  - Immune-mediated HA
  - Drug-induced HA
  - Family history
  - PNH
  - Hypersplenism
  - By immune complexes
  - By autoantibodies
  - By hapten
  - ABO incompatibility
  - Mixed
  - Cold antibodies
  - Warm antibodies
  - Autoimmune HA
  - By immune complexes
  - By autoantibodies
  - By hapten
  - ABO incompatibility
  - Mixed
  - Cold antibodies
  - Warm antibodies
  - Autoimmune HA

- Congenital HA
- Enzymopathies
- Hemoglobinopathies
- Membranopathies
- Big vessels
- Small vessels
- Infectious
- Exogenous
- Endogenous
Red Cell Survival Study
To prove that the life span of RBC is reduced than normal (about 120 days), then the study labeling the red cells with 51Cr can be done and fall in radioactivity over days to weeks can be measured. Nonradioactive isotope 15N can also be used now in place of radioactive substance.

Algorithm for the Evaluation and Diagnosis of Hemolytic Anemia
Anemia—present
Indirect hyperbilirubinemia—present
Reticulocytosis—present

Then look for hemolysis: CBC, reticulocyte count, LDH, indirect bilirubin, haptoglobin, and peripheral blood smear

- If negative: Consider alternative diagnosis (other causes of normocytic anemia like chronic kidney disease, hemorrhage, chronic disease)
- If positive:
  - Spherocytes, positive DAT (Flowchart 1)—Immune hemolysis: lymphoproliferative disorder/malignancy, autoimmune diseases, drugs, infections, transfusion.
  - Spherocytes, negative DAT, family history—Hereditary spherocytosis.
  - Schistocytes—Microangiopathic hemolytic anemia PT/PTT, LFT, KFT, Blood Pressure TTP, HUS, DIC, Eclampsia, Preeclampsia, malignant hypertension, prosthetic valve.
  - Hypochromic microcytic anemia Thalassemia-Hemoglobin electrophoresis.
  - Sickle cells-Sickel cell anemia—Hemoglobin electrophoresis.
  - Infection/drug exposure—G6PD activity.
  - Fever/recent travel—Thick and thin smears, Babesia serology, bacterial cultures.

Conclusion
Hemolytic anemias can be diagnosed on the basis of detailed history, general and physical examination. Laboratory investigations and peripheral smear are helpful to confirm the hemolysis and to guide further tests to look for etiology.

Suggested Readings
Abstract

Beta-Thalassemia can be broadly classified into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT). The primary management in these patients is regular blood transfusion and adequate iron chelation. Some patients may require splenectomy and hematopoietic stem cell transplantation (HSCT). Majority of the complications arise because of transfusion related iron overload and include cardiac failure, arrhythmias, endocrine complications like hypogonadism, hypoparathyroidism, diabetes, hypothyroidism, delayed growth, osteoporosis, renal complications, and infections. Early initiation of chelation therapy can reverse the process of iron deposition in tissues and salvage or prolong the development of complications. This chapter highlights the challenges which arise in managing patients of beta-thalassemia and summaries the clinical approach in preventing various complications which arise out of iron load in these patients.

Introduction

The disease β-Thalassemia includes many different conditions like β-thalassemia major, β-thalassemia intermedia, and HbE/β-thalassemia. They can be broadly classified into transfusion-dependent thalassemia (TDT), which includes severe forms of HbE/β-thalassemia and β-thalassemia major and non-transfusion-dependent thalassemia (NTDT), which includes thalassemia intermedia and milder forms of HbE/β-thalassemia (Fig. 1). TDT are patients who require regular transfusion of blood to survive whereas NTDT will require blood transfusions in certain conditions like pregnancy, surgery, and infections.1

Management

The management of TDT requires regular blood transfusion and adequate iron chelation. In some patients, splenectomy and hematopoietic stem cell transplantation (HSCT) are required. Table 1 summarizes the current therapies available for TDT.

Complications and Management Issues

Majority of the complications in TDT arise because of transfusion related iron overload and include cardiac failure, arrhythmias, endocrine complications like hypogonadism, hypoparathyroidism, diabetes, hypothyroidism, delayed growth, osteoporosis, renal complications, and infections. Some complications like silent cerebral infarction and pulmonary hypertension are more common in patients with NTDT.

Cardiac Complications

They include cardiomyopathy congestive heart failure, arrhythmias, peripheral vascular disease, and pulmonary hypertension. Persistent hypoxia, high levels of abnormal hemoglobin, and high iron levels in tissues along with
### TABLE 1  Treatment options for beta thalassemia²

<table>
<thead>
<tr>
<th>Treatment modality</th>
<th>Merit</th>
<th>Demerit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfusion</td>
<td>Helps by suppressing ineffective erythropoiesis, prevents bone marrow expansion</td>
<td>• Every 2–4 weeks’ transfusion required for life long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Transfusion associated complications like infections, iron overload, and alloimmunization</td>
</tr>
<tr>
<td>Iron chelation</td>
<td>• Decreases iron overload in myocardium, liver, pituitary, etc.</td>
<td>• May not be effective in every patient</td>
</tr>
<tr>
<td></td>
<td>• Improves endocrine functions and cardiac health</td>
<td>• Side effects are common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Parenteral formulation often lead to non-compliance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cost of therapy is high</td>
</tr>
<tr>
<td>Deferoxamine: Parenteral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deferasirox: Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deferiprone: Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyurea (It is a cytotoxic antimetabolite that helps to increase fetal hemoglobin levels)</td>
<td>• Some hematological parameters may improve in NTDT patients</td>
<td>• Lack of clear evidence of its use in TDT</td>
</tr>
<tr>
<td></td>
<td>• Cost of therapy is low</td>
<td></td>
</tr>
<tr>
<td>Splenectomy</td>
<td>• The quality of life and growth may improve</td>
<td>• Risk of infection and sepsis increases</td>
</tr>
<tr>
<td></td>
<td>• It may improve hemoglobin concentration</td>
<td>• The risk of venous thrombosis and pulmonary hypertension increases</td>
</tr>
<tr>
<td></td>
<td>• The frequency of transfusions may decrease</td>
<td>• It also reduces the ability to scavenge toxic-free iron species</td>
</tr>
<tr>
<td>HSCT</td>
<td>• Treatment of choice for patients younger than 14 years</td>
<td>• May not be useful for all patients, only a subset of patients qualify for this treatment</td>
</tr>
<tr>
<td></td>
<td>• Others may benefit depending on donor availability and iron load after this age</td>
<td>• Young age</td>
</tr>
<tr>
<td></td>
<td>• The survival rate is up to 90% and the disease-free survival rates reaches up to 80%</td>
<td>• Donor should be a compatible sibling</td>
</tr>
<tr>
<td></td>
<td>• The quality of life is improved</td>
<td>• Risk of 5–10% mortality</td>
</tr>
<tr>
<td></td>
<td>• Long-term cost-effective</td>
<td>• Preparation for this treatment requires myeloablative conditioning and can cause impairment of fertility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Highly trained centers are required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Very high cost</td>
</tr>
</tbody>
</table>

HSCT, hematopoietic stem cell transplantation; NTDT, non-transfusion-dependent thalassemia; TDT, transfusion-dependent thalassemia
infection with cardiotropic viruses contribute to initiation and progression of cardiomyopathy in these patients. Presenting features are dyspnea, fatigue, or palpitations. Cardiomyopathy in thalassemia presents with two different phenotypes:

- Dilated cardiomyopathy where the left ventricle is dilated and contractility is reduced. This progresses to congestive heart failure.
- Restrictive cardiomyopathy where the left ventricle filling is restrictive. This phenotype can lead to pulmonary hypertension, dilatation of right ventricle, and subsequent heart failure.

Measurement of amino-terminal pro-B-type natriuretic peptide is more reliable in early diagnosis than Doppler echocardiography. Cardiac MRI provides better estimate of iron burden than serum ferritin and it has been found to be a better predictor of heart failure and arrhythmia in different studies.4

Rhythm Disturbances

They are atrial fibrillation, atrial flutter, intra-atrial re-entrant tachycardia, ectopic atrial tachycardia, and ventricular arrhythmias. Prolonged QT interval and repolarization abnormalities occur because of iron overload leading to torsades de pointes and sudden cardiac death (SCD).5

Endocrine Complications

They include hypogonadism, growth retardation, diabetes mellitus, hypoparathyroidism, and hypothyroidism.6

Gonadal Axis

Hypogonadism can be primary (hypogonadotropic hypogonadism) due to iron deposition in gonads or secondary (hypogonadotropic hypogonadism) due to iron affecting the gonadotrophs in anterior pituitary. Secondary hypogonadism is more common in patients with TM. Primary amenorrhea precedes secondary amenorrhea in most women with thalassaemia major. Screening for hypogonadism should be done annually. Careful history should be taken which includes erectile function, libido, spontaneous erections in males and libido, vasomotor symptoms, and menstrual history in females. The patient’s genitalia should be examined and loss of secondary sexual characters should be noted.

Investigations:

- Serum fasting testosterone, SHBG, LH, FSH, and hCG in males and estradiol, LH, and FSH in females.

Management:

- Females: Treatment is given to relieve from symptoms of estrogen deficiency like sweats, hot flushes, mood changes and vaginal atrophy. Hormone replacement therapy (HRT) is helpful to overcome them and prevent osteoporosis.
- Males: Testosterone replacement in the form of transdermal gel or intramuscular injections every 2 weeks helps to improve libido, erectile dysfunction, and may improve bone mineral density and muscle mass.

Growth Hormone Axis

Many factors contribute to delayed growth in patients, which are iron overload, chronic anemia, hypersplenism, hypothyroidism, hypogonadism, chronic liver disease, malnutrition, stress, and growth hormone (GH) deficiency. GH deficiency is common in patients with TM and results in short stature and delayed growth. GH deficiency can have varied presentations like classic GHD, GH resistance or a combination of both.7 Clinical Assessment should
be done preferably at regular interval of 6 monthly. It includes:

- Patient’s height (standing and sitting) and weight
- Measurement of upper/lower segment ratio and calculation of BMI and annual growth velocity (GV)
- Recording of parental heights at the first visit (calculate mid-parental height)
- Accurate measurement of standing and sitting heights, weight, and head at each visit; measurement of the head circumference especially during the first 2 years of life
- Plotting growth data on ethnically adjusted charts or international (WHO) adjusted charts
- Calculating annual growth velocity (GV), body mass index (BMI), and upper/lower segment ratio at every visit
- Assessment of pubertal status as per Tanner stage (development of breast in girls and testicular volume in boys)
- Assessment of bone age

Investigations:

- Serum IGF-1, Serum TSH, and free T4, LH, FSH, and sex steroids along with X-ray of wrist and hand for bone age.

Treatment:

- Children who are short with low IGF-1 levels and normal GH secretion to stimulation tests may benefit from IGF-1 or GH-IGF-1 treatment.
- If the bone age is 10 years or greater, then priming with sex steroids is done before initiating any treatment for GH deficiency.
- Treatment of other diseases like diabetes and hypothyroidism has to be done simultaneously.
- There must be psychological evaluation and support for conditions that are non-treatable like constitutional delay of puberty and growth, familial short stature, etc.
- Treatment with rhGH should start with low doses and then titrated according to IGF-1 levels and growth rate.

Adrenal

Patients with thalassemia are often asymptomatic for adrenal insufficiency and may have only biochemical evidence. A study of 56 children suffering from thalassemia had shown presence of adrenal insufficiency in about 37.5% asymptomatic patients. Acute stress can precipitate adrenal insufficiency and symptoms can be feeling of lack of energy, fatigue, decreased appetite, and muscular weakness. Adrenal androgen deficiency can sometimes lead to decreased pubic and axillary hairs.

Investigations:

- Serum cortisol level, adrenocorticotropic hormone (ACTH) stimulation test or rarely insulin stimulation test (ITT) can be done to assess adrenal insufficiency.

Treatment:

- Acute adrenal crisis is not common in these patients, and hence steroids should not be used routinely to cover asymptomatic individuals. Glucocorticoids can be used in acute stressful conditions.

Thyroid

Primary hypothyroidism is present in 4–29% of patients with TDT and is much common than central or secondary hypothyroidism. Prevalence of hypothyroidism is directly proportional to iron load. With the increase in serum ferritin the prevalence of hypothyroidism increases. Also hypothyroidism is more frequent in splenectomized patients than non-splenectomized patients. The reason may be because of the fact that intact spleen acts as reservoir of excess iron and functions as a scavenger for free iron fraction.

Classification of hypothyroidism:

- **Sub-biochemical hypothyroidism**: The response to TRH test is exaggerated and TSH and FT4 are normal.
- **Subclinical hypothyroidism**: The TSH is high (>4.2 mIU/L and <10 mIU/L) and FT4 levels is normal.
- **Overt (clinical) hypothyroidism**: The TSH is high (TSH >10 mIU/L) and FT4 is low.

Investigations:

- Assessment of serum-free T4 and TSH should be done annually from the age of 9 or earlier if hypothyroidism is suspected. For early diagnosis, TRH stimulation test can be used. In patients with iron overload, there is exaggerated TSH response to TRH, which may evolve into subclinical or clinical hypothyroidism. In clinical hypothyroidism, there are the FT4 and basal TSH gradually decrease over time.

Treatment:

- Early detection and adequate chelation therapy may reverse the thyroid dysfunction. Patients with overt hypothyroidism are treated with L-thyroxine.
Hypoparathyroidism

It is an uncommon and a late complication in TM patients. Deposition of iron in parathyroid glands results in hypoparathyroidism. Hypocalcemia and paraesthesia occur in mild cases and severe cases may present with tetany and seizures.

Investigations:
- Measurement of calcium, phosphate and parathyroid hormone (PTH) levels should be done. In hypoparathyroidism there is low serum calcium, high phosphate, and low PTH levels.

Treatment:
- Supplementation with calcium and vitamin D is done. Calcitriol 0.25 µg twice daily brings the calcium and phosphate levels to normal. In some patients with high phosphate levels, phosphate binders may be used. In severe hypocalcemia, phosphate binders may be used. In severe hypocalcemia, tetany, and cardiac failure, intravenous calcium has to be given.

Thalassemia Bone Disease

Patients with thalassemia can suffer from bone diseases, which include osteopenia, osteoporosis, bone deformity and fractures. Risk factor for bone disease in these patients includes concurrent presence of hypogonadism and deficient growth hormone. Other contributory factors include chronic anemia and marrow expansion, chelator toxicity, hypercalciuria, and renal dysfunction from deferasirox, liver disease, and advancing age.

Investigations:
- Serum calcium, serum phosphorus, alkaline phosphatase, and 25-hydroxy vitamin D
- PTH, LH, FSH, testosterone levels
- Osteocalcin
- C-terminal telopeptide
- 24-h urinary calcium
- X-ray of spine (AP and lateral views)
- MRI spine: to exclude an intervertebral disc degeneration
- Dual-energy X-ray absorptiometry (DEXA) scan for assessing bone mineral density (BMD)

Treatment:
- Treatment with calcium and vitamin D can be started although there is not much evidence of improvement in BMD.

- Bisphosphonates like zoledronic acid have the advantage of intravenous administration and longer duration of action lasting more than 12 months and should be initiated earlier.
- Other drugs helpful in bone disease include teriparatide, denosumab, and zinc.

Glucose Intolerance and Diabetes Mellitus

Patients with thalassemia are susceptible to develop diabetes. The etiology is multifactorial including genetic predisposition, β-cell destruction because of iron overload, insulin resistance, insulin deficiency, chronic liver disease, and viral infections. Initially patients may present with impaired glucose tolerance because of insulin resistance and later develop insulin deficiency. Some characteristics of insulin dependent diabetes in thalassemics are:
- Ketoacidosis is an uncommon presentation
- Islet cell antibodies are usually negative
- HLA haplotypes like DR4, B8-DR3, and BW15 have no association

Investigations:
- Fasting blood sugar levels, 2-h oral glucose tolerance testing (OGTT) and measurement of insulin levels should be started at the age of 10 years. HbA1c is altered in hemoglobinopathy and cannot be used as a reliable marker for monitoring. Instead serum fructosamine levels may be used for monitoring long term glycemic control.

Treatment:
- Dietary counseling and weight reduction in metabolically obese patients.
- Chelation therapy should be more intensive. There is increase in insulin secretion and decrease in insulin resistance with intensive chelation therapy.
- Data on use of oral antidiabetic drugs is limited. Insulin is needed in late presentation and complications.

Conclusion

The management in thalassemia requires a team effort and a multidisciplinary involvement. Management of TDT mainly depends on lifelong transfusion therapy. The complications arise because of the iron deposition in various organs and infections from frequent blood transfusions. It has been proven in studies that early initiation of chelation therapy can reverse...
Contd...

the process of iron deposition in tissues and salvage or prolong the development of complications. Pregnancy, fertility, and psychosomatic stress are other associated issues in managing patients of thalassemia and require treatment for enhancing quality of life in these patients.

References

Abstract

Thrombocytopenia is a common medical problem encountered by physicians in their day-to-day OPD as well as IPD practice. Anxiety is at peak for patients as well as young physicians when dealing with case of severe thrombocytopenia. Thorough history, physical examination, and directed investigations lead to correct cause and resolution of thrombocytopenia. Causes range from benign disease like ITP and megaloblastic anemia to serious diseases like leukemia, lymphoma, and TTP. Drugs are culprit in many cases and sometimes misleading drug history leads to unnecessary battery of tests. Once properly diagnosed, correct and timely treatment can resolve thrombocytopenia. Platelet transfusion is not always required and should be reserved for severe thrombocytopenia or with bleeding patients.

Introduction

By the time you shall be reading this chapter, we hope that world will be on the path of recovery from COVID pandemic crisis and message like “stay safe” will be less frequently used.

Thrombocytopenia is a common medical problem encountered by physicians in day-to-day practice. It creates panic among general people as well as practicing physicians, which is not always justified.

We will describe how to approach a case of thrombocytopenia with brief about treatment options of common diseases.

Thrombocytopenia is defined as a platelet count below the 2.5th lower percentile of the normal platelet count distribution. Results of the third US National Health and Nutrition Examination support the traditional value of 150 × 10^9/L as the lower limit of normal; however, counts between 100 × 10^9/L and 150 × 10^9/L do not necessarily indicate disease if they have been stable for more than 6 months, and the adoption of a cut-off value of 100 × 10^9/L may be more appropriate to identify a pathologic condition.1-3

Low Platelets—How Serious It is?

Platelets play an important role in vessel wall integrity and so low platelet leads to primary hemostatic defects. Its relevance in an individual patient is variable and depends upon the clinical presentation.

Clinically significant bleeding does not occur unless platelets are less than 10–20,000 × 10^9/L, while mild to moderate thrombocytopenia becomes significant when there is additional bleeding risk like surgery, trauma or when higher cut-off to be met as when treatment for hepatitis C or chemotherapy is indicated.

In some situation, finding of thrombocytopenia points toward serious disease like HIV or Myelodysplastic syndrome and also sometimes it indicates disease activity like in thrombotic thrombocytopenic purpura (TTP).

How Important is Setting of Thrombocytopenia?

In OPD settings, mostly thrombocytopenia is isolated and asymptomatic and diagnosis is straightforward while in inpatient, or in ICU settings, multisystem
involvement leads to thrombocytopenia and diagnosis and management is sometimes challenging (Table 1). Pregnancy with thrombocytopenia has to be approached differently as it has significance in the care for mother as well as the newborn.

A structured approach involves clinical details and support of lab along with other medical disciplines.

**Mechanism of Thrombocytopenia**

Major mechanism of thrombocytopenia is reduced production as in aplastic anemia, Myelodysplastic syndrome (MDS), or chemotherapy induced thrombocytopenia and platelet destruction as in disseminated intravascular coagulation (DIC) or TTP.

Less common mechanism is platelet sequestration as in congestive splenomegaly and hemodilution as in excessive fluid or platelet poor component transfusion.

There are conditions like ITP and hepatitis C, where multiple mechanisms play role.

**History and Physical Examination—How does it Help in Evaluation?**

As for any medical disorder history is very important for thrombocytopenia evaluation and question should be directed toward bleeding history, associated symptoms like fever, jaundice, joint pain, medication history, dietary habits, addiction and history to rule out autoimmune disease and malignancy.

Physical examination should be directed to look for bleeding, organomegaly, or skeletal abnormality.

**Lab Investigations—What Battery of Tests Required?**

Even today, initial and basic investigation for thrombocytopenia evaluation is complete blood count (CBC) and peripheral smear. Routine tests, like liver function and renal function test, are always required. Chest X-ray is required to look for focus of infection and mediastinal mass and USG abdomen is done to look for any evidence of chronic liver disease or lymphadenopathy. Autoimmune workup is required in suspected cases to rule out SLE/APLA syndrome. Infective workup is required in patients who present with classical features. Immature platelet fraction or reticulated platelets help in differentiation from bone marrow failure (low percentage) and hyper-destructive thrombocytopenia (higher percentage). Flowchart 1 shows algorithm for thrombocytopenia on the basis of peripheral blood smear findings.

Artefactual thrombocytopenia and conditions like Harris platelet syndrome are not so rare problem encountered in our clinical practice and it has huge psychological impact on the patient, their immediate care giver, and physicians. They need only counseling that they should not worry and need no intervention. We will discuss common conditions leading to thrombocytopenia in our practice.

**TABLE 1** Clinical scenario and most common causes of thrombocytopenia

<table>
<thead>
<tr>
<th>Outpatient</th>
<th>Inpatient</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITP</td>
<td>Multisystem illness/ICU</td>
</tr>
<tr>
<td>DITP</td>
<td>Infections</td>
</tr>
<tr>
<td>Infections: HIV, Hep C, CMV, H. Pylori, Dengue, and other recent viral infection</td>
<td>DITP</td>
</tr>
<tr>
<td>Connective tissue disorder</td>
<td>TTP/HUS</td>
</tr>
<tr>
<td>Vaccinations</td>
<td>DIC</td>
</tr>
<tr>
<td>Congenital thrombocytopenia</td>
<td>Liver disease/BM disorders</td>
</tr>
<tr>
<td>Common variable immunodeficiency disease</td>
<td>HIT</td>
</tr>
<tr>
<td>MDS</td>
<td>MAS</td>
</tr>
<tr>
<td></td>
<td>CIT</td>
</tr>
</tbody>
</table>

CIT, chemotherapy-induced thrombocytopenia; DITP, drug-induced ITP; MAS, macrophage activation syndrome; MDS, myelodysplastic syndrome.
**Isolated Thrombocytopenia**

It is defined as thrombocytopenia in the absence of RBC or WBC abnormality and without signs or symptoms of systemic illness. Common examples are ITP and D-ITP.

**Immune Thrombocytopenia (ITP)**

(terms idiopathic and pupura have been removed)

Even being the commonest cause of isolated thrombocytopenia, there is no single test, which confirms ITP as diagnosis and it remains a diagnosis of exclusion. Box 1 lists all blood investigations in a suspected case of ITP. Antiplatelet antibody assay is not very sensitive, but specificity approaches 90%. Table 2 enumerates morphologic features and pointers towards different causes of thrombocytopenia. Bone marrow is not required unless patients are more than 60 years or with atypical features.

**BOX 1** List of basic tests required as per international consensus report

- Complete blood count
- Peripheral blood smear
- Reticulocyte count
- Quantitative Ig level measurement
- BM examination (in patients >60 years)
- Blood group (Rh)
- Direct antiglobulin test
- *H. pylori*
- HIV
- HCV

ITP patients are divided into newly diagnosed (thrombocytopenia is of less than 3 months duration), persistent (thrombocytopenia extends from 3 months to
TABLE 2  Showing morphological findings on PBS in different causes of thrombocytopenia

**Platelets**

- **Platelet clumping**
  - Platelet clumping caused by EDTA-dependent platelet autoantibodies is a common cause of artifactual thrombocytopenia. It occurs in about 1 in 1000 normal adults and is not associated with bleeding or thrombosis.
  - Platelet size and granularity
    - Consistently large platelets suggest hereditary macrothrombocytopenia. Large platelets with a gray color on Wright-Giemsa stain define the gray platelet syndrome, an autosomal-dominant macrothrombocytopenia associated with bleeding tendency due to absent or greatly reduced α-granules.
    - In thrombocytopenia due to peripheral destruction, large platelets or giant platelets are often seen in addition to platelets of normal size.
    - When thrombocytopenia is due to reduced platelet production (e.g., after chemotherapy), platelets are of normal size. In myelodysplastic syndromes, platelets have variable size (giant platelets may be seen) and are frequently hypogranular. In Wiskott-Aldrich syndrome, and X-linked thrombocytopenia, both caused by mutations of the WAS gene, platelets are small.

**WBCs**

- **Leukemic cells**
  - Malignant hematological disorders (leukemias and lymphomas) are often associated with thrombocytopenia, which is almost never an isolated finding.
  - Other abnormalities of WBCs, including leukocyte inclusions
    - A constellation of nonspecific abnormalities of WBCs are common to many conditions (e.g., neutrophilia, lymphocytosis, leukopenia, etc.) and may be associated with thrombocytopenia. The presence of hypolobulated neutrophils (Pelger-Huet anomaly) suggests a myelodysplastic syndrome. Dark coarse granules (toxic granulations) found in neutrophils suggest sepsis. Atypical lymphocytes suggest viral infection. The presence of WBC inclusion (Dohle-like bodies) should be investigated carefully when platelets are mostly large (MYH9-related congenital macrothrombocytopenia).

**RBCs**

- **Schistocytes**
  - The presence of RBC fragments known as schistocytes is indicative of a thrombotic microangiopathy (TTP/HUS) or DIC.
- **Size and other morphological features.**
  - Microspherocytes may suggest Evans syndrome, but may also be present along with schistocytes in thrombotic microangiopathies. Macrocytosis (and hypersegmentation of neutrophils) suggest vitamin B12 or folate deficiency. Dacrocytes (teardrop-shaped cells) suggest myelofibrosis. Nucleated RBCs suggest hemolytic anemia, myelofibrosis, or an infiltrative process of the BM.
- **Parasites**
  - The presence of intracellular parasites (e.g., in malaria) is diagnostic of infection.

12 months), and chronic when thrombocytopenia is for more than 1 year.

In ITP patients with emergency (intracranial bleed, massive GI bleed) IVIG, High dose methylprednisolone, and anti D (in Rh positive patients) are options.

For asymptomatic or patients with minor bleeding, observation and local bleeding control are best options, as most of the morbidity in ITP patients are due to treatment than due to low platelet count. Good counseling can reduce need of drugs and give better quality of life to patients. It is important to explain to patients that even with very low platelets, severe or life threatening bleeding is rare in ITP.

Options in symptomatic patients are steroid (short course), immunosuppressants (azathoprine, MMF), Dapsone, MAB (rituximab), Thrombopoietin mimetic (wltrombopag, romiplostim), and splenectomy. Other less commonly used options are danogen, vincristine, cyclosporine, and cyclophosphamide.

**Drug-induced Immune Thrombocytopenia (D-ITP)**

The list of drugs leading to thrombocytopenia is ever increasing one. The pathophysiological mechanism of D-ITP is due to formation drug dependent antibody against epitope of platelet glycoprotein created by their interaction with the drug.
Sometimes D-ITP can be confused with ITP, and only good history can help to differentiate.

Diagnosis often is difficult as sometimes not only drug but even food and beverages can lead to thrombocytopenia.

Diagnosis is mostly empirical with recovery of platelet count after discontinuation of drug. Lab diagnosis is by demonstration of drug dependent antibody by various methods.

An overall score of 5 or more is compatible with overt DIC. A score less than 5 is suggestive of non-overt/low-grade DIC.

**Thrombocytopenia in Hospitalized Settings**

Approximately 1% of admitted patients in acute care hospital are thrombocytopenia but only 30% of them have bleeding manifestation. As expected it is more common in ICU, where 8–68% are thrombocytopenic on admission and 13–44% during stay in the unit. Many potential etiologies like sepsis, DIC, drugs, CABG coexist and it is often difficult to elucidate cause of thrombocytopenia. ITP should always be considered as it is seen in about 20% of these and antibiotics are the major culprit. Discontinuation of such drug should always be based on clinical criteria.

**Disseminated Intravascular Coagulation**

DIC is a consumptive coagulopathy characterized by activation of intravascular coagulation with microvascular thrombi formation, thrombocytopenia, depletion of clotting factors, bleeding complications, and end organ damage. It can be acute or chronic. International society on thrombosis and hemostasis divides DIC into overt (decompensated hemostatic system) or non-overt (compensated coagulopathy).

Acute DIC is seen in sepsis, septic shock, after trauma (neurotrauma), after surgery, after obstetric complications and in APML. Chronic DIC is seen in solid tumors and large aortic aneurysm. Table 3 enumerates factors and scoring to diagnose overt or non-overt DIC.

**Thrombocytopenia in Cardiac Patients**

Open heart surgery is an important cause of thrombocytopenia. Nadir is typically seen on days 2–3 and recovery starts rapidly thereafter. Causes of low platelet after surgery are multiple.

Severe thrombocytopenia has been observed in 0.1–2% of patients after exposure to gp IIb/IIIa inhibitors (abciximab, tirofiban, eptifibatide). Peculiarity is sudden onset, usually within hours after surgery, and used to resolve by day 10. This has been attributed to presence of naturally occurring antibody to neoepitopes exposed by gp IIb/IIIa inhibitors.

**HIT**—It occurs in 1–3% of patients receiving heparin beyond the first postoperative week and in 10% of patients after ventricular assist device implantation. HIT described in Table 4 helps in diagnosis of heparin induced thrombocytopenia.

**Pregnancy with Thrombocytopenia**

About 6–15% of women develop thrombocytopenia (<1.5 lakhs/mm³) at the end of pregnancy but platelets less than 1 lakh/mm³ are seen in only 1% of patients. Most common cause of thrombocytopenia is gestational thrombocytopenia (GT) (70%), preeclampsia (21%) ITP (3%).

**Gestational Thrombocytopenia (GT)**

GT is seen in mid second to third trimester of pregnancy and it is extreme variation of normal physiological fall of platelet count. Platelet count usually remains above 70 k/mm³ and if falls to lower than 70 k, then alternative diagnosis should be considered. Diagnosis is

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Diagnostic score of DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>0</td>
</tr>
<tr>
<td>Platelet count</td>
<td>&gt;100 × 10⁹</td>
</tr>
<tr>
<td>Elevated fibrin degradation products</td>
<td>No increase</td>
</tr>
<tr>
<td>PT more than ULN</td>
<td>&lt;3S</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>&gt;1 gm/L</td>
</tr>
</tbody>
</table>
by excluding other causes and there may similar history of thrombocytopenia in previous pregnancy and fetus/newborn remains unaffected (normal platelet) and recovery takes 1–2 months after delivery.

**ITP in Pregnancy**

ITP in pregnancy is seen in about 1–2 of 1,000 pregnancies. It is the most common cause of thrombocytopenia in first and early second trimester. One third of patients are diagnosed first time during pregnancies and rest has history of ITP.

Differentiating GT from ITP is difficult and it is more important for neonatal management as 9–15% of neonates of ITP mother can have thrombocytopenia and 1–2% can have intracranial hemorrhage.21

Whenever platelet is less than 50 k/mm³, diagnosis is ITP by default, though as in medicine always it should be correlated in clinical context.

**COVID-19 and Thrombocytopenia**

SARS-CoV-2 leading to present COVID-19 pandemic present commonly with fever, cough, and breathlessness. Common abnormality in hematological parameters includes leukopenia, lymphocytopenia, and thrombocytopenia. Thrombocytopenia is seen in about 5% of patients at admission and overall about 36% of patients showed thrombocytopenia, most of which are significant in severe cases. The proposed mechanism is multifactorial due to cytokine storm, direct hematopoietic stem cell injury, increased autoimmune destruction and lung injury.22-25

Bleeding due to thrombocytopenia is rarely seen.

**What are the Indications, Dosing of Platelet Transfusion?**

- Any patient with bleeding due to thrombocytopenia or severe thrombocytopenia (less than 30 k/mm³ even without bleeding) or patient with moderate thrombocytopenia (30 k to 1 lakh/mm³) and due for surgery or has other associated bleeding risk need platelet transfusion.
- Higher threshold (more than 1 lakh/mm³) is required for patients with life threatening bleeding (ICH) or planned for neurosurgery or ophthalmic surgery.
- Exact cut-off depends upon primary disease leading to thrombocytopenia.
- In aplastic anemia and acute leukemia, platelet transfusion should be done when platelet count is less than 10 k/mm³ without fever and less than 20 k with fever, for APML threshold is higher due to excess bleeding risk.
- Disease like TTP and HIT are conditions where platelet transfusion is always avoided and common disease like ITP rarely needs platelet transfusion.
- Physicians should think twice before ordering for platelet transfusion in asymptomatic thrombocytopenia patients.
Dose for adult patient is single donor platelet or 6 unit random donor platelet (1 RDP per 10 kg). One SDP leads to increase of platelet by 40–50 k and RDP increase platelet by 5–10 k unless reasons for platelet refractoriness are absent.

**Conclusion**

Thrombocytopenia is a common problem encountered by physicians in day-to-day in IPD as well as OPD practice. Common causes are ITP, D-ITP, and infections. Good history, physical examination, and directed investigations can lead to correct diagnosis of thrombocytopenia.

Delay in diagnosis and blind treatment leads to unnecessary intervention in many patients, which should always be discouraged. Platelet transfusion is required in emergency cases and should not be misused, just to overcome fear and anxiety of low platelet.

We thank our patients for having faith on us and giving us opportunity to understand “thrombocytopenia” and all supporting staff including nurses who help us in patient management.

**References**

Management of CML in Resource-limited Settings

Hemant Malhotra, Naveen Gupta, Ajay Yadav

Abstract
After the development, testing and global availability of BCR/ABL targeted tyrosine kinase inhibitors (TKIs), the prognosis of patients with CML in the chronic phase has improved to point that the majority of patients can expect a multi-decade survival which is now close to that of age and sex matched subjects without the disease, at least in the developed world. However, the situation in the low- and middle-income countries (LMIC), like India, may not be as encouraging. Many hematological cancers in developing countries, including CML, have subtle and not so subtle differences in incidence, age of onset, stage at presentation, phenotype, stage-for-stage response rates, and prognosis as compared to their counterparts in the developed world. Several reasons have been postulated for this and these include; socioeconomic factors, genetic differences, environmental factors (infections, particularly, viral infections, exposure to pesticides, etc.), nutritional factors and factors related to availability of drugs, means for monitoring the disease and option for the use of second generation agents. Generic first generation TKIs (imatinib) and also second generation ones are available in many parts of the world but several challenges still remain in providing optimal treatment to the patients with CML in resource-poor countries. These include availability of optimal and high-quality BCR/ABL testing, availability and cost of second and third generation TKIs (nilotinib, dasatinib, bosutinib, and ponatinib) and hematopoietic stem cell transplantation, compliance and toxicities of drugs and ensuring minimal standard-of-care treatment and monitoring for each and every patient diagnosed with CML. Some of these issues will be reviewed and highlighted in this article.

Introduction
Chronic myeloid leukemia (CML) is a clonal hematopoietic disorder of a pluripotent stem cell that leads to increased proliferation of cells of the myeloid lineage. Its hallmark is the presence of reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11), which brings the BCR (breakpoint cluster region) gene into proximity to the ABL1 (Abelson murine leukemia viral oncogene homolog 1) gene, forming a new fusion chimeric gene—BCR-ABL1—which is the oncogene responsible for the pathogenesis of the disease. The truncated chromosome so formed is termed the Philadelphia chromosome, owing its name to the city where it was first described.

Our understanding of the pathophysiology of this disease led to the development of targeted therapies in the form of tyrosine kinase inhibitors, which have a specific action to inhibit the BCR-ABL1 fusion oncogene. The agents were rightly dubbed “magic bullets” and revolutionized the practice of oncology. They have led to a paradigm shift in the management of CML, and now most patients with newly diagnosed disease can look forward to a multi-decade survival as opposed to the 4–5 years survival in old times.

In this chapter we have discussed the practical aspects of usage of these agents with special focus on optimum use in a setting of resource constraints.
Clinical Features, Diagnosis, and Risk Stratification

A significant proportion of patients are nowadays detected in asymptomatic stage with incidentally detected leukocytosis. Patients in more advanced stages of the disease present with signs and symptoms of gross splenomegaly (abdominal fullness and discomfort with feeling of early satiety), symptomatic anemia, and constitutional symptoms like fever, fatigue, or weight loss. Hemogram shows leukocytosis, which may be accompanied with thrombocytosis and/or anemia. The differential leukocyte count shows prominence of immature precursors of the granulocytic series, like myelocytes and metamyelocytes. Circulating blasts are also seen. Basophilia is a consistent feature. The absolute eosinophil and monocyte counts are usually increased, although the percentages are not elevated. Small numbers of nucleated red blood cells and mild reticulocytosis may be seen. Clinical chemistry may reveal hyperuricemia and hyperuricosuria, elevated serum lactate dehydrogenase (LDH), increased serum vitamin B12-binding capacity, and increased serum B12 levels. Low or absent neutrophil alkaline phosphatase activity is seen in 90% of patients.

Bone marrow (BM) examination usually shows marrow hypercellularity up to 75–90% with marked increase in myeloid precursors. Blasts usually represent less than 5% of cells in CP CML. Presence of more than 10% blasts indicate transformation to AP. Megakaryocytes may be hypolobated and typically dwarf forms are seen.

The definitive diagnosis of CML is established by demonstrating the presence of the characteristic translocation and its resultant transcript. This can be done by conventional cytogenetics, fluorescence in situ hybridization (FISH) or by polymerase chain reaction (PCR).

The disease has a triphasic clinical course through chronic phase (CP), accelerated phase (AP), and blast phase (BP). Without treatment, the disease inevitably progresses from CP to AP/BP, which have high morbidity and mortality. Risk stratification helps in predicting survival and it can be done using the Sokal score or ELTS score.

Treatment

The standard goals of therapy were relief of symptoms and prevention of progression of the disease. The classical understanding was that treatment has to be continued lifelong for continued suppression of the BCR-ABL1 transcript. But with tyrosine kinase inhibitors (TKIs), we have managed to achieve such deep suppression of the disease activity that it is possible in a certain cohort of patients to discontinue TKI after a few years and attain a state of treatment-free remission (TFR) and in fact, TFR has now become one of the standard goals of therapy.

Tyrosine Kinase Inhibitors

TKIs are orally administered agents, which act by binding to the ATP binding site in the BCR-ABL1 protein and inhibit its kinase activity. These agents are able to provide much deeper and sustained responses and can provide near normal life expectancy in a large proportion of patients. Imatinib was the first TKI approved for the use in CML after showing markedly better results than the previous standard therapy of interferon plus low dose cytarabine in the pivotal IRIS trial. Acquired mutations in the kinase domain can render imatinib ineffective in certain proportion of cases. Second generation TKIs, which include nilotinib, dasatinib, and bosutinib, can be effective in such a setting. These agents are also approved as first line agents where they have demonstrated superior activity than imatinib with respect to rapidity and depth of response, although survival outcomes are similar (ENESTnd, DASISION, and BFORE trials). The T315I is a unique mutation that renders the disease resistant to all four of these TKIs. The 3rd generation TKI ponatinib is effective in such cases. These TKIs are summarized in Table 1.

Monitoring Response to Therapy

Initiation of therapy leads to resolution of symptoms, splenomegaly, and normalization of blood counts. Complete blood count (CBC) needs to be monitored every 2 weeks till a state of complete hematological response is noted. Subsequent depth of response is monitored using techniques that detect the characteristic chromosomal translocation using karyotyping or FISH or by detecting the BCR-ABL1 transcript using PCR. Monitoring using PCR based assays is the current gold standard as it allows for detection of minute quantities of the transcript, and hence responses can be monitored to a degree of depth, which is not possible by any of the other techniques.

There can be dramatic variations in the absolute transcript value across laboratories and standardization...
### TABLE 1  Tyrosine kinase inhibitors for CML

<table>
<thead>
<tr>
<th>Generation</th>
<th>Imatinib</th>
<th>Nilotinib</th>
<th>Dasatinib</th>
<th>Bosutinib</th>
<th>Ponatinib*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>400 mg OD</td>
<td>300 mg BD/400 mg BD</td>
<td>100 mg OD/70 mg BD</td>
<td>400 mg OD/500 mg OD</td>
<td>45 mg OD (May initiate at lower dose)</td>
</tr>
<tr>
<td>2nd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse effects</td>
<td>Myelosuppression</td>
<td>Myelosuppression</td>
<td>Myelosuppression</td>
<td>Myelosuppression</td>
<td>Diarrhea</td>
</tr>
<tr>
<td></td>
<td>GI intolerance</td>
<td>Hyperglycemia</td>
<td>Pleural effusion</td>
<td>Myelosuppression</td>
<td>Myelosuppression</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>Hepatotoxicity</td>
<td>Pulmonary hypertension</td>
<td>Raised ALT</td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>Cardiotoxicity</td>
<td>Pancreatitis</td>
<td>Thrombotic events</td>
<td>Arrhythmias</td>
</tr>
<tr>
<td></td>
<td>Skin changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muscle cramps</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not available in India at the time of writing.

### TABLE 2  Response definitions

<table>
<thead>
<tr>
<th>Test</th>
<th>Frequency of monitoring</th>
<th>Definition of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical examination and complete blood counts</td>
<td>Every 15 days until CHR, then every 3 monthly unless otherwise required</td>
<td>Complete hematological response: • Platelets ≤450 × 10⁹/L • WBC count ≤10 × 10⁹/L • Differential without immature granulocytes and with ≤5% basophils and • Non-palpable spleen</td>
</tr>
<tr>
<td>BCR-ABL1 quantitative PCR (ratio of transcript/housekeeping gene expressed on IS)</td>
<td>Three monthly</td>
<td>• Major Molecular Response (MMR): ≤0.1% • MR²: ≤0.01% • MR²⁺: ≤0.0032% • MR³: ≤0.001% • Deep molecular response (DMR)—MR² or deeper • Molecurally undetectable leukemia—Undetectable BCR-ABL1 mRNA transcripts</td>
</tr>
<tr>
<td>Cytogenetics (chromosome banding analysis of bone marrow metaphase cells)</td>
<td>Alone not sufficient for monitoring To be done in patients with atypical translocations, atypical transcripts of BCR-ABL1 not quantifiable by PCR, at failure or progression to document ACA</td>
<td>• Complete (CCyR): No Ph+ chromosome • Partial (PCyR): 1–35% Ph+ metaphase • Minor (mCyR): 36–65% Ph+ metaphase</td>
</tr>
</tbody>
</table>

Techniques are employed in order to homogenize the results. The BCR-ABL1 transcript value is described as a ratio of the transcript value to that of a housekeeping gene like ABL1 or GUSB. This ratio is further converted to the International scale (IS). The baseline 100% was defined as the median transcript value measured in 30 pooled samples of patients of newly diagnosed CML CP enrolled in the IRIS trial. Reference materials were generated using this pool of samples and laboratories can use this to generate specific conversion factors to convert their results into IS values. The results are defined on a log scale; 1%, 0.1%, 0.01%, 0.0032%, and 0.0001% correspond to 2, 3, 4, 4.5, and 5 log reductions respectively from the baseline value of the IRIS trial. BCR-ABL1 quantitative PCR is recommended to be performed every 3 months. The standard response definitions are described in Table 2.

Effective therapy leads to progressive decline in the BCR-ABL1 IS and target values at different timepoints are defined in order to ascertain the efficacy of treatment. The
molecular timepoints as per the European LeukemiaNet 2020 guidelines are described in Table 3. In case of suboptimal response or failure of therapy there is a need to determine if any mutations are present in the kinase domain. Detection of these mutations serves as a guide for choosing the most optimum second line therapy. Mutation analysis was conventionally done using Sanger sequencing but Next-generation sequencing has proved to be more sensitive and has become the recommended technique.

Imatinib was the first TKI to be approved for use in CML based on the results of the IRIS trial. The starting dose is 400 mg once a day but higher doses of 600 mg and 800 mg have also been tried. The second generation TKIs were compared with imatinib as the frontline agent in newly diagnosed CML CP in various trials and were found to induce faster and deeper responses as compared to imatinib, but there was no significant superiority in progression-free and overall survivals in any of these studies. No head-to-head comparison is available for any of the 2G-TKIs. Hence, the choice of frontline TKI depends on a combination of various factors including risk stratification, cost, availability, adverse effect profile, comorbidities, and individual preferences of the physician and the patient. The choice of agent for second line use also depends on similar factors but also take into account mutation status.

Treatment of patients with failure of two previous TKIs is not so straightforward. Allogeneic stem cell transplant should be considered in this setting. TKIs (2G and 3G) can be used but responses are seldom durable. If effective, TKIs can be used as an effective bridge to transplant.

Advanced phase CML (AP and BP) can present either de novo or as progression from CP. TKIs are effective in TKI-naïve patients with de novo AP. Imatinib at higher dose of 600 mg or any of the 2G TKIs may be considered in this setting. Patients with AP who have additional cytogenetic abnormalities or who have progressed after TKI failure respond poorly with second line TKIs and allogeneic stem cell transplant should be considered. CML BP can be either myeloid-type or lymphoblastic-type and this should be determined by immunophenotyping. The outcomes of CML BP remain poor and the only modality that can provide long-term cure in setting of BP is allogeneic stem cell transplant. Patients are usually treated with TKI along with acute myeloid leukemia (AML)-like or acute lymphocytic leukemia (ALL)-like chemotherapy to bring the disease into chronic phase or hematological remission prior to proceeding with transplant.

When TKIs were first introduced two decades ago it was thought that they have converted CML into a chronic disease with near normal life expectancy and lifelong treatment. But long-term experience with TKIs showed that it is possible to go even a step further. These drugs are able to achieve sustained deep molecular responses when used over a period of few years and it is possible to discontinue the drug in selected patients, providing them with the equivalent of a “cure.” This concept of TFR was initially explored with the use of the older agent interferon. The STIM1 and TWISTER trials demonstrated that TFR can be achieved in patients on long-term imatinib. Subsequently a large number of trials have been done with both first line and second line TKIs demonstrating the utility and feasibility of TFR. Although all studies have used different criteria for patient selection, a few common criteria have emerged. Ideally the patient should be in first CP, on first line TKI for prolonged duration (5 years or more), having achieved optimal response at all milestones and having achieved a sustained deep molecular response (DMR) for 2 years or more. Patients on TFR should be motivated and have access to regular monitoring (monthly for initial 6 months, 2 monthly for next 6 months, and subsequently 3 monthly for life). The success rates of TFR in almost all trials have been in the range of 40–50%. Failure of TFR is considered when there is a loss of MMR. Most failures occur in the initial 6 months but thankfully nearly all patient regain MMR following reinitiation of TKI. The success of TFR has led to its incorporation as one of the standard goals of therapy.
Managing CML in Resource Poor Settings

Although major advances have been made in our understanding, treatment, and monitoring of CML, these advances have not universally permeated into clinical practice, and this is particularly true for low and middle income countries. Patients and physicians in these regions of the world face unique challenges at every step of disease management. Efforts are needed to ensure that our patients are able to obtain similar benefits of treatment as those in developed nations.\textsuperscript{17}

An algorithm for guidance in resource-limited setting is provided in Flowchart 1.

Specific Issues Related to CML Treatment in Resource-limited Settings

- \textit{Delays in diagnosis:} A majority of CML patients in India are diagnosed late and have a higher disease burden and higher risk score at the time of diagnosis.\textsuperscript{18,19} This is related to poor awareness of the disease and inadequate access to diagnostic modalities in peripheral centers. Presentation with a higher risk score leads to inferior survival outcomes.

- \textit{Age at diagnosis:} Patients of CML in Asian countries present a decade earlier than those in the west.\textsuperscript{20} This translates to a longer course of the disease and subsequently a longer need for TKIs and monitoring, which adds to the overall financial burden.

- \textit{Investigations:} Molecular and cytogenetic methods for diagnosis and monitoring are not readily available in smaller cities. Even with availability, the cost of these investigations ends up posing a hindrance to their usage. The standard recommendations on the monitoring frequency (once in 3 months) are seldom, if ever, followed in India. Clinicians have to devise their own modifications to these guidelines in order to ensure that the patient remains well monitored yet not be overburdened by the cost.

- \textit{Drugs:} The cost of imatinib at the time of launching was prohibitively expensive, but three major developments have ensured that today they are within the reach of nearly every patient who needs it. In this regard,

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**Flowchart 1:** Algorithm for CML-CP treatment in resource-limited settings

![Flowchart 1](image-url)

Allo SCT, allogenic stem cell transplantation; CP CML, chronic phase CML; TKI, tyrosine kinase inhibitor
massive strides have been made in the last two decades. Firstly, the Glivec International Patient Assistance Program (GIPAP), in association with the Max Foundation, which sought to make imatinib available free of cost to patients particularly in low and middle income countries. India has been the largest beneficiary of this program, and GIPAP has provided free of cost imatinib to more than 12,000 patients (25% of all GIPAP beneficiaries) from 2002 onward. The program stopped enrolling new patients in 2016. Secondly, a large number of Indian pharmaceutical companies started production of generic imatinib, which is available to patients at a much cheaper rate. Thirdly, several public funded institutions provide generic imatinib free of cost. These benefits are not restricted to imatinib alone. Nilotinib is available at a concessional cost as a part of a patient assistance program. Generic formulations of Dasatinib and Bosutinib were launched in early 2020. These have ensured that even the 2G TKIs are much more accessible to patients than what they were previously and have led to increased use of these agents both as first line and second line.

- **Adherence to treatment:** Poor adherence to treatment is a major barrier to obtaining favorable long-term outcomes. Various factors non-adherence relevant to the Indian context are financial constraints, lack of social support, and poor patient awareness about the disease and treatment. Treating clinicians must pay adequate attention to addressing these issues. Adequate counseling at diagnosis and reinforcement of the same at each hospital visit is essential. Various government and non-government aid regarding provision of TKIs go a long way in ensuring uninterrupted treatment.

**Conclusion**

CML is a chronic myeloproliferative neoplasm characterized by t(9;22)(q34;q11) and the resultant chimeric BCR-ABL1 oncogene. Effective targeted therapy is now available in the form of TKIs, which can induce deep and prolonged molecular responses in a large number of patients. TFR has shown moderate success and has become one of the major goals of treatment. Challenges remain in low and middle income countries related to accessibility and affordability of investigations and treatment. Various governmental and non-governmental schemes along with availability of generic medications have gone a long way in ensuring our goal of providing treatment for all.

**References**


CHAPTER 230
Disability-free Hemophilia in India—Myth or Reality

Sunita Aggarwal, Ranvijay Singh, Sandeep Garg

Abstract
Hemophilia is a rare X-linked genetic disorder where a patient bleeds because of lack of clotting factors essential for coagulation. Based on the type of clotting factor deficiency, hemophilia is classified into hemophilia A, which is deficiency of factor VIII and deficiency of a clotting factor IX is called hemophilia B. Currently, replacing the deficient clotting factors with plasma derived and recombinant clotting factor is the widely accepted treatment modality for hemophilia. In many countries, advanced treatment prophylaxis is the accepted modality of treatment, which allows persons living with hemophilia (PWH) to lead a disability-free life. According to recent survey, India has 10% of all the PWH in the world. Yet most of the patients have not been adequately treated because of various factors. A void in awareness, unavailability of clotting factors, high treatment costs, poor economic status, and lack of home therapy along with low frequency of physiotherapy exercises are causing big dent in the Indian PWH’s aspiration in achieving a disability-free life. In India, access to prophylaxis and optimal dosages of CFCs is still not up to the established standards of care. This review describes the current scenario of hemophilia care and the challenges faced by PWH in India.

Introduction
Hemophilia is a mostly inherited genetic bleeding disorder, first found in the literatures of 2nd century’s Jewish-Talmud (Jewish religious script). A more detailed explanation was given by the 11th century physician Albucasis. The word “hemophilia” is derived from the Greek word “haima” which means “blood” and “philia” which means “love or attraction.” In the late 18th and early 19th century, the understanding of hemophilia evolved and conceptualized. The European royal families suffered during the same period, and it led the world to recognize the disease and brought it into prominence. The two types of hemophilia, that is, Hemophilia A and Hemophilia B (Christmas disease) were recognized in the mid-20th century. Hemophilia is transmitted as X-linked recessive pattern where males are usually affected and females remain as asymptomatic carriers. The lack of heterogeneous transformations of the coagulating factor qualities because of the mutation F8C gene and F9 gene causes Hemophilia A and B, respectively. Hemophilia A constitutes to about 80–85% of the hemophilia patients (Table 1).

Clinical Features
Hemophilia is X-linked recessive disease where male is clinically affected and women are almost asymptomatic. Family history is absent in ~30% of the cases, attributing to de novo mutation. Severe the hemophilia earlier the presentation of first bleeding episode, which can present as early as at birth and majority of them are spontaneous bleeds. New born can present as cephalohematoma, central nervous system (CNS) bleed, or excessive bleeding during medical intervention like venipunctures and circumcision. As child starts walking, joint bleed, bruising...
and musculoskeletal bleed becomes more common. In older children and adult, presentation is mainly joint and muscle bleed. Hemarthrosis can affect every joint but commonly involves knee, elbow, ankle, shoulder, and hips. However CNS, oropharyngeal, and retroperitoneal bleed are life threatening and require immediate therapy.

**Complications**

**Hemophilic arthropathy:** Most common and develops in ~50% of patients with severe hemophilia. Mechanism of arthropathy is multifactorial including chronic or episodic synovitis, loss of cartilage, subchondral cyst formation, bone cysts, erosion and joint space narrowing leading to contractures, pain, and limitation of motion.

**Infection:** Found relatively more in plasma-derived products compare to recombinant factor products and elimination of Parvovirus B19, hepatitis A and prion diseases like Creutzfeldt-Jakob disease is still a challenge.

**Pseudotumor:** Unique to hemophilia, pseudotumor is a potentially disabling condition that results from inadequately treated soft tissue bleeds usually in muscle adjacent to bone, which can be secondarily involved.

**Inhibitors:** The cumulative incidence (i.e., lifetime risk) of inhibitor development in severe hemophilia A is in the range of 20–30%, approximately 5–10% in moderate or mild disease and hemophilia B has 1–5% lifetime risk of inhibitor development.²⁻⁴

Inhibitors are neutralizing alloantibodies developed following the host immune response to the infusion of clotting factor concentrate, seen by host immune system as foreign protein. Inhibitors development is highest during the first 20 exposure days (ED) to factors. Frequent screening is required during initial exposure days, screening every 5ED until 20ED are reached and after 150ED frequency of screening can be reduced. Inhibitors are detected either during routine screening or it is suspected when patient fails to respond to replacement of CFC. High titer inhibitor (HTI) and low titer inhibitor (LIT) are inhibitor classes quantified by Bethesda units (BU).

High titer inhibitor (≥5BU) and low titer inhibitor (<5BU) behave differently and consequently are managed differently. LIT may be transient and disappear spontaneously without specific treatment but significant proportion of it gets converted into HTI; hence, demands close monitoring. Treatment of bleed in LTI include factor infusion at higher dose, porcine rFVIII, which is not quickly inactivated unlike human FVIII and Desmopressin (DDAVP) in mild hemophilia A, which releases endogenous FVIII.⁵

HTI are persistent and completely resistant to factor concentrates; hence, their management requires avoidance of further FVIII exposure until immune tolerance induction (ITI) is commenced where infusions of variable doses of FVIII and FIX given over a period of time to tolerize the immune response. Use of bypassing agents (rFVIIa and aPCC) and monoclonal antibody emicizumab can be opted.¹,⁵ Emicizumab (Hemlibra) is registered in over 50 countries around the world for prophylactic use for hemorrhagic episodes in adult and pediatric hemophilia A patients with inhibitors.⁶ In addition, this drug has recently been approved by FDA for prophylaxis in patients with hemophilia A without FVIII inhibitors.⁶

**Treatment Modalities**

Hemophilia is treated by replacing the deficient clotting factors. Based on source, there are plasma derived clotting factors concentrates (PDCFCs) and recombinant clotting factors (rFVIII/rIX). Recombinant clotting factors are inherently safer as the risk of transfusion transmitted diseases is much less compared to plasma derived CFCs. There are two modalities in treatment based on timing of the therapy, i.e., episodic/on demand therapy and prophylaxis therapy.⁷
On Demand Therapy

Assuming the baseline factor of persons living with hemophilia (PWH) as 0%, the therapy should raise the clotting factor to a certain minimum level in order to stop the bleeding. Although on demand, treatment can stop bleeding, reduce the pain to an extent and restore joint movement. It will not protect the PWH from arthropathy. The target dose of CFC is calculated with the help of bleeding site specific desired levels and approximate raise of plasma factor levels (Tables 2 and 3).

Prophylaxis Therapy

Here the treatment is given by intravenous injection of factor concentrate to prevent anticipated bleeding. It prevents life threatening bleeding, joint destruction and preserves normal musculoskeletal function. Prophylaxis has been a standard of care in developed countries. Two well-studied and documented clinical protocols for prophylaxis are being followed across the world:

- **Malmo protocol**: (high dose: 25–40 IU/kg/dose)
- **Utrecht protocol**: (intermediate dose: 15–30 IU/kg/dose)

For hemophilia A, the factors are administered thrice a week and for hemophilia B, the factors are administered twice weekly. In countries with resource constraints lower dose of prophylaxis may be given as an interim measure.

On Demand versus Prophylaxis Therapy

Manco-Johnson et al. conducted a randomized controlled study and found prophylaxis is more effective in preventing hemarthrosis and is also efficacious in decreasing bleeding and joint damage compared to on demand therapy. Another randomized controlled study, ESPRIT study, concluded by saying prophylaxis was more effective when started at age 36 months or less with PWH having fewer joint bleeds and no radiologic signs of arthropathy versus patients treated on demand. The MUSFIH study concluded, on demand therapy failed to preserve musculoskeletal functions in PWH. All these studies are stressing the importance of prophylactic replacement of clotting factors over the on-demand therapy. Even in India, several pilot studies conducted across India have shown the evidence of the advantage of prophylaxis over on-demand.

Hemophilia Scenario in India

India has currently 20,321 registered PWH. 17,606 with hemophilia A and 2,715 with hemophilia B. Majority of Indian PWH fall into 19–44 years of age. However, as per the incidence and prevalence estimates, India should have had approximately 100,000 PWH. Many of the patients go undiagnosed or unregistered because of lack of awareness and lack of diagnostic facilities.

Among the registered patients, a significant percentage of them suffer from joint disease due to recurrent joint bleed. In a study of 148 severe hemophilia A patients from 5 centers across India, Kar et al. found that about 94% had some form of disability. Patients from the age group 25+ were the most affected with 0% of them being free from disability. This pervasive disability has turned hemophilia from a mere bleeding disorder to a chronic musculoskeletal disorder. Many of these patients in India do not complete education, are not gainfully employed and become a burden to their families and the society.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Type</th>
<th>1 unit/kg factor VIII/IX</th>
<th>Dose required (in units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A</td>
<td>Raise the plasma FVIII level approximately 2%</td>
<td>[Body weight x Desired level]/2</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Raise the plasma FIX level approximately 1%</td>
<td>[Body weight x Desired level]</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Site of bleed</th>
<th>Desired level (IU/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle/joint</td>
<td>40</td>
</tr>
<tr>
<td>Iliopsoas bleed</td>
<td>60</td>
</tr>
<tr>
<td>Throat</td>
<td>50–80</td>
</tr>
<tr>
<td>Neck</td>
<td></td>
</tr>
<tr>
<td>GI bleed</td>
<td></td>
</tr>
<tr>
<td>Genitourinary bleed</td>
<td></td>
</tr>
<tr>
<td>Surgery (Postoperative)</td>
<td></td>
</tr>
<tr>
<td>Surgery (Preoperative)</td>
<td>100</td>
</tr>
<tr>
<td>Intracranial bleed</td>
<td></td>
</tr>
</tbody>
</table>
contrast, in most developed countries, PWH enjoy good joint health, are not burdened with disability and lead a normal and productive life.

This stark contrast among the quality of life led by PWH in India and other countries may be ascribed to several factors:

- **Lack of optimum management of bleeds**: Bleeds are not treated with optimum dosages in many cases. In India, mean per capita factor VIII and IX usage is 0.230 and 0.063 IU/population while global mean is 2.40 and 0.37 IU/population. Also the time from onset of bleed to factor administration is rarely less than 2 hours. This leads to increased blood in the joint space, increased inflammation and increased time to recovery and in the long-term results in joint arthropathy.

- **Lack of prophylaxis**: Only about 13% of Indian PWH have access to prophylaxis. Some of the reasons could be lack of awareness, difficulty in venous access in young children, training the parents of PWH and in some cases inadequate supply of clotting factors. Moreover, psychosocial barriers like handling the myths, misconception, apprehensions about prophylaxis and willingness to endure repeated venipunctures play a vital role.

- **Lack of home therapy**: Self-administration at home setting demands skills and expertise with proper training of the family members. Lack of motivation among patients and administrative hurdles reduce the efficiency of home therapy.

- **Lack of diagnostic facilities**: Only a few laboratories across the country can perform factor and inhibitor assays. This may be one of the reasons why only small portion of the PWH are identified.

However, there has been a significant improvement in the hemophilia care in India in the recent years. Out of all the genetic disorders and hemoglobinopathies hemophilia have attracted a fair share of support and funding from the government. Over the last few years treatment of hemophilia in India has evolved from whole blood transfusion, fresh frozen plasma and cryoprecipitates to plasma derived and recombinant clotting factor concentrates. It is also encouraging to note that hemophilia is now listed in “The Rights of Persons with Disabilities Act, 2016”. The government is allocating funds, purchasing good quality clotting factors and bypassing agents. Many hemophilia treatment centers (HTCs) are opening across the country to help PWHs. Most HTC have well qualified treating physicians, nurses, lab technicians and physiotherapists with adequate experience of handling PWH. HTCs are mainly supported by respective State Governments and in some cases by Central Government as well. Some hospitals also serve as tertiary care centers for hemophilia care where advanced management for inhibitors and even surgeries are provided. However, the number of such surgeries is abysmally low. Some of the tertiary centers have started prophylaxis and home therapy as pilot projects, albeit at smaller scale.

The Hemophilia Federation of India (HFI) is a patient support group, working to help PWH in India in various ways. It also performs a vital task of maintaining a national hemophilia registry for all PWH.

**Conclusion**

Persons living with hemophilia (PWH) in India still face many issues in terms of comprehensive care, inhibitor management, optimum dosage in acute bleeds and optimum prophylaxis regimen. Paving our way toward WFH’s vision of “Treatment for All”. Awareness, access to safe factors, prophylaxis and home therapy should be the focus areas for all the concerned stakeholders to work in a coordinated fashion and make the treatment accessible to all the PWH. With such an approach of comprehensive hemophilia care and tireless efforts of medical experts, government, health administrators, and even patients, we can aspire for a disability-free hemophilia in India.

**References**


Abstract
Cancer immunotherapy is rapidly evolving and is gaining a major role in current oncological practice. Advances in understanding of immune surveillance and tumor biology have opened up new therapeutic strategies that can be used for the treatment of many cancers. Immune evasion is a hallmark of property of cancer and cancer immunotherapy uses strategies to augment body’s immunity against cancer. A subset of cancer patients dramatically benefit from this approach and identifying a right patients through a biomarker is need of the hour. In addition, knowledge on tumor response to immunotherapy and toxicity profile of these agents are important as they differ from traditional cytotoxic chemotherapies by virtue of its unique mechanism of action.

Introduction
Advances in understanding of the interlink between immune surveillance and tumor biology have opened up new therapeutic strategies that can be used for treatment of many cancers. Nobel prize for physiology/medicine in 2018 was awarded to James P. Allison for the discovery of cytotoxic T-lymphocyte associated protein (CTLA-4) and to Tasuku Honjo for programmed cell death protein 1/programmed cell death protein ligand 1 (PD-1/PD-L1), a landmark discoveries in the field of immunotherapy. Cancer immunotherapy is based on the principle of strengthening the host immune system to combat against the cancer.

Evasion of Immune Surveillance
Evasion of the immune surveillance is one of the hallmark of cancer. Adaptive immunity directed at the cancer antigens is one of the defense mechanism which helps to fight against the cancer. There are several mechanisms by which the cancer cells escape this natural defense known as “immune evasion.” Immune evasion helps in tumor growth despite having a normally functioning host immune system. Various mechanisms for immune escape include:
- Up regulation of immune checkpoints such as PD-1 and PD-ligand 1 (PD-L1)
- Alteration of antigen presentation mechanism by loss of MHC class 1 expression or cellular mechanisms that help in transportation of tumor antigens for T cell recognition
- Promotion of immune tolerance by alteration of cytokines like IL-6, IL-10, and TGF-beta

Proper understandings of these mechanisms have been exploited as the basis of immunotherapy in current clinical practice.

Approaches to Cancer Immunotherapy
A number of therapeutic approaches are in practice or under investigation to strengthen the body’s immune system fight against cancer. Various immunotherapy approaches in current practice are enumerated in the Box 1.
SECTION 19

Hematology and Oncology

Checkpoint Inhibitors

Programmed Cell Death 1 (PD-1) and PD-Ligand 1/2

Programmed cell death-1 (PD-1), a transmembrane protein expressed on immune cells like T cells, B cells, and NK cells. Ligand of PD-1 (PD-L1) is usually expressed on many tissues including tumor cells. Interaction of the PD-1 with its ligand acts as check point and resists the cell death by inhibition of apoptosis. Up regulation of PD-L1 expression is seen in many tumor cells and drugs targeting PD-1/PD-L1 pathway known as check point inhibitors are an important class of immunotherapy in current oncological practice. The interaction of the PD-1 with its ligand is depicted in the schematic Figure 1.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is another immune check point, which helps in down regulation of the immune responses against tumor cells. Monoclonal antibodies targeting CTLA-4 can also clear these “breaks” in immune surveillance. Ipilimumab, an anti-CTLA-4 antibody was the first immune checkpoint inhibitor to be approved in patients with malignant melanoma.

The various check point inhibitors currently in use are listed in Table 1 with the approved indications and doses.

Immunotherapy Response Criteria

The pattern of response to immunotherapies mainly checks point inhibitors that can differ from that of classical cytotoxic chemotherapeutic agents by the virtue of difference in mechanism of action of these agents. Unlike chemotherapy the responses may take longer time to become apparent. The immune infiltration of the tumors can lead to an apparent increase in the size of the lesion initially known as immune confirmed progressive disease (IUPD) commonly known as pseudoprogression. In such situations the clinical status of the patient should be taken into consideration in deciding the further course of action.

To address these issues many response criteria have been developed like immune-related response criteria (irRC), immune response evaluation criteria in solid tumors (iRECIST), immune-modified response evaluation criteria in solid tumors (imRECIST). Detailed description of these criteria is beyond the scope of this chapter.

Toxicities Associated with Checkpoint Inhibitor Immunotherapy Immune-related Adverse Events (irAEs)

The toxicities depend on the class of immunotherapeutic agents. Check point inhibitors are the most commonly used class of immunotherapy in clinical practice. Though these drugs are well tolerated in most of the cases they are known for unique side effects related to their mechanism of action. These are known as immune-related adverse events (irAEs). Virtually these can affect any organs. However, the important organs affected and the manifestations are depicted in the Figure 2. The usual timeline of appearance of these side effects are shown in the Figure 3.

The management of irAEs depends on the severity of manifestations. It varies from close monitoring,
interruptions in treatment, dose reductions, and other pharmacological management. The pharmacological agents like steroids, infliximab, may be used in the management of irAEs.\textsuperscript{13}

Other Forms of Immunotherapy

Manipulating T Cells

Immune enhancement by manipulating the T cells is another emerging mode of immunotherapy.

Chimeric Antigen Receptors (CAR) T Cells

CAR-T cell therapy involves the modification of the patient’s own T cells to recognize the cancer cells more effectively and to destroy them. Chimeric antigen receptors (CARs) are the engineered proteins that give T cells this new acquired ability. These proteins are chimeric as they are engineered by combining antigen-binding and T cell activating functions into a single receptor.\textsuperscript{14} So CAR-T cell therapy utilizes these T cells engineered with CARs as a therapeutic strategy.

CAR-T cells are studied in various solid and hematological malignancies and have shown a great result in B-cell acute lymphoblastic leukemia (B-ALL). Tisagenlecleucel and axicabtagene-ciloleucel were the first two CAR-T cell therapy receiving FDA and EMA approval for ALL (tisagenlecleucel) and diffuse-large B-cell lymphoma – DLBCL (tisagenlecleucel and axicabtagene ciloleucel).\textsuperscript{15}

CD3-Directed Therapies—Bispecific T-cell Engagers

Bispecific T-cell engager antibodies (BiTEs) act as linker between T cells and specific target antigens. These consist of a protein, which contains two separate variable regions,
one recognizing CD3 on T cells and the other recognizing target antigen. Thus, activating cytotoxic T cell-mediated tumor damage. BiTEs functioning in an MHC independent manner and don’t require patient specific processing enabling it to administer to all the patients irrespective of human leukocyte antigen (HLA) type.16

**Blinatumomab** is a BiTE having specificity for CD19 found on B cells and the Fc region of the CD3 receptor on T lymphocytes and is approved for Philadelphia-chromosome negative B-ALL.17

**Oncolytic Viruses**

Several viruses are genetically engineered to infect the cancer cells preferentially and to present the tumor associated antigens to immune system. Many virus backbone like attenuated herpes simplex virus 1, adenovirus, reovirus have been studied in clinical trials. talimogene laherparepvec (T-VEC) uses an attenuated HSV-1 to over express granulocyte macrophage colony-stimulating factor (GM-CSF) and mediates the antigen presentation through dendritic cells. Intratumoral injections of T-VEC have shown durable responses in Melanomas.18

**Therapeutic Cancer Vaccine**

Therapeutic cancer vaccines are a form of immunotherapy, which educates the immune system about how cancer cells look like so that it can fight against it. Vaccine antigen is the most important component of a cancer vaccine,
Interferon (IFN) alfa-2b has also been used in Interleukin-2 (IL-2): The initial approaches of immunotherapy were mainly based on alteration of cytokines that influence the immune system. Cytokines

- Interleukin-2 (IL-2):
  - IL-2 plays an important role in activation of immune system and is an approved treatment for renal cell carcinoma and melanoma.\(^{20}\)
  - Immunomodulators like lenalidomide and pomalidomide are an established therapy in multiple myeloma act by destruction of Ikaros family proteins, which results in inhibition of IL-2 secretion.\(^{21}\)
- Interferon (IFN) alfa-2b has also been used in malignant melanoma.

Conclusion

The immune-oncology has transformed the care of cancer patients. These act in a targeted manner minimizing the side effects. Checkpoint inhibitors are the mainstay of current immunotherapy practice in oncology. CAR-T cell therapies and personalized cancer vaccine have limited utility at present, which will be added to the immune-oncology armamentarium for wider use in near future.

References

Abstract
Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder characterized by partial or complete deficiency of glycoprophosphatidylinositol anchored proteins (GPI-AP). PNH can present with varied clinical manifestations. PNH can masquerade as an intravascular hemolysis or thrombosis. It can be associated with acquired bone marrow failure. Early diagnosis of PNH is crucial for appropriate clinical management. However, the rarity and diverse clinical manifestations complicate an early diagnosis. Though various tests are available, flow cytometric PNH analysis is considered the gold standard because of its rapidity and high sensitivity. A high index of suspicion and choice of appropriate high sensitivity assay helps in timely diagnosis and prevention of fatal complication in patients.

Introduction
Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder of hematopoietic stem cells caused by a somatic mutation in X-linked PIGA gene resulting in partial or absolute deficiency of all glycoporphosphatidylinositol (GPI) linked proteins.1,2 The GPI anchored proteins (GPI-AP) have different functions as enzymes, receptors, adhesion molecules, or complement regulators.3 Examples of GPI-AP, which inhibit the complement are CD55 (decay accelerating factor-DAF) and CD59 (membrane inhibitor of reactive lysis-MIRL). PNH can cause myriad manifestations ranging from the expected intravascular hemolysis to presentation with thromboses or bicytopenia/ pancytopenia due to bone marrow failure. In addition, some patients of PNH present with abdominal pains and leg cramps with compensated hemolysis.

Clinical Manifestations and Pathogenesis2,5
Complement-mediated Intravascular Hemolysis
PNH red cells are vulnerable to complement mediated lysis due to reduction or absence of CD59 and CD55. CD55 (DAF) accelerates destruction of membrane bound C3 convertase where as CD59 (MIRL) inhibits membrane attack complex (MAC) induced lysis. In PNH there is low-level continuous activation alternate complement pathway, which in association with GPI-AP causes chronic intravascular hemolysis. Classically patients have paroxysms of hemolysis at night, a morning dark colored urine and gradual resolution over the day. It is primarily the CD59 molecule that is responsible for inhibiting complement mediated lysis.

Thromboses
• NO (nitric oxide scavenging)—Free hemoglobin released into plasma following intravascular hemolysis scavenges NO. This leads to endothelial dysfunction and platelet activation. NO depletion can manifest in abdominal pain, dysphagia, erectile dysfunction, and thrombosis.
• Platelet activation by procoagulant microparticles released from GPI-AP deficient cells.
• Deficiency of GPI-AP like u-PAR (urokinase like plasminogen activator receptor) and TFPI (tissue factor
pathway inhibitor) that lead to reduced inactivation of the thrombotic pathway.

Bone Marrow Failure

Approximately 30–45% of PNH patients have associated aplastic anemia or myelodysplastic syndrome (MDS). A second hit following PIGA mutation causing immune destruction of normal stem cells and clonal selection of PNH stem cell may explain coexistence of PNH clones in acquired aplastic anemia.

PNH has been subclassified by International PNH interest group into three categories:
- The classical PNH—clinical and laboratory signs of intravascular hemolysis without evidence of other diseases of bone marrow failure
- PNH associated with AA/MDS
- Subclinical PNH with a minor PNH clone usually less than 1%

We will discuss three case scenarios (Table 1) to elucidate the manifestations of the disease and identify patients who should be tested for PNH.

### TABLE 1: Details of the three clinical cases

<table>
<thead>
<tr>
<th>Case details</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/Sex</td>
<td>21/Male</td>
<td>28/Female</td>
<td>31/Male</td>
</tr>
<tr>
<td>Primary complaints</td>
<td>symptomatic anemia, intermittent jaundice, leg cramps</td>
<td>Jaundice, Abdominal pain</td>
<td>Petechiae, Anemia requiring transfusions</td>
</tr>
<tr>
<td>Duration of illness</td>
<td>2 years</td>
<td>1 month</td>
<td>2 months</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.1</td>
<td>10.2</td>
<td>5.1</td>
</tr>
<tr>
<td>TLC (×10^9/L)</td>
<td>5.08</td>
<td>8.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Platelets (×10^9/L)</td>
<td>150</td>
<td>99</td>
<td>18</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>115.3</td>
<td>96.5</td>
<td>91.5</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>13.4</td>
<td>3.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Additional information</td>
<td>-</td>
<td>-</td>
<td>ANC-120/μL</td>
</tr>
<tr>
<td>Bilirubin total/Indirect (mg/dL)</td>
<td>5.2/4.8</td>
<td>2.2/1.9</td>
<td>0.8/0.2</td>
</tr>
<tr>
<td>LDH (U/L; Normal reference 220–420)</td>
<td>890</td>
<td>550</td>
<td>230</td>
</tr>
<tr>
<td>Peripheral blood smear</td>
<td>Macrocyes, polychromasia, hypersegmented neutrophils</td>
<td>Normocytic normochromic; mild polychromasia; Normal platelets</td>
<td>Pan cytopenia with normocytic normochromic red cells</td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
<td>Megaloblastic erythroid hyperplasia; giant myeloid forms</td>
<td>Normoblastic erythroid hyperplasia with adequate megakaryocytes</td>
<td>Paucicellular with predominantly lymphocytes and plasma cells</td>
</tr>
<tr>
<td>Perl's stain</td>
<td>Absent iron stores</td>
<td>1+</td>
<td>4+</td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
<td>Hypercellular; megaloblastic erythroid hyperplasia</td>
<td>Normocellular with normoblastic erythroid hyperplasia</td>
<td>Cellularity 10% consistent with hypoplastic anemia</td>
</tr>
<tr>
<td>Other lab tests</td>
<td>Negative G6PD, incubated osmotic fragility test, Direct Coombs test</td>
<td>AST&gt;ALT, Negative Direct Coombs test; Thrombophilia workup normal including Antiphospholipid antibody syndrome and JAK2V617F mutation</td>
<td>Chromosomal fragility test negative</td>
</tr>
<tr>
<td>Radiology</td>
<td>-</td>
<td>Hepatic vein thrombosis, mild hepatomegaly and mild ascites</td>
<td>-</td>
</tr>
<tr>
<td>Summary</td>
<td>Unexplained hemolytic anemia with secondary folate deficiency</td>
<td>Hepatic vein thrombosis with evidence of hemolysis</td>
<td>Aplastic anemia</td>
</tr>
</tbody>
</table>
When to Suspect PNH?\textsuperscript{5-10}

The rarity and diversity of clinical manifestations complicates the early diagnosis of PNH. Patients may present with anemia, fatigue, dyspnea, chronic kidney disease, abdominal pain, pulmonary hypertension, erectile dysfunction, dysphagia, thrombosis or hemoglobinuria. Many of these signs and symptoms are so common that every patient with anemia or thrombosis cannot be screened for PNH. Thus, PNH testing should be advised when some clinical or laboratory findings raise a suspicion.

A PNH test should be ordered in any patient who presents with:

- Intravascular hemolysis (~25% patients present with hemoglobinuria) as evidenced by hemoglobinuria, elevated LDH, reticulocytosis, and elevated plasma hemoglobin.
- Significant hemoglobinuria is usually present in classical PNH, whereas in cases associated with AA or MDS it may be absent due to a small PNH clone.
- Evidence of unexplained hemolysis with accompanying iron-deficiency, or abdominal pain or esophageal spasm, or thrombosis, or neutropenia and/or thrombocytopenia.
- Other acquired Coombs’ negative, non-schistocytic, non-infectious hemolytic anemia.
- Thrombosis with unusual features:
  - Unusual sites—Hepatic veins (Budd-Chiari syndrome)/Other intra-abdominal veins (portal, splenic, splanchnic)/Cerebral sinuses/Dermal veins
  - With signs of accompanying hemolytic anemia
  - With unexplained cytopenia
  - Young patients
- PNH is the second most common cause of intra-abdominal vein thrombosis after myeloproliferative neoplasms. Thrombosis is the leading cause of mortality in PNH. About 29–44% of PNH cases experience at least one thromboembolic event during course of the disease. An early diagnosis and management of PNH associated thrombosis is paramount.
- Evidence of bone marrow failure:
  - Suspected or proven aplastic or hypoplastic anemia: Up to 70% patients with acquired AA have a PNH clone and 40% PNH evolves from AA.

New Delhi of 1501 AA patients, detected PNH clone in 39.7% cases at diagnosis.\textsuperscript{9}

Various studies have mentioned an excellent response of AA to immunosuppressive therapies in presence of minor PNH clones. Emergence of PNH clones and expansion of pre-existing PNH clones post ATG is known. However, clinically relevant PNH clones are seen in less than 5% cases of AA post ATG causing significant hemolysis.

- Refractory cytopenia with unilineage dysplasia, hypoplastic MDS: Up to 50% patients with MDS test positive for a PNH clone. Testing for PNH in MDS is recommended in cases with refractory anemia subtype, MDS with evidence of hemolysis, or evidence of bone marrow failure such as hypoplastic MDS.
- Other cytopenias of unknown etiology after adequate workup.

Diagnosis

Diagnosis of PNH is done by detection of the PNH clones. Different methods can be adapted.

Complement-based Test—Modified Ham’s Test, Sucrose Lysis Test\textsuperscript{5,11,12}

These tests are based on the sensitivity of PNH cells to activated complement proteins. Though inexpensive, these tests are laborious, technically challenging, and not accurate quantitatively. As these tests are RBC based, spurious results may be obtained during hemolytic episodes and post-blood transfusion. Also, autoimmune hemolytic anemia and congenital dyserythropoietic anemia may give false positive reports. Hence, these tests are no longer recommended as tests of choice for the diagnosis of PNH.

Gel Card-based Tests\textsuperscript{5,11,13}

Gel card-based test is based on antigen-antibody reaction. If GPI linked proteins, like CD55 and CD59, are not present on RBCs there will be no reaction with anti-CD55 and anti-CD59. Minor clones and subclinical clones cannot be identified using this technique. This test is non-quantitative. Just like complement-based tests, this test also has limited ability to detect PNH clone during hemolytic episodes and in post-transfusion samples.
**Flow Cytometric (FCM) Detection of PNH Clones**

This is a rapid, highly sensitive assay. FCM for PNH is the gold standard test for detection of PNH clones. FCM can pick up subclinical clones and can delineate type I, type II, type III RBCs with normal expression, partial deficiency, and complete deficiency of GPI linked protein, respectively. Patients with more than 20% type III red cells are likely to manifest as hemolysis whereas patients with majority type II clone without significant type III cells usually do not show hemolysis. Unlike previous tests, PNH can detect PNH clones in RBCs and neutrophils. As in multiparametric FCM one can acquire more events/cells and analyze multiple antigen characteristics of a cell, it can help detecting clones as low as 0.01%. More importantly monitoring of patients can be done by highly sensitive technique like FCM.

However, proper knowledge of monoclonal antibodies to be used, their expression, sample requirement, gating strategy, and sensitivity of the assay is required.

**Sample Requirement**

Most preferred specimen is peripheral blood in EDTA. Bone marrow sample is not recommended as immature myeloid cells may have changes in GPI-linked protein expression and in MDS patients there may be altered expression of some GPI-linked proteins in neutrophils and monocytes.

A minimum of 1 mL sample is required. But in cases with pancytopenia sample as much as 3 mL is required for higher cell acquisition to detect small subclinical PNH clones.

Though sample can be stored for 7 days at 4°C for RBC analysis, it is preferable to process sample within 48 hours as alteration in scatter and antigen expression in neutrophils over time is known.

Any commercial lysing agent with fixative can be used for WBC analysis (ammonium chloride with fixative may be used).

**RBC or WBC**

Goal of RBC analysis is to diagnose and quantify cells lacking GPI-linked protein (type I/II/III cells). However, testing only RBCs may not be adequate in cases having active hemolysis or in case of transfusion where it underestimates clone size. So, determining WBC clones is more important for appropriate quantification of PNH clones.

**Choice of Antibodies**

GPI-linked protein—simultaneous two GPI-linked proteins for two lineages are recommended.

- **CD55 and CD59 analyses in neutrophils** are not recommended as they give a higher false positivity rate for PNH.

- **FLAER directly binds to GPI anchors and detects wide range of GPI-linked structures in different WBCs.**

- **CD157 is brightly expressed on both neutrophils and monocytes and can replace two different antibodies for neutrophils and monocytes and is cost effective.**

- **Testing for lymphocytes is not recommended because of their longer life span, which may give erroneous results in new onset PNH.**

**TABLE 2** Antibodies and panels used for the diagnosis of PNH by flow cytometry

<table>
<thead>
<tr>
<th>Lineage of cells</th>
<th>Gating marker</th>
<th>Lineage marker</th>
<th>GPI-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>CD235a</td>
<td>CD55, CD59</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>Neutrophils</td>
<td>CD45</td>
<td>CD15</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>CD45</td>
<td>CD64</td>
</tr>
<tr>
<td>PANELS</td>
<td>RBC</td>
<td>3-color CD235a/CD55/CD59</td>
<td>CD14, CD157, FLAER</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>6-color CD45/CD15/CD64/CD24/CD14/FLAER</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-color CD45/CD15/CD64/CD157/FLAER</td>
<td></td>
</tr>
</tbody>
</table>
**Quantification**\(^{14,16}\)—A laboratory specific lower limit of detection and lower limit of quantification should be established. In a high-sensitive FCM-PNH assay, minimum 50 PNH cells should be obtained to appropriately quantify. A minimum of 1,00,000 RBCs, 50,000 CD15 positive cells, and 10,000 CD64 positive cells are recommended to be acquired for sensitivity of 0.05%, 0.1%, and 0.5%, respectively in high sensitive assays. High sensitivity assays are not required for classical PNH but small clones in AA/MDS.

**Interpretation**\(^{11}\)—
- >1% clone—PNH clone
- 0.1–1%—Minor PNH clone
- <0.1%—Rare cells with PNH phenotype

**Monitoring/Retesting**\(^{7,18}\)—Monitoring of PNH clones may be done annually or more frequently if there is worsening of symptoms. Frequent monitoring may also be needed in eculizumab therapy. Patients with aplastic anemia need serial monitoring as minor clone can progress to a hemolytic PNH. Patients with no detectable clone should be screened every 6 months, decreasing to yearly if no clone appears in the first 2 years. If a clone is present or appears, patients should be screened every 3 months until the clone size is shown to be stable for 2 years.

**Outcome of the three Cases**

See Table 3.
Fig. 2: Case 2. Both neutrophils and monocytes exhibit predominant type II PNH clones with reduced expression of GPI anchor and related proteins. Type II clones are sometimes clearly apparent on the CD57/FLAER plots in neutrophils and monocytes.
**Fig. 3: Case 3 at the time of diagnosis and follow up.** Neutrophils gated on the CD15-SSC plot after refinement of initial gate on CD45-SSC plot show a 0.6% PNH clone while monocytes gated on CD64 vs. SSC plot show a 0.4% PNH clone at diagnosis using a high-sensitivity assay. Red cells do not show any PNH clone on a routine sensitivity assay. After 5 years, PNH immunophenotyping was repeated when cytopenias recurred. PNH clone in neutrophils and monocytes had increased to 28% and 25.8%, respectively while a small clone of 2.9% was seen in red cells, mostly type II. The reason for lower red cell clone was attributed to ongoing packed cell transfusions and mild hemolysis.
Conclusion

PNH is a rare and life-threatening disorder, has varied presentation like hemolysis, thrombosis at abnormal locations or bone marrow failure. Knowledge of different presenting symptoms and high index of suspicion is needed for an early diagnosis. Reliable testing and reporting procedures matter. Laboratory testing for high sensitivity PNH analysis by FCM in WBCs and RBCs should be preferred.

References

12. Lima M. Laboratory studies for paroxysmal nocturnal hemoglobinuria, with emphasis on flow cytometry. Pract Lab Med. 2020;20:e00158.

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**TABLE 3**
PNH clones and clinical course in the three cases

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary</td>
<td>Unexplained hemolytic anemia with secondary folate deficiency</td>
<td>Hepatic vein thrombosis with evidence of hemolysis</td>
</tr>
<tr>
<td>PNH flow cytometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophil clone (Fig. 1)</td>
<td>77.3%</td>
<td>36.7%, type II: 27.2%</td>
</tr>
<tr>
<td>Monocyte clone</td>
<td>65.5%</td>
<td>47%, type II: 37.1%</td>
</tr>
<tr>
<td>Red cell clone</td>
<td>29%, Type III: 28%</td>
<td>Not done</td>
</tr>
<tr>
<td>Clinical course</td>
<td>Due to unavailability of eculizumab in India and lack of response to steroids, the patient underwent matched sibling donor transplant; remains asymptomatic 2 years post-transplant</td>
<td>Patient was treated with full anticoagulation but succumbed to death due to a second thrombotic episode</td>
</tr>
</tbody>
</table>

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Abstract

Myeloproliferative disorders include polycythemia vera, primary myelofibrosis, and essential thrombocytosis. These disorders have an increased risk of thromboembolic complications and may progress post fibrotic state or transform into acute myeloid leukemia. Understanding of the molecular pathogenesis has resulted in newer targeted therapies and better risk stratification of patients.

Introduction

We are entering an era of molecular medicine. All of us were earlier taught about the variability of disease presentation, severity, and varied responses to treatment. We are now aware that variability even in infectious diseases and much more so in non-infectious diseases has a genetic basis. We know that different mutations, polymorphisms of genes, and even the allele burden are implicated in diseases. Knowledge about JAK2 mutation associated diseases is increasing. The classic JAK2 diseases are myeloproliferative conditions—polycythemia vera (PV), essential thrombocytosis (thrombocythemia) (ET) and primary myelofibrosis (PMF). But the JAK2 mutation has been found in many other conditions and to make things more complicated the classic conditions of PV, ET, and PMF may also occur without JAK2.

JAK2 Mutation

When we speak of JAK2 mutation we commonly refer to JAK2 (V617F) mutation (Fig. 1). This mutation is acquired, and is present in the myeloid lineage of the hematopoietic cells. JAK2 mutation has been associated with a wide variety of myeloproliferative/myeloid disorders—such as PV, PMF, chronic myelomonocytic leukemia (CMML), myelodysplastic syndrome (MDS), etc. (Table 1). The presence of the mutation is not diagnostic of any disorders; it has to be taken in context with other clinical and laboratory abnormalities. To make a diagnosis we need to evaluate the clinical presentation and hematological parameters. Many of the conditions typically associated with JAK2 can also arise without JAK2 mutation.
What are the Implications of JAK2?

JAK2 associated diseases are a wide variety of chronic diseases in the myeloproliferative neoplasms (MPN) or MDS group of diseases. They are characterized in MPN by increased production of red blood cells, platelets, or one type of white blood cell; or in MDS by increased production of dysplastic hematopoietic cells, resulting in cytopenias. They are associated with constitutional symptoms, high symptom burden, poor quality of life, and decreased life expectancy and risk of transformation to acute myeloid or other leukemia (Fig. 1).

Why Do Various Conditions Evolve from the Same Mutation?

The JAK2 gene is a signaling molecule for many cytokines including: INF-γ, erythropoietin (EPO), prolactin, thrombopoietin, G-CSF, GM-CSF, and IL-3 via activating many signaling pathways like: MAPK, PI3, ERK, and importantly STAT. JAK2 abnormalities present with different genetic alterations (Table 2), the interplay of various genetic and cytokine abnormalities results in the clinical disease state and its severity.

What is JAK2?

The JAK2 gene is a member of a family of genes that includes four Janus kinases 1, 2, 3, and tyrosine kinase 2. The protein group was named Janus kinases after the Roman God Janus with two faces, as the non-receptor kinases have two similar “active” and “inactive” domains. The JAK gene has domains, which bind to type 1 cytokine receptor, it plays a role in trafficking of the erythropoietin (EPO) receptor (EPOR). The JAK2 protein in contact with the cytoplasmic domain of the receptor catalyses tyrosine phosphorylation and leads to activation of signal transducer and activator of transcription (STAT) molecules that act as transcription factors and modifies other key regulatory pathways and cytokine signaling (diagram illustrating functional JAK STAT pathway—Fig. 2).

The V617F mutated JAK2 spontaneously activates downstream STAT mediated transcription, and activation of ERK/MAP kinase and PI3K/AKT pathways. The wildtype JAK2 has an autoinhibitory activity, and does not mediate such events. The hematopoietic stem cells in patients of myeloproliferative/myeloid disorders are extremely sensitive to growth factors; proliferation is

---

**TABLE 1** Conditions associated with JAK2 V617F mutation

<table>
<thead>
<tr>
<th>Conditions associated with JAK2 V617F</th>
<th>Frequency of JAK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia vera (PV)</td>
<td>PV: &gt;90%</td>
</tr>
<tr>
<td></td>
<td>JAK2 exon 12 = PV: 2–3%</td>
</tr>
<tr>
<td>Essential thrombocytosis/thrombocytosis (ET)</td>
<td>ET: 60%</td>
</tr>
<tr>
<td>Primary myelofibrosis (PMF)</td>
<td>PMF: 60%</td>
</tr>
<tr>
<td>Systemic mastocytosis (SM)</td>
<td>25%</td>
</tr>
<tr>
<td>Chronic neutrophilic leukemia (CNL)</td>
<td>17–33%</td>
</tr>
<tr>
<td>Hypereosinophilic syndrome (HES)</td>
<td>0–2%</td>
</tr>
<tr>
<td>Myelodysplastic syndrome (MDS)</td>
<td>1.5–5%</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia (CMML)</td>
<td>3–13%</td>
</tr>
<tr>
<td>Unclassified MPD (UN)</td>
<td>20%</td>
</tr>
<tr>
<td>HVOTO/ Budd-Chiari Syndrome (BCS)</td>
<td>Varied</td>
</tr>
</tbody>
</table>

**TABLE 2** Types of JAK2 abnormalities and pathogenesis

<table>
<thead>
<tr>
<th>Rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2 can be rearranged with other genes:</td>
</tr>
<tr>
<td>- TEL/ETV6: t(9;12) (p24;p13) reported in myeloproliferative neoplasm (MPN), also in T-cell ALL</td>
</tr>
<tr>
<td>- BCR: t (9;22) (p24;q11.2) reported in few MPN</td>
</tr>
<tr>
<td>- PCM1: t (8;9) (p22p24) reported in MPN, also in AML and ALL</td>
</tr>
<tr>
<td>- NF-E2: der (9 t9(12) (p24.q13) reported in MDS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Point mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>V617F G &gt; T at nucleotide 1849 on exon14, the classical MPNs T875N reported in AML (M7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deletions/Insertions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon12: In 4% PV patients there are &gt;8 reported mutations including deletions and insertions in codon 538 to 543</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Numerical</th>
</tr>
</thead>
<tbody>
<tr>
<td>It can present as trisomy (+9) or be overexpressed due to amplification.</td>
</tr>
</tbody>
</table>

**Abbreviations:** CMML, chronic myelomonocytic leukemia; CNL, chronic neutrophilic leukemia; ET, essential thrombocytosis/thrombocytosis (ET); HES, hypereosinophilic syndrome; MDS, myelodysplastic syndrome; PMF, primary myelofibrosis; PV, polycythemia vera; SM, systemic mastocytosis; UN, unclassified MPD.
triggered by JAK2 signaling. Patients who are homozygous for JAK2 mutation have advanced disease and increased risk of progression to secondary myelofibrosis compared to heterozygous patients.3-6

**Types of JAK2**

JAK2 V617F occurs more frequently in a specific JAK2 haplotype, named JAK2 46/1 or GGCC, which is tagged by rs10974944 (C/G), rs12343867 (T/C), and rs4495487 (T/C). Other JAK2 mutations are more commonly associated with Budd Chiari and other disorders.6-9

**Allele Burden and Disease**

Allele burden has different implications in different diseases, a high V617F allele burden in PV is associated with a more aggressive phenotype, but a low allele burden in myelofibrosis is associated with reduced survival.10

**JAK2 as a Target for Therapy**

We now have agents, which are targeted inhibitors JAK2, and also has inhibits JAK1. This is approved for therapy of patients with intermediate- or high-risk MF, including primary MF (PMF), post-polycythemia vera MF (PPV-MF), and post-essential thrombocytopenia MF (PET-MF), and PV patients with hydroxyurea (HU) failure or intolerance. Newer more specific JAK2 inhibitors are in phase 3 trials—fedratinib.

**Clinical Features of MPN**

The MPN are characterized by increased counts and constitutional symptoms (Table 3). These may occur secondary to other conditions, so exclusion of renal, pulmonary causes of increased hemoglobin, or causes of reactive thrombocytosis etc. are necessary. Myelofibrosis is often missed or under diagnosed as the huge splenomegaly may be confused with other medical conditions. The bone marrow fibrosis results in massive splenomegaly, hepatomegaly, and extramedullary hematopoiesis as a compensatory mechanism. In India, patients with lower hemoglobin levels than standard criteria may be recognized; the other molecular tests and bone marrow evaluation are useful to identify them so that they can receive appropriate therapy. MPN patients particularly PV may show iron deficiency, this may be due to many reasons, but one important factor is disorder of iron metabolism, with the inflammation caused by PV counteracting Hepcidin suppression.11 Iron therapy is controversial as the HU used in cytoreductive therapy in high-risk PV patients is more effective in iron-deficient than in iron-replete patients.

The diagnosis of MPNs is made in a symptomatic patient and requires a combination of parameters and tests.12,13

**Diagnostic Criteria**

The diagnosis of MPNs is made in a symptomatic patient and requires a combination of parameters and tests. The usual criteria followed are the WHO 2016 diagnostic criteria. The diagnosis of MPN should be based on the 2016 WHO diagnostic criteria.12 Criteria include specific findings from the CBC, blood smear, bone marrow analysis, correlated with clinical history, as well as the presence of certain molecular markers and the exclusion of other disorders.

**Laboratory Tests**

All patients should have a CBC, differential count test; this should be repeated to ensure no fallacy. Tests to fulfill diagnostic criteria of WHO criteria12 should be done. In PV serum erythropoietin levels and evaluation to exclude causes of secondary polycythemia are required. In all patients, a detailed peripheral smear with evaluation for blasts, base line tests for RFT, LFT, evaluation for comorbidity, history of smoking, medications, etc., should be performed. Cardiovascular risk assessment is essential.
Testing for BCR-ABL1 mutation is required as by definition these conditions are BCR-ABL negative. Then evaluation for other MPN mutations should be performed. Molecular testing is used to assess clonality and to detect MPN-specific mutations. First testing for JAK2 V617F mutation should be performed in all MPN suspected patients; it is the most common mutation and occurs more than 95% patients with PV.

If the JAK2 V617F mutation is not detected, testing for CALR and then MPL mutations should follow for patients with ET or PMF. If PV is still suspected in those with negative JAK2 V617F mutation results, testing should be performed for JAK2 exon 12 mutations. In those with ET or PMF but without JAK2, MPL, or CALR mutations (triple-negative MPNs), testing for mutations in ASXL1, CBL, CSF3R, DNM3TA, EZH2, IDH1, IDH2, LINK/SH2B3, SF3B1, SRSF2, TET2, TP53, and U2AF1 genes should then be considered. A comprehensive next generation sequencing (NGS) myeloid panel can be useful for this, instead of single-gene tests and provides details of additional prognostic genes.
Bone Marrow

Bone marrow examination is very important and a bone marrow aspiration and biopsy is necessary to confirm a diagnosis of MPN, and for prognosis by assessment of blast percentage and evidence of fibrosis.\textsuperscript{12,13}

Cytogenetics

Cytogenetic analysis is important in distinguishing MPN subtypes. The chromosome analysis also serves to provide evidence of clonality and clonal evolution, it may be repeated if concerns of progression exist.

Prognosis

The overall survival of patients with MPNs is variable depending on the subtype. Several scoring systems and prognostic models have been developed for the risk stratification of patients with MPN. The dynamic international prognostic scoring system (DIPSS) is commonly used for its ease and ability to stratify patients into risk groups.\textsuperscript{14} Treatment decisions are made per the risk stratification of the patient, higher risk patients are more likely to develop progression from their baseline MPN this can mean transformation to acute myeloid leukemia or post-fibrotic myelofibrosis. These secondary cases are more difficult to treat and have poorer prognosis.

Therapy

These patients must get a hematology consultation periodically due to their risk of thrombosis, progression, and symptom load. Shared care with primary care physicians is possible and desirable for low-risk patients. Therapy of MPN depends on the diagnosis, age, risk classification, and whether complications of thrombosis have occurred or not.\textsuperscript{15-18}

Patients of PV and ET require low-dose aspirin to decrease the risk of thrombosis. Younger PV patients can be well managed by phlebotomy, and/or interferon alpha, but older patients or those with history of thrombosis require hydroxyurea (HU). HU is titrated to response; those who fail or are intolerant to HU can be given ruxolitinib (a JAK2 inhibitor). In ET along with low-dose aspirin, additional medications to reduce the platelet counts like hydroxyurea, anagrelide, and interferon alpha are used.\textsuperscript{17,18}

Myelofibrosis low-risk patients may be given HU, thalidomide-prednisone or ruxolitinib. High-risk patients should be offered allo-hematopoietic stem cell transplant as a curative option. Patients with low-risk disease will have a longer life and supportive treatments like hydroxyurea and ruxolitinib are acceptable.\textsuperscript{15,16} All of these treatments are palliative. For high-risk patients curative options of allogeneic hematopoietic stem cell transplant (bone marrow transplant, BMT) should be discussed and planned.

Glossary

- A gene is polymorphic if more than one allele occupies the gene’s locus within a population. A polymorphic variant of a gene can lead to the abnormal expression or abnormal form of protein. These may cause or be associated with diseases.
- An allele is any of the possible forms in which a gene for a specific trait can occur. In humans, two alleles for each gene are inherited, one from each parent. The variant alleles arise by mutation and are found at the same place as the original gene on the chromosome.
- Wildtype gene is in its normal state, mutated gene has acquired or inherited alterations.
- Allele burden One, called the gene-dosage hypothesis, postulates a correlation between disease phenotype and the proportion of JAK2 (V617F) mutant alleles introducing the concept of allele burden, that is, the ratio between mutant and wildtype JAK2 in hematopoietic cells.
- Haplotype—A haplotype is a group of genes, which is inherited together by an organism from a single parent.

Conclusion

JAK2 associated conditions of PV, ET, and PMF may be underrecognized and underdiagnosed. The rare conditions associated with JAK2 are also often missed. High index of suspicion, appropriate tests, and timely referral can change the scenario. Correct diagnosis and treatment can prevent serous thrombotic complications and allow access to recommended treatment will help the patient.

References

2. Reilly JT. Pathogenetic insight and prognostic information from standard and molecular cytogenetic studies in the BCR-
A 59-year-old male teacher reported with painful erythematous finger swelling and excessive itching without any rash of 3-week duration. Physical examination shows multiple excoriations, splenomegaly, and erythematous swelling of hands. Vitals were normal. Complete blood count shows Hb 17 g/dL, MCV 87 fL, TLC 14 × 10⁹/L, and platelet 780 × 10⁹/L. Biochemistry shows normal LFT and urea, creatinine while serum EPO was 2.4 U/L (range 7–20 U/L). He had JAK2V617F mutation and was started on regular phlebotomy and Tab Aspirin 75 mg PO OD. His hand swelling and pruritus improved.

Approach to a Case of Polycythemia: More Blood May Be Bad….

Tuphan Kanti Dolai, Prakash Singh Shekhawat, Malini Garg

Abstract

A 59-year-old male teacher reported with painful erythematous finger swelling and excessive itching without any rash of 3-week duration. Physical examination shows multiple excoriations, splenomegaly, and erythematous swelling of hands. Vitals were normal. Complete blood count shows Hb 17 g/dL, MCV 87 fL, TLC 14 × 10⁹/L, and platelet 780 × 10⁹/L. Biochemistry shows normal LFT and urea, creatinine while serum EPO was 2.4 U/L (range 7–20 U/L). He had JAK2V617F mutation and was started on regular phlebotomy and Tab Aspirin 75 mg PO OD. His hand swelling and pruritus improved.

Introduction

Polycythemia means an abnormal increase in hemoglobin or hematocrit. It is categorized as absolute and relative. Absolute polycythemia has an increased red blood cell mass (RCM), the classical example being polycythemia vera (PV). There is a mild rise in hematocrit without raised RCM in cases of relative polycythemia like severe dengue fever, severe diarrhea, or other conditions with a moderate increase of hematocrit secondary to reduced plasma volume. Absolute polycythemia is categorized into primary and secondary. PV is the standard example for primary absolute polycythemia. Secondary absolute polycythemia has hypoxia, because of chronic lung disease, carboxyhemoglobinemia due to smoking and renal cell carcinoma producing erythropoietin. PV is a clonal malignant disorder arising from a mutation in multipotent hematopoietic stem cells with an increase in blood cell production independent of cytokine. It is the commonest myeloproliferative neoplasm (MPN) in the United States and has a male preponderance with median age of diagnosis in the seventh decade. Annual incidence ranges from 1–2.5 per 100,000 persons in different countries.

Etiology and Pathogenesis

Primary polycythemia is caused by dysregulation in erythropoietin sensing mechanism and is mainly associated with low erythropoietin levels. Primary familial and congenital polycythemia has an EPOR gene mutation, which disrupts down regulation of JAK2 pathways. PV is due to an acquired, somatic mutation of multipotent hematopoietic stem cell triggering clonal proliferation plus suppression of normal polyclonal hematopoiesis. Most common mutation is JAK2V617F found in 95–98% of cases. It causes persistent activation of EPO receptor signaling. In vitro development of erythroid colonies in absence of EPO is characteristic of PV. PV can occur at any age because JAK2V617F expression is age-independent.

Secondary polycythemia has increased erythropoietin production because of hypoxia. Secondary familial and congenital polycythemia may have mutations that exist in genes encoding hypoxia inducible factor (HIF), von Hippel-Lindau (VHL) proteins, or prolyl-hydroxylase domain (PHD) enzymes, which regulate renal oxygen sensing and EPO production. Secondary acquired causes have increased EPO levels either secondary to tissue hypoxia (pulmonary illness, CO poisoning, high
altitude, high-affinity binding hemoglobinopathy) or due to overproduction (stenosis of renal artery, kidney cyst, tumors with ectopic EPO production). Smoking history or occupational exposure to hydrocarbon may lead to increased carboxyhemoglobin and increased EPO levels. Separation of primary and secondary polycythemia is important as primary polycythemia has risk of leukemic/fibrotic transformation. Phlebotomy is rarely needed in secondary polycythemia unlike primary polycythemia.

**Diagnosis**

Polycythemic patients are often diagnosed incidentally having elevated hemoglobin or hematocrit found in CBC during baseline evaluation of other complaints. History of smoking, high altitude stay, congenital heart disease, alcohol, and drug abuse along with clinical examination becomes very important to distinguish secondary from primary causes.

Clinical spectrum varies from no symptoms to life threatening thrombosis. Genesis of these symptoms could be related to increased RCM or the underlying disease process. Increased RCM may cause hyperviscosity resulting in symptoms like vertigo, tinnitus, headache, visual disturbance, and hypertension. Both usual and unusual types of arterial and venous thrombosis can be seen. Thrombotic symptoms depend upon the site and the extent of thrombus. Polycythemic patients may have characteristic ruddy complexion.

Although aquagenic pruritus and peptic ulcer disease are common associates of polycythemia, commonly they may have signs and symptoms of the underlying disease like chronic obstructive airway disease (COAD) or cyanotic heart disease. Apart from the signs of the underlying disease patient may have splenomegaly, which is rarely seen in early phase of the disease.

Pretreatment correct diagnosis is essential. Overt PV is easily revealed by clinical findings. Splenomegaly is seen in 75% of the cases and is rare in other varieties. Around 30% cases have hepatomegaly. Pruritus occurs in 40% of PV cases and is rare in other varieties of polycythemia. Associated thrombocytosis, leucocytosis, or basophilia indicates the diagnosis of polycythemia vera. Panmyelosis is seen in bone marrow. Diagnostic marrow studies commonly show reticulin fibrosis and absent iron stores. Bone marrow cytogenetics shows clonal markers in 13–31% of untreated patients. In patients lacking definite evidence of PV the differential diagnosis includes early or mild polycythemia vera, primary pure erythrocytosis or secondary erythrocytosis. The history and physical examination may give clues to the presence of a cause for secondary erythrocytosis. Additional laboratory testing is often helpful. An oxygen saturation < 92% indicates hypoxemia as the cause for polycythemia. HPLC and oxygen dissociation curve can pick high oxygen affinity hemoglobins which usually has a family history. Renal ultrasound, intravenous pyelography, or computed tomography is essential to rule out suspected renal lesion. In selected patients hepatic lesion is diagnosed by ultrasound, CT scan, or radionuclide scan. Similarly, for cerebellar hemangioblastoma a CT brain with stress on the posterior fossa is helpful and can determine the source of ectopic EPO production. Commercially available serum erythropoietin radioimmunoassay is helpful in differential diagnosis. Overall, increased erythropoietin suggests secondary erythrocytosis, whereas a normal value is uncertain as occasionally erythropoietin may be increased in secondary erythrocytosis. Erythropoietin assay may be most useful in those lacking obvious clinical sign of PV and no apparent cause for secondary erythrocytosis.

In PV patients endogenous colony formation occurs without added erythropoietin, whereas for other causes of polycythemia exogenous erythropoietin should be added. Flowchart 1 shows the approach to polycythemia.

WHO defines polycythemia vera as a myeloproliferative neoplasm with hemoglobin level >165 g/L (16.5 g/dL) for males and >160 g/L (16 g/dL) for females or hematocrit levels >49% in men or >48% in women or increased red cell mass (RCM) >25% from baseline (Table 1).

**Treatment of Polycythemia Vera**

Age, sex, initial presenting symptoms along with blood picture must be considered for individualized therapy. Goal of therapy is to decrease symptoms, reduce likelihood of thrombus, and prevent/delay transformation.

**Phlebotomy:** Initially phlebotomy is needed in all patients to keep hematocrit <45% to reduce the risk of thrombotic and cardiovascular complications. In young fit, a 450 mL phlebotomy is safely done on alternate days till this goal is reached. In older, unfit patients smaller phlebotomies approximately 200–300 mL twice weekly can be considered. A standard unit of phlebotomy
**Flowchart 1:** The approach to polycythemia

- **Major criteria**
  - Persistent elevated Hb/Hct/RBC count
  - Absolute erythrocytosis
  - ABG/O2 saturation
  - MPN panel: 1st BCR ABL, 2nd JAK2 V617F, 3rd JAK2 Exon 12
  - Serum EPO
  - Bone marrow studies

- **Minor criteria**
  - Mutation in JAK2V617F or JAK2 exon

- **Diagnosis**
  - All major criteria or any two major criteria and minor criterion

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**TABLE 1** WHO 2016 PV diagnostic criteria

<table>
<thead>
<tr>
<th>Major criteria</th>
<th>Minor criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb &gt;16.5 g/dL in men (or Hct &gt; 49%), Hb &gt;16 g/dL in females (or Hct &gt;48%), or &gt;25% increase in red cell mass</td>
<td>Serum EPO level below the normal reference range</td>
</tr>
<tr>
<td>Bone marrow biopsy showing characteristic panmyelosis and pleomorphic megakaryocytes</td>
<td></td>
</tr>
<tr>
<td>Mutation in JAK2V617F or JAK2 exon</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2** Polycythemia vera risk stratification

<table>
<thead>
<tr>
<th>PV risk stratification</th>
<th>Age ≤60 years</th>
<th>Age &gt;60 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>No history of thrombosis</td>
<td>Low risk</td>
<td>High risk</td>
</tr>
<tr>
<td>History of thrombosis</td>
<td>High risk</td>
<td>High risk</td>
</tr>
</tbody>
</table>

*Cardiovascular risk factors and arterial thrombus: Aspirin BD dose

Decreases Hct by 3%. Polycythemia Vera Study Group (PVSG) recommends use of myelosuppressive treatment. Cytoreduction is usually not needed in low risk group whereas it is used in high risk group (Table 2).
Hydroxyurea (HU): HU, a ribonucleotide reductase inhibitor, is effective and first-line recommendation for cytoreduction in high risk group. Need of cytoreduction or other therapy should be individualized, and this reminds us of Dameshek. “There is a tendency in medical practice—by no means limited to hematologists—to treat almost any condition as vigorously as possible. In hematology, this consists in attempting to change an abnormal number—whether this number is the hematocrit, white cell count, or platelet count to get normal values, whether the patient needs it or not!”

Overall hydroxyurea is the commonest cytoreductive drug used for ET and PV cases, based on the results of the PT-1 randomized controlled trial in 809 high-risk patients with ET, in which HU proved superiority to anagrelide in the rates of serious hemorrhage, arterial thrombosis, and myelofibrosis progression.

Interferons: Interferon-α is an important drug if HU is no longer suitable for a patient. Newer longer acting pegylated-α-2a is available and can allow less frequent administration. IFN-α has anti-clonal activity and also helps in histological improvement.

Although it does not have leukemogenic effect and is safe for long-term use but flu-like symptoms and mood changes often limit its use.

Ruxolitinib: The JAK1/2 inhibitor ruxolitinib is approved therapy for PV cases where HU is intolerant or refractory, based on RESPONSE and RESPONSE-2 studies in patients with or without splenomegaly, respectively. It is given in 15–20 mg BD doses for symptom control.

Treatment of Secondary Polycythemia

Cause and underlying mechanism will help in deciding treatment for polycythemia, especially secondary causes. Aggravating factors like smoking and dehydration must be rectified. Smokers must be encouraged to refrain from tobacco usage. Diuretics can reduce plasma volume, increase hyperviscosity, and thus are avoided where possible. Drugs like Androgens must be carefully discontinued or lower doses used if feasible. Smoking cessation helps in reversal of polycythemia related to carboxyhemoglobinemia and it can improve chronic obstructive pulmonary disease associated polycythemia.

Continuous low flow oxygen therapy may reduce hematocrit and it improves status of hypoxic patients due to sleep apnea and chronic obstructive pulmonary disease. Reducing weight may be helpful in obesity-hypventilation syndrome. Surgical removal of EPO producing lesions usually causes improvement and resolution of the polycythemia. Likewise, polycythemia reversal may be seen after correcting underlying benign renal lesion. Some secondary polycythemics require phlebotomy. Preoperative elective phlebotomy should be considered in these cases. The ideal hematocrit level in these patients is a tough call, to maximize the oxygen carrying capacity, and minimize the deleterious side effects of hyperviscosity.

The ideal hematocrit differs with the main disorder and usually differ amongst patients with the same illness. Usually hematocrit >60% is expected to be harmful and they must undergo phlebotomy, especially when sign/symptoms of poor oxygenation are seen. There is decrease arteriovenous oxygen difference, improvement in pulmonary artery resistance, better right ventricular function along with improved hemodynamics and exercise tolerance. Maintaining hematocrit between 50–55% is likely to help hypoxic lung patients. Acute hemodynamic effects of phlebotomy can be managed by isovolumic phlebotomy. Myelosuppressive treatment is best avoided in cases of secondary erythrocytosis.

Treatment of Relative Polycythemia

Numerous factors are responsible for the relatively increased hematocrit. Abstaining from alcohol intake and smoking may be helpful. Strict hypertension management is essential. Hydration status should be well supported. Phlebotomy is not recommended in cases of relative polycythemia.

Conclusion

Polycythemia included variety of causes from smoking, high altitude, dehydration, congenital heart disease to the clonal polycythemia vera, which has a risk of leukemic progression. They have an increased likelihood of vascular manifestation and risk of thrombosis. Goal is to keep Hct <45% and a cardiovascular risk factor modification. Phlebotomy and aspirin forms important part of treatment along with hydroxyurea or interferons in selected cases. Ruxolitinib is approved for HU resistant or intolerant cases.
References