ABSTRACT
Polycythemia literally means too many cells in the blood and is initially classified into relative and absolute. In absolute polycythemia the red cell mass is increased whereas in relative it is not. Since automated blood counts are done commonly many asymptomatic patients with polycythemia are detected. Hematocrit value above 51% for males and 48% for females requires further evaluation. Values above 60% in males and 55% in females almost establish absolute polycythemia. Absolute polycythemia is classified into primary and secondary. The most important cause of primary polycythemia is PV, a clonal disorder of hemopoietic stem cell which shows selective growth advantage (erythroid cell line). Recently JAK2 mutation has been discovered in majority of patients with PV. Important secondary causes are smoking, chronic lung disease, renal and hepatic tumors and high altitude. Chuvash polycythemia is a hereditary polycythemia found in Chuvash population of the Russian republic. It is a primary polycythemia but also has features of secondary variety (the Epo level is increased). More information is now available regarding the erythropoietin (Epo) receptors and its regulations. Epo receptors are present on the erythroid progenitor and precursor cells (Fig 1). Immediately after Epo binding, JAK2 interacts with the Epo receptor. JAK2 phosphorylates itself, the Epo receptor and other proteins such as STAT5. This starts JAK2/STAT5 signalling and ultimately results in erythroid progenitor proliferation and differentiation. This process is self-regulatory. After appropriate signalling, molecules like HCP (Hemopoietic Cell Phosphatase) interact with the C terminal of the Epo receptor. HCP dephosphorylates Epo and turns off the signalling. In primary familial and congenital polycythemia the C terminal of Epo receptors is truncated. (Fig 2)

INTRODUCTION
Polycythemia is a literal translation from Greek meaning “too many cells in the blood. Polycythemia can be initially classified as relative and absolute. (Table 1) Absolute polycythemia is associated with an increase in the red blood cell mass. The classical example in this group is polycythemia vera, a clonal neoplastic disorder. In relative polycythemia there is a modest elevation of the hematocrit without an increase in the red cell mass. The classical example in India is severe Dengue fever in which there is a moderate elevation of the hematocrit due to a decreased plasma volume which results from capillary leak.

Absolute polycythemia is further classified into primary and secondary. The classical example of the primary absolute polycythemia is polycythemia vera (PV). The examples of secondary absolute polycythemia are hypoxia produced by chronic lung disease, carboxy hemoglobinemia as in smoking and renal cell carcinoma, which produces erythropoietin (see classification of polycythemia Table 1). The term erythrocytosis is sometimes used when the increase in hematocrit is not associated with increased white blood cells and platelets. Very rarely there is a mixed primary and secondary polycythemia. The example is Chuvash Polycythemia (CP), which is an autosomal recessive congenital polycythemia first described in the Chuvash population of the Russian republic. The EPO concentration of the most of the affected individuals is elevated; thus CP has features of both primary and secondary polycythemia.

NEWER CONCEPTS
More information is now available regarding the erythropoietin (Epo) receptors and its regulations. Epo receptors are present on the erythroid progenitor and precursor cells (Fig 1). Immediately after Epo binding, JAK2 interacts with the Epo receptor. JAK2 phosphorylates itself, the Epo receptor and other proteins such as STAT5. This starts JAK2/STAT5 signalling and ultimately results in erythroid progenitor proliferation and differentiation. This process is self-regulatory. After appropriate signalling, molecules like HCP (Hemopoietic Cell Phosphatase) interact with the C terminal of the Epo receptor. HCP dephosphorylates Epo and turns off the signalling. In primary familial and congenital polycythemia the C terminal of Epo receptors is truncated. (Fig 2)
The HCP cannot bind to any structure on Epo receptor and the EpoR is left in the activated position resulting in the uncontrolled erythroid proliferation and elevated red cell mass. The negative control on EpoR is lost. Epo production is mediated by hypoxia within the red cells.

Hypoxic stimulation in the kidney produces HIF-1 (Hypoxia Inducible Factor), a major factor in the transcriptional activities of the Epo gene. When normoxia is attained to HIF-1 is degraded and this is mediated by ubiquitin. This reduces the stimulation for additional Epo production. This degradation of HIF-1 by ubiquitin requires von Hippel-Lindau (VHL) protein, O2 and a unique iron requiring proline hydroxylase enzyme. The whole complex is termed as the O2 sensor. There are a number of humeral factors other than Epo production that can directly stimulate proliferation of the erythroid progenitors in vitro. They are insulin like growth factor I, and angiotensin II of the RAS system. Use of ACE inhibitors could lead to anaemia.

The members of the Janus Kinase family of tyrosine kinase

### Table 1: Classification of Polycythemia

<table>
<thead>
<tr>
<th>I. Absolute (true) polycythemia (increased red cell volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Primary polycythemia</td>
</tr>
<tr>
<td>1. Acquired</td>
</tr>
<tr>
<td>a. Polycythemia vera</td>
</tr>
<tr>
<td>2. Hereditary</td>
</tr>
<tr>
<td>a. Primary familial and congenital polycythemia</td>
</tr>
<tr>
<td>1. Erythropoietin receptor mutations</td>
</tr>
<tr>
<td>2. Unknown gene mutations</td>
</tr>
<tr>
<td>B. Secondary polycythemia</td>
</tr>
<tr>
<td>1. Acquired</td>
</tr>
<tr>
<td>a. Hypoxemia</td>
</tr>
<tr>
<td>1. Chronic lung disease</td>
</tr>
<tr>
<td>2. Sleep apnea</td>
</tr>
<tr>
<td>3. Right-to-left cardiac shunts</td>
</tr>
<tr>
<td>4. High altitude</td>
</tr>
<tr>
<td>5. Smoking</td>
</tr>
<tr>
<td>b. Carboxyhemoglobinemia</td>
</tr>
<tr>
<td>1. Smoking</td>
</tr>
<tr>
<td>2. Carbon monoxide poisoning</td>
</tr>
<tr>
<td>c. Autonomous erythropoietin production</td>
</tr>
<tr>
<td>1. Hepatocellular carcinoma</td>
</tr>
<tr>
<td>2. Renal cell carcinoma</td>
</tr>
<tr>
<td>3. Cerebellar hemangioblastoma</td>
</tr>
<tr>
<td>4. Pheochromocytoma</td>
</tr>
<tr>
<td>5. Parathyroid carcinoma</td>
</tr>
<tr>
<td>6. Meningioma</td>
</tr>
<tr>
<td>7. Uterine leiomyoma</td>
</tr>
<tr>
<td>8. Polycystic kidney disease</td>
</tr>
<tr>
<td>d. Exogenous erythropoietin administration (“Epo doping”)</td>
</tr>
<tr>
<td>e. Complex or uncertain etiology</td>
</tr>
<tr>
<td>1. Post renal transplant (probable abnormal angiotensin II signalling)</td>
</tr>
<tr>
<td>2. Androgen/anabolic steroids</td>
</tr>
<tr>
<td>2. Hereditary</td>
</tr>
<tr>
<td>a. High-oxygen affinity hemoglobins</td>
</tr>
<tr>
<td>b. 2,3-Bisphosphoglycerate deficiency</td>
</tr>
<tr>
<td>c. Congenital methemoglobinias (recessive, i.e., cytochrome b5 reductase deficiency, dominant globin mutations)</td>
</tr>
<tr>
<td>d. Recessive high erythropoietin polycythemias not due to von Hippel-Lindau gene mutations</td>
</tr>
<tr>
<td>e. Autosomal dominant high erythropoietin polycythemias not due to von Hippel-Lindau gene mutations</td>
</tr>
<tr>
<td>C. Mixed primary and secondary polycythemia</td>
</tr>
<tr>
<td>1. Proven or suspected congenital disorders of hypoxia sensing</td>
</tr>
<tr>
<td>a. Chuvash polycythemia</td>
</tr>
<tr>
<td>b. High erythropoietin polycythemias due to mutations of von Hippel-Lindau gene other than Chuvash mutation</td>
</tr>
<tr>
<td>II. Relative (spurious) polycythemia (normal red cell volume)</td>
</tr>
<tr>
<td>A. Dehydration</td>
</tr>
<tr>
<td>B. Diuretics</td>
</tr>
<tr>
<td>C. Smoking</td>
</tr>
<tr>
<td>D. Gaisböck syndrome</td>
</tr>
</tbody>
</table>

Fig. 1: Erythropoietin receptor signaling. In polycythemia vera, JAK-STAT is constitutively hyperactivated by gain-of-function JAK2 mutation.

Fig. 2

Normal EPO receptor

EPO receptor truncated in FCP
receptors (JAK1, JAK2, JAK3 and tyrosine kinase 2 TyK2) derive the name after a Roman god with two faces, ending and beginning. The important JAK2 receptor contains two symmetrical kinase like domains. They are the C terminal JAK homology 1 (JH1) domain processing the tyrosine kinase function. Immediately adjacent is the JH2 domain which is enzymatically inactive but has the important role of negatively regulating the activity of JH1 (fig 1). The JAK2 receptors are controlled by a JAK2 gene located in chromosome 9 (9p24).

In normal hemopoietic cells, signalling is initiated when cytokines like erythropoietin (Epo) and thrombopoietin bind to and activate their cell surface receptor. For example when the cytokine Epo binds to the Epo receptor the Epo receptor gets activated and initiates signalling (Fig 1). JAK2 associated with Epo receptor becomes activated by auto phosphorylation. The phosphorylated JAK2 then phosphorylates the Epo receptor which recruits and activates many other molecules including STAT (signal transducer and activator of transcription). The activated STAT move to the nucleus from the cytoplasm, bind to the DNA and finally results in the proliferation of the erythroid cells completing the signalling pathway. The entire process of this signalling pathway is tightly controlled at multiple levels by different mechanisms. One of the structures which negatively control this process is the JH2 domain of the JAK2 receptor. In 2005 an acquired JAK2 mutation (termed JAK2V617F) was reported in association with polycythemia vera and related myeloproliferative disorders.[1]

JAK2V617F mutation is a somatically acquired G to T nucleotide shift at position 1849 in exon 14 (of chromosome 9) which results in a valine to phenylalanine substitution at codon 617 located in the JH2 pseudo kinase domain of JAK2 receptor. As a result, the auto inhibitory control of JAK2 is lost. The mutated JAK2 is in a constitutively phosphorylated state, independent from the binding of the Epo to the Epo receptor. In simple words the mutated JAK2 remains in the phosphorylated form all the time irrespective of the binding of Epo to the Epo receptor. This results in continuous signalling by STAT leading to uncontrolled proliferation of the erythroid cells.

When this mutation is introduced into erythroid cell line, growth of erythroid cells occur independent of Epo. This may be the explanation of endogenous erythroid colony formation (ECC). JAK2V617F mutational frequency is found in more than 95% of PV, 60% of ET or PMF, 40-50% in refractory anemia with ringed sideroblasts and thrombocytosis. On the other hand it is very rare in AML or MDS. Other types of JAK2 mutations have been discovered in PV who is negative for the classical mutation. One of these is the exon 12 mutation. This seems to be specific for PV. Whether JAK2V617F is actually the cause of the disease or only a disease modifier is not yet clearly known. How a mutation of a single gene can be responsible for three different clinical phenotypes is also not entirely understood. JAK2 is now a target for development of new treatments for the myeloproliferative disorders.

In the 2008 WHO classification of myeloid neoplasm PV is included under a new heading called myeloproliferative neoplasm (MPN) which includes the following entities:

- 3.1 Chronic myelogenous leukemia (CML) BCR-ABL1 positive
- 3.2 Polycythemia vera (PV)
- 3.3 Essential thrombocytopenia (ET)
- 3.4 Primary myelofibrosis (PMF)
- 3.5 Chronic neutrophilic leukemia, (CEL)
- 3.6 Chronic eosinophilic leukemia not other wise classified (CEL-NOS)
- 3.7 Mastocytosis
- 3.8 Myelo proliferative neoplasm unclassified (MPN-u)

MECHANISMS OF POLYCYTHEMIA

The mechanism of polycythemia in primary familial and congenital polycythemia (PFCP) is due to the truncated EpoR (genetic mutation) in which there is no inhibition of signalling pathways. (Fig 2) In all conditions of hypoxia HIF-1 is responsible for the polycythemia. Some patients with chronic lung disease or congenital cyanotic heart disease do not develop polycythemia in spite of hypoxia, the mechanism of which is not very clear. Polycythemia in smokers is due to increased blood carbon monoxide (CO). CO displaces one molecule of O2 from hemoglobin and converts it to carboxy hemoglobin (COHb). COHb has 200 times greater affinity than oxygen. This results in not only occupation of one of the heme groups of haemoglobin but also increase in the oxygen affinity of the remaining heme group resulting in tissue hypoxia. Polycythemia accompanying kidney and liver diseases and neoplastic disorders is usually associated with increased Epo production. In tumours Epo production is shown to be autonomous of hypoxic stimuli.

The molecular basis of post transplantation erythrocytosis (PTE) remains unclear. It is found in 5-10% of renal allograft recipients developing within 8-24 months following a successful renal transplantation. It resolves spontaneously within 2 years in about 25% of patients.

In congenital secondary polycythemia, mutations in the haemoglobin can lead to increased oxygen affinity leading to decreased oxygen delivery and compensatory polycythemia. A rare mechanism in this group is 2, 3 BPG (previously called 2, 3 DPG) deficiency. This compound is synthesised in red blood cell and binds to haemoglobin reducing its affinity for oxygen. Its absence leads to increased affinity of haemoglobin for oxygen resulting a life long hypoxic stimulus and erythrocytosis. The foetal haemoglobin has high oxygen affinity and many of the neonates may have markedly elevated hematocrits.

Polycythemia vera rises from the transformation of a single hematopoietic stem cell with a selective growth advantage that gradually becomes the predominant myeloid progenitor. Recently
a somatic mutation is detected in a gene on chromosome 9p in
majority of polycythemia vera patients. This gene encodes for
tyrosine kinase JAK. This somatic mutation transforms this kinase
into a constitutively active form and seems to be responsible for
the uncontrolled proliferation of the erythrocyt cells.

The first phase of polycythemia vera is a phase of erythrocytosis
characterised by an increase in the hematocrit, white blood cells
and the platelets. After a few years the patient passes into a spent
phase when the disease frequently becomes inactive. This phase
is also called post polycythemic myeloid metaplasia (PPMM)
which is not distinguishable from another MDP, the idiopathic
myelofibrosis. Finally a good number of patients eventually, go
on to develop acute myeloid leukaemia. This orderly transition
occurs only in some patients. Rest of them can directly transit
from the polycythemic phase directly into an acute leukemia or
a myelodysplastic disorder.

**CLINICAL APPROACH TO POLYCYTHEMIA**

Differential diagnosis- Since automated blood counts are easily
available it is common to find an elevated haemoglobulin or
hematocrit on routine complete blood count. The symptoms of
polycythemia are very non specific like headache, weakness,
pruritis, dizziness, sweating and visual disturbances. Some of the
patients are seen initially with complications of polycythemia like
thrombosis (cerebral, peripheral) and haemorrhage. Thrombosis
may occur at unusual sites like hepatic vein (Bud Chiari syndrome).
Polycythemia may be diagnosed when Bud Chiari syndrome is
being investigated. Hematocrit values above 51% in males and
over 48% in females requires further evaluation.

**HISTORY**

A detailed history is very important in differentiating the causes
of polycythemia. Primary familial and congenital polycythemia is
suggested when there is history of polycythemia from childhood
or many members of the family are having polycythemia. History
of residing in high altitude, liver diseases, chronic respiratory
diseases, congenital heart diseases, smoking, renal tumours,
renal transplantation, and EPO use especially by athletes are all
important.

**PHYSICAL EXAMINATION AND INVESTIGATIONS**

A thorough physical examination goes a long way in differentiating
primary and secondary polycythemias. Splenomegaly suggests PV.
Abnormalities of the respiratory system (chronic lung diseases)
congenital cyanotic heart diseases, evidence of hepatic or renal
tumours point towards secondary polycythemias.

Investigations help to differentiate the different causes of
polycythemias. If the hematocrit values are more than 60% in
males and 55% in females are almost always associated with
absolute polycytheamias rather than relative. If the values are
between 51 and 60 in males and between 48 and 55 in female
blood volume and red cell mass studies are necessary for a definite
differentiation. Unfortunately these studies are done only in very
selective centres.

If absolute polycythemia is diagnosed PV should be differentiated
from secondary causes. Since PV is a panmyelosis an increase
in the white blood cell and platelet is suggestive. ABG (Arterial
blood gas) picks up conditions of hypoxia producing secondary
polycytheamias. Adjunctive laboratory findings for PV are increased
leukocyte alkaline phosphate activity (LAP score), elevated serum
B12 levels and B12 binding protein. The bone marrow biopsy
shows hypercellularity with trilineage hyperplasia.

Since smoking is a common cause of secondary polycythemia it
is wise to estimate the carboxy haemoglobin levels early in the
investigations. The Epo level helps in differentiating PV from other
causes of polycythemia. Elevation of Epo is indicative of a hypoxic
state whereas a low level of Epo is virtually diagnostic of PV.
Additional tests which are not usually done to prove PV are the
ability of the bone marrow cells to form erythroid colonies in the
absence of exogenous Epo and decrease in C-Mpl receptor levels
on platelets and megakaryocytes. Recently an elevated expression
of polycythemia rubra vera 1 (PRV-1) m RNA in granulocytes has
been suggested to be diagnostic of PV.

Diagnostic criteria laid down by PVSG (polycythemia vera study
group) and WHO require demonstration of an elevated red cell
mass as a must. This is practically not possible in most centres.
So WHO has revised the criteria (2008) for the diagnosis of PV.
Accordingly there are 2 major and 3 minor criteria.

**Major criteria**

1. Hemoglobin level above 18.5 g/dl for men and 16.5 g/dl for
   females OR Hemoglobin or hematocrit > 99th percentile of
   reference range for age, sex, or altitude of residence OR
   elevated red cell mass >25% above mean normal predicted
   value.
2. Presence of JAK2 gene mutation (V617F) or other function-
   ally similar.

**Minor criteria**

1. Bone marrow showing hypercellularity for age and tri-line-
   age growth (panmyelosis)
2. Subnormal Epo level
3. EEC (endogenous erythroid colonies)

Diagnostic combinations - Major criteria + one minor criterion
and first major criterion + 2 minor criteria

**MANAGEMENT OF POLYCYTHEMIA**

The management depends on the cause. All secondary causes
should be appropriately treated. Congenital cyanotic heart
disease should be surgically corrected. If the polycythemia is due
to smoking the habit should be stopped. Tumours producing Epo
should be surgically removed and the polycythemia disappears
after this treatment. Lowering the hematocrit to normal or
Polycythemia vera- The aim of the treatment is to ameliorate the symptoms and to reduce the risk of thrombosis and haemorrhage. This is done by reducing the blood count.

Phlebotomy- The initial treatment in most of the patients in the plethoric phase is phlebotomy\(^9\). On an average 350ml of blood is removed twice weekly till the hematocrit is normalized. The removed blood is discarded and is not used for transfusion as it may contain the clonal neoplastic cells. As the hematocrit is normalized symptoms like headache gets better. Phlebotomy normalises the viscosity and reduce the risk of thrombosis. The advantage of phlebotomy is that it carries low risk and simple to perform. The disadvantages are that it does not control the thrombocytosis and leucocytosis. Hematocrit should be maintained at 45% in males and 42% in females.

Myelosuppression - The main indications are

a. When the need of phlebotomy is more than one every one or two months.

b. When the platelet counts are more than 800-1000,000/ cumm as there is risk of thrombosis and bleeding.

c. Patients having severe pruritis

Hydroxyurea is the most common drug used. The dose should be titrated between 500mg and 2000mg. This drug is very effective in controlling the erythrocyte, leukocyte and the platelet counts and decreasing the risk of thrombosis during the first few years of therapy. Since it is a short acting drug it is better used as a continuous rather than intermittent regimen. The risk for leukaemia transformation is very low for this drug\(^1,12\). The other myelosuppressive drugs like chlorambucil, busulfan and radio active phosphorus are rarely used now. Pipobroman is effectively used in many countries but risk of leukaemia is relatively high.

Interferon alpha in a dose of 3 million unit three times weekly is effective in 50% of patients but it is inconvenient and costly. Interferon therapy is found to be very useful in patients with pruritis and in pregnant women\(^1\). There is a possibility of less risk of leukaemia and myelofibrosis.

Anagrelide, an excellent drug to reduce the platelet count may be used when the platelet count is very high\(^1\). The dose is 1-2mg per day.

Imatinib mesylate a tyrosine kinase inhibitor, very effective in CML is only having minimal effects in PV\(^1\).

Pruritis which is annoying for some patients is not relieved by myelo suppression. Photo chemotherapy with psoralen and ultraviolet light has been found to be helpful. Interferon therapy is useful for some patients.

Low dose aspirin 75-150mg is recommended in all PV patients without history of major bleeding or gastric intolerance, based on the results of the ECLAP study\(^16\).

Patients with PV should be properly hydrated when they develop gastrointestinal disorders. The spent phase occurs after about 15-20 years when the phlebotomy requirement decreases and the patient develops anaemia. The marrow fibrosis increases and spleen becomes greatly enlarged. The treatment during this phase is purely symptomatic including blood transfusions.

Other treatment modalities tried are splenectomy, thalidomide and marrow transplantation in younger patients. In the future we may have new JAK2 targeted inhibitors to treat PV\(^17,18\). Some patients may get transformed into acute leukaemia. Any form of treatment during this phase is not at all satisfactory.

Currently management of PV depends on the risk stratification

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Age &gt;60yrs or history of thrombosis</th>
<th>Cardiovascular risk factors(^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Intermediate</td>
<td>NO</td>
<td>Yes</td>
</tr>
<tr>
<td>High</td>
<td>Yes</td>
<td>-</td>
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</tbody>
</table>

\(^6\)Hypertension, hypercholesterolemia, diabetes, smoking.

Phlebotomy is the corner stone of low risk patients aimed at reaching and maintaining a target hematocrit of 45% in males and 42% in females. Low dose aspirin may be added to the treatment. High risk patients should receive myelosuppressive treatment in addition to phlebotomy. The drug of choice is hydroxyurea.

PV may infrequently occur during child bearing years and pregnancy. There is increased incidence of abortion in about 30% of cases. Pre-eclampsia is also common. It is very interesting that some of the women may even reduce their hematocrit. Their phlebotomy requirement is also found to be decreased. The possible explanations are erythropoietic suppressive effect of the high oestrogen levels, expansion of the plasma volume and nutritional deficiencies. If needed, the patient should be treated with phlebotomy, low dose aspirin or interferon\(^1\). After delivery the blood count will drift back to the original polycythemic level.

**SUMMARY**

Polycythemia is uncommon. Hematocrit value above 51% in male and 48% in female requires further evaluation. Since automated blood counts are becoming quite common it is always a good practice to see the hematocrit value because many patients with polycythemia are asymptomatic. Values above 60% in males and 55% in females clearly indicate absolute polycythemia. There are primary and secondary causes of polycythemia. Polycythemia could be hereditary and acquired. The most important cause
of primary polycythemia is PV an acquired disease which arises from the transformation of a single hemopoietic stem cell which shows selective growth advantages (the erythroid line). It is very important to differentiate between primary and secondary types of polycythemia. JAK2 mutation is found in a majority of patients with PV. Revised criteria by WHO makes diagnosis of PV simple. The basic principle in management is keeping the hematocrit of the patient at 45% in males and 42% in females by phlebotomy to prevent complications such as thrombosis and hemorrhage. Secondary type of polycythemia should be investigated and treated. For PV myelosuppressive drugs like hydroxyurea should be added to control the white cell and the platelet count. This should be done according to the risk category. Low dose aspirin may be useful. PV has a good prognosis but after an average period of 10-15 years they transform into a spent phase (post-polycythemia myeloid metaplasia PPMM) and a good number into acute leukaemia. The future is bright as new targeted treatments are in the horizon.

REFERENCES