Introduction
Pallor, as a sign of disease, is perhaps recognized from the ancient times. It is the clinical counterpart of anemia, which means less blood in the body. Anemia is defined as a clinical condition resulting from a significant decrease in the total body erythrocyte mass, reducing the oxygen carrying capacity of the blood. Free oxygen, the plant’s unique gift to mankind, is highly reactive and dangerous on one hand, and the only security to free flow of ATP for all intermediary metabolism of body, on the other. All pathological damage due to anemia is caused by hypoxia. The ultimate goal of all speciality is to prevent hypoxia; for cardiologists and pulmonologists this is at the gross mechanical level and for hematologists this is at the molecular level. Hypoxia arises either at the physicochemical level of hemoglobin or at the biochemical level of housekeeping erythrocyte membrane.

Anemia is a common clinical problem, which is very simple to detect but very difficult to solve. For radical cure the condition requires etiological diagnosis, and that is, most of the time, not easy to establish.

Points to remember in evaluation of anemia
1. Anemia may exist as a laboratory finding in a subjectively healthy individual, because the body can compensate, within limits, for decreased red cell mass. The compensation is effected by:
   a. Increased delivery of oxygen to the tissues by decrease of oxygen affinity of hemoglobin due to stimulation of 2,3 DPG production.¹
   b. Redistribution of blood ow to vital organs from skin and kidney by selective vasoconstriction.
   c. Increased cardiac output by compensatory tachycardia, decreased peripheral vascular resistance, decreased blood viscosity, so that B.P. is not increased.
Depletion of whole blood volume produces symptoms when 20% of red cell mass is depleted but when anemia develops gradually, it is not uncommon that the patient does not report till red cell mass is depleted up to 70% i.e. Hb% is 4 gm/dl.²
2. Symptoms of anemia at early stage are very much non-specific like fatigue, insomnia, or lethargy. When breathlessness on exertion or ankle edema appears, interfering daily activities,
usually the patient first reports for medical advise. By the time the condition becomes so much advanced that transfusion therapy may be needed, which further masks the appropriate etiology.

3 In developing countries like ours, where tropical climate prevails, food habits and cooking process are two determining factors for development of anemia. Greater proportion of carbohydrate in relation to protein in the diet provides excess phytate and fiber, which interfere with absorption of iron, the essential element for formation of hemoglobin. The process of cooking i.e. excess frying and boiling cause denaturation of protein and removal of essential elements for synthesizing hemoglobin. All these may perpetuate the anemic process even when other etiological factor is corrected. For evaluation of a case of anemia, socioeconomic and dietary history is, therefore, very much important.

4 Etiology of anemia is multi-dimensional and may involve any organ-system of the body like kidney, thyroid, liver, besides hemopoietic or lymphoreticular system, which is the seat of blood cell formation. The underlying pathological process may be also diverse e.g. infection, inflammation, malignancy or organ failure. The effects of anemia are also multiple e.g. cardiomegaly and congestive cardiac failure, relative myocardial ischemia, cerebral anoxia, diminished renal perfusion. Thus the clinical picture may be confusing and diagnosis is difficult to be established.

Clinical Evaluation of Anemia Requires Careful History Taking and Careful Clinical Examination

1. Age of Onset
   Early childhood: Genetic disorder of red cells or hemoglobin producing hemolysis.
   Prepubertal age: Nutritional deficiency as nutrition lags behind growth spurt.
   Young people: Chronic infection, chronic in ammation, malignancy of lymphoreticular system, in ammatory bowel disease.
   In elderly people: Chronic renal failure, malignancy of G.I. tract, multiple myeloma.

2. Sex
   Female of reproductive age: Nutritional deficiency due to extra demand put by menstruation, pregnancy and lactation.
   Middle-aged female: Hypothyroidism, chronic in ammatory disease, including in ammatory bowel disease.
   Elderly female: Chronic renal failure, malignancy of genital tract.

3. Source of Bleeding
   Frank bleeding, e.g. bleeding piles, fresh bleeding with stool, hematuria, menorrhagia.
   Occult bleeding, e.g. drug induced gastric erosion, undetected esophageal varices or congestive gastropathy as well as worm infestation, which may also produce malabsorption of nutrients.

4. Fever
   Associated with night sweat, lymphadenopathy, splenomegaly – Chronic infection, chronic in ammation, malignancy of lymphoreticular system.
   Petechiae, purpura, lymphadenopathy, splenomegaly – hematological malignancy.

5. History of
   i. Rheumatoid arthritis- anemia of chronic in ammatory disease or that due to methotrexate therapy.
   ii. Hepatitis or evidence of portal hypertension-anemia of chronic liver disease, hypoplastic anemia, or blood loss from esophageal varices.
   iii. Kidney disease or stigmata of chronic renal disease including hypertension – renal anemia.
iv. Anorexia, persistent dyspeptic symptoms in spite of continuous treatment in previously healthy individual – gastric malignancy.

v. Multiple sexual exposure, chronic diarrhea, weight loss – HIV infection.

vi. Lethargy, poverty of thought, sleepiness, feeling of cold – hypothyroidism.

vii. Gastrointestinal operation, chronic inflammatory bowel disease, may produce deficiency of intrinsic factor or vitamin B\textsubscript{12}.

6. Drug History

i. Prolonged use of analgesic, NSAID, steroid may produce gastropathy and occult blood loss from G.I. tract.

ii. Use of cytotoxic chemotherapeutic agents may produce myelosuppression.

iii. Methyldopa may produce immune hemolytic anemia.

iv. Folate antagonists e.g. Methotrexate may produce folate deficiency.

v. Purine and pyrimidine inhibitors like 6-mercaptopurine or azathioprine and 5- urouracil may produce folate deficiency.

7. Occupational Exposure

i. Lead may produce interference of bone marrow hemopoiesis, inhibition of hem synthesis or red cell membrane damage.

ii. Naphthalene may produce hemolytic anemia.

Physical Examination

1. Confirmation of anemic state – Dorsum of tongue or lower bulbar conjunctiva are not always reliable sites, as local in amniation may interfere with the finding, especially glossitis is very much common due to associated avitaminosis. The surest sign of anemia is the pallor found on palmar creases, when the hand is hyperextended. This usually occurs if Hb level is below 8 gm%.

2. Careful examination of respiratory system may exclude COPD, which may produce relative polycythemia and may mask anemic state.

3. Careful examination of heart not only helps to detect functional or murmur but also other organic murmur which may produce bacterial endocarditis and may be a source of anemia.

4. Evidence of portal hypertension, hepatosplenomegaly, must be sought for.

5. Careful palpation of all lymph glands is mandatory.

6. Inspection of skin for petechiae, purpura, and spider angioma as well as a look for jaundice is essential.

7. Measurement of BP and assessment of kidney size must be done to exclude renal disease.

8. Careful palpation of thyroid and relaxation of ankle jerk must be noted in relevant cases.

9. Rectal examination should be done to exclude piles, polyp, or inflammatory changes in mucosa, as evident on proctoscopy, especially if patient has got such complaints.

Etiological Diagnosis

Etiological diagnosis is dictated by results of history and physical examination. If clinical examination raises suspicion of abnormality of a definite organ-system, then investigation may be first directed towards that line e.g.

Kidney disease: Serum urea and creatinine, USG evidence of chronic kidney disease, serum erythropoietin.

Thyroid disease: Serum \( T_3 \), \( T_4 \), TSH.

Liver disease: Liver function tests, upper G.I. endoscopy.

Fresh bleeding per rectum: Colonoscopy

These investigations should always follow routine stool and urine examination to detect any occult
source of bleeding and worm infestation or evidence of nephritis, as well as chest X-ray to detect any chronic infection. In most of the cases, usually investigations are directed towards typing of anemia. For the purpose of planning laboratory investigations, anemia is classified as follows.

Cytometric: Normocytic, normochromic or microcytic, hypochromic.
Erythrokinetic: Hemorrhagic or hemolytic.
Biochemical/Molecular: DNA point mutation producing amino acid substitution in hemoglobin beta chain or red cell membrane enzyme.

Cytometric Parameters
These are easier to perform and less expensive, so they are performed first, of course, they are next to Complete Blood Count (CBC) and Peripheral Blood Smear examination (PBS). Parameters are:

- MCV= Hct/ RBC = 80-90
- MCH= Hb/ RBC = <27 pg
- MCHC (g/l) = Hb/Hct x 0.1 = <30g/l
- Hct (%) = MCV x RBC x 0.1

RBC, Hb, MCV can be directly measured by automated instruments or can be calculated from measured values of Hb, Hct, RBC.

Normocytic normochromic: Anemia of acute hemorrhage, Aplastic anemia, Anemia of chronic disease.

Hypochromic microcytic: Iron deficiency anemia, Thalassemia, Anemia of chronic disease (30% of cases due to impaired iron utilization).

Macrocytic normochromic: (MCV> 95fl should prompt further investigations) Single or combined deficiency of folate or Vitamin B12, Myelodysplastic syndrome, Hemolytic anemia with elevated reticulocyte count.

1. Reticulocyte count and indices: Reticulocytes are stained by supravital staining. Typical normal range is 0.5-1.5%. The count depends on total RBC count. The index is the corrected value in relation to total red cell mass and Hb%. Increased count indicates increased red cell turn over.

2. If reticulocyte count is high other evidences of hemolysis should be searched for e.g. serum heptoglobin and hemopexin, which are degraded hemoglobin bound complexes, and become untraceable in acute intravascular hemolysis. Serum unconjugated bilirubin and urine urobilinogen concentration should be also measured. Unconjugated hyperbilirubinemia in absence of urobilirubinogen in the urine is indicative of hemolysis.

3. Red cell distribution width (RDW): It is determined by automated cell counter. High RDW means that there is a defect in erythropoiesis. If it is associated with low serum ferritin concentration the probability is that it is due to iron deficiency anemia. The sensitivity of the test is 90% and specificity is only 50%. RDW is the measured counterpart of morphological variation of RBC, as found on microscopical examination of stained blood film.

4. Discriminant function
   DF= [MCV- (5xHb conc.)- RBC-K], where k is 3.4 if the Hct is corrected for plasma trapping. DF may be greater than 1.0 in iron deficiency anemia and less than 1.0 in thalassemia.

5. Serum ferritin versus ESR and CRP: Serum ferritin (SF) and ESR or CRP, which are markers of in ammation, increase in both iron deficiency anemia and anemia of chronic disease, but the increase is less in iron deficiency anemia.

6. Serum ferritin (SF), if less than 12 micro gram/l, is a substantive evidence of iron deficiency anemia but if it is more than 80 microgram/l, it excludes the possibility of iron deficiency. SF
more than 40 micrograms/l may occur in elderly, even in presence of iron deficiency anemia.  
7. When the results of plotting of SF Vs. CRP are of doubtful significance of iron deficiency anemia, TIBC and serum iron should be estimated. TIBC is reduced in patients with anemia of chronic disease but it is usually increased in iron deficiency. 
8. Erythrocyte zinc protoporphyrin level helps to differentiate iron deficiency anemia, in which it is increased from thalassaemia, in which it is normal. It is also increased in lead poisoning.  
9. Serum transferrin receptors are increased in iron deficiency anemia, thalassemia, and myelodysplasia but decreased in anemia of chronic disease and aplastic anemia.

Complete Blood Count (CBC)  
This is helpful to diagnose aplastic anemia, to find out associated feature of white blood cell or thrombocyte, which may provide diagnostic clue.

Severe aplastic anemia is characterized by:
1. Neutrophils < 500/ microlitre
2. Platelets < 20,000/ microlitre
3. Reticulocytes <1%
Combined with marrow hypocellularity showing < 30% of cells being hemopoietic, any two of the above mentioned features gives the diagnosis of aplastic anemia.

Leucocytosis in the leukemic range of 50,000-100,000/ microlitre with <10% immature forms may indicate hematological malignancy as a cause of anemia (due to suppression of erythropoietic elements) and may prompt bone marrow examination.

Peripheral Blood Smear Examination  
Much useful information about the etiological diagnosis of anemia is obtained by examining Romanowsky stained blood film.

1. Hypochromia: The intensity of orange pink color of hemoglobin gradually decreases from periphery to center and the central pallor occupies more than one third of cell diameter—indicates less hemoglobinization.
2. Polychromasia: This refers to diffuse basophilic hue in red cells and correlates well with increased reticulocyte count. This is found in myelodysplastic syndrome or megaloblastic anemia.
3. Spherocytes have abnormally increased central staining as found in hereditary spherocytosis.
4. Target cells have a small normally stained center surrounded by a hypochromic ring, finally a normally stained rim, which is found in thalassemia, iron deficiency anemia, liver disease, as well as after splenectomy.
5. Howell Jolly bodies are purple black, usually round chromatin fragments, being more deeply staining than basophilic stippling, found as small discrete dots in lead poisoning. H-J bodies are found in hemolytic anemia or after splenectomy.
6. Macrocytes are immature erythroid precursors, sometimes; they are even blast like with chromatin in large nuclei. They are formed due to defect of DNA synthesis, and asynchronous cytoplasmic nuclear maturation. Presence of hypersegmented neutrophils with 5 or more lobes may be the first indication of megaloblastic anemia.
7. Burr cells are fragmented erythrocytes formed in renal failure due to microangiopathy.
8. Myelodysplastic disorders should be suspected if dimorphic anemia i.e. a mixture of normochromic cells with hypochromic microcytes is present. Ring sideroblasts may be found in some of these cases.

Bone marrow smear examination under the microscope and test for stainable iron in bone marrow by Prussian blue gives confirmatory diagnosis in hematological malignancy, aplastic anemia, megaloblastic anemia, and differentiates iron deficiency states from anemia of chronic disease. Decreased sideroblastic
iron in the face of increased histiocytic iron shows impaired iron transport in anemia of chronic disease. Finally hemoglobin electrophoresis may help in typing of hemoglobinopathy and serum B$_{12}$ estimation or absorption test as well as red cell folate help to identify the cause of megaloblastic anemia.

Conclusion
Diagnosis of anemia entirely depends on physician’s knowledge and skill in planning the proper test against the background of particular clinical set up. Peripheral Blood Smear (PBS) and Complete Blood Count (CBC) are two bedside procedures. These should be followed by Cytometric tests that are also simple to perform, and are mandatory. Other tests are planned on information gathered from the previous tests. The clinical evaluation always lies behind.

References