

Anaemia is defined as a fall in hemoglobin (Hb) below once individual baseline value. Often, such baseline value is unavailable. In this situation, physician utilizes age-specific, sex-specific and race-specific reference ranges. There are large number of different diseases that are included in the World Health Organization (WHO) list regarding causes of anaemia. Our today's goal is to provide a simple approach to anaemia which is based on clinical and simple laboratory tests. In clinical practice, the standard method of taking a good history followed by proper examination supported by laboratory parameters still forms the best approach<sup>1</sup>. We have tried to propose a hierarchical and logical way to reach a diagnosis as quickly as possible by properly managing the medical interview, physical examination, appropriate laboratory tests including bone marrow examination and other complementary tests.

There are various ways of classifying anaemia. None is perfect. The approach to find the cause of anaemia differs between men and women, children and adults and also between different ethnic groups. Various algorithms exist. It is important to approach the problem carefully. One should not miss treatable disorders like nutritional deficiencies, GI bleeding, hemolysis and anaemia of renal origin. The traditional approach of sub categorizing anaemia in to microcytic, normocytic and macrocytic subtypes is still probably the best. The basis of this subtyping is Mean Corpuscular Volume (MCV). At the same time, one must remember that this is just a starting point. Subsequently, each category has to be deciphered using a stepwise approach which utilizes various laboratory tests.

### CLASSIFICATION

One can approach the problem of anaemia from three angles i.e. based on :

1. Pathogenesis
2. Clinical presentation
3. Red cell morphology and indices

**Table 1 : Causes of microcytic anaemia (MCV < 80 fl)**

|                                   |
|-----------------------------------|
| Iron deficiency anaemia (IDA)     |
| Thalassaemia & Hemoglobinopathies |
| Anaemia of chronic disease (ACD)  |
| Sideroblastic anaemia             |
| Lead intoxication                 |

Pathogenic mechanisms involved in the production of anemia include

1. Inadequate production
2. Excessive destruction (hemolysis)
3. Blood loss (bleeding)

### CLASSIFICATIONS BASED ON PATHOGENESIS

1. Hypo-regenerative: Here blood production is decreased. This could be due to lack of nutrients (iron, vitamin B<sub>12</sub> or folic acid), paucity of stem cells, defective marrow function or marrow infiltration
2. Regenerative: Here marrow is normal and it responds appropriately to anaemia by increasing production of erythrocytes.

### CLASSIFICATIONS BASED ON CLINICAL PRESENTATION

1. Acute (bleeding or hemolysis)
2. Chronic (primary marrow disorders and various chronic diseases)

### CLASSIFICATION BASED ON RED CELL MORPHOLOGY AND INDICES

In practice, classification based on red cell morphology & indices chiefly mean corpuscular volume (MCV) is very useful. From this angle, anaemia is classified as microcytic, normocytic or macrocytic.

Red cell morphology as assessed by microscopic examination of peripheral blood film, MCV as analysed by electronic cell counter together with reticulocyte count which differentiates between regenerative and hypo-regenerative anaemias can give an accurate diagnosis in most of the patients

We will now look at various causes of anaemias based on MCV

### MICROCYTIC ANAEMIA

MCV below 80 fl is considered as microcytosis. Common causes of microcytic anaemia are listed in Table 1.

#### Iron deficiency anaemia (IDA)

Iron deficiency is the commonest cause of microcytic anaemia. It has one specific symptoms i.e. pica. Pica relates to persistent and compulsive craving to eat non-food items. It comes from a Latin word for a bird known for its indiscriminate appetite (Figure 1). Pica includes eating of dirt, clay, paint chips, plaster, chalk, cigarette ashes, soap, toothpaste, burnt match heads, cigarette butts, ice, glue, hair, buttons, paper & sand etc. IDA has a specific sign



**Fig. 1 : Pica (A bird with indiscriminate appetite)**



**Fig. 2 : Koilonychia**

i.e. Koilonychia (Figure 2). Differentiating iron deficiency anaemia (IDA) from anaemia of chronic disease (ACD) is important. The tests used for differentiating these are listed in Table 2. A diagnostic test for IDA is low S. ferritin (SF). This finding is diagnostic and specific of iron deficiency state.<sup>2</sup> Raised S. ferritin, specially above 100 ng/ml is very unusual in iron deficiency, however, ferritin is an acute phase reactant and hence it may be normal or high despite iron deficiency if person has infection, inflammation or malignancy. Serum Iron (SI) is low in both IDA and Anaemia of Chronic Disease (ACD). Total iron binding capacity (TIBC) is high only in iron deficiency and hence TIBC also helps. Soluble Transferrin Receptor (STfR) is elevated in IDA and hence the ratio of STfR to SF is high in IDA as against ACD.

A gold standard to diagnose iron deficiency state is to document deficiency of iron in bone marrow, however, this is not necessary in day-to-day practice. Also, this may also be inaccurate due to certain technical problems<sup>3</sup>.

**Table 2 : Investigations to differentiate IDA (iron deficiency anaemia) from ACD (Anaemia of chronic disease)**

| Test                                | IDA | ACD    |
|-------------------------------------|-----|--------|
| S. Iron                             | ↓   | ↓      |
| Total Iron Binding Capacity (TIBC)  | ↑   | ↓ or N |
| Transferrin saturation              | ↓   | ↓      |
| S. Ferritin (SF)                    | ↓   | N or ↑ |
| Soluble transferrin receptor (STfR) | ↑   | N      |
| Ratio of STfR / SF                  | > 2 | < 1    |
| Cytokine levels                     | N   | ↑      |

N : Normal; ↑ Increase ; ↓ Decrease

Another condition that needs to be differentiated from IDA is thalassaemia minor. Increased red cell distribution width (RDW) is associated with IDA. In thalassaemia minor, RDW is normal. For a given level of Hb, RBC count is relatively higher in thalassaemia minor. Peripheral smear examination in IDA shows pencil cells which are quite characteristic. As against this, in thalassaemia, one has basophilic stippling and target cells. Also, thrombocytosis is often associated with iron deficiency. Lastly, a therapeutic trial with iron supplementation is both cost-effective and definitive way to diagnose IDA.

In each case of IDA, underlying cause must be detected. Nutritional deficiency is the commonest while bleeding from GI tract or menorrhagia accounts for most important underlying disorders. Other conditions include celiac disease (gluten enteropathy) etc. Although, Hcpidin is important in iron metabolism, its assay is neither clinically available or required.

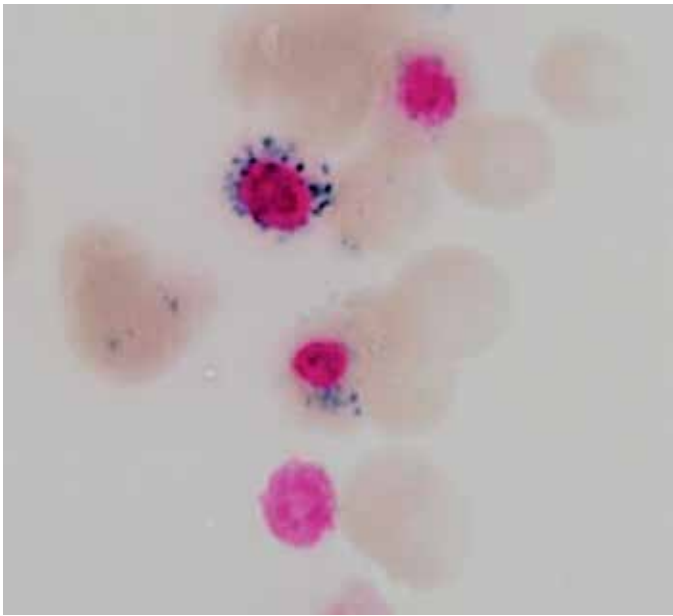
### Thalassaemia and hemoglobinopathies

In India, in community practice, thalassaemia minor (heterozygous state or carrier state or trait) is the second most common cause of microcytic anaemia. It is  $\beta$ -thalassaemia minor which is common and more important than relatively rare and clinically insignificant  $\alpha$ -thalassaemia trait. The definitive test is documenting raised Hb-A<sub>2</sub> fraction which is usually above 3.8%. Normal individuals have Hb-A<sub>2</sub> between 2.0 and 3.5 % while in IDA, it decreases. These tests can be carried out by HPLC (High Pressure Liquid Chromatography) or Hb Electrophoresis, the first being more reliable. In case of difficulty, molecular techniques are required. As stated above, thalassaemia trait will have normal RDW and RBC count is much higher for a given level of Hb while peripheral smear shows morphological changes in red cells as mentioned above.

Common hemoglobinopathies in India include Hb-S, Hb-E, Hb-D, Hb-Q etc. Detailed description of diagnosing these is beyond the scope of this write-up

### Anaemia of chronic disease (ACD)

Third important cause of microcytic anaemia is ACD. It is not uncommon and probably the most common cause in hospitalised patient. Usually, ACD is normocytic, however, various chronic disorders e.g. tuberculosis,



**Fig. 3 : Bone marrow showing ring sideroblasts**

**Table 3 : Causes of macrocytic anaemia (MCV > 100 fl)**

|                              |
|------------------------------|
| Anaemia with reticulocytosis |
| Megaloblastic anaemia        |
| Drug induced disorders       |
| Alcohol related anaemia      |
| Primary marrow disorders     |
| Liver disorders              |
| Spurious macrocytosis        |

rheumatoid arthritis, other collagen vascular diseases & even malignancies are accompanied by microcytosis. The microcytosis of ACD is mild. RDW is usually normal. Peripheral blood smear is unremarkable. Diagnosis is often suspected on clinical grounds. Bone marrow examination is hardly ever required. Many patients are febrile or have other constitutional symptoms due to raised cytokines (Table 2).

#### Acquired sideroblastic anaemia

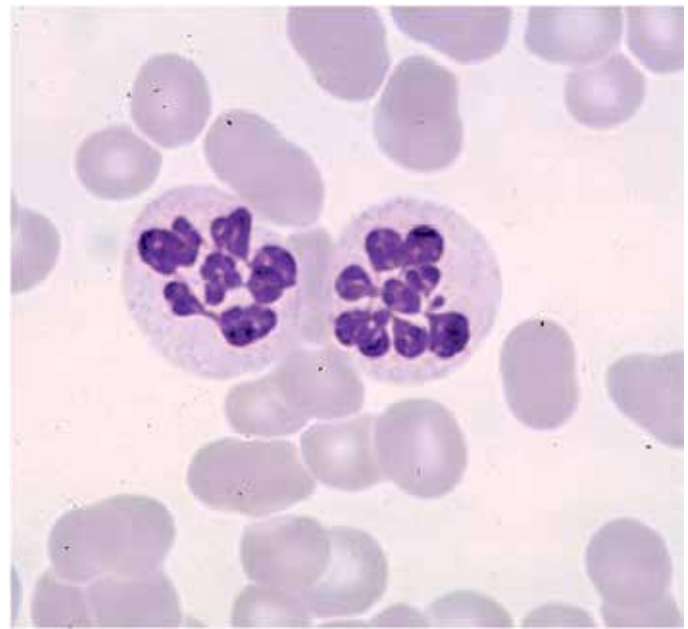
This is a rare disorder which is often drug induced. An important cause is low grade myelodysplastic syndrome (MDS). Here, peripheral smear shows dimorphic morphology, RDW is high and marrow shows ring sideroblasts (Figure 3).

#### Lead intoxication

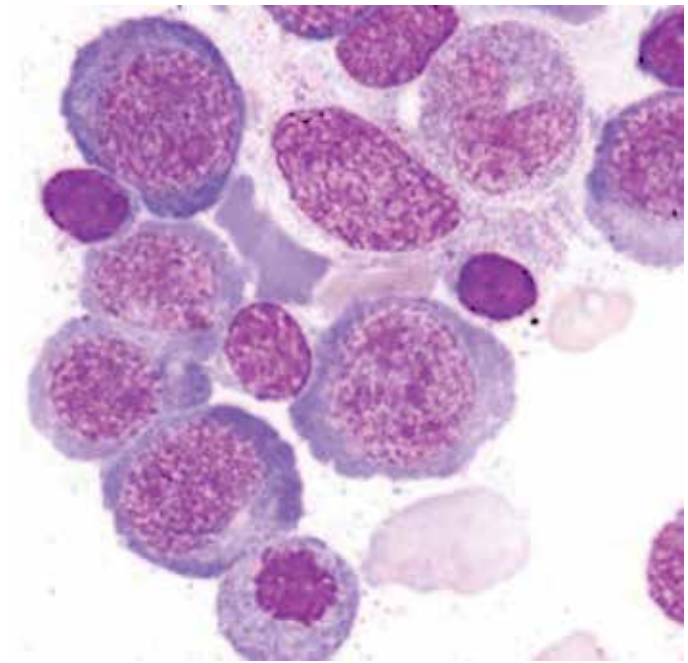
This is not an unusual cause of microcytic anaemia, specially in India. Unlike the causes mentioned in hematological text books, ayurvedic medicines containing "Bhasmas" is a common etiology. Clinically, patients may have characteristic blue line over the gums, peripheral smear shows punctate stippling and blood lead levels are high

#### MACROCYTIC ANAEMIA

MCV above 100 fl is required to consider anaemia as macrocytic. Common causes of macrocytic anaemia are listed in Table 3. One should first look for reticulocytosis



**Fig. 4 : Hypersegmented neutrophils**



**Fig. 5 : Bone marrow showing megaloblasts**

as the cause of mild macrocytosis.<sup>4</sup> This is reflected as polychromasia in peripheral blood film. If present, it will suggest a regenerative anaemia i.e. hemolysis or hemorrhage. If there is no reticulocytosis, the next thing is to look for red cell morphology to differentiate between macro-ovalocytosis which is seen in megaloblastic anaemia vs round macrocytosis which is common with remaining disorders. In patients with round macrocytosis, it is important to ask for history of alcohol and drugs e.g. hydroxyurea, methotrexate, trimethoprim, zidovudine etc.

In India, nutritional vitamin B<sub>12</sub> and/or folic deficiency is common.<sup>5</sup> Often, these patients have pancytopenia as vitamin B<sub>12</sub> and folic acid are required for formation of all nucleated cells. Neutrophils are often hypersegmented

(Figure 4). Platelet count may be low. Homocystein levels are high. S. Vitamin B<sub>12</sub> or RBC folic acid level should be ordered. Although not common in practice, S. methylmalonic acid level is high in vitamin B<sub>12</sub> deficiency.<sup>5</sup> Often, a bone marrow examination may be needed to differentiate between non megaloblastic macrocytic anaemia vs megaloblastic anaemia (Figure 5). This is also important as many primary bone marrow disorders produce mild to moderate macrocytic anaemia. These include aplastic anaemia, myelodysplastic syndrome and others. If vitamin B<sub>12</sub> deficiency is confirmed, one has to search for its etiology. In India, many vegetarians have nutritional vitamin B<sub>12</sub> deficiency, specially if they do not take milk which is an important dietary source of vitamin B<sub>12</sub> for vegetarians. It is also important to look for pernicious anaemia by testing for intrinsic factor antibodies. Schilling test is not so widely available. Lastly, various primary intestinal malabsorption disorders e.g. tropical sprue, celiac disease, inflammatory bowel diseases etc. should be considered and if required, investigated.

**Table 4 : Causes of normocytic anaemia (MCV : 80-100 fl)**

|                                  |
|----------------------------------|
| Dimorphic anaemia                |
| Anaemia of renal insufficiency   |
| Haemolytic anaemia               |
| Anaemia of chronic disease (ACD) |
| Primary marrow disorders         |

## NORMOCYTIC ANAEMIA

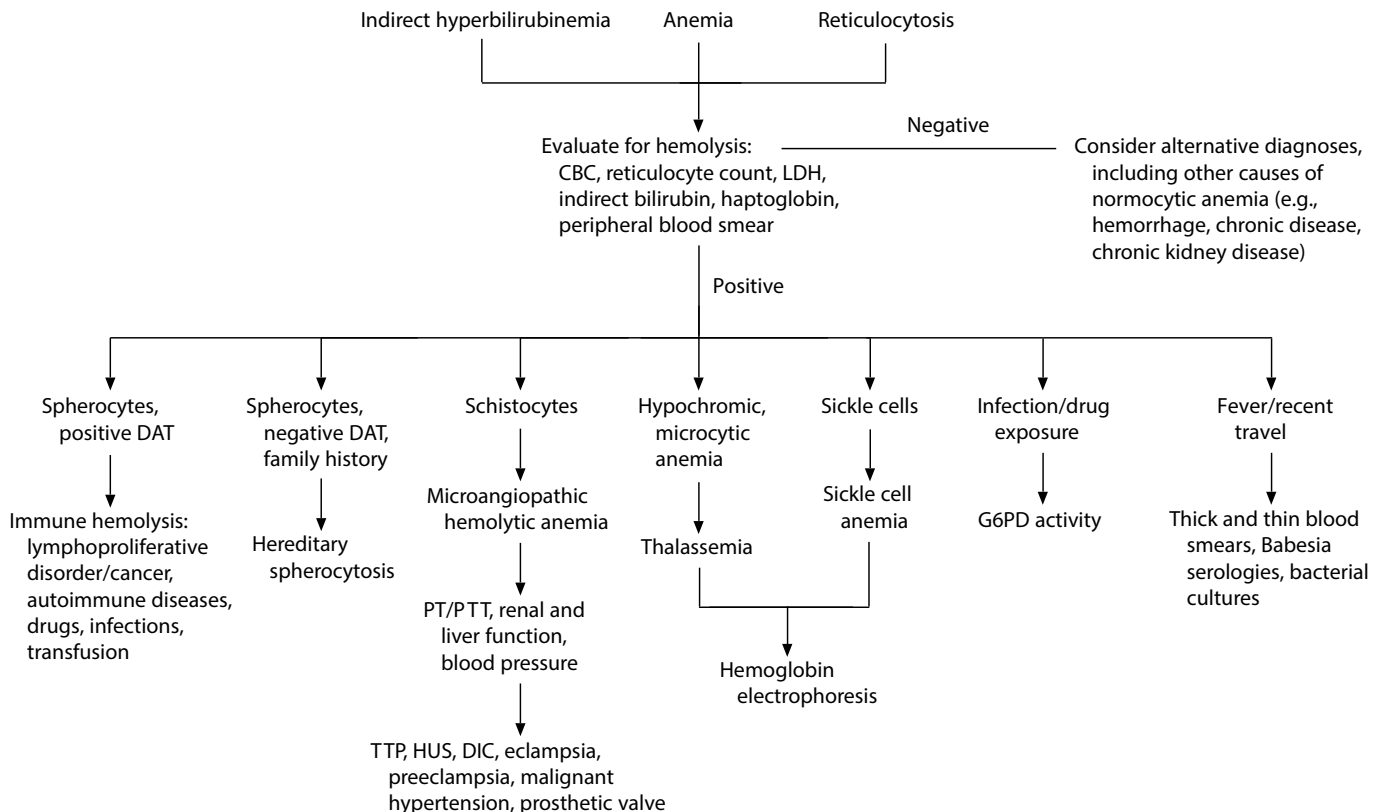
Anaemia with MCV between 80-100 fl are called normocytic. Table 4 enlists the common causes. Dimorphic anaemia (combined deficiency of iron and vitamin B<sub>12</sub> / folic acid) is a common condition. Anaemia of renal origin has a unremarkable hemogram with normal looking peripheral blood smear. Here, anaemia is proportional to the degree of renal failure. Marked anaemia will invariably be associated with high S. Creatinine level. Once again, both ACD & anaemia secondary to marrow disorders are often normocytic.

Lastly, haemolytic anaemias are usually normocytic (Figure 6). These disorders will have clinical features of hemolysis i.e. icterus and splenomegaly. Laboratory evidence will include indirect hyperbilirubinemia, raised LDH, decreased haptoglobin & erythroid hyperplasia in the marrow. There is reticulocytosis and polychromasia (Figure 6).

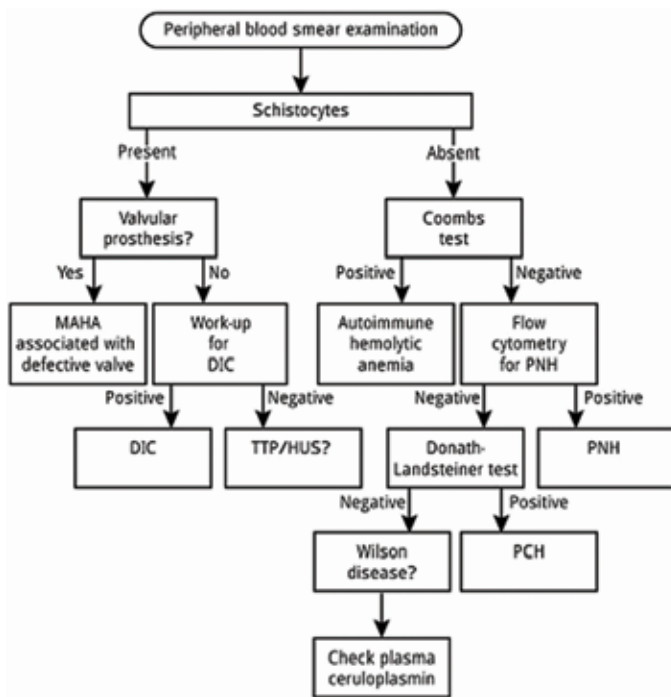
Once haemolytic anaemia is suspected, it's cause has to be found. Peripheral smear is an important test in assessing the etiology of hemolysis (Figure 7). In addition, doing coombs test differentiates between immune hemolysis and others (Figure 8).

In patients without hemolysis, a large number of bone marrow disorders feature and hence marrow examination become a must. This will give diagnosis of important primary marrow disorders e.g. Aplastic anaemia,

## Hemolytic Anemia



**Fig. 6 : An approach to haemolytic anaemia**



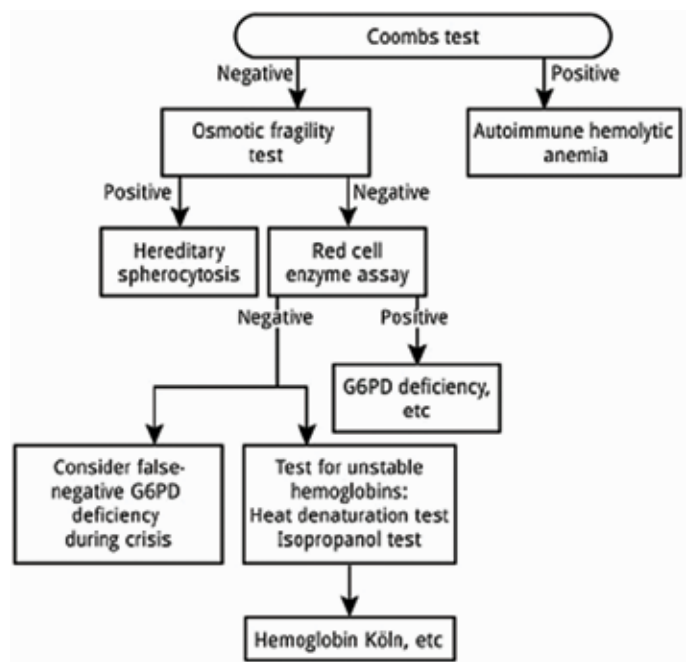
**Fig. 7 : Importance of peripheral blood smear examinations in assessing the etiology of haemolytic anaemia. DIC = disseminated intravascular coagulation; HUS = hemolytic uremic syndrome; MAHA = microangiopathic hemolytic anemia; PCH = paroxysmal cold hemoglobinuria; PNH = paroxysmal nocturnal hemoglobinuria; TTP = thrombotic thrombocytopenic purpura**

acute leukaemia, myelodysplastic syndrome, multiple myeloma, infiltrative disorders etc.

## CONCLUSION

The human mind is a marvelous albeit complex tool. It is difficult to understand how it works. Clinicians are no exception in their varied approach to any clinical problem. The guidelines outlined above have to be used in the context of good history and clinical examinations. Over 100 diseases cause anaemia. It is important to look at various points in history and examination which cannot be dissociated from laboratory investigations. A simple example is a patient with clinical frank features of myxoedema requiring no other investigations except TSH. Similarly, history of recent blood transfusions can vitiate many results.

Laboratory tests such as complete blood count obtained from an electronic cell counter, peripheral smear examination & reticulocyte count are the first line investigations. These give a guideline for selecting further tests. Serum iron studies and S. ferritin assay are sufficient



**Fig. 8: An approach to hemolytic anaemia based on coombs tests and other investigations. G6PD - Glucose-6-Phosphate dehydrogenase**

to in arriving at the diagnosis of IDA. For macrocytic anaemia, smear examination and reticulocyte count form the first line of investigation. Based on the results, further laboratory tests should be ordered i.e. S. Vitamin B<sub>12</sub>, RBC folic acid, hemolytic profile or marrow examination.

If ACD is suspected, various tests e.g. S. Creatinine, TSH, ANA, Anti CCP Antibody & tumor markers may be needed.

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