# What Doctors Must Learn
# Volume 3

## Testing a test


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**Preface**

Advancing technology and easy availability of investigatory modalities including imaging, genetic and immunological tests have made many doctors depend on them for diagnosis of diseases, avoiding detailed history and physical examination. Test results must be correlated with clinical impression and hence are not meant to diagnose diseases but only to confirm, support or rule out the provisional clinical diagnosis. Test results are not always right and dependable. A positive test may be seen without an active disease and a negative test may not rule out the disease. Without a provisional diagnosis, test results could add to confusion besides wasting time and money. There is nothing like “routine tests”. Doctors must know what they expect out of a test rather than treat what they see in the test results. Rationality demands minimum and appropriate tests based on provisional clinical diagnosis.

## Section 1 – General view

### 1.1 Introduction

In evidence-based modern medicine, ideally, clinical bedside diagnosis should be confirmed by relevant laboratory tests. However, itmay not be necessary for every patient and it may not be possible for various reasons. Rational practice demands provisional clinical diagnosis before ordering relevant laboratory tests. Tests are not meant to search the diagnosis but just to confirm, support or rule out clinical bedside provisional diagnosis. Test results must be correlated

with clinical profile and not considered in isolation. Test results are at times confusing and there may be a disparity between test results and clinical judgment that must be cautiously addressed. Hence, **a test must be tested** for its worth in a given situation.
Clinical bedside diagnosis of any disease consists of four components – anatomy (site of disease), pathology (type of disease), etiology (cause of disease) and functional status of an affected organ (normal, compensated or decompensated function). The tests should be planned accordingly to confirm either or all the components of the diagnosis as per the need.

## 1.2. Tests for anatomical diagnosis

A particular system or organ involved in the disease is often evident clinically but further localisation may not be clear. For example, symptoms of respiratory disease may arise from the airways, lung parenchyma, interstitium or pleura. Imaging modalities help in the evaluation of microanatomy. Images must be interpreted in correlation with a clinical profile which is often not shared with a radiologist. Abdominal USG is commonly ordered in case of vague abdominal symptoms.The presence of lymphnodes and fluid in the peritoneal cavity in an abdominal USG are normal findings but the size and other characteristics of lymph nodes and the amount of peritoneal fluid decide whether they are abnormal. Besides, normal abdominal USG may not rule out an abdominal disease. The same is true about other imaging modalities such as X-ray, CT or MRI scans.Normalimages don’t exclude diseases as imaging modalities show only the structural changes and diseases do exist with functional

deterioration without obvious structural abnormalities. Video imaging modalities are able to study the function of an affected organ as in the case of swallowing incoordination. An abnormal imagemay be an artifact, a normal variant and hence needs cautious interpretation. A 2D echocardiogram is commonly used in the diagnosis of heart diseases and may also evaluate heart functions besides structural changes. Similarly, liver disease may be localised either to hepatocyte, biliary tract, venous system or reticuloendothelial system. Selective biochemical tests may be able to pinpoint the microanatomy of liver disease. The same is true about other organs. Thus, it may be necessary to confirm the microanatomical diagnosis by relevant tests.

### 1.3. Tests for pathological diagnosis

Imaging modalities may help to a certain extent to guess the pathology such as edema, caseation, necrosis, fibrosis, consolidation, emphysema or tumor etc. Histology of a biopsied or an aspirated sample detects pathology more clearly as in case of inflammation, caseation or atrophy and helps in the probable etiological diagnosis. Histopathology is most important in the diagnosis of tumors. All such test results have to be correlated with clinical profile to make a certain etiological diagnosis as many pathological abnormalities are shared by different etiology. For example, histopathology of tuberculosis and lymphoma may not be easy to differentiate and at times, both diseases may coexist. Biopsied or aspirated samples can also be subjected to etiological diagnosis of infections. Neutrophilic leucocytosis

suggests inflammation but fails to define the cause. Urinalysis can reveal glomerular inflammationas in case of nephritis and stool microscopy can show mucus or blood as evidence of inflammation. But they do not give etiological diagnoses. Acute phase reactants as ESR or CRP are also markers of inflammation and stool calprotectin of intestinal inflammation. Intra-cellular enzymes leak out into the blood when cells are damaged as happens with hepatocyte damage leading to an increase in ALT / AST. The level of increase corresponds with the degree and acuity of damage. The same is true about muscle damage that leads to an increase in CPK – creatine phosphokinase or LDH denoting cellular damage.

### 1.4. Tests for etiological diagnosis

Infection is the common cause of diseases in routine practice. Culture must be attempted in bacterial infections that may also reveal antibiotic sensitivity against a causative organism. This assumes far more importance in the case of infections that carry a high risk of antibiotic-resistant strains as in the case of tuberculosis, UTI or typhoid fever. However, culture may not be possible in every infection for genuine reasons. For example. Brucella, leptospirosis or rickettsia are difficult to culture. If etiology cannot be confirmed in such infections, at least, supportive tests should strengthen the possible diagnosis as done by antibody tests in these infections. Antibody tests are not ideal because they continue to be positive well after the disease is cured. IgM antibody suggests recent infection and IgG antibody the past infection. Even IgM antibodies may persist in the blood for a few months and

hence by themselves, do not suggest active infection. However, confirmative or supportive tests may not be necessary for every infection as in classical acute tonsilitis, acute bacillary dysentery orcommon viral infections such as measles or varicella. It is because clinical diagnosis is near certain and progress of such a disease is possible to monitor clinically. On the other hand, laboratory tests are mandatory when the choice of drugs depends on accurate etiological diagnosis or progress and complete control of the disease cannot be monitored clinically as in the case of UTI (urinary tract infection), meningitis or tuberculosis. It is clear that antibiotics should not be prescribed without confirmation of an infection, for which provisional diagnosis is a must prior to testing, the exception being an emergency situation as in a very sick patient. Rapid antigen tests are substitutes for culture but have limitations. The antigen may persist for a long time after the disease has been cured and it detects both live and dead organisms and hence may not suggest active infection. The advantage of an antigen test is the fact that the test results are available immediately and prior antibiotic therapy does not interfere with the test results. PCR – polymerase chain reaction – is a molecular test that can detect infection in an early stage when organisms are small in number and the test is available now. It is most accurate and hence more useful. PCR is also used in the diagnosis of genetic disorders. A blood smear may pick up malarial parasites and also LD bodies in kala azar, which are confirmative tests. Similarly, stool microscopy can confirm the diagnosis of amoebiasis or giardiasis and

other intestinal parasitic infections.
Bone marrow examination can pick upleukemia and further special tests can diagnose the type of disease. Diagnosis of autoimmune disorders can be supported by antibody tests and immune deficiency diseases can be diagnosed by specific tests of immune functions. Obviously, such diseases demand accurate diagnosis by laboratory tests to an extent possible prior to starting specific therapy, though the exact etiology of many diseases remains obscure.While the etiological diagnosis should always be attempted, it may not be always possible for genuine reasons andin such cases, supportive tests must be ordered.

## 1.5. Tests for functional diagnosis (to assess functional integrity)

Functional impairment of an organ may exist without structural changes and are therefore not evident on routine imaging or histopathology but are detected with biochemical abnormalities. Commonly employed blood tests include blood sugar, LFT (liver function tests), RFT (renal function tests), thyroid profile, intestinal absorptive functions, electrolytes and acid-base, ABG (arterial blood gas).2D echocardiogram and pulmonary function tests are also commonly used. EEG (electroencephalogram) for epileptic focus and EMG (electromyogram) and nerve conduction for neuromuscular disorders, BERA (brainstem evoked response auditory) for hearing assessment and VER (visual evoked response) for visual acuity are some other tests used in special situations.  Degree of functional abnormality

correlates with the severity of the disease to differentiate between organ dysfunction and organ failure.

### 1.6. Tests for monitoring the progress of diseases

When clinical improvement alone cannot guarantee a complete cure, laboratory tests are necessary. ESR or CRP are often used in such situations. In the case of Rheumatic fever, the patient becomes asymptomatic within a few days but aspirin is continued for a few more weeks till ESR comes down to normal indicative of control of inflammation. In acute bacterial infections such as typhoid, fever may continue longer in which case, the appearance of eosinophils in CBC would suggest an improving condition and rule out antibiotic resistance. (Absence of eosinophils is characteristic of active infection). Similarly, a patient suffering from meningitis may improve clinically but antibiotics may have to be continued longer if repeat CSF has not come back to normal. Chronic infections such as tuberculosis, chronic organ dysfunctions and other chronic diseases such as malignancy need repeat tests to ensure desired control or cure of the disease.

### 1.7. Concept of sensitivity and specificity

It is important to measure the reliability of a given test in terms of accurate prediction of a suspected disease. “Gold standard” is one where the test is 100% reliable. Detection of a malarial parasite in a blood smear is “gold standard” for the diagnosis of malaria and the reliability of every other test would be compared with this gold standard test.
Sensitivity refers to the ability of a positive test to predict the

presence of disease accurately while specificity is the ability of a negative test to predict the absence of disease accurately. Thus, a positive test with 80% sensitivity can diagnose 80% of patients accurately but misses the diagnosis in 20% of patients. Similarly, a negative test with 80% specificity rules out the disease accurately in 80% of the patients but the other 20% may have a disease. Ideally, the test should be 100% sensitive and 100% specific but no test is that accurate. Thus, the physician must keep in mind the probability of false +ve or false -ve test results and should always correlate the test result with the clinical profile. For example, a positive bacterial culture may be due to contamination of a sample or the presence of a commensal organism – one that is normally present but not the cause of the disease as happens in the case of alpha or non-hemolytic streptococci reported in throat culture. It is the beta-hemolytic streptococci that are pathogenic. AFB – acid-fast bacilli (tubercle bacilli) in sputum can be detected only if the number of bacilli in the sample is as high as 5000-10000, culture is positive if the number of bacilli are between 50-100 while GeneXpert can detect even 5 bacilli in the sample. Blood thick smear should be screened thoroughly for the presence of malarial parasites and then a thin smear can differentiate between vivax and falciparum. However, as parasites are present in the blood for a short time that may coincide with the onset of high fever and then disappear from the blood into the spleen. Thus, the timing of a blood smear may influence detection. That is why all the laboratory reports end with a statement “please correlate clinically”

especially when a blood smear does not show malarial parasite.

In summary, modern medical science has evolved with advances in the field of diagnostics. Evidence-based medicine demands proof of the diagnosis for rational treatment. However, there are many limitations of investigatory modalities. Laboratory tests should be used to confirm, support or rule out the provisional diagnosis made on the bedside and should not be used to search the diagnosis. Unfortunately, tests are being misused in place of clinical bedside medicinewhich is vanishing fast. This trend must be reversed and judicious use of modern technology is the need of the hour.

**MCQs**

Q 1 Generalised weakness (feeling tired) may be a result of this system(anatomical diagnosis)

A) Haematological
B) Cardiac
C) Respiratory
D) All of the above

Q 2 Chest x-ray is not ideal to diagnose the disease involving this anatomical site

A) Lungs
B) Pleura
C) Bronchi
D) Mediastinum

Q 3 Laboratory tests fail to diagnose this condition

A) Alzheimer disease B) Irritable bowel syndromeC) Migraine
D) All of the above

A 4 This test is not diagnostic of active disease

A)Mantoux test for tuberculosis B) Reducing substance (sugar) in stool for lactose intolerance
C) Rapid antigen test for malaria
D) All of the above

Q 5 Which of the following statement is WRONG? Positive blood culture may

A) suggest active disease
B) may indicate recently cured diseaseC) may be a commensal D) may be a contaminant

**Answers to MCQs**

Q 1 D, Q 2 C, Q 3 D, Q 4 D, Q 5 B

**Section 2 – Commonly ordered tests**

**2.1 ABC of CBC**

**Back to basics**Complete blood count includes estimation of haemoglobin and haematocrit, number of red blood cells, white blood cells (neutrophils, lymphocytes, monocytes, basophils and eosinophils) and platelets. It is not enough to count the number of different blood cells but equally important is to study the characteristics of the cells in terms of size, shape, colour, maturity including abnormal cells in **peripheral blood smear (PS).**It is an important part of CBC.In fact, cell count must be corroborated with peripheral smear.

PS is used to screen various types of diseases such as infections and blood disorders and monitor their progress.

**RBCs (erythrocytes) and haemoglobin**Normal RBC count is 4.5 to 5.5 million/ ml and Hb 12-15 Gm%. Normal value of RBC count and haemoglobin vary as per the age, especially in early life. Haemoglobin level at birth is as high as 16-18 Gm% which gradually comes down to 10 Gm% at about 3 months of age and then increases to around 12 Gm% at one year of age. Thus, degree of anaemia in infants must be corelated with normal values at that particular age.Low level suggests anaemia. RBCs contain haemoglobin that carries oxygen to the tissues that turns into energy to keep the body healthy and also help in eliminating carbon-dioxide from the body via lungs. RBCs do not have nucleus that makes them change shape which helps smooth travel through blood stream. Anaemia is mainly due to deficiency of iron or B12 / folate but may also be due to haemolysis of RBCs or deficient production by bone marrow. RBC indices would indicate type of anaemia.Polycythemia refers to increased haemoglobin level and is seen in severe hypovolemia as in case of capillary leak syndrome triggered by dengue viral infection and also in cyanotic heart diseases due to chronic hypoxia. Hematocrit–It refers to packed cell volume. Normal level is 35-45%, varies a little with age and gender. Low hematocrit means less number of RBCs.

**WBCs(leucocytes)**Normal WBC count is between 4500 – 11,000/ml. Differential WBC count consists of 50-60% neutrophils, 30-40% lymphocytes, 2-8% monocytes, 2-4% eosinophils and 0-1% basophils. WBCs are the part of

immune system as they fight against infections and defend the body against foreign material. Neutrophils are the first to respond to bacterial ror viral infection. Eosinophils fight against parasitic infections and also respond to foreign material such as pollen or dust mite. Thus, it has a role in allergic disorders. Basophils produce non-specific immune response and also play a role in asthma. Lymphocytes are of two types – B cell and T cell. B cells are responsible for humoral immunity by producing antibodies while T cells kill the organisms. Monocytes are like garbage trucks as they help clearing the dead cells.Neutrophilic leukocytosis suggests acute inflammation that may result from acute bacterial infection, acute viral infection during initial 1-2 days and acute non-infective inflammation as in case of rheumatological disorders. However, typhoid fever, brucella and rickettsia are acute bacterial infections that present with leucopenia. Leucopenia is also a feature of acute viral infections beyond initial 1-2 days. Lymphocytosis is seen in acute viral infection beyond initial 1-2 days, typhoid fever (also monocytosis), chronic infections including pertussis, tuberculosis and lymphatic leukemia and also during recovering bacterial infections. Eosinopenia is seen in acute infections including typhoid fever and normalization of eosinophil count indicates recovery from such infections. Eosinophilia denotes allergic disorders including in case of worm infestations.

**Platelets (thrombocytes)**Normal platelet count is 1– 4 lakhs/ml. Thrombocytopenia is a feature of typhoid fever, vital infections and malaria while thrombocytosis is seen in severe inflammation. Platelets play a major role in control of

bleeding by clamping together to form a clot that plugs the site of bleeding. In case of severe inflammation with oozing of blood, platelet count increases (thrombocytosis) as happens in autoimmune disorders and also in severe destructive bacterial infections. In typhoid, viral infections and malaria, platelet count goes down to moderate level (thrombocytopenia). However, as quality of platelets is good, usually there is no bleeding in such cases. When platelets are reduced to very low level (usually <50000), there exists a risk of bleeding while when they are increased, there is a risk of thrombosis obstruction blood flow. Thrombocytopenia results either from defective production as in case of bone marrow diseases or excessive peripheral destruction.as in hypersplenism or antibody mediated destruction as in immune thrombocytopenia (ITP). Thrombocytosis is seen in iron deficiency anemia, malignancy or severe inflammation.

**Methods of cell counting**Hemocytometer is a counting chamber (Neubauer counting chamber) that was used commonly in clinical practice that involved manual counting. Automated counters are now-a-days used in most laboratories. They provide quick and smooth counting. These counters recognize blood cells by their size – WbCs are larger than RBCs in size, which are larger than platelets. Thus, if RBCs are smaller than normal as in case of iron deficiency anemia, counter counts such small RBs as platelets and hence platelet count is reported to be high. On the other hand, if there is a small blood clot in the sample, platelets are consumed and platelet count reported to be low. Such technical issues must be considered in the interpretation. Ideally, in case of doubt (when clinical profile does not match with the result), results

of platelet count as reported by the counter should be checked with peripheral blood smear. Roughly, number of platelets counted manually in one high power field multiplied by 10,000 should be total platelet count. Automated counters also cannot differentiate monocytes, basophils and eosinophils and they are clubbed together as others. Technician studies peripheral smear and assigns approximate value to these three types of WBCs. Latest generation of automated counters are now available that can recognize all types of WBCs. Besides blood cell count, automated counters evaluate more than 50 parameters either directly or indirectly by calculating the values such as hematocrit and RBC indices.

**MCQs**

Q 1 CBC / PS should be ordered on D1 of fever in this condition

A) Acute tonsillitis
B) Pneumonia
C) Malaria
D) All of the above

Q 2 Ideal time to order CBC in case of undiagnosed feveris on

A) First day  B) After 2-3 days  C) Either of the two
D) Only after D 4

Q 3 Which of the following statement is WRONG?
Bacterial infection may present with

A) Leukocytosis
B) Leucopenia
C) Normal WBC count
D) None of the above

Q 4 Eosinopenia is a feature of

A) Malaria
B) Tuberculosis
C) Rheumatological disorders
D) Typhoid

A 5 Automated counter may mislead in case of

A) RBC count
B) WBC count
C) Hemoglobin
D) Platelets

**Answers to MCQs**

Q 1 C, Q 2 B, Q 3 D, Q 4 D, Q 5 D

## 2.2 CBC in short duration fever

**Basics revisited** CBC is the most common test ordered in routine practice in case of fever. Ideally, it should be ordered 48 hours after onset of fever as it takes some time for the bone marrow to respond appropriately. Therefore, it is important to consider the timing of the test in relation to the duration of the fever. Results must be correlated with clinical profile and not interpreted in isolation. Clinical profile of the disease for interpretation of CBC report includes age, nutritional and immune status of the patient, drugs administered prior to the test and severity of the diseaseProvisional differential diagnosis is a prerequisite to ordering any tests. Random testing without probable clinical diagnosis is likely to confuse more than help in the final diagnosis. Besides total WBC count, differential cell count assumes importance. It is not

only neutrophils and lymphocytes, but eosinophils and platelet count are relevant for interpretation. A peripheral blood smear may also offer clues to the diagnosis. It is important to realise that CBC results can be variable even in the same disease and may have a different interpretation. For example, an acute bacterial infection often presents with neutrophilic leucocytosis but typhoid fever or worsening severe bacterial infection may suppress bone marrow with resultant leucopenia. Similarly, eosinopenia is a feature of acute infection but in a child with an allergy who has increased eosinophils, it may not reveal eosinopenia and confuse the doctor about interpretation. Thus, every part of CBC needs careful assessment and clinical correlation is vital.

Following case scenarios will help in the rational interpretation of CBC report.Note abnormalities, correlate with clinical profile to arrive at a probable diagnosis and find answersat the end.

**Case 1**

2 years old child presented with a high fever, no other complaints for 3. days. Physical examination showed a sick look, high fever, no other localising signs
Hb 10 Gm%, WBC 18000 P 75 L 23 M 2 E 0 Pl 2.8 L

**Case 2**

2 years old childpresented with high fever and cold for a day. Physical examination showed no localising signs.
Hb 9 Gm%, WBC 16000 P 70 L 27 M 3 E 0 Pl 1.1 L

**Case 3**

6 years old child presented with high fever for 3 days, no other complaints. Physical examination showed no localsing signs.
Hb 10 Gm%, WBC 18000 P 78 L 16 M 3 E 3 Pl 2.9 L

**Case 4**

8 years old child presented with fever for 4 days, no other complaints. Physical examination showed sick look with high fever, no other signs
Hb11 Gn%, WBC 3200 P 42 L 48 M 10E 0 Pl 0.8 L

**Case 5**

4 years old child presented with high fever for 3 days, no other complaints. Physical examination showed not a sick child, no abnormality.
Hb 7 Gm% WBC 7400 P52 L 43 M 2 E 3 Pl 0.8 L

**Answers with explanation**

**Case 1**
High fever without localization on D3 in a younger child, neutrophilic leukocytosis (acute inflammation – either infective or non-infective) eosinopenia (acute infection), normal platelets (unlikely acute viral infection), sick child without localization (hidden acute bacterial infection)
Diagnosis – look for **acute UTI** (confirm with urine culture)

**Case 2** High fever and cold (nasal symptom) on D1 in a younger child, neutrophilic leukocytosis, (acute inflammation – infective or non-infective) eosinopenia (acute infection), thrombocytopenia (likely viral infection), nasal symptoms favor viral infection. Diagnosis – **acute viral infection**. Note that neutrophilic leukocytosis on D1may also be an acute viral

infection that may change to lymphocytic response and leukopenia over next 2-3 days.

**Case 3**

High feverwithout localization on D3 in an older child, mild anemia, neutrophilic leukocytosis (acute inflammation – infective or non-infective), normal eosinophils (unlikely an acute infection), normal platelets (unlikely viral infection)
Diagnosis –**systemic inflammatory disease**.
Note the difference in terms of normal eosinophilsthat makes acute infection unlikely. Systemic inflammatory disease may show increasing neutrophilic leukocytosis with thrombocytosis and often decreasing hemoglobin.

**Case 4**

High fever without localization on D4 in an older child, leukopenia, lymphocytosismonocytosis, eosinopenia and thrombocytopenia. All these findings may suggest acute viral infection, However, most vital infections improve over 3-4 days and those who don't improve present with other symptoms and signs. Besides, this child looks sick on D4 that is more likely to be acute bacterial infection Thus, it may be type of acute bacterial infection that responds with leucopenia
Diagnosis – **Typhoid fever**

**Case 5**

High fever in non-sick child without localising signs on D3, low hemoglobin (anemia), normal WBC and differential count (rules out acute inflammatory diseases – both infective and non-infective) thrombocytopenia.
Diagnosis – **Malaria** It should be confirmed with peripheral blood smear

Following table summarizes interpretation of CBC in the diagnosis of common diseases

| Hb | TC | P | L | E | Pl | Diseased |
|---|---|---|---|---|---|---|
| N | +++ | +++ | 0 | N | Acute bacterial infection | |
| N | ++ | ++ | 0 | N | Acute viral infection | |
| N | +++ | +++ | N | high | Systemic inflammation | |
| N | low | ++ | 0 | low | Typhoid fever | |
| N | +/- | +/- | +/- | N | N | Chronic infection or |
| N | Low | +/- | +/- | +/- | N | low Malaria |
| Low | +++ | +++ | N | low | Acute leukemia | |
| High | ++ | ++ | 0 | low | Dengue capillary leak | |

## 2.3 Laboratory diagnosis of anemia

### Basics revisited

Hemoglobin concentration in health differs in relevance to age, gender and hydration state. Hb less than the lower limit of normal for a particular age denotes anemia. In neonates, Hb is 16-17 Gm% which slowly reduces to around 10 Gm% by the age of 3 months. It rises slowlyover the next few years to attain a normal level of 13-15 Gm% in children > 5 years of age and adults, Hb between 11.9-10 Gm% is considered as mild anemia, between 9.9-7Gm% as moderate and< 7 Gm% as severe anemia. Anemia may be caused by deficiency of major nutrients (iron, B12, folate), hemolysis of RBCs (congenital or acquired) or bone marrow disorders (aplasia or infiltration). One or more of these three factors are responsible for anemia of chronic infections, inflammatory diseases and chronic organ disorders (renal, liver, thyroid). Chronic

persistent hemorrhage presents as deficiency anemia while acute severe hemorrhage manifests as shock. RBC indices and peripheral blood smear examination help to narrow down the probable type of anemia. Further tests are necessary to pinpoint final diagnosis.

RBC indices measure MCV (mean corpuscular volume – ratio of haemoglobin % and RBC count – normal value 80-100 femtoliter), MCH (mean corpuscular haemoglobin – ratio of haemoglobin in Gm and RBC count – normal value27-31 picogram/cell), MCHC (mean corpuscular haemoglobin concentration – haemoglobin in Gm and haematocrit – normal value 32-36 grams/decilitre) and RDW (red cell diameter width – ratio of MCV and standard deviation of the mean cell size – range of red blood cell volume – normal value 12-16%) MCV and RDW together can help diagnosis of type of anaemia.
Low MCV N RDW – thal trait, anemia of chronic diseaseLow MCV, H RDW – iron def, thal major, other hemolytic anemias, vit C, E deficiency, copper deficiency
H MCV, N RDW – aplastic anemia, bone marrow disease, liver disease, hypothyroidismH MCV, H RDW – megaloblastic anemia Increased RDW indicates active bone marrow and so also increased platelet distribution width.
Low MCV, MCH and MCHC in iron deficiency anemia High MCV,MCH and normal MCHC in megaloblastic anemia High MCHC in spherocytosis, sickle cell and autoimmune hemolyticanemia.

## Peripheral blood smear

It is an important test in hematological disorders that reveals abnormalities if any in various blood cells. Test results can be altered by technical issues such as delay in making a smear, extremes of temperature exposure, clotted blood or inexperienced technician. Even prior blood transfusion does alter test results. RBCs - It gives information about size (anisocytosis – normal, microcytes or macrocytes), shape (poikilocytosis – normal, spherocytes, sickle-shaped) and color (pink or pale) of RBCs,It can reveal premature cells such as normoblasts, if more than 1% indicating hyperactive bone marrow as in hemolytic anemia, burr cells in renal disease, fragmented RBCs - schistocytes in microangiopathic hemolytic anemia, tear-drop cells in myelofibrosis and myeloproliferative disorders,acanthocytes or spur cells with thorny projections in liver disease, helmet cells in intravascular hemolysis, rouleaux formation meaning stack of RBCs stuck together as in connective tissue disorder, multiple myeloma, diabetes and allergic diseases, Howell-jolly bodies in splenectomy and B12 / folate deficiency, Heinz bodies denoting denatured Hb clumped in RBCs. A confirmative test for malaria is demonstrating the parasite in RBCs, a thick smear helps to pinpoint the diagnosis and a thin smear can recognize the type of parasite – vivax or falciparum. It can also indicate severity by calculating the parasitic index. WBCs – observed band cells are a premature form of polymorphonuclear cells and if increased > 10% in peripheral smear indicates acute bacterial infection, also referred to as "shift to left". Band cells have curved nuclei as against oval in neutrophils. Toxic granules in neutrophils indicate acute bacterial infection. Hypersegmented neutrophils (increase in a

number of lobes) denote B12 deficiency anemia, myelofibrosis, chronic renal or liver disease or leukemia. Premature WBCs – atypical lymphocytes > 5% are seen in EB viral infection, blast cells are seen in leukemia.Basophilic stippling is seen in lead poisoning. Platelets – it is ideal to count the number of platelets in one high power field in a blood smear and correlate it with the platelet count reported by the automated counter. Roughly,the number of platelets in one high power fieldmultiplied by 10,000 should be the expected total platelet count. Such a correlation takes care of technical issues in counter-reporting. Large size platelets – mega platelets suggest hyperactive bone marrow. Immature platelet fraction is the ratio of immature platelets to the total number of platelets and increases in IPF suggests recovering thrombocytopenic condition.

**Clinical application in the diagnosis of anemia**

Here are a few laboratory reports for you to interpret. Answers with explanations are given at the end.

**Case 1**

Hb 8 Gm%, RBC 3.7 PS – microcytic hypochromic, MCV 60, MCH 22. MCHC 27, RDW 18%, WBC 7800, platelet 4.5

**Case 2**

Hb 8 Gm%, RBC 3.5 PS – macrocytic hypochromic, MCV 110, MCH 22, MCHC 34, RDW 18% WBC 3200, hypersegmented neutrophils, platelet 0.7 lakh

**Case 3**

Hb 6 Gm%,RBC 2.7, PS – microcytic hypochromic, normoblasts, target cells, MCV 62, MCH 23, MCHC 25, RDW 24%, WBC 18,000, platelet 2.3 lakh

**Case 4**
Hb 7 Gm%, RBC 2.9, PS – microcytic, hyperchromic, spherocytes, MCV 72, MCH 28, MCHC 40, RDW 12%, WBC N, platelet N

**Case 5**
Hb 8 Gm%, RBC 3.4, PS – macrocytic hypochromic, MCV 110, MCH 33, MCHC 34, RDW 14%, WBC 2500, platelet 0.7 lakh

**Answers with explanation**

**Case 1**Microcytic hypochromic anemia, RBC indices are low except RDW is high. Platelets are high because microcytes (small size RBCs are counted as platelets by automated counter)
Diagnosis – **iron deficiency anemia**

**Case 2**
Macrocytic hypochromic, high MCV and RDW, hypersegmented neutrophils are characteristic of megaloblastic anemia, low WBC and platelet count – pancytopenia is due to suppressed bone marrow that is due to lack of DNA maturation as B12 is necessary for DNA maturation,
Diagnosis – **B12 deficiency anemia**
It simulates aplastic anemia except RDW is high in B12 deficiency while normal in aplastic anemia.

**Case 3**
Microcytic hypochromic, all RBC indices are low and RDW is very high, normoblasts suggest hyperactive bone marrow denoting hemolytic anemia, WBC count is high because normoblasts are bigger than RBCs and hence counted as

WBCs by automated counter (it is necessary to calculate corrected WBC count by subtracting normoblast percentage from total RBCs)
Diagnosis – **Thalassemia Major**

**Case 4**
Microcytic hyperchromic, high MCHC, spherocytes, normal WBC and platelets
Diagnosis – **hereditary spherocytosis**

**Case 5**
Macrocytic hypochromic, high MCV but normal RDW, low WBC and platelet count – pancytopenia
Diagnosis – **aplastic anemia**

## 2.4 Acute phase reactants (ESR, CRP, PCT)

### Back to basics

Acute phase reactants are markers of inflammation that are evident by the change in their serum concentration during inflammation. ESR is an exception as it is not measured in serum, it depends on the sedimentation rate of erythrocytes. Cytokines such as IL6 (interleukin 6), IL1, tumor necrosis factor-alpha(TNF-alpha) and gamma interferon (IFN-gamma) induce the production of acute-phase reactants. Thus, the more the cytokines produced, the higher will be acute phase reactants. Acute phase reactants are classified as positive (increased concentration) or negative (decreased concentration). Positive acute phase reactants include commonly used CRP, PCT (procalcitonin) and also ferritin,

fibrinogen, ceruloplasmin. Negative acute phase reactants include albumin, transferrin, antithrombin.

**ESR** It is measured by mounting blood in a vertical standing tube and allowing RBCs to settle down. During inflammation, RBCs clump together and so descend fast to the bottom. The higher the distance they travel within an hour, the higher the ESR at the end of one hour. Normal value 0-20 mm/at the end of one hour. It is not a good screening test except a three-digit value that suggests serious disease - severe bacterial infection, inflammation or malignancy. ESR depends on fibrinogen level and RBCrouleauxformation. It is increased in case of increased fibrinogen level and macrocytic anemia. ESR is low in case of low levels of fibrinogen (as in HLH), microcytosis, polycythemia, hypergammaglobulinemia, hyperviscosity and high WBC counts.Females tend to have higher ESR up to 20 mm, in men up to 15 mm.There are technical problems such as tilted tube causes elevation of ESR while less anticoagulant with clotting of blood leads to low ESR. ESR rises slowly and has too many variables dependent on it. ESR may have some value as a sickness index - prognosis in a suspected disease. High ESR in an otherwise normal person needs repeat tests after some time before embarking on other tests.

**CRP**

Normal valueis <10 mg/L. It is produced by the liver in response to IL6, it starts rising between 4-12 hours of stimulation and peaks by 24-48hours. It has a long half-life and so it takes several days to come to a baseline after the

stimulus has disappeared. It has a wide range of reference values, thus sequential records are more useful than a single value.Several factors decide CRP polymorphism - such as genetic and phenotypic variables besides environmental and lifestyle issues. Including dietetic factors. Such as high transfat consumption increases CRP level, amount and type of carbohydrates, fiber, protein especially from meat and micronutrients also affect CRP.Different cytokines exert variable triggers to CRP, IL 8 releases CRP from hepatocytes the most. It is elevated in many conditions including traumatic conditions, infarctions, serotonin syndromes (caused by interaction with some drugs that increase serotonin levels).If a high CRP level does not come down within 3 days of treatment, the situation needs a review. The higher the elevated CRP, the more is the likelihood of a bacterial infection. CRP in viral infection is mildly increased. Low platelet with high CRP is seen in malaria, low platelet with normal CRP in dengue viral disease.
In SLE (systemic lupus erythematosus), ESR is high but CRP is low. This is because of the development of antibodies against CRP. While in diseases with low fibrinogen such as HLH (hemolymphocytic phagocytosis), CRP is high but ESR is low. Thus, in specific conditions, both ESR and CRP are important.

**Procalcitonin**– normal level 0.05 ng/ml. It is attenuated by interferon-gamma in response to viral infections and hence it is low in viral infection and is a specific marker of bacterial infection. The level of PCT correlates with the severity of the bacterial infection and also decreases as infection comes under control. It is detectable within 3-4 hours of infection

and peaks at 6-12 hours and has a half-life of 12 hours. In health, it is < 0.05 microgram/L,infection is unlikely if PCT is between 0.1 to 0.5,local infection is often seen with PCT between 0.5 to 2. However, besides likely infection, trauma, surgery and shock of other types are likely to produce such a response. Systemic infection typically results in PCT between 2-10 microgram/L and if >10 it suggests sepsis and septic shock. In localised infections including empyema or subacute bacterial endocarditis, PCT may be normal. PCT is used for initiation, withdrawal or escalation of antibiotic therapy. In primary care, PCT >0.25 microgram/L justifies antibiotic therapy.

**MCQs**

Q 1. This is the earliest detectable acute phase reactant

A) ESR
B) CRP
C) Procalcitonin
D) All of the above

Q 2. CRP level peaks at this time

A) Between 12-24 hours
B) Between 24-48 hours
C) Between 48-96 hours
D) Beyond 96 hours

Q 3. ESR is a parameter to stop drug treatment in this disease

A) Acute bacterial pneumonia B) Tuberculosis
C) Rheumatic fever
D) Nephrotic syndrome

Q 4. This test is commonly used at birth

A) ESR
B) CRP
C) Procalcitonin
D) Any of the above

Q 5. ESR is high but CRP is low in this disease

A) Tuberculosis B) Rheumatic fever C) Nephrotic syndrome D) SLE (systemic lupus erythematosus)

**Answers to MCQs**

Q 1 C, Q 2 B, Q 3 C, Q 4 B, Q 5 D

## 2.5 Urinalysis

Macroscopic (volume, colour, odour, transparency), chemical (urine strips for pH, specific gravity, glucose, proteins (picks up only albumen), bilirubin, nitrites, ketones, RBCs, leukocyte esterase enzyme denoting WBC in urine – 10 or more WBCs/c.mm is pyuria , the intensity of colour change proportionate to the concentration of each compound) and microscopic (cells – RBCs in infections, WBC casts in interstitial nephritis and severe infections, tumour, stone, pyuria in infections, RBC casts in nephritis, granular casts in acute tubular necrosis, waxy casts in chronic renal disease, fatty casts in nephrotic syndrome (lipid particles seen in the protein matrix), crystals in stones, organisms – done on the centrifuged sample under light microscopy but can be done under phase-contrast microscopy that offers more details

and also fluorescence flow cytometry) analysis. Also included are urinary electrolytes, drugs and poison testing, pregnancy testand microbiological cultures.

Volume of urine – In routine practice, one depends on history by asking a patient or parent of a child whether usual amount of urine is passed or not. However, one may have to measure the exact volume in extreme conditions such as oliguria or polyuria. < 0.5 ml per kg of body weight per day is considered as oliguria and > 4 ml per kg of body weight per day as polyuria. Urine volume is not easy to measure and may need collection by catheterisation in young children or sick adults. Unless it is likely to offer information that would change the management, it should be avoided due to fear of infection. Older children and adults can collect urine during 24 hours. Oliguria results from dehydration and glomerular diseases while polyuria is commonly due to diabetes mellitus or diabetes insipidus as well as due to renal tubular disorders.
Proteinuria–Normally proteins are not filtered by the kidney and if small molecular proteins leak out through glomeruli, they are reabsorbed by tubules. Thus, a very small amount of protein (< 150 mg/day) is present in urine. Transient proteinuria may be present in dehydration, fever or intense exercise. Strongly acidic urine and concentrate urine gives false +ve results while alkaline urine and dilute urine gives false -ve results. 24 hour urinary protein estimation is most reliable and can be assessed in a spot sample by urinary protein creatinine ratio – normal < 0.2, abnormal > 0.5 and nephrotic range > 2. Orthostatic or postural proteinuria refers to elevated protein excretion in standing position but

not in lying down position and is due to compression of renal vein between aorta and left superior mesenteric artery. Ideally first morning fresh sample must be examined. Haematuria –red urine may be due to haemoglobinuria or myoglobinuria, drugs like rifampicin or methyldopa and beetroot consumption. Frank red colour suggests bleeding from collecting system as in case of renal stone (fresh RBCs) while cola-coloured urine indicates glomerular disease such as nephritis (crenated RBCs). Few RBCs may be seen in urine due to fever or use of ibuprofen. However one must follow this finding to rule out any significant renal pathology. Endothelial glomerular disease (acute post-streptococcal nephritis) presents with mild oedema, oliguria and hypertension in a mildly sick child besides haematuria while interstitial glomerular disease presents with isolated haematuria. Epithelial glomerular disease per se does not produce haematuria unless pathology has extended to endothelium. Urine culture– Urinary tract infection should be confirmed by urine culture because increased WBCs in urine may also result from non-infective inflammatory conditions such as renal stone, tumour or nephritis. Ideally, mid-stream specimen must be collected after cleaning external part and if possible,the sample should be collected in the laboratory itself so as to avoid contamination and delay in processing. Colony count > $10^6$ is considered significant of UTI.

Here are a few laboratory reports for you to interpret. Answers with explanations are given at the end.

**Case 1**Small volume, urine macroscopy – cola colored, chemical examination - proteins +,urine protein / creatinine ratio 0.3 microscopy – RBCs +, crenated, RBC casts +, WBCs 4-5/HPF

**Case 2**Normal volume, urine microscopy - bright red-colored, chemical examination – proteins +, urine protein / creatinine ratio 0.4, microscopy – RBCs ++, fresh cells, no casts, WBCs 6-8/HPF

**Case 3**
Urine macroscopy – N, chemical – proteins +++, urine protein/creatinine ration 4.5, microscopy – fatty casts

**Case 4**Turbid urine, chemical examination of urinel – proteins +, urine protein / creatinine ration 0.6, nitrites and leukocyte esterase enzyme + on a urinary strip, microscopy – WBCs 50-60/HPF, RBCs +, culture -ve

**Case 5**
Urine macroscopy – N, chemical – proteins +, urine protein / creatinine ratio 0.7, microscopy – WBCs 4-5/HPF, culture E.coli colony count > $10^6$

**Answers with explanation**

Case 1
Cola colored urine suggests the glomerular endothelial or interstitial origin of blood, PC ratio (protein/creatinine) is normal (no significant proteinuria), crenated RBCs and RBC casts suggest glomerular endothelial pathology
Diagnosis – **glomerulonephritis**

**Case 2**

Bright red colour suggests fresh blood coming from collecting system and not kidneys, no significant proteinuria, a high number of fresh RBCs without casts, WBCs in small number that is not significant of pyuria

Diagnosis – **ureteric (stone) or bladder (tumour) pathology**

**Case 3**

Macroscopy N, protein-creatinine ratio 4.5 suggests severe proteinuria of nephrotic range, fatty casts denote increased lipids in urine

Diagnosis – **Nephrotic syndrome** (proteinuria and hypercholesteremia)

**Case 4**

Turbid urine suggests probable infection, no significant proteinuria, nitrites and leukocyte esterase enzyme +ve on urine strip denotes probable infection, a high number of WBCs with few (insignificant) RBCs indicate severe pyuria indicating probable infection. However, culture is -ve.

Most probable diagnosis – **UTI** UTI can be confirmed only with +ve urine culture. When culture is -ve, one must look at clinical presentation (high fever, sick child, backache suggestive of upper UTI, frequency and burning of micturition indicative of lower UTI) and if highly suggestive of UTI, one may consider repeating the culture and start an antibiotic. Negative culture may be a technical error or may also be due to prior antibiotic therapy.

**Case 5**Macroscopy, chemical examination and microscopy are all normal but culture is +ve (significant colony count)

Diagnosis – **asymptomatic bacteriuria**
In such a situation, one must look at the clinical profile and if normal, the best way is to repeat urine culture as the previous result could be due to technical error. However, asymptomatic bacteriuria should not be treated with antibiotics but the patient should be closely observed for any new symptoms or signs.

## 2.6Stool examination

It is mainly indicated in gastrointestinal disorders such as infections, non-infective inflammatory disorders such as inflammatory bowel disease, bleeding disorders due to local causes and maldigestion or malabsorption. A stool sample is collected in a clean container and sent to the laboratory without delay. Tests include macroscopic examination (colour, consistency, odour, volume, presence of mucus or blood), microscopic examination (RBCs, WBCs, macrophages, ova or cyst of parasites, fungal spores, fat globules), chemical analysis (guaiac test for occult blood, reducing substance for sugar malabsorption, calprotectin for inflammation, stool pH - acidic in sugar malabsorption) and culture for bacteria or fungi. Rectal swabfor stool culture is reserved for specific conditions such as immune deficiency or chronic persistent diarrhoea.

### Clinical application

Stool examination in acute diarrhea- In routine practice, it is rarely justified. Viral diarrhea is usually a small intestinal

infection, seen in young children and presents as a large volume of watery stools often resulting in dehydration. Bacterial infection is usually a large intestinal infection and presents as frequent but small volume stools with mucus and / or blood with abdominal pain and high fever. So the distinction between viral and bacterial acute diarrhea is clinical and there is no need for stool examination. Rarely, acute watery diarrhea may be due to cholera – that is rare now. It presents as a large volume of frequent watery stools and the patient mostly presents in shock due to severe dehydration. Hanging drop preparation is necessary to confirm cholera.

Stool examination in chronic diarrhea– It is strongly indicated. Chronic diarrhea may be caused by chronic infections (bacterial, parasitic or fungal), especially in immune-compromised patients in whom malabsorption of multiple nutrients (carbohydrates, proteins and fats besides vitamins and minerals), allergy (especially to animal proteins), bile acid irritation and drug toxicity are additional factors. Thus, chronic diarrhea in such a situation is multifactorial. Besides stool microscopy, one may need chemical tests, tests for malabsorption and culture for various organisms.

**Stool examination for malabsorption** Fat malabsorption
Stool is greasy or oily in case of fat malabsorption and is typically a feature of cystic fibrosis. Stool microscopy shows fat globules in other pancreatic disorders and also in case of excess of fat consumption. In giardiasis. The stool appears to

be greyish white, is large in quantity and has a very foul smell. Ideally, excretion of stool fat over 24 hours in 1-3 days period after ingestion of measured amount of fat can be studied to judge the degree of fat malabsorption. It may assume importance to differentiate various conditions resulting in fat malabsorption. For example, bile acid deficiency and bacterial overgrowth in the intestines may result in a small excess of fat in stools, a moderate amount of fat is lost in stools in celiac disease and severe steatorrhea is seen in pancreatic diseases. Though routinely such tests are not required and mere microscopic presence of stool fat globules in conjunction with clinical profile serves the purpose. Carbohydrate malabsorption– It presents as watery stools with perianal excoriation due to acidic stools and stool shows the presence of reducing substance evident of sugar malabsorption. It is important to realise that reducing substances in the stool should not be tested in case of acute diarrhea. This is because lactase is an enzyme in the most superficial layer of the intestinal mucosa and is the first to be destroyed in case of any intestinal insult. When lactase enzyme is not available, lactose is not absorbed and it is seen as a reducing substance in the stool sample. In acute diarrhea, such a change is very transient and self-limiting. It does not call for a change in diet. The same is true in a breast-fed infant in whom,asbreast milk contains a large amount of lactose, reducing substance in stool has no relevance and so should not be tested. However, in chronic diarrhea, it is not only lactose but also other carbohydrates that are not absorbed and hence test for reducing substance

in stool is relevant. Other testsmay be necessary to substantiate intestinal malabsorption.D-xylose absorption test measures xylose in urine and blood after oral consumption and a low level would suggest intestinal malabsorption of sugar. Specific lactose malabsorption may be found by hydrogen breath test. Unabsorbed lactose enters the colon where it is absorbed and resultant hydrogen is excreted in the breath. Similarly, the Schilling test is used for vitamin B12 malabsorption. Protein malabsorption is evident by low serum protein levels. However low serum protein may be caused by many other diseases and hence not specific to protein malabsorption. Barium study of intestines or other imaging modalities may offer a non-specific clue to intestinal malabsorption. Endoscopy and intestinal biopsy may help in confirming specific diagnosis such as celiac disease or inflammatory bowel disease. Intestinal tuberculosis rarely presents with diarrhea because the disease affects submucosa and not intestinal mucosa. Once the healing starts either naturally or induced by treatment, it results in subacute intestinal obstruction due to scarring and presents as constipation as the main symptom.

Stool examination in stools with mucus and / or blood – Such a condition may be of short duration as in case of acute bacillary dysentery or of long duration as in case of parasitic infections (commonly amoebiasis and giardiasis) or inflammatory bowel disease. Blood in the stool may not be visible by the naked eye. Microscopic examination may prick up blood (also by guaiac test) besides ova or cysts of parasites. Calprotectin in stool is a marker of inflammation.

Rectal bleeding may occur without loose stools and it may result from hard stools as in severe constipation. It may also be a result of hemorrhoids, intestinal polyps, diverticulosis, vascular malformations or abnormal gastric mucosa in the small intestine as in Meckel's diverticulum. For small bleeders in the intestine, radioactive technetium study may be necessary. Refractor anemia is often a presentation of such occult intestinal bleed.

**MCQs**

Q 1 This microscopic findingdifferentiates bacillary dysentery from other non-infective causes

A) RBCs B) WBCs C) Macrophages D) All of the above

Q 2 Microscopic stool examination is normal in this acute bacterial infection

A) Tuberculosis B) Dysentery C) Typhoid fever D) Cholera

Q 3 Stool must be checked for reducing substance in this condition

A) Viral diarrhoea
B) Every case of diarrhoea
C) Chronic diarrhoea
D) All of the above

Q 4 Stool must be checked for fat globules in this condition

A) Liver disease
B) Pancreatic disease

C) Intestinal disease
D) All of the above

Q 5 Stool culture is indicated in this condition

A) Bacillary dysentery B) Chronic persistent diarrhea
C) Typhoid fever
D) Tuberculosis

**Answers to MCQs**

Q 1 C, Q 2 D, Q 3 C, Q 4 D, Q 5 B

## 2.7 Antibody, antigen and PCR

### Back to basics – antibody tests

The body responds to infection by producing antibodies to fight infection. IgM antibody is the initial response that may appear in the first 5-7 days and disappears over the next few weeks (IgM antibodies against dengue and brucella may remain for 2 months, CMV for 4 months, toxoplasma and rickettsia for many months) followed by long-lasting IgG antibodies for a variable time (months or years). Though detection of IgM antibody suggests recent infection, the test may be negative early in the course of the disease and the test may remain positive well after the disease is cured. Besides, there is a possibility of cross-reacting antibodies to other infections.Fourfold rise of antibodies over 7-10 days from the initial level offers high sensitivity but for which one has to wait for that length of time and so it is impractical. IgG antibodies occur a few weeks after infection and persist for a

long time hence they indicate past infection. The presence of antibodies also depends on the patient's ability to mount an appropriate response. Thus, the antibody test is less dependable for diagnosis and test results need cautious interpretation. There are different methods of antibody testing such as agglutination or Elisa, the latter being more reliable. It is ideal to prove infection with culture, antigen detection or molecular test and only when it is not possible that antibody test is an alternative. However, culture must be ordered prior to starting antibiotic therapy. Organism captured on culture may be a contaminant or a commensal and not responsible for the disease. Antigen tests are also available, the results of which are not interfered with by prior antibiotic therapy but they can't differentiate between active and dead organisms. Molecular diagnosis has high sensitivity and specificity but is costly and may not be available. Finally, clinical correlation is important for rational interpretation.

**Indications for antibody tests**

Those viral infections that have a non-specific clinical profile and have a short viraemic period are best diagnosed with IgM antibodies. While PCR can detect viral infections but results may take time and it is not cost-effective. Thus, the IgM antibody test is commonly used to detect viral infections such as EBV, herpes, dengue, chikungunya, CMV as well as measles, rubella and mumps. Few bacterial infections such as leptospirosis,rickettsia and brucella are also detected byspecific IgM antibody tests as these organisms are difficult to culture.

**Commonly employed antibody tests and their limitations**

Widal test–It is widely used in clinical practice though single test result is not dependable and detection of the four-fold rise of antibody though diagnostic is impractical. Theoretically, O antibody suggests recent infection while H antibody denotes past infection. Prior typhoid vaccine may alter results unless the vaccine uses Vi antigen.The tube test is better than the slide test, thus, the Typhidot test is not recommended, it should not be used for the diagnosis of typhoid fever. Besides, many other intestinal gram-negative organisms also show cross-reaction to widal test and even malarial parasites also cross-react. Thus, blood culture is the only way to confirm the diagnosis of typhoid.

Antibody tests for hepatitis – IgM HAV strongly supports the diagnosis of active hepatitis A infection but IgG antibody denotes past infection or immunisation with HAV vaccine. Hepatitis B virus has three antigens, two of them (S and E) in blood and C antigen in the liver. IgM HbC (antibody to core antigen) denotes active infection while antibody against S and E antigen indicates recovering or past infection. Antibody to S alone in absence of HbS antigen is due to prior vaccination. The presence of S antigen suggests of infection that may be active or silent while e antigen represents high infectivity. Other viruses such as CMV, EBV or HIV can also be detected by antibody tests.

ASLO (Anti-streptolysin O) antibody test – it has similar limitations in that positive test suggests recent or prior exposure to such an infection. Streptococcal infections are so

common in the community and it is necessary to know antibody titre in the community. Unless the test shows a very high titre compared to one that is prevalent in the community, the diagnostic value of this test remains limited. In fact, a negative test may rule out recent as well as past streptococcal infections

Leptospira, Rickettsia and Brucella antibody tests – These tests also have similar limitations but as these organisms are difficult to culture, antibody tests are widely used in conjunction with clinical correlation.

Dengue antibody test – IgM antibody becomes positive only after the first few days and so not useful for early diagnosis. However, the presence of both IgG and IgM antibodies in febrile child suggest a second episode of dengue with a risk of immune-mediated complications.

TB antibody test – it should never be used. Even Mantoux test and Interferon-gamma assay such as Gold test are not recommended for diagnosis as they only suggest exposure to infection anytime in the past but not necessarily a recent disease.

Antibody tests for rheumatological and immune disorders– Such tests are specialised tests, rarely ordered in routine practice and should not be used randomly, best left to specialists. They are often not very specific with the exception of Anti-dsDNA (part of ANA) that is diagnostic of SLE (systemic lupus erythematosus). ANA may be normal in 5% of the population and per se is not diagnostic of any disease. Similarly, RA factor is present in polyarthritis in older

female child and should not be ordered in every joint disease. Absence or low level of IgG or IgA deficiency denotes immune deficiency disorder.

**Antigen tests**

These tests are more specific and useful than antibody tests. They have the advantage of quick results available in minutes that are not interfered by prior antibiotic therapy but cannot differentiate live from dead bacilli and antibiotic sensitivity cannot be assessed. Dengue NS1 antigen test is routinely used but may not be positive during the first 24 hours of the illness. A throat swab showing strep antigen may be reliable to diagnose bacterial pharyngitis but is not representative of pneumonia. Urinary antigen tests have been tried in the diagnosis of respiratory infections including pneumonia but have not been found to be reliable. Antigen tests on CSF samples are also used in the diagnosis of bacterial meningitis

Rapid antigen test for malaria – Though blood smear microscopy is the gold standard for the diagnosis of malaria, it needs skill and patience to perform the test. In absence of such feasibility, a rapid antigen test is useful. Glutamate dehydrogenase test and lactase dehydrogenase testis positive in case of active infection by either vivax and falciparum and histidine rich protein test is positive only in falciparum but cannot differentiate live from dead parasites. Antigen test may remain positive for a month or more even after control of infection.

NS1 antigen test for dengue virus – This test is useful for early diagnosis as it is positive in first 24-48 hours. Though, in

5% of patients, it may be negative in the initial days. It is more specific for the diagnosis of dengue fever as compared to antibody tests.

Rapid antigen test for streptococcal throat infection – it is a fairly reliable test, the result of which is available in few minutes as against the culture that takes 2-3 days. However, this test is specific only to streptococcal infection and antibiotic sensitivity is not available. As viral infections are common, this test can decide the need for antibiotic therapy.

Rapid antigen test for pneumonia – Sensitivity and specificity of such a test is not high and result also depends on the collected sample (blood or urine).

Rapid antigen test for meningitis–Latex particle agglutination test (LPA) in CSF is considered to be a useful test in the diagnosis of meningitis as compared to gran staining and culture. Test can screen common organisms causing meningitis such as streptococcus pneumoniae and haemophilus influenza.

Antigen test for Covid 19 infection – they are less sensitive as compared to PCR but test result is available immediately. However, in a symptomatic patient, positive antigen test may be used for diagnosis.

**PCR – Polymerase Chain Reaction (also known as real time PCR or reversed transcription PCR – rtPCR, quantitative PCR)**

The test detects DNA (genetic material that contains information for all living things) or RNA (information copied from DNA and involved in making proteins) of an infectious

agent or abnormal cells in the sample. PCR besides being accurate, becomes positive in very early stage of the disease, even before symptoms appear, when there are not enough existing pathogens or antibodies for detection. Test employs multiplication of small amount of genetic material several times known as amplification that makes it easy to detect.

PCR is useful in detection of infectious diseases, genetic disorders and malignancy. Test can be done on any tissue including blood or saliva. The sample will show DNA besides patient's own DNA. Polymerase enzyme is added to the sample to produce copies of existing genetic material and the process is repeated several time to produce large number of copies that helps detection of abnormal DNA.
Certain viruses are made up of RNA instead of DNA. In such cases, RNA has to be changed into DNA before copying (rtPCR). Amount of genetic material can be counted in the same sample (qPCR).Multiplex PCR can screen several pathogens at a time in a given sample and is used in the etiological diagnosis of pneumonia or meningitis.

False -ve results in PCR may be due to insufficient sample, very small pathogen load and their dynamics and variability in techniques. However, chance of false -ve test results is extremely small. False +ve results are negligible, though may be caused by contamination.

GeneXpert test for TB – Test is ideally done on a sputum sample but lymph node or other biopsied tissues can also be subjected to this test. Positive yield from CSF sample in TB meningitis is low and further lower in pleural fluid in TB

pleural effusion. CB-NAT – cartridge based nucleic acid amplification test is used in India. The test also detects rifampicin resistance if any. The test is positive even if a sample has 10-15 bacilli per ml as against culture that needs 50-100 bacilli per mlwhile smear needs 5000-10000 bacilli per ml in the sample. Besides, test also picks up dead bacilli and so test may be positive in recently treated patient. GeneXpert Ultra is a further modification that can detect even small number of bacilli in the sample. However, as sensitivity increases, specificity goes down a bit and so there can be false +ve results.

**MCQs**

Q 1 IgM antibody denoting recent infection may persist for

A) 4-5 days
B) 1-2 weeks
C) 2-3 months
D) Any of the above

Q 2 This disease is diagnosed in routine practice only by antibody tests

A) Typhoid fever B) Corona virus C) Leptospirosis D) Malaria

Q 3 Typhoid fever may be confirmed by

A) Typhidot test B) Widal slide test
C) Widal tube test
D) None of the above

Q 4 Streptococcal antigen test for pharyngitis is not used in India because

A) It is not reliable
B) Clinical diagnosis is certain
C) It is not cost-effective
D) None of the above

Q 5 Which of the following statement is WRONG ?.
GeneXpert test for tuberculosis can be done on this sample

A)Sputum
B) Gastric aspirate
C) Blood
D) Lymph node

**Answers to MCQs**

Q 1 D, Q 2 C, Q 3 D, Q 4 C, Q 5 C

## 2.8 Liver function tests

### Back to basics

Liveris one of the largest organs in the body and is unique in terms of ability to react to damage and repair as well as regenerate. It performs multiple functions such as synthesis of plasma proteins such as albumen and clotting factors, production of bile, excretion of bile, cholesterol, hormones and drugs, metabolism of proteins, fats and carbohydrates, enzyme activation, storage of glycogen, vitamins and minerals, detoxification and purification of blood. In routine

clinical practice, few blood tests are commonly used in the evaluation of liver diseases.

**Initial screening tests**

Urinalysis– High coloured urine with presence of bile salts and pigments suggests direct bilirubinaemia.
Blood tests -These tests include bilirubin (decides the **extent** of the disease), – conjugated and unconjugated fractions,proteins – albumen and globulin (evaluates **chronicity** of the disease), enzymes(assesses **acuity**of the disease) – SGOT (AST), SGPT (ALT), alkaline phosphatase and gamma-glutamyl transferase (**GTT – specific to bile duct disease)** and prothrombintime (estimates **seriousness** of the disease).

Serum bilirubin – normal level 0.2-1.2 mg%, of which direct (conjugated) fraction is < 0.3 mg%. Generally, icterus is not visible in the eyes unless total bilirubin is > 2 mg%. In case of increased bilirubin level, if direct fraction is > 20% of the total bilitubin, it is considered as conjugated bilirubinaemia. In such a case, urine is high coloured (dark yellow) and chemical examination of urine shows bile salts and bile pigments. If direct fraction is < 20% of the total bilirubin, it is considered as indirect (unconjugated) bilirubinaemia. In such a case, urine is not high coloured (pale yellow) due to urobilinogen. Increased direct bilirubin is due to liver cell (hepatocyte) disease or biliary tract disease. Itching in a non-sick child with higher degree of jaundice is characteristic of biliary tract disease as compared to sick child with proportionately lesser degree of jaundice suggests hepatocyte disease. Increased

unconjugated bilirubinaemia is due to haemolysis of RBCs that generates bilirubin in amount that liver cannot conjugate and hence retained in the blood. It is not water soluble and so cannot be excreted in urine. Besides, heaptovyte or biliary tract disease and haemolysis of RBCs, congenital enzyme defects may also result in jaundice – either conjugated (Dubin-Johnson or Rotor syndrome) or unconjugated (Criggler-Najjer or Gilbert syndrome). Such enzyme defects are benign except Criggler-Najjer type 1 that may result in brain damage. They manifest with jaundice without abnormal liver functions or anemia.

SGOT / SGPT(AST / ALT) – normal level 5-40 units. SGPT is more specific to liver pathology while SGOT is raised in muscle diseases including heart muscle and also intestinal mucosa and high WBC count. When both are increased but SGOT is higher than SFPT, it suggests systemic disease (typhoid,malaria) involving multiple organs in which liver is also diseased while SGPT is higher than SGOT denotes primarily liver disease (viral A hepatitis). Marked increase in thousands denotes acuity of hepatocyte damage as seen typically in viral A hepatitis while moderate increase is seen many other hepatocyte diseases. Enzymes may be very low if most of the hepatocytes are already destroyed as happens in late stages of liver failure, however, by then all other liver functions are severely abnormal.

Alkaline phosphatase and gamma-glutamyl transferase
Normal Alkaline phosphatase is up to 150 IU (varies with laboratories) and GTT up to 40 IU. Alkaline phosphatase is

increased inhepatic/biliary tract and bone diseases but gamma GT is more specific to biliary tract disease.

Serum albumin and globulin -Normal total protein 6-7 Gm% of which albumen is 3.5-4 Gm%and remaining is globulin. A low level of albumin with normal or increased globulin(reversal of albumen-globulin ratio) suggests chronic hepatocyte disease.

Prothrombin time – Normal 11-13 seconds. Most of the time, result is given as INR (international normalised ratio – calculation based on PT), normal value is between 1 and 1.5.Increased INR suggests liver cell failure.

**Other tests**

Blood tests - glucose and ammonia are considered in suspected liver cell failure. Infection can be proved by serological tests such as IgM HAV for acute hepatitis A disease and so also other infections (hepatitis B and C, CMV, EBV). Extra-hepatic infections may also result in hepatitis (typhoid, leptospirosis, malaria) for which specific tests may be necessary. Diseases caused by inborn errors of metabolism require relevant tests (serum ceruloplasmin for Wilson disease or hypoglycemia in glycogen storage diseaseand specific metabolic tests as in case of tyrosinosis).
Imaging study – USG helps to evaluate hepatomegaly and liver cell architecture, gall bladder and biliary tract, portal venous system, splenomegaly and ascites. MRI can assess degree of iron overload and fibrosis as well as fat in the liver.
Liver biopsy – helps to diagnose cirrhosis and offer clues in

support of inflammatory disorders (autoimmune hepatitis and other types of hepatitis) and storage disorders.

In summary, urinalysis for bile salts and pigments along with serum bilirubin, proteins, enzymes and INR can evaluate micro-anatomy and pathology of the disease as well as functioning level of liver cells. Specific tests would be necessary for further etiological workup.

**MCQs**

Q 1 Total bilirubin is 2 mg%, direct fraction is 0.8 mg%, indirect fraction is 1.2 mg% It is suggestive of

A) Indirect bilirubinemia
B) Direct bilirubinemia
C) Both together
D) Any of the above

Q 2 Total bilirubin 4 mg% Direct 3 mg%, SGPT 1200 SGOT 400 Serum proteins 6.5 Gm% Alb 3.8 Gm%. It mostly suggests

A) HAV B) HBV C) HBC D) Any of the above

Q 3 Total bilirubin 4 mg%. Direct 3 mg% SGPT 300 SGOT 450 Serum proteins 6 Gm% Alb 3.5 Gm%, Alkaline phosphatase 30 units. Hb 7 Gm% It mostly suggests

A) Hepatitis B B) Cholangitis
C) Malaria
D) None of the above

Q 4 Total bilirubin 32 mg% Direct 22 mg% SGPT 30 SGOT 40 Alkaline phosphatase 40, Serum proteins 5 Gm% Alb 2.2 Gm% INR 2.8 It is most suggestive of

A) Acute hepatitis
B) Acute hepatitis with failure
C) Chronic hepatitis
D) Chronic liver disease with failure

Q 5 Total bilirubin 12 mg% Direct 10 mg% SGPT 50 SGOT 30 Alkaline phosphatase 600 Serum proteins 6.5 Gm% Alb 4 Gm% INR 1.8 It is mostly suggestive of

A) Acute hepatitis with failure
B) Chronic hepatitis with failure
C) Biliary obstruction
D) Any of the above

**Answers to MCQs with explanation**

Q 1 B – direct bilirubin level more than 20% of the total or more than 2 mg% is considered direct bilirubinemia.

Q 2 A – markedly increased SGPT suggests acute liver disease such as hepatitis A. Such a high SGPT does not indicate serious disease. In fact, mild increase in bilirubin in this child suggests mild disease.

Q 3 C – SGOT > SGPT suggests extra-hepatic disease that has caused hepatitis. Low Hb level may denote malaria as possible cause. Such findings are also seen in typhoid but not low Hb.

Q 4 D – markedly high bilirubin level suggests severe disease, low proteins and albumin indicates chronic disease, high INR denotes liver cell failure in which as liver cells are destroyed, there is no more enzymes available to leak out in blood. It is

important to note that higher bilirubin with increased INR with normal enzymessuggest bad prognosis.

Q 5 C – moderate increase in bilirubin with marked increase in alkaline phosphatase and normal protein level suggests primary biliary tract obstructive disease.

## 2.9 Renal function tests

### Back to basics

Kidney has four major functions. They include filtration (cells, proteins and large molecules are retained), reabsorption (water and small molecules), excretion (waste products) and secretion(H+, K+, NH3, urea, creatinine, histamine, drugs like penicillin, hormones such as renin, erythropoietin, calcitriol). These functions maintain homeostasis in the body.Filtration is the glomerular function and reabsorption the tubular function.

### Initial screening tests

Urinalysis is a simple and useful screening test. Macroscopic, microscopic and chemical examination offer a clue to a probable diagnosis. (Details of urinalysis can be found in another chapter). GFR – glomerular filtration rate – tests filtration function and serum urea and creatinine reflect the same. Serum proteins – albumin and globulin, calcium and phosphorous may represent abnormal urinary loss and so also serum electrolytes and blood gas. In most of the renal diseases, glomerular dysfunction goes hand in hand with

tubular functions.Isolated tubular dysfunction needs special tests but it is not common in routine practice.

eGFR – Estimated GFR is a rough measure of glomerular filtration function but is adequate for monitoring progress in slowly progressive renal diseases. It is calculated by the following formula. eGFR = 0.55 X height in cm divided by serum creatinine in mg% (0.55 as a constant is used > one year of age, in neonate it is 0.35 and in infants 0.45). Normal eGFR varies between 80 and 120 ml/min. eGFR < 60 ml/min suggests renal disease.

Serum creatinine – It is a measure of glomerular filtration. Normal value varies a lot with age. Most laboratories have a printed normal value that represents adult value in which 1.2 mg% is considered a higher limit of normal. For practical purposes, three-fourths of height in cm is a number that should be considered as a higher limit of normal serum creatinine with change in decimals. For example, if a child's height is 100 cm, then three-fourths is 75 and so the normal level of serum creatinine in such a child should be < 0.75 mg%. It is important to realise that serum creatinine increases beyond the normal limit only when eGFR decreases to less than 30 ml/min. Thus, abnormal serum creatinine is a late sign of renal disease and eGFR should be used for monitoring chronic progressive glomerular disease.

Blood urea nitrogen (BUN) – It is a product of dietary protein metabolism as against creatinine is a product of muscle protein metabolism. Blood urea includes nitrogen and other molecules and is approximately double the amount of BUN.

The normal level of BUN is 7-10 mg%. Both BUN and serum creatinine (Cr) are increased in renal dysfunction. The normal ratio of BUN / Cr varies between 12-20. If > 20, it suggests prerenal disease (dehydration or hypoperfusion). This is because BUN is reabsorbed by tubules in prerenal conditions. If the ratio is < 12, it may be due to renal disease or liver disease (ammonia is not converted into urea) or malnutrition due to protein deficiency.

Serum proteins – albumin and globulin– Albumin is lost in the urine in case of glomerular epithelial disease, such as nephrotic syndrome.It results in hypoalbuminemia and compensatory increase inalpha-2 macroglobulin to maintain osmotic pressure in the blood. Thus, albumin–globulin ratio is reversed. (It is also reversed in chronic liver disease)

Other blood tests - Excessive loss of electrolytes in urine results in lower level while abnormal retention leads to higher level. Results are corroborated with urinary electrolytes. Such tests include serum sodium, potassium, chlorides, bicarbonate, ammonia, calcium, phosphorous, uric acid etc. Anion gap is the difference between (Na + K) and (Cl + HCO3). Arterial blood gas detects disturbance in acid-base metabolism. Low anion gap metabolic acidosis is seen in renal diseases and also in diarrhoea while high anion gap metabolic acidosis is seen in diabetes and sepsis.

Imaging tests – Structural abnormalities are picked-up by abdominal USG as in obstructive uropathy. Micturating cystourethrogram (MCU) detects vesico-ureteric reflux. CT,

MRI, angiography and radionuclide scans are other imaging modalities used for specific purposes.

Renal biopsy – It may be necessary in chronic medical renal diseases that helps planning therapy and monitoring progress.

In summary, urinalysis including microscopy, chemical examination and bacterial culture along with blood urea nitrogen and serum creatinine are basic investigations for diagnosis of common renal diseases. Abdominal USG is useful for diagnosis of surgical renal disorders and occasional medical renal diseases.

**MCQs**

Q 1 eGFR is an ideal test in this condition

A) Acute nephritis
B) Nephrotic syndrome
C) Obstructive uropathy
D) None of the above

Q 2 Higher limit of serum creatinine in a normal 4 year old child is

A) 0.4 mg%
B) 0.5 mg%
C) 0.6 mg%
D) 0.7 mg%

Q 3 Ratio of blood urea and serum creatinine> 20 suggests

A) Prerenal disease B) Acute renal disease C) Chronic renal D) Post-renal disease

Q 4 Arterial blood gas is an important test in

A) Glomerular disease
B) Tubular disease
C) Either of them
D) Both of the them

Q 5 These imaging tests are necessary in an infant suffering from urinary tract infection

A) Abdominal USG
B) Radionuclide scan
C) Micturating cystourethrogram
D) All of the above

**Answers to MCQs**

Q 1 C, Q 2 D, Q 3 A, Q 4 B, Q 5 D

## 2.10 Hormonal tests

### Back to basics

The thyroid function test is routinely used in office practice. Other hormonal tests are selectively used such as growth hormone, sex hormones, adrenal and parathyroid hormones. Hypothalamus-pituitary axis controls the production of thyroid, parathyroid and adrenal hormones while the pituitary itself generates growth hormone and anti-diuretic hormone besides LH and FSH.

**Initial screening tests**

Thyroid function tests include T3, T4 , FT4 and TSH. Normal– TSH 0.5-5 mu/L, T3 80-200 nanogram/dL, T4 5-12 microgram/dL, FT4 07-1.5 microgram/dLMost of T3 and T4 are both bound to different proteins and so interpretation of their value should consider available protein binding sites. As T4 is converted into T3, free T4 is considered as the early indicator of hypothyroid state. TSH is increased in the hypothyroid state and decreased in the hyperthyroid state. The incidence of iodine deficiency has decreased with the use of iodised salt though it still exists in some parts of our country. It presents as an endemic goiter with low T4 but high T3 as a result of an adaptive response of conversion of T4 and TSH s often normal. Congenital hypothyroidism is seen in 1 out of 3500-4000 neonates and leads to developmental delay if diagnosed late. Hence neonatal screening is strongly recommended. TSH is estimated in cord blood as a screening test for congenital hypothyroidism.

Growth hormone is a pulsatile hormone-dependent on many variables and hence a single estimation is of no use. IGF 1 – insulin-like growth factor 1 level in blood correlates well with growth hormone and hence used in clinical practice. Growth hormone can be estimated by a challenge test in which its production is stimulated by other factors such as insulin. In case of the absence of an expected increase, GH deficiency is diagnosed.

Adrenal functions – cortex produces steroids (glucocorticoids, mineralocorticoids, progestins, androgens

and estrogens) while medulla produces adrenaline and noradrenaline.
Urine steroid profile, serum electrolytes, ACTH stimulation test and imaging studies help to diagnose adrenal disorders.

Parathyroid function–It helps to maintain calcium homeostasis by increasing absorption from intestines, retention of calcium by kidneys and maintaining blood level of calcium by moving calcium from bones.
Initial screening test includes serum calcium, phosphorous and alkaline phosphatase and serum parathormone (PTH). X-ray of bone helps to define rarefaction if any.

## 2.11 Blood glucose

### Back to basics

When demand for glucose and energy is met, the remaining glucose is converted into glycogen by insulin and stored in the liver. Glycogenolysis can provide glucose only for a few hours and if fasting continues, the brain uses ketone bodies formed from the breakdown of fatty acids to provide energy.Initial screening testNormal fasting blood sugar <100 mg%, post-prandial <140 mg% A1C <5,7and fasting insulin< 25mIU/L which comes back to the same level at the end of 3 hrs during glucose tolerance test.C-peptide is another hormone produced by the pancreas and its normal level is between 0.5-2 nanogram/mL and it correlates well with insulin level. Thus, C-peptide level is used to differentiate between type 1 (insulin deficiency and so low C-peptide level) and type 2 (insulin resistance so high C-peptide)
Hyperglycemia (fasting blood sugar > 125 mg%) can result

from insulin deficiency or resistance. Hypoglycemia (less than 55 mg%) may be caused by hyperinsulinism, failure of glycogenolysis or neoglucogeneis and defective fatty acid metabolism.

## Section 3 – Other tests

### 3.1 Tests for musculoskeletal disorders

#### Back to basics

Muscle disorders may be inflammatory (myositis), degenerative (myopathy and muscular dystrophy), neurological (paresis).
The most common skeletal disorders are vitamin D deficiency (rickets in children and osteomalacia in adults) and osteoporosis. Other disorders include inflammatory (osteomyelitis, arthritis), tumors and skeletal dysplasia.

#### Initial screening tests

CPK - creatine phosphokinase – normal value 20-120 U/L
High value is seen when muscles are damaged as the enzyme leaks out into the blood and it could be as high as thousands. However, as the muscle damage increases, CPK level goes down as there are not many muscles left for the enzyme to leak out. CPK may increase even with a prick while collecting a blood sample.
Other tests include electromyography, muscle biopsy and genetic tests.

Vitamin D–It is important for bone health and also contributes to immune function and helps other organs. The best source of vitamin D is sunlight (Ideally half an hour

between 11 am and 1 pm with open body surface as much as possible) though some of the food items such as fish, egg, cheese etc do provide a small amount. Hence in western countries, many food items including milk are fortified with vitamin D. Deficiency is supported by high alkaline phosphatase and low phosphorous in blood and x-ray of bones but may be confirmed by low blood level of 1:25 OH cholecalciferol. However, normal value of 1.25 OH D > 18 picogram/mL

Other tests include DEXA scan for estimating bone density and imaging modalities.Rheumatological disorders need specific antibody tests and HLA B12 for spondyloarthritis.

### 3.2 Tests for bleeding disorders

#### Back to basics

In case of injury, the blood clot is formed at the site to stop bleeding through the interplay between many clotting factors and platelets. Deficiency of any of these factors or platelets results in excessive bleeding. Besides, vasculitis may also lead to the oozing of blood out of the blood vessels.

#### Initial screening tests

Bleeding time, platelet count, PT- prothrombin time and aPTT – partial thromboplastin time help to differentiate between platelet and coagulation factor deficiencies. Bleeding time is prolonged in platelet disorders (thrombocytopenia, DIC, Won Willebrand disease and late stage of liver cell failure). PT is prolonged in vitamin K deficiency, early stage of liver cell

failure and DIC) and aPTT is prolonged in hemophilia, late stage of liver cell failure and DIC.

### 3.3 Pulmonary function test

Back to basics

Airways are responsible for ventilation (moving air in and out) and lung parenchyma helps in oxygenation and removal of carbon dioxide. Oxygen diffuses from the alveolar membrane into the blood and carbon dioxide diffuses back into the alveoli. In health, all segments of the lungs are ventilated and perfused by blood to facilitate the transfer of gases.

**Initial screening tests**

Tests for ventilation – spirometer is used to assess ventilatory function. It assesses lung volume, lung capacity, rate of flow. It can differentiate (FEV1 / FVC) between obstructive and restrictive airway disease. Bronchodilator challenge can show reversibility of airway obstruction. The peak flow meter can measure peak expiratory flow rate (PEFR) and is used to monitor the improvement of obstructive airway disease on treatment.

Tests for gas exchange – Arterial blood gas measures $PaO_2$ and $PaCO_2$ and evaluates acid-base disturbances. Alveolar-arterial oxygen gradient (A-a $O_2$ gradient) can be calculated that evaluates the presence of a shunt if any (difference between ventilation and perfusion)  The pulse oximeter is a part of clinical bedside measurement that estimates oxygen

saturation in capillary blood. Such a measurement has limitations but is an easy non-invasive method.

### 3.4 Immune function test

Back to basics

We are born with innate immunity that consists of mechanical barriers such as skin, mucus membranes, stomach acidity as well as neutrophils, monocytes, macrophages and natural killer cells (NK cells). Adaptive immunity is provided by B lymphocytes through the production of antibodies and T lymphocytes through cellular immunity. IgM antibody is an initial response but short-lived while IgG antibody develops later but lasts long. IgA antibodies work at the mucosal level.

### Initial screening tests

T cell function is assessed by CBC (absolute lymphocyte count), flow cytometry (CD4 and CD8 cells), Mantoux test and ADA – adenosine deaminase and molecular tests
B cell function is evaluated by serum immunoglobulins (IgG, IgM, IgA, IgE), flow cytometry (CD 19 and CD 20)
Phagocyte function is assessed by CBC (neutropenia), NBT (nitro blue tetrazolium) and DHR (dihydrorhodamine)
Leucocyte adhesion defect presents with leucocytosis with non-healing ulcer without pus formation.
Complement function is evaluated by CH 50 (classic pathway) or AH 50 (alternate pathway)

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