CHAPTER 5

Plasma Enzymes and Proteins including Immunoglobulins and Tumor Markers

Plasma contains a large number of enzymes and proteins with different functions. Evaluation of plasma for activities of different enzymes, total protein, certain groups of proteins or individual proteins, has become quite important in diagnosis and management of a large number of diseases. Enzymes may be measured in terms of their activities or by immunochemical methods. Ordinary chemical methods are used for estimation of total protein and its albumin fraction. Different electrophoretic techniques are used to separate different plasma/serum proteins. Immunochemical and immunonephlometric methods are now available for almost any specific plasma protein, including hormones and also for enzymes.

ENZYMES

Enzyme activities or concentrations in serum may be elevated by a number of factors. It may be increased production. Level of alkaline phosphatase (ALP) increases when production of this enzyme (in liver or by osteoblasts in bone) increases. It may be leakage of an enzyme from certain damaged cells. In hepatic damage ALT, AST and LDH, which normally, remain inside cells, are released into circulation because of increased permeability of the damaged cells. Similarly certain enzymes leak out of myocardial cells, when these cells are damaged in myocardial infarction (MI). In acute pancreatitis there is necrosis of pancreatic tissue. This leads to activation and leakage of pancreatic enzymes into circulation. Certain enzymes catalyze reactions in plasma itself. These are produced in some tissues but enter circulation for their actions. Lecithin cholesterol acyl transferase (LCAT) for example, is synthesized in liver and functions in blood. In liver disease, as its synthesis is reduced, its level in blood decreases.

To determine activity of an enzyme present in serum, most commonly, serum is added to the specific substrate at a concentration, much higher than Km, and incubation started. Enzyme activity is calculated from the initial rate of reaction in kinetic assays. In end point assays, the incubation is completed for a fixed period and enzyme activity is calculated from either decrease in concentration of the substrate or from increase in concentration of the reaction product. Problems in enzyme assays include, rapid inactivation at room temperature and need for transport/storage at a suitable temperature, need to avoid hemolysis of the sample (the leaked red cell enzymes may make results inaccurate), non-availability of control material and need for meticulous control of conditions of reaction. Also see Chapter 1.

Immunochemical assays

Immunoassays are used for determination of enzyme protein levels. These assays are primarily used to determine the levels of specific isoenzymes of an

enzyme and take advantage of difference in amino acid sequence between different isoenzymes. Isoenzymes of CK have been studied by these methods.

Aminotransferases

These are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Muscle tissue (skeletal, cardiac) does not contain ALT which comes mainly, from liver. AST is found in muscle, liver and red cells (hemolysis, however, significantly increases only LD and acid phosphatase). AST along with CK increases in muscle disorders. AST is increased (along with reticulocytosis and low levels of haptoglobin) in hemolytic disorders. 25% of cases of pulmonary embolism (important in differential diagnosis of myocardial infarction) have raised levels of AST. LD increase (total) is associated with all above referred disorders and is thus nonspecific. In liver ALT is a cytoplasmic enzyme while AST is both cytoplasmic and mitochondrial. In viral hepatitis there is greater rise of ALT than AST. Reverse happens in most other diseases of hepatocellular damage. Delay in analysis of sample can increase level of AST.

In congestive heart failure (due to hepatic cell necrosis secondary to hepatic congestion), LD and AST increase along with ALP and with slight increase of bilirubin. In pulmonary embolism/infarction, there is increase of LD, bilirubin (in about 20% patients) and occasionally of AST.

Lactate dehydrogenase (LDH/LD)

Total LD lacks specificity. LD isoenzymes offer greater specificity and have greater clinical importance. In myocardial infarction levels of LD_1 and LD_2 are important. These can be estimated electrophoretically. One may estimate total LD and the heat stable LD_1 (65°C for 30 min). LD_1 can also be estimated as α-hydroxy butyrate dehydrogenase (HBD) activity (using α-hydroxy butyrate as the substrate instead of lactate). Normal LD/HBD ratio is 1.2 to 1.6. In myocardial infarction, it is 0.8 to 1.2. LD_1 can also be determined by an immuno chemical (more expensive) method.

In hepatic diseases LD_5 is increased (also see under liver disease). Quite high levels of LD (LD_1 and LD_2) are seen in megaloblastic anemias. In hemolytic anemias LD levels increase proportional to extent of hemolysis. LD levels are impressively increased in metastasizing tumors (not in localized, small ones) and these can be used to indicate prognosis or response to treatment.

While interpreting the significance of raised levels of LD it should be kept in mind that both hypoxia and shock cause rise in LD levels. The samples, also, should not be delayed.

Creatine phosphokinase (CPK) or creatine kinase (CK)

CK has three isoenzymes, BB (CK_3), MM (CK_1) and MB (CK_2). In brain 90% of CK is BB, in skeletal muscle 99% is MM and in cardiac muscle 80% is MM and about 20% is MB type.

For diagnosis of acute MI, CK or CK-MB may be analyzed. As CK-MB is mostly present in cardiac muscle, increase in levels of this isoenzyme has higher specificity for diagnosis of MI. However, in conditions of rhabdomyolysis, along with rise of CK-MM, there is sufficient rise of CK-MB to give a false positive for MI. Significant rhabdomyolysis occurs in hypothermia, hyperthermia, uremia, diabetic ketoacidosis and septic shock. Thus for proper diagnosis of MI one should know the level of CK-MB and also the fraction it forms of total CK. CK isoenzymes are assayed immunochemically. For use of CK and CK-MB in diagnosis of MI the samples should be obtained between 4 to 72h of the acute episode. Ratio of CK-MB activity: CK activity > 6% is highly suggestive of MI.

In about 5% of acute MI patients, especially old individuals, presence of peak (increase followed by decrease) of CK-MB is the only abnormality found as total CK and CK-MB levels vary only in the normal range. Thus it is best to take multiple samples at 8 to 12h intervals and study CK-MB in all these samples. If source of CK or CK-MB is skeletal muscle a plateau pattern is seen (no peaking occurs). Besides, above mentioned disorders with associated rhabdomyolysis, CK is also increased in muscular diseases (muscular dystrophies, myopathies, polymyositis), hypothyroidism, cardiac catheterization, electric cardioversion, stroke, trauma, convulsions and prolonged immobilization.

Alkaline phosphatase (ALP)

Liver and bone are two major tissues which produce ALP and the enzyme helps diagnosis of many diseases related to these tissues (Table. 5.1). Other tissues which produce ALP are intestinal epithelial cells and placenta (some tumors also produce ALP). Because of placental origin, the fetal ALP level is increased in pregnancy (third trimester). The high level, at birth, comes down rapidly, still remaining 2 to 3 times the adult level and this is maintained till a moderate rise, coinciding with pubertal growth spurt, occurs. The higher levels of these age groups are because of more rapid bone turnover at these ages, compared to adults. Osteoblasts are the source of ALP and the amounts produced relate to their activity.

All above referred tissues (liver, bone, intestinal epithelium, placenta, tumors) produce different isoenzymes. These isoenzymes can be separated by electrophoresis or by the use of heat inactivation. The placental isoenzyme and one tumor related isoenzyme (Reagen isoenzyme) are more stable at 65°C than other isoenzymes. Another way of confirming hepatic origin of ALP is to, simultaneously study some other liver specific enzyme (Table. 5.2).

Indications of estimations of ALP are (Table 5.1): i) diagnosis of bone disease; ii) differential diagnosis of jaundice; iii) serial measurements to monitor healing of fractures or progress of certain diseases (Paget's disease). ALP level is also increased in inflammatory bowel disease. Levels are reduced in arrested bone growth, cretinism and achondroplasia. Genetic deficiency of ALP, in hypophosphatasia, causes rickets or osteomalacia.

Table 5.1. Pathological conditions with increased levels of ALP in serum

Quite high levels (often 5 to 6 times higher than upper normal limit):

- Paget's disease of bone.
- Rickets/osteomalacia.
- Extrahepatic cholestatic liver disease.
- Primary biliary cirrhosis, post necrotic cirrhosis.
- Drug induced cholestasis.

Levels are high but less than in the first group:

- Bone disorders in which osteoblastic activity is increased (healing fractures, bone tumors, renal osteodystrophy, osteomyelitis, hyperparathyroidism with bone involvement).
- Liver disorders (acute hepatitis, cirrhosis, acute fatty liver).
- Primary and metastatic liver cancer; infiltrative liver disease
- Shock, congestive heart faiilure and drug hepatitis.

- There is no rise of ALP in a bone disease if bone formation is not stimulated (multiple myeloma and osteoporosis without a fracture).
- Serial estimations of ALP help monitoring of healing of osteomalacia, renal osteodystrophy and paget's disease.
- ALP estimation also helps to differentiate cholestatic liver disease from other types of liver disorders.

Table 5.2. Biochemical findings which increase specificity of ALP of hepatic origin

If there is increased activity of 5´-Nucleotidase

If there is increased level of serum bilirubin (cholestasis stimulates synthesis of ALP by bile ductules and amphipathic nature of bile salts facilitates release of ALP from membrane bound sites).

- In hepatic malignancy or hepatic infiltrative disease, high levels of ALP may occur without much increase in bilirubin.

If there is also increased level of γ-GT

- γ-GT is very sensitive to detect cholestatic liver disease. Both, in intrahepatic (viral, drug induced) and extrahepatic cholestatic liver diseases, levels increase 2-5 times (increase is more in extrahepatic cholestasis) the upper normal limit.
- γ-GT increase is from moderate to high in chronic alcoholics.
- Other conditions with less marked increase include pancreatic, prostatic and renal diseases; slight increase may also occur in obesity and diabetes mellitus.

Acid phosphatase (ACP)

50 to 75% of patients of cancer prostate, in which tumor is extending beyond the capsule, show high levels of ACP. This enzyme helps to detect metastatic carcinoma of prostate but has no role in diagnosis of resectable tumor, confined within the capsule. Other sources of ACP are red cells and platelets and hemolysed sample of blood is not assayed for ACP. ACP from prostate (and platelets) is inhibited by tartrate but not that from red cells. In diagnosis of cancer prostate, tartrate inhibited ACP is more useful than the total ACP. After collection

of the sample, assay of ACP should not be delayed as its activity in the sample declines rapidly. Any pressure on prostate during rectal digital examination can cause release of ACP into circulation. The sample for assay of ACP should be drawn before such an examination or instrumentation.

A prostate specific protease also called prostate specific antigen (PSA) and located in acinar and ductal cells and present in seminal fluid, is also present in serum and can be estimated using ELISA technology. Its level is raised in prostatic disorders (both hypertrophy and cancer). If level of this marker is raised, search should be made for prostatic malignancy.

In two other conditions ACP estimation has been used to help diagnosis. In hairy cell leukemia there is rise in serum level of ACP (tartrate resistant). Rape can be confirmed by demonstrating high level of ACP in vaginal secretions (seminal fluid is rich in ACP). In Paget's disease and many cancers metastasizing in bone, increased osteoclastic activity, increases ACP level (ACP released from osteoclasts).

Gamma glutamyl transferase (γ-GT)

It is one of the most sensitive indicators of the liver disease. It is a microsomal enzyme, widely distributed in tissues including liver. There is enzyme induction in liver disease. Alcohol also induces γ-GT. Its level is increased in alcoholics even when there is no involvement of liver. In acute hepatic damage levels of γ-GT and aminotransferases run in a parallel fashion. Rise of γ-GT alongwith that of ALP suggests that ALP has arisen from the biliary tract.

Amylase

Normally this enzyme is mostly derived from salivary glands and pancreas. Because of its low mol. wt. it is readily excreted in urine. In normals, amylase - creatinine clearance ratio is 1-5% and rises to 8% in acute pancreatitis. The main use of estimation of serum amylase lies in diagnosis of acute pancreatitis as a cause of acute abdomen. In this condition rise in serum level of the enzyme may be five to ten times the upper limit of the reference range. In a few other conditions, some times, a similar high rise may occur and these conditions, should be borne in mind. These conditions are, chronic renal failure (due to reduced GFR), severe diabetic ketoacidosis, and perforated peptic ulcer. Same can also occur in macroamylasemia in which amylase is bound to a high mol. wt. protein leading to reduced clearance.

Salivary amylase activity is strongly inhibited by a wheat germ protein. This inhibition forms basis of a method used to study, specifically, pancreatic type of amylase raised in acute pancreatitis and chronic renal failure. For study of amylase, a fresh sample should be used or the sample should be immediately refrigerated.

Angiotensin converting enzyme (ACE)

The main sources of this enzyme are endothelial cells of pulmonary artery, testes and brain. Serum levels are increased in leprosy and sarcoidosis especially pulmonary sarcoidosis, when disease is active. ACE may be used in diagnosis of sarcoidosis but 5% of the positive cases turnout to be false positives because of elevated levels occuring in granulomatous conditions of the lung (tuberculosis, mycotic infections and berylliosis) and other disorders. Enzyme levels are also elevated in Gaucher's disease, amyloidosis, primary biliary cirrhosis and hyperthyroidism. CSF studies of this enzyme have indicated neuronal dysfunctions of Alzheimer disease. Radioimmunoassay and automated methods are available for the enzyme assay.

Cholinesterase (CHS)

This enzyme (CHS) is also called pseudo-CHS and is derived from liver. It is different from acetyl-CHS or true CHS which is an intracellular enzyme present in erythrocytes and nerve cells. There is difference in substrate preference between the two enzymes, and that is the basis of their differential analysis. Serum CHS (or pseudo-CHS) levels are reduced in different hepatic diseases but the enzyme is not used for diagnosis of these disorders. Serum CHS is also depressed in poisoning by organophosphorous compounds (insecticides) and is used for diagnosis of this condition.

Succinyl choline (scoline) is a muscle relaxant

often used during anaesthesia. The enzyme CHS inactivates succinyl choline to limit its duration of action during anaesthesia. An individual may have CHS in one of the three forms given below. The three forms have different activities towards succinyl choline as mentioned below.

Normal CHS	78% activity inhibited by dibucaine (dibucaine number 78).	Inactivates scoline rapidly
Atypical heterozygous	Dibucaine number 60.	Inactivates scoline less rapidly
Atypical homozygous	Dibucaine number 16	Poor inactivation activity

If scoline is used in a patient who is homozygous for the atypical enzyme the action of scoline is prolonged and he may develop prolonged apnea. Thus before using this muscle relaxant the enzyme form of the patient should be properly defined.

Table 5.3. Levels of different enzymes after myocardial infarction

	Behaviour of enzyme after chest pain		
	Appears	Peaks	Disappears
CK-MB	4-8h	12-24h	72h
CK(total)	4-8h	18-36h	4-5d
AST	Follows CK	48h	5d
LD	Follows AST	72h	>10d

- CK-MB rises early, rises sharply (mean rise more than 5 times the upper normal limit (UNL); declines sharply. Often estimation of this isoenzyme is enough.
- For CK, the peak level is more than 4 times UNL and for AST peak level is less than 3 times UNL. Both the enzymes decline sharply.
- For LD peak level is less than 3 times UNL. Decline is very gradual.

Diagnosis of myocardial infarction (MI)

In majority of cases of MI, study of serum enzymes is not required for diagnosis of the condition (clinical features and the ECG changes are enough for the purpose). In less than 30% of cases when clinical features are not typical (in elderly patients there may be no pain) or when ECG changes are not helpful (intramural, posterior or lateral infarct; ECG changes masked by bundle branch block or residual changes from a previous infarct), the enzyme studies become essential.

As already mentioned myocardial tissue contains about 80% of CK as CK-MM and about 20% as CK-MB. Following MI both isoenzymes rise in serum. CK-MB appears within 4 to 8h, peaks at 12 to 24h and comes down within 3 days. CK-MM remains elevated for 4 to 5 days. CK-MM alone has poor specificity for MI since exercise, trauma, surgery or even intramuscular injection can cause rise of CK-MM (originating from muscle tissue). Time courses of different enzymes, for appearing, peaking and disappearing from serum are given in Table. 5.3.

Protocols using CK-MB, CK and CK-MB together and CK-MB in serial samples were mentioned earlier. Another protocol is shown in Table 5.4. Generally, however, LD (total and isoenzymes) and AST are only, occasionally, useful in samples which are available late.

Pulmonary embolism may simulate an attack of MI. ECG may however, show right axis deviation and there will be a source of embolus. It may be followed by pulmonary infarction with findings in the chest. Commonly, 24h after chest pain there is elevated LD; AST may or may not be increased; CK-MB is not elevated.

Table 5.4. Use of CK-MB, LD_1 and LD_2 in diagnosis of myocardial infarction (MI)

Sample on admission (earlier than 12h after chest pain)	Sample at 24h	Sample at 48h
Absence of CK-MB is 100% predictive of absence of MI	CK-MB present; $LD_2 > LD_1$ (MI or some other cause)	CK-MB present and $LD_1 > LD_2$ (100% predictive of MI)

- In MI both LD_1 and LD_2 enter blood from cardiac tissue but more of LD_1 than LD_2. Thus there is more of LD_1 than LD_2 (flipped LD). This pattern appears within 12 to 24h and within 48h is present in sera of 80% cases of MI. This a highly specific finding in diagnosis of MI.

MI may get complicated with shock or congestive heart failure. In the latter condition both LD and aminotransferases may be elevated. Shock can also lead to increase in levels of AST.

The enzyme estimations are also useful in diagnosis of extension or recurrence of infarction during convalescence. Other uses are to judge prognosis and to select patients for thrombolytic therapy and also to monitor success of this therapy. Marked elevation of serum enzymes with increased incidence of ventricular arrhythmia, shock and heart failure indicate poor prognosis. Thrombolytic therapy should be given within 4-6h of the acute episode. Thus there was search for some test which could indicate cardiac damage rather early.

CK-MB from cardiac muscle ($CK\text{-}MB_2$) and not that from skeletal muscle is cleaved by lysine carboxy peptidase to produce $CK\text{-}MB_1$. $CK\text{-}MB_2$: $CK\text{-}MB_1$ ratio of >1.5 at 6h after the episode, has sensitivity of 97% and specificity of 94% for diagnosis of MI. Total CK-MB at 6h has sensitivity of only 48%.

Myoglobin has also been found useful in diagnosis of MI. Myoglobin can be estimated in serum by an ELISA assay and has been shown to rise in serum very early after MI since it is released from the heart muscle within two hours after the episode (and its level normalizes in 24h). It could help as a screening test and could also be helpful in selecting the patient for thrombolytic therapy. It should however, be realized that specificity of myoglobin estimation in diagnosis of MI is quite low since it is present in all types of muscles and any myolytic condition would increase its level in serum.

Cardiac-specific-troponins T and I are different from skeletal muscle troponins. These are new serum cardiac markers when differentiation is required between skeletal muscle damage and MI and are considered superior to CK-MB or CK. These markers increase in serum as early as CK-MB but their peaks are much sharper and thus have much greater sensitivity than CK-MB. Further troponin I may remain elevated for 7-10 days and troponin T for 10-14 days. These markers are also useful in selecting patients for thrombolytic therapy and predicting prognosis of patients of unstable angina. In these patients, the levels of these troponins may increase (indicating probable microinfarction) while levels of CK or CK-MB do not increase.

Once thrombolytic therapy is given its success is monitored by levels of CK-MB or myoglobin (as reperfusion is established there is rapid rise in levels of these markers).

Diagnosis of liver disease

Important enzymes used in diagnosis of liver disease are, AST, ALT, ALP and γ-GT. Ornithine transcarbamoylase is most specific to liver but is not much used since its method of assay is complex. Both AST and ALT levels rise in liver cell damage but AST is more sensitive indicator of liver damage. Advantage of ALT is that it is more specific for liver involvement. For liver cell damage γ-GT is as good as AST. In infective hepatitis rise in levels of these enzymes occurs before rise of bilirubin.

In cholestatic liver disease there is greater rise in level of ALP than in hepatitis. 5′-Nucleotidase is a sort of hepatic isoenzyme of ALP, but it is more difficult to estimate. γ-GT is more sensitive than ALP in diagnosis of biliary obstruction. It is not altered in bone disease.

Significant, isolated elevations of ALP and LD suggest presence of space occupying lesion in liver. Studies of serum enzymes greatly help: i) to differentiate liver disease from myocardial infarction or other conditions accompanied with pain in upper abdomen/chest; ii) to differentiate between hepatic cell damage and cholestasis; iii) to follow course of chronic hepatitis.

Serum enzymes in muscle disorders (Table 5.5)

In the dystrophic group there are a number of inherited diseases with difference in age of onset and the muscles involved. The commonest is Duchenne muscular dystrophy. In these disorders (especially Duchenne dystrophy) muscle tissue associated enzymes, CK, aldolase, LD and AST are raised. CK-MM isoenzyme is most specific for these disorders. Electromyography is helpful to differentiate these myopathies from neurotropic muscle disorders.

Table 5.5. Diagnostic features of certain skeletal muscle disorders

HEREDITARY MYOPATHIES

Muscular dystrophies (These disorders are progressive in nature. Individual diseases differ in age of onset and the muscle groups involved. The commonest condition is Duchenne dystrophy.):

- CK levels are 20 to 100 times the normal at birth and decline with age due to loss of muscle tissue.
- EMG (differentiates between dystrophic, atrophic and disorders due to receptor or channel defects).
- Muscle biopsy (differentiates atrophic, dystrophic and muscles affected in connective tissue disorders).
- For definitive diagnosis of Duchenne dystrophy, dystrophin deficiency is demonstrated in muscle biopsy by Western blot analysis.
- Mutant DNA analysis can also be done on peripheral leucocytes for definitive diagnosis. This procedure can also be used for carrier detection and for prenatal diagnosis.

Congenital myopathies (It is a non-progressive group of hereditary myopathies with early appearance of clinical features. One disorder in this group predisposes the patient to malignant hyperthermia).

- EMG and muscle biopsy are used for diagnosis of these disorders:
- CK levels are usually normal.

METABOLIC MYOPATHIES

Glycogen storage diseases (producing exercise intolerance): It includes glycogenesis type V (most common, muscle phosphorylase deficiency), type VII (phosphofructokinase deficiency), type IX (phosphoglycerate kinase deficiency), type X (phosphoglycerate mutase deficiency) and type XI (lactate dehydrogenase deficiency).

- All these disorders have autosomal recessive inheritance except type IX, which is X-linked recessive.
- Manifestations of these disorders generally start in adolescence.
- After a short severe bout of exercise muscles become stiff and painful. There is myoglobinuria and increase of CK.
- There is associated hemolytic anemia in type VII (mild) and type IX (severe).
- Type IX, more commonly, presents with mental retardation and seizures than with exercise intolerance.
- In the forearm exercise test, there is impaired increase in venous blood pyruvate and lactate (in type XI there is increase of pyruvate but not lactate)
- For confirmation, enzyme levels are studied in the muscle biopsy samples.

Carnitine palmitoyl transferase deficiency[1]: This also produces a myopathy which starts in teenage and is the most common cause of recurrent myoglobinuria (more common than glycogen storage disorders).

- Muscle pain and myoglobinuria occurs after prolonged severe exercise (fasting predisposes to the trouble).
- CK level increases only during the episode of myoglobinuria.
- In the forearm exercise test there is normal increase in venous lactate level.
- For confirmation, enzyme level is studied in the muscle biopsy sample.

Myoadenylate deaminase deficiency

- This enzyme regulates ATP level in skeletal muscle.
- Deficiency may cause exertional faigue, myalgia and myoglobinuria in some cases; but often there are no symptoms.

MYASTHENIA GRAVIS AND CHANNEL RELATED DISORDERS

Myasthenia gravis: Diagnosis is based on special groups of muscles involved and demonstration of improved muscle strength with edrophonium chloride (the anticholinesterase test). One should look for other disorders which may aggravate the disease (thyroid disorders, occult infection, certain drugs) or which interfere in treatment of disease with anticholinesterase drugs or immunosuppressives (tuberculosis, diabetes, peptic ulcer, renal disease, hypertension, asthma, osteoporosis).

- The anticholinesterase test is commonly used for diagnosis; other tests are:
- The repetitive nerve stimulation test.
- Acetyl choline receptor assay.
- Single fiber EMG.

Familial periodic paralysis and related disorders: In these disorders episodic (for less than an hour to several hours duration) muscle weakness or flaccid paralysis occurs but not during vigorous activity. The disorders manifest in the first two decades of life.

Contd.....

- Serum K^+ is often high (hyperkalemic periodic paralysis) or low (hypokalemic periodic paralysis) at the time of attack.
- Attacks can be provoked by lowering of serum K^+ (glucose and insulin administration; a heavy carbohydrate meal) or by loading with K^+.

Polymositis-dermatomyositis, scleroderma:

- These may require differentiation from peripheral neuropathy with the help of EMG.
- Biopsy may help to confirm diagnosis.

Myopathies associated with endocrinal disorders: Both increased and reduced levels of thyroid, parathyroid, and adrenal hormones may be associated with muscle weakness. Primary hyperaldosteronism produces hypokalemia which can produce muscle weakness. Muscle weakness is also associted with diabetes and vitamin D deficiency.

- CK levels are normal or reduced.
- Establish the hormone status of the patient.

Post-infective/toxic myopathies:

- CK level is increased in acute state and reduced in chronic state.

Muscular atrophies (secondary to lower motor neuron damage):

- CK level is reduced.

Crush injuries:

- CK level is increased.

[1] A similar but less common myopathy is "Myopathic Carnitine deficiency", with overlapping features of muscular dystrophy and polymyositis. Carnitine is deficient in muscle but not in blood. CK is increased. Muscle biopsy shows lipid accumulation.

- For muscle disorders useful enzymes are CK, CK(MM) and aldolase. CK(MM) and aldolase are equally sensitive but aldolase is less specific. Less commonly used enzymes are LD (LD_3 and LD_4) and AST. Muscle tissue does not contain ALT.
- Liver glycogenoses are grouped as: i) with hepatomegaly and hypoglycemia (type Ia, defective glucose-6-phosphatase, Ib, glucose-6-pO_4 translocase defect; IIIa, liver and muscle debrancher defects, IIIb, liver debrancher defect only; type VI, liver phosphorylase defect and the liver phosphorylase kinase defect (formerly called type VIa or type IX)) and ii) with cirrhosis and hepatomegaly (type IIIa and IIIb; type IV brancher defect).
- Muscle glycogenoses are grouped as: i) with muscle energy impairment (type V, type VIII, phosphofructokinase/ phosphoglycerate kinase/phosphoglycerate mutase/lactate dehydrogenase/muscle phosphorylase kinase defects) and ii) with progressive skeletal muscle weakness, atrophy and/or cardiomyopathy (type II, lysosomal acid α-glucosidase defect; type IIIa and type IV, brancher and cardiac specific phosphorylase kinase defects).

In the group of endocrine myopathy, diagnosis needs evaluation of thyroid, adrenal, parathyroid and vitamin D status of the patient. Also serum levels of K^+; Ca^{2+} and Mg^{2+}. In elderly, often hypokalemia, hypomagnesemia, vitamin D and other deficiencies develop over time and may cause muscle weakness. In case of toxic myopathy history of drug intake is important. Alcohol, steroids and thiazide diuretics may produce this type of muscle weakness. Diabetes and renal, hepatic, pulmonary and cardiac diseases can also produce muscular weakness.

Some myopathies can be recognized from their special features. Myasthenia gravis is a disorder of neuromuscular junction. There is weakness of muscles of lids (causing ptosis), ocular muscles (causing diplopia), and muscles of chewing, swallowing and speech. There is improved muscle strength with endrophonium (a short acting anticholinesterase inhibitor). Familial periodic paralysis is a rare disorder in which attacks of severe muscle weakness are associated with low serum K^+ level. A high carbohydrate diet may induce an attack by promoting K^+ entry into cells.

Some myopathies arise in diffuse connective tissue disorders with muscle weakness and inflammatory changes in muscle and skin (polymyositis - dermatomyositis, scleroderma). Test for rheumatoid factor is positive and there are antibodies to certain nuclear antigens. EMG helps to differentiate from peripheral neuropathy. Levels of aminotransferases, LD and aldolase indicate disease activity. MRI can also reveal active myositis noninvasively. These connective tissue disorders may be secondary to some malignancy. Search for that is important.

Glycogen storage disease may also produce myopathy. In these cases confirmation of disorder

requires study of certain enzymes in muscle biopsy. The investigated enzymes are phosphorylase, phosphofructokinase, lactate dehydrogenase and others. Myopathies with mitochondrial enzyme defects (and mitochondrial inheritance) are also known (objective type question 25, Chapter 18).

Post viral/bacterial myopathy may also occur. In acute stage, CK is raised but is reduced in chronic stage.

Progressive muscular atrophies (motor neuron disease) are due to progressive damage to lower motor neurons. These are more common, late in life (dystrophies occur in childhood), and commonly affect distal muscle groups. Muscles in process of atrophy show fasciculations (not seen in dystrophic muscles). EMG differentiates dystrophic from atrophic muscles. It can also demonstrate abnormality at neuromuscular junction. Muscle biopsy can differentiate atrophic, dystrophic and muscles involved in connective tissue disorders. The tissue can also be studied for activities of certain enzymes of some other substances. Features of important myopathic groups are summarized in Table 5.5.

Crush injuries

Such injuries result in leakage of CK, myoglobin and K^+ from muscle cells into the blood. Generally, there is simultaneous loss of blood and sequestration of fluid in damaged tissue. Hypovolemia and myoglobinuria can result in acute renal failure (acute tubular necrosis). Also read under acute renal failure in Chapter 4.

Leakage of cellular contents (see above) from damaged muscle cells is called rhabdomyolysis. Besides trauma significant rhabdomyolysis occurs in hypothermia, hyperthermia, uremia, diabetic ketoacidosis and septic shock. Quite high serum levels of CK-MM are seen in these disorders. Also read under 'haptoglobin'.

PLASMA PROTEINS (Table 5.6)

Low level of total protein in plasma provides information about status of nutrition or about some severe organ disease (protein losing state, liver disease). It may also indicate overhydration if it develops, rather rapidly. Raised level indicates presence of some paraprotein and patient should be further investigated. It may also indicate dehydration if develops rapidly. Albumin levels are more useful as indicators of nutritional status. This investigation is also important in a chronic liver disease and nephrotic syndrome. In septicemia and general inflammatory disorders, hypoalbuminemia can occur due to leakage of albumin into interstitial compartment. Albumin level alongwith total protein level gives indication of the globulin level.

Table 5.6. Feature of certain plasma proteins[§]

†1.	Prealbumin (62,000), (0.15-0.36)
2.	Albumin (66,000), (39-51)
3.	α_1-Antitrypsin (54,000), (2.0-4.0)
4.	α_2-Macroglobulin (725,000), (1.5-3.5)
5.	Haptoglobulin (1,000,000), (0.4-2.9)
6.	Transferrin (80,000), (2.0-4.0)
7.	C3 — (185,000), (0.6-1.4)
8.	Fibrinogen (340,000), (1.0 -4.0)
††9.	Ceruloplasmin (132,000), (20-30)
10.	Hemopexin (70,000), (50-120)
11.	Gc-Globulin (51,000), (20-55)
‡12.	C-reactive protein (118,000-144,000), (470-1340)

†Nos 1 to 8: (Mol. wt.), (Conc. in g/L).

††Nos 9 to 11: (Mol. wt.), (Conc. in mg/dL).

‡No.12: (Mol. wt.), (Conc. in ng/mL in adults), conc. in children 170 ng/mL.

§Nos. 3,5,8,9,10 are acute inflammatory reactants. Others are α_1-acid glycoprotein and ferritin as well as ESR and neutrophils.

Protein electrophoresis is carried out on serum, since plasma contains fibrinogen which migrates in β region and its band could be mistaken for a myeloma band. For electrophoresis cellulose acetate and agarose produce better separations than paper. Bands of albumin, and α_1, α_2, β and γ globulins are seen. Occasionally, discrete bands of C-reactive protein and α-fetoprotein may be seen if these proteins are present in excess. The prealbumin band may be seen depending on the method used. Paraproteins (or M-proteins), if present, are often seen as discrete bands (called M-bands).

The most important use of electrophoresis is in study of paraproteins. It can also easily display deficiency of IgG by reduced density of the γ-globulin band. IgA and IgM changes are not revealed since the amounts of these immunoglobulins are rather small and these proteins might migrate with other globulins. The electrophoretic studies are also important in long

term follow up of cases of myeloma, cirrhosis, nephrotic syndrome and extensive burns.

Some specific protein studies are also important in certain clinical disorders. α_1-Antitrypsin (genetic deficiency causes emphysema in childhood and neonatal hepatitis progressing to cirrhosis), transferrin (important in diagnosis of hematological disorders), C3 (to monitor rheumatic disease activity), fibrinogen (in cases of hemorrhagic diathesis and intravascular coagulation), ceruloplasmin (in diagnosis of Wilson's disease), the C-reactive protein (to monitor the active phase of certain autoimmune disorders), haptoglobulin (in hemolytic disorders) and α-fetoprotein (for diagnosis of hepatoma and neural tube defects), are the important examples (discussed later). For most of these nephlometric methods (Fig. 5.1) are used in automated immunochemistry analyzers instead of previous radial immunodiffusion measurements.

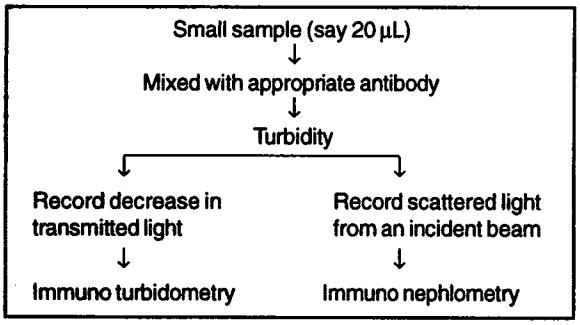

Fig. 5.1. Immune nephlometry/immune turbidometry.

- The major advantage of these methods is that these are easily automated but there is interference from lipemic or turbid sample (due to repeated freezing and thawing).

Albumin

It is synthesized in liver. Fetal liver synthesizes α-fetoprotein which has regions of homology with albumin. The two are synthesized from the same ancestral gene. In an inborn error, modified albumin is synthesized and in heterozygotes for the modified albumin, two albumin bands are seen, because of difference in rates of migration of normal and mutant albumin. Another mutant albumin has much higher affinity for thyroid hormones.

Albumin is main determinant of plasma oncotic pressure. However, in congenital absence of albumin (analbuminemia), there is no peripheral edema, unlike other causes of low albumin (malnutrition, malabsorption, chronic liver disease, ascites, protein losing enteropathy and nephrotic syndrome).

Albumin has multiple binding sites to bind thyroxine, bilirubin, cortisol, estrogen, FFA, Ca^{2+}, Mg^{2+}, heme and certain drugs. Albumin acts as a carrier of amino acids from liver to peripheral tissues.

Normally, 8% of albumin is glycosylated while in diabetes upto 25% may be glycosylated. As half life of albumin is 17 days, glycosylated albumin can be used to monitor diabetics over a shorter period compared with glycosylated Hb.

An immunologic measurement is used to study microalbuminuria of diabetes for early detection of diabetic complications. Table. 5.7 presents clinical situation in which study of serm albumin/albuminuria may be helpful.

Table 5.7. Clinical significance of altered serum albumin levels and albuminuria

- Low levels indicate malnutrition/malabsorption. It is a good parameter to indicate protein nutritional status in chronic malnutrition, chronic illness and chronic malabsorption but not a good parameter to monitor nutritional intervention.
- Low levels occur in nephrotic syndrome (increased α_2-macroglobulin level partly compensates for the reduced plasma oncotic pressure).
- Low level in septicemia is due to leakage of albumin into interstitial compartment.
- In liver diseases serum albumin levels may determine prognosis of the disease.
- In Rh incompatibility cases, low albumin increases risk of kernicterus.
- Low serum albumin levels may interfere in proper interpretation of levels of those hormones which bind to albumin.
- Low serum albumin levels are produced by enteropathies, burns, hemorrhage and catabolic states like fever/septicemia/trauma/wide spread cancer.
- Rapidly produced changes in serum albumin level may indicate changes in plasma volume. Same is also true of rapidly produced changes in hematocrit, Hb and total protein levels.
- Glycosylated albumin level (normal about 8%) has been used to monitor diabetic control.
- Microalbuminuria is studied to monitor progression of chronic complications of diabetes (with time micro albuminuria of diabetes changes into albuminuria).

Pre-albumin

It migrates faster than albumin in electrophoresis. It binds to retinol binding protein and prevents its loss in urine. It is a marker for nutritional state of an individual. Its half life is only about two days. In nutritional intervention, it is considered better than albumin and transferrin which have longer half lives and are also affected by certain factors other than nutrition. It also binds thyroxine. When CSF protein electrophoresis is done to detect a monoclonal band, absence of band of pre-albumin is used to confirm that the sample is that of CSF. It is best quantitated by immunologic assay (nephlometric).

α_1-Antitrypsin (ATT)

In electrophoretic separation, it migrates in α_1-globulin fraction. ATT acts as a natural defence against protease action. In an inflammatory reaction or on exposure to irritants, leukocytes release proteases, which could cause tissue damage (in lungs emphysema and in liver, cirrhosis can be produced), and ATT gives protection against this damage.

There are at least 75 different alleles for ATT. Normal genotype is MM. The most common abnormal allele is called Z and some others are F and S. Individuals with genotype ZZ are at great risk of developing the above referred diseases. Individuals with genotype MZ (heterozygous) have about 60% of normal ATT activity and have no risk of developing the disorders. Similarly individuals with genotypes, MS, SS, MF or FF are not at increased risk.However, those with genotype SZ have some risk.

Risk of developing emphysema is greatly increased by smoking. Smoke oxidizes thiol group at active site of ATT and makes it ineffective.

Screening of family members and the antenatal diagnosis is possible. Isoelectric focussing is used for study of different phenotypes of ATT. Antenatal screening based on DNA analysis, is also available. DNA obtained from chorionic villus sampling is first amplified using PCR, before analysis.

Fibrinogen

It is most abundant of the coagulation factors. It is an acute phase protein and its level is directly related to elevation of ESR. Level also rises in pregnancy and with use of oral contraceptives. Low level of fibrinogen along with increased level of fibrinogen split products is an index of intravascular coagulation. There are many inherited variants of fibrinogen (dysfibrinogenemia) in which clotting is excessive (thrombotic tendency) or impaired (hemorragic diathesis). In electrophoresis of plasma, it appears as a distinct band between β and γ regions and looks like a monoclonal band).

Transferrin

It is the major β-globulin protein which transports iron from stored ferritin of mucosal and other cells, to bone marrow. In normal serum transferrin protein level is 240-480 mg/dL (App. B). It is also measured as total iron binding capacity of serum. A more useful allied parameter is percent saturation of transferrin. In iron deficiency anemia, transferrin synthesis is increased and serum iron is reduced. Thus percent saturation is greatly reduced. In idiopathic hemochromatosis and transfusional siderosis percent saturation of transferrin is very high (Chapter 6). There is deposition of iron (same is also true in congenital deficiency of transferrin) in tissues.

In iron deficiency, increased level of transferrin leads to a sharp band on electrophoresis simulating a monoclonal band.

Ceruloplasmin

It is also an acute phase protein but unlike fibrinogen, its level does not, directly, influence ESR. It may be involved in transferring iron from ferritin to transferrin since it can oxidize ferrous iron to ferric. In Wilson's disease, ceruloplasmin level is reduced and there is impaired excretion of Cu in bile (and Cu is deposited in tissues). Wilson's disease is discussed in Chapter 6. A simple colorimetric assay using p-phenylenediamine as the substrate (which is oxidized) is available besides the immunochemical method, for ceruloplasmin estimation.

Complement

C3 is a sub fraction of β-globulin. C3 and C4 (not seen in electrophoresis), are easily measured by

Table 5.8. Some primary immune deficiency diseases and AIDS

Congenital X-linked agammaglobulinemia:

- Increased susceptibility to pyogenic infection in second year of life (staphylococcal skin abscesses, pneumonia, meningitis) but not to viral infection.
  - Deficiency of B-cells and IgG, IgA and IgM.
  - Peripheral lymphocyte count is normal.
  - Skin tests are normal.

Selective IgA deficiency

- It is common in Caucasians (1 in 500) and is associated with inheritance of certain class III histocompatibility antigens. Increased susceptibility to respiratory tract or gastrointestinal tract infections, is produced. Selective IgM deficiency makes patient more susceptible to blood borne infections (septicemia). In another pattern, there is reduced/absent IgM and IgA but normal to increased IgG.

Common variable immunodeficiency:

- Patient is prone to secondary immune deficiency and he may present in this way or he may present as a case of late onset (adult form) of primary hypogammaglobulinemia. There is high incidence of autoimmune diseases (pernicious anemia/hemolytic anemia). He may also have malabsorption due to giardia infection. There may be reduced level of any class of Ig (generally, IgA).

Hypogammaglobulinemia of infancy (transient):

- In these cases there is increased incidence of bacterial infections, for a limited period, after birth. Prophylactic IgG treatment helps.

X-linked immunodeficiency with increased IgM:

- Patient suffers from pneumocystis pneumonia and often has associated cyclic neutropenia. Normal to elevated levels of IgM and absent IgA and IgG.

Severe combined immunodeficiency[1]:

- It is caused by IL-2 receptor defect (X-linked inheritance) and a number of other inherited defects including the one caused by adenosine deaminase deficiency. There is stem cell defect which leads to impairment of both T-cell and B-cell functions. About 50% of infants with autosomal recessive form of the disease have deficiency of adenosine deaminase. The detection of this deficiency helps prenatal diagnosis of these cases. The affected infants suffer from both bacterial and severe viral infections. If the infants are given small pox or BCG vaccines they may develop severe forms of the respective diseases.

Hyper IgE syndrome (Job - Buckley syndrome)[1]:

- T-cells defect is poorly defined. There are severe staphylococcal abscesses of skin.

Thymic hypoplasia (DiGeorge's syndrome)[2]:

- It is a primary T-cell deficiency. There is characteristic facies, heart lesions and hypoparathyroidism. There is increased susceptibility to viral, fungal and bacterial infections
  - Peripheral lymphocyte count is low, and there are no T-cells in peripheral blood.
  - Response to skin tests is poor.
  - Igs may be normal or low depending on degree of T-cells deficiency.

Nezelof syndrome[2]:

- Congenital fault in thymic embryogenesis.
  - Specific antibody response is poor.

Wiskott-Aldrich syndrome[1]:

- It is an X-linked combined T and B-cell functional deficit. The condition is associated with eczema and thrombocytopenia. There is high incidence of lymphoreticular malignancy.
  - IgM level is low. IgA and IgE levels are often elevated.
  - Skin tests are impaired to fungal antigens..
  - Decrease, in vitro, response to phytohemagglutinin stimulation

Ataxia-telangiectasia associated immunodeficiency (A chromosomal repair defect)[1]:

- It is an autosomal recessive disorder. There is ataxia and choreoathetoid movements in infancy and telangiectasia (face, conjunctiva etc.) seen a few years afterwards.
  - IgE and IgA deficiencies are seen commonly along with T-cell deficit.

Acquired immune deficiency syndrome (AIDS):

- Hallmark of HIV infection is progressive deficiency of Helper or inducer - T-cells (HIV, a retrovirus passes through lysogenic pathway inside these cells). When the number of these (CD4 + T-cells) declines below a certain level, the patient suffers from AIDS - related disorders including opportunistic diseases particularly infections and neoplasms. All these diseases cannot be explained completely by immunosuppressive effects of HIV infection. This is especially true for disorders like Kaposi's sarcoma and neurologic abnormalities. Thus multiple pathogenic mechanisms (which may vary with phase of the disease) are involved in the disease process.
- After HIV infection the course of the disease is divided into acute HIV syndrome with widespread virus dissemination and seeding (9 to 12 months), the clinically latent period (about 8 years), constitutional symptoms (low fever, diarrhea), opportunistic diseases and death.
- The tests used in diagnosis of HIV infection include: i) antibody detection by ELISA, the test is highly sensitive but less specific (false positive test is caused by antibodies to class II antigens, autoantibodies, hepatic disease and recent influenza vaccination); ii) Western blot test (WB), in which viral antigens are separated and transferred to a filter paper and reacted with test serum followed by addition of enzyme-linked antihuman globulin antibody to reveal the separated antigens (this test is highly specific and the commercial kits contain viral antigens both from HIV-1 and HIV-2); iii) polymerase chain reaction (PCR) is used to study viral RNA and proviral DNA. RNA PCR is more commonly used to detect HIV infection at an early stage before antibodies become detectable; iv) detection of p24 antigen by an ELISA type assay. This test is positive in about 50% of patients before development of antibodies and the test is quite simple.
- The following sequence of testing may be followed in HIV infection: i) do ELISA; if positive, on two separate occasions, confirmation is done by WB (if negative, there is no infection); ii) if WB is negative but there is strong suspicion of HIV infection, do PCR to take final decision from the results.

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[1] Combined T-cell, B-cell deficiency syndromes (primary).

[2] Primary T-cell deficiency syndromes: Rest are primary B-cell deficiency syndromes.

- Besides the above conditions, T-cell functional deficiency can be produced by inosine phosphorylase deficiency, by non-expression of CD_3 on T-cells and other causes. Primary immune deficiency may also be produced by C_3 deficiency (mimics antibody deficiency syndromes), leukocytic defects, and other causes.

nephlometry to monitor rheumatic disease activity (both C3 and C4 are reduced in autoimmune disorders, SLE and rheumatoid arthritis). Increased levels of C3 and C4 have very little clinical significance except as indicators of acute phase response.

Haptoglobin

One of the major proteins migrating in α_2-region, in electrophoresis. Blood may contain different molecular forms. In one major form the structure resembles that of immunoglobulins. Because of presence of different forms, haptoglobin, estimation (immunologic method or method based on Hb binding capacity) presents difficulty. Thus its study is most useful when required in serial estimations, otherwise, only those levels are helpful which are quite away from the normal range.

It is an acute phase protein and level increases in stress, infection, inflammation or tissue necrosis. Its level is reduced in cases of hemolysis as haptoglobin - Hb complexes are cleared from circulation. Change may be sharp after a massive hemolytic episode. It may also be useful to follow cases with slow but steady hemolysis (mechanical heart valves). Haptoglobin - Hb complex migrates differently from haptoglobin and may simulate a M-band.

A red coloured fluid from hemolysis will show test for pseduo-peroxidase (the reagent strip urine test for heme), low level of haptoglobin and presence of LD_1. On the other hand a red solution from rhabdomyolysis will be pseudoperoxidase positive, with no change in level of haptoglobin and presence of LD_5. Level of haptoglobin can also be low in hepatic disease (reduced synthesis) and in cases of hereditary deficiency.

Hemopexin also binds Hb when level of free Hb exceeds binding capacity of haptoglobin. Reduced level of this protein also helps diagnosis of intravascular hemolysis. This protein migrates

in β-globulin region and is quantitated by immunologic methods.

α_2 - Macroglobulin

One of the largest serum non-immunoglobulin. It is an endogenous protease inhibitor like AAT but its low level is not related to any disease. Level of this protein greatly increases in nephrotic syndrome (its synthesis is increased), and it assumes role of maintaining oncotic pressure.

Some other proteins are, Gc-globulin (a transport protein for vitamin D which is lost in urine in nephrotic syndrome alongwith vitamin D), α_1-acid glycoprotein which is very rich in carbohydrate content, and is excreted in urine and binds progesterone, may be for its transport (level increases in pregnancy) and C-reactive protein (CRP). The last mentioned protein increases in serum in conditions of tissue necrosis. It is a γ-region migrating protein (it may form a monoclonal type band, when its level is high). It is used to monitor the active phase of certain autoimmune disorders. It can also be used to distinguish between bacterial (rise occurs) and viral (no rise) infections. It can bind many substances like different lipids, polysaccharides, DNA and nucleotides. It appears to be a general scavenger molecule.

Quantitative analysis of CRP is better than ESR, as non-specific indicator of intensity of inflammatory reactions. Generally changes in CRP parallel those in ESR except that unlike ESR, CRP does not change in anemia, congestive heart failure and hypergammaglobulinemia. CRP is most useful in monitoring a rheumatic disease.

PROTEINS CONCERNING THE IMMUNE SYSTEM

Proper assessment of immune system requires, evaluation of cell mediated immunity, humoral immunity, the complement system and the granulocyte functions. Table 5.8 gives important primary immune deficiency disorders. Acquired immune deficiency syndrome, although not a primary disorder has been included in the Table. Table 5.9 mentions important causes of secondary immune deficiency.

Table 5.9. Some factors which may cause secondary immuno deficiency

- Viral infections (especially Epstein-Barr virus and HIV) may occur in utero and produce immune deficiency state in fetus.
- Nutritional deficiencies of calories, protein and micronutrients (especially biotin, zinc and selenium).
- After irradiation or use of immunosuppressive drugs.
- Certain malignant disorders (chronic lymphoid leukemia, multiple myeloma and Hodgkin's disease).
- In old age impaired immune system functioning leads to increased chances of autoimmune diseases besides reduced T-cell functions.
- Patients remain in relative immune deficiency for several days in the post operative period.

Proper evaluation of immune system requires a careful history and physical examination. This may suggest the immune system component needing further exploration. Table 5.10 lists some points which should be borne in mind while examining the patient.

Investigations start with total and differential counts. An absolute lymphocyte deficiency represents T-cell deficiency, since only 12% of total lymphocytes are B-lymphocytes. More specifically, number of small lymphocytes (T-cells) should be normally, more than 1200/cmm. The skin tests are done to qualitatively, assess cell mediated immunity.

Table 5.10. Some features suggestive of primary immune deficiency disorders

- Frequent infections with extracellular pyogens (B-cell defect).
- An ordinary benign virus causing a severe infection (T-cell defect).
- Infections caused by opportunistic organisms (T-cell defect).
- Live virus/attenuated bacterial vaccine produces systemic illness or severe untoward effect.
- Chronic/recurrent osteomyelitis or abscesses of skin, liver or lymph nodes.
- X-ray changes in ribs, scapulae and vertabrae.
- Neonatal tetany and uncommon face.
- Eczema and thrombocytopenia.
- Telangiectasia of sclerae and ears.
- Short limb dwarfism.
- Family history of recurrent infections.

Skin sensitivity is noted after intradermal injection of certain antigens against which humans are frequently sensitized. However, skin tests are reliable only after 3 to 5 years of life (after the infant has sufficient previous exposure to the employed antigens). The reliability is in doubt when need for testing T-cell immunity is maximum.

Humoral immunity is assessed by determination of serum immunoglobulins. For the first few months of life IgG (placental transfer from mother) is the major immunoglobulin Ig. Thus for diagnosing panhypoglobulinemia presence of IgM and IgA is a good evidence against the condition. Panhypogamma globulinemia is the usual presenting form of X-linked or autosomal primary hypogammaglobulinemias. As immunoglobulin levels increase with age the levels in patients should be reported with age related controls.

Besides panhypoglobulinemia, a careful note should be taken of: i) absent IgA with normal or high IgM; ii) a marked increase of IgE; iii) low IgM with marked elevation of IgA. IgG subclass deficiencies also occur but may go undetected (total IgG may remain normal).

More useful than simple immunoglobulin levels, is measurement of specific antibody response to previously administered or ubiquitous naturally occurring antigens. Thus isohemagglutinin titres and febrile titres should be assessed. If the child has received usual immunizations, their titres should be assessed. If not, immunization response after vaccine administration should be measured. However, no live virus vaccine should be given, if immune status of the child is under doubt.

See Table 5.8 for diagnosis of acquired immune deficiency syndrome (AIDS).

A note on techniques for studying immunoglobulins will be presented in the next section.

Polyclonal gammopathy and paraproteins in disease

In a number of diseases there is polyclonal increase of immunoglobulins (Igs), seen as diffuse increase in the γ-globulin band on ordinary electrophoresis. It may be related to antigenic stimulation or loss of immunoglobulin regulation. The finding, however is, generally, of very little help in diagnosis. Polyclonal increase is seen in chronic liver disease, chronic infections (leprosy, all Igs, helminthic infections, IgE or all Igs, chronic granulomatous disease of childhood, all Igs, infectious mononucleosis, IgM or all Igs and others), certain immune deficiency diseases, autoimmune diseases, sarcoidosis (all Igs), amyloidosis (all Igs), down's syndrome (all Igs), narcotic addictions (IgM) and others.

Increase of monoclonal immunoglobulins or fragments of immunoglobulins (called paraproteins or M-proteins) are also associated with a number of diseases (multiple myeloma, Waldestroms macroglobinemia, B-cell neoplasm, chronic lymphatic leukemia, lymphomas and monoclonal gammopathy of undetermined significance (MGUS). Patients of chronic liver disease and those of autoimmune disorders may also have monoclonal immunoglobulin (paraprotein) increase. Monoclonal immunoglobulins appear as sharp discrete bands in electrophoresis (Tables. 5.11 & 5.12).

Laboratory evaluation of Ig deficiencies or for presence of paraprotein

Generally, simple electrophoresis is first performed. The agarose medium results in better separations than paper or cellulose acetate. Based on results of this investigation other investigations are undertaken. Some times the specialized investigations

Table 5.11. About estimation of serum proteins and electrophoresis

- Assumption of upright posture and application of tourniquet before venepuncture lead to increased diffusion of fluid from vascular compartment into interstitial compartment and increase in serum protein concentration.
- In electrophoresis, serum (and not plasma) is used since fibrinogen, migrating in β–γ region, may produce a sharp band which may be mistaken as a paraprotein.
- Presence of acute phase proteins, haptoglobin and ATT increase the size of α_1/α_2 band, and reduce albumin.
- Presence of bilirubin or drugs binding with albumin, modify migration of albumin band. Thus one may see two albumin bands in such conditions.
- Hb when present free in serum (after saturating haptoglobin) migrates in β or α_2 region. Hapto-globulin Hb complex travels separate from free Hb.

Table 5.12. Proteins which may appear as an M-band in serum electrophoresis

- Paraproteins (from abnormal plasma cells)
- Haptoglobin-hemoglobin complexes
- C3
- β-Lipoprotein
- Transferrin
- Fibrinogen (when plasma is used as the sample)
- Immune complexes
- α_1-Macroglobulin
- C-reactive protein (CRP)

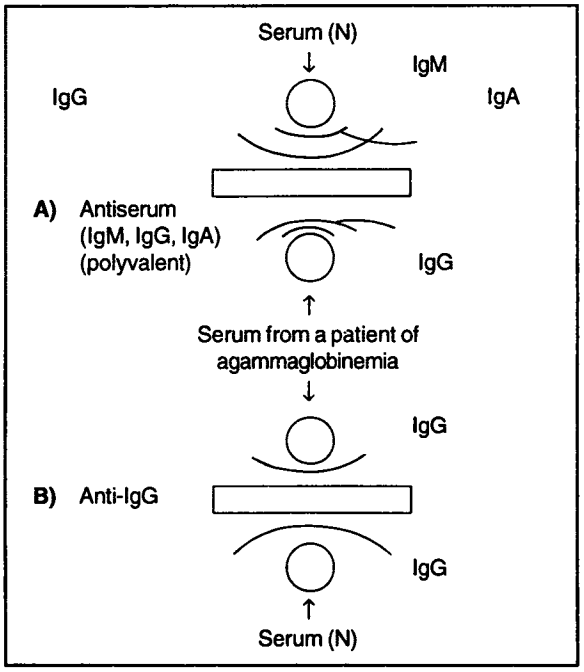

Fig. 5.2. Serum immune protein analysis by immunoelectrophoresis.

- In the round wells cut in the gel are added normal/test sera, as shown. In the elongated gutters antisera, polyvalent or against a particular Ig (anti-IgG, anti-IgM, anti-IgA, anti-K, anti-γ) are added. The test serum of this patient contains reduced amount of only IgG.

may be directly performed depending upon clinical symptoms and signs of the patients.

Immunoelectrophoresis is the most useful screening method for evaluation (semiquantitative) of IgG, IgA or IgM and for identifying abnormal immunoglobulin molecules such as myeloma proteins (Fig. 5.2). Immunologic nephlometry (Fig. 5.1) and radial immunodiffusion techniques are used to quantitate individual Igs and other proteins. In radial immunodiffusion method, circular wells are cut in a gel plate impregnated with specific antiserum directed against a single immunoglobulin class (or Ig component). A circular precipitin ring will form after the sample protein placed in the well diffuses through the gel. The radius of the precipitin ring is proportional to the concentration of the serum immunoglobulin. In the immunofixation technique, test serum replicates are subjected to electrophoresis on agarose gel (the number of replicates depends upon the number of antisera to be used). Six replicates will be required if five antisera have to be used. After electrophoresis, five gels are reacted with five antisera (anti-IgG, anti-IgA, anti-IgM, anti-K and anti-γ) one for each gel. Next, the gels are washed and then stained for proteins. The sixth gel, after electrophoresis, is neither reacted with any antiserum nor washed. It is directly stained for proteins. In this gel the position of the monoclonal band is revealed by comparison with other gels.

In autoimmune diseases antigen is known and serum is tested for autoantibodies by methods like, tanned red cell hemagglutination, radioimmune precipitation, immunofluorescence microscopy and complement fixation.

Different autoantibodies and other investigative parameters used in detection and follow up of chronic rheumatic diseases/collagen diseases are ANA (antibody, nuclear antigen), Anti-DNA, ANCA (antibody, neutrophil cytoplasmic antigen), C3, C4, C-reactive protein and ESR.

Multiple myeloma

It is a malignant disorder of plasma cells with peak incidence after 60 years. The malignant cells produce the same immunoglobulin, meaning thereby, that the tumor represents clone of a single B-cell. The immunoglobulin produced, thereby, is monoclonal. The paraprotein belongs to one of immunoglobulin type (with one or other of the two light chains). The light chains may be produced in excess and because of small size, pass in urine. These Ig light chains in urine are called Bence Jones protein. This protein is present in urine in about 50% of myeloma cases and in about 20%, only light

chains are synthesized and Bence-Jones proteinuria occurs without the myeloma band in simple serum electrophoresis.

With malignant proliferation of plasma cells (single clone), in bone marrow, there is increased synthesis of abnormal Ig, reduced synthesis of normal Igs, and reduced formation of red cells, white cells and platelets. There is often anemia and increased susceptibility to infections.

The presymptomatic phase of multiple myeloma (may last many years) may show: unexplained proteinuria, elevated ESR, the myeloma protein in serum and/or urine, increased incidence of infections and amyloidosis.

Table 5.13 indicates the systems involved and the basis of investigations, in multiple myeloma. Some cases of B-cell lymphoma and chronic lymphatic leukemia also produce paraproteins. In some old individuals a benign paraprotein band may be seen, in electrophoresis. It however, does not increase in size with passage of time and is not accompanied with Bence Jones protein in urine. Further there is no decrease in normal Igs (Table 5.14). Also read Case history 5.1.

The factors determining the tumor mass or stage of the disease, with values indicated for stage III (high tumor mass) are as follows: i) Hb (<8.5 g/dL); ii) corrected serum calcium (>12 g/dL); Bence-Jones proteinuria (>12 g/d); serum IgG and IgA (>7 g/dL and >5 g/dL) and bone involvement (generalized lytic lesions in bones).

Myeloma needs treatment (with cytotoxic drugs) when paraprotein level rises above 5 g/dL or progressive bone lesions develop.

Table 5.13. Systems involved with relevant investigations in multiple myeloma

Growth of malignant cells in bone marrow:

- Marrow biopsy shows a large number of malignant plasma cells.
- Osteolytic lesions in X-ray.
- Increased serum calcium and alkaline phosphatase levels.

Immune system:

- Reduced levels of normal serum immunoglobulins.
- M-Band in electrophoresis.
- Bence Jones protein in urine.

Kidney:

- Increased serum levels of urea, uric and creatinine.

- Plasma cell neoplasms are often associated with primary or secondary amyloidosis. Amyloid material arises from abnormal immunoglobulin products. Renal involvement may cause glomerular damage (nephrotic syndrome), renal tubular acidosis, or diabetese insipidus. Chronic renal failure occurs in about half of the patients with renal involvement.

Table 5.14. Monoclonal gammopathies of uncertain significance (MGUS)

- More common than myeloma (in 1% of population above 50 years of age; upto 10% above the age of 70 years).
- Features compared to those of myeloma;
  - Bone marrow plasma cells <10% (>10% in myeloma).
  - M-component level <30g/L.
  - No Bence Jones protein.
  - No lytic bone lesions, no anemia, no renal failure.
  - In bone marrow cells, labeling index <1% (cells exposed to radioactive thymidine).
  - Low plasma cell acid phosphatase and β-glucuronidase.
  - Salmon calcitonin stimulation test negative.

- In long term follow up about 25% of patients with MGUS develop myeloma or some other B-cell malignancy.

Waldenstroms macroglobinemia

The condition is less common than multiple myeloma. It is a varient of chronic lymphatic leukemia with greater number of plasma cells. It is characterized by presence of monoclonal IgM detected by immunoelectrophoresis. Vascular blockage due to high viscosity of serum may result in retinal hemorrhages, visual impairment and transient neurological problems. The macroglobulin binds with red cells to produce hemolytic anemia and with coagulation factors to produce hemorrhages. The macroglobulins may show features of cryoglobulins (precipitation on cooling to 4°C) and may be associated with Raynaud's phenomenon (episodic vasoconstriction of small arteries and arterioles of fingers/toes, causing pallor and/or cyanosis). Cryoglobulin formation is more common in this condition than in multiple myeloma. Cryoglobulinemia also occurs in other conditions associated with abnormal globulins (SLE). In Waldenstroms macroglobinemia, unlike multiple myeloma, there are no bone pains and no osteolytic lesions in bones, on X-ray.

Heavy chain disease

It is a rare disorder in which abnormal serum and urine protein is homogeneous α, γ or μ chain. Presentation in γ-chain disease is more like lymphoma (lymph adenopathy, hepato-splenomegaly, fever and propensity to infection) than multiple myeloma. In the α-chain disease, there is abnormal plasma cell infiltration of lamina propria of small gut resulting in severe diarrhea and malabsorption. Immunoelectrophoresis is needed for correct diagnosis, as ordinary electrophoresis may only show hypogammaglobulinemia.

Amyloidosis

Primary amyloidosis is a paraprotein disorder with monoclonal L-chain deposits in tissues and there is associated Bence-Jones proteinuria. Secondary amyloidosis (deposition of amyloid A protein) with Bence-Jones proteinuria, occurs in chronic infections (tuberculosis, leprosy), chronic inflammation (including rheumatic disorders), neoplasms or as a familial disorder. Amyloid deposits in the heart produce congestive heart failure, in kidney, proteinuria and azotemia, in intestinal tract, malabsorption and in nerves, peripheral neuropathy and other functional impairments.

Diagnosis is established by demonstration of amyloid in tissues. In primary amyloidosis monoclonal proteins can be demonstrated in serum and urine.

TUMOR MARKERS

Mitogenesis is a multistep process, starting with activation of a growth factor receptor. This in turn leads to activation of other membrane and cytosolic proteins and second messenger molecules, that transduce the mitogenic signal to the nucleus. Defects in the components of this intracellular cascade result in loss of cellular control on the mitogenic pathway. Generally, lesions in a number of components of this pathway are required for malignant change in the cell; defects in one or two components may not be enough. The normal components of the cascade are encoded by proto-oncogenes and oncogenes formed from these proto-oncogenes encode abnormal protein components (oncoproteins). With loss of control over mitogenesis, there is rapid cell proliferation, in tumor cells. Besides mitogenesis, the process of differentiation is equally important in the process of growth. Control of this process is less well understood but lesions in the components of the control process of differentiation (leading to de-differentiation) are very important in malignant transformation of tumors. The third fundamental property of malignant tissue is related to its capability to metastasize. Mechanism by which this property is acquired is not well understood. *Information of malignant tissue, as explained above, new proteins are produced corresponding to the changes of the transformation process.*

The molecules involved in the above mentioned process of malignant transformation may act as tumor markers. The transformed cells may also produce certain substances which are unusual for the normal tissue. These will also out as tumor markers. Thus tumor markers are produced related to the process of malignant transformation, cell de-differentiation, rapid cell proliferation and other tumor associated events. Related to malignant transformation, c-erbB-2 protein (p185) and its cleaved product have been studied. Serum level of cleaved product of p185 correlates with change in concentration of many other major tumor markers in serum.

Proteins encoded by mutated suppressor genes (for suppressing cell growth) may become tumor markers for certain tumors. Mutant p53 proteins belong to this group. Other suppressor genes of interest are BRCA-1 and BRCA-2. Their mutations are of interest in cancer breast. However, their encoded proteins cannot be studied at present.

Carcinoembryronic proteins are produced in the dedifferentiation process. Substances like hCG, LD, AP, HVA and 5-HIAA may be produced in increased amounts in relation to rapid cell proliferation process. In relation to metastases, tumor cells produce certain proteolytic enzymes. Activities of various tissue specific glycosyltransferases are also altered in tumor cells. This would lead to alteration in structures of certain glycoproteins, mucins, blood group substances and AFP.

Most human cancers are monoclonal in origin. But as the clone develops, further mutations occur and this gives rise to cell heterogeneity in tumors. For the same reason the antigenic profile of tumor cells and epitope profile of tumor antigens goes on changing with time.

Some tumor markers are discussed below.

Human chorionic gonadotropin

It is glycoprotein hormone, made up of noncovalently linked α and β subunits. Both malignant and non-neoplastic trophoblast cells produce intact hCG as well as free α and β subunits. Assay of hCG is specific for β subunit. Further the free α and β subunits can also be assayed separately.

Hydatidiform mole is a potentially malignant proliferation of trophoblastic tissue which is treated by uterine curettage. If some tissue is left behind there is risk of developing chriocarcinoma (it is malignant proliferation of trophoblastic tissue). hCG is an excellent tumor marker for this tumor. Free β -subunit is more useful in detection of recurrence, as intact hCG level may remain normal. It is useful in monitoring response to treatment as well.

α-hCG (free) is a tumor marker for pituitary endocrine tumors. Use of hCG, as a tumor marker, for germ cell tumors, is discussed separately.

Prostatic specific antigen (PSA)

It is perhaps the best tumor marker discovered so far. It is synthesized by epithelial cells of prostate gland and its usefulness is because of high tissue specificity, although, there is lack of cancer specificity. PSA levels are increased in both prostatic hypertrophy and prostatic cancer. Together with rectal digital examination or transrectal ultra-sound, it may be used for screening of clinically significant prostatic cancer. Major amount of PSA, in serum is present as PSA-antichymotrypsin complex. Further the complexed fraction increase is higher in cancer cases than those of prostatic hypertrophy. Thus assay of complexed form has higher specificity for detection of cancer prostate. PSA assay is particularly useful in monitoring surgical prostatectomy. Measurable PSA level after the operation means residual prostatic tissue or metastases.

Table 5.15. Superiority of monoclonal assays over polyclonal assays

- Give better reproducibility.
- Give better specificity as there is reduced non-specific cross reactivity.
- Give wider linear concentration range in assay.
- Better agreement between results of different kits.

- The tumor markers CA 19-9, CA 125 and CA 15-3 detected by monoclonal antibodies are more specific and sensitive than CEA detected by using polyclonal assay.

Carbohydrate antigens (CA) markers

CA 19-9, CA 125 and CA 15-3 (detected by monoclonal antibodies) are much more sensitive and specific (Table 5.15) than CEA (using polyclonal assay) for pancreatic, ovarian and breast carcinomas. These markers have been used with CEA and other tumor markers to increase both sensitivity and specificity for tumor diagnosis (Table 5.16). CA 125 is expressed by more than 80% of non-mucinous epithelial ovarian carcinomas. CA 125 is found in most serous, endometriod and clear cell carcinomas of ovary. Ovarian tumors also have a strong familial incidence. Thus CA 125 estimation along with ultrasound examination and clinical examination can be used to screen relatives of a patient of an ovarian tumor.

Alpha-fetoprotein (AFP)

It is a major fetal serum protein and also one of the major carcinoembryonic proteins. It resembles serum albumin in many physiochemical properties. It is the most useful marker for diagnosis and management of hepatocellular carcinoma (HCC). But its level is also increased in pregnancy and many benign hepatic diseases. AFP has been used for screening population for hepatoma in China and certain nearby countries (also Eskimos). There is high incidence of this tumor in these parts of the World. Increased fucosylation of AFP in primary HCC (determined by lentil lectin reactivity of serum AFP) is used to differentiate the condition from benign liver disorders and also to indicate beginning of hepatoma in cirrhosis. Use of AFP in screening for neural tube disorders is discussed elsewhere (Chapter 18).

Table 5.16. Use of some tumor markers in different malignancies

hCG	Choriocarcinoma Gonadal germ cell tumours (D,P,MT,R)	ACP, PSA	Cancer prostate (D,MT,R)
		Paraprotein	Myeloma (D.MT,R)
AFP	Hepatoma, Gonadal germ cell tumor (D,P,MT,R)	Calcitonin	Medullary carcinoma thyroid(S.D,MT,R)
CEA	Colorectal carcinoma (MT,R)	CA-125	Ovarian carcinoma (D,MT,R)

Some other important tumor markers are catecholamines for pheochromocytoma, CD-30 for Hodgkins disease and anaplastic large cell lymphoma, CD-25 for hairy cell leukemia, neuron specific enolase for neuroblastoma and small cell cancer lung.

- Some substances act as nonspecific but sensitive markers of activity of many tumors (lactic dehydrogenase, lipid associated sialic acid, β2M, tennessee antigen, tissue polypeptide antigen). These can be used in monitoring efficacy of tumor therapy.
- Some tumor makers are produced when tumor becomes highly de-differentiated and highly malignant. Some examples are AFP (gastrointestinal, renal, bladder, breast and ovarian carcinomas), free α-hCG (colorectal and pancreatic endocrine tumors) and chromogranin A (medullary thyroid carcinoma and certain other endocrine tumors).

S = screening, D = diagnosis, P = prognosis (higher the levels more disseminated the tumor) MT = monitoring treatment (successful treatment lowers the levels), R = recurrence, after levels were once normalized.

For abbreviation of tumor marker, see the text.

Table 5.17. The screening procedures for certain malignancies

Cervical cancer	• Pappanicolaou smear, every 1 to 3 years between 18 to 65 years.
Ovarian Cancer	• Abdominal examination, transvaginal ultrasound and CA-125 level in plasma in high risk groups.
Breast cancer	• Clinical examination of breast, every year, over 50 years in females. • Mammography between 50 to 75 years, every 1 to 2 years. • Some clinicians recommend breast self examination every month.
Cancer prostate	• Evaluation of PSA level every year in men over 50 years of age.
Cancer colon	• Fecal occult blood, periodically, in people over 50 years. • Periodic sigmoidoscopy.

- It should be clear from this table that tumor markers are not recommended for population screening of malignancies except probably for cancer prostate which is also controversial. Screening, however, is recommended in high risk groups (CA-125 for ovarian cancer, AFP for hepatoma in certain regions of China. Also see Table 5.16.

Use of AFP, as a tumor marker for germ cell tumors is discussed separately.

Carcinoembryonic antigen (CEA)

It is the first carcinoembryonic protein and still most widely used tumor marker. Its levels are increased in cases of colorectal cancer, especially when hepatic metastases are present. Table 5.16 indicates its utility in cases of colorectal cancer.

Population screening or screening of high risk groups is recommended for certain malignancies (Table 5.17).

Tumor markers for germ cell tumors

These tumors arise from gonads or from extragonadal sites (retroperitoneum, mediastinum, pineal gland and others). About 50% of the germ cell tumors of testis are non-seminomatous type (pure embryonal type without much differentiatioin, endodermal sinus tumors arising from yolk sac elements, choriocarcinoma and teratomas including different cell types) and commonly present in the third decade and are associated with elevated levels of hCG and AFP. The rest 50% of the testicular germ cell tumors are seminomatous type (mor-

differentiated and less malignant) and are associated with elevated levels of only hCG.

In female the germ cell tumors constitute 75% of all ovarian tumors in women under 30 years of age. The ovarian germ cell tumors are: i) the benign tumors (usually it is dermoid cyst lined by epidermis bearing hair. It may contain teeth or calcified bone which may be seen in pelvic X-ray); ii) malignant tumors arising from dermoid cysts; iii) primitive malignant germ cell tumors (dysgerminoma, yolk sac tumors, immatue teratomas, embryonal carcinoma and choriocarcinoma).

AFP and hCG are useful in diagnosis, knowing prognosis and management of the germ cell testicular tumor and the malignant ovarian germ cell tumors. Chemotherapy is very effective against these gonadal tumors.

CEREBROSPINAL FLUID

Cerebrospinal fluid (CSF) is produced at a rate of about 500 mL/d. 70% of this fluid is derived by ultrafiltration and secretion from the choroid plexuses, and the rest from ependymal lining of the ventricles and cerebral subarachnoid space. It leaves the ventricular system through the medial and lateral formina of the fourth ventricle, into the subarachnoid space. Resorption of CSF occurs at the arachnoid villi. It circulates nutrients and collects wastes from central nervous system. It has also functions of lubricating and cushioning the brain.

Subarachnoid space contains CSF around the brain and spinal cord. This space extends as perivascular space around blood vessels entering and leaving the brain. In meningeal inflammatory diseases, proteins and cells enter CSF via these perivascular spaces. Resorption of CSF occurs at the arachnoid villi into venous sinuses and other veins. In the region of spinal cord, it is also resorbed into venous plexuses surrounding the duramater. Spinal tumors may press on this drainage system leading to increase in pressure of CSF below the tumor site and also leakage of proteins from congested vessels. The spinal cord ends near the first lumbar vertebra and the spinal subarachnoid space is punctured between the third and fourth lumbar vertebrae to obtain a sample of CSF.

Any space occupying lesion of the brain will press the surface of brain against the vault and interfere in resorption of CSF leading to increase in CSF pressure. In this case collection of CSF may cause herniation of brain tissue through foramen magnum and should be avoided. A dramatic drop in CSF pressure after removal of 1 to 2 mL of CSF, suggests herniation or spinal block above the puncture site and no further fluid should be withdrawn in such a case.

CSF should be submitted separately, for different studies: i) chemistry and immunology studies; ii) microbiological examination; iii) total and differential cell counts (for this purpose do not use glass tubes to avoid cell adherence and process quickly to avoid cell degradation); iv) cytology, if malignancy is suspected. The four major categories of diseases in which CSF examination is generally required are; meningeal infection, subarachnoid hemorrhage, CNS malignancy and demyelinating diseases. (Table 5.18)

In meningeal infections there is increase in cell count. In pyogenic cases, there is granulocytic cell reaction; in viral cases, there is lymphocytic cell reaction and in tubercular cases there is mixed cell reaction. In areas of inflammation, there is leakage of proteins (mostly albumin) form the vessels and the infecting organisms may consume CSF glucose. Changes in these two biochemical parameters, are often, not seen in viral cases. In postinfective polyneuritis, there is disproportionate increase in CSF protein, compared to cellular reaction because of hypersensitivity reaction.

Blood in CSF may indicate subarachnoid or intracranial hemorrhage. In such cases, xanthochromia (yellow colour of supernatant after centrifugation of sample of CSF) may also occur. No xanthochromia will be seen if hemorrhage is fresh due to puncture of some vein by the lumbar puncture needle. A commercially available, latex agglutination immunoassay for cross-linked fibrin derivative D-dimer is specific for fibrin degradation and is negative in traumatic taps. False positive, however, can be expected in DIC, fibrinolysis or trauma from repeated lumbar punctures.

In case of meningeal tumors, the drainage of

CSF is blocked and leakage occurs from distended veins. There is greatly increased CSF pressure, not affected by jugular vein compression. There is also a large increase in CSF protein. There is only slight increase in mononuclear cells while CSF glucose is reduced by metabolic activity of the tumor cells. Some times carcinoma elsewhere causes diffuse involvement of meninges (carcinomatosis meningitis). The clinical condition runs a chronic course with mild mixed cell reaction, increase of CSF protein and decrease of CSF glucose.

In degenerative states like multiple sclerosis and neurosyphilis, there is mild hypersensitivity reaction to the damaged tissue components, leading to mononuclear cell pleocytosis, an elevation of total immunoglobulin and presence of oligoclonal IgG. There is selective production of IgG in CNS. Thus CSF IgG is increased while total protein level is normal. The CSF IgG index is used to distinguish locally synthesized IgG from serum IgG, that may have entered CSF across a disruptive blood brain barrier. It is calculated by dividing CSF/serum IgG index (CSF IgG/serum IgG) by CSF/serum albumin index (CSF albumin/serum albumin). If the levels of IgG and albumin are expressed in mg/dL in CSF and g/dL in serum, 0.8 may be taken as upper normal limit. IgG index is abnormal multiple sclerosis, neurosyphilis, brain tumors and after cerebrovascular accidents.

One or more oligoclonal bands of CSF IgG may be demonstrated by agarose gel electrophoresis in multiple sclerosis. Simultaneous serum study will exclude a systemic origin of the bands. These oligoclonal bands are absent at onset of the disease and their number goes on increasing with time.

TRANSUDATES AND EXUDATES

Excess of fluid present in the pleural space is called pleural effusion and in the peritoneal space,ascites. The fluid, if derived by filtration across the capillary endothelium by altered systemic factors, is called a transudate and if formed in response to some local factors like infection or malignant involvement, is called an exudate. Exudates are distinguished from transudates by higher protein concentration of the former.

Table 5.18. Some CSF findings in normals and in certain disorders

Parameter (normal values)	Meningitis			Multiple sclerosis	Subarachnoid hemorrhage
	Pyogenic	Tubercular	Viral		
Pressure (50-180 mm water)	↑	↑/↓ with spinal block	N	N	↑
Cells (0-4 lymphocytes/mL)	(1000-10,000) (PMNs)	(100-5000) (Lymphos.)	10-2000 (Lymphos.)	0-100 (Lymphos.)	Large no. (Red Cells)
Glucose (40-70 mg/dL) (2/3rd of blood glucose level)	↓	↓	N	N	N
Protein (20-50 mg/dL)	↑	↑	N/↑	N/↑	↑
IgG (0.9-5.7 mg/dL)/total protein)% (<13%)	-	-	-	↑	-
IgG index (0.29-0.59)	-	-	-	↑	-

- A CSF to blood glucose conc. ratio <0.23, CSF protein >2.2g/L and >1180 x 10^6 neutrophils/L favour diagnosis of pyogenic than any other form of meningitis.
- Gram staining and CSF culture should also be done in pyogenic cases.
- Low neutrophil count in pyogenic meningitis carries poor prognosis.
- Certain other tests used in diagnosis of bacterial meningitis include levels of C-reactive protein, and TNF (both levels ↑), certain bacterial antigens and bacterial DNA after amplification by PCR.
- In certain viral infections viral DNA/RNA is studied (after PCR) for proper diagnosis.
- Newer tests for tuberculous meningitis include studies of adenosine deaminase, Mycobacterium tuberculosis antigens/antibodies and bacterium DNA after amplification. The last mentioned test holds maximum promise and is likely to become most useful test in paucibacillary forms of pulmonary tuberculosis and extrapulmonary disease, in near future.

Pleural effusion

The exudative effusion should have at least one of the following three characteristics: i) pleural fluid protein/serum protein ratio >0.5; ii) pleural fluid LDH/serum LDH ratio >0.6; iii) pleural fluid LDH more than two-thirds of normal upper limit for serum. The pleural effusion is a transudate if none of the three characteristics is there. Three important causes of transudative pleural effusion are, congestive heart failure, hepatic cirrhosis and hypoproteinemia (fourth cause is pulmonary embolism and infarction).

Important causes of exudative pleural effusions are tuberculosis and other infections (pulmonary), malignancy (metastatic and pulmonary), pleural involvement in SLE and rheumatoid disease, pancreatitis and ruptured esophagus. Important tests to arrive at the cause are as under; pleural fluid (PF) pH, and levels of glucose and amylase; gram staining, cytology and culture of PF. Needle biopsy of pleura may be needed.

Any of the following is an indication for tube thoracostomy (for drainage of fluid): presence of gross pus; organisms revealed by Gram staining; glucose level <50 mg/dL and pH<7.0 or 0.15 units lower than arterial pH.

Ascites

Important causes of ascites include, cirrhosis, congestive heart failure, nephrosis, tuberculosis and disseminated carcinomatosis. It is important that the cause of ascites is firmly established before treatment is started. For example ascites due to cirrhosis may get worsened by superadded tuberculous infection and in that case simple treatment with salt restriction and diuretic may appear ineffective.

It is best to characterize ascites on basis of serum-ascites albumin gradient. In exudative type it is <1.1 (pyogenic, tuberculous, neoplasm and pancreatitis). In transudative type it is >1.1 (cirrhosis, congestive heart failure). In nephrosis although the ascites is transudative type the gradient is <1.1 because of low serum albumin level. Also see Table 5.19.

Table 5.19. Ascitic fluid characteristics in certain diseases

Condition	Protein (g/L)	Cells(per µL)	Other tests
Cirrhosis	<25	Predominantly mesothelial cells(<250)	
CHF	15-50	Mesothelial, mononuclear (<1000)	
Nephrosis	<25	Mesothelial, mononuclear (<250)	May be chylous
Pyogenic	>25 (if purulent)	Predominantly polymorphonuclear	Gram stain: Culture
Tuberculous	>25	Lymphocytes (>1000) Also red cells	Stain for acid fast bacilli: Peritoneal biopsy
Malignancy	>25	Variable cell types (>1000) Also red cells	Cytology: Peritoneal biopsy
Pancreatitis	Often >25 (variable)	Variable May be blood stained	Increased amylase level in peritoneal fluid/blood: Chylous

- Serum - ascites albumin difference or gradient >1.1 in cirrhosis, CHF and <1.1 in the rest.
- Blood stained fluid with protein level >25 g/L often in malignancy, tuberculosis. In pancreatitis it may be chylous/blood stained.

CASE HISTORY: 5.1

A 62 year old patient presented with complaints of bone pains, loss of weight and increased frequency of intercurrent infections. Patient had proteinuria, better demonstrated by sulfosalicylic precipitation method than by the dipstick method. There was suspicion of multiple myeloma.

➢ How to confirm the diagnosis?

➢ What is the cause of increased frequency of infections?

- Three important investigations used in diagnosis of multiple myeloma are: i) demonstration of Bence Jones protein in urine and/or a paraprotein band (M-band) in serum electrophoresis.

Bence Jones protein is found in urine in about 50% of the patients and in about 20%, this finding occurs without presence of the M-band; ii) excess of plasma cells in bone marrow biopsy; iii) X-ray evidence of typical bone lesions. Two of the three findings are considered sufficient for diagnosis. Many patients have the initial finding of increased serum globulin level. Subsequently, M-band would be demonstrated on electrophoresis.

- As bone marrow is dominated by plasma cells synthesizing abnormal immunoglobulin, synthesis of normal immunoglobulins is reduced. This explains increased frequency of infections.

CASE HISTORY: 5.2

A 54 year old employee of a hospital developed breathlessness soon after his lunch. He also complained of dizziness. His blood pressure was 110/70 (previously recorded level was 170/100). ECG record indicated a recent myocardial infarction. Within 2h of the episode he was admitted to ICCU.

➢ Comment on clinical presentation of the patient.
➢ What is role of enzyme studies at the time of admission?
➢ What supportive meausres and drugs are used to limit the infarct size?
➢ What are biochemical indiations of multple organ failure in case of severe shock following AMI?

- Clinical presentation indicates early left ventricular failure due to myocardial infarction. Lowering of blood pressure and dizziness are pointers to the episode. Many times especially, in old individuals there is no chest pain.
- The earliest enzyme change is increase of CK-MB in serum, occurring at 4 to 8h and peaking at 12 to 24h. Thus no enzymes changes can be expected at this stage. However, it may be useful to establish baseline values for enzymes at this stage. About the protocols for use of serum enzymes in diagnosis of MI, it may be said: i) CK-MB is better than CK; ii) CK-MB and CK together (to express CK-MB as % of CK) are better than CK-MB alone; iii) CK or CK-MB estimatioins in multiple samples (to look for peak levels) are better than single sample studies; iv) AST/LDH are occasionally useful in late samples.
- It is done by protecting ischemic myocardium by proper O_2 administration, pain control, minimizing tachycardia, controlling hypertension and treating congestive heart failure. Drugs used for thrombolysis, anti-thrombotic agents, β-blockers and ACE inhibitors are employed for restricting the size of infarct.
- Prolonged severe hypotension produced by cardiogenic shock, (hypovolemic shock is much less damaging) can produce multi-organ failure syndrome and is associated with high mortality. The shock stage is followed by a period of hypermetabolism of 7-10 days showing hyperglycemia; lactic acidemia and polyuria with excessive nitrogen excretion in urine (>15 g/d). During this period evidence of lung injury and hepatic failure appear. After a progressive increase of bilirubin for about a week evidence of renal failure appears (progressive increase of serum creatinine). This is followed by encephalopathy, consumption coagulopathy, gastrointestinal bleeding and failure of immune system.

With progressive increase of blood glucose, serum lactate and urinary N-excretion, there is decrease in levels of serum albumin and other liver proteins.

Poor prognostic features are initial mean arterial pO_2/FIO_2 ratio of <250 (normal = 400) and blood lactate of ≥3.4 mg/dL (normal <1.5 mg/dL) on day 2. Subsequently rapidly developing hepatic and renal failures confirm a bad prognosis.

CASE HISTORY: 5.3

A 58 year old patient was admitted with complaints of pain in upper abdomen, loss of weight and mailase. There was long history of excessive alcohol intake. Examination showed dullness on both the lung bases. There was no jaundice. There was only modest increase of ALT and AST. Levels of ALP and γ-GT were 2080 U/L and 940 U/L, respectively.

In this case there is no parallel increase in bilirubin and ALP levels and at the same time there is only a small increase in levels of ALT and AST. This combination of findings strongly suggests presence of primary or secondary hepatic malignancy. This combination of findings can also occur in infiltrative conditions of liver (amyloidosis). In case of secondary liver malignancy, one should look for the primary tumor. Also the enlarged liver is hard and irregular. Hepatocellular carcinoma may develop in a patient of cirrhosis or chronic hepatitis following HBV infection and following hepatic injury produced by various carcinogens including aflatoxins. High level of γ-GT in the present case indicates that the hepatic damage could be because of chronic alcoholism, which in turn could be a factor in development of hepatocellular carcinoma.

α-Fetoprotein is a good marker for hepatocellular carcinoma. Liver biopsy and fetoprotein level are two investigations which will help confirmation of diagnosis.

OBJECTIVE TYPE QUESTIONS

Pick out the incorrect statement

1. Myocardial infarction

i) CK-MB is more specific for diagnosis of myocardial infarction than total CK.
ii) In pulmonary embolism, levels of both AST and LDH may increase.
iii) In a hemolysed sample level of CK will increase due to its release from red cells.
iv) γ-Glutamyl transpeptidase (γ-GT) levels increase in chronic alcoholics.
v) In a patient without jaundice, high level of ALP may indicate hepatocellular carcinoma.

2. Myocardial infarction

i) A silent episode of MI may subsequently be detected by ECG.
ii) Three commonly used enzymes for diagnosis of MI are CK, LDH and AST.
iii) CK rises most rapidly of the three and peaks at 24h.
iv) Raised CK levels can be demonstrated even 1h after an attack of MI.
v) Increase of serum enzymes in MI, in isolation, cannot be used to indicate size of infarct.

3. Transaminases

i) Serum ALT, primarily, arises from liver.
ii) Serum AST can arise from different tissues like heart, muscle, kidney, brain, besides liver.
iii) ALT is cytosolic enzyme.
iv) AST is present in cytosol as well as mitochondria.
v) In viral hepatitis AST/ALT ratio is generally, more than 1.0.

4. Plasma proteins

i) Increase of serum proteins is usually due to increase of globulins.
ii) A serum with high globulin level may show a paraprotein band.
iii) All patients of multiple myeloma show presence of Bence-Jones protein in urine.
iv) In multiple myeloma there may be evidence of bone lesions in X-ray examination.
v) Multiple myeloma may show presence of large number of plasma cells in bone marrow biopsy.

5. Diagnostic use of certain specific proteins

i) Reduced ceruloplasmin levels occur in patients of Wilson's disease.
ii) α_2-Macroglobulin level, in plasma, increases, in nephrotic syndrome.
iii) C-reactive protein increases in acute infections.
iv) Haptoglobin levels are reduced in hemolytic disorders.
v) Transferrin level gives an idea of iron stores.
vi) Antitrypsin deficiency may cause pulmonary disease.

6. Electrophoresis

i) The most important use of electrophoresis is to study the presence of M-band.
ii) If plasma is used for electrophoresis, fibrinogen may appear as a separate band which may be mistaken as a M-band.
iii) In the presence of bilirubin or drugs binding with albumin, one may see two bands for albumin.
iv) The M-band and Bence Jones protein in urine are also features of MGUS.
v) Haptoglobin-hemoglobin complexes may also appear as a M-band.

7. Electrophoresis

i) In hemolytic condition, hemoglobin appears as a sepatate band in electrophoresis, only after haptoglobin has been depleted.
ii) In acute phase response, electrophoresis shows increased level of haptoglobin and reduced level of albumin.
iii) In chronic response to stress, there is increase of haptoglobin, greater decrease of albumin (than acute phase response) and increase in size of γ-globulin band due to polyclonal increase of immunoglobulins.
iv) Once a myeloma band is seen in electrophoresis, in a patient of myeloma, there is no need to quantitate immunoglobulins.
v) In cirrhosis, besides decrease of albumin, there is polyclonal increase of all immunoglobulins causing increase of γ–globulin and β-globulin, causing β–γ bridging.
vi) In adults hypogammaglobulinemia (poor γ–globulin band on electrophoresis) is commonly seen in lymphorticular disorders and also after chemotherapy for malignancies.

8. Immunodeficiency (primary)

i) Absolute lymphocyte count is primarily related to T cell function and not B cell function.
ii) Presence of IgG in first few months of life rules out X-linked or autosomal primary panhypoglobulinemia.
iii) Congenital X-linked agammaglobulinemia is

familial and is caused by mutation in Bruton tyrosine kinase gene.

iv) Common variable immunodeficiency and IgA deficiency may be two different clinical forms of the same underlying gene defect in a large number of patients.

v) In some primary B cell deficiency states levels of some immunoglobulins may be reduced while of others increased.

vi) Severe T cell abnormalities with or without hypogammaglobulinemia are often treated with bone marrow transplantation.

vii) Human immunoglobulin replacement therapy should be used only in IgG deficient patients with recurrent bacterial infections.

9. Immunodeficiency

i) In DiGeorge syndrome there is primary T cell deficiency with heart lesions and hypoparathyroidism.

ii) In DiGeorge syndrome, the immune defect usually, spontaneously, improves.

iii) In ataxia-telangiectasia there is DNA repair defect and cells are highly susceptible to radiation induced chromosomal damage.

iv) In ataxia-telangiectasia there is high incidence of lymphoreticular malignancy.

v) In primary T cell deficiency states immunoglobulin levels are not affected.

10. Myeloma (bone lesions)

i) Myeloma cells produce osteoclastic activity factor (OAF) which stimulates osteoclasts to produce bone lesions.

ii) Myeloma bone osteolytic lesions are not followed by osteoblastic (bone formation) activity.

iii) Bone lesions are not used in staging myeloma.

iv) In patients of MGUS, there are no bone lesions.

v) The salmon calcitonin simulation test is positive in myeloma but not in MGUS.

11. Myeloma (renal involvement)

i) Over half the patients of myeloma suffer from some renal pathology.

ii) Renal failure occurs in about 25% of myeloma patients.

iii) Myeloma associated hypercalcemia and hyperuricemia contribute to renal failure.

iv) Glomerular deposits of amyloid also contribute to renal failure.

v) L-chains passing through filtration membrane, produce glomerular injury.

vi) Kidney infection may also occur as there is general increased tendency to infections.

12. Multiple myeloma

i) The M-component is cationic leading to decreased anion gap.

ii) Increased plasma protein level may produce pseudohyponatremia.

iii) Interaction between M-component and the clotting factors may produce clotting abnormalities.

iv) The M-component may form cryoglobulins producing Raynaud's phenomenon and hyperviscosity syndrome.

v) Besides change in bone marrow, plasma cell expansion often leads to enlargement of spleen and lymph nodes.

13. Tumor markers

i) AFP and hCG are tumor markers for testicular teratoma.

ii) Very high serum level of AFP in teratoma, means poor prognosis.

iii) Management of teratoma may require surgical excision followed by chemotherapy.

iv) Steady increase in post treatment low level of AFP means recurrence.

v) Tumor markers are not used for screening purpose.

14. Tumor markers

i) Tumor markers may be hormones/proteins/enzymes secreted by the tumor cells.

ii) Tumor markers may be certain tumor antigens.

iii) A positive pregnancy test in a male is always a false positive.

iv) Prostatic specific antigen (PSA) has high tissue specificity but lacks cancer specificity.

v) CEA is used for monitoring success of treatment in colorectal carcinoma.

15. CSF examination

i) Early diagnosis of meningeal disorders is important to reduce mortality/morbidity.

ii) In bacterial meningitis, the rapid bacterial antigen tests have become quite popular because of high sensitivity and ease of use.

iii) Early diagnosis of tubercular meningitis from CSF examination, at present, is quite difficult.

iv) Increased IgG index and presence of oligoclonal bands are highly diagnostic of multiple sclerosis.

v) Diagnosis of viral meningitis is mostly a matter of exclusion.

ANSWERS

1. No.(iii). Hemolysis increases serum LD and acid phosphatase but does not affect levels of serum amylase, ALP and ALT. Hemolysis is visible in serum at Hb level of >20 mg/dL.
2. No.(iv). Increases at 4 to 8h.
3. No.(v). In viral hepatitis AST/ALT ratio is generally less than 1.0. In most other types of hepatic damage this ratio is generally more than 1.0.
4. No.(iii). Only 50% of the myeloma patients show this abnormality.
5. No.(v). Ferritin level gives an idea of iron stores.
6. No.(iv). There is no Bence-Jones proteinuria.
7. No.(iv). It is required to provide a baseline to monitor response to treatment during management of the patient.
8. No.(ii). It is presence of IgM and IgA which rules out primary panhypoglobulinemia. IgG may be there because of transfer from mother.
9. No.(v). T-cells help β-cell function.
10. No.(iii). Staging of myeloma to predict prognosis is based on hemoglobin, calcium and paraprotein levels, degree of skeletal involvement, and total body tumor burden.
11. No.(v). The L-chains present in tubular fluid are reabsorbed and catabolized by the tubular cells. The process leads to damage to these cells. This process produces Fanconi type syndrome and not renal failure.
12. No.(v). Plasma cells mostly remain in bone marrow. In solitary bone plasmacytoma there is a single bone lesion. In extramedullary plasmacytoma there is involvement of lymphoid tissue in regions of nasopharynx or paranasal sinuses.
13. No.(v). In certain familial malignancies these may be used to screen rest of the family members.
14. No.(iii). It may indicate a testicular tumor.
15. No.(iv). Positive predictive value of the test is largely dependant upon degree of clinical suspicion. Thus the tests have a complementary value only.