# D.M. BRANCH VIII – CLINICAL HAEMATOLOGY

India must produce specialists in Haematology who are able to integrate the laboratory aspects and clinical management of the patient with haematologic disorders. There have been numerous advances in Haematology over the past two decades which makes it necessary for the country to have post-graduate training in the speciality. The Haematology laboratory is now expected to provide rapid, accurate and reproducible results for large numbers of samples and this is possible with automation. Molecular techniques are now no longer research tools but necessary for ante natal diagnosis and clinical decision making. Blood banking has come a long way with component therapy and single donor apheresis. Bone Marrow transplantation now provides a cure for many hitherto incurable diseases and a competent haematologist is necessary to offer this type of treatment. The doctor who undergoes post-graduate training in haematology should possess the necessary clinical and laboratory skills to be able to manage patients with primary haematological problems and interact as a consultant for haematology problems from other specialities and be competent in laboratory haematology and transfusion medicine.

Departments involved in the training programme:

i) Clinical Haematology
ii) Immuno Haematology and Transfusion Medicine
iii) Biochemistry
iv) General Pathology
v) Nuclear Medicine
vi) Radiotherapy

## I. **Laboratory Haematology Training:**

### A. General Laboratory Haematology:

a) Proper use and care of common laboratory instruments such as the light microscope, Centrifuge, water baths, freezers, weighing balance etc.,

b) Weighing of solids, preparation of molar and Normal solutions, preparation and use of buffers. Familiarization with the practical concepts of pH, molarity, normality, osmolality, normal values and reference ranges.

c) The nature and uses of distilled and deinoized water.

d) Blood collection of samples venupuncture and finger prick methods of sample collection, types of anticoagulants, containers and the effects of delay in processing and storage.

e) Determination of blood counts (Haemoglobin, haematocrit, total WBC and platelets) manually and calculation of red cell indices.

i) Use of automated electronic blood cell counters including principles and practice.

ii) Interpretation of peripheral blood counts.

iii) Preparation of blood films - manual and automated techniques.

iv) Staining of peripheral blood films with Romanowsky and other dyes by manual and automated techniques.

v) Review of normal and abnormal blood films with emphasis on

a) Morphology of red cells, White cells and Platelets.
b) Performance of WBC differential count.
c) Subjective assessment of platelet count.
d) Diagnostic interpretation of abnormal films.

vi) Preparation of smears of bone marrow aspirates and biopsy imprints (touch preparations) .

vii) Preparation and staining of thin and thick blood films for malaria parasites.

viii) Supravital staining of reticulocytes; manual and automated counting of reticulocytes.

ix) Performance of bone marrow aspiration and trephine needle biopsy.

xi) Staining (Romanowsky dyes and Prussian Blue for iron) and diagnostic valuation of smears of bone marrow aspirate.

xii) Performance and interpretation of Hbs (sickle haemoglobin) solubility test, screening for red cel G6PD activity and its interpretation.

xiii) Neutrophil function assays.

B. Cyto Chemistry:

Performance of the following staining procedures viz. Kleihauer acid elution technique for HbF: PAS: Sudan Black B, Myeloperoxidase, specific and non-specific and dual esterases, acid phosphatase and iron staining.

C. Laboratory Investigation of Haemolytic Anaemias with particular reference to the Haemoglobinopathies (including the thalasaemias) red cell Enzymopathies. Red cell membrane disorders and Immune Haemolytic anaemias;

(A)
i) HbS solubility test.
ii) Screening for unstable hemoglobin (heat instability and Isopropanol tests).

iii) Supravital staining for HbH inclusions.
iv) Principles and practice of separation and identification of normal and abnormal hemoglobin by electrophoresis and chromatography.
v) Quantitation of normal HbA, HbF and HbA2 and abnormal HbS, D,E,C etc. haemoglobin by densitometry and chromatography – HPLC.
vi) Quantitation of HbF by alkali destruction and Cellular distribution of HbF by the Kleihauer elution technique.
vii) Heinz body preparation.
viii) Screening for red cell G6PD deficiency and quantitative estimation of red cell G6PD activity.
ix) Screening for red cell pyruvate kinese (PK) Deficiency and essay of red cell G6PD activity.
x) Screening for other red cell enzymopathies and assay of Red cell PK activity.
xi) Standard hypotonic saline osmotic fragility test, acid Glycerol lysis, time (AGLT) and autohaemolysis tests.
xii) Sucrose lysis and Ham's acidified serum tests for PNH, Urine hemosiderin.
xiii) Direct and indirect antiglobulin (Combs) tests, warm and cold autoantibody (Cold agglutinin) titre, Donath Land-stainer cold auto antibody screening and titration.

B) Miscellaneous bio chemical tests on red cells, Plasma and Urine:

i) Principles of procedures for estimation of plasma bilirubin and haematolobin and significance of results, screening for methamelbumin, methemoglobin and Sulphaemoglobin.
ii) Screening for cryoglobulins and cryofibrinogen; principles of immunoglobulin estimation and immuno electrophoresis.
iii) Examination of urine for Hb, red cells, haemosiderin, urobilinogen and bilirubin.
iv) Principles of estimation and significance of serum ferritin, Iron and TIBC.
v) Principles of estimation and significance of red cell folate, Serum folate and serum cobalamin.

D) Cytogenetics:

Familiarization with cytogenetic techniques, understanding the principles of cytogenetics and appreciating the relevances and significance of chromomes in diagnostic haematology, interpreting the results of chromosome preparation of haemopoletic cells.

E) Laboratory Investigation of Bleeding Disorders:

a) Platelets

i) Performance of Ivy bleeding time, template bleeding time and platelet count; study of platelet morphology.
ii) Principles, practice and interpretation of platelet aggregometry tests.
iii) Platelet associated immunoglobulin (PlAIg) and circulating antiplatelet antibodies.

b) Screening and coagulation factor abnormalities:

i) Prothrombin time and Stypven time.
ii) Activated partial thromboplastin time.
iii) Thrombin time and reptilase time.
iv) Plasma fibrinogen.
v) Correction studies with normal plasma, adsorbed plasma, aged serum and factor deficiency plasmas.
vi) FDP and D- Dimers.
vii) Assays of clotting factors particularly factors viii and ix.
viii) urea solubility test for factor XIII.

c) Euglobulin lysis time and other relevant tests of plasma, fibronolytic activity.

F) Laboratory Investigation of Thrombotic disorders:

i) Assays of plasma AT III, protein C, protein S.

ii) Screening for lupus anticoagulant and activated protein C resistance - principles of screening tests and interpretation of results.

iii) Laboratory monitoring of anticoagulant (heparin and oral anti-coagulant) therapy.

iv) Techniques for the detection of anticardiolipin antibodies;

G) Transfusion Medicine:

1. a) ABO blood grouping (forward and reverse), Rh typing (Phenotypes and genotypes), screening of antibody in sera of donors and recipients, antibody identification following elution by various techniques.

   b) Blood group compatibility (cross matching testing).

   c) Investigation of ABO, Rh and other immunohaemolytic diseases of the new born.

   d) Investigations of platelets refractoriness.

   e) Practical aspects in the selection of blood for normal exchange transfusion.

2. Donor recruitment.
3. Clinical evaluation and laboratory screening of donors prior to phlebotomy.
4. Phlebotomy of donors.
5. Blood component preparation and storage.
6. Practical and administrative procedures involved in issuing and transfusing blood.
7. Principles of the mechanic of the cell separator and its use for blood component preparation and therapeutic apheresis.

8. Practical steps in the laboratory investigation of transfusion reactions.

H. Flow Cytometry:

A working knowledge of the principles and practice of flow cytometry, sample preparation and interpretation of the clinical significance of common leucocyte immunophonotypes as well its use and relevance in the evaluation of red cell and platelet disorders.

I. Laboratory Organizations:

a) Laboratory space distribution, ordering, location and installation of laboratory equipment; work flow procedures and handling of samples.

b) Staffing - technical and non-technical.

c) Use of computers and generation of laboratory statistics.

d) Health and safety measures - personnel safety.

e) Waste disposal.

f) Quality assurance (Internal and External) measures.

i) Pre-analytical variables : request forms, patient information, patient preparation, effects of medication and blood transfusion, sample collection, anticoagulants, containers, sample labeling, identification, transport, processing and storage.

ii) Analytical variables inter laboratory harmonization, data handling and statistical analysis.

iii) Post analytical variables : computer inter facing security and recording of results, turn around time.

j) Laboratory Equipment:

A working knowledge of the mechanics of the various laboratory instruments including their operation, calibration and basic maintenance is desirable.

II. Histopathology module:

Practical laboratory training and related theory should cover the following areas:

a) General processing of tissues.
b) Techniques of cytology including cytopin in relation to body fluid of patients with haematological disorders.

c) Immunocytochemistry relevant to haematology.
d) Electron microscopy of haemopoietic cells.
e) Anatomical pathology of the bone marrow - review of biopsy material.

III. Bio-chemistry Module:

Laboratory Techniques - Practical hands on experience and related theoretical background in the following:
a) Separative procedures - Electrophoretic techniques, chromatography.
b) Immunochemical methods.
c) Radio Immunoassays.

IV. HLA module for haematologists:

Demonstration and understanding the principles of:

a) Separation of lymphocytes using density gradient centrifugation.
b) The microlymphocytotoxicity test and its application in HLA typing, cross matching and antibody screening.
c) DNA based HLA typing.
d) HLA antibody identification.
e) Miscellaneous investigations (on request) including mitogen and antigen induced lyphocyte transformation.

V. Molecular Biology :

Understanding the principles involved in the molecular diagnosis of Haematological disorders :

a) DNA and RNA extraction.
b) PCR - Polymerase Chain Reaction.
c) RT and RQ PCR.
d) RFLP and other techniques to evaluate polymorphisms.
e) Mutation detection principles and techniques.
f) Sequencing.

VI. Nuclear Medicine:

a) Measurement of blood volume, red cell mass, red cell survical studies.
b) Screening techniques applicable to blood disorders.
c) Use of PET and gallium scans.

VII. Medical Statistics:

a) Study design, statistical methods, survival curves, data collection and storage, interpretation and analysis.

## II. Clinical Haematology Training:

With appropriate guidance and under supervision, the post-graduate student will be responsible primarily for the acquisition of knowledge in all areas of Haematology and Transfusion Medicine. Such knowledge will be acquired and demonstrated through Seminars, case presentations, Journal clubs, Tutorials, proper use of the library for 'Suggested Reading' and formal reviews of selected major topics. Faculty should be present at these various exercises so as to provide the appropriate input. When necessary, faculty may be required to review certain subjects in the form of formal lectures, however lectures will not play a dominant role in the theoretical component of the training Programme. Clinical experience will be acquired by the trainee by day to day management of all patients admitted to the haematology service. Faculty will be involved in teaching of trainees in the ward rounds and out patient clinics.

Red Cell Disorders:

Clinical evaluation of a patient with anaemia, history, physical examination, appropriate laboratory investigations and management, Comparative epidemiological significance of `nutritional' and other anaemias in the population and the national programmes for control.

1. Iron deficiency anaemia:

Epidemiology, iron deficiency as a community health program, causes in the population, control strategies in the population. Evaluation of the individual patient interpretation of serum iron, TIBC, transferrin, ferritin, indications for and interpretation of ferrokinetic studies, management including iron replacement.

2. Megaloblastic anaemia:

Clinical and laboratory evaluation, clinical recognition, evaluation and management of complications of vitamin B12 deficiency, investigation of aetiology and management. Understanding the role of Vitamin B12 and folate in cellular metabolism and the interaction of disease and drugs with the metabolism of folate.

3. Hemolytic anaemia:

Evaluation of a patient with haemolysis and investigation of its courses;

i) Thalassaemia: Principles of control of the thalassaemia syndromes in the population, screening strategies, antenatal diagnosis, genetic counselling, clinical and laboratory diagnosis of alpha and beta thalassaemia syndromes. Management of thalassaemia intermedia and major transfusion regimes, chelation, role of splenectomy and bone marrow transplantation.

ii) Sickle cell disease: Evaluation management of the steady state, management of painful of crises, management of chronic complications, clinical and haematological features of

the various sickle cell diseases, clinical and haematological effects of the interaction of thalassaemia with sickle cell anemia; therapeutic role of bone marrow transplantation.

iii) Inherited enzymopatheis (Red Cell G6PD deficiency) evaluation and management of acute haemolytic crises.

iv) Acquired haemolytic disorders, immune haemolytic anaemia management with immunosuppression, role of intravenous immunoglobulin, plasmapheresis, splenectomy, Clinical and laboratory evaluation (including aetiological diagnosis) of patients suffering from acquired intravascular haemolysis.

4. Aplastic anaemia:

Aetiology, evaluation and management including immunosuppression (antilymphocyte globulin etc) and supportive therapy. Role of bone marrow transplantation in treatment of the individual patient; preparation for bone marrow transplantation.

5. Red Cell Aplasia:

Diagnostic evaluation and treatment of congenital and acquired forms. Transient erythroid aplasia including the pathogenetic role and biology of the human B19 parvovirus.

III. White Cell Disorders :

1) Neutropenia : Clinical evaluation of neutropenic patient, role of surveillance microbiology, antimicrobial therapy in neutropenia, role of growth factors, principles in providing a sterile environment for the neutropenic patient.

2) Functional disorders of neutrophils: Neutrophil function, laboratory tests for evaluation and management of patient with chronic neutrophil dysfunction, role of growth factors and bone marrow transplantation.

3) Leukaemia: Clinical evaluation, diagnostic confirmation by morphology, immunophenotyping, special stains, cytogenetics and electronmicroscopy. The trainee must be familiar with the principles of leukemia management and the various protocols available. He/she should be familiar with the statistical tools used to evaluate therapy protocols, survival curves etc. He/she should be thoroughly familiar also with the pharmacology of antimitotic drugs and their toxicity and very well versed in the supportive and management of patients with all types of leukaemia.

4) Myeloproliferactive disorders (MPD):

Classification, systematic diagnostic evaluation of erythrocytosis including polycythaemia vera, interpretation of blood volume studies with radionuclides, familiarity with current management strategies of MPD including the use of interferons.

5) Lymphome: Classification of lymphomas - principles in staging, Management of the different types of lymphomas.

6) Immuno deficiency disorders: Trainees must be able to order systematically the appropriate investigative scheme for a patient with congenital or acquired immuno-deficiency, they must understand the principles of management with immunoglobulin replacement, interferons, bone marrow transplantation and be familiar with the haematological manifestations (and their therapy) of AIDS.

7) Multiple Myeloma and other paraproteinaemia: Clinical and laboratory evaluation of a patient with a monoclonal gammopathy. Interpretation of quantitative immuno-globulin levels, serum protein electrophoretic strips and immunoelectrophoresis patterns, concept of monoclonel gammopathy of undetermined significance, management of myeloma and Walden strom's macroglobulinaemia.

IV) HAEMOSTASIS - (Trainees should be thoroughly grounded in the general clinical (history and physical signs) approach to the patient with a bleeding tendency.

1. Thrombocytopenia:

Thorough understanding of platelet kinetics and evaluation with radionuclides. Evaluation and investigation of the aetiology of thrombocytopenia. The student should be conversant with the spectrum of management including immunosuppression, intravenous immunoglobulin.

2. Inherited platelet function disorders:

Clinical evaluation, laboratory diagnostic strategies and management.

3. Inherited coagulation factor deficiencies:

Laboratory diagnosis of haemophilia, genetics and antenatal diagnosis, principles of factor replacement factor replacement schedule in a patient with haemophilia who needs surgery, management of complications. Principles management of patients with inhibitors.

4. Acquired bleeding disorders:

Vitamin K deficiency and supplementation; DIC its course and management, management of haemorrhegic complications of liver disease and renal failure.

5. Thrombotic disorders:

Classification and laboratory diagnosis of inherited thrombotic disorders, evaluation of haemostasis in the acquired thrombotic disorders, clinical use and monitoring of anticoagulants.

6. Transfusion Medicine:

1. Blood component preparation and clinical use:

Collection of blood, correct techniques for venupuncture, plastic systems, anticoagulants and additives and their effect on storage stability, centrifugation, preparation of platelets fresh frozen plasma and cryoprecipitate, storage of components, principles of fractionation. Quality assurance in transfusion medicine. A thorough understanding of the clinical indications for the proper use of specific blood components.

2. Diagnosis and management of transfusion - related complications:

Febrile transfusion reactions - laboratory investigations, diagnosis, management of prevention diagnosis and management of haemolytic transfusion reactions. Infections transmitted by transfusion complications of transfusion.

3. Cell Separation principles:

The trainee must be able to perform cell separation and plasmapheresis. Principles of the plasmapheresis. Principles of the machine, continuous versus intermittent flow techniques, replacement fluids for plasmapheresis, current status and indication in various diseases should also be known and understood.

4. Techniques of leucodepletion:

Problems related to white cells in donor and techniques of removal. Principles of filter design and use.

5. Irradiation of blood components:

Biology of irradiation of blood and components; transfusion graft versus host disease (GVHD) indications for irradiation of blood. Use of equipment.

6. Management of alloimmunisation in relation to transfusion :

Techniques for prevention of alloimmunisation; role of ultra violet radiation and photosensitizers, management of patients with red cell and platelet allo antibodies.

V. Bone Marrow Transplantation:

1) Current Indications: The student should be familiar with the current indications and results of bone marrow transplantation in various diseases.

2) Donor Selection: HLA typing issues in stem cell transplantation. Issues related to matched unrelated donor and cord blood transplants.

3) Conditioning regimens: The trainees must be familiar with the different conditioning regimes, principles of their use in different disorders and complications.

4) Harvesting and manipulation of bone marrow: Bone marrow collection, red cell, or plasma reduction, peripheral blood stem cell mobilization, collection and cryopreservation. Transfusion of marrow, purging of marrow - T cell depletion.

5) Transplantation immunology: Histocompetibility, graft versus host disease – diagnosis and management, Immune reconstitution following transplantation.

6) Management of the post transplant patient.

VI) Haemetological Oncology:

1) Cell Cycle - Cell Kinetics
2) Principles of Chemotherapy
3) Oncogenesis
4) Cytogenetics in relation to haematological malignancy
5) Use of growth factors

VII) Consultation Haematology:

1) Haematological complications of pregnancy and the interactions of the pregnant state with disorders of the Haemopoietic system.
2) Haematological complications of systemic disease.
3) Haematological problems of the intensive care patient.

VIII) Bone Marrow Aspiration and Trephine Biopsy:

Accumulation and consolidating experience in this practical procedure and in diagnostic interpretation.

IX) Neonatal Haematology:

The candidate should be familiar with haematological problems in the new born and should be able to interact with the neonatologist regarding management.

**D.M. Clinical Haematology Examination Scheme**

**THEORY**

| | | |
|---|---|---|
| Paper – I | Basic Sciences<br>Structure and function of haemopoietic System, Molecular biology and Genetic aspects of haemopoiesis | 100 |
| Paper – II | Laboratory Haematology | 100 |
| Paper – III | Clinical Haematology | 100 |
| Paper - IV | Recent Advances in Haematology | 100 |

PRACTICAL – 2 days

| | | | |
|---|---|---|---|
| CLINICAL | Long case<br>Short cases<br>Ward Round | 1<br>2 | 200 |
| LABORATORY HAEMATOLOGY | | | 100 |
| | : haemostasis<br>: haemolytic<br>: blood banking<br>: Morphology Peripheral blood and Bone Marrow | | |
| VIVA VOCE | | | 100 |
| | | TOTAL | 800 |

The candidate should obtain a minimum of 50% separately in theory, clinical and laboratory subjects.

**Marks required to pass the examination**

| | MAX | PASS |
|---|---|---|
| THEORY | 400 | 200 |
| CLINICAL | 200 | 100 |
| PRACTICAL | 100 | 50 |
| VIVA VOCE | 100 | 50 |
| TOTAL | 800 | 400 |