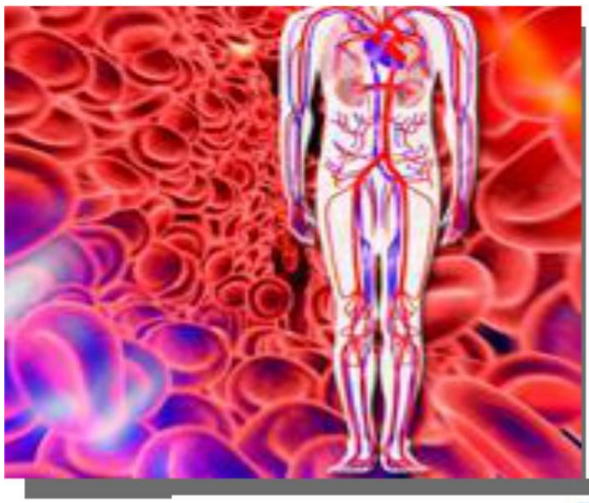
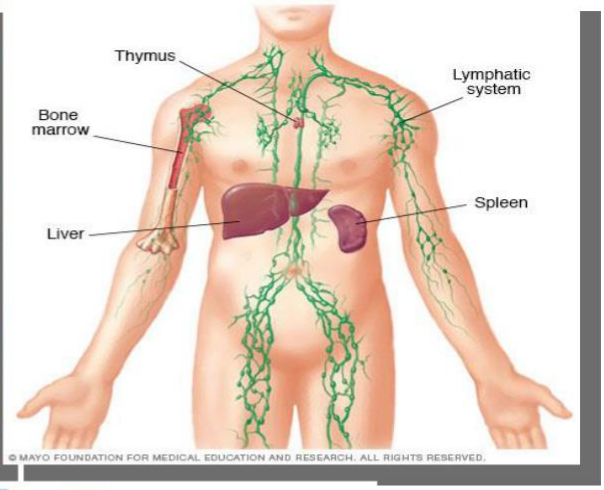
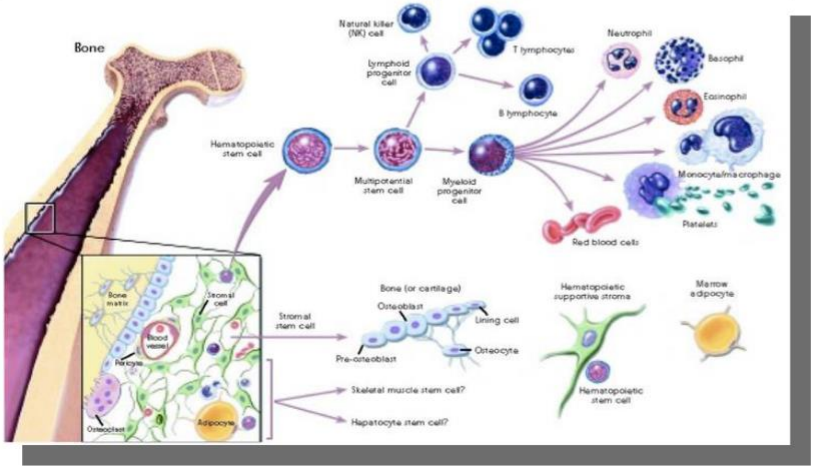
Jordan University of Science and Technology
Faculty of Medicine
Hematopoietic and Lymphoid System (HLS) Module
Integrated System Course MED272
Study Guide







Hematopoietic and Lymphoid System (HLS) Module (MED272)

Coordinator: Dr. Sohaib M. Al-Khatib **Email:**smkhatib4@just.edu.jo

Credit Hours: 6 credit hours
Duration: 4 Calendar weeks

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	THE CORRESPONDING INSTRUCTOR BY: The third week of the module	

COURSE DESCRIPTION:

(MED 272) Hematolymphoid System (6 credit hours, 4 calendar weeks)

This is an interdisciplinary integrated module of hematolymphoid system. Basic sciences of anatomy, biochemistry microbiology, pathology, pharmacology, and physiology of the hematolymphoid system correlated with clinical disorder of this system. The goal of this integrated course is to provide the medical student with comprehensive knowledge about hematolymphoid system components related to clinical manifestations of diseases. The teaching methods include lectures, labs as well as seminars and small group discussions of clinically oriented problems to enhance self-directed learning. This Knowledge is supported by skills-developing laboratory activities and clinically oriented activities. Research ideas with specific embedded objectives are also included to emphasize social responsibility, evidence-based medicine, community service, and innovative thinking.

Participating Staff Members:

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Recommended Text Books and Atlases:

Subject	Book (Resources):
Anatomy	Text of Histology by Gartner, latest edition. Clinical anatomy by Snell, latest edition.
Physiology	Textbook of Medical physiology. By Guyton and Hall, 13th edition.
Biochemistry	Thomas M. Devlin. Textbook of Biochemistry with clinical Correlations, 7th Edition
Pharmacology	Lippincott's Illustrated Reviews: Pharmacology, 5th edition.
Pathology	Basic Pathology. By Kumar, Cotran and Robbins, 9th edition.
Microbiology	Medical Microbiology. An Introduction to infectious Diseases. By Sheries, latest edition.
Community Medicine	Dr. Hashim Power point presentations and assignment.

GENERAL OBJECTIVES:

Upon completion of this course, students should be able to:

1. Describe the constituents of blood, their origin and function.
2. Discuss the structure and function of the lymph-reticular system.
3. Describe the important aspects of hemoglobin genetics and abnormal hemoglobin.
4. Understand the basic classification systems of anemia, their laboratory and clinical features, public health aspects, and their management.
5. Understand the classification of neoplastic diseases of hematopoietic cells, methods for their diagnosis and their natural history and general guidelines for their management.
6. Describe the regulatory mechanisms of normal Hemostasis, abnormalities that lead to bleeding disorders, pathologic aspects that cause thrombotic disorders and how are these conditions being treated.

ASSESSMENT AND DISTRIBUTION of GRADES:

Grade Categories	Grade %
Attendance & Behavior*	**5%**
Research/Predetermined Activities	**5%**
Midterm Theory Exam	**55%**
Final Exam on: Labs, Cases, and Clinical Lectures	**35%**
Total	**100%**

- All exams are online and in integrated form.
- The final exam will be at the end of the module course, NOT at the end of the semester.

*Any act of misconduct as determined by the course coordinator would be substituted by Attendance & Behavior grades.

SPECIFIC LEARNING OBJECTIVES:

After studying the material covered in lectures, practical sessions, clinical seminars and case presentations of this course, and after using his/her private self-learning time in a productive way, the student is expected to achieve the following specific objectives mentioned in the table for each lecture and lab:

A-Lectures:

Lecture Title	Lecture Objectives
Introduction to Hematopoietic system	1. Understand the general outline of the module. 2. Be familiar with the modalities of teaching throughout the course. 3. Acknowledge the important relation between normal and abnormal structure and function. 4. Appreciate the importance of basic sciences in clinical application.
Characteristics of Blood	1. Describe the composition of blood. 2. Understand the functions of blood. 3. Understand factors affecting viscosity of blood.
Porphyrins, Heme And Hemoglobin	1. Describe synthesis of porphyrins 2. Describe regulation of heme synthesis 3. Know types and causes of porphyrias 4. Describe degradation of heme and jaundice 5. Know structural features of human hemoglobin 6. Know different types of human globin genes 7. Know different types of normal hemoglobin 8. Know the developmental expression of globin genes 9. Identify some abnormal hemoglobins.
Heme-Lymphoid tissue I/II/III	1. Describe briefly the microscopic morphology of various blood cell types 2. Discuss the development of various blood cell types 3. Understand the origin and composition of lymphatic fluid. 4. Explain the circulation of lymph in the body 5. Describe the major lymph vessels in the body 6. List the major lymph nodes groups in the body 7. Describe the microscopic structure of lymph node (mucosa and other associated lymphoid tissue). 8. Describe the microscopic structure of spleen 9. Describe the microscopic structure of Tonsils 10. Describe the microscopic structure Thymus
Hematopoiesis	1. Understand the concept of Hematopoiesis. 2. Name organs responsible for Hematopoiesis in the fetus

	and list the developmental stages of Hematopoiesis both prenatally and postnatal. 3. Understand the concept of pluripotent hematopoietic stem cell, and the growth factors involved. 4. Understand the nutritional requirement for Hematopoiesis specifically, B12, folic acid and iron. 5. Describe cellular stages of hematopoiesis
Hemoglobin Structure and Function	1. Describe the mechanism of oxygen binding to myoglobin and hemoglobin. 2. Describe the conformational differences between deoxygenated and oxygenated hemoglobin. 3. Define the concept of cooperativity in oxygen binding to hemoglobin. 4. Describe the Bohr effect and its role in modulating the binding of oxygen to hemoglobin. 5. Explain how 2,3-bisphosphoglycerate interacts with hemoglobin and influences oxygen binding. 6. Summarize the processes by which carbon dioxide is transported from peripheral tissues to the lungs.
Physiology of RBCs/WBCs	1. Describe RBCs structure & its structure-function relationship. 2. Understand the different functions of RBCs. 3. Understand structure-function relationship of RBCs cell membrane like fluidity. 4. Identify the physiological factors that affect RBCs count. 5. Understand the life span of RBCs & its relationship to blood donation. 6. Recognize the different structural types of WBCs & their physiological functions. 7. Define the life span of WBCs & the physiological implication of that. Differentiate between migrating & circulating pools of WBCs.
Anemia Classification/Diagnosis	1. Name and describe the maturational sequence of erythroid cells in the bone marrow using the terms: proerythroblast, erythroblast, normoblast and reticulocyte. 2. Classify anemia according to pathophysiologic criteria. 3. Classify anemia according to mean corpuscular volume (MCV) and give three examples of each type. 4. Discuss the reticulocyte count, corrected reticulocyte count, and diseases associated with high and low numbers.
Drugs Used in Anemia	1. Describe the normal mechanism of regulation of iron in the body. 2. List the major forms of iron used in the therapy of anemias.

	3. List the anemias for which iron supplementation is indicated and those for which it is contraindicated. 4. Describe the acute and chronic toxicity of iron. 5. Describe the major hazards involved in the use of folic acid as sole therapy for megaloblastic anemia. 5. Describe the major bone marrow colony stimulating factors.
Molecular Basis Of Hemoglobin Disorders	1. Understand the molecular basis of sickle cell disease and trait 2. Understand the molecular basis of beta thalassemia . 3. Know molecular basis of alpha thalassemia & types 4. Know what is hemoglobin Lepore and how it is produced 5. Understand the molecular basis of delta-beta thalassemia.
Hemolytic Anemia I/II	Identify the different types of hemolytic anemia (Intravascular Vs. Extravascular/ Intracorpuscular Vs. Extracorpuscular), their clinical presentation, pathologic findings and relevant Laboratory tests and appropriate diagnostic approach.
Blood Coagulation	1. Understand the structure, function & life span of platelets. 2. Understand the interaction of platelets, blood vessels and plasma coagulation factors in homeostasis. 3. Understand the role of the liver in normal homeostasis. 4. Understand the process and stages (cascade) of blood coagulation and its significance. 5. List and understand the role of factors involved in blood coagulation.
Congenital Bleeding Disorders and DIC	1.For each of von Willebrand disease, hemophilia A and hemophilia B, describe: A- heritance B-Etiology C-Clinical presentations D-Laboratory findings E-Treatment 2. Understand the correct usage and significance of abnormalities of each of the following: A. Prothrombin time (PT) B. Partial thromboplastin time (PTT) C. B-Thrombin time (TT) D. Platelet count *For Disseminated Intravascular Coagulation (DIC), describe: A. Etiology B. Clinical presentations and complications
Drugs Used in Coagulation Disorders I/II	1. Compare the oral anticoagulants with heparin in terms of their pharmacokinetics, mechanisms, and toxicities. 2. Understand the pharmacological aspects of thrombolytic preparations. 3. Understand the pharmacological aspects of antiplatelet

	drugs. 4. List three different drugs used to treat disorders of excessive bleeding.
Inherited Disorders of Platelet Function, ITP and TTP	1. Describe the etiology, pathogenesis, clinical findings, laboratory results and patient management of adult and pediatric ITP. 2. Identify the mechanism of neonatal and post transfusion thrombocytopenia. 3. Describe the clinical findings and laboratory results of TTP.
Community Health Aspects of Anemia	1. Categorize socio-economic variables that influence community dietary practices with special reference to anemia. 2. Describe the major nutritional risk factors in the determination of anemia
Blood Grouping and Transfusion	1. Understand the principles of ABH blood group system. 2. Understand the principles of Rh blood group system. 3. Understand the principles of the HLA system
Non-Hodgkin Lymphoma	1. Understand the general characteristics of NHL, with reference to pathogenesis, classification and procedures used to diagnose them. 2. Describe the grading systems of NHL. 3. Compare the histopathologic, immunologic and clinical features of NHL. 4. List three chromosomal translocations associated with NHL; describe the oncogenes associated with them. 5. Describe the morphology of: a. small lymphocytes b. small cleaved cells c. mantle cells d. immunoblasts e. prolymphocytes f. small non-cleaved cells g. lymphoblasts
Hodgkin Lymphoma	1. Describe the appearance of Reed-Sternberg cells and identify the significance of their presence. 2. Define the meaning of “background” appearance of Hodgkin’s disease and how it is used in diagnosis and classification of this disease. 3. Describe the staging system of Hodgkin disease. 4. List the four types Hodgkin’s disease; describe their clinical presentations, general guidelines for patient evaluation and management.
Bacterial Infection I/II [Salmonella typhi (Enteric fever), Brucella, & others]	1. Understand bacterial classification and characteristic properties. 2. Understand bacterial virulence factors and pathogenesis. 3. Outline clinical presentation and differential diagnosis. 4. Understand laboratory diagnosis including specimen collection, culture techniques, and sensitivity to

	antibiotics. 5. Describe management options including specific therapeutic agents. 6. Describe epidemiological aspects including modes of transmission, prevention & control measures.
Blood Culture	1. Understand clinical indications. 2. Describe specimen collection including aseptic techniques, timing, volume, and number of bottles. 3. Differentiate conventional vs. automated techniques. 4. Be able to interpret results and specific identification properties. 5. Identify alternative methods.
Anti-Neoplastic Drugs I/II	1. Recognize the general principles of cancer therapy. 2. Understand the three main lines of cancer therapy. 3. Understand methods of administration of cytotoxic drugs and the rules for combination therapy. 4. Understand the terms: adjuvant therapy, growth fraction and cell cycle. 5. Understand the mode of drug action either phase-specific or non- specific. 6. Classify cytotoxic drugs and explain their mechanisms of action. 7. Recognize the major adverse effects of cytotoxic drugs.
Clinical management of lymphoma/Leukemia	1. Understand what are the different modalities of treatment of hemato-lymphoid malignancies: *Chemotherapy (adjuvant and neoadjuvant). *Surgery. *Radiotherapy. *Monoclonal antibodies *Bone marrow transplantation 2. Identify features that put patients at increased risk of complications of treatment. 3. Describe the common complications of chemotherapy and how to prevent and treat such complications. 4. Describe the clinical presentations, complications and patient management of acute leukemias.
Acute Leukemia I/II	1. Understand the classification of acute leukemias (AML/ALL). 2. Define the term “blast”. 3. Describe the normal phenotypic changes seen in differentiating myeloblasts and lymphoblasts 4. List chromosomal abnormalities associated with acute leukemias. Identify the oncogenes associated with them and their effects on prognosis.
Viral Infections [Cytomegalovirus (CMV), Epstein-Barr Virus (EBV),	1. Viral classification and characteristics properties. 2. Growth, multiplication, and pathogenesis. 3. Clinical presentation and differential diagnosis.

& Parvovirus (B19)]	4. Laboratory diagnosis including specimen collection. 5. Management including specific therapeutic agents. 6. Epidemiology including modes of transmission, prevention & control measures.
Parasitic Infections [Malaria, Babesiosis, Trypanosomiasis, Visceral Leishmaniasis, & Filariasis]	1. Parasitic classification and characteristic properties. 2. Life cycle and pathogenesis. 3. Clinical presentation and differential diagnosis. 4. Laboratory diagnosis including specimen collection, and microscopic findings. 5. Management including specific therapeutic agents. 6. Epidemiology including modes of transmission, prevention & control measures.
Immunosuppressant Agents	1. Recognize the general principles of Immunosuppressant therapy. 2. List the common drugs, which have an immunosuppressive effect. 3. Understand the mode of action, Pharmacokinetics and toxicities for these agents. 4. Understand the new modalities of immunosuppressant therapies.
Bone Marrow Transplantation	1. Apply the knowledge given in the blood grouping system in blood transfusion. 2. Apply the knowledge given in the blood grouping system into organ transplantation. 3. Understand how to use this knowledge in the clinical practice.

B-Practical Laboratory Sessions:

#	Lab. Title	Objectives
1 and 2	**Histology of blood smear And Histology of lymphoid tissue**	1. Review criteria for identifying blood cells. 2. Examine a blood smear under the light microscope to identify different blood cells. 3. Examine histological sections of lymphoid organs under light microscope.
3	**RBCs & WBCs count**	1. To examine a sample of blood and determine the percentage of each type of white blood cells in the sample.

		2. To be able to determine if there is any WBC deficiencies or excesses, which are suggestive of certain illnesses. 3. To be able to determine the hematocrit and to determine Red Blood Cells (RBC) indices.
4	**Hb, PCV, RBCs, WBCs, & differential**	1. To determine hemoglobin (Hb) levels in the blood. 2. To be able to determine the blood type according to ABO and Rh systems.
5	**Anemia and acute leukemia**	Identify the morphologic abnormalities of peripheral blood and bone marrow in: 1. Iron deficiency anemia 2. Megaloblastic anemia 3. Thalassemia 4. Sickle cell anemia 5. Microangiopathic Hemolytic Anemia (MHA) 6. G6PD hemolytic anemia 7. Autoimmune hemolytic anemia 8. Hereditary spherocytosis 9. Lymphoblasts 10. Myeloblasts 11. Promyelocytes 12. Prolymphocytes 13. Auer rods 14. Identify the diagnostic microscopic changes of: 15. Acute myeloid leukemia 16. Acute lymphoblastic leukemia& its clinical significance 17. Chronic myeloid leukemia 18. Chronic lymphocytic leukemia 19. Hairy cell leukemia
6	**Lymph node enlargement and lymphomas**	1. Non-Hodgkin's Lymphoma Follicular lymphomas Mantle cell lymphoma Small lymphocytic lymphoma Large cell lymphoma 2. Hodgkin's disease and its subtypes 3. Follicular hyperplasia
7	**Gram staining of representative bacterial growths from blood cultures**	1. Appreciate the value of Gram staining (Gram positive, Gram Negative) in blood cultures examination 2. Be familiar with Gram stain technique 3. Distinguish the potential pathogens by their stain affinities, morphologies, and arrangements.

D-Small Group Discussions:

A. AML Vs. TMD CASE:

CLINICAL HISTORY:

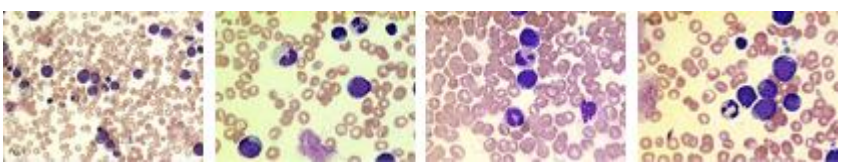
The patient was a 12- day old female who was diagnosed with Downs Syndrome. Her karyotype showed trisomy 21. She was noted to have mild cyanosis with symptoms of mild hypoxemia. Her initial blood count demonstrated severe anemia and thrombocytopenia. Flow cytometry, performed on her peripheral blood showed a >20% population of myeloblasts. A bone marrow smear and aspirate was subsequently ordered. All her other blood work including coagulation studies were normal.

PERIPHERAL BLOOD COUNTS AND MORPHOLOGIC FINDINGS:

CBC with differential

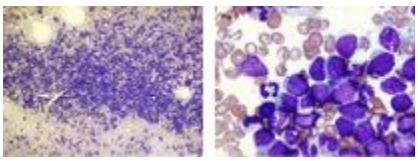
	Patient Value		**Normal Range**
Blasts	73%		<5%
WBC	138.9 x 10^9/L		5.0 – 21.0x 10^9/L
RBC	3.18 x 10^{12}/L		4.5 – 9.57 x 10^9/L
Hemoglobin	11.7 g/dL		12.9 – 16.9 g/dL
Hematocrit	33.2 %		37.0 –42%
MCV	104.1 fL		88.6 – 126.0 fL
MCH	31.1 pg		27.8 – 33.4 pg
MCHC	35.1g/dL		32.7 – 35.5 g/dL
RDW	18.8 %		11.5 – 15.2 %
Platelets	95x 10^9/L		150 – 450 x 10^9/L
Eosinophils	2 %	0.05 x 10^9/L	0.00 – 0.40 x 10^9/L

PERIPHERAL BLOOD:



As illustrated in Figures 1-4, the peripheral smear demonstrated many blasts with a small amount of blue cytoplasm, and prominent vacuoles. The nuclei are enlarged, with clumped chromatin and some folds. There was also some toxic granulation of neutrophils.

BONE MARROW ASPIRATE:



The bone marrow smear showed a cellular aspirate. There were large clusters of cells with enlarged nuclei, clumped chromatin and scant blue cytoplasm similar to the peripheral blood. There were in addition decreased levels of erythroid precursors and an absence of megakaryocytes.

FLOW CYTOMETRY:

38% of cells were CD34/CD 33 positive 45-50% of cells were CD 117 positive
Weak expression of CD 4,10
CD 61+ accounted for <1% of cells

CYTOGENETICS:

Cytogenetic studies revealed a male with trisomy 21 karyotype.

FINAL DIAGNOSIS:

MACROCYTIC ANEMIA AND THROMBOCYTOPENIA, ACUTE MYELOGENOUS LEUKEMIA VERSUS TRANSIENT MYELOPROLIFERATIVE DISORDER

DISCUSSION:

Patients with Downs syndrome have approximately a 1/100 incidence of Acute myelogenous leukemia (AML) with a relative risk of 10-20 fold as compared to cytogenetically normal individuals (1,4). The majority of these patients >50% have acute megakaryoblastic leukemia (AMKL), which represents a 46 fold increase in relative risk.

Transient myeloproliferative disorder (TMD) is a unique, neoplasia specific to Down's syndrome (DS). It affects approximately 10%of DS neonates. In 20-30% of cases, it reoccurs as progressive acute megakaryoblastic leukemia (AMKL) at 2-4 years of age. Morphologically the blasts of TMD and AMKL can appear identical. Immunophenotypically, the cells have similar profile (2,3,4,5). The blasts are usually CD 45, CD 34, CD 117,CD 38, CD 33 positive. Furthermore, CD61 and CD41 used to differentiate megakaryocytic lineage may be positive in both. Given the transient nature of TMD versus the potential lethal nature of AMKL, there is an important distinction to be made.

The exact nature of molecular events in both AMKL and TMD are unknown, however it is believed that mutations in the GATA1 gene are a key event. GATA1 is a transcription factor located on the X chromosome involved in the maturation of erythroid and megakaryocyte lines (2,3). However, mutations are non- specific and found in both. Therefore, they cannot be used in differentiating on from the other. Interestingly enough though, identical mutations of GATA can be found in TMD's and AMKL's which subsequently evolve from them. This suggests a clonal evolution of some TMD's to delayed AMKL's, but differences in early AMKL's and TMD's.

Currently, there are some efforts under way using microarray technology to differentiate early AMKL and TMD's (1). One recent study showed a significant increase in the amount of CDKN2C in AMKL but not TMD. This of interest since CDKN2C is a negative effector of GATA and an increased quantity may point to a loss of GATA inhibition in AMKL. There was an inverse correlation with levels of MYCN (neuroblastoma protooncogene). The latter was more abundant in TMD. MYCN belongs to the myc family of oncogenes. It is seen in 25% of neuroblastomas. Lastly, there is an increased expression of preferentially expressed in melanoma gene (PRAME) in AMKL but not TMD (1,6). This might make it a good candidate for differentiating the two.

It should be noted, however that none of these strategies differentiate between TMD which progress to delayed AMKL's and those that do not. Nor do they differentiate between TMD and non AMKL leukemia. Ultimately, the only way to differentiate the two is to watch and observe the patient for several weeks

REFERENCES:

1. Suzanne McElwaine, Claire Mulligan, Jurgen Groet, Monica Spinelli, Andrea Rinaldi, Gareth Denyer and Afua Mensah, Microarray transcript profiling distinguishes the transient from the acute type of megakaryoblastic leukaemia (M7) in Down's syndrome, revealing PRAME as a specific discriminating marker, British Journal of Haematology, 125, 729-742
2. Gamis, A.S. & Hilden, J.M. (2002) Transient myeloproliferative disorder, a disorder with too few data and many unanswered questions: does it contain an important piece of the puzzle to understanding hematopoiesis and acute myelogenous leukemia? Journal of Pediatric Hematology/Oncology, 24, 2-5.
3. Groet, J., McElwaine, S., Spinelli, M., Rinaldi, A., Burtscher, I., Mulligan, C., Mensah, A., Cavani, S., Dagna-Bricarelli, F., Basso, G., Cotter, F.E. & Nizetic, D. (2003) Acquired mutations in GATA1 in neonates with Down's syndrome with transient myeloid disorder. Lancet, 361, 1617-1620.
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5. Homans, A.C., Verissimo, A.M. & Vlacha, V. (1993) Transient abnormal myelopoiesis of infancy associated with trisomy 21. American Journal of Pediatric Hematology and Oncology, 15, 392-399.
6. Ikeda, H., Lethe, B., Lehmann, F., van Baren, N., Baurain, J.F., de Smet, C., Chambost, H., Vitale, M., Moretta, A., Boon, T. & Coulie, P.G. (1997) Characterization of an

antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity, 6, 199-208.
7. Karandikar, N.J., Aquino, D.B., McKenna, R.W. & Kroft, S.H. (2001) Transient myeloproliferative disorder and acute myeloid leukemia in Down syndrome. An immunophenotypic analysis. American Journal of Clinical Pathology, 116, 204-210.
8. Mundschau, G., Gurbuxani, S., Gamis, A.S., Greene, M.E., Arceci, R.J. & Crispino, J.D. (2003) Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis. Blood, 101, 4298-4300.

B. ANEMIA CASE

CLINICAL HISTORY:

A Caucasian man in his 50s presented to the emergency department with increasing exertional dyspnea, generalized weakness, lethargy, and anemia. The patient underwent orthotopic cardiac transplant approximately six months earlier for ischemic cardiomyopathy secondary to a previous myocardial infarction. The anemia was first noted a few weeks postoperatively.

A bleeding duodenal ulcer was discovered approximately one month after the transplant. Endoscopic repair was performed and the patient received antibiotic treatment for Helicobacter pylori. The anemia did not resolve, although repeat endoscopy one month after the repair demonstrated that the ulcer was healing. The continuing anemia was attributed to an elevated tacrolimus (ProGraf®, FK507) level; tacrolimus was subsequently discontinued, and cyclosporin (Neoral®) was then used as the patient's primary immunosuppressant. The medication changes were made approximately one month prior to the presentation at the emergency department.

The patient's medications included cyclosporin (Neoral®), mycophenolate mofetil (CellCept®), valganciclovir (Valcyte®), atorvastatin (Lipitor®), pantoprazole (Protonix®), trimethoprim-sulfamethoxazole, levothyroxine, and prednisone.

PERIPHERAL BLOOD COUNTS AND MORPHOLOGIC FINDINGS:

CBC with differential

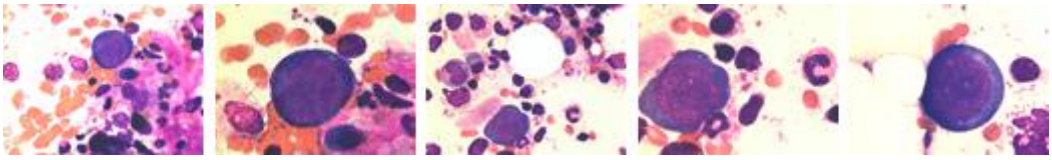
	Patient Value	**Normal Range**
WBC	2.3 x 10^9/L	3.8 – 10.6 x 10^9/L
RBC	2.38 x 10^{12}/L	4.13 – 5.57 x 10^9/L
Hemoglobin	7.4 g/dL	12.9 – 16.9 g/dL

Hematocrit	20.7 %	38.0–48%
MCV	87.1 fL	82.6– 97.4 fL
MCH	31.1 pg	27.8– 33.4 pg
MCHC	35.7 g/dL	32.7– 35.5 g/dL
RDW	13.7 %	11.8– 15.2 %
Platelets	254 x 10^9/L	156 – 369 x 10^9/L
Reticulocytes	0.008 x 10^{12}/L	0.018 – 0.158 x 10^{12}/L
Reticulocytes	0.3 %	0.8 – 2.0 %

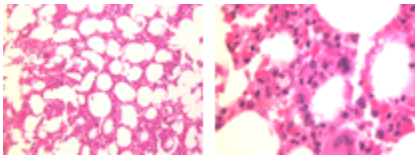
	Patient Value (Percentage)	**Patient Value (Absolute)**	**Normal Range (Absolute)**
PMNs	58 %	1.33 x 10^9/L	2.24 – 7.68 x 10^9/L
Bands	1 %	0.02 x 10^9/L	0.10 – 0.80 x 10^9/L
Lymphocytes	23 %	0.53 x 10^9/L	0.80 – 3.65 x 10^9/L
Atypical Lymphocytes	10 %	0.23 x 10^9/L	
Monocytes	6 %	0.14 x 10^9/L	0.30 – 0.90 x 10^9/L
Eosinophils	2 %	0.05 x 10^9/L	0.00 – 0.40 x 10^9/L

Rare plasmacytoid lymphocytes were identified; review of the peripheral blood smear was otherwise unremarkable.

BONE MARROW ASPIRATE AND BIOPSY FINDINGS:



Examination of the bone marrow aspirate revealed near absence of erythroid precursors. The rare pronormoblast forms identified were very large and megaloblastic, as shown in Figures A, B, C, D, and E. Myeloid maturation was complete but showed slight to moderate megaloblastoid changes. Some megakaryocytes were dysplastic with separated nuclei.



Examination of the bone marrow biopsy revealed a hypocellular marrow (20%) with very few identifiable erythroid forms, as shown in Figures F and G.

FLOW CYTOMETRY:

Flow cytometric immunophenotyping did not reveal an abnormal cell population. A slight increase in CD3-positive natural killer cells was detected; predominant heterogeneous T-lymphocytes and polyclonal B-lymphocytes were identified.

CYTOGENETICS:

Cytogenetic studies revealed a normal male karyotype (46, XY).

IMMUNOHISTOCHEMICAL STAINS

Positive for Parvovirus.

FINAL DIAGNOSIS:

Peripheral blood:

Anemia, neutropenia, and lymphopenia

Bone marrow biopsy and aspirate:

Marked **erythroblastopenia** associated with **parvovirus infection**

The bone marrow also demonstrated dyspoietic changes in the granulocytic and megakaryocytic lineages. These changes could be related to cyclosporin therapy or an underlying myelodysplastic process but could not be accurately assessed during active parvovirus infection.

Note: The term *pure red cell aplasia (PRCA)* is used to describe severe anemia associated with absence of reticulocytes, absence or near absence of bone marrow erythroid precursors, and presence of normal numbers of granulocytic and megakaryocytic elements that are morphologically normal. In this case, the more inclusive term erythroblastopenia was used to describe the dyserythropoiesis; the dysplastic changes seen in the granulocytic and megakaryocytic lineages, which are most likely unrelated to parvovirus infection, do not fit the more specific diagnosis of pure red cell aplasia.

DISCUSSION:

Pure red cell aplasia (PRCA) is a condition of severe anemia associated with absence of reticulocytes and virtual absence of erythroid precursors in the bone marrow. The other hematopoietic lineages seem morphologically normal and are present in normal numbers. The disease can be either acquired or congenital; the congenital form is associated with other anomalies and is called Diamond-Blackfan syndrome.

Multiple causes of acquired PRCA have been described.

Causes of Acquired Pure Red Cell Aplasia	
Drugs Azathioprine Chloramphenicol Isoniazid Phenytoin Zidovudine Recombinant erythropoietin **Idiopathic** **Infection** HHV-6 HIV Parvovirus B19 Viral hepatitis	**Immune Disorders** Systemic lupus erythematosus Rheumatoid arthritis Sjogren's syndrome **Malignancies** Chronic lymphocytic leukemia LGL leukemia Hodgkin lymphoma Non-Hodgkin lymphoma Prodrome to myelodysplastic syndrome Lung cancer Thymoma

In PRCA, suppression of normal erythroid maturation has been demonstrated to occur at a point between progression of the Colony Forming Unit erythroid (CFUe) cell to the pronormoblast stage. Several mechanisms have been described, including neutralizing immunoglobulins (described with recombinant erythropoietin administration), suppression mediated by T-lymphocytes (described with thymoma), and direct toxicity (described with phenytoin and chloramphenicol). Direct attack and toxicity is also the mechanism of PRCA associated with parvovirus B19 infection.

Human parvovirus B19 is a small, non-enveloped, single-stranded DNA virus belonging to the Parvoviridae family. It was discovered in 1975 while screening units of blood from asymptomatic donors for hepatitis B virus. It is mainly transmitted by respiratory secretion and, rarely, by blood products. B19 selectively infects and replicates within erythroid precursor cells; the blood group P antigen system serves as the receptor by which the virus enters the pronormoblast. Rare individuals whose erythrocytes lack P antigens (p phenotype) are inherently resistant to parvovirus B19 infection.

Neither cytotoxic immunoglobulins nor evidence of T-lymphocyte-mediated suppression have been described with parvovirus-associated PRCA. Severe anemia is most likely to occur in immunocompromised individuals or patients with an underlying hemolytic disease, such

as sickle cell disease, hereditary spherocytosis, thalassemia, or deficiencies of glucose-6-phosphate dehydrogenase or pyruvate kinase.

PRCA associated with parvovirus B19 infection often produces giant pronormoblasts in the marrow, which is a characteristic morphologic finding useful for diagnosis. It is thought that these giant pronormoblasts represent very early regenerating erythroid precursors that appear after clearance of the virus. Positive immunohistochemical staining for parvovirus in an affected erythroid precursor, or virocyte, is represented by a nuclear inclusion surrounded by a dense ring of chromatin. In non-immunocompromised patients, stainable virocytes are present only very briefly in the early phase of the infection and are rapidly cleared.

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STUDENT BASED ACTIVITIES

Students Responsibilities:

Students are requested to attend lectures sitting on their dedicated seats.
Students are requested to attend laboratories and small group discussions.

During the course of the module, students will be divided into small groups conducting research activities or presentations. These activities are intended to enhance student active participation in learning and they will be subject to evaluation.

Student Activities:

In addition to two student-based presentations covering two different topics (Please see above), these activities will include:

1. Writing short essays on the most recent advancements in translational Medicine and or up-to-date clinical guidelines.
2. Performance of small-scale research projects.
3. Open research/innovative ideas

ACTIVITY # 1: WRITING SHORT ESSAYS:

Herein, students are requested to submit a brief summary (four pages long, double spaced, narrow margins A4 sized, Times New Roman font) on the most recent advancements in translational medicine, and up-to-date clinical guidelines of the diagnosis and management of defined conditions related to the topics covered in the module. Topics of interest are listed below, and student groups will be randomly assigned to each of the topics by the course coordinator. This activity aims at empowering the students with the most relevant research skills, in addition to potentiating the concept of continuous medical education in the students.

#	Topic	Instructor
1	Plasma cell myeloma targeted therapy.	Pharmacology
2	Management of fungal infection in immunosuppressive patients.	Pharmacology
3	2016 WHO updates in Primary myelofibrosis Disease	Pathology
4	Write an assay on the development of bone marrow elements	Anatomy
5	2016 WHO updates in follicular lymphoma classification	Pathology
6	The illness porphyria	Biochemistry
7	Porphyria a chromosomal disorder	Biochemistry
8	Write a brief Review about B12/Folate metabolism	Physiology
9	Write a short summary of novel insights about thromopoietin signaling	Physiology
10	Updates about prevention and management of	Physiology

	GVHD	
11	Write a short summary about blood transfusion complications	Physiology
12	Write a short summary endothelium role in blood coagulation	Physiology
13	Write a brief Review about vitamin rule in coagulation	Physiology
14	What disease did Mad King George have	Biochemistry
15	Diagnostic methods for hemoglobinopathies	Biochemistry
16	2016 classification of EBV positive large b cell lymphoma	pathology
17	Recent advances in the management of Myeloproliferative neoplasm Hodgkin lymphoma	Medicine
18	Gene polymorphism and treatment response in DLBCL	Pathology
19	Recent diagnostic technologies in malaria	Microbiology
20	H1N1 in Jordan	Microbiology
21	Advances in prevention of H1N1	Microbiology
22	Correlate the histology of gallbladder with the pathology of chronic Cholecystitis.	Anatomy
23	Autologous Bone marrow transplantation concept and indications	Medicine
24	Molecular basis of thalassemia	Pediatrics

EVALUATION CRITERIA:

Student based presentations will be evaluated based on the following criteria:

ITEM	SCORE
English Language	------- out of 1
Flow of Logic	-------- out of 1
Effective Use of Illustrations	-------- out of 0.5
Originality (No plagiarism)	-------- out of 1
Proper Citations	-------- out of 1
Adherence to Format Guidelines	-------- out of 0.5
Total (out of 5)	-------- out of 5

ACTIVITY # 2: SMALL SCALE RESEARCH PROJECTS:

Herein, Students are requested to fulfill a pre-defined task related to the HLS system, as assigned to them by the course coordinator. Topics of interest are listed below. This activity

aims at encouraging the students' expression of innovative abilities in addition to enhancing their eventual integration into the clinical setting.

#	Topic	Instructor
1	What can trigger porphyria	Biochemistry
2	Era of Stem cell uses in modern medicine	Anatomy
3	Heparin induced thrombocytopenia: etiology, pathophysiology and management	Pharmacology
4	Short video about the surgical removal of appendix	Anatomy
5	How does porphyria affect the nervous system	Biochemistry
6	Single nucleotide polymorphism in AML	Pathology
8	Single nucleotide polymorphism in DLBCL	Pathology
9	Advances in transfusion medicine	Physiology

EVALUATION CRITERIA:

Student based presentations will be evaluated based on the following criteria:

ITEM	SCORE
English Language	------- out of 1
Flow of Logic	-------- out of 1
Effective Use of Illustrations	-------- out of 0.5
Originality (No plagiarism)	-------- out of 1
Proper Citations	-------- out of 1
Adherence to Format Guidelines	-------- out of 0.5
Total (out of 5)	-------- out of 5

HISTOLOGY LAB:

Lab: Examination of histological slides using the light microscope.

The slides will cover the microscopic structure of the:

1. Spleen
2. Thymus
3. Tonsils
4. Lymph nodes

PHYSIOLOGY LAB:

Lab 1: Differential White Blood Cell Count, hematocrit and Red Blood Cell indices

Lab 1 objectives:

1. To examine a sample of blood and determine the percentage of each type of white blood cells in the sample.
2. To be able to determine if there is any WBC deficiencies or excesses, which are suggestive of certain illnesses.
3. To be able to determine the hematocrit and to determine Red Blood Cells (RBC) indices.

Differential White Blood Cell Count Procedure:

1) Obtain a prepared blood cell smear and scan the slide on low power to find an area where blood cell distribution is best (cells are evenly spread out and not clustered together). Avoid the outside edges of the smear where, in some slides, the cells have an abnormal appearance.

2) Bring the slide in focus under oil immersion. Scan the slide in a systematic manner until you've counted and identified 100 leukocytes.

3) Record your results in a table to calculate the percentage of each WBCs.

Hematocrit Procedure:

1. Draw well-mixed anticoagulated blood into two microhematocrit tubes by capillary action avoiding air bubbles. Wipe off excess blood with a kimwipe or gauze.
2. Seal one end of each tube with a small amount of clay material at 90° angle. Be sure the seal has a perfectly flat bottom.
3. Securely fasten the flat lid on top of the capillary tubes.
4. Centrifuge for 5 minutes at a set speed (11000 rpm).
5. Allow the centrifuge to stop on its own.
6. Promptly read the hematocrit reader.

Lab 2: Hemoglobin determination and blood grouping

Lab2 Objectives:

1. -To determine hemoglobin (Hb) levels in the blood.
2. To be able to determine the blood type according to ABO and Rh systems.

Hb determination procedure:

1. Add 5 ml of Drabkin's reagent to a test tube.
2. Add 20ul blood to the test tube using Hb pipette or micropipette.
3. Mix blood with Drabkin's and wait for 5 to10 minute for the reaction to take place.
4. Measure the absorbance of the sample against blank (Drabkin's reagent) at 540 nm by spectrophotometer.
5. According to the absorbance, get the Hb concentration by using standard Hb curve.

Blood grouping procedure:

1. Obtain a drop of blood from a finger prick in each of the circles of the disposable blood group slides.
2. Add one drop of Anti A (blue) and a drop of Anti B (yellow) and a drop of Anti D sera in the proper circles on the disposable blood group slide.
3. Using clean (uncontaminated) glass rod to mix the blood with antiserum.
4. Tilt the slide from side to side occasionally, and after 2 minute read macroscopically for agglutination.
5. Any apparently negative tests should be read microscopically after 5 minute.

***A detailed manual will be provided to the students by the lab's supervisors**

MICROBIOLOGY LAB:

Lab 1: Gram staining of representative bacterial growths from blood cultures

Lab objectives:

1. Appreciate the value of Gram staining in blood cultures examination
2. Be familiar with Gram stain technique
3. Distinguish the potential pathogens by their stain affinities, morphologies, and arrangements

Method of instruction: Demonstration & hands-on experiment

-Demonstration of turbid blood culture bottle
-Collection of bacterial suspension from blood culture bottle -Preparation and fixation of glass slide
-Gram staining of different slides by students
-Viewing under compound light microscope using oil immersion lens

Gram stain technique:

-Stain; Crystal violet
-Stain fixation; Iodine
-Decolorizer; Alcohol
-Counterstain; Safranin