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HAEMATOLOGY CASE STUDIES

WITH BLOOD CELL MORPHOLOGY AND PATHOPHYSIOLOGY

Haematology Case Studies with Blood Cell Morphology and Pathophysiology

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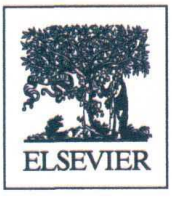

ACADEMIC PRESS
An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN 978-0-12-811911-2

Publisher: Mica Haley
Acquisition Editor: Tari K. Broderick
Editorial Project Manager Intern: Gabriela D. Capille
Production Project Manager: Punithavathy Govindaradjane
Cover Designer: Greg Harris

Typeset by SPi Global, India

Haematology Case Studies with Blood Cell Morphology and Pathophysiology

Foreword

The aim of this book is to present the haematology morphology of a Romanowsky-stained peripheral blood smear which may be used in conjunction with a text book to assist with the diagnosis of blood cell diseases.

"Case Studies with Cell Morphology and Pathophysiology" is divided into very useful and well-thought-out sections with excellent photographic examples of the morphology in disease and an array of the "typical" haematology results and pathophysiology for each condition. Even with major advances in haematology automation and molecular diagnostics, examination of a properly made and stained peripheral smear is still considered to be the "old standard" in diagnosing blood cell disorders. Sadly, this appears to be a diminishing skill for many scientists.

This reference is a valuable adjunct to the libraries of those beginning students in haematology morphology, experienced morphologists, to the multi-disciplinary scientists wishing to enhance their morphology skills and to clinicians alike.

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Preface

This book is written for students of laboratory medicine and haematology both at undergraduate and postgraduate level as well as for people who work in the clinical or hospital diagnostic laboratories and want to increase their knowledge in haematology, particularly blood cell morphology and associated pathophysiology of haematological disorders. This book will provide basic information about how to recognize and diagnose some haematological conditions that are frequently observed in the laboratories. The population of technicians and scientists who will benefit most from this book include those working in core laboratories including biochemistry or blood bank and are rotated around various disciplines as part of shift and weekend work. Moreover, it can be used as a reference book by technicians, scientists, and haematologists alike because it includes information relevant to every level of expertise in diagnosing haematological disorders. Most haematology books or atlases available in the market are either very in-depth and long or very basic with blood cell images with minimal explanation. We have compiled in this book specific case studies with general and specific information about various haematological disorders with Full Blood Examination (FBE or CBC), blood film images, pathophysiology of the conditions, and further confirmatory evaluation with their expected results for final diagnosis. This is what makes our book unique but simple. This book is the result of demand from industry for all in one simple reference book for teaching morphological skills to new trainees and upgrading the skills of senior workers starting rotation between various disciplines of laboratory as increasingly seen in the current economic situation.

Acknowledgements

This book has taken 7 years to complete and we are grateful for the input and encouragement of everyone along the way. A special mention must be made to RMIT for the use of their blood film collection and Ms. Janine Myers from Royal Melbourne Hospital. Dr. Gasim Dobie took the images of the blood films as part of his Masters at RMIT and Janine was his co-supervisor who reviewed the material and helped Gasim to incorporate some pathophysiology which became part of this book. Other people to be mentioned are Ms. Pamela Taylor whose attention to detail helped with editing the text and the images and Ms. Quirine O'Loughlin who took time out of her busy schedule to review the completed text. Many thanks also to all of our colleagues for their suggestions and discussions about the clinical conditions included in the book.

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Chapter 1

Introduction

THE IMPORTANCE OF THE BLOOD FILM IN THE DIAGNOSIS OF HAEMATOLOGICAL DISORDERS

Diagnostic information can be obtained from the blood smear examination if done by an experienced laboratory scientist or haematologist. Aside from the fact that it allows certain organisms to be directly visualized, peripheral blood film morphology gives helpful information on the aetiology, severity, host susceptibility, and systemic impact. It assists us to see the typical footprints left by different infections on blood cell morphology and this gives cytological clues in diagnosis such as in the case of Dohle bodies in haemophagocytosis. Physicians usually request a blood smear following perceived clinical features or irregularity of a previously done full blood count. Several factors determine whether a smear will be reviewed or not. These include the sex and age of the patient, whether such a request is an initial or subsequent one and the degree of clinical improvement from the previous result. It is possible to make a definitive diagnosis from the blood smear such as in the case of haemolytic anaemia, myelodysplastic syndrome (MDS), leukaemia, and lymphoma [1]. The morphological features of the red blood cell (RBC) and white blood cell (WBC) are important in the diagnosis of haemolytic anaemia, leukaemia, and lymphoma. The aetiology and confirmation of such diseases is then possible. Differential diagnosis can be considered which may make further investigation necessary. This booklet identifies the major roles of the blood smear in the diagnosis and differential diagnoses of anaemia, thrombocytopenia, as well as the determination and characterization of lymphoma and leukaemia. Furthermore, it highlights the further tests that should be done to confirm the diagnosis and the pathophysiology behind the condition.

MICROCYTIC ANAEMIA, HAEMOGLOBINOPATHIES, AND BLOOD FILM MORPHOLOGY

Most cases of microcytic anaemia are diagnosed by a combination of red cell indices, inflammatory markers, serum ferritin level, and clinical suspicion. However, the presence of Pappenheimer bodies and dimorphism of RBCs are helpful in the case of sideroblastic anaemia, basophilic stippling in lead poisoning, and some types of thalassemia. In the case of iron deficiency anaemia,

Haematology Case Studies with Blood Cell Morphology and Pathophysiology. http://dx.doi.org/10.1016/B978-0-12-811911-2.00001-5

the presence of elongated cells is the most diagnostic feature. The blood smear is also important in the diagnosis of sickle cell trait as compound heterozygosity of haemoglobin S and haemoglobin C may have a normal haemoglobin level and thus may be confused with sickle cell trait. The blood smear of a compound heterozygote usually shows irregular contracted cells, target cells, and boat-shaped cells with few classic sickle cells. So, the blood smear combined with sickle solubility test permits an accurate diagnosis [1].

BLOOD FILM MORPHOLOGY AND DIAGNOSIS OF MACROCYTIC ANAEMIA

Hypersegmented neutrophils, macrocytes, as well as macroovalocytes are seen in macrocytic anaemia induced by Vitamin B12 or folic acid deficiency. In the severe form of this, one may also see red cell fragments and tear drop poikilocytes in the blood film. The blood smear is important here in that it provides provisional speedy diagnosis and allows for commencement of therapy while awaiting results. It is also useful in that it eliminates problems of false negativity of patients with remarkable B12 deficiencies that however have normal assay result. This occurs because a lot of the B12 being assayed for is bound to haptocorrin while the effective B12 bound to transcobalamin less contributes to the total B12 assay. Hepatic disease and alcohol intake usually give rise to macrocytic anaemia. In this case, the blood smear shows round (not oval) macrocytes without any hypersegmented neutrophils. However, stomatocytes and targets may occasionally be present [1]. In older individuals, macrocytosis may be caused by myelodysplastic syndromes with the blood smear often revealing hypolobulated and hypogranular neutrophils. Other morphological features include Pappenheimer bodies, blast cells, and giant platelets. Sometimes hypochromic microcytes are present which makes the smear dimorphic. In macrocytosis brought about by blood loss or recent haemolysis, RBCs usually have polychromasia due to reactionary reticulocytosis. Significant poikilocytosis is seen sometimes in macrocytic anaemia [1].

PERIPHERAL BLOOD FILM AND DIAGNOSIS OF HAEMOLYTIC ANAEMIA

The shape of the RBC is important in the diagnosis of haemolytic anaemia. Certain types of haemolytic anaemia yield distinctive blood smears which can be enough for diagnosis. These include hereditary spherocytosis, hereditary pyropoikilocytosis, as well as South East Asia ovalocytosis. In hereditary elliptocytosis, the blood smear shows several elliptocytes as well as a smaller number of ovalocytes. In hereditary pyropoikilocytosis, one can see numerous poikilocytes including ovalocytes, elliptocytes, and fragmented cells. South East Asia ovalocytosis reveals poikilocytosis with macroovalocytes seen after staining specimens with May-Grunwald Giemsa stain [1]. Hyperchromic small cells

are seen in conditions such as microangiopathic haemolytic anaemia burns as well as spherocytic haemolytic anaemia. The diagnosis of conditions such as disseminated cancer, haemolytic uremic syndrome (HUS), pregnancy-induced hypertension, and thrombotic thrombocytopenic purpura (TTP) is aided with the detection of a microangiopathic haemolytic anaemia. Furthermore, the blood film becomes important in diagnosing acute haemolysis brought about by oxidative damage. Morphologically, one would be able to see bite cells or keratocytes. Blister cells as well as irregularly contracted cells are also seen. Oxidative stress leading to haemolysis is seen in anaemia of G6PD deficiency. In the case of G6PD deficiency, G6PD assay can be normal as seen in acute haemolysis in G6PD-deficient individuals who usually are men of African-American origin or those females who are carriers. In these two categories, a repeat assay is indicated after the period of acute haemolytic episode. The red cell in G6PD deficiency is also polychromatic and irregularly contracted. For hereditary spherocytosis, there are numerous, hyperchromic cells with a regular spherocyte outline. The blood film in diagnosing G6PD becomes more important even when G6PD assay is normal [1].

BLOOD FILM MORPHOLOGY, THROMBOCYTOPENIA, AND THROMBOCYTOSIS

Blood film examination is also important in individuals with thrombocytosis to check for the possibility of a myeloproliferative disorder change such as giant platelets or basophilia. The basophilia is not reliably detected by an automated counter and therefore requires clarification by examination of a blood smear. Falsely low platelet counts can be seen with platelet clumping and satellitism as well as in small clots, whereas falsely high platelet counts may be the result of red cell fragments, leukaemic cell fragments, and fungi being counted as platelets. The presence of fibrin strands suggests that thrombocytopenia may be factitious [1].

BLOOD FILM MORPHOLOGY, LEUKAEMIA, LYMPHOMA, AND BONE MARROW FAILURE

In the case of unexplained monocytosis, leucocytosis, and lymphocytosis, it is important to check the blood film. Pancytopenia in which all the cell lines are low is also an indication for blood film analysis. This may be due to an acute leukaemia, bone marrow infiltration by malignant cells, hairy cell leukaemia, or aplastic anaemia. The blood smear helps to indicate which further tests should be carried out. This also provides a morphologic basis in the absence of which sophisticated investigations and immunophenotyping cannot be interpreted. In acute promyelocytic leukaemia and Burkett's lymphoma, blood smears can help make rapid diagnosis and specific treatment can thus be initiated rarely

without further flow results. In Burkett's lymphoma, the blood smear shows basophilic vacuolation in lymphocytic cells while Auer rods are seen in acute promyelocytic leukaemia [1].

BRIEF ABOUT LEUKAEMIA

Leukaemia is a disease that affects blood-forming cells in the body. It is a cancerous condition characterized by an abundance of abnormal blood cells including leucocytes, erythrocytes, and thrombocytes blood cells in the body. Leukaemia begins in the bone marrow and spreads to other parts of the body. Both children and adults can develop leukaemia. The other condition that can affect the white blood cells is lymphoma which originates from the lymphatic system [2]. The direct causes related to the development of the malignancy are unknown; however, there are some conditions that may cause leukaemia. Environmental toxins can induce genetic changes, leading to the malignancy; exposure to radiation is also known to lead to malignancy. Viruses such as Epstein–Barr virus may play a pathogenetic role in causing a lymphoid malignancy. Additionally, it is considered that some alkylating agents and other chemotherapy that are used to treat various forms of cancer can cause hematologic malignancy as they induce DNA damage in hematopoietic cells. Generally, genetic changes or mutations could be the reason behind the leukaemia regardless of the causes of these mutations. The classification and diagnosis of leukaemia under the FAB system were based largely on morphologic characteristics and cytochemistry. However, there is a movement towards another classification to be more precise because of recurring chromosomal and genetic lesions that were found in many patients. These lesions are related to disruption of oncogenes which are genes that cause dominant-acting cancer mutation; tumour suppressor genes which code for proteins that help cells resist malignant transformation; and other regulatory elements that control apoptosis (programmed cell death), proliferation, maturation, and other vital cell functions. In 2001 the WHO (world health organisation) included or added the chromosomal translocation to the old criteria. The study of chromosomal translocation in hematopoietic malignancies has taught us how a single mutation or series of mutations may result in malignant transformation by disrupting the molecular machinery of the cell. There are many chromosomal translocations occurring in leucocyte malignancies. For example, t(9:22) in chronic myelogenous leukaemia which was the first genetic lesion found in human cancer, whereas the second one was t(8:14) in Burkett's lymphoma [2].

EXPLANATIONS FOR SOME TERMS USED IN THIS BOOKLET

Provisional diagnosis is a temporary and most likely diagnosis that needs to be confirmed by further appropriate tests.

Differential diagnosis means that there is a possibility that the diagnosis could be something else such as other disorders which may share the same

symptoms of the provisional diagnosis and they should be ruled out by further tests to reach the actual diagnosis.

Further tests are a number of tests that should be done to confirm the provisional diagnosis and exclude the differential diagnosis.

Pathophysiology is the study of the changes of normal mechanical, physical, and biochemical functions, either caused by a disease or resulting from an abnormal syndrome.

CYTOGENETICS

Cytogenetics is the study of the structure and the inheritance of the chromosomes (r408). Normally, there are 46 chromosomes found in each cell [2]. Each chromosome is composed of a short arm (p) and a long arm (q) separated by a region known as the centromere. The classification of chromosome disorders can be done as structural or numerical and involve the gain, loss, or the rearrangement of the chromosomes [2].

Normal chromosome number (46) = diploid, 23 pairs and there are two arms: short (p) and long (q).

(t) Translocation or exchange between two or more chromosomes.

For example; t(12:21) (p13:q22) → (t) represents a translocation and the two numbers in the brackets represent the number of the chromosome, (p) represents the short arm and (q) represents the long arm.

(Hyperdiploidy) → gain of a chromosome—trisomy
(Hypodiploidy) → loss of a chromosome—monosomy
(del) → deletion—loss of part of a chromosome
(inv) → inversion—rearrangement within an individual chromosome

CYTOCHEMISTRY

Cytochemistry is the study of chemical elements found in the cells. These elements can be enzymes, lipids, or glycogen. It can be used in differentiating hematopoietic malignancies, especially acute and chronic leukaemias [2]. There are many stains that are used in cytochemistry tests.

MYELOPEROXIDASE (MPO)

MPO is a peroxidase enzyme present in primary granules of neutrophils, eosinophils, and to a certain extent of monocytes. Lymphocytes do not exhibit MPO activity [2]. So, immunohistochemical staining for myeloperoxidase used to be administered in the diagnosis of acute myeloid leukaemia to demonstrate that the leukaemic cells were derived from the myeloid lineage. It differentiates between acute myeloid leukaemia and acute lymphoblastic leukaemia [2].

SUDAN BLACK B (SBB)

SBB is a lysochrome diazo dye used for staining of lipids, such as neutral triglycerides, sterols, and phospholipids [2].

In differentiating haematological disorders Sudan black will stain myeloblasts but not lymphoblasts, because these lipids are found in the neutrophil granules and in the monocyte lysosomal granules [2]. So, it differentiates acute myeloid leukaemia (AML) from acute lymphoblastic leukaemia (ALL).

CHLOROACETATE ESTERASE

The aim of this stain is to demonstrate the presence of granulocytes. The activity of chloroacetate esterase is found in the neutrophils and their precursors [3]. It differentiates between acute myeloid leukaemia and acute lymphoblastic leukaemia.

PERIODIC ACID SCHIFF (PAS)

PAS staining is mainly used for staining structures containing a high proportion of carbohydrate including glycogen, which is often found in hematopoietic cells [3]. This stain is positive in the majority of acute lymphoblastic leukaemias and negative in the majority of myeloid and monoblastic leukaemias [3]. Thus it differentiates ALL from AML.

TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE STAIN (TdT)

The TdT stain differentiates between acute lymphoblastic leukaemia and acute myelogenous leukaemia as it is positive in ALL [2].

NEUTROPHIL ALKALINE PHOSPHATASE (NAP)

The NAP score is used to differentiate between chronic myelogenous leukaemia (CML) and other myeloproliferative neoplasms (MPN) as it is low in CML and normal or high in other MPN, and between CML and leukemoid reaction as it increases in leukemoid reactions [3].

TARTRATE-RESISTANT ACID PHOSPHATASE STAIN (TRAP)

The TRAP stain is primarily used to identify the cell of hairy cell leukaemia cells [2].

ACID PHOSPHATASE

Acid phosphatase is usually positive in T-cell leukaemia [3].

ALPHA NAPHTHYL ACETATE ESTERASE (ANAE)

Alpha Naphthyl Acetate Esterase confirms monocytic differentiation [3].

IMMUNOPHENOTYPING

Immunophenotyping is the analysis of heterogeneous populations of cells for the purpose of identifying the presence and proportions of the various populations of interest. Antibodies are used to identify cells by detecting specific antigens expressed by these cells, which are known as markers. These markers are usually functional membrane proteins involved in cell communication, adhesion, or metabolism. Immunophenotyping using flow cytometry has become the method of choice in identifying and sorting cells within complex populations, for example, the analysis of immune cells in a blood sample. Applications of this technology are used both in basic research and clinical laboratories. Cell markers are a very useful way to identify a specific cell population. However, they will often be expressed on more than one cell type. Therefore flow cytometry staining strategies have led to methods for immunophenotyping cells with two or more antibodies simultaneously. By evaluating the unique repertoire of cell markers using several antibodies together, each coupled with a different fluorochrome, a given cell population can be identified and quantified. Many immunological cell markers are CD markers and these are commonly used for detection in flow cytometry of specific immune cell populations and subpopulations. CD (Cluster of Differentiation) markers are a group of special molecules on the surface of the cells in our body. There are several types of CD molecules. All cells in our body have one or more of them, but they are most useful for classifying WBC (white blood cells) malignancies as every linage expresses specific antigens. For example, the myeloblast is characterized by expression of immature cell markers CD34, CD38, HLA-DR, and stem cell factor receptor CD117. Pan-myeloid markers, CD13 and CD33, present on all myeloid progeny are first expressed at this stage. In monocytic lineage, bright expression of CD64 and HLA-DR antigens persists throughout monocytic maturation. In addition, Glycophorin A is present on reticulocytes and erythrocytes whereas CD41 and CD61 appear as the first markers of megakaryocytic differentiation. In regard to lymphoid lineage, the lymphoid progenitors express CD34, terminal deoxynucleotidyl transferase (TdT), and HLA-DR as the blast cells. CD19, CD22, and CD79 are expressed in the B-lineage whereas CD2, CD3, and CD7 appear in the T-cell lineage [2].