Role of platelet indices in differentiating immune thrombocytopenic purpura from other causes of thrombocytopenia

Thesis

Submitted for Partial Master Degree
In **Clinical and Chemical Pathology**

By

Rasha Ramadan Osman Ibrahim

M. B., B. Ch. Ain Shams University

Supervised by

Professor / Dahlia Ahmed Zaki El Sewefy

Professor of Clinical and Chemical Pathology
Faculty Of Medicine, Ain Shams University

Doctor / Botheina Ahmed Thabet Farweez

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Faculty Of Medicine
Ain Shams University
2014

بسم الله الرحمن الرحيم

وقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ
ورَسُولُهُ والْمُؤْمِنُونَ

صدق الله العظيم

سورة التوبة آية (105)

*First, thanks are all due to **Allah** for Blessing this work until it has reached its end, as a part of his generous help throughout our life.*

*My profound thanks and deep appreciation to **Prof. Dahlia Ahmed Zaki El Sewefy**, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for her great support and advice, her valuable remarks that gave me the confidence and encouragement to fulfill this work.*

*I am deeply grateful to **Dr. Botheina Ahmed Thabet Farweez,** Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams university for adding a lot to this work by valuable supervision, co-operation and direction that extended throughout this work.*

*I am want also to thank **my family and my husband** for supporting me throughout my life.*

Rasha Ramadan Osman Ibrahim

List of Contents

List of Abbreviations

AA : Aplastic anemia
Aptt : Activated partial thromboplastin time
ATG : Antithymocyte globulin
BFU-MK: Burst-forming unit-megakaryocyte
cAMP : Cyclic adenosine monophosphate
CAMT : Congenital Amegakaryocytic Thrombocytopenia
CFUM : Colony-forming unit-megakaryocyte
CMP : Common myeloid progenitor
CXCR4 : Chemokine type 4 receptors
DIC : Dissiminated intravascular coagulopathy
DMS : Demarcation membrane system
FA : Fanconi anemia
FDP : Fibrin degradation products
GP : Glycoproteins
HCV : Hepatitis C virus
HIPA : Heparin-induced platelet aggregation test
HIT : Heparin-induced thrombocytopenia
HIV : Human immune deficiency virus
HP : Helicobacter pylori
HPA : Anti-Human Platelet Antigen-1a
HPP-CFU-MK : High proliferation potential-colony-forming unit-megakaryocyte
HSC : Hematognenic stem cell
HUS : Hemolytic uremic syndrome
IL8 : Interleukin 8
ITP : Immune thrombocytopenic purpura
KMS : Kasabach-Merritt Syndrome
LDH : Lactate dehydrogenase
MACE : Modified antigen capture ELISA
MAHA : Microangiopathic haemolytic anaemia

List of Abbreviations (Cont.)

MAIPA	:	Monoclonal antibody –specific immobilization of platelet antigens assay,
MDS	:	Myelodysplastic syndrome
MEP	:	MK-erythroid progenitor
MK	:	Megakaryocyte
MPV	:	Mean platelet volume
NAP-2	:	Neutrophil activating peptide 2
NMB	:	New Methylene Blue
PAIgG	:	Platelet-associated immunoglobulin G
PDW	:	Platelet size deviation width
PIFT	:	Platelet immunoflouresence test
P-LCR	:	Platelet large cell ratio
PT	:	Prothrombin time
PTP	:	Post transfusion purpura
SLE	:	Systemic lupus erythematosus
TAR	:	Thrombocytopenia-absent radii
TF	:	Transcription factors
TMA	:	Thrombotic microangiopathies
TTP	:	Thrombotic thrombocytopenic purpura
TXA2	:	Thromboxane A2
VWF	:	Willibrand factor

List of Figures

List of Tables

List of Tables (Cont.)

INTRODUCTION

Platelet counts below normal values define thrombocytopenia. However, platelet counts alone do not reveal the underlying pathomechanism. Reduced platelet count is associated with diverse diseases and generally associated with a bleeding risk. Thrombocytopenia is often divided into two major causes, hypoproductive thrombocytopenia and hyperdestuctive thrombocytopenia ***(Staub et al., 2011).***

Thrombocytopenia due to defective platelet production (hypoproductive thrombocytopenia) is found in many conditions such as bone marrow failure, including Fanconi anemia (FA), aplastic anemia (AA), myelodysplastic syndrome (MDS), or thrombocytopenia-absent radii (TAR) syndrome, malignancy involving the bone marrow, folate and vitamin B deficiency, liver cirrohsis, and also following the use of certain drugs, the most common example is following chemotherapy for malignant conditions ***(McMIillian, 2007).***

Thrombocytopenia due to increased platelet breakdown (hyperdestuctive thrombocytopenia) is found in many conditions such as dissiminated intravascular coagulopathy (DIC), immune thrombocytopenic purpura (ITP) and hypersplenism ***(McMIillian, 2007).***

Quite often patients with ITP present with severe thrombocytopenia or even with hemorrhagic events. In some cases, this constitutes an emergency, since there is a significant risk of serious bleeding complications such as intracranial or gastrointestinal hemorrhage. Within a few hours, the diagnosis of ITP should be reached with the aid of medical history, physical examination, complete blood count and examination of peripheral blood smear.

However, such a diagnosis is a diagnosis of exclusion. There is no available test for the positive diagnosis of ITP. Thus, in many instances, clinicians proceed with corticosteroid

or intravenous immunoglobulin administration with a little feeling of uncertainty ***(Ntaios et al., 2008).***

In evaluating the mechanism of thrombocytopenia, it is necessary to know which is more dominant, hypo-productive thrombocytopenia or hyper-destructive thrombocytopenia. For this purpose, bone marrow aspiration, platelet-associated immunoglobulin G (PAIgG), and molecular markers for DIC are often evaluated ***(George et al, 1996; Mak et al, 2000; Marsh et al., 2003).***

In fact, the necessity for both bone marrow aspiration and PAIgG in ITP was not accepted in the routine guidelines (British Committee for Standards in Haematology General Haematology Task Force, 2003). However, these two diagnostic approaches are actually overused in the diagnosis of ITP ***(Kaito et al., 2005).***

Advances in automated blood cell analysers have made it possible to measure various blood cell parameters automatically. Among these parameters, platelet indices, such as mean platelet volume (MPV), platelet size deviation width (PDW), and platelet large cell ratio (P-LCR) provide some important information ***(Threatte, 1993; Niethammer & Forman, 1999; Park et al, 2002).***

If these indices really are informative regarding platelet kinetics, they might become very useful laboratory measures for diagnosis of isolated thrombocytopenia ***(Kaito et al., 2005).***

Three recent studies have shown that platelet indices are helpful in discrimination between hypoproductive and hyper destructive thrombocytopenia ***(Kaito et al., 2005).***

AIM OF THE WORK

To assess sensitivity and specificity of platelet indices, mean platelet volume (MPV), platelet size distribution width (PDW), and platelet large cell ratio (P-LCR) to differentiate immune thrombocytopenic purpura (ITP) from hypoproductive thrombocytopenia.

Platelet Formation and Physiology

Platelets (thrombocytes) are colorless blood cells that play an important role in blood clotting. They stop blood loss by clumping and forming plugs in blood vessel holes ***(Mullally et al., 2010).***

Platelets are extremely small and discoid anucleated cells 2 to 3μm in diameter. They circulate at a concentration of 150. 000-400. 000 cells/μl blood with a mean volume of 7-11 fL. The glycoproteins of the surface coat are particularly important for the platelet interactions of adhesion and aggregation which are the initial events leading to initial plug formation during haemostasis ***(Hoffbrand et al., 2006).***

I-Platelet formation:

Megakaryopoiesis and Thrombopoiesis:

Hematopoiesis is a complex process that takes place in the yolk sac in the first few weeks then occurs in liver and spleen in the period from 6 weeks until 6-7 months of life. Liver and spleen continue to produce blood cells until 2 weeks after birth ***(Stohlawetz et al., 2001).***

After birth, the major site of hematopoietic activity shifts gradually from the liver and spleen to the bone marrow cavities of nearly all bones of the axial and appendicular skeleton. By early adulthood, the long bones no longer bear red marrow and the primary sites of red marrow are confined to the sternum, ribs, vertebrae and pelvis ***(Emerson et al., 2002).***

Megakaryocytopoiesis involves the commitment of haematopoietic stem cells, and the proliferation, maturation and terminal differentiation of the megakaryocytic progenitors ***(Varda et al., 2006).***

The process of megakaryopoiesis and platelet production is complex, with the potential for regulation at multiple stages.

Mature Megakaryocytes are polyploid cells that assemble a unique set of organelles, including alpha granules, dense bodies, and an extensive system of internal membranes ***(Roy et al., 2001).***

Megakaryocytes are derived from the hematopoietic stem cell through successive lineage commitment steps, and they undergo a unique maturation process that includes polyploidization, development of an extensive internal demarcation membrane system, and finally formation of pro-platelet processes. Platelets are shed from these processes into vascular sinusoids within the bone marrow. Megakaryocyte differentiation is regulated both positively and negatively by transcription factors and cytokine signaling illustrated in Fig. (1) ***(Nezam et al., 2010).***

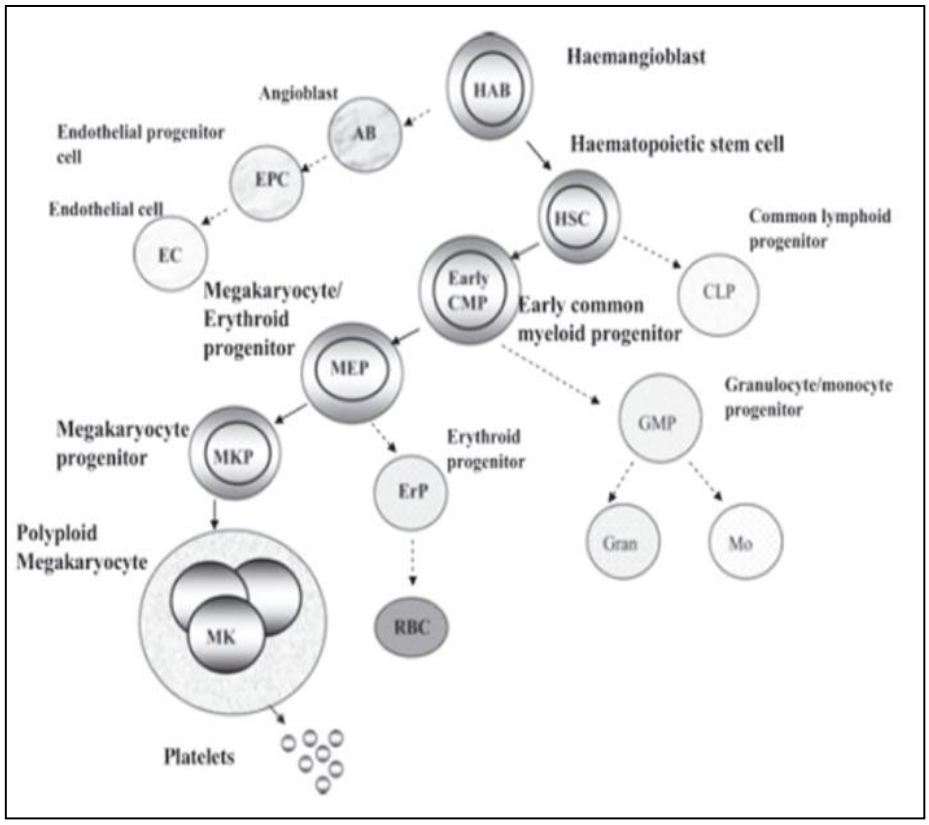

Fig. (1): The megakaryocytopoietic developmental pathway ***(Varda et al., 2006).***

Development:

The HSC gives rise to the early common myeloid progenitor (CMP) that can be cloned as the multi-lineage (granulocyte, erythrocyte, MK and monocyte) colony-forming unit (CFU-GEMM). Erythroid and MK lineages arise from a common MK-erythroid progenitor (MEP) derived from the early CMP. CMP differentiation is orchestrated by molecular signals controlled by regulatory transcription factors (TF). Two major TF involved in CMP differentiation are GATA-1, which drives differentiation of MEP and PU. 1, which regulates granulocyte-monocyte precursors ***(Nutt et al., 2005).***

The downregulation of PU. 1 expression in the CMP is the first event associated with the restriction of differentiation to erythroid and MK lineages. In response to environmental factors, cytokines and chemokines, the bipotential MEP can develop into the highly proliferative, early MK burst-forming unit (BFU-MK), or the more mature smaller CFU-MK, which both express the CD34 antigen ***(Pang et al., 2005).***

Alternatively, MEP can progress to early and late erythroid progenitors, the BFU-E and CFU-E. The proliferating diploid MK progenitors (megakaryoblasts) lose their capacity to divide, but retain their ability for DNA replication (endoreduplication) and cytoplasmic maturation ***(Schulze et al., 2004).***

The most definite primitive progenitors of MKs are the high proliferation potential-colony-forming unit-megakaryocyte (HPP-CFU-MK). A more differentiated progenitor is the burst-forming unit-megakaryocyte (BFU-MK) that gives rise to colony-forming unit-megakaryocyte (CFU-MK) **(*Wickrema et al., 2009).***

Megakaryocyte progenitor cells are responsible for the expansion of megakaryocyte numbers and proliferation in response to a number of mitotic growth factors ***(Long et al., 2000).***