

FLOW CYTOMETRIC RETICULOCYTE ANALYSIS

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Thesis

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By

Sahar Samir Abd El-Maksoud



Under the supervision of

51189

Prof. TARIF HAMZA SALLAM

Professor of Clinical Pathology



Dr. AZZA SADEK EL-DANASOURY

Lecturer of Clinical Pathology

Dr. MANAL HASHEM FAYEK

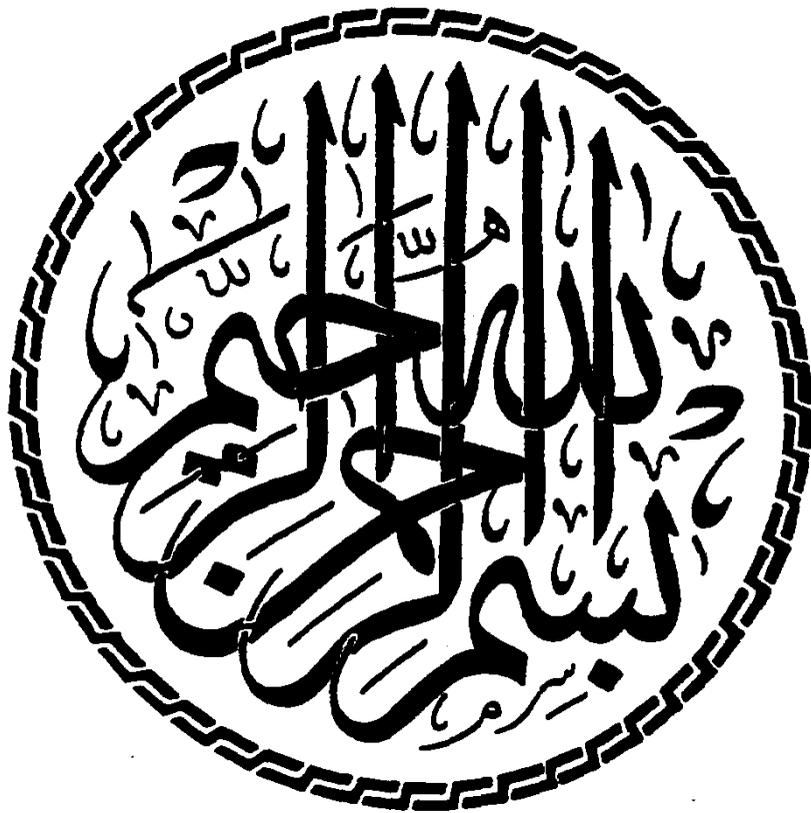
Lecturer of Clinical Pathology



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LIST OF ABBREVIATIONS

- AO: Acridine orange.
- BCB: Brilliant cresyl blue.
- BFU-E: Burst forming unit-erythroid.
- BM: Bone marrow.
- c-AMP: Cyclic adenosine monophosphate.
- CFU-E: Colony forming unit-erythroid.
- CFU-GEMM: Colony forming unit-granulocyte-erythrocyte-monocyte-macrophage.
- Da: Dalton unit.
- DNA: Deoxyribonucleic acid.
- Epo: Erythropoietin.
- FCM: Flow cytometry.
- FDA: Food drug administration.
- HFR: High fluorescing reticulocyte.
- LFR: Low fluorescing reticulocyte
- NMB: New methylene blue.
- RBCs: Red blood cells.
- RDW: Red cell distribution width.
- RMI: Reticulocyte maturity index.
- RNA: Ribonucleic acid.
- RPI: Reticulocyte production index.
- TO: Thiazole orange.

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INTRODUCTION AND AIM OF THE WORK

Reticulocyte enumeration remains one of the more useful, yet inexpensive assays for the evaluation of patients with hematologic disorders resulting in anemia. Determination of reticulocyte percentage and the absolute reticulocyte count provides information about the effectiveness of the erythropoietic response in an anemic individual (Davis, 1993).

Reticulocytes can be simply defined as those non-nucleated erythroid cells, newly released from the hemopoietic tissues containing higher levels of cellular ribonucleic acid (RNA) than mature red blood cells (Gilmer and Koepke, 1976).

The methodology to quantify reticulocytes has remained a manual microscopic technique in most clinical laboratories (Peebles et al., 1981 and Davis and Bigelow, 1991). However, the combination of the recent availability of stable commercial fluorescent reagents for reticulocyte enumeration, discriminating red cell populations based on RNA content, and of flow cytometric (FCM) instrumentation within clinical laboratories should allow flow cytometric reticulocyte analysis to rapidly become the standard and preferred method for this hematologic laboratory assay (Davis, 1993).

Introduction and Aim of The Work (1)

Flow cytometric reticulocyte analysis has potential advantages over the relatively subjective manual microscopic technique which has been repeatedly shown to have inherent inaccuracies (Peebles et al., 1981 and Savage et al., 1985).

Additionally, flow cytometry, by virtue of its ability to quantitate fluorescence intensity measurements, offers the ability to provide a new parameter of erythropoietic activity through the generation of a reticulocyte maturity index (RMI) based on the relative fluorescence intensity of the reticulocyte population (Davis and Bigelow, 1989).

The clinical utility of RMI determinations, independent of mere reticulocyte enumeration has been shown in bone marrow transplantation patients (Davis and Bigelow, 1989). It also has potential clinical utility in a number of other clinical situations (Davis, 1993).

The purpose of this study is to evaluate reticulocyte analysis by FCM using thiazole orange (TO) as a reticulocyte labeling dye.

REVIEW OF LITERATURE

ERYTHROPOIESIS

Cells of the peripheral blood (erythrocytes, leucocytes and platelets) are constantly destroyed or removed from the circulation and incapable of self renewal. The replication of these cells depends on the function of less differentiating hematopoietic cells with proliferative capabilities. These are the stem cells. A stem cell is a self renewing cell in which the progeny (daughter cells) of cell division are identical, in appearance and potential, to the mother cell. It also has the ability to produce progeny destined to differentiate. The hematopoietic stem cell being capable of both proliferation and differentiation will lead to renewal of the mature non-dividing compartments (Lajtha et al., 1962; Winkelstein and Boggs, 1971).

HIERARCHY OF HEMATOPOIETIC PROGENITORS

A hierarchy of hematopoietic progenitors can be proposed as shown in figure (1). The most primitive progenitor, detectable after extreme hematopoietic damage, is a totipotent cell from which lymphocytes and pluripotent stem cells are derived. The CFU-S of Till and McCulloch (1961) appears to be such a cell, and is capable of pluripotent differentiation to myeloid, erythroid, and megakaryocytic cells, as well as self-renewal. The overall schema depends on pluripotent cells that give rise to

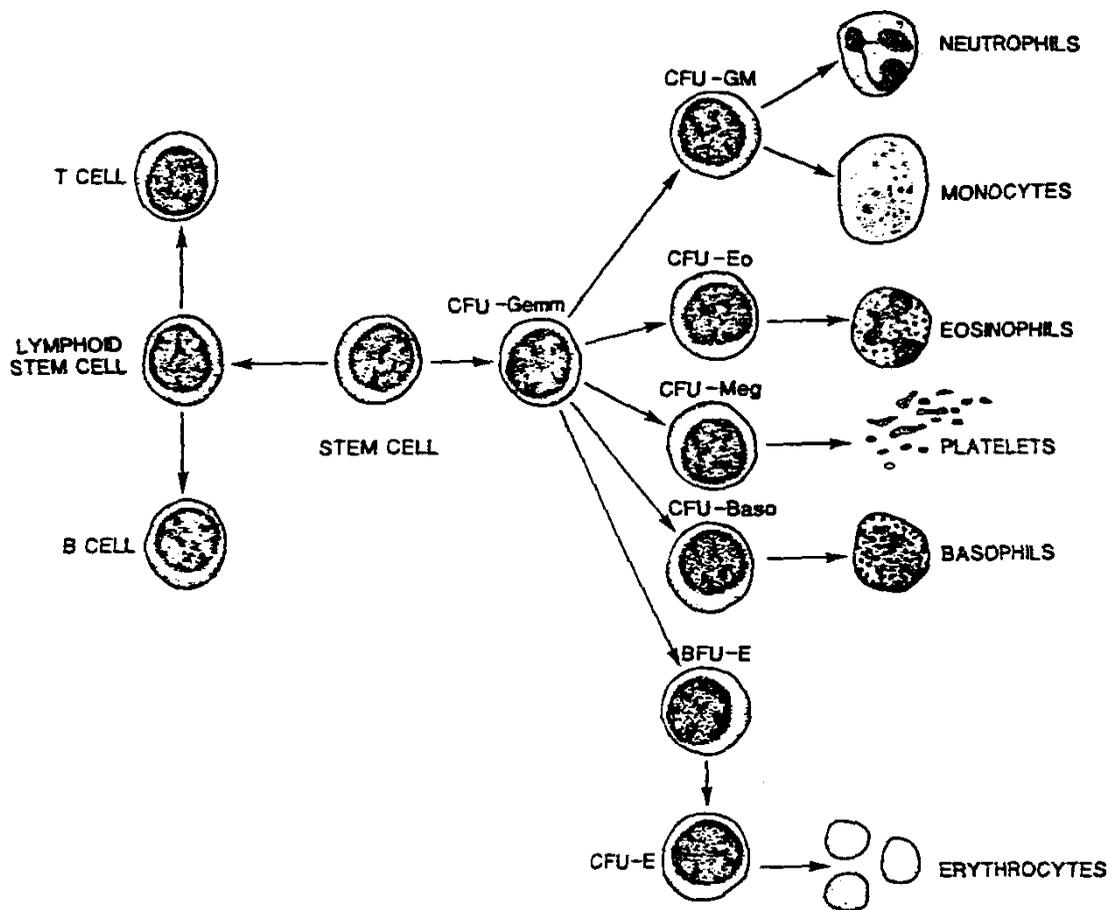


Figure (1): Hierarchy of hematopoiesis. The various progenitors are schematically represented. CFU-GEMM, multipotential progenitor for granulocytes, macrophages, erythroid cells, megakaryocytes; CFU-GM, progenitor for neutrophils and monocytes; CFU-Eo, progenitor for eosinophils; CFU-Baso, progenitor for basophils; CFU-Mega, progenitor for megakaryocytes; BFU-E, most primitive committed progenitor for erythroid line; CFU-E, more mature progenitor for erythroid line (Rothstein, 1993).

progressively more committed progenitors, not stem cell-like, in that they lack capacity for self-renewal. The most pluripotential of those identified in vitro is the CFU-GEMM, from which are derived fully committed precursors for granulocytes, macrophages, megakaryocytes and erythroid cells. The morphology of most of these cells is not known, but most evidence suggest the CFU-S is a mononuclear cell that morphologically resembles small or medium sized lymphocytes (Hoffbrand and Pettit, 1993).

Factors that affect commitment of stem cell progeny into a specific differentiation pathway are poorly understood and generally undefined. Commitment and differentiation are generally accepted as irreversible events. A differentiated cell can not regress to an undifferentiated stage or change into another differentiation pathway under normal circumstances. Once commitment occurs, differentiation proceeds fully to the stage of mature cell, which, in the case of blood cells, has a limited life span. Thus, differentiation is a process that leads to cell death (Dessypris, 1993).

Three major theories address the process of commitment of stem cell progeny into a specific differentiation pathway. According to the stochastic theory, commitment is a