# LABORATORY EVALUATION OF PLATELET SIZE MEASUREMENT GENERATED BY THE COULTER COUNTER MODEL S-PLUS JUNIOR

Thesis
submitted for the partial fulfilment
of M.D. Degree in Clinical and Chemical Pathology

By


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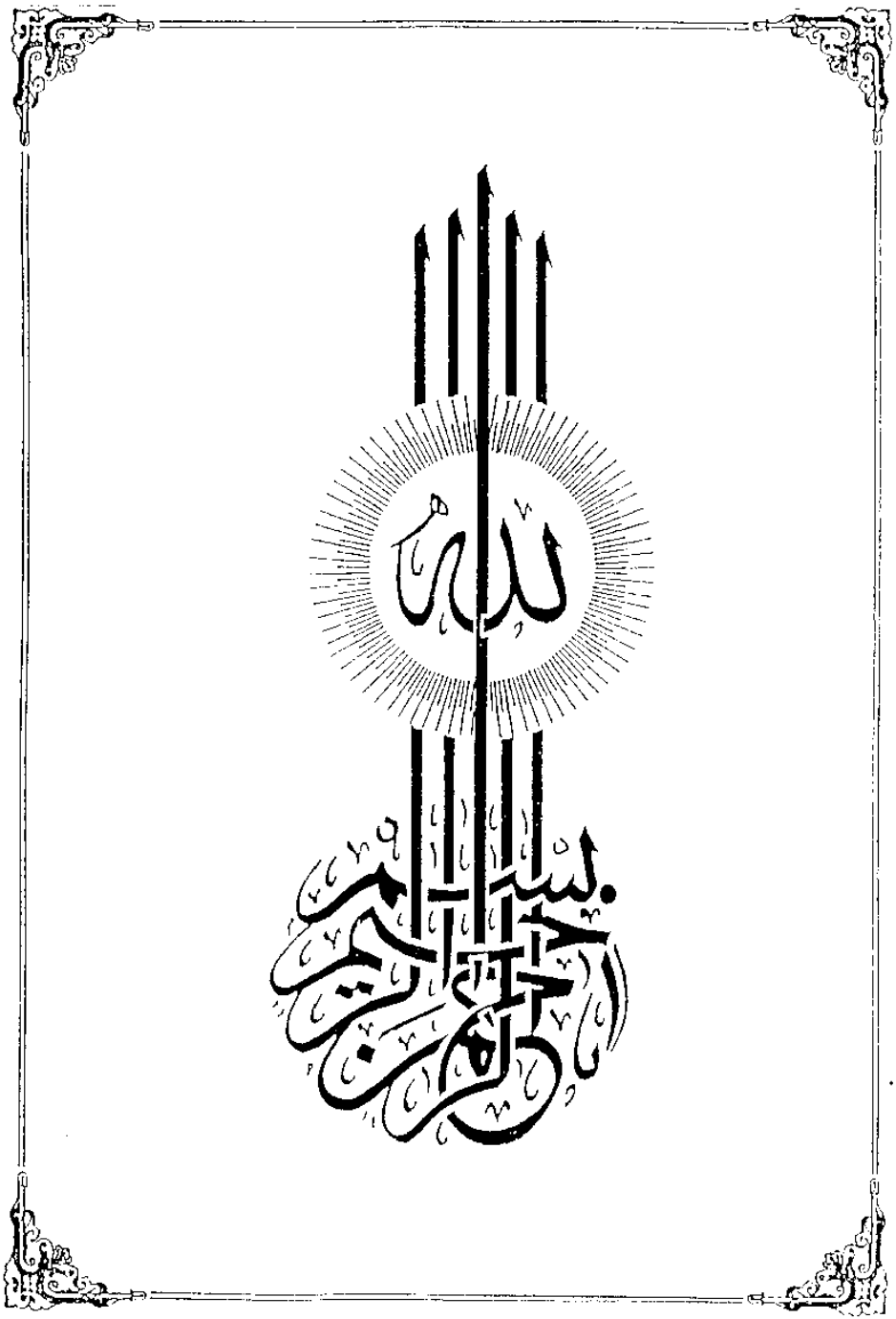


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Finally, thanks to Coulteronics for their maintenance service of the cell counter during the period of the study.

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# INTRODUCTION

The introduction of new instruments and new measurements is a challenge to the practicing physician when some of the new measurements are novel in concept and, superficially, in the first instance at least, do not appear to possess clinical utility.

In addition, the replacement of admittedly imperfect but well respected tests by alternatives which again appear to give no additional information, must provoke criticism.

Although in the past thirty years, automated quantitation of the blood elements, as well as red cell indices, hemoglobin concentration, and the hematocrit has supplemented routine microscopic examination of the blood film.

It is only very recently that tentative attempts have been made to relate variation of platelet volume distribution to the pathological factors. Platelet parameters derived from Coulter counter S-plus series as, mean platelet volume (MPV) plateletcrit (Pct) and platelet distribution width (PDW) are not in fact so new, each one been the subject of considerable study over many years and may have been introduced to clinical use earlier if satisfactory technology for performing the test existed.

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## AIM OF THE WORK:

To evaluate platelet count and platelet parameters as platelet distribution width, plateletcrit and mean platelet volume on ground scientific bases in order to study their clinical and functional utility.

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# THROMBOPOIESIS

## Origin of Cells:

Platelets originate from the pluripotent hematpoietic stem cell, that is capable of differentiating into a variety of hematopoietic lineages. This pluripotent stem cell is also capable of extensive self renewal (Visser et al., 1984 and Spangrude et al., 1988). The origin of platelets from hematopoietic stem cell had been proved by in vivo and in vitro studies. In vivo studies using the murine assay for pluripotent stem cell (CFU-S), have been shown to contain megakaryocytes in addition to cells belonging to other hematopoietic lineages. Recently, a number of groups have developed in vitro assays for a hematopoietic progenitor cell, the colony forming unit blast (CFU-Bl), that forms colonies that are composed primarily of blast cells (Rowley et al., 1987 and Brandt et al., 1988). These blast cells have both an extensive capacity for self renewal and the ability to differentiate into a number of different hematopoietic cell types. While the relationship between the CFU-Bl and the pluripotent hematopoietic cell is not well defined it appears that these cells may be closely related. Such experiments whether in vitro or in vivo indicate the ultimate origins of megakaryocytes from a cell common to other hematopoietic elements (Brandt, 1988).

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Megakaryocyte progenitor Cells:

Recently, a variety of semisolid assay systems have been used to detect megakaryocyte progenitor cells (Mazur, 1987). These progenitor cells have the ability to form in vitro colonies composed exclusively of megakaryocytic elements. At present, at least three classes of megakaryocyte progenitor cells have been identified: the burst forming unit megakaryocyte (BFU-Mk), the colony forming unit megakaryocyte (CFU-Mk), and the light density megakaryocyte progenitor cell (LD-CFU-Mk) (Long et al., 1985 and Chatelain et al., 1988). The BFU-Mk and CFU-Mk have each been detected in human and rodent systems while the LD-CFU-Mk has only recently been defined in the mouse (Chatelain et al., 1988).

The BFU-Mk first described by Long et al. (1985) is the most primitive progenitor cell committed to the megakaryocyte lineage. BFU-Mk derived colonies require longer incubation times to appear in vitro (21 days for BFU-Mk and 12 days for CFU-Mk). BFU-Mk derived colonies are composed of multiple clusters of megakartocytes and contain larger numbers of megakaryocytes than the primarily unifocal CFU-Mk derived colonies (Briddell et al., 1988).

BFU-Mk and CFU-Mk have recently been shown to differ with regard to their sensitivity to pretreatment with the chemotherapeutic drug 5-fluorouracil (5-FU) (Briddell et

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al., 1988). The cloning efficiency of the CFU-Mk is markedly reduced by such pretreatment while the cloning efficiency of the BFU-Mk is unaltered. The ability to survive at exposure to such chemotherapeutic agents has been shown to be characteristic of primitive hematopoietic progenitor cells (Hoffman, 1989).

Recently, Chatelain et al. have described a megakaryocyte progenitor with properties indicating that it is more differentiated than CFU-Mk. These investigators have termed this cell the light density megakaryocyte progenitor or LD-CFU-Mk. This nomenclature is based upon the separation of megakaryocyte progenitor cells according to density sedimentation. Over 99% of megakaryocyte progenitor cells were reported to sediment above 1.05 g/ml, while a small number of megakaryocyte progenitors sediment below 1.05 g/ml; the so called LD-CFU-Mk. The constituent cells composing such colonies were found to contain megakaryocytes of a higher ploidy class than megakaryocytes composing CFU-Mk derived colonies (Chatelain et al., 1988). The LD-CFU-Mk appears to reside at a stage of development close to where the megakaryocyte progenitor cell ceases to undergo mitosis and acquires the capacity of endomitosis (Hoffman, 1989).

Some progress has been made in phenotyping the various megakaryocyte progenitor cell. Studies by a variety of groups have indicated that the CFU-Mk expresses the major

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histocompatibility type II locus (HLA-DR antigen) (Long et al., 1985 and Ishibashi et al., 1986), the $DR_{3,4}$ antigen which is known to be present a wide variety of human hematopoietic progenitor cell (Lu et al., 1988) and the expression of the platelet membrane glycoproteins (Levene et al., 1985). On the other hand, the BFU-Mk expresses only non detectable quantities of the HLA-DR antigen (Briddell et al., 1988).

Transitional Immature Megakaryocyte:

Small mononuclear cells that express platelet-specific phenotypic markers, but are not morphologically identifiable as megakaryocytes, have been identified in both rodent and human bone marrow (Jackson, 1973 and Mazur et al., 1981). These cells have been thought to represent a transitional stage of development in megakaryocytopoiesis, which bridges the gap between the progenitor cells and morphologically identifiable megakaryocytes (Long et al., 1982 and Young and Weiss, 1987). These transitional cells are characterized by their small size and round, indented nuclei, as well as the presence of acetylcholinesterase activity in the mouse and by expression of platelet membrane glycoproteins in man. They represent approximately 5% of marrow megakaryocytic elements (Long and Henry, 1979 and Rabellino et al., 1981). These transitional cells are not able to form colonies in vitro, thereby distiguishing them from the LD-CFU-Mk (Rabellino et al., 1981 and Chatelain et al., 1988). In the

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presence of appropriate growth factors, such transitional elements mature into morphologically identifiable megakaryocytes (Long et al., 1982 and Young and Weiss, 1987). Sixty-eight percent of these transitional cells have a ploidy of 8N, while 80% of the remaining cells have a ploidy of 6N, suggesting that they are actively undergoing endomitosis (Hoffman, 1989).

The frequency of such transitional cells in marrow can be altered by variation in peripheral platelet numbers. Creation of thrombocytosis by platelet transfusion in rodents results in a 50% reduction in number of transitional cells, while thrombocytopenia is accompanied by the appearance of greater numbers of such cells (Jackson, 1973; Long and Henry, 1979; Lepore et al., 1984 and Stranev et al., 1986).

Such data suggest that important regulatory factors may possibly exert their effect on megakaryocytopoiesis at this level of development.

Promegakaryoblast:

It had been first isolated and cloned from the bone marrow of long-Evans rat by Cicoria and Hempling in (1979) (Cicoria and Hempling, 1979 and 1980). This cell was shown by Weinstein et al. (1981) to exhibit a low degree of polypoidy while expressing such platelet specific markers as

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von Willebrand's factor, fibrinogen, acetylcholinesterase activity, and platelet factor 4.

Megakaryoblast:

It can be identified because of the unique ultrastructural features not present in primitive marrow erythroid or granulocytic precursors. The cytoplasmic membrane of isolated megakaryoblasts forms characteristic blunt protrusions, and the cytoplasm contains many polyribosomes and clear vacuoles up to 0.25 um in diameter. The central area of the cell contains mitochondria and strips of rough endoplasmic reticulum. The nucleus occupies much of the volume of the cell and has prominant nucleoli. The Golgi complex, composed of characteristic vacuoles, lamellae, and vesicles, occupies the paranuclear area of the cytoplasm.

High resolution immunohistochemistry confirms the identity of these cells as megakaryocyte precursors. The indirect antiplatelet antibody reaction, which uses peroxidase or ferritin, labels the cell membrane of mature giant polyploid cells that are unequivocal megakaryocytes, as well as immature precursors, but does not label erythroid, granulocytic or stromal cells (Willingham et al., 1971).

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Intermediate Megakaryocyte:

In early part of the intermediate stage of development, the megakaryocyte increases further in volume. The cell membrane often shows the characteristic protrusions seen in the megakaryoblast. The peripheral cytoplasm has a rich complement of polyribosomes and some rough endoplasmic reticulum (RER), which is required for the formation of cytoplasmic granules. The nucleus is now lobed but still shows both minimal margination of chromatin around the nuclear membrane and prominent nucleoli.

An elaborate network of membranes appear in the cytoplasm and is early evidence of the demarcating membrane system (DMS), which is connected with the cell membrane system (Behnke, 1968 and Shaklai and Tavassoli, 1978).

In the next stage of maturation, the late intermediate stage, the megakaryocyte has all the structural components of the mature cell, but not present in final arrangement or quantity. Cell volume increases and a peripheral cell margin is often present, devoid of organelles other than polyribosomes. The central zone of the cytoplasm contains abundant mitochondrial strips of RER, and characteristic granules. The nucleus is multilobed and shows pronounced margination of the chromatin, with lucent pore areas near the nuclear membrane (Colman et al., 1987).

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Mature Megakaryocyte:

It is the only polyploid hematopoietic cell. The maturation of megakaryocytes from their most immature recognizable stage to mature, platelet producing cell is accompanied by considerable morphologic change and increase in size.

Levin in (1988) classified them into three stages according to cytoplasmic characteristics, increasing segmentation or lobulation of the single nucleus, and the cell size.

The maturation of the cytoplasm is associated with the appearance of the demarcation membrane system (DMS), which is in continuity with the surface membrane (Tavassoli, 1980). There is a little evidence that this DMS demarcates the individual platelet areas (Tavassoli, 1980 and Radely and Haller, 1982).

Immature megakaryocytes must reach 4N state to be recognizable by light microscope (Odell and Jackson, 1968). Importantly, it has been shown that megakaryocyte with ploidy levels of 8N and higher are capable of maturation and producing platelets and conversely, each stage of maturation contains more than one ploidy class (Odell and Jackson, 1968; Odell et al., 1970 and Paulus, 1970).

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