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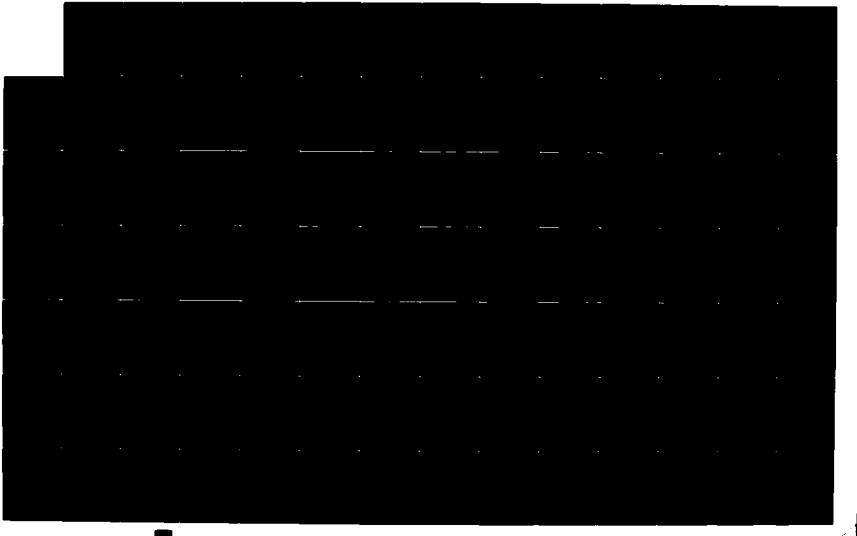

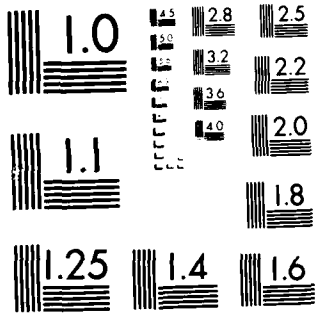

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MEAN PLATELET VOLUME AS AN INDICATOR OF PLATELET REJUVENATION FOLLOWING BONE MARROW TRANSPLANTATION

A THESIS

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE UNIVERSITY OF MINNESOTA

BY

DANNY GLENN SEANGER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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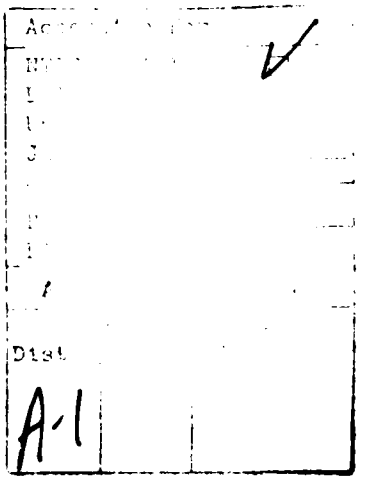
ABSTRACT

Thrombocytopenia of unpredictable duration and severity is an expected outcome of the radiation/chemotherapy protocols performed prior to bone marrow transplantation. Serial evaluation of the platelet count and mean platelet volume of patients diagnosed with acute leukemia demonstrated the mean platelet volume to increase into reference limits 24 to 48 hours prior to a rise in the platelet count in those patients whose bone marrow sucessfully responded to induction chemotherapy. Serial platelet counts and measurements of mean platelet volume were performed on 31 patients following bone marrow transplantation. Numerous platelet transfusions, together with sustained thrombocytopenia, inhibited accurate assessment of 29 of 31 patients. Two patients; however, demonstrated a rise in the mean platelet volume prior to an increase in the platelet count. Both of these patients received no platelet transfusions during the period preceding or following the rise in the platelet count. It was proposed that the serial evaluation of the mean platelet volume may assist practitioners in the decision making process of deciding whether platelet transfusions are required, or an increase in the number of circulating platelets is imminent. A decision not to transfuse would have the direct benefit of decreasing patient costs, in conjunction with eliminating a potential source for the development of an antibody against platelets. Mean Platelet Volume as an Indicator of Platelet Rejuvenation Following Bone Marrow Transplantation, Seanger, Danny G., Captain, United States Air Force, 1986, 183 pages, Master of Science in Medical Technology, University of Minnesota.

DEDICATION

I dedicate this thesis to my family; especially my father, Glenn, and my mother, Marjorie, for their continued love and understanding throughout my life; and to my daughters, Shelly, Cindy, and Jenny, for their patience during this endeavor; and to my wife, Rosie, for the love, life, and faith that we share together have made this work possible.



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I extend appreciation for the advice and suggestions made by Dr. Mary E. Dempsey and Dr. Karen S. Karni, who together with Dr. Brunning, comprised my committee.

I acknowledge all of the members of Dr. Brunning's laboratory who have been my friends and assisted me greatly with the collection of data; particularly, Peter Houlihan for our conversations concerning the interpretation of hematological data and the writing of this thesis.

To the faculty of the University of Minnesota who accepted me into this program, and graduated me out, I thank you. A very special thanks goes to Karen Lofsness who assured me I was sane, that I would survive the educational process and the Minnesota winters, and that I could depend on her friendship as a source of strength whenever I needed it.

To my friends, Cheri, Curt, and Brian for being there when I needed an ear to bend, I say thanks. Cheri convinced me there is more than one lake in South Dakota, Curt assured me Iowa was not just fields of corn, and Brian showed me the finer side of Wisconsin.

Finally, I wish to express my sincere appreciation to my family, near and extended. The hugs and kisses from my daughters, Shelly, Cindy, and Jenny gave me the strength to continue. The love, patience, and gentle encouragement I received from my wife Rosie, gave me no choice but to succeed. Without the love and encouragement I have received throughout this study from all my family members I am quite sure this page would not have been written.

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STATEMENT OF PURPOSE

Circulating platelets have been shown experimentally to be markedly heterogeneous in size and density (Charmatz et al.,1974; Corash et al.,1978; Karpatkin,1969A; Penington et al.,1976A). Dense/large platelets have been shown to be more active metabolically (Karpatkin,1969A) and to survive substantially longer when reinfused (Corash et al.,1978). These results have led Karpatkin and his co-workers to propose that platelet age is related to platelet size and density, with young platelets being larger and denser.

An alternate proposal, led by Penington and co-workers, suggest that platelet heterogeneity is determined during megakaryocyte production. Variations in the ultrastructure of platelets of various density populations were similar to the variations in the ultrastructural anatomy of differing ploidy classes of megakaryocytes (Penington et al.,1976A). Penington et al. proposed that the various ploidy classes (8N,16N,32N) of megakaryocytes produce platelets of differing characteristics. Platelets from 8N megakaryocytes are denser, containing more granules and less surface connecting canalicular system than the platelet progeny from 32N megakaryocytes.

Thrombocytopenia of unknown duration and severity is an expected outcome of the current radiation/chemotherapy protocols prior to bone marrow transplant. Bessman (1982) demonstrated that changes in thrombopoiesis occur sequentially with respect to the mean platelet volume (MPV) and platelet count. Patients diagnosed with acute myelogenous leukemia undergoing chemotherapy were found to demonstrate a fall in the MPV prior to a decrease in the platelet count, with the MPV remaining below normal for most of the

thrombocytopenia. Early evidence of platelet return following myelosuppression was found to be predictable by a rise in the MPV into or above the normal range on an average of 1-2 days prior to an increase in the platelet count. As the platelet count increased the MPV decreased into the normal range. It was proposed by Bessman that serial evaluation of the MPV may assist practitioners in the decision making process of deciding whether platelet transfusions are required or an increase in the number of circulating platelets is imminent.

The purpose of this study is to determine if the findings of Bessman can be extended to include patients receiving bone marrow transplant; in particular, does the serial evaluation of the MPV have any predictive value with respect to a future increase in the number of circulating platelets following bone marrow transplantation.

LITERATURE REVIEW

A. PLATELET PRODUCTION

Within the hierarchy of hematopoietic differentiation, there exists a group of cells capable of giving off clones restricted to a given cellular series. These precursor cells are committed to a given maturational sequence and have a relatively high degree of proliferative potential, in contrast to mature cells undergoing a process of terminal differentiation. These cells are known as the committed progenitor cells. They are the progeny of pluripotential stem cells that are thought to be capable of developing into each of the three myeloid series: granulocytic-macrophage, erythrocytic, or megakaryocytic series (Fauser et al., 1979). Since these early progenitor cells are morphologically unrecognizable, they are functionally defined by their ability to give rise to a clone of series restricted daughter cells in short-term in vitro assays. When these cells differentiate to form colonies of mature cells, they are referred to as colony-forming units (CFU). These in vitro assays require the presence of a semi-solid matrix and the presence of one or more hematopoietic regulatory factors referred to as colony stimulatory activity (CSA).

The committed megakaryocytic progenitor cells (CFU-MK) are believed to be the immediate precursor of the immature megakaryocytes (Long et al., 1982). The committed megakaryocytic progenitor cell differs from the immature megakaryocyte in its ability to proliferate into colonies of mature megakaryocytes. In vivo and in vitro studies have shown that the immature megakaryocyte is a small cell approximately 8-18 microns in diameter. They are subdivided into three classes based on their nuclear shape; either round, indented, or lobed (Long et al., 1982B). A majority of the evidence suggesting that this small cell is the

precursor to the megakaryocyte series originates from animal studies. In certain species, particularly the mouse, these cells along with megakaryocytes, are the only hematopoietic cells containing acetylcholinesterase (Zajicek,1954). These small acetylcholinesterase positive cells have been shown to follow disturbances of megakaryocytopoiesis. Following transfusion-induced thrombocytosis, they were found to be decreased in number, and elevated following immunothrombocytopenia (Jackson,1973; Levin,1980; Long et al.,1979). These small acetylcholinesterase cells were also found in in vitro megakaryocyte colonies where the full spectrum of megakaryocyte maturation would be expected (Williams et al.,1978). They have been separated and enriched from bone marrow and shown to develop into mature, single megakaryocytes in the presence of thrombopoietic stimulatory activities. In addition, early megakaryocytic colonies were found to be comprised exclusively of these immature cells, indicating that small immature megakaryocytes were the progeny of the committed progenitor cell (Long et al., 1982A). Unfortunately, acetylcholinesterase cannot be used as a specific marker for human immature megakaryocytes since the majority of the erythroid series also contains this enzyme. Cells of similar size and morphology have been identified in human bone marrow using antibodies to Platelet Factor 4, Factor VIII antigen, and human platelet glycoproteins (Levin, 1980; Levin et al., 1982; Mazure et al., 1981; Rabellino et al., 1979&1981). These researchers have indicated the presence of small lymphoid-like cells in human bone marrow which may be analogs of the murine immature megakaryocytes.

In vitro studies of the ability of murine immature megakaryocytes to respond to thrombopoietic stimuli have found these cells to develop into single, mature megakaryocytes (Long et al., 1982B). They do not possess the ability to divide, forming daughter cells that could develop into megakaryocytes. In response to thrombopoietic stimulatory activity the

youngest form of immature megakaryocyte, delineated by the presence of a round nucleus, has been shown to be acted upon. Following platelet depletion it was found that the round nuclear shaped immature megakaryocyte would undergo additional ploidy, resulting in an increase in the number of 32N and 64N megakaryocytes in conjunction with an overall larger size. Maximum increase in 32N and 64N ploidy cells was between 32 and 72 hours post platelet depletion, with near normal ploidy counts by 120 hours. In conjunction with increased cell size and ploidy, beginning about 36 hours post stimulation and lasting for about 72 hours, was a rapid increase in platelet count. The average platelet size rose and returned to normal within 60 hours (Long et al., 1982B; Odell et al., 1974 & 1976). Under normal steady state megakaryocytopoiesis the 8N megakaryocyte is the predominant ploidy class present in humans (Levin,1980). The immature megakaryocyte upon stimulation matures into recognizable megakaryocytes by Romanowsky type staining. Over the years various investigators have published classification systems based on Romanowsky-staining characteristics. The system proposed by Ebbe and Odell correlated megakaryocyte staining characteristics with maturational status. Levine extended this morphological approach to correlate cytological parameters with the functional-maturational status of the megakaryocyte. Levins' classification is composed of four stages, I-IV (Levin et al., 1982; Williams et al., 1982A).

The stage I megakaryocytes are cells measuring about 6-25 microns in diameter, with basophilic cytoplasm, high nucleus:cytoplasm ratio, with a relatively euchromatic nucleus which is slightly indented to lobulated in shape. By electron microscopy, demarcation membranes can be delineated along with a few granules. Its suggested name is the megakaryoblast. The stage II megakaryocytes are moderately larger being 14-30 microns, with

an increased amount of cytoplasm, the presence of a few perinuclear azurophilic granules and a lobulated to horseshoe shaped nucleus that is beginning to show nuclear condensation. The central cell area is pinkish colored. By electron microscopy the demarcation membranes proliferate towards the cell center and the granules begin to increase in number. The suggested name for the stage II megakaryocyte is the promegakaryocyte. Stage III megakaryocytes increase in size to between 16 and 50 microns in diameter, have abundant azurophilic granules, and a multilobulated nucleus. The cell becomes progressively more pink than blue. By electron microscopy, the demarcation membranes are extensive but asymmetric and great numbers of granules are present. Its suggested name is the granular megakaryocyte. The final stage of development, called stage IV or the mature megakaryocyte, are wholly azurophilic cells, 20-50 microns in size, whose granules are organized into platelet fields. The nucleus is compact but highly lobulated. By electron microscopy, demarcation membranes are evenly distributed and the granules are organized into platelet fields. Following formation of distinct platelet fields, clumps of platelets called proplatelets are released into the marrow sinusoids. Proplatelets then either break apart into individual platelets or may circulate in the peripheral blood, possibly traveling to the spleen or lungs. In addition, an unknown number of mature megakaryocytes enter the circulation prior to platelet release (Kaufman et al., 1965A & B).

The exact regulatory factors controlling megakaryocytopoiesis are unknown. Two levels of regulation are believed to exist (Williams et al., 1982B). The first is directed to the point at which the progenitor cells are maintained and stimulated to divide. A second level of regulation seems to be mediated by factors that are responsible for terminal differentiation. In vitro studies have found that at least two factors are required for optimal development of

megakaryocyte colonies. While both factors are required to sustain colony growth, lack of one inhibits colony formation. The first factor, termed megakaryocyte colony stimulating activity, is required for cellular proliferation. A second factor, termed megakaryocyte potentiator activity, affects megakaryocyte maturation. Megakaryocyte colony stimulating activity factor is needed in the early phases of colony development to stimulate cell division; thus its role is to regulate the number of daughter cells formed. The megakaryocyte potentiator regulates aspects of cell differentiation leading to the development of mature, platelet-shedding megakaryocytes by increasing individual cell size, enzyme concentrations and DNA content, those changes resulting in transformation of immature megakaryocytes to stage IV mature megakaryocytes (Long et al., 1982A & B; Williams et al., 1982A & B).

The majority of the information on the regulatory role of thrombopoietin originates from animal studies. In vivo studies of acute thrombocytopenia have shown increased megakaryocyte size and DNA content following a rise in thrombopoietin, but no changes in megakaryocyte numbers. These studies indicate thrombopoietin has an effect on the maturation of megakaryocytes by increasing DNA content (ploidy), cell size, and cytoplasmic content of the developing megakaryocyte. Thus the in vivo activity of thrombopoietin is mimicked in vitro by megakaryocyte potentiator activity. Whether these two factors are the same protein remains unknown. In vivo counterparts to the megakaryocyte colony stimulating activity found in vitro, has not been isolated in vivo.

The maturation time for the complete sequence of megakaryocyte maturation is estimated to be 4 to 5 days for humans and slightly shorter for the rabbit (Cooney et al., 1965). The daily rate of production can only be estimated indirectly by studying platelet turnover in blood. Platelets are released from bone marrow megakaryocytes as well as megakaryocytes

found in the lungs. Estimates of 35,000 plus or minus 4,300 and 43,000 plus or minus 3,000 platelets are produced per microliter per day in human beings (Branehog et al., 1975; Harker et al., 1969), of which no more than 7 to 17 percent are the result of pulmonary megakaryocytes (Kaufman et al., 1965B). Evidence of a limited reserve of platelets to provide an immediate compensation following platelet depletion has been found in humans, but this may be the result of platelets released from the spleen rather than new production (Zucker et al., 1961). The maximum level to which platelet production can be raised is not agreed on. Studies of platelet turnover in humans with chronic idiopathic thrombocytopenic purpura, indicate that platelet production may be increased up to 5-to-8-fold, although, the average was about a 2.4-fold increase; however, evidence for suppressed platelet production also exists (Branehog et al., 1975; Harker et al., 1969; Baldini, 1978).

B. PLATELET ANATOMY

Platelets in the peripheral blood appear as round or slightly oval, anucleate structures with a characteristic biconvex or lentiform shape. Random indentations are present on the platelet surface, manifesting sites of communication between the open canalicular system (OCS) or surface-connected canalicular system and the exterior of the cell. When viewed by phase-contrast or light microscopy the internal morphology appears relatively simple. A few organelles are randomly dispersed in the cytoplasm of discoid cells and become concentrated centrally during aggregation. When examined under electron microscopy the ultrastructural anatomy of platelets is more complex and detailed towards function.

The ultrastructural anatomy is best reviewed by dividing the platelets into four regions based on functional and biochemical activities. The peripheral zone, located toward

the exterior of the platelet has the primary function of adhesion. The organelle zone, located centrally in the platelet has the primary function of secretion. Located between these two zones is the sol-gel zone which is primarily responsible for platelet contraction. The final region, termed the membrane systems, traverse throughout the platelet. (see Figure 1 for location of anatomical components of platelets, page # 13).

1. Peripheral zone: The peripheral zone is composed of the exterior coat, unit membrane, and sol-gel matrix or submembrane region. The exterior coat or glycocalyx is in immediate contact with the surrounding plasma. The coat material is 15-200 angstroms in thickness and covers the exterior of the platelet membranes and the lumens of the surface connected canalicular systems. Chemical constituent of the exterior coat provide the receptor site for stimuli triggering platelet activation. It contains at least eight different glycoproteins (Ia, Ib, Ic, IIa, IIb, III, IV, V) in addition to protein antigens and thrombin-binding sites (George, 1978B; Norden et al., 1978; Phillips et al., 1980; White, 1965).

The platelet unit membrane provides a physical barrier between extracellular and intracellular constituents. An important component of the membrane are the Na/K ATPase and the Ca/Mg ATPase pumps which aid in appropriate transmembrane ionic gradients. The unit membrane is a typical trilaminar unit membrane originating from endoplasmic vessicles of the parent megakaryocyte which is rich in asymmetrically distributed phospholipids which are essential for coagulant protein function. The outer layers of the trilaminar membrane are composed of protein, while the inner membrane layer is predominantly lipid. The surface membrane normally has a negative charge, largely due to the presence of sialic acid. Glycopropteins IIb and III are also present and believed to be the site of platelet aggregation (Phillips et al., 1980). Platelet factor 3 is believed to reside in the lipoprotein-rich area of the

trilaminar membrane (Marcus et al., 1964). The unit membrane plays a key function in protecting the internal integrity of the platelet, in platelet adhesion/aggregation, contraction, and is the coagulation site for thrombin and coagulation factors V and VIII (Marcus et al., 1966; Nachman et al., 1967; Phillips et al., 1980; Silver, 1965).

The areas immediately interior to the unit membrane, constituting a transition between the peripheral and sol-gel zone is the submembrane region or sol-gel matrix. The submembrane area is considered part of the peripheral zone because its structural elements appear closely associated with changes in the cell surface during viscous metamorphosis. Located within the submembrane area, peripheral to the band of circumferential band of microtubules, are fine filaments. These filaments are believed to originate from damaged microtubules of the circumferential band and from hyaloplasmic microfilaments (Nachmias, 1980; Zucker-Franklin, 1970). Their proximal relationship to the cell membrane and association to fibrous elements of the sol-gel zone suggest the submembrane filaments may function to support the platelet discoid shape by acting as stress fibers between the circumferential tubules and the cell membrane, participation in formation and stabilization of pseudopodia, and to contribute towards retraction of surface pseudopods during viscous metamorphosis (White, 1969C).

2. Sol-gel zone: The sol-gel zone is comprised of masses of fibrous tubular elements termed submembrane filaments, microtubules, and microfilaments. Each microtubule is in itself a fibrous system composed of 12 to 15 subfilaments arranged in parallel association (White, 1968E). The subfilaments are more stable than the microtubules and are indistinguishable from microfilaments and filaments found in the submembrane area (Nachmias, 1980; White, 1968A). The degree of polymerization, aggregation, and location within the cell

may be the only difference in these fiber systems.

Microtubules have functional roles in both maintenance of platelet shape and contraction. The location of the circumferential band of microtubules just beneath the cell wall in discoid shaped platelets suggest its participation in cytoskeletal support. Two experimental approaches have been used to offer further proof of this function. Platelets are known to become irregular and relatively spherical when exposed to low temperatures for short periods of time. When rewarmed to 37 degrees Celcius, discoid shape is returned. Loss of discoid shape is associated with the disappearance of the circumferential band of microtubules and recovery is associated with reformation of the circumferential band to its normal position (White et al., 1967). Colchicine, vincristine, and vinblastin were found to dissolve platelet microtubules in addition to inhibiting mitosis by dissembling or preventing the formation of microtubules. The effect of these agents on the circumferential band of microtubules results in the loss of platelet discoid shape (White, 1968A). These studies support the concept that the circumferential microtubules play a role in the maintenance of the discoid platelet shape.

The relationship between the circumferential band of microtubules and the contractile activity following activation was proposed in the mid-1960's, based on the findings that the circumferential band of microtubules constrict into a tight ring around centrally clumped organelles in activated platelets (White, 1968C). The central movement of the circumferential band was only observed in platelets after exposure to agents which stimulate a physiological response. Therefore, the marginal bundle of microtubules appear to play a role in regulating the internal contraction of platelets resulting in central movement of organelles, which appear to facilitate the secretory process.

Microfilaments comprise a second system of fibers in the platelet sol-gel zone.

Depending on the concentration, duration of exposure, sensitivity to, and nature of the stimulus, the randomly dispersed organelles are moved into a compressed mass in the central region of the platelets where they are encircled by the circumferential band of microtubules and a tightly fitting web of microfilaments (White, 1968C). After the secretory organelles have discharged their contents, the microtubules become dispersed, leaving a mass of microtubules centrally located within the cell.

3. Organelle zone: The organelle zone is composed of a variety of particulate elements and formed organelles embedded within the sol-gel matrix, performing vital roles during normal platelet function. Additionally, glycogen particles occurring singly or in packets are found throughout the platelet matrix. Stacks of flattened saccules resembling a Golgi apparatus are occasionally found depending on the plane of section. Rarely, giant granules, nuclear remnants, centrioles, and bits of endoplasmic reticulum are identified (White, (1968E).

The constituents of the organelle zone are of particular interest because they are numerous and have been found to be an important source of products secreted subsequent to platelet activation, leading to viscous metamorphosis. Granules are primarily round or oval, although variations in shape are frequently observed. Individual granules are enclosed by a unit membrane (White et al., 1965). Two zones of differing opacity make up the internal matrix. Often the internal matrix is separated from the unit membrane by a clear zone. Within the matrix of granules, tubular elements of unknown significance have been identified (White, 1968E). Granules have been found to contain different chemical constituents, some being relatively abundant while others are of limited quantity. Alpha-granules have been found to contain thrombosis-sensitive protein, beta-thromboglobulin, fibrinogen, platelet factor 4, and platelet-derived growth factor (Marcus et al., 1966; Nachman et al., 1967). Peroxisome

granules contain catalase, whereas the group of lysosome granules contain several hydrolytic enzymes, including beta-glucoronidase, cathepsin, and acid phosphatase.

A specific population of organelles, the dense bodies, are characteristically electron-dense. Dense bodies have an intensely opaque internal content, which is often separated from an enclosing membrane by a clear space. Dense bodies in platelets are relatively few in number but are believed to participate in the hemostatic function of platelets.

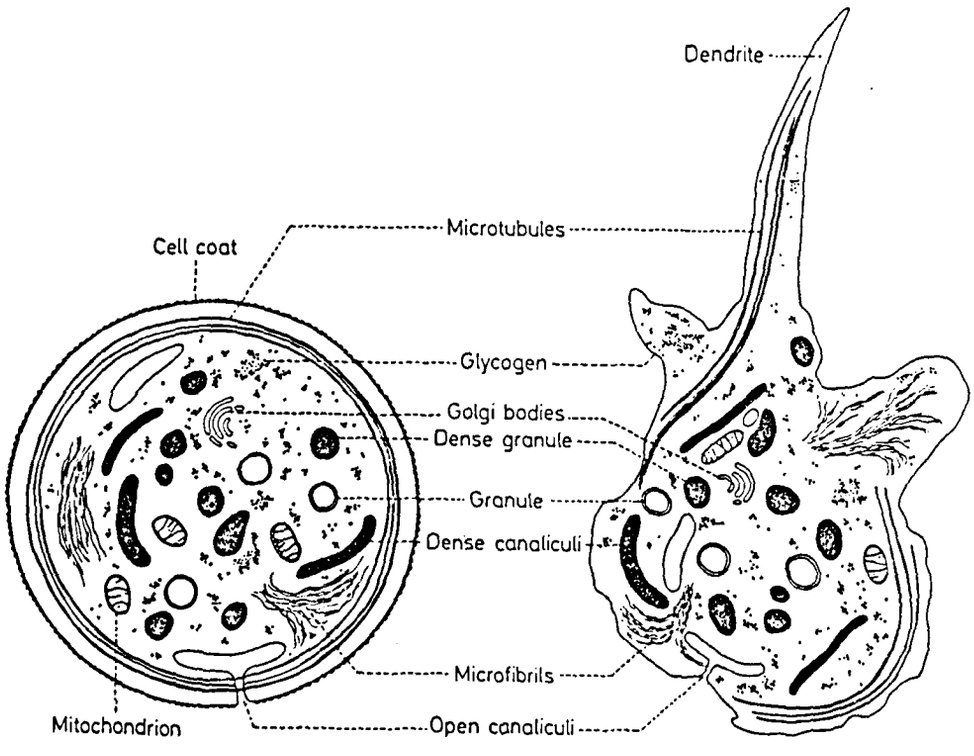

Figure 1. Ultrastructural platelet anatomy (Bessis, M., 1973).

Dense bodies are located throughout the platelet cytoplasm. Occasionally they appear to be near the cell edges, with tails extending into the exterior coat beyond the plasma membrane. Other times they appear to have long tails which extend across the platelet surface. Dense bodies are believed to originate from granules following the uptake of serotonin, an energy requiring reaction. Electron opacity is believed to result from nucleation of calcium (Davis et al., 1968; Tanzen et al., 1966; White, 1968D). In addition to calcium and serotonin, dense bodies are the storage site for the non-metabolic pool of adenine nucleotides, catecholamines and pyrophosphate (Holmsen et al., 1969; Kaplan, 1979).

Platelet mitochondria are few in number. They function to contribute significantly to the metabolic pool of ATP required during activation and viscous metamorphosis.

4. Membrane systems: Two membrane systems, the surface-connected open canalicular system (OCS) and the dense tubular system (DTS) comprise the membrane systems of platelets. The OCS are extentions of the outer membranes forming tortuous invaginations of the cell wall, meandering in a serpentine fashion throughout the platelet cytoplasm. The channels are covered with an amorphous material similar to that of the exterior coat, but the lumen of the channels remain clear (White, 1972). The channels are continuous in activated/aggregated platelets as well as in normal circulating platelets. Canaliculi of the OCS greatly increases the total surface area of the platelet exposed to plasma. They function as a conduit for particulate or chemical substances to reach the central area of the platelet and also as a channel for extrusion of granule substances from platelets during the release reaction following contraction of the sol-gel zone during viscous metamorphosis.

The second membrane system of platelets, originating from the rough endoplasmic reticulum of the parent megakaryocyte is the dense tubular system (White, 1972). Channels of

the DTS are filled with an amorphous material, similar in density to the surrounding cytoplasm, making the DTS easily distinguishable from the clear canaliculi of the OCS.

The two membrane systems are not totally isolated from each other. Canaliculi of each system form intimate contact in virtually all cells, but areas of contact are predominantly limited to one or two areas of the cytoplasm and are usually eccentrically located. In areas of contact, channels of the OCS gather in groups or clusters. Interspersed within channels of the OCS are closely approximated small channels of the DTS. The relationship of the OCS and DTS is easily observed in platelets stained for peroxidase activity. The dense reaction products delineate channels of the DTS and outline clearly their proximal relationship to clustered channels of the OCS (Breton-Gorius et al., 1972). High magnification reveals that elements of the DTS are the only structures interspersed between channels of the OCS. Membranes of the two systems are located practically in apposition near areas of fenestrations of the OCS.

Calcium accumulation is associated with the function of a DTS calcium pump. Platelets appear to be able to pump calcium out of the cytoplasm into the DTS and perhaps to the platelet exterior. The calcium pump activity is essential to monitoring a low cytoplasmic concentration in order to keep the microtubules polymerized, maintaining the platelet in its normal discoid shape (Marcus et al., 1966; White, 1972).

The role of the DTS in the synthesis of thromboxane A2 via oxidative enzymes is not totally clear. It is believed that arachidonic acid, a fatty acid in the platelet membrane, is converted to prostaglandin endoperoxidase (PGG2 and PGH2). Both PGG2 and PGH2 under the influence of thromboxane synthetase are converted to thromboxane A2, a powerful platelet aggregant. Thromboxane A2 acts as an ionophore and transports calcium from the DTS into the platelet cytoplasm. The rise in cytoplasmic calcium results in platelet contraction

(Gerard, 1976).

C. PLATELET FUNCTION

Platelets have the critical function of arresting bleeding from injured blood vessels. They accomplish their hemostatic function by forming plugs in any hole of the endothelium of blood vessels and providing the phospholipid surface required for protein coagulation. Morphological changes occur upon contact with various aggregating agents both in vitro and in vivo. Morphological changes can best be viewed by electron microscopy study at selected intervals as the aggregation proceeds. Progress of the platelet reaction caused by various aggregating agents can be followed by use of an aggregometer, an instrument that records changes in platelet shape and aggregation by measuring changes in the intensity of light transmission through the reaction mixture. Thus, morphological and physiological changes can be related to time.

Two waves of changes classically occur between contact with the aggregating agent and clot formation, although specific aggregants have been found which may result in a single wave of aggregation. Individual aggregants will be reviewed following discussion of two wave aggregation.

Platelets normally circulate as flattened biconvex discs. The lentiform of platelets can be preserved for "in vitro" aggregation studies by collection with either heparin or citrate anticoagulant and maintained at 37 degrees Celcius. Addition of various aggregants, at various concentrations to platelet rich plasma (PRP) results in either a one or two wave aggregation. The first wave of aggregation is termed the primary wave. The primary wave is characterized by internal reorganization. Organelles are moved toward the cell center due to

movement of the circumferential bundle of microtubules shifting from their normal location just beneath the cell membrane towards the center of the platelet. The degree of reorganization varies from cell to cell, with the centrally clumped organelles becoming loosely packed. Concurrently, shape changes occur as the normally disc shaped platelets are transformed into an irregularly shaped sphere with multiple pseudopods randomly occurring across the cell membrane. Adjacent platelets may form loosely associated clumps or on occasion a few may become molded to one another. Depending on concentration, length of exposure, and type of aggregating agent, reversal may occur at this point or aggregation may proceed through a secondary wave. If no further aggregation is to occur, platelets may return to their normal discoid shape.

In contrast to primary aggregation, changes which result from contact with an aggregating agent in the platelet environment, secondary aggregation results due to contact with products which are actually secreted from the platelet. Secondary aggregation is virtually irreversible and affects a majority of the platelets present. During the trough between primary and secondary aggregation, constituents of the dense bodies are released. The primary constituents include ATP, ADP, calcium, catecholamines, and serotonins (Holmsen et al., 1969; Kaplan, 1979). The release of serotonin, ADP, and calcium, initiates the secondary wave of aggregation. Individual cells become more closely molded in conjunction with further internal changes. Centrally grouped organelles are compressed tightly together, being surrounded by a close fitting band of microtubules and a meshwork of microfilaments surrounding individual granules. As a result of granule compression by microfilaments, centrally located granules and dense bodies are crushed together, releasing their contents that include calcium, serotonin, ATP, ADP, fibrinogen, platelet factor 4, catalase, and

beta-thromboglobulin, to name a few. Release of granules results in fragmentation of the circumferential band of microtubules, enhanced stickiness of peripheral membranes, and adhesiveness. Granule contents are believed to be extruded via the surface-connected canalicular system (Holmsen et al., 1969; Kaplan et al., 1979; White, 1968C). The peripheral cytoplasm becomes less electron-dense as a result of organelle displacement. Membrane pseudopodia are more pronounced, appearing as either filaments or scalloped bulbs. Parallel groups of microfilaments are present in nearly all pseudopods, with microtubules in a few. Channels of the surface-connected open canalicular system (OCS) continue to dilate, but remain intact to the peripheral cell membrane (White et al., 1967B). The periphery of aggregated platelets become more electron-transparent and central areas are more electron-dense as additional platelets aggregate. Swollen pseudopodia surround the margin of the aggregates. Pseudopodia deeper in the cellular mass become intermingled and lodged together. Glycogen masses disappear and mitochondria appear more dense than prior to aggregation due to increased output of ATP to fuel the aggregation process. The compaction force of contraction within the cellular aggregate results in loss of cellular integrity of individual platelets and fusion by surrounding platelets. These changes are late and represent the end of viscous metamorphosis or clot retraction. Thus, a centrally oriented wave of contraction dominates the physical alterations of large secondary aggregates as it does individual platelets.

The dense bodies are the only structural elements which disappear to a significant extent from platelets during late primary and early secondary aggregation, but not all the dense bodies disappear (White, 1968B). Degranulation is not associated with primary aggregation. This is consistent with gradual evolution of the release reaction corresponding

to physical changes during secondary aggregation. Although release of dense body constituents is associated with pre-secondary aggregation, some dense bodies are known to be entrapped centrally during circumferential microtubule contraction (Holmsen et al., 1969). Several dense bodies are found at the periphery, often having long tails positioned toward the platelet interior, or in some cases toward the cell wall. On occasion the unit membrane surrounding the dense body appears to be in continuity with the surface membrane of the platelet (White, 1968B). The method of secretion of dense body constituents is not totally known, but may be directed through the platelet membrane. The extracellular space between aggregated platelets is not sealed and it is conceivable that the surface-connected canalicular system and gaps between aggregated platelets may serve as the major route of secretion of granular constituents (White, 1973). Release of dense body constituents, serotonin and ADP, are known platelet activators leading to continued aggregation of platelets, acting similar to that of a catalyst.

Many agents are responsible for both in vivo and in vitro platelet aggregation.Typical examples would be collagen, ADP, thrombin, epinephrine, norepinephrine and serotonin. The extent of the aggregation reaction is based on the type of activator, concentration, and duration of exposure.

Thrombin and ADP can cause primary and secondary aggregation or a reversible primary aggregation. Low concentrations of either thrombin or ADP result in primary aggregation, but no release of granular constituents. Without granule constituent release, activated platelets may revert back to their normal characteristic shape. Larger concentrations result in both primary and secondary aggregation. The secondary wave is the result of granule and dense body constituents.

Serotonin can produce either a single wave of aggregation (O'Brien, 1966) or a typical double wave pattern if present in low concentrations, but the double wave aggregation is not seen in all individuals evaluated (White, 1970).

Collagen produces only a single massive wave of aggregation, whereas epinephrin and norepinephrin produce unique responses (O'Brien, 1966). Platelets aggregate without an increase in volume or loss of discoid shape, with only a few narrow pseudopodia formed. The first-wave aggregated platelets gradually change appearance until they are indistinguishable from thrombin and ADP aggregates found between first and second waves of aggregation. Alterations during the second wave are promoted by catecholamines and are indistinguishable from those caused by serotonin or ADP, except that they occur more rapidly (Thomas, 1969). In all cases though, the transformation is essentially identical, differing only in rate and extent to which physical alterations become evident.

The characteristics of platelets to undergo reversible physical transformation from disc to spherical and back to disc forms is of critical importance. Platelets circulating in the blood are constantly exposed to a wide variety of stimuli. If contact between platelets and stimuli resulted in an all-or-none reaction, the individual would be in constant danger of thromboembolism. The ability of the platelet to respond to a degree proportional to the stimulus provides a rapid response to vascular injury as well as protection against over reaction.

D. PLATELET HETEROGENEITY

1. General characteristics: Within the peripheral circulation can be found a vastly heterogeneic population of platelets. Several methods have been devised to separate this

heterogeneic population into more uniform populations for comparison with respect to function, age, and size.

A popular method has been separation based on density. Following density separation, large platelets have been found to be of a higher proportion in the dense platelet population with the less dense population of platelets being smaller in size (Karpatkin et al., 1969A; Karpatkin, 1978A; Leone et al., 1979; Rand, 1981). The heavy/large platelet population compared to the lighter/smaller platelets have been found to contain greater quantities of glycogen, orthophosphate, lactate dehydrogenase, adenine nucleotides, protein and sialic acid with an increased rate of glycogenolysis, glycolysis, protein synthesis and glycogen synthesis. No difference in total lipid or lipid synthesis was observed. The quantity of glycoproteins measured per cell was greater for large platelets (Blajchman et al., 1981; Karpatkin, 1969A & B, 1978A; Rand et al., 1981; Thompson et al., 1982).

Heterogeneity of platelet function has also been related to density, size and age. Karpatkin (1969A) found heavy/large platelets to have a greater resistance to osmotic shock as measured by ATP and ADP release. Also, the extent of the primary wave of aggregation induced by ADP, collagen, or epinephrine was directly proportional to platelet volume, particularly when correlated to the number of large platelets. Blajchman (1981) found in vivo bleeding times to be shorter for young platelets, which were found to be larger than older platelets.

Over the years two hypotheses have evolved from continued study of platelets in an attempt to determine the most accurate source for platelet heterogeneity.

2. Theories for Origin of Platelet Heterogeneity: Two hypotheses have been proposed to account for the origin of size and density heterogeneity of circulating platelets.

One hypothesis, spirited by Karpatkin and co-workers suggest that platelet heterogeneity may reflect platelet aging in the circulation, whereas the second hypothesis championed by Penington and co-workers contends that the primary determinant of platelet heterogeneity is megakaryocyte heterogeneity. The majority of the work supporting the hypothesis that platelet heterogeneity results from an aging process in the circulation is based on animal studies where the populations were separated by density gradient methods and were either subsequent or prior to separation labeled with radioactive markers. Ideally, an isotopic cohort should satisfy several criteria. The isotope should not be neutralized. The survival of the isotope in the circulation should be short when not bound to the platelet as compared to the platelet bound survival time. All megakaryocytes should be synchronized to incorporate isotope and produce platelets at the same stage of maturation. These criteria for the ideal experiment cannot be totally fulfilled with presently available isotope markers; some reutilization of isotope does take place and megakaryocytes cannot be synchronized to incorporate isotope and release platelets.

Free Se^{75}-selenomethionine and S^{35}-methionine have been shown to be rapidly cleared from plasma, so the criteria for no reutilization is nearly achieved with the amount of reutilization of these labels being relatively low (Amorosi et al., 1971; Karpatkin, 1978B). Human platelets were separated into two extreme density populations by centrifugation in specific inert oil media. The heavy/large platelets were shown to be enriched with large platelets and to incorporate considerably more of the cohort isotope, Se^{75}-selenomethionine, during the early stages of the experiment compared to light platelets which were shown to be composed of smaller platelets which showed specific activity late in the experiment. During the late testing points of the experiment, relatively less label

was found in the heavy platelets compared to light platelets. These data were interpreted to indicate that heavy, dense platelets found to be composed of predominantly larger sized platelets, were released relatively early into the peripheral circulation and hence were considered young platelets (Amorosi et al., 1971). It was also suggested that heavy/large platelets become lighter or less dense and smaller platelets with time.

The density gradient separation of platelet populations was further refined by the use of an albumin density gradient. In comparison to the inert oil density gradient, the albumin gradient gave the advantage of greater resolution and better resuspension of isolated populations. When separated into two populations, the heavy population was found to be predominantly large platelets, with 2.3 times more platelets with diameters of 2 microns, and 2 times less platelets with a 1 micron diameter in comparison to the light population. Platelets with a diameter of 3 or more microns were found exclusively in the heavy fraction. Following in vivo labeling of megakaryocytes with Se^{75}-selenomethionine on days 1-2, the heavy:light labeling ratio was 3.7 and declined to 1.4 on days 3-4 and to 0.7 by day 6 (Chamatz et al., 1974). This study supported the findings of Amorosi et al. that indeed heavy/large platelets are young platelets.

The suggestion that heavy/large platelets become lighter/smaller platelets as they age in the circulation is supported by non-human platelet studies using rabbits. The rabbit platelet membrane has three main glycoproteins, I, II, III, similar to humans. Labeling with diazotized (I^{125})-diiodosulfanilic acid (125 DD-ISA) has been shown to be specific for the exposed membrane glycoproteins. The fate of the platelet membrane can be studied in vivo by labeling glycoproteins with 125 DD-ISA and compared to the fate of cytoplasm which can be selectively labeled with $chromium^{51}$ (George et al., 1976). Labeling platelets with either

label or both was shown to have no affect on the amount of recovered radioactivity. Thirty minutes after infusion, 125 DD-ISA was found to be bound to plasma membrane protein. After 3 hours of circulation, 125 DD-ISA disappearance became more rapid than the linear chromium^{51} clearance. Loss of 125 DD-ISA was specific for platelets circulating in vivo since it was demonstrated that loss of 125 DD-ISA from platelets in vitro parallels the loss of chromium^{51}. The loss of 125 DD-ISA was equal among all three major glycoproteins. George et al. (1976) postulated that the loss of 125 DD-ISA was due to loss of pieces of plasma membrane as a result of reversible adhesion encountered in the peripheral circulation as part of the hemostatic process.

In order to prove whether portions of platelet membrane were lost during hemostatic function, similar experiments were conducted using known platelet activators and inhibitors of normal platelet function (George et al., 1978A). Inhibition of platelet function by aspirin and dipyridamole slowed the normally rapid exponential loss of membrane bound 125 DD-ISA so that it paralleled the loss of chromium^{51}. In contrast, initiation of platelet hemostatic function by thrombin infusion, accelerated the loss of 125 DD-ISA relative to chromium^{51} loss. These findings were interpreted to show that 125 DD-ISA labeled membrane glycoproteins are shed from platelets during hemostatic processes, which are increased by thrombin and decreased by aspirin-dipyridamole. In addition, the finding that inhibition of platelet function by aspirin and dipyridamole did not prolong the time for chromium^{51} disappearance, indicates that factors other than hemostatic function and membrane loss are involved in platelet aging and removal from the circulation.

Further studies using sulfate^{35} label found that as dense platelets became less dense due to aging in the circulation, a corresponding loss of sialic acid and protein occurred

(Rand et al., 1981). The decreased protein and sialic acid content was believed to be due to loss of proteins and glycoproteins from the surface of the platelet or loss of internal granules, or both. The sulfate^{35} cohort platelet was incorporated into megakaryocyte proteoglycans, followed by distribution into forming platelets. Any detectable activity of sulfate^{35} represented newly formed platelets as little to no reutilization of sulfate^{35} was recognized. These studies found that the sulfate^{35} label appeared more rapidly in the dense platelets and that the label concentration found in the dense platelet population reached a maximum between 48 and 72 hours. The specific activity of the dense population rapidly declined following its peak in conjunction with a rise in specific activity of sulfate^{35} in the less dense platelet population through 120 hours, indicating that the more dense platelets were being converted to less dense platelets. Dense platelets were found to be 1.2 times the size of lighter platelets and contained 1.5 and 1.3 times more sialic acid and protein respectively, than the less dense platelet population.

The aging hypothesis is furthur supported by platelets labeled with Se^{75}-selenomethionine , followed by separation into five different platelet density fractions on each of seven days by repetitively centrifuging the same sample of platelet rich plasma at increasing gravitational force. The heavy platelet population was found to be enriched with large platelets and lighter populations with smaller platelets. The dense:light specific activity ratio of the isotope declined with time following the incorporation of isotope, which further supports the theory that young platelets are larger and more dense than old platelets (Karpatkin, 1978B).

In an effort to eliminate the effect of different megakaryocyte maturation stages during cohort labeling with isotopes, a method utilizing arabinogalactan density gradient

separation of platelets prior to labeling was developed (Corash et al., 1978). Rhesus monkey density-dependent populations believed to represent cells of different ages were isolated and labeled with chromium^{51}. The different density populations corresponded to earlier studies showing heavy platelets to be larger than lighter platelets. This study clearly showed a difference in the life-span between light and heavy platelets following infusion. The light platelet populations mean survival was 74.6 hours, compared to 313.6 hours for the heavy platelets. In all animals tested, the survival of the heavy platelet population was 2.5-3.0 times that of the light platelet survival. Serial density gradient analyses following infusion of either the labeled heavy or light platelet population showed that it was possible to reisolate a platelet population with high specific activity from the recipient. When radioactive heavy platelets were reinfused they were gradually replaced by non-labeled platelets in the most dense fraction, with a concurrent increase in labeled platelets in the less dense fractions as the activity decreased in the heavy gradient fraction. In contrast, when labeled light platelets were infused they remained in the light region of the gradient with no transfer to heavier regions of the gradient.

The findings of Corash contrast the findings of Penington and co-workers, which are reviewed below. Penington and co-workers report no density related aging effects in their studies and relate density heterogeneity to megakaryocyte heterogeneity. However, Corash points out that these investigators used platelets isolated from whole blood by silica-polyvinylpyrolidine (silica-PVP) gradients without first removing leukocytes, which if not removed can contaminate density separated platelet populations. Use of in vivo Se^{75}-selenomethionine results in leukocyte labeling as well as megakaryocytes, and hence lymphocyte contamination of the platelet gradient would markedly mask a primary platelet

effect. Corash also contends that the platelet labeling observed in vitro by Penington and co-workers was possibly due to leukocyte labeling rather than platelet labeling. Due to these technique problems, Corash concludes that data from Penington and co-workers are not sufficient to negate the platelet observations of Karpatkin and co-workers.

While the present studies of Corash indicate an aging process in vivo of platelets in the peripheral circulation, they do not exclude the possibility that changes in thrombocytopoiesis at the megakaryocyte level may affect the initial size distribution of platelets. Corash proposes the hypothesis that megakaryocyte heterogeneity and platelet aging in the peripheral circulation contributes to platelet heterogeneity.

In opposition to Karpatkin and co-workers hypothesis, Penington and co-workers contend that the major determinant for platelet heterogeneity is megakaryocyte heterogeneity. In experiments designed to compare the 8N, 16N, and 32N megakaryocytes ultrastructurally, they were found to differ in their concentration of cytoplasmic constituents which would be expected to influence the buoyant density of their platelet progeny. Similar ultrastructural studies of various density separated platelet populations were found to be similar to the findings of the various megakaryocyte populations.

The 8N megakaryocyte in comparison to the 32N cell is similar in size, contains greater numbers of granules and mitochondria, and contains less surface-connected canalicular system. When comparative studies were performed on three density separated platelet populations, the lighter platelets which were small in size were found to contain a greater amount of surface-connected canalicular system and less mitochondria and granular substance than the more dense population of platelets which were larger, containing more mitochondria and granular substance, and less surface-connected canalicular system

(Penington et al., 1976A). From these experiments Penington et al. postulated that in a normal steady state of health, the more dense, larger platelets were the progeny of 8N megakaryocytes and the smaller, less dense platelets were the progeny of 32N megakaryocytes, with an intermediate population of platelets overlapping each group, being the progeny of the 16N megakaryocytes. Penington et al. further speculated that the differences found for each population with respect to function was the direct result of the ultrastructural findings for each population. The 8N megakaryocyte progeny with a greater content of granules and mitochondria with less canalicular structure may function more specifically to surface contact, whereas the 32N cell progeny with more canalicular structure may respond more rapidly to changes in the plasma environment.

This hypothesis was further supported by platelet sizing studies of human and rat platelets following buoyant density separation using colloidal silica-polyvinylpyrolidone methods (Penington et al., 1976B). Following separation into three density specific populations, each population was sized using a Coulter Counter, Model B (Coulter Electronics, Hialeah, Florida). The three populations were found to be heterogeneous in size. The most dense platelets ranged in size from 3 to greater than 21 femtoliters for both species. The lighter platelets ranged in size up to 13 femtoliters for both species. These findings correspond to Karpatkins' in that the denser platelets tended to be larger platelets, but exception is taken to the fact that even the intermediate and heavy platelets also contain small sized platelets, with heterogeneity most remarkable in the dense population. Cohort labeling studies of rat platelets with Se^{75}-selenomethionine, followed by monitoring for 5 days failed to show selective incorporation into any one fraction, although slightly more activity was noted in the dense population. Penington contends his findings strongly suggest that

heavy, intermediate density, and light platelets are released concurrently from different megakaryocytes, and that these populations are each very heterogeneous in size, with the exception of the light platelets. Penington points out that even though there may be an appearance of movement from heavy to light platelets resulting from in vivo hemostatic activities as claimed by Karpatkin and co-workers, it could also be easily explained by increased susceptibility of aged dense platelets to the manipulation involved in density gradient techniques. With respect to increased incorporation into more dense platelets than lighter platelets as shown by Karpatkin and co-workers for both man and rabbits, Penington and co-workers attribute this to increased protein synthesis in dense platelets compared to light platelets. In vitro studies of dense and light platelets found a seven-fold greater rate of incorporation of labeled leucine into dense rather than light platelets, when calculated as activity per platelet, whereas the ratio of the total platelet content was only 2.58 to 1 (Karpatkin et al., 1969A; Penington et al., 1976). In the current study, Penington found the rate of protein synthesis to be higher in dense than light platelets, although not as great as Karpatkins' study, indicating a greater protein turnover corresponding to an increase in initial incorporation of label into dense platelets. Penington contends that evidence of varying protein synthesis activity of different platelet populations invalidates the premise for using the ratio of specific activity in dense and light platelets as evidence for conversion of dense platelets into intermediate or light platelets by an aging process in the circulation.

Additional studies by other investigators add support to Peningtons' hypothesis. Experiments have produced evidence indicating heavy platelets are not necessarily the youngest platelets (Boneu et al., 1973). This study found no variation in the distribution of chromium^{51} between heavy and light fractions in human studies, suggesting survival of each

platelet population to be the same. Following injection of Se^{75}-selenomethionine into sheep, a concurrent appearance of three different density populations were also found, indicating megakaryocytopoiesis may play an important role in platelet heterogeneity.

Additional support for platelet heterogeneity resulting from release of platelets from megakaryocytes of varying heterogeneity come from human studies of individuals with hyperdestructive syndromes (Paulus, 1975). If macrothrombocytosis in hyperdestruction is due only to the young age of the circulating platelet population, a decreased dispersion of volumes should be found since young platelets have a smaller age heterogeneity than do normal populations of platelets of all ages, but the opposite was found. Platelet size was not only increased, but was also more heterogeneous in hyperdestructive syndromes, with small as well as large platelets present. Although the findings of Paulus do not exclude the possibility that some decrease in size occurs during aging in the circulation, it does demonstrate that aging alone does not provide an adequate explanation for the heterogeneic population of platelets found in destructive processes.

In summary of the two hypotheses for platelet heterogeneity, Penington and co-workers contend heavy, intermediate density, and light platelets originate from 8N, 16N, and 32N megakaryocytes respectively and within each density population there is broad heterogeneity in size with the greatest size variation found amongst the dense platelets. They support the hypothesis that platelets may age in the circulation, decreasing in size, and they believe platelets remain predominantly within their original density class. They dispute the hypothesis that young platelets are dense platelets and due to aging migrate into less dense platelet populations. Futhermore, Penington contends there are three major criteria necessary to support a correlation between platelet age and density, which were not totally

observed in his experiments. These criteria are, a delay in the incorporation of label into light platelets, a rise in the proportion of labeled light platelets with a corresponding decline in heavy platelet activity, and a shorter life-span for lighter platelets than heavy platelets.

Karpatkin and co-workers contend recent studies using refined density gradient separations following cohort labeling offers an answer as to which hypothesis is correct. Asplenic and eusplenic rabbits were studied following injection of a tritiated L-amino acid mixture. An arabinogalactan density gradient method was used to separate platelets into four populations (Corash et al., 1982). Substantial preferential incorporation of label into heavy platelets compared to light platelets was found with a rapid increase and decline by day 2 of specific activity in the heavy platelets followed by a rise in the specific activity in the light platelets on day 3 and 4. During the first 2 days, specific activity of the heavy platelets was twice that of the light platelets, and vice versa during days 3 and 4. Variation of activity was not only limited to the extreme classes of platelets, for comparison of the intermediate density classes showed a sequential pattern of preferential early label in all platelet classes of increased density compared to the next lightest population.

The finding that labeled platelets within each density population increased sharply to peak activity and subsequently declined rapidly, is consistent with the migration of label from one density population to another. The appearance and persistence of the peak activities for the two opposite density populations does not appear to be consistent with the hypothesis of staggered platelet release from different megakaryocyte ploidy classes. The finding of some overlap between labeling of the subpopulations may be due to the wide distribution of platelet densities and is most likely the result from a series of overlapping of normal density subpopulation in which the mean density of the youngest platelets is greater than the oldest,

but in which substantial overlap in the middle population can be found.

Specific activities were also calculated per protein content. The present study calculated activity based on total content of platelet protein expressed as dpm per microgram of protein so that differences in cell size alone would not account for the findings. A ratio of 1.17 to 1 for protein content per cell for heavy to light platelets was found, indicating protein content does not explain the increased specific activity found in more dense platelets. This finding is supported by earlier experiments of heavy platelets which found that the most dense platelets do contain some endoplasmic reticulum, while intermediate density platelets contain relatively infrequent endoplasmic reticulum (Corash et al., 1978). Hence the increased label content of intermediate density platelets compared to the lightest platelets cannot be explained by virtue of more ribosomal complexes, rather the label must be incorporated at an earlier time. In addition, the least dense platelets which have equal specific label activity as dense platelets contain little or no endoplasmic reticulum so their accumulation of label must result through transformation of dense platelets to light platelets. Further, it was found in two of the three asplenic rabbits that the light platelet specific activity was higher than the activity of heavy platelets, which is inconsistent with Peningtons' finding that light platelets have lower specific activity because of less active protein synthesis.

These studies and those reviewed earlier primarily support the hypothesis of Karpatkin and co-workers that platelets age during peripheral circulation under nonstressed conditions. However, it cannot be stated that aging accounts totally for platelet heterogeneity. Megakaryocyte growth and platelet release undoubtedly have an effect on platelet properties and should be included as an additional determinant of platelet heterogeneity in conjunction with aging in the peripheral circulation.

3. Regulation of platelet volume:

a. Intrinsic regulation: Conventionally, the regulation of cellular volume is believed to reflect a balance of active and passive ion movement across a cell membrane, with the colloid osmotic pressure of the intracellular macromolecules being counterbalanced by the exclusion of sodium from the cells. In addition, the forces that offset the osmotic effects of the intracellular cytoplasm must be dealt with. These counterbalancing forces include the compartmentalization of solutes and water within the cell, the osmotic activity of the extracellular medium, and any property the cell may have to oppose swelling.

Only a few studies have been reported on the regulation of cell volume in platelets. Platelets have been found to contain membrane sodium-potassium- ATPase and calcium-magnesium-ATPase, but their discovery was found in studies centering around platelet function as opposed to volume regulation. The complete answer for platelet intrinsic volume regulation is unknown.

b. Extrinsic regulation:

(1). Platelet sequestration: Two exchangeable platelet storage pools in humans are known: a splenic pool storing an estimated 1/3 of the total platelet mass and a nonsplenic pool of unknown origin. The splenic pool is known to preferentially store larger sized platelets, but the size of platelets stored in the nonsplenic pool is unknown. Several anatomical sites for the nonsplenic pool have been proposed which include the reticuloendothelial organs, primarily the lungs, and the endothelial vascular surface (Freedman et al., 1977; Kaufman et al., 1965).

The presence of a platelet storage pool which preferentially holds larger sized

platelets implies that the size of the circulating platelets is controlled by factors regulating the passage of platelets in and out of the pool. The splenic pool is known to be mobilized following exercise or epinephrine administration, whereas the nonsplenic pool is mobilized by exercise (Branehog et al., 1973; Freedman et al., 1972).

(2). Platelet aging in the circulation: Two areas of study to determine the aging process of platelets in the circulation have been pursued. One centers on density changes while the second relates to membrane loss. Whether or not platelets decrease in density with circulation has been a source of controversy (Karpatkin et al., 1977; Penington et al., 1976A & B). Evidence supporting this concept includes studies which found labeled light platelets injected into animals were recovered by density fractionation only in the same original fraction, whereas labeled heavy platelets which are initially located in the dense region progressively migrate into lighter regions during their life-span. In addition, the life-span for light platelets is shorter than heavy platelets (Corash et al., 1978; Karpatkin, 1978B).

It is fairly well established that platelets shrink as they age in the circulation (George et al., 1976 & 1979). The decrease in platelet size is believed to result from loss of membrane. Animal studies revealed that platelet membrane surface glycoproteins, labeled with diazotized [I^{125}] diiodosulfanilic acid (125 DD-ISA) disappeared more rapidly than platelets labeled with chromium^{51}, a selective platelet cytoplasmic label. The conclusion that the loss of 125 DD-ISA label resulted from loss of membrane rather than specific proteins from the membrane surface is upheld by the observation that 125 DD-ISA decreased proportionally in all labeled fractions of membrane glycoprotein. The idea of membrane loss with aging was further supported by studies which show the rate of loss of 125 DD-ISA is decreased by inhibitors of

normal platelet function and accelerated by induction of intravascular coagulation. These findings are consistent with the premise that the platelet membrane is lost during normal circulation, possibly resulting from reversible contact interactions during the process of hemostasis (George et al., 1978A). A more detailed review can be found under the subtitle, Theories for platelet heterogeneity.

(3). Megakaryocyte maturation and heterogeneity: Platelets are produced as a result of a fragmentation process of megakaryocytes. Normally they mature to a point of cytoplasmic disintegration in three principle ploidy classes: 8N, 16N, 32N. Following platelet depletion, immature megakaryocytes undergo additional ploidy, resulting in an increase in numbers of 32N and 64N megakaryocytes in conjunction with an overall increase in cell size. Maximum increase in 32N and 64N ploidy cells occured between 32 and 72 hours post platelet depletion, with a return to normal ploidy counts by 120 hours. In conjunction with increased ploidy and cell size, beginning about 36 hours post stimulation and lasting about 72 hours was a rapid rise in platelet count. The average platelet size was found to rise and fall within 60 hours. These findings suggest that the circulating platelet mass may be compensated during thrombocytopenic periods by increases in ploidy and cytoplasmic size of parent megakaryocytes (Odell et al., 1976; Long et al., 1982B). A more detailed review can be found under the subtitle, Theories for platelet heterogeneity.

(4). Total platelet mass: In normal steady-state conditions the regulatory system in humans for platelet concentrations appears to be under precise control as levels remain constant for long periods of time (Brecher et al., 1953). In principle, regulation of the circulating platelet mass could occur either prior to or following release of platelets from the parent megakaryocyte into the circulation. Hence the circulating platelet

mass may reflect the influence of preferential sequestration of platelets according to platelet volume in peripheral storage pools, or age-dependent processes that alter platelet volume in the circulation, or megakaryocyte maturation and heterogeneity, or a combination of the three. Investigation into the relationship between platelet count and mean platelet volume has found a non-linear, inverse relationship during normal health and in several disorders to be reviewed below (Levior et al, 1983; Giles, 1981).

E. HETEROGENEITY OF PLATELET SIZE IN HUMAN HEALTH

Over the years, clinical meaning has been attributed to platelet sizing by many researchers using a variety of techniques. One of the major difficulties with determining clinical relevance of these studies results from failure of standardization. The use of different anticoagulants, storage temperature, length of time between specimen acquisition and analysis, method of size determination, and the interpretation of results have all led to confusion and contention.

For the purpose of this thesis a cross section review of the literature was performed; separating the various studies into the broad catagories as having either an inherited or an acquired origin. Disagreement exists as to which anticoagulant is the best for use with platelet size determinations, but the vast majority of researchers used ethylenediamine tetraacetic acid. Size determinations were either based on volume or diameter. Diameter measurements were usually performed using an ocular micrometer, whereas volume determinations were determined by use of one of the various Coulter instruments. The Coulter method, based on the principle of aperture-impedance, and the effect of specimen handling prior to analysis is reviewed below in detail. Prior to a review of size variations found in various inherited and acquired conditions of health, a review of the normal platelet distribution is required.

a. Normal platelet distribution: Over the years virtually every conceivable means for reporting platelet size has been used. A majority of the studies performed are found to report platelet size for the individual group being studied, which is then compared to a normal size as determined from a control population. Historically, the control groups have been normal healthy individuals, oftentimes from the laboratory staff who generally have normal platelet counts. Through the evolution of continued study it was discovered that platelet size was inversely proportional to the platelet count. The higher the platelet count, the smaller the size. During the late 1970's, the marketing of the Coulter model S-Plus series of instrumentation revolutionized platelet size determinations, along with a revised interpretation of normal platelet size distribution. Researchers demonstrated that although there exists an inverse relationship between platelet count and size, that relationship is non-linear (Bessman et al., 1982A; Giles, 1981). The inverse, non-linear relationship was demonstrated for both normal and abnormal conditions of health. Platelet counts below $100x10^9$/L were found to show a non-linear increase in platelet volume as the count decreased. Platelet counts between 100-$850x10^9$/L showed a steady decline in platelet size as the count increased and platelet counts above $850x10^9$/L showed less of a decrease in size as counts increased.

These studies demonstrated that there is no single normal size for platelets, but rather a series of normal ranges which vary with the platelet count. In order to depict a normal mean platelet volume (MPV) visually, Bessman developed a MPV/Platelet count nomogram which plots the mean platelet volume versus the platelet count graphically.

The direct result of these studies is two-fold. First, when evaluating platelet volumes, comparison of normal ranges must be correlated with respect to the platelet count. Secondly, they may offer a possible reason for the variation in results found in the literature, since many

of these studies compared abnormal individuals against a normal population, with platelet counts in the normal range.

b. Platelet Size Variation in Hereditary Disorders: Over the years platelet size studies for several hereditary diseases have been characterized by the findings of abnormal platelet counts and size. The most notable disorders demonstrating an increased platelet size would be the Bernard-Soulier Syndrome or the May-Hegglin Anomaly, with the Wiskott-Aldrich Syndrome being the most notable disorder with characteristically small platelets. The majority of the other hereditary disorders listed inTable 1, are extremely rare with a few being isolated to single families. Several of these findings may be considered coincidental and of little diagnostic value, whereas others may aid in the clinical management of afflicted individuals.

1. Bernard-Soulier Syndrome (BSS): This syndrome has an autosomal recessive mode of inheritance. BSS is a rare disorder which has been characterized by thrombocytopenia with giant platelets on blood smear examination and a prolonged bleeding time, resulting in a bleeding tendency. BSS platelets lack binding sites for von Willebrands Factor, preventing platelet adhesion to damaged blood vessels. Early investigators proposed that the platelets from BSS were normal in size while in the circulation, but during the preparation of blood smears they became unusually large due to excessive spreading. It was proposed that excessive surface-connected canaliculi system membrane became extruded during preparation of blood smears (Frojmovic et al., 1978). Recent studies using electron microscopy have disproved these early proposals. BSS platelets were found to be 2 to 3 times larger than normal within the circulation, containing normal amounts of surface-connected canaliculi system (White et al., 1984). They were also found to be less resistant to deformability, which lead White et al. to propose that this unusual lack of

deformability was the cause for increased spreading during whole blood smear preparation.

Table 1. Platelet sizes found in subjects with inherited disorders (A/C=anticoagulant; ACD=Acid citrate dextrose; E.M.=Electron microscopy; I=increased; D=Decreased; V=variable; N=normal).

Disorder	A/C	Method	Count	Size	Reference
Bernard-Soulier Syndrome	citrate	volume	D	I	Holme et al., 1981A & B
	citrate	diameter	?	I	Frojmovic et al., 1978
	citrate	volume	?	N	Frojmovic et al., 1978
	citrate	volume	D	I	White et al., 1984
Congenital Thrombo-	?	diameter	V	I	Niewiarowski et al., 1969
cytopenic Thrombocytopathy	?	volume	V	I	Niewiarowski et al., 1969
	citrate	diameter	D	I	Grottum et al., 1969A
		diameter	D	I	Evenson et al., 1974
	?	diameter	D	I	Cullum et al., 1967
	?	volume	D	I	Baadenhuijsen et al., 1971
Familial Bleeding Tendency	?	E.M.	N	D	Maurer et al., 1971
Familial Thrombocytopenia-					
Family E	ACD	volume	D	N	Murphy et al., 1972
Family R	ACD	volume	D	D	Murphy et al., 1972
Connective Tissue Disorders					
Ehlers-Danlos Syndrome		diameter	N	I	Estes, 1968
Marfan Syndrome		diameter	N	I	Estes, 1968
Mucopolysaccharidosis		diameter	N	I	Estes, 1968
Osteogenesis imperfecta		diameter	N	N	Estes, 1968
Montreal Platelet Syndrome	?	diameter	?	I	Milton et al., 1979
Hemoglobinopathies					
Sickle cell anemia	EDTA	volume	N	V	Levin et al., 1983
Thallasemia	EDTA	volume	N	V	Levin et al., 1983
May-Hegglin Anomaly	citrate	volume	D	I	Goodwin et al., 1974
Mediterranean Macro-					
thrombocytopenia	EDTA	volume	D	I	von Behrens, 1975
Wiskcott-Aldrich Syndrome	EDTA	diameter	?	D	Rivard et al., 1975
	citrate	diameter	D	D	Grottum et al., 1969B
	ACD	volume	D	D	Murphy et al., 1972
pre-splenectomy	?	volume	D	D	Corash et al., 1979
post splenectomy	?	volume	N	N	Lum et al., 1980

2. Congenital Thrombocytopenic Thrombocytopathy: This group of platelet disorders is found in the literature with the following titles: Congenital Macrothrombocytic Thrombopathia (Niewiarowski et al., 1969), Congenital Thrombocytopenia with Giant Platelets (Grottum et al., 1969A), Familial Bleeding Disorder with Thrombocytopenia and Giant Blood Platelets (Evenson et al., 1974), Familial Thrombocytopenic Thrombocytopathy with deficiency of Platelet Factor 3 (Baadenhuijsen et al., 1971). The most common findings between these investigators was an increase in the size of circulating platelets, often 2-3 times larger than normal, thrombocytopenia, and a reduction on the concentration of Platelet Factor 3. One investigator reported periodic thrombocytopenia (Niewiarowski et al., 1969). In patients where the bone marrow was examined, megakaryocytes appeared normal in number and appearance (Cullum et al., 1967; Grottum et al.,1969A). Electron microscopy revealed that the submarginal band of microtubles appeared incomplete and the microtubules were randomly distributed (Evenson et al., 1974;Niewiarowski et al., 1969). The consensus of the investigators was that the hemostatic defect originated at the platelet membrane, primarily a sialic acid and Platelet Factor 3 deficiency. Patients commomly exhibited easy bruising, spontaneous echymosis, and profuse bleeding during minor surgery. Cullum, Grottum, and Evenson report splenectomy to be of little value. The platelet count was found to rise in some individuals, but little clinical improvement was noted. Family studies indicated the mode of inheritance to be autosomal dominant.

Two platelet disorders, Glanzmans thrombasthenia and the May-Hegglin Anomaly should not be confused with this disorder. In Glanzman thrombasthenia, platelet adhesiveness, aggregation, and clot retraction are absent in the presence of ADP or thrombin. In addition, the concentration of Platlet Factor 3 is within normal range. Just the

opposite is true for Congenital Thrombocytopenic Thrombocytopathy. In May- Hegglin Anomaly, normal concentrations of Platelet Factor 3 are associated with an increase in platelet size, which is just the opposite for this disorder which is lacking in Platelet Factor 3.

3. Familial Bleeding Tendency associated with microcytic platelets: Reported cases are insufficient to establish the inheritance mode for this platelet disorder. Three members (mother and two daughters) of this family were all characterized by easy bleeding and abnormal platelet function studies. All three family members suffer from hematuria, easy bruising and recurring epistaxis. Coagulation studies found a lack of platelet response to a suspension of connective tissue and thrombin. Disaggregation of platelets following primary aggregation due to the initial presence of ADP, indicated failure of continued aggregation was the result of ADP not being released from internal granules during primary aggregation. Electron microscopy revealed two of the three family members have microcytic platelets,with the average surface area about 1/2 the normal size. These smaller sized platelets may be the result of production of defective platelets which are lacking in ADP or have accelerated platelet aging, or increased platelet survival, or possibly the selective destruction of larger, presumably younger platelets. In summary, this disorder is isolated to three members of one family, two daughters and their mother, all of which have a bleeding tendency, an abnormal ADP release following primary platelet aggregation, and microcytic platelets in two of the three affected family members.

4. Familial Thrombocytopenia- Family E: Affected members of Family E include the father and three children (1 son,2 daughters). The disorder is believed to have an autosomal dominant mode of inheritance (Murphy et al., 1972). The two males have no hemorrhagic symptoms as opposed to the two females who suffer from persistent

menorrhagia. Following splenectomy, relief from persistent menorrhagia was achieved, although the platelet count rose in only one individual. Prior to splenectomy the subjects presented with thrombocytopenia, increased circulating megathrombocytes, and normal platelet life-span and aggregation studies. Bone marrow biopsy showed an increased number of megakaryocytes, with normal morphology. This disorder has several characteristics similar to Bernard-Soulier Syndrome and Idiopathic Thrombocytopenic Purpura (thrombocytopenia, megathrombocytes, increased bone marrow megakaryocytes) but can be distinguished by their normal survival in the peripheral circulation.

5. Familial Thrombocytopenia-Family R: Family R is characterized by thrombocytopenia, decreased platelet size and survival within the peripheral circulation, and is associated with a sex-linked inheritance mode, similar to the Wiskott-Aldrich Syndrome (WAS). Due to the similarities to the WAS, Family R is believed to be a variant form of this syndrome (Murphy et al., 1972). Three members from the second generation and two from the fourth generation are symptomatic. The female carrier in the third generation is symptom free with a normal platelet count. Members of the fourth generation were characterized by easy bruising, mild eczema, thrombocytopenia, decreased platelet size, shortened platelet life-span, and normal immunologic studies. Both affected members of the fourth generation underwent splenectomy which resulted in a subsequent increase in platelet count and clinical improvement. Bone marrow biopsy prior to splenectomy revealed a normal number of megakaryocytes with normal morphology.

Individuals with this disorder should not be misdiagnosed with the classic Wiskott-Aldrich Syndrome. Although inheritance and clinical manifestations mimic the WAS, the classical WAS triad of recurrent infections, eczema, and thrombocytopenia is not present

in combination.

6. Inherited Disorders of Connective Tissue: Platelet size was evaluated in 31 families with the following disorders: Osteogenesis imperfecta (autosomal dominant), Marfan syndrome (autosomal dominant), Mucopolysaccharidosis syndrome (sex-linked), Ehlers-Danlos syndrome (autosomal dominant), Pseudoxanthoma elasticum (autosomal recessive). A total of 117 subjects were investigated (Estes, 1968). The presence of large platelets was found in all with the exception of Pseudoxanthoma elasticum, although not all affected individuals possessed large platelets. Platelet counts were within normal limits. The presence of megathrombocytes was not a common finding, but among individual families where large platelets were found it was a consistent finding within that family. The precise finding of increased platelet size in several afflicted families is difficult to assess due to the inconsistent finding of large platelets from one family to another; however, they are relatively consistent within families. The benefit of platelet sizing as an aid in diagnosing these disorders remains questionable and unknown.

7. Montreal Platelet Syndrome (MPS)-Hereditary Giant Platelet Syndrome: MPS is a poorly characterized hereditary giant platelet syndrome which has many characteristics similar to the Bernard-Soulier syndrome (BSS). Similar findings are thrombocytopenia, normal sized platelets within the circulation but abnormally large platelets following blood smear preparation, prolonged bleeding times and spontaneous platelet aggregation. The mode of inheritance is autosomal dominant, as opposed to autosomal recessive found in BSS. MPS platelets were found to have normal size in the circulation while in a native discoid form, but following physical and biochemical stimulation the disc shaped platelets are transformed into abnormally large spheroidal forms. The defect in MPS is believed to be at the site of the

platelet membrane, primarily membrane deformability, which results in abnormal hemostatic properties following thrombin or ADP activation.

8. Hemoglobinopathies (sickle cell anemia and thalassemia): In a series of 18 subjects with homozygous Hemoglobin S, 11 were found to have a mean platelet volume (MPV) within the normal range and 7 subjects with an increased MPV. The increase in platelet volume was not related to the platelet count.

In a series of 50 subjects with Thalassemia, 30 were found to have a normal MPV, 19 an increased MPV, and one with a decreased MPV. The study population showed an increasing MPV as the platelet count decreased. No difference was noted between alpha-or-beta-thalassemia or to ethnicity (Levin et al., 1983).

Variations in the MPV appears to have little diagnostic value with respect to Sickle Cell Anemia or Thalassemia, as more definitive diagnostic tests exists. Data revealing if any of these subjects were splenectomized, which would account for the increase in platelet size, were not presented.

9. May-Hegglin Anomaly: The May-Hegglin Anomaly (MH) is an autosomal dominant inherited disorder characterized by giant platelets, Dohle body inclusions within leukocytes, and commonly by thrombocytopenia and minor bleeding episodes. The platelet life-span is normal, bleeding time prolonged with poor clot retraction, and normal adhesiveness and aggregation studies. Among reported cases, 43% have had abnormal bleeding symptoms which include easy bruisability, gingival bleeding, menorrhagia, recurrent epistaxis, and excessive hemorrhage following minor surgery or trauma. Approximately 1/2 of the affected individuals are asymptomatic. A majority of the symptomatic subjects are thrombocytopenic, whereas the majority of the asymptomatic subjects have a normal to

slightly decreased platelet count. Bone marrow studies reveal the presence of a normal number of megakaryocytes. Godwin et al. (1974) proposed that the defect with regards to platelet count and size may be the result of a disorderly fragmentation of megakaryocytes into variously sized platelets.

10. Mediterranean Macrothrombocytopenia: This disorder is a benign variant of platelet production which can be easily confused with the May-Hegglin Anomaly (MH) or Idiopathic Thrombocytopenic Purpura (ITP). The majority of individuals afflicted by this benign disorder reside or have direct familial lineage to either the Italian or Balkan peninsulas or their adjacent islands. Characteristic findings reveal a reduced platelet count with an increased mean platelet volume. The platelet population functions normally, with the afflicted individuals having normal hemostatic function. Light microscopy studies find platelets which exceed the size of erythrocytes. Electron microscopy demonstrates a large platelet whose band of microtubules are randomly disorganized. It is of importance that these healthy individuals not be confused with either MH or ITP subjects who have similar blood smear morphology findings, but in conjunction with bleeding tendencies (von Behrens, 1975).

11. Wiskott-Aldrich Syndrome (WAS): WAS is a sex-linked deficiency disorder associated with a triad of eczema, thrombocytopenia, and recurrent infections. Severe hemorrhage and overwhelming sepsis are the most threatening acute symptoms. The recurrent bacterial infections are relatively easy to control by antibiotics, but hemorrhage due to severe thrombocytopenia remains a difficult problem in some subjects. Bleeding is attributed to severe thrombocytopenia, characterized by small platelets. This microthrombocytopenia was originally believed to be the result of a primary defect in platelet production until splenectomy was found to normalize the platelet count in several subjects

(Lum et al., 1980). Prior to splenectomy, platelets were commonly found to be 1/2 normal size, but returned to normal after splenectomy. The decreased size of platelets was the result of increased platelet antibodies of the immunoglobulin G class, resulting in the premature destruction of platelets by splenic macrophages. Following splenectomy, antibody titers were found to decrease within 2 weeks, with a concurrent rise in platelet volume into normal limits (Corash et al., 1981).

C. Platelet Size Variation in Acquired Disorders: The literature contains a large number of articles relating platelet size to various acquired health conditions. The tables found on the following pages review the overall findings from several studies, a few of which are found to conflict with each other. Several possible reasons can be listed for these discrepancies, with specimen processing, data interpretations, and limited numbers of subjects studied, the predominant ones. Review of these studies does reveal consistent findings in several disorders, although additional research is required before the clinical relevance of these parameters (platelet count and size) can be established.

1. Anemia: Several researchers have evaluated the platelet count and size in several anemias, much of which shows random variability (see table 2). Patients with aplastic anemia are found to have reduced platelet counts with a corresponding low mean platelet volume (MPV). Those subjects who go into remission, with platelet counts on the low side of normal retain a predominance of smaller sized platelets. A constant finding can not be predicted for iron deficiency anemia. Platelet counts have been found to be normal to increased with platelet size showing variability from small to large, with no predictive pattern. In a series of iron deficient patients evaluated prior to and post treatment, the MPV was found to show little change, indicating iron levels may be of little significance as a determinant of

platelet size (Levin et al., 1983). In megaloblastic anemia a similar result is found from one study to another. A majority of investigators report a decreased platelet count corresponding to an increased MPV, athough variability exists with both.

Table 2. Platelet sizes found in subjects diagnosed as being anemic (A/C=anticoagulant; EDTA=ethylenediamine tetraacetic acid; D=decreased; I=increased; V=variable; N=normal).

Disoder	A/C	Method	Count	Size	Reference
Aplastic anemia	EDTA	volume	D	D	Bessman et al., 1982
	EDTA	volume	?	D	Bessman, 1984
-at diagnosis	?	?	D	D	Bessman, 1985
-in remission	?	?	V	D	Bessman, 1985
	EDTA	diameter	D	D	Garg et al., 1971
	EDTA	volume	D	D	Tomita et al., 1980
Iron deficiency anemia	EDTA	diameter	V	I	Garg et al., 1971
	EDTA	volume	I	N	Giles, 1981
	EDTA	diameter	?	D	Rivard et al., 1975
	EDTA	diameter	N	N	Zeigler et al, 1978
-before and during therapy	EDTA	volume	N	N-I	Levin et al., 1983
-following therapy	EDTA	volume	N	N-I	Levin et al., 1983
Megaloblastic anemia	EDTA	volume		D	Bessman et al., 1982
	EDTA	volume	V	I	Bessman, 1984
	?	?	N-D	I	Chatteriiet al., 1971
	EDTA	diameter	D	I	Garg et al., 1971
	EDTA	diameter	D	I	Garg et al., 1969
	?	volume	?	I	Rowan, 1979A

2. Acute leukemia/Infiltrative bone marrow tumor: The severity of thrombocytopenia and variation in the MPV is the result of the degree of bone marrow involvement or as a side effect of chemotherapy. Variation in study findings within the literature exists, but the consensus agrees that the platelets found in acute lymphoblastic leukemia (ALL) subjects are decreased in number and size, whereas the platelets from acute myelogenous leukemia (AML) subjects tended to be normal in size when correlated to the platelet count (see table 3).

The exception was found with acute myelomonocytic leukemia where a large percentage of the subjects were found to be thrombocytopenic with an elevated MPV.

Table 3. Platelet size variation in Leukemias/Infiltrative Bone Marrow Tumors (A/C=anticoagulant; EDTA=ethylenediamine tetraacetic acid; D=decreased; I=increased; V=variable; N=normal; *=platelet count compared to MPV).

Disorder	A/C	Method	Count	Size	Reference
Acute leukemia	EDTA	volume	D	N	Giles, 1981
	EDTA	volume	D	D	Tomita et al., 1980
Acute leukemia/Solid tumor	EDTA	volume	D	N-D	Bessman, 1980
	EDTA	diameter	D	I	Zeigler et al., 1978
-receiving no chemotherapy	EDTA	volume	*	N	Levin et al., 1983
-receiving chemotherapy	EDTA	volume	*	D	Levin et al., 1983
Acute granulocytic leukemia	citrate	volume	D	N-I	Nouvel, 1978
Acute myelogenous leukemia					
-at diagnosis	EDTA	volume	*	N	Bessman et al., 1982
-during chemotherapy	EDTA	volume	*	D	Bessman et al., 1982
-in remission	EDTA	volume	*	N	Bessman et al., 1982
-during chemotherapy	EDTA	volume	*	D	Bessman,1984
Acute myelomonocytic leukemia	citrate	volume	D	I	Nouvel, 1978
Acute myelogenous/lympho-blastic leukemia	citrate	volume	D	N-D	Nouvel, 1978
Acute lymphoblastic leukemia	citrate	volume	D	N-I	Nouvel,1978
Chronic lymphocytic leukemia	EDTA	volume	*	N	Bessman, 1982A
Chronic myelogenous leukemia	EDTA	volume	*	I	Bessman, 1982A
	EDTA	diameter	V	I	Branehog et al., 1982
Myeloproliferative disorders	citrate	diameter		I	Holme, 1981A
	citrate	volume		N	Holme, 1981
	EDTA	diameter	V	I	Cortelazzo, 1980
	EDTA	volume	*	N	Levin et al., 1983
	EDTA	volume	I	I	Small, 1981
Myeloproliferative syndromes	EDTA	diameter	V	I	Zeigler et al., 1978

Random platelet size analysis appears to have little diagnostic benefit as opposed to serial analyses of AML patients which has been proposed to be of predictive value for the imminent return of platelets following chemotherapy (Bessman et al., 1982). The MPV was

found to fall during chemotherapy to levels below normal reference ranges. Prior to return of the platelet count, a rise in the MPV to levels above normal was found to occur. The rise in the MPV preceded the rise in the platelet count by 24 to 48 hours. It was proposed that by monitoring the MPV, a rise would signal the forth coming return of the platelet count, thereby aiding practitioners in the decisions concerning further chemotherapy or the need for platelet transfusion.

3. Myeloproliferative disorders/Chronic lymphocytic leukemia: The majority of studies have found the size of platelets in myeloproliferative disorders (chronic myelogenous leukemia, essential thrombocythemia, myelofibrosis with myeloid metaplasia, polycythemia vera) to be increased, with the platelet count being normal to increased. Volume measurements correspond to microscopic diameter analysis, with increased numbers of hypogranular megathrombocytes found in myeloproliferative disorders. Variations in actual platelet counts and volume exist within the literature.

The platelet count and volume varies from subject to subject with chronic lymphocytic leukemia, but the MPV has a tendency to fall into the normal range when correlated to the platelet count.

4. Idiopathic thrombocytopenic purpura: The term idiopathic thrombocytopenic purpura (ITP) has been used to name a clinical disorder of unknown etiology associated with thrombocytopenia and purpura. The disorder was found to be either acute, chronic, or recurrent with periods of remission. In the early 1950's it was established that the major cause of the thrombocytopenia was a result of reticuloendothelial destruction of antibody coated platelets. Approximately 65% of subjects diagnosed with ITP were found to possess an increased platelet antibody titer, leading to the proposal that these subjects should be

diagnosed as having autoimmune thrombocytopenic purpura (ATP). For the purposes of this review, ITP and ATP will be grouped since their platelet studies are similar (see table 4).

Table 4. Platelet size studies from subjects diagnosed as Idiopathic (autoimmune) thrombocytopenic purpura (A/C=anticoagulant; D=decreased; I=increased; N=normal; *=platelet count as correlated with MPV).

Diagnosis	A/C	Method	Count	Size	Reference
Thrombocytopenic purpura					
-autoimmune	EDTA	volume	D	I	Friedhoff et al., 1978
-idiopathic	EDTA	volume	D	I	Bessman, 1980
-idiopathic (acute)	EDTA	volume	*	N	Levin et al., 1983
-idiopathic (chronic)	EDTA	volume	*	N	Levin et al., 1983
-idiopathic	EDTA	diameter	D	I	Zeigler et al., 1978
-idiopathic	EDTA	diameter	N	I-N	Zeigler et al., 1978
-idiopathic	citrate	volume	D	I	Holme, 1981A
-idiopathic	EDTA	diameter	?	I	Rivard et al., 1975
-idiopathic	EDTA	volume	D	I	Bessman, 1984
-idiopathic (chronic)	EDTA	volume	D	I	Boneu et al., 1982
-idiopathic					
(prior to splenectomy)	citrate	volume	D	I	Boneu et al., 1982
(post splenectomy)	citrate	volume	N	N	Boneu et al., 1982
-idiopathic (acute)	EDTA	volume	D	N	Tomita et al., 1980
-idiopathic (chronic)	EDTA	volume	D	I	Tomita et al., 1980

Megathrombocytes are routinely found in this entity on peripheral blood smear or by volume measurement. The volume of the megathrombocytes are typically 1.5 times normal. The degree of megathrombocytes has been found to parallel the increase in size and number of bone marrow megakaryocytes (Garg et al., 1971). Although the output of platelets is increased, typically a low platelet count persists as a result of short survival within the circulation. Splenectomy results in a return to a normal platelet count and size in approximately 70% of splenectomized subjects.

Table 5. Platelet size variation found in acquired conditions (A/C=anticoagulant; D=decreased; I=increased; N=normal; V=variable).

Disorder	A/C	Method	Count	Size	Reference
Alcoholic thrombocytopenia	?	diameter	D	D	Sahud, 1972
Cardiopulmonary bypass	EDTA	volume	D	D	Bailey et al., 1982
	Citrate	volume	D	D	Laufer et al., 1975
Hypersplenism	EDTA	volume	D	N-D	Bessman, 1980
	EDTA	volume	D	D	Karpatkin et al.,1978B
	EDTA	volume	D	D	Levin et al., 1983
	EDTA	diameter	D	N	Zeigler et al., 1978
Infection	EDTA	volume	V	V	Giles, 1981
Newborns-Term	EDTA	volume	D	I	Kippen et al., 1982
-Premature	EDTA	volume	D	I	Kippen et al., 1982
-Term	EDTA	volume	N	N	Kippen et al., 1982
-Premature	EDTA	volume	N	N	Kippen et al., 1982
Pregnancy	EDTA	volume	N	N	Giles, 1981
-pre-eclampsia with edema and proteinuria	EDTA	volume	D	I	Giles, 1981
SLE	?	?	D	I	Chatterii, 1971
SLE	EDTA	diameter	D	I	Garg et al., 1971
SLE -megakaryocyte compensated	EDTA	diameter	V	I	Garg et al., 1971

5. Alcoholic thrombocytopenia: Six subjects admitted for thrombocytopenia and acute alcoholism were evaluated daily for platelet count and size. All subjects were free from chronic liver disease. At the time of admission, platelet counts ranged from 18-47x10^9/L and increased into a range of 88-212x10^9/L after 7 days of alcohol abstension. At the time of admission the mean platelet size was smaller than normal and increased into larger than normal range after 7 days of treatment, reflected by an increase in megathrombocytes of three to four times normal. It was proposed that the rise in mean platelet size was indicative of megakaryocyte recovery following alcohol induced suppression (Sahud, 1973).

6. Cardiopulmonary bypass: Prior to cardiopulmonary bypass, circulating platelets were found to have a normal mean platelet volume for the corresponding platelet count. Following 10 minutes of extracorpeal circulation the MPV decreased to 85% of the prebypass level, followed by a further decrease to 75% of the prebypass levels by 50 minutes of extracorpeal circulation. The intial fall in MPV remained for 24 to 48 hours at which time the MPV became elevated above pre-op values, although the platelet count showed only a small increase. Three to four days post-op the platelet count began to increase with a corresponding decrease in the MPV (Bailey et al., 1982; Laufer et al., 1975).

7. Hypersplenism: Patients with hypersplenism invariably have a decreased platelet count due to platelet sequestration. The MPV is also decreased, as larger sized platelets are preferentially sequestored by the spleen. Typical platelet distributions of hypersplenic patients are skewed to the left with a narrow distribution, indicating a circulating population of small platelets with little variation in size.

8. *Infection*: In subjects with infection, the platelet count and MPV show much variability. In a study of 278 subjects with overt infection, 34.1% had increased platelet counts as opposed to 6.5% who were thrombocytopenic. Within this group the MPV was variable with a majority having normal size with respect to platelet count (Giles, 1981).

Viral and bacterial infections both have been implicated to cause thrombocytopenia. Elevations in MPV have been reported in approximately 50% of subjects with septicemia, whose platelet counts were normal to decreased. No relationship was made between infecting microorganism and the increase in MPV. The elevated MPV was present at the time of the first positive blood culture and returned to normal after one week of appropriate antibiotic treatment (van der Lelie, 1982).

9. Newborn infants: Premature and term infants with normal platelet counts were found to have a MPV within reference ranges for adults. Thrombocytopenic premature or term infants were found to have an increased MPV for their corresponding platelet counts. These infants proved to have bacterial sepsis, enterocolitis, or disseminated intravascular coagulation. Whether the increase in the MPV was a characteristic of these disorders or the increase in MPV was an indication of increased platelet production could not be stated with certainty. It was proposed that the elevated MPV was an indicator of a compensatory action of the bone marrow megakaryocytes to increase production of circulating platelets (Kippen et al., 1982).

10. Systemic lupus erythematosus (SLE): In general, thrombocytopenia in conjunction with an increase in megathrombocytes is found in SLE. In subjects whose platelet counts were found to be in the normal range, an increase in megathrombocytes was present, leading investigators to propose that these subjects were in a compensatory thrombocytolytic state, which was varified by an increase in bone marrow megakaryocytes (Garg et al., 1971).

11. Miscellaneous clinical findings: A number of conditions have been studied for both variation of platelet count and size. Several of these findings can be considered coincidental and of little diagnostic value. Table 6 lists the results of several of these studies, together with their findings with respect to platelet count and size. Their precise diagnostic role, if any, remains to be identified.

Table 6. Unrelated conditions for which platelet size has been reported (A/C=anticoagulant; D=decreased; I=increased; N=normal; *=platelet count correlated to platelet volume).

Diagnosis	A/C	Method	Count	Size	Reference
Diabetes mellitus	EDTA	volume	*	N	Bessman et al., 1982
-with retinopathy	EDTA	diameter	?	I	Garg et al., 1972
Rheumatic heart disease					
-severe valve impairment	EDTA	diameter	?	I	Garg et al., 1972
-prosthetic heart valves	EDTA	diameter	?	I	Garg et al., 1972
Renal transplants					
-receiving azathioprine	EDTA	volume	*	N-I	Levin et al., 1983
-not receiving azathioprine	EDTA	volume	*	N	Levin et al., 1983
Rheumatoid arthritis	EDTA	volume	V	N	Giles, 1981
Schizophrenia	EDTA	volume	*	N	Bessman et al., 1982

F. PLATELET SIZE DISTRIBUTION ANALYSIS

1. Introduction: Platelets have been the last cellular component of the peripheral blood to benefit from automation. The microscopic counting method despite several modifications is tedious, lacking in precision, and is not adapted for the evaluation of a large number of samples.

In the early 1950's, electronic counters were developed to detect the small sizes of platelets. Early instrumentation was semi-automated with a leukocyte and erythrocyte free specimen requirement. Today, automated instrumentation is able to simultaneously count platelets, erythrocytes, and leukocytes from a whole blood specimen.

Until recently measurement of platelet volume was limited to research laboratories. Platelet sizing methods originated through the microscopic evaluation of blood smears prepared from either whole blood or platelet-rich plasma. These procedures were time consuming and subject to several inherent procedural errors. Many of the early manual microscopic methods have been replaced by multiparameter hematology analyzers.

Normal values for platelet size vary from one method to another. Variations in normals not only result from the method used for platelet sizing, but also by specimen handling prior to analysis. The anticoagulant, storage temperature, and time interval between specimen acquisition and analysis have all proven to play a critical role in the size of the platelet population being studied.

A detailed review of all the various methods for platelet sizing is beyond the scope of this review. This study used EDTA anticoagulated specimens which were analyzed by the Coulter Model S-plus IV, an aperture-impedance method for counting and sizing platelets. This methodology for sizing and counting platelets, together with the effect of EDTA on platelets will be reviewed in detail, preceded by a brief review of several alternative methods for sizing a population of platelets.

2. Manual platelet sizing methods: Early methods for platelet sizing ranged from rather crude determinations of platelet size by comparison of platelets to their surrounding erythrocytes (Estes, 1968), to the tedious measurement of large numbers of platelets using an ocular micrometer. Relatively simple procedures called for the counting of platelets above a certain diameter size, for example 2.5 or 3.0 microns, and reporting their percentage of the total population (Cortelazzo, 1980; Garg, 1971; Zeigler et al., 1978). These early procedures lead to more detailed sizing of the overall platelet population, the classification of platelets into size groups of 0.5-1.0 microns, 1.0-1.5 microns, 1.5-2.0 microns and so fourth (Holme et al., 1981A). In an effort to simplify the classification of platelets into size groups, a procedure was developed where platelet size was compared to beads which were previously spread over the blood smear and allowed to air dry. The beads were of two sizes, 2.0 microns and 3.5 microns, and provided a rapid method for sizing platelets into three groups (Rivard et al., 1975).

All of these methods have several characteristics in common. They are time consuming, tedious, and lacking in desirable accuracy and precision. Results from one study to another can be compared qualitatively but not quantitatively, since data interpretation from one study to another was based on different sizing criteria.

3. Automated platelet sizing methods: Since the early 1960's semi-automated and fully automated analyzers have been available for platelet counting. Automated platelet counting and sizing provides a better determination of normal platelet values and variations from normal. This is particularly evident in the comparison of the actual number of platelets counted when the platelet count is low. It was estimated that when the platelet count is below $20x10^9/L$, the Coulter S-plus counts about 1,200 platelets as compared to manual methods which count approximately 40 platelets (Dalton et al., 1980).

Many of the early semi-automated counters required an erythrocyte free specimen prior to analysis. Several studies have been published reporting the differences in platelet populations found in whole blood versus platelet-rich plasma. Whole blood platelet counts were found to be 5% higher when counted on the Coulter S-plus, than corresponding counts using platelet-rich plasma, counted by phase microscopy (Bessman, 1980). In contrast to these results, no difference between whole blood and platelet-rich plasma platelet counts or MPV were found for patients with a platelet count range of $5\text{-}1{,}000x10^9/L$ (Levin et al., 1983). In a study of platelet size and distribution, no difference was found between platelet-rich plasma or the platelet-rich plasma interface, when Wright stained smears were examined (Roper et al., 1977). In a subsequent study, the MPV was found to have a correlation of variation of 0.95 when comparing whole blood to platelet-rich plasma MPV samples (Bessman, 1980). Although the loss of platelets during the preparation of platelet-rich plasma

has not been proven conclusively, the use of whole blood in the latest generation of automated instrumentation avoids excessive specimen handling which may result in either falsely elevated or decreased platelet counts and size.

The vast majority of information present in the literature with respect to platelet size has originated from studies using aperture-impedance instrumentation, primarily those instruments marketed by Coulter Diagnostics, Incorporated. Recently the Technicon Corporation has marketed a fully automated hematology analyzer, the Technicon H-1, which can perform a standard eight-parameter complete blood count in addition to determining the MPV from a platelet distribution histogram. The Technicon H-1 uses a lazer light source to optically detect and size individual platelets based on the amount of light scattering resulting from passage of a fine stream of cells through the lazer beam. Three other instruments, the Sysmex CC-800 marketed by TOA Medical Electronics Incorporated, the MK-4/Haema Count marketed by the Baker Intrumentation Corporation, and the ELT-8000 marketed by the Ortho Diagnostics Incoporation are also available for routine evaluation of platelet size and count. These instruments count and size platelets either by light-scattering or aperture-impedance, and are relatively new on the market with little reference material available judging their performance.

Over the past decade the vast majority of studies investigating the variations in platelet volumes as found in various conditions of health have been accomplished with the use of aperture-impedance instrumentation. Aperture-impedance counters are marketed in a variety of configurations depending upon the amount of automation of sample processing, and data reduction desired; however, the essential structure of the aperture-impedance method is common to all instruments using this methodology for sizing and counting of

platelets.

In general, particles such as blood cells are suspended in an electrically conducting medium which is forced to flow through a small aperture that has an immersed electrode on either side. When a particle passes through the aperture it changes the resistance between the electrodes, producing a current pulse of short duration. The current pulses are amplified and enters a threshold circuit whose level is adjustable. If the pulse equals or exceeds the set level, it is counted. By setting a lower threshold, electrical noise can be edited and use of an upper threshold eliminates the counting of cells larger than the desired population size.

The height of the electrical pulse is not directly proportional to the volume of electrolyte displaced in the aperture by the particle, but rather is produced by the passage of the particle through the aperture, resulting in both a displacement of conducting liquid and a disturbance of electrical lines within and around the aperture orifice. The path the particle takes through the aperture also has an effect on the pulse height. The density of the current is significantly higher at the peripheral edges of the entrance and exit of the aperture, than in the center. In addition, the velocity of the electrolyte stream is higher in the center of the aperture than it is at the periphery. Particles approaching the aperture obliquely will travel close to the wall of the aperture and will be subjected to higher shear forces found in the peripheral flow path. These particles move more slowly than those passing through the center of the aperture, traversing zones of higher current density and may undergo shape-distortions as a result of the higher shear forces found near the aperture periphery. Compounding the problem are particles which become trapped at the exit side of the aperture, re-entering the sensing zone to produce a low amplitude pulse that is of an extended duration.

These limitations for size measurement can be overcome by the application of one or more modifications to the counting sytem. The first and most effective method is the use of a finely focused stream of particle suspension (Mundschenk et al., 1976). A steady flow of diluent is pulled through the aperture with the particle suspension injected into the flow of diluent close to the aperture entrance. Proper focusing of the flow of diluent through the central region of the aperture results in the elimination of particles approaching the zones of high current near the periphery of the aperture. Development of commercial instrumentation using this system has met practical difficulties though. In lieu of a practical method for focusing a stream of particles to be counted, early commercial instruments adopted a process of editing. As the particles pass through the aperture, pulses resulting in passage through the central portion of the aperture are counted and processed. Particles passing through the non-central regions of the aperture, producing abnormal pluses, are selectively edited from those counted and sized.

A second limitation for correctly sizing particles in an aperture-impedance system is imposed by the shape of the particle. The electrical lines of force in a current field around a rigid sphere have a fusiform shape. In the case of a rigid sphere this electrical "shadow" in the field produces a pulse nearly 1.5 times the actual volume of the sphere. Particle shapes other then rigid spheres create electrical "shadows" which vary in size due to shape and orientation of the particle as it passes through the aperture.

Two methods can be employed to correct for the electrical "shadow" produced by a particle as it passes through the aperture. First and most difficult would be to calculate a correction factor for any non-spherical particles, correcting for "shadow" error added to the true particle volume. This would be virtually impossible since the orientation of the particle

may change as it traverses the aperture, resulting in various electrical "shadows" being produced. A second method for determining true volume would be to convert the individual particles to spheroidal forms whose measured volume is known to be very close to 1.5 times their true volume (Mundschenk et al., 1976). In the case of platelets, whole blood anticoagulated with EDTA results in the conversion of normally discoid shaped platelets into spherical forms. The affect of EDTA on platelets is reviewed in detail below.

The Coulter Model S-plus IV Hematology Analyzer (S-plus IV) was used throughout this study for specimen analyses. The methodology utilized by this instrument for cell counting and sizing is based on the aperture-impedance methodology previously reviewed, with several refinements for accurate and precise counting and sizing of blood cells.

The S-plus IV flushes fluid behind the aperture to prevent recirculation of counted platelets or erythrocytes. Individual pulses arising from non-central movement through the aperture are eliminated by a pulse editor. Platelets and erythrocytes are simultaneously counted. Pulses in the range of 2 to 20 femtoliters (fl) are counted as platelets. If the number of pulses counted is below a set number for the initial counting cycle, the counting period is extended for not more than four additional (4 second) periods in order to assure the platelet population is accurately reflected by the platelet count and size-distribution histogram. The individual pulses are sorted and stored in a 64-channel size-distribution histogram. A log-normal curve is then fitted to the raw data histogram by the least square method. The platelet count reported is the area under the curve, extrapolated to 70 fl. To prevent erroneous results, several checks have been built into the system. Each sample is counted by three different apertures, of which two have to be within a set statistical range of each other prior to the analyzer accepting the sample data. In addition, the platelet size-distribution

histogram must have a mode between 3 and 15 fl, a minimum point at both the ascending and decending end of each curve, the platelet distribution width must be less than 20, and the log-normal curve must be positive. Providing the above criteria are met and the platelet count is above $20x10^9$/L, the platelet data is reported. If any of the above criteria are not met, platelet data can be retrieved from the instrument manually, but the platelet count, and the MPV will be representative only of those particles within the raw data size-distribution curve. The MPV is derived from the total number of platelets within the size-distribution histogram, whether one or more cycles are counted. The MPV is calculated from the sum of the individual platelet volumes divided by the total number of platelets counted.

It is of extreme importance that the platelet histogram be closely monitored for distribution abnormalities when the platelet count is below $20x10^9$/L. Invasion into the platelet channels by microspherocytes, or leukocyte fragments, or giant platelets can be identified by failure of the histogram to return to baseline at or near the 20 fl point. Identifying the return of the histogram to baseline also assures a majority of the platelets have been counted. If the histogram tails up at the 20 fl point, the platelet count should be correlated with either a manual platelet count, or examination of a peripheral blood smear for the identification of the interfering population. Subjects with hemolytic hereditary elliptocytosis, severe iron deficiency, or subjects receiving chemotherapy with circulating leukocyte fragments, have all been found to have falsely high platelet counts (Bessman et al., 1982; Cornblee. et al., 1985; Gilmer et al., 1982; Savage et al., 1985).

4. Effects of sample handling on the platelet count and size: Sample handling plays an essential role in the accuracy of the platelet count and size. Platelets normally circulate in the peripheral circulation in a discoid form. Several factors can result in their transformation

from disc forms to spiny spheres. Platelet sphering can result from normal activation of platelets due to traumatic collection or from exposure to certain temperatures or anticoagulants.

Sphering of platelets is known to result from contact with tissue fluid or delay of adequate mixing with anticoagulant immediately following venipuncture. In order to compare results of the MPV from day to day, one of two criteria must be assured. Either care has to be taken to guarantee specimens are collected in such a manner as to assure their native discoid shape is maintained or following collection the platelets which have been activated to spheroidal forms must either be returned to their native discoid shape, or all the platelets present must be transformed to spheroidal forms.

The effect of syringe drawn specimens compared to specimens drawn directly into vacutainers is a prime example of the effect of platelet activation on MPV studies. Whole blood specimens drawn by syringe and injected into tubes containing the anticoagulant acid citrate dextrose (ACD) and maintained at 37 degrees Celcius resulted in an MPV 15% greater than specimens collected directly into vacutainers contining ACD, when analyzed within 30 minutes of collection. The difference could be reduced to 10% if the syringe drawn specimens were incubated for 2 hours at 37 degrees Celcius prior to analysis. Syringe drawn specimens transferred to EDTA-containing vacutainer tubes resulted in a 31% increase in MPV over specimens collected in citrate containing vacutainer tubes when analysed 30 minutes post collection.

The increase in MPV in syringe drawn specimens either resulted from platelet contact with the syringe barrel, resulting in activation, or due to delayed exposure to the anticoagulant (Threattle et al., 1984).

There are three specimen handling protocols which require standardization in order to evaluate patient results on a daily basis. The type of anticoagulant to be used, the storage temperature, and the time between specimen acquisition and analysis, must all be established in order to compare data on a daily basis. Two studies have been published relating these properties to platelet size analysis. The first study evaluated four anticoagulants; 15% trisodium-EDTA (EDTA), 0.105 molar buffered citrate solution (BC), acid citrate dextrose solution (ACD), and 0.01 molar pyridoxal-5'-phosphate in 0.1 molar sodium citrate (PPC), maintained at 37 degrees Celcius. Specimens were analyzed immediately following venipuncture and after intervals of 0.5, 1, 2, 3, or 6 hours. Due to the specimen acquisition time, an average time lapse of 7.5 minutes passed between specimen acquisition and initial analysis. Determinations of the MPV immediately following specimen acquisition in vacutainers containing BC, ACD, or PPC showed no significant differences. Following 3 hours of incubation at 37 degrees Celcius these same specimens showed increases from the initial MPV determinations of less than 3%. In addition, ACD and PPC specimens incubated at room temperature showed an 18-20% increase in MPV over those stored at 37 degrees Celcius. If these specimens were allowed to incubate at 37 degrees Celcius for 30 minutes, the increase in MPV dropped to 3-5%.

The most dramatic changes occured in specimens exposed to EDTA. Specimens collected in EDTA and stored at room temperature showed a 23% increase in MPV over specimens collected in BC at the immediate analysis. When compared to BC specimens at the 0.5, 1, 2, and 3 hours, EDTA specimens were 35%, 42%, 46%, and 50% greater, respectively. EDTA specimens maintained at 37 degrees Celcius showed similar increases in the MPV (Threattle et al., 1984).

A second comprehensive study was performed on specimens anticoagulated by Na_2EDTA, K_3EDTA, Heparin, NaCitrate, ACD(15%), ACD(11.9%), and ACD/NA_2EDTA, analyzed immediately post collection and after 2,4,6, and 8, hours of incubation at room temperature. All specimens anticoagulated by ACD produced MPV's consistantly lower than the MPV's measured in either Na_2EDTA or K_3EDTA anticoagulated specimens. The anticoagulants, heparin and NaCitrate produced intermediate MPV's.

When evaluated over time, both the ACD(15%) and ACD/Na_2EDTA showed variations in the MPV from 99.1-100% and 99.5-100.3% from the mean value, respectively. For the other 5 anticoagulants, high variations over the first two hours were noted. In the Na_2EDTA, K_3EDTA and 11.9% ACD specimens, the initial increase in MPV over the first two hours was followed by a stable value for the remainder of the test period. Heparin and Na Citrate specimens showed erratic values, with the MPV decreasing during the late hours of analysis.

Platelet counts were simultaneously determined. All anticoagulants, with the exception of heparin, demonstrated platelet counts within instrument limitations. During the middle hours , the platelet count from the heparinized specimen fell, followed by an increase in the late hours. The NaCitrate specimens from some subjects showed decreasing platelet counts with a 6.5% measurement variation.

All specimens were analysed for leukocyte count, erythrocyte count and mean corpuscular volume, in conjunction with platelet anaiyses. Na_2EDTA and the ACD anticoagulants all produced similar WBC aand RBC counts over time. NaCitrate was less accurate in measuring both WBC and RBC counts. The MCV value for all anticoagulants with

the exception of NaCitrate showed less than a 1% variation over time, but the Na_2EDTA, heparin, and NaCitrate anticoagulated specimen showed a consistently lower MCV than the ACD anticoagulated specimens.

Both Na_2EDTA and K_3EDTA anticoagulated specimens showed an increase in MPV over the first two hours, with the increase due to K_3EDTA being greater than the Na_2EDTA. Presumably this is due to the fact that potassium is an intracellular cation whereas sodium is an extracellular cation. In studies comparing potassium to sodium effect on platelet swelling, potassium resulted in a 3.3% greater increase in MPV (Thompson, 1983C).

Electron microscopy studies have revealed that platelets maintain their discoid form in whole blood anticoagulated by citrate if incubated at 37 degrees Celcius. If the sample is chilled to either room temperature or lower, platelets lose their discoid form and develop into irregular spheroidal forms as a result of a disorganization or disappearance of the marginal band of microtubules. Incubation at 37 degrees Celcius results in the reorganization of the marginal band of microtubules and return to discoid forms. Following incubation at 37 degrees Celcius, reversal has resulted from platelets stored at 0 degrees Celcius for up to 5 hours (Salzman et al., 1969).

The consequence of exposing platelets to the anticoagulant EDTA is well documented. The exact amount of MPV increase over time is disputed, but a general consensus agree that a majority of the shape changes take place within the first half hour and within 2 hours there is a leveling off in MPV, which last through 6 hours post venesection at a minimum.

It is well known that the anticoagulant effect of EDTA results from the irreversible

binding of calcium, blocking the coagulation cascade at several points, but exactly how its presence results in the increase in the MPV, transforming platelets from discoid forms to spiny spheroidal forms, is not totally known.

When platelets are subjected to EDTA anticoagulant, they rapidly lose their discoid form by transformation to irregularly swollen spheres with multiple pseudopodia formation (Zucker & Borelli,1964). During the initial 15 minutes of EDTA exposure a vast majority of platelets undergo extensive structural transformations. The smooth, contoured lentinform platelets are transformed to irregular forms. Thin pseudopods consisting primarily of microfilaments, rarely microtubules, protrude irregularly from the cell surface. Internally, many channels of the canalicular system swell and appear as large vacuoles or cavities on electron microscopy. Following 30 minutes of exposure virtually all platelets appear altered. The primary transformation is in the cell surface canalicular system. The tube-like channels of the canalicular system become extensively dilated with interspersed areas of narrow channels. The narrow channels often become elongated and extend in one direction for long distances, taking on a serpentine appearance, meandering through the hyaloplasm. Although these areas of narrow channels interspersed within the dilated channels gives the appearance of more than one canalicular system in platelets, the evidence is that the two channels are connected (White,1968B).

Although the canalicular system and cell surface are extensively transformed during the first 30 minutes of EDTA exposure, little effect to the hyaloplasm ground substance is noted. Granules are pushed aside by the swelling canalicular system, but are not moved towards the central area of the platelet. The marginal bundle of microtubules appears deeply placed under the surface of platelets which exhibit severe cell wall irregularities, but the vast

majority maintain their peripheral cellular location (White,1968B). Between 60-120 minutes of exposure, granules of the hyaloplasm begin to develop changes in appearance. They begin to break down, which corresponds to further dilation of the canalicular system. The extensively dilated channels appear to fuse with each other and with the swollen canalicular system membranes surrounding them, leaving areas of irregular appearing vacuoles or cavities, which often contain granular debris. In severely damaged platelets the circumferential microtubules appear disorganized(Firkin,1965; White,1968B).

These physical changes in the platelets appear to be due to the direct effect of EDTA on the membranous surface of the cell wall and canalicular system, as opposed to triggering contractile mechanisms found during normal platelet activation. Since EDTA does not enter living cells (Gretle. 1962), it is believed that the canalicular system acts as a passageway into the cell for EDTA and a passage out for granular debris (Behneke,1965 & 1967).

MATERIALS AND METHODS

A. PATIENT POPULATIONS:

1. Whole Blood MPV versus Platelet-rich Plasma MPV Study: Thirty peripheral blood specimens with known platelet counts of less than 50 x 10^9/L were chosen at random from specimens submitted to the Hematology Department for analysis.

2. Effect of EDTA on MPV: This study consisted of two populations of patients. The first group consisted of 30 peripheral blood specimens obtained in conjunction with the clinical evaluation of normal, healthy subjects undergoing study by the Cancer Detection Center at the University of Minnesota Hospital. The second group consisted of peripheral blood specimens from 30 bone marrow transplant candidates undergoing cytological and immunological reduction protocols with irradiation and chemotherapy prior to transplant.

3. Post Bone Marrow Transplant MPV Study: Thirty one bone marrow transplant recipients at the University of Minnesota Hospital were evaluated daily for a minimum of 60 days, providing they remained on an in-patient status and adequate specimens were available for analysis. The study population consisted of the following diagnostic populations: 9 acute lymphoblastic leukemias, 6 acute myelogenous leukemias, 8 chronic myelogenous leukemias, 5 aplastic anemias, 2 neuroblastomas, and 1 acid mucopolysaccharidosis (for more details of the populations see Table #7).

B. TECHNICAL METHODOLOGIES:

1. Specimen Acquisition and Processing:

a. Whole blood MPV versus platelet-rich plasma MPV: Specimens were collected by venipuncture, anticoagulated with Na_2EDTA, and stored at room temperature. All size determinations were performed between 2.5 to 4 hours post acquisition. Aliquots of whole blood were placed into 10 x 77 millimeter test tubes and allowed to sediment by gravity

Table 7. Bone marrow transplant study population (ALL=acute lymphoblastic leukemia; AML=acute myelogenous leukemia; CML=chronic myelogenous leukemia; AA=aplastic anemia; NRB=neuroblastoma; MPS=mucopolysaccharidosis).

SUBJECT	IDENTIFICATION #	SEX	AGE	DIAGNOSIS	DONER
ALL-1	15125091	M	47	ALL	allogous
ALL-2	15204409	M	5	ALL	allogous
ALL-3	13415049	M	12	ALL	autologous
ALL-4	15186143	M	45	ALL	allogous
ALL-5	15183603	M	32	ALL	allogous
ALL-6	15220223	F	11	ALL	allogous
ALL-7	15118815	M	13	ALL	allogous
ALL-8	15226691	F	18	ALL	allogous
ALL-9	15125240	F	37	ALL	autologous
AML-1	15151782	F	8	AML	allogous
AML-2	15083365	F	26	AML	allogous
AML-3	15168414	F	14	AML	allogous
AML-4	15125422	M	26	AML	allogous
AML-5	15046826	F	8	AML	allogous
AML-6	15175492	M	39	AML	allogous
CML-1	13655222	M	26	CML	allogous
CML-2	15196274	F	12	CML	allogous
CML-3	15098215	F	45	CML	allogous
CML-4	15180633	F	14	CML	allogous
CML-5	15134440	F	47	CML	allogous
CML-6	15083878	M	36	CML	allogous
CML-7	14988770	M	49	CML	allogous
CML-8	14611877	M	23	CML	allogous
AA-1	15209861	M	30	AA	allogous
AA-2	15140579	M	29	AA	allogous
AA-3	15161039	M	15	AA	allogous
AA-4	15158332	M	12	AA	allogous
AA-5	15183397	F	37	AA	allogous
NRB-1	15038955	M	3	NRB	autologous
NRB-2	15000021	F	2	NRB	autologous
MPS-1	15043716	M	5	MPS	allogous

for 20 minutes, producing a platelet-rich plasma (PRP). The PRP was removed from the erythrocytes by syringe and injected into the platelet counting dilution chamber of the S-plus IV. The PRP was then immediately diluted with isoton into a platelet count range of 225 to 300 x 10^9/L. The MPV of the PRP was determined in duplicate, followed by the MPV determination of the original whole blood specimen.

b. Effect of EDTA on MPV: Specimens obtained in conjunction with the Cancer Detection Center were collected by venipuncture using a Na_2EDTA containing vacutainer tube and were stored at room temperature. Whole blood MPV determinations were performed at 0.5, 1, 2, 3, 4, 5, and 6 hours post collection. The specimens obtained from the bone marrow transplant candidates were collected by syringe from a Hickman indwelling catheter, immediately transferred to a Na_2EDTA containing vacutainer tube, and stored at room temperature. MPV determinations were determined at 0.5, 1, 2, 3, 4, 5, and 6 hours post collection.

c. Post bone marrow transplant MPV study: Whole blood specimens were collected by syringe from a Hickman indwelling catheter, immediately transferred to a Na_2EDTA containing vacutainer tube, and stored at room temperature. Complete blood counts in addition to the platelet count, MPV, and platelet crit were determined between 2.5 to 5 hours post collection.

2. Specimen Analysis:

a. Coulter S-plus IV method: The S-plus IV counts and sizes platelets by the principle of aperture-impedance. Platelets and erythrocytes are counted simultaneously from the same dilution with pulses corresponding to a size range of 2 to 20 fl classified as platelets and pulses corresponding to a size range of 36 fl or greater being counted as erythrocytes. The cells are suspended in an isotonic, electrically conducting medium which is forced to flow through a small aperture that has an immersed electrode on either side. As the particle passes through the aperture it causes a change in resistance between the two electrodes, producing a current pulse of short duration. The individual pulses are sorted and stored in a 64-channel size-distribution platelet histogram. A log-normal curve is then fitted to the raw data histogram by the least square method. The platelet count reported is the area under the curve,

extrapolated over a size range of 0 to 70 fl. To prevent erroneous results, several checks have been built into the system. Each sample is counted in triplicate, of which at least two have to be within a set statistical range of each other prior to the analyzer accepting the sample data. In addition, the platelet size-distribution histogram must have a mode between 3 and 15 fl, a platelet distribution width less than 20, a minimum point at both the ascending and decending end of each curve, and the log-normal curve must be positive. Providing the above criteria are met and the platelet count is greater than 20 x 10^9/L, the platelet data are reported. If any of the above criteria are not met, platelet data can be retrieved from the instrument manually, but the platelet count and the MPV will be determined only from those particles within the raw data size-distribution curve. The MPV is calculated from the sum of the individual platelet volumes divided by the total number of platelets counted.

b. Coulter S-plus IV performance limitations: Table 8 is a review of the performance limitations of the S-plus IV.

Table 8. Operating range linearity limits for the Coulter S-plus IV.

Parameter	Linearity Range	Limits
WBC x 10^9/L	0 to 99.9	0.2 or 3.0% (whichever is greater)
PLT x 10^9/L	0-999	10 or 7% (whichever is greater)
MPV fl	5.0 to 20.0	5%

c. Statistical analyses: Standard statistical analyses were used to evaluate the results obtained. All statistical analyses (linear regression, coefficient of correlation, standard deviations, means, medians, ranges, ect.) were performed with the aid of the Apple Macintosh microcomputer, utilyzing the StatWorks program for statistical analyses.

RESULTS

A. Whole Blood MPV versus Platelet-rich Plasma MPV Study:

The accuracy of the Coulter Counter model S-plus IV to determine accurate mean platelet volumes (MPV) from whole blood specimens which have platelet counts below $50x10^9/L$ was found to correlate well with the MPV determinations from a corresponding platelet-rich plasma sample. Thirty specimens were analyzed, of which two were eliminated from statistical analyses due to failure of their platelet size-distribution histograms to return to baseline at the 20 fl point. The ascending curve near the 20 fl point was determined to represent the presence of a population of erythrocyte fragments by peripheral blood smear examination.

The platelet counts of the remaining 28 whole blood specimens ranged from $7 \times 10^9/L$ to $48 \times 10^9/L$. The median platelet count was $27 \times 10^9/L$ and the mean platelet count was $28 \times 10^9/L$.

Linear regression analysis comparing the MPV of the whole blood specimen to a corresponding platelet-rich plasma sample demonstrated random scatter on either side of the line of identity (figure 2). The coefficient of correlation was 0.95 for the whole blood versus the platelet-rich plasma mean platelet volume. With the exception of one data point, all were within two standard deviations of the line of identity.

Comparison of the whole blood group to the platelet-rich plasma group found both groups as a whole to have identical mean platelet volumes of 8.2 fl and standard deviations of 0.9 fl.

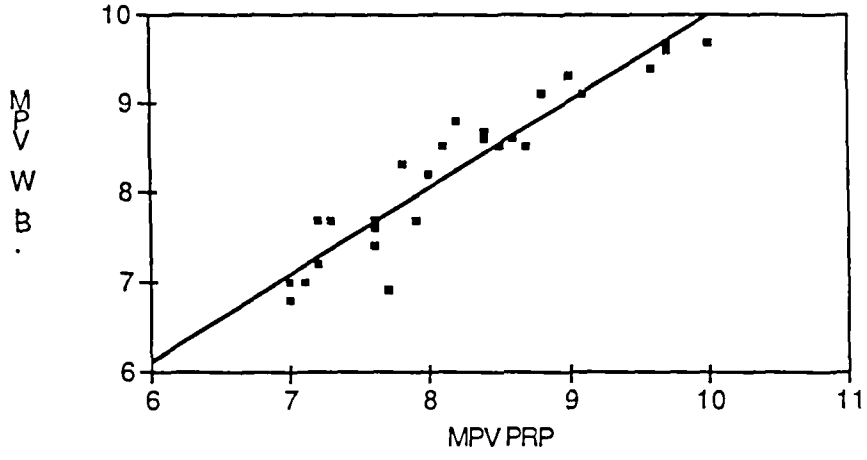

Figure 2. Plot of whole blood MPV versus the platelet-rich plasma MPV. The solid line is the line of identity.

B. Effect of Na_2EDTA on the MPV:

The anticoagulant disodium ethylenediamine tetraacetic acid (Na_2EDTA) was found to have a similar influence on the volume of platelets for both the normal, control population (Cancer Detection Center specimens), and subjects undergoing preparation for bone marrow transplantation. The platelet count varied between the normal, control population and the pre-bone marrow transplant populations (table 8). No correlation linking platelet count to the degree of increase in platelet volume following Na_2EDTA exposure was demonstratable.

The platelet volume from both populations demonstrated an increase in the platelet volume during the first 2 hours following Na_2EDTA exposure (Table 9). This initial increase in the platelet volume preceded a plateau period between 2 to 6 hours post Na_2EDTA exposure, during which time the platelet volume remained relatively stable (Figure 3). The median MPV during this plateau period for both populations was 9.0 fl. Due to the logistics of

Table 8. Platelet count statistics for the normal, control population and the pre-bone marrow transplant population (ALL=acute lymphoblastic leukemia, AML=acute myelogenous leukemia, CML=chronic myelogenous leukemia, AA=aplastic anemia).

Population	Range (x 10^9/L)	Median (x 10^9/L)	Mean (x 10^9/L)
Normal (n=30)	167-359	254	252
Pre-transplant	29-607	150	184
-ALL (n=9)	36-211	125	120
-AML (n=6)	48-198	142	129
-CML (n=8)	128-607	340	350
-AA (n=4)	29-86	41	49

Table 9. Effect of Na_2EDTA on the MPV over a time span of 6 hours. The unit of measurement for the MPV is femtoliters (fl).

	Normal, control population (n=30)			Pre-bone marrow transplant group (n=30)		
Exposure (hr)	Range	Median	Mean	Range	Median	Mean
0.5	7.2-11.5	8.8	8.8	6.1-9.7	8.4	8.2
1.0	7.5-11.9	9.0	9.0	6.5-10.9	8.8	8.7
2.0	7.4-12.2	9.0	9.2	6.6-11.5	9.0	8.8
3.0	7.6-12.4	9.0	9.2	6.6-11.2	8.9	8.8
4.0	7.5-12.4	9.0	9.3	6.5-10.7	9.0	8.8
5.0	7.5-11.9	9.0	9.2	6.7-11.1	9.0	8.8
6.0	7.5-12.0	9.0	9.2	6.5-10.8	9.0	8.8

specimen acquisition, analysis prior to 0.5 hours post Na_2EDTA exposure was not possible, eliminating an accurate assessment as to whether the original size of the platelets act as a determinant of the degree of platelet swelling following exposure to the anticoagulant Na_2EDTA.

Subclassification of the pre-bone marrow transplant group by diagnosis demonstrated variability in both the platelet count (Table 8), and the MPV following exposure to Na_2EDTA (Table 10). Figure 4 graphically demonstrates the median MPV for the acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and the aplastic anemia (AA) groups to be essentially the same, in contrast to the median MPV for the acute myelogenous leukemia (AML) group which was approximately 11% less than the above groups. The cause for the decrease in the median MPV for the AML group could not be correlated to either the platelet count, nor the pre-bone marrow transplant protocols for irradiation and chemotherapy, since the protocols for the ALL, AML, and CML are essentially the same. All of the groups demonstrated a relatively steady median MPV between 2 to 6 hours post Na_2EDTA exposure with minimal variation over time, probably the result of the low number of subjects studied in each diagnostic group.

The combined groups of the normal control population and the pre-bone marrow transplant subjects demonstrate that Na_2EDTA has a common effect on platelets resulting in an immediate increase in MPV which levels off approximately 2 hours post Na_2EDTA exposure, lasting a minimum of 6 hours post exposure. As a result of this plateau period, a 2.5 to 5.0 hour post Na_2EDTA exposure window for specimen analysis of individuals undergoing

bone marrow transplantation was established.

Table 10. Effect of Na_2EDTA on the MPV over a 6 hour time span for the pre-bone marrow diagnostic subgroups. The MPV is measured in fl.

	Acute lymphoblastic leukemia (n=9)			Acute myelogenous leukemia (n=6)		
Exposure (hr)	Range	Median	Mean	Range	Median	Mean
0.5	7.8-8.8	8.4	8.3	7.1-8.5	7.6	7.7
1.0	8.1-9.7	8.9	8.9	7.6-8.7	8.0	8.0
2.0	8.1-9.6	9.0	9.0	7.6-8.9	8.2	8.2
3.0	8.1-9.6	9.2	9.0	7.6-8.8	8.2	8.2
4.0	8.2-9.5	9.2	9.0	7.7-8.9	8.4	8.3
5.0	8.0-9.7	9.1	9.0	7.7-8.9	8.3	8.2
6.0	8.2-9.7	9.1	9.0	7.4-9.0	8.2	8.2
	Chronic myelogenous leukemia (n=8)			Aplastic anemia (n=4)		
Exposure (hr)	Range	Median	Mean	Range	Median	Mean
0.5	7.5-9.7	8.8	8.6	7.9-9.3	8.8	8.7
1.0	7.5-10.2	9.0	8.9	8.8-10.9	9.2	9.5
2.0	7.4-10.3	9.1	9.0	8.9-11.5	9.2	9.7
3.0	7.5-10.3	9.2	9.0	8.9-11.2	9.2	9.6
4.0	7.6-10.3	9.2	9.1	8.9-10.7	9.2	9.5
5.0	7.6-10.1	9.2	9.0	8.6-11.1	9.3	9.6
6.0	7.6-10.3	9.2	9.0	8.8-10.8	9.2	9.5

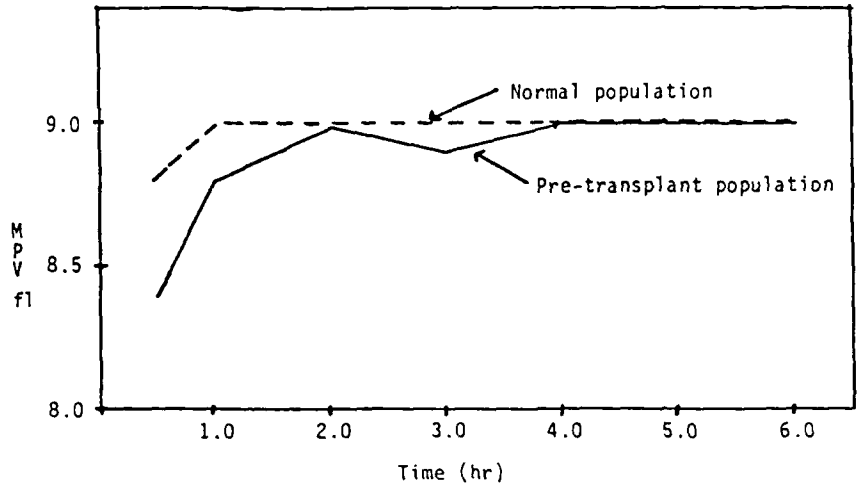

Figure 3. Effect of Na_2EDTA on the MPV over time for the normal, control population versus the pre-bone marrow transplant population.

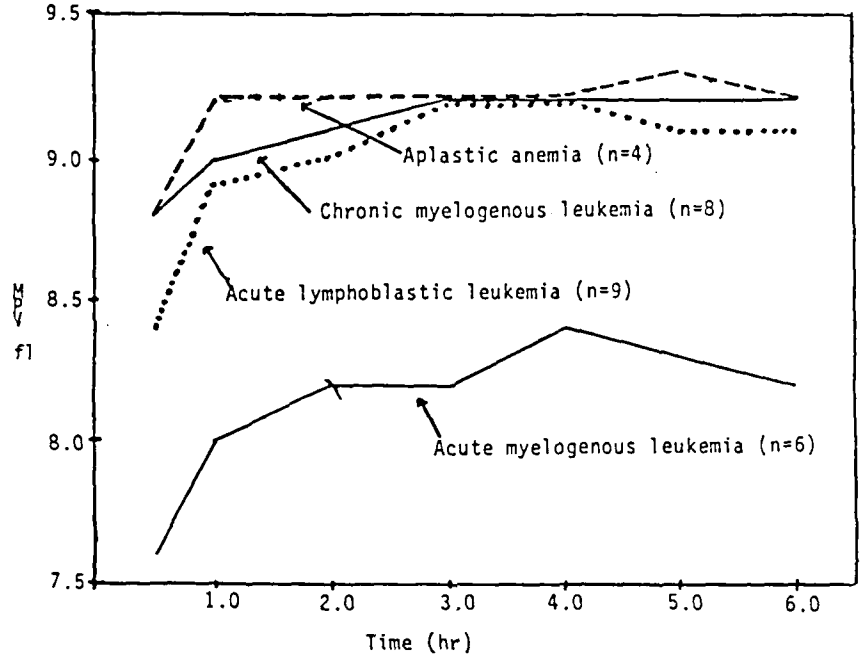

Figure 4. Effect of Na_2EDTA on the MPV over time for the pre-bone marrow transplant subgroups.

C. Post Bone Marrow Transplant Study:

Prior to a review of the results of the serial analyses of bone marrow transplant recipients, two patients diagnosed with acute myelogenous leukemia (AML) and two with acute lymphoblastic leukemia (ALL) will be reviewed to demonstrate both a sucessful bone marrow regeneration and a failure of the bone marrow to regenerate following induction chemotherapy. Subject LEU-1, diagnosed with AML clearly demonstrated a rise in the MPV prior to a rise in the platelet count (figure 5). The MPV began to rise on day 21, reached a peak on day 24, and then gradually decreased through day 30. The platelet count began to increase on day 24, corresponding to the peak in the MPV and continued to rise through day 30 with the most significant increases occurring between day 25 and day 28. The leukocyte count was found to increase at the same time as the MPV. Bone marrow biopsy on day 21 following therapy found the marrow to be moderately hypocellular with a slightly reduced number of megakaryocytes. In contrast to the above subject, Subject LEU-2, also diagnosed with AML, failed to respond to induction chemotherapy and following 43 days of treatment was discharged to his hometown hospital. Review of Figure 6 shows the platelet count and MPV to have several increases from one day to another. Correlation to platelet transfusions show the increases to correspond to transfusions. The leukocyte count shows minimal evidence of regeneration with the count remaining below 2.1×10^9/L. Bone marrow biopsy 28 days post therapy revealed a moderately hypercellular marrow with a markedly increased number of atypical megakaryocytes. Bone marrow biopsy 39 days following therapy demonstrated a moderately hypocellular marrow with a moderate increase in atypical megakaryocytes.

Subject LEU-3 diagnosed with ALL, went into remission, but the rise in the MPV

prior to an increase in the platelet count was not as evident as Subject LEU-1 above. On day 18 the MPV began to rise, reaching a peak on day 21 and then gradually decreased through day 26 (Figure 7). The platelet count began to increase on day 24 and continued to rise through day 27. Whether the rise in the MPV on day 18 through 22 was an indicator for a future increase in the platelet count or was the result of platelet transfusions on day 18 and19, can not be determined with absolute certainty, but the rise in the MPV does appear to be steady with a further increase in the MPV for 2 days following the last platelet transfusion. Bone marrow biopsy 28 days post therapy found the marrow to be moderately hypocellular with a normal to slightly decreased number of megakaryocytes. In contrast to Subject LEU-3, Subject LEU-4, also diagnosed with ALL, failed to enter remission following induction chemotherapy and expired on day 29. Review of Figure 8 clearly demonstrates the failure of the bone marrow to regenerate following induction chemotherapy. Peaks are present in both the platelet count and the MPV which correspond to platelet transfusions. The leukocyte count showed no evidence of recovery, failing to rise above $0.7x10^9$/L. Bone marrow biopsy 28 days post therapy found the marrow to be markedly hypocellular with a moderate decrease in the number of megakaryocytes.

These four subjects demonstrate both successful induction chemotherapy, with two of the subjects entering remission, and also the opposite, with two patients failing to recover from induction chemotherapy. Data from subject LEU-3 is not totally conclusive due to platelet transfusions occurring early in the rise of the MPV, but the transfusions occured prior to an increase in the platelet count. Overall the two subjects who went into remission can be used as a general model for comparison, when evaluating bone marrow transplant recipients.

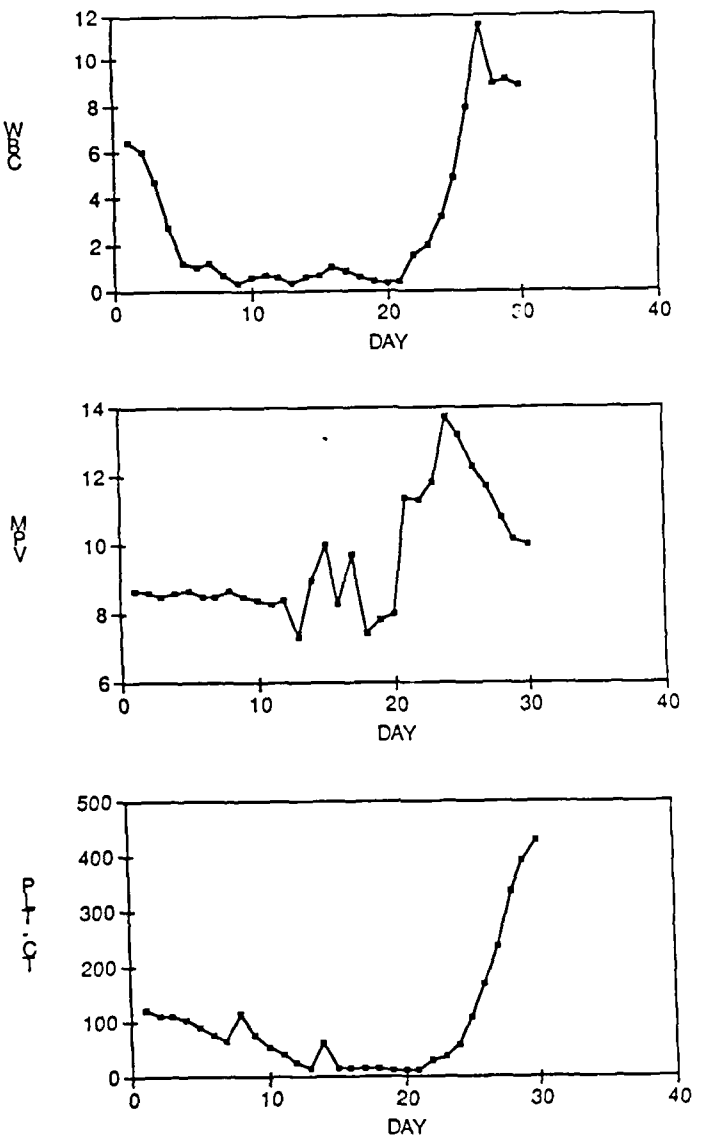

Figure 5. Subject LEU-1 serial results of the WBC,MPV,and platelet count during induction chemotherapy for AML.

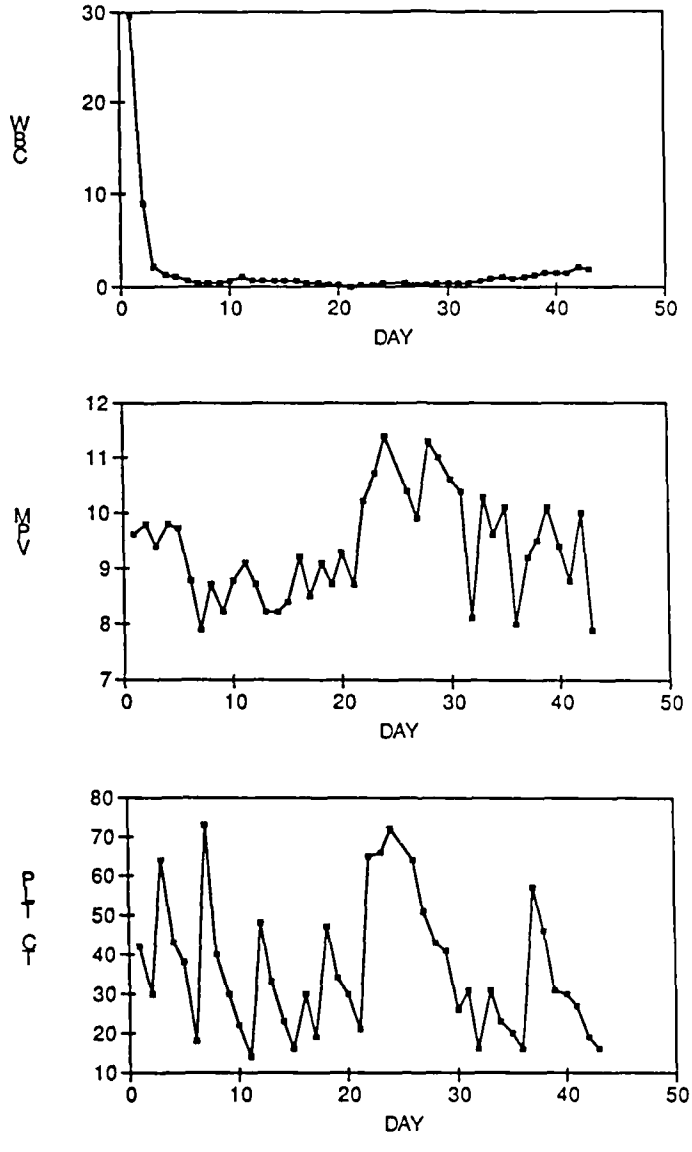

Figure 6. Subject LEU-2 serial results of the WBC, MPV, and platelet count during induction chemotherapy for AML.

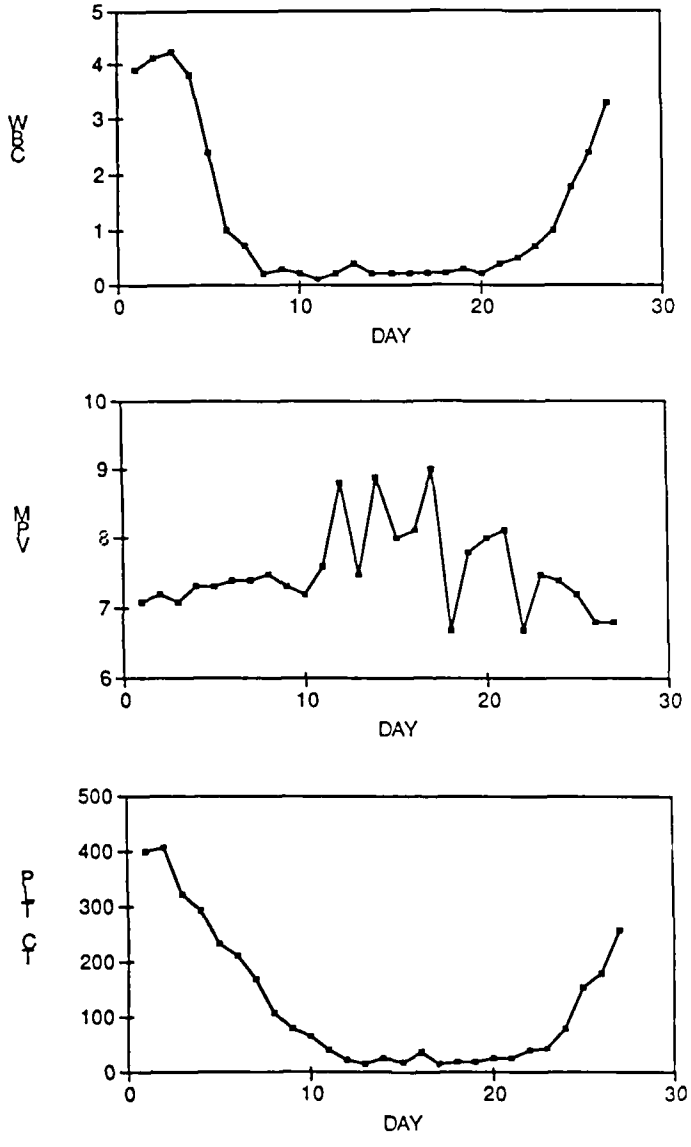

Figure 7. Subject LEU-3 serial results of the WBC,MPV, and platelet count during induction chemotherapy for ALL.

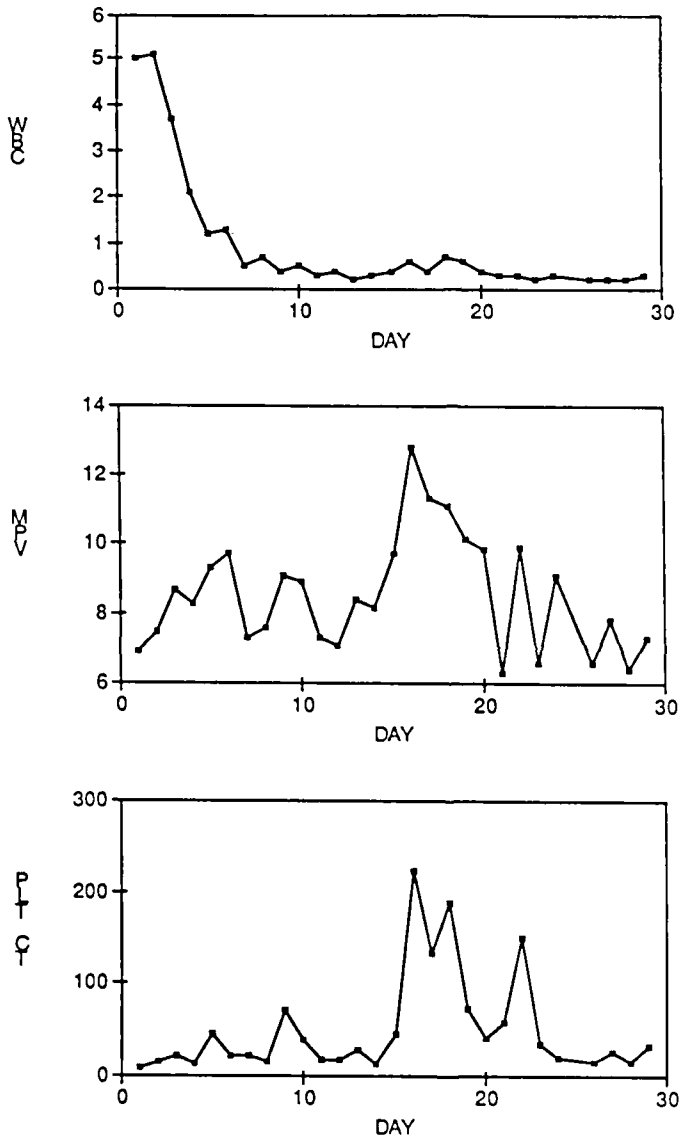

Figure 8. Subject LEU-4 serial results of the WBC, MPV, and platelet count during induction chemotherapy for ALL.

Hematology data collected from the bone marrow transplant study group is presented in Appendix 1. Graphic display of representative examples from each diagnostic subgroup is presented below. Three parameters, the white blood cell count, the MPV, and the platelet count are graphically displayed in the back of this section for the subjects reviewed. Caution is required when comparing one graph to another since there is deviation in the y-axis coordinates from one subject to another.

The platelet crit, the product of the platelet count times the MPV, divided by 10,000, will not be graphically displayed since it was found to add no additional useful information. Overlaying the platelet count with the platelet crit graphically showed them to shadow each other. Unlike the hematocrit which remains relatively constant over a range of normal erythrocyte volume and count, the platelet crit varies substantially within the normal range of the platelet count and volume. The non-linear, inverse relationship of the platelet count to the MPV is most pronounced at low platelet counts, resulting in a variation in the platelet crit as the platelet count rises, since the change in the MPV is not sufficient to result in a constant platelet mass over time.

Subject ALL-5 diagnosed as having acute lymphoblastic leukemia, received an allogeneic bone marrow transplant. Review of the daily white blood cell count shows a return of the circulating leukocytes approximately on day 13 post transplant (Figure 9). The MPV failed to show any indication of a future rise in the platelet count. The platelet count remained relatively stable while the MPV showed a greater amount of variation. Bone marrow biopsy evaluation on day 28 and 56 following transplant showed the marrow to be mildly to moderately hypocellular with a moderately reduced number of megakaryocytes.

Subject ALL-4 diagnosed as having acute lymphoblastic leukemia, also received an

allogeneic transplant. Review of Figure 10 shows the leukocyte count to rise on approximately day 12 post transplant. The platelet count failed to rise above $61x10^9$/L, remaining relatively stable from day 10 through 34 post transplant, while the MPV showed marked variation, with no indication of a future increase in the platelet count. Bone marrow biopsy evaluation on day 14 found the bone marrow to be moderately hypocellular with a moderately reduced number of megakaryocytes. The day 72 post transplant biopsy showed a variably cellular marrow which was normal to slightly hypocellular with a slightly reduced number of megakaryocytes.

Subject AML-4 diagnosed as having acute myelogenous leukemia, received an allogeneic transplant. The leukocyte count began to rise approximately 20 days post transplant, reaching a count slightly below normal reference limits (Figure 11). Close review of the platelet count and MPV graphics show a steady increase in the platelet count between days 39-48 which coincided with a steady decrease in the MPV which had peaked earlier on day 35. This pattern resembles the two leukemic subjects reviewed earlier, who went into remission following induction chemotherapy, although the changes in MPV and platelet count are spread out over a longer period of time. The extent of the increase in the platelet count is unknown since the patient was discharged, but the rate of increase appears to be substantial. Bone marrow biopsy 28 days post transplant demonstrated the marrow to be slightly to moderately hypocellular with a slight to moderately reduced number of megakaryocytes.

Subject AML-3 also received an allogeneic transplant for acute myelogenous leukemia. Similar to the previous case the leukocyte count began to rise approximately 20 days post transplant, peaking on day 31 prior to falling to a count of less than $1.0x10^9$/L on day 39, which then rebounded into normal reference limits (Figure 12). Correlation of a rise in

the platelet count to a preceding peak of the MPV was not evident, possibly being masked by the daily requirement of platelet transfusions throughout the study period for this subject, resulting in an eventual increase in the platelet count to above $100x10^9$/L. Day 42 and 100 post transplant bone marrow biopsies showed the marrow to be moderately to markedly hypocellular on day 42 and slightly hypocellular on day 100. The number of megakaryocytes appeared moderately reduced in number on both biopsies.

Subject CML-3 diagnosed with chronic myelogenous leukemia received an allogeneic transplant. This patient was the exception among this diagnostic group as her leukocyte count rose into the low side of normal reference limits prior to discharge (Figure 13). The leukocyte count began to rise on day 30 following transplant. No platelets were transfused during the 10 days prior to discharge during which time the platelet count remained stable at a count of approximately $50x10^9$/L. During this period the MPV remained stable, giving no indication of a future increase in the platelet count. Bone marrow biopsy on day 28 post transplant found the marrow to be moderately hypocellular with a moderate to markedly reduced number of megakaryocytes. Biopsy on day 100 post transplant revealed a slightly hypocellular marrow with a moderately reduced number of megakaryocytes.

Subject CML-1 is more representative of the chronic myelogenous leukemia group. Review of Figure 14 demonstrates the minimal regeneration of both the leukocyte and platelet populations. Bone marrow biopsy evaluation on day 28 post transplant found the marrow to be moderately to markedly hypocellular with a moderate reduction in the number of megakaryocytes present. Bone marrow biopsy 41 days post transplant showed a moderately hypocellular marrow with a slight to moderate reduction in the number of megakaryocytes.

Subject AA-3 diagnosed as having aplastic anemia received an allogeneic bone

marrow transplant. Similar to the other three patients with aplastic anemia who showed evidence of granulocyte regeneration, the leukocyte count began to rise between day 16-20 post transplant (Figure 15). Following the last platelet transfusion on day 19 the platelet count steadily fell to a low on day 34 which was followed by a steady increase through day 37. As the platelet count rose between day 34-37, the MPV steadily fell from a peak of 9.6 fl on day 34 to its smallest value on day 37 of 7.5 fl. The day 34 MPV peak was preceded by a steady increase in the MPV from a low of 7.9 fl on day 31. Additional data beyond day 31 post transplant is not available since the patient was discharged. Bone marrow biopsy 28 days post transplant found the marrow to be moderately hypocellular with a markedly reduced number of megakaryocytes.

Two patients diagnosed as having neuroblastoma received autologous transplants. Graphic display of the platelet count and MPV showed no indication for predicting a rise in the platelet count.

The study population included one patient, (MPS-1), diagnosed as having acid mucopolysaccharidosis, Sanfilippo's type, received an allogeneic bone marrow transplant. Review of Figure 16 is rather unremarkable, demonstrating the relative constancy of the platelet count and MPV over time. Between day 40-92 no platelets were transfused. During this period of time the platelet count remained below normal, ranging from 50 to $100x10^9/L$, while the MPV remained relatively constant, indicative of a steady marrow output of a relatively homogeneous sized population of platelets, giving no indication of a future increase in platelet output. Bone marrow biopsy 28 days following transplant revealed an essentially normocellular marrow with a moderately reduced number of megakaryocytes. Bone marrow biopsy on day 101 post transplant showed a moderately hypocellular marrow with a

moderately reduced number of megakaryocytes.

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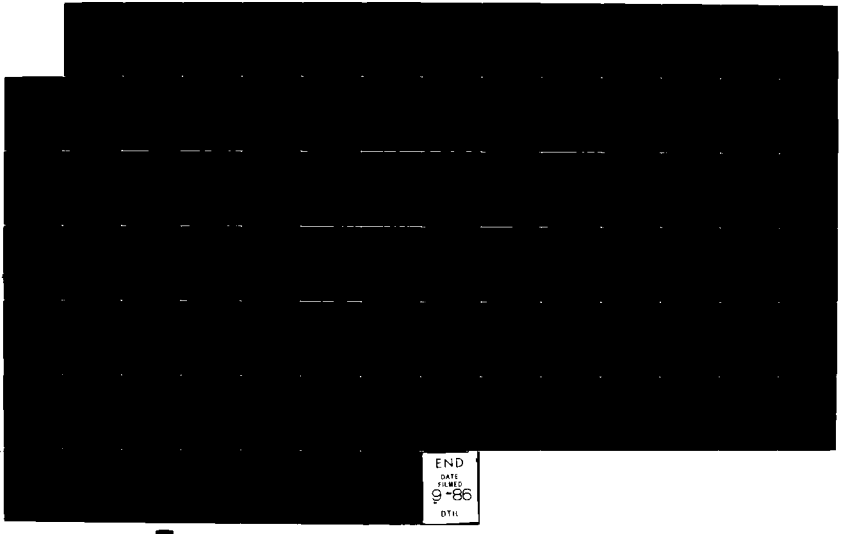

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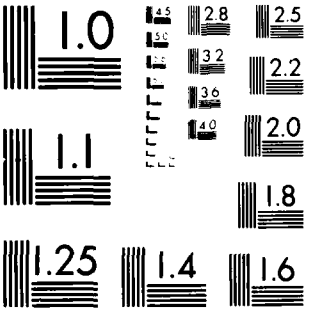

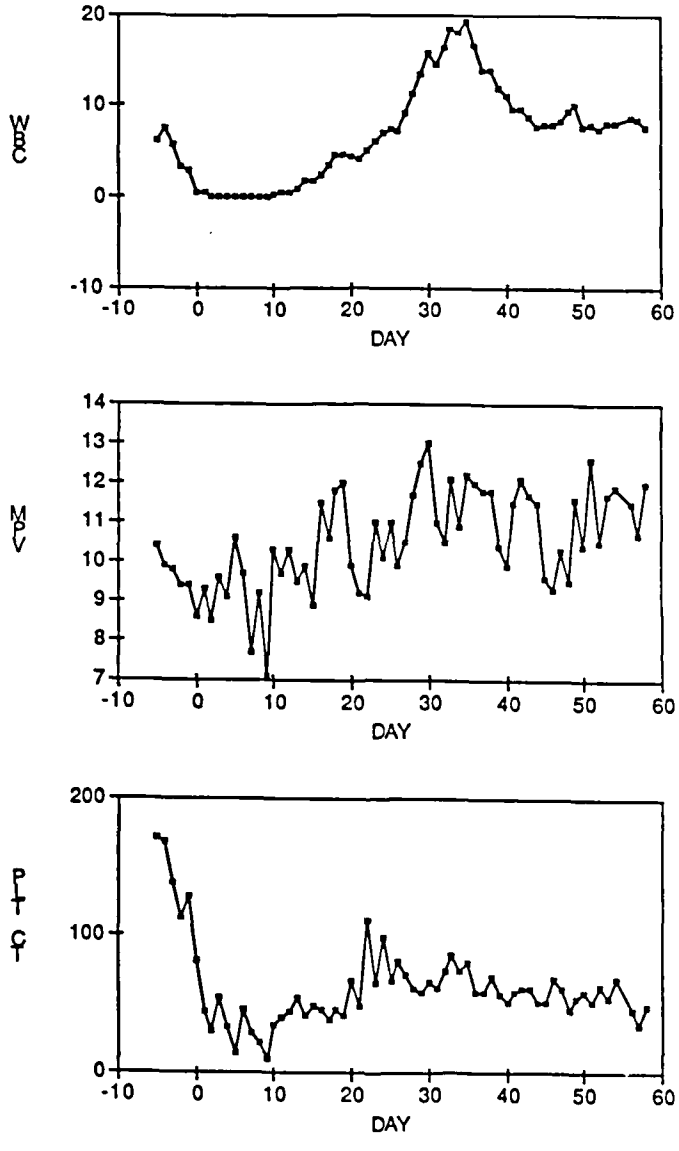

Figure 9. Subject ALL-5 serial results of the WBC, MPV, and platelet count following bone marrow transplant for acute lymphoblastic leukemia.

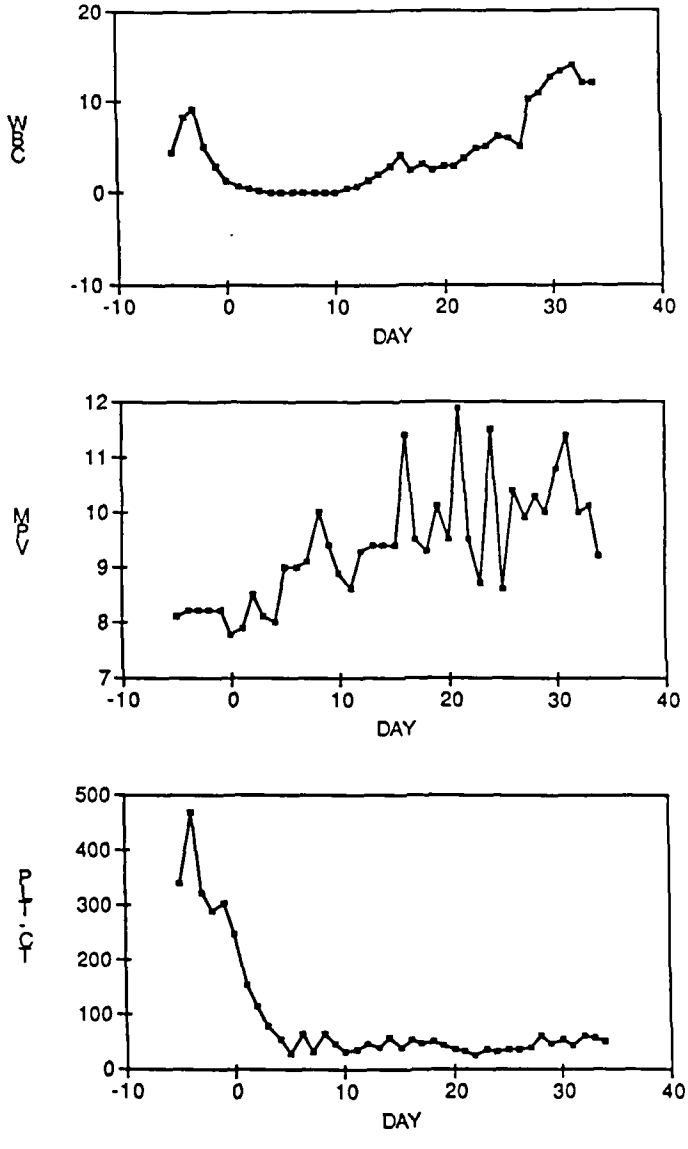

Figure 10. Subject ALL-4 serial results of the WBC, MPV, and platelet count following bone marrow transplant for acute lymphoblastic leukemia

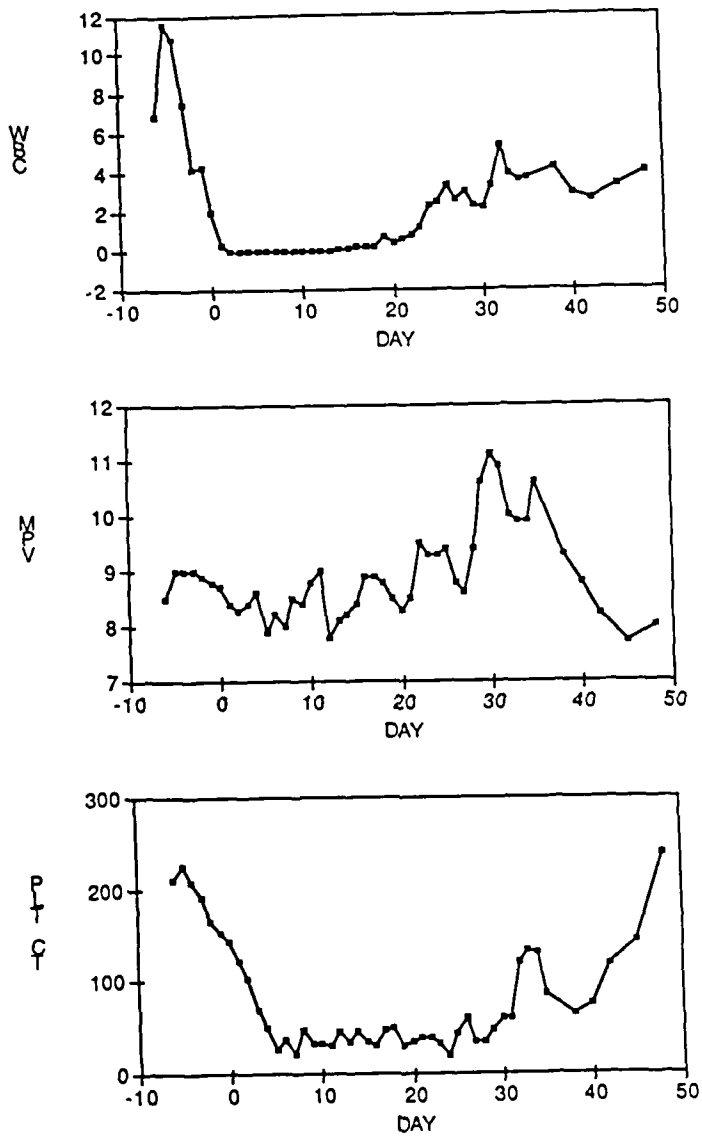

Figure 11. Subject AML-4 serial results of the WBC, MPV, and platelet count following bone marrow transplant for acute myelogenous leukemia.

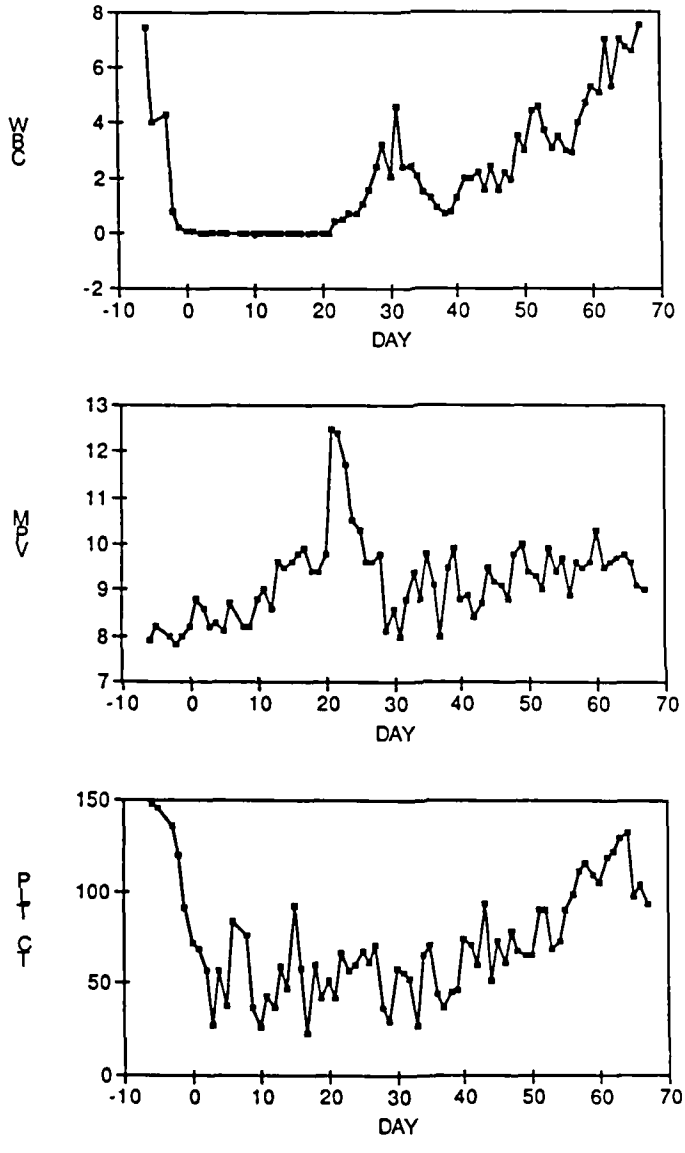

Figure 12. Subject AML-3 serial results of the WBC, MPV, and platelet count following bone marrow transplant for acute myelogenous leukemia.

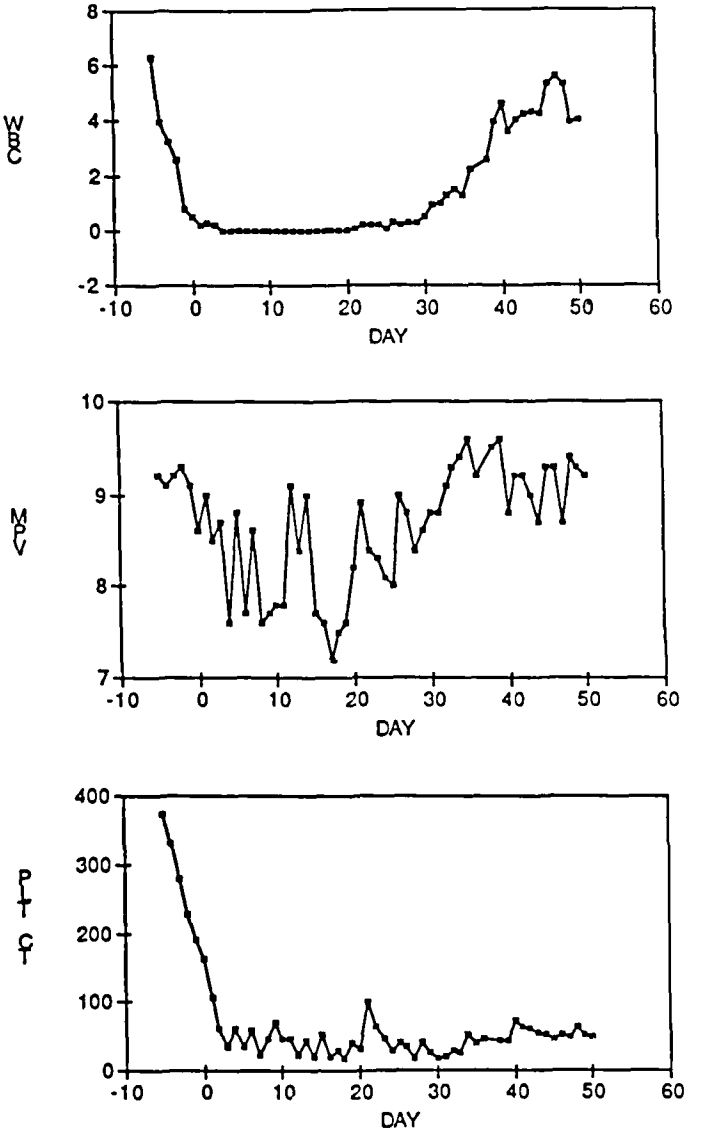

Figure 13. Subject CML-3 serial results of the WBC, MPV, and platelet count following bone marrow transplant for chronic myelogenous leukemia.

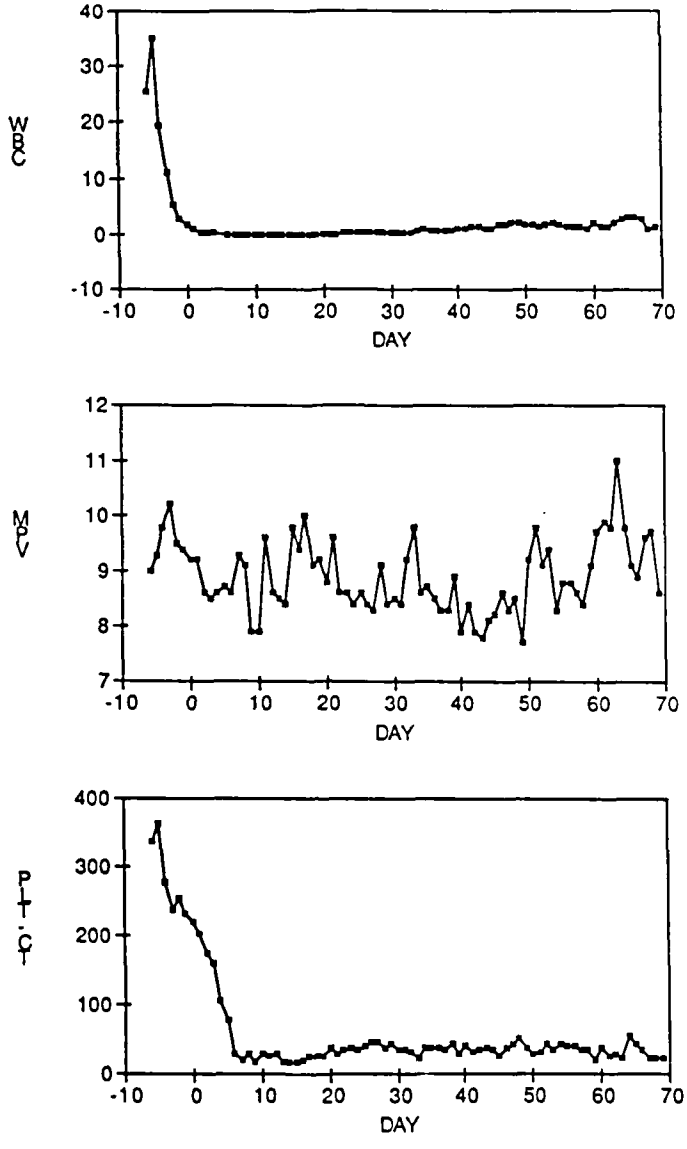

Figure 14. Subject CML-1 serial results of the WBC, MPV, and platelet count following bone marrow transplant for chronic myelogenous leukemia.

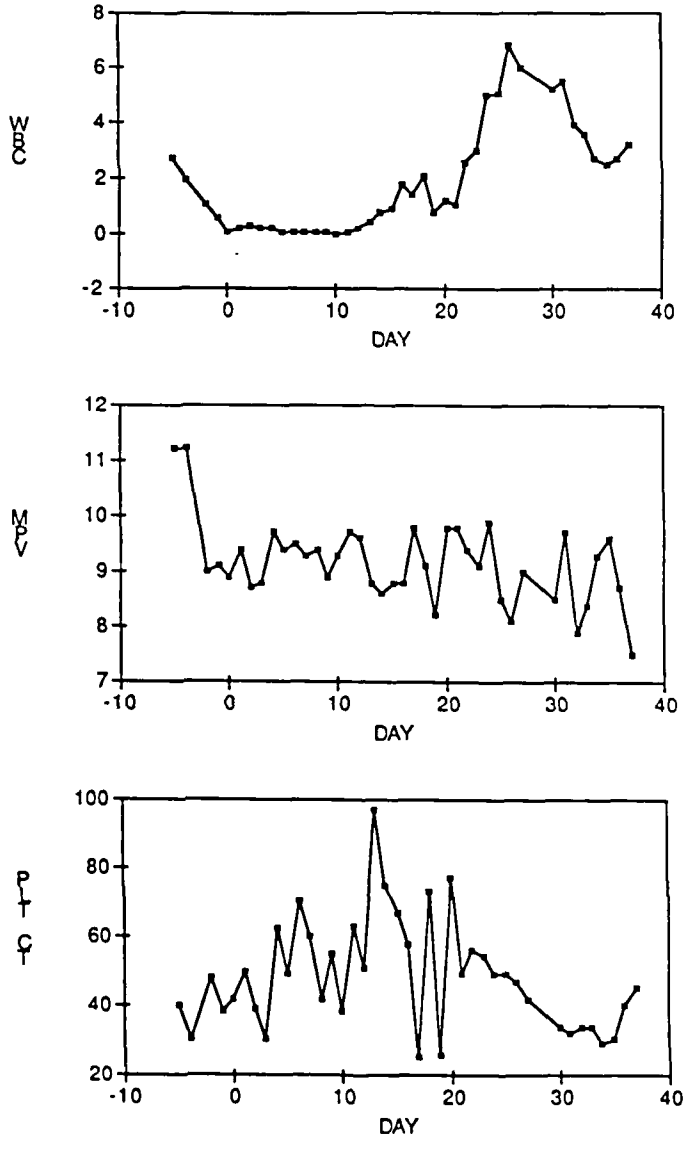

Figure 15. Subject AA-3 serial results of the WBC, MPV, and platelet count following bone marrow transplant for aplastic anemia.

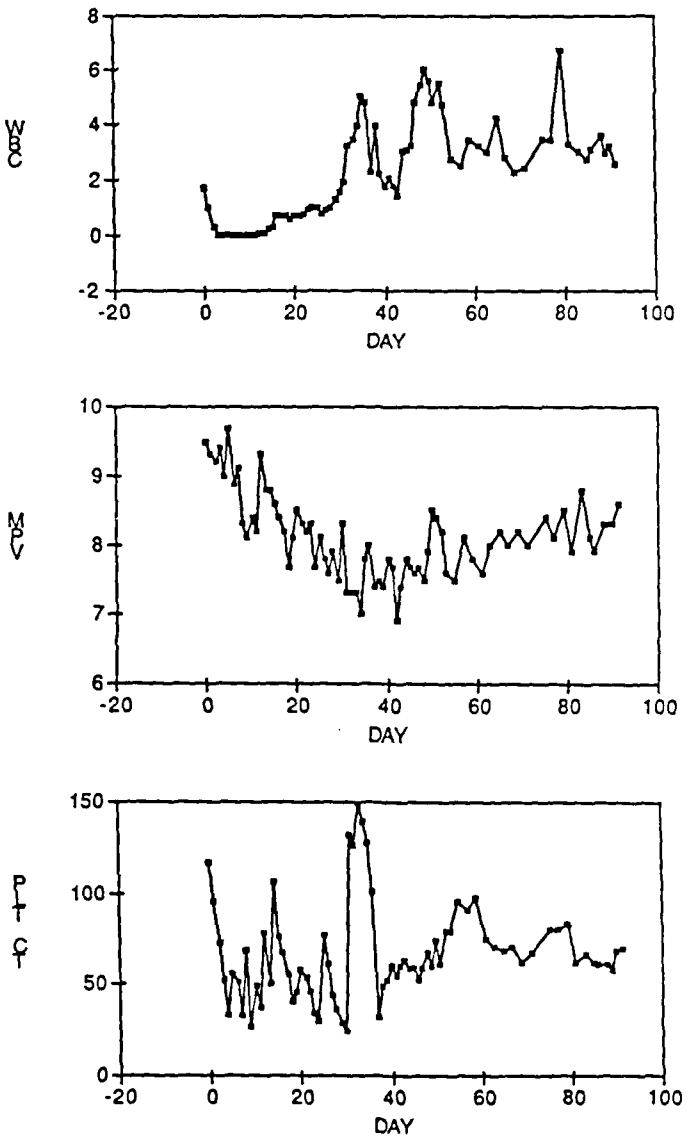

Figure 16. Subject MPS-1 serial results of the WBC, MPV, and platelet count following bone marrow transplant for acid mucopolysaccharidosis.

DISCUSSION

The Coulter Counter model S-plus IV (S-plus IV) proved to provide accurate mean platelet volume (MPV) determinations from thrombocytopenic specimens. Figure 2 (page 73) clearly demonstrates a random scatter of the data points comparing the whole blood MPV to the platelet-rich plasma MPV. Additional specimen handling during the preparation of the platelet-rich plasma showed no demonstratable effect on the volume of platelets when compared to the corresponding whole blood samples.

Review of the whole blood platelet histograms of the above subjects, found two of the thirty specimens to clearly demonstrate the presence of a non-homogeneous population of platelets. Both subjects showed a variation from the standard log-normal platelet distribution, with a skew or plateau towards larger sized platelets. This was indicated by an ascending curve as opposed to the normally descending curve at approximately 20 fl. The invading population was found to consist of erythrocyte fragments by peripheral blood smear evaluation. The finding of an invasion of the platelet histogram as depicted by an ascending curve at the 20 fl point demonstrates the critical importance of a visual inspection of the platelet histogram prior to acceptance of the platelet data from thrombocytopenic patients. Spherocytes, elliptocytes, erythrocyte fragments, and leukemic blast fragments have all been found to cause a failure of the distribution curve to return to baseline (Bessman et al., 1981; Savage et al., 1985). The presence of an abnormal platelet histogram curve is an indicator to the operator that an inherent error in both the platelet count and MPV may be present, warranting confirmation of results by an alternative manual method.

The effect of the anticoagulant disodium ethylenediamine tetraacetic acid

(Na_2EDTA) corresponded to previous reports in the literature (Levin et al., 1983; Roper et al., 1977; Salzman et al., 1969). The logistics of specimen acquisition prevented the determination of the MPV prior to 30 minutes post Na_2EDTA exposure, a time period during which a majority of the swelling occurs. Levin et al., (1983) reported the MPV to increase by 21 to 30% within 5 minutes of exposure to EDTA, followed by a further increase of 10 to 16% during the next 2 hours, and then remained constant for up to 16 hours. The majority of the increase occurred during the first 30 minutes post exposure, followed by a decreasing amount through 2 hours post EDTA exposure. The increase in the median MPV of the normal,control population of this study was 2.2% between 30 minutes and 2 hours compared to a slightly higher increase in the pre-bone marrow study group of 6.6%. The 4.4% difference between the two populations is not significant based on instrument limitations. In addition, the median MPV for both populations are within published normal MPV limits. Both groups showed no additional increase in MPV following 2 hours of Na_2EDTA exposure, a critical factor with respect to the present study. As a result of this finding a time window ranging from 2.5 to 5 hours post acquisition for specimen analysis was established during which comparable daily data could be collected.

The median platelet count for the normal, control population was 254 x 10^9/L with a range of 167 to 359 x 10^9/L. The median platelet count for the pre-bone marrow transplant population was moderately lower, being 150 x 10^9/L with a marked variation in the platelet count range of 29 to 607 x 10^9/L. Part of this variation correlates directly to the subjects diagnosis and an unknown factor resulting from the pre-bone marrow transplant protocols of irradiation and chemotherapy. As expected the median platelet counts for the aplastic anemia

population was the lowest (41 x 10^9/L) and the median platelet count for the chronic myelogenous leukemia group was the highest (340 x 10^9/L). The two acute leukemias, lymphoblastic and myelogenous, were both on the low side of normal. The influence of the irradiation and chemotherapy on the platelet count and size is not totally evident when evaluating the individual diagnostic groups. The cytological and immunological reduction protocols for five of the nine acute lymphoblastic leukemia subjects consisted of total body irradiation (TBI) followed by cyclophosphamide. The remaining four received cytosine arabinoside in place of cyclophosphamide. The acute and chronic myelogenous leukemia subjects all received cyclophosphamide followed by TBI. The aplastic anemia subjects received cyclophosphamide followed by total lymphoid irradiation (TLI) as opposed to TBI. The two neuroblastoma subjects received a regiment of TBI, followed by cis-platinum and etoposide (VP-16), followed by cyclophosphamide. The acid mucopolysaccharidosis subject received a regiment of myeloran and cyclophosphamide. At the time prior to the pre-transplant irradiation and chemotherapy the leukemia subjects were all in remission. During the course of the cytological and immunological reduction protocols the platelet count demonstrated a steady decrease, with either a steady or slightly decreasing mean platelet volume.

Animal studies have resulted in a proposed model for platelet recovery, which is tied to the MPV. Several animal studies have been performed (Odell et al., 1976; Penington et al., 1975), but ethical considerations have limited human studies, although a few have taken place. Bailey et al., (1982) serially followed the platelet count and MPV of subjects undergoing extracorpeal circulation during open heart surgery. Following the initial postoperative fall in the MPV, a significant increase in MPV occurred at 24 to 48 hours,

although the platelet count showed only a minimal increase. During the third and fourth day the MPV fell below preoperative values while the platelet count rose to preoperative counts. Similar findings were reported by Bessman (1982) from studies of myelosuppressed subjects undergoing chemotherapy, whose MPV was found to increase 1 to 2 days prior to a rise in the platelet count. As the platelet count increased the MPV decreased.

This model of a peak in the MPV 1 to 2 days prior to an increase in the platelet count was demonstrated during the serial analyses of subjects undergoing induction chemotherapy for leukemia, although the findings were not evident among all subjects studied. Serial analyses of the MPV and platelet count of Subject LEU-1, who successfully went into remission following induction chemotherapy for acute myelogenous leukemia, clearly supports the findings of Bessman (1982). The MPV peaked and then steadily declined into normal ranges with a corresponding rise in the platelet count as the MPV fell (Figure 5, page 80). Subject LEU-3 (Figure 7, page 82) also revealed a peak in the MPV prior to an increase in the platelet count as the MPV fell. In comparison to Subject LEU-1 the peak in the MPV was not as dramatic, but the general pattern exists to indicate that the MPV of treated acute lymphoblastic leukemias may be indicative of a future increase in the platelet count.

Serial daily determinations of the MPV and platelet count of bone marrow transplant recipients revealed a negative correlation with respect to the MPV predicting a future increase in the platelet count in 29 of 31 subjects studied. Only two subjects, AML-4 and AA-3, demonstrated an increase in the MPV prior to an increase in the platelet count, although these findings are not totally conclusive.

Subject AML-4 (Figure 11, page 91) demonstrated a peak in the MPV on day 35 followed by a steady decline through day 45 with a coinciding increase in the platelet count.

Evaluation of the MPV prior to day 35 is questionable due to a platelet transfusion on day 33. Although the general model of the MPV peaking prior to an increase in the platelet count appears to be present, the change in the two occur over several days compared to those shown in the above model. Subject AA-3 (Figure 15, page 95) clearly resembles the model above. Close review of the MPV and platelet count over the last 6 days of study shows the MPV to increase from 7.9 fl on day 32 to 9.6 fl on day 35, which then fell to 7.5 fl on day 37. On day 35 the platelet count began to rise, increasing from $30x10^9$/L to $45x10^9$/L over a 48 hour period. Additional data beyond the 48 hours post MPV peak is not available since the patient was discharged; a time period which would confirm the model should the platelet count continue to increase into reference limits. These two subjects suggest that an increase in the MPV may precede an increase in the platelet count for bone marrow transplant recipients, but due to a lack of confirmatory data and an increased time period for these changes to occur in one of the subjects, conclusive evidence is not present to support this statement.

Bone marrow biopsy following bone marrow transplantation found the number of megakaryocytes present to correlate with the overall marrow cellularity. The marrow cellularity ranged from slight to markedly hypocellular, with the megakaryocytes being slightly to markedly reduced in number. Subject LEU-1, whose platelet count rose into the upper limits of normal, also revealed a moderately hypocellular marrow, with a slightly reduced number of megakaryocytes. This finding suggests that perhaps the ploidy of the megakaryocytes may play a more important role in platelet production, rather than the actual number of megakaryocytes present. Determination of the megakaryocyte ploidy was not included in this study.

Serial analyses of the white blood cell count was found to be the earliest indicator of bone marrow engraftment. Variation was observed between the time period from bone marrow transplant and the first noticeable rise in the leukocyte count, depending upon the original diagnosis. The variation in time span prior to return of circulating leukocytes most likely originated from the radiation-chemotherapy reduction protocols prior to transplant which varies between diagnostic groups. All subjects who showed evidence of engraftment by bone marrow biopsy demonstrated a rise in the leukocyte count prior to the platelet count. The opposite, an increase in the platelet count prior to the leukocyte count, was not found.

The hematocrit and the mean corpuscular volume (MCV) offer little additional value in the prediction of marrow engraftment which is not otherwise available. With the exception of one subject with aplastic anemia, one with neuroblastoma, and one with acid mucopolysaccharidosis, the MCV was found to steadily decrease following radiation-chemotherapy. Daily variation was minimal, which can be expected since the erythrocyte mean life-span is approximately 120 days (Figure 17). Several subjects showed an indication of an ascending curve late in their study, but correlation to a reticulocyte count was not included in this study. Serial hematocrit determinations showed variability between daily analyses (Figure 18). Several factors can influence the hematocrit, with erythrocyte transfusions, hemorrhage, dilution of blood specimens by intravenous transfusions, and lipemia, all resulting in a variation in the hematocrit. The daily determination offers little predictive value for a future increase or decrease in the circulating erythrocyte mass, but rather may be used in conjunction with the hemoglobin concentration to determine the requirement for erythrocyte transfusion.

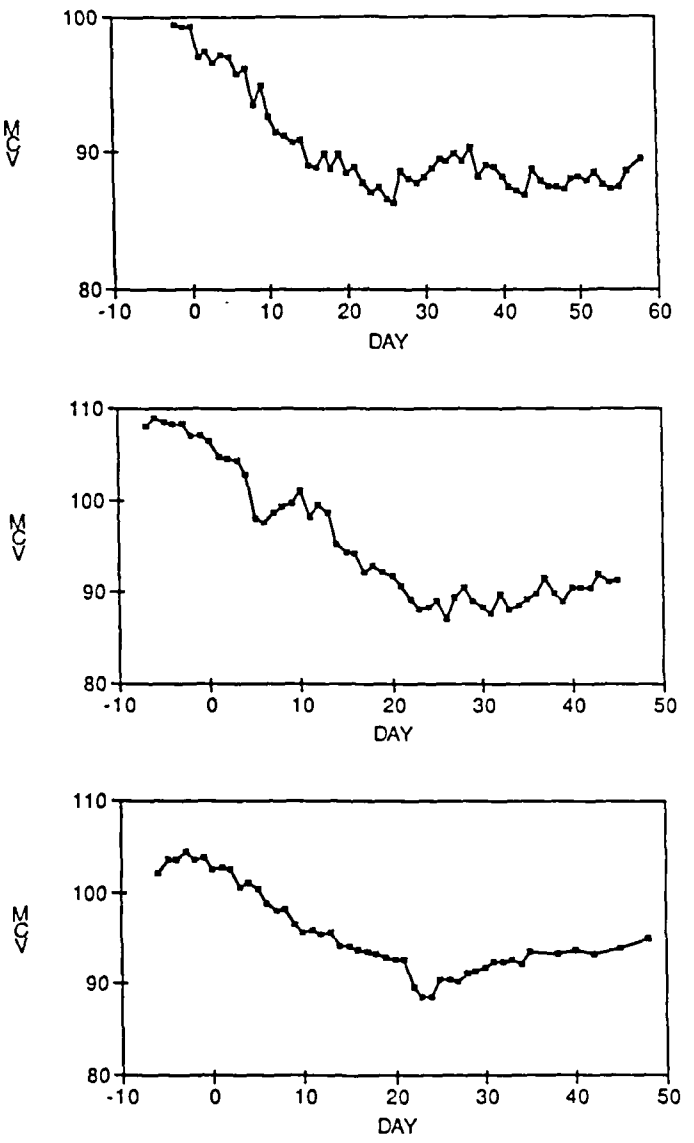

Figure 17. Serial MCV determinations. Top: Subject CML-7 who received erythrocyte transfusions on day7,9,14-16,21-23,25-28,30-32,36,37,39,42,44,54. Center: Subject CML-8 who received erythrocyte transfusions on day 5,13,15,21,29,43. Bottom: Subject AML-4 who received erythrocyte transfusions on day 8,12,13,17,21.

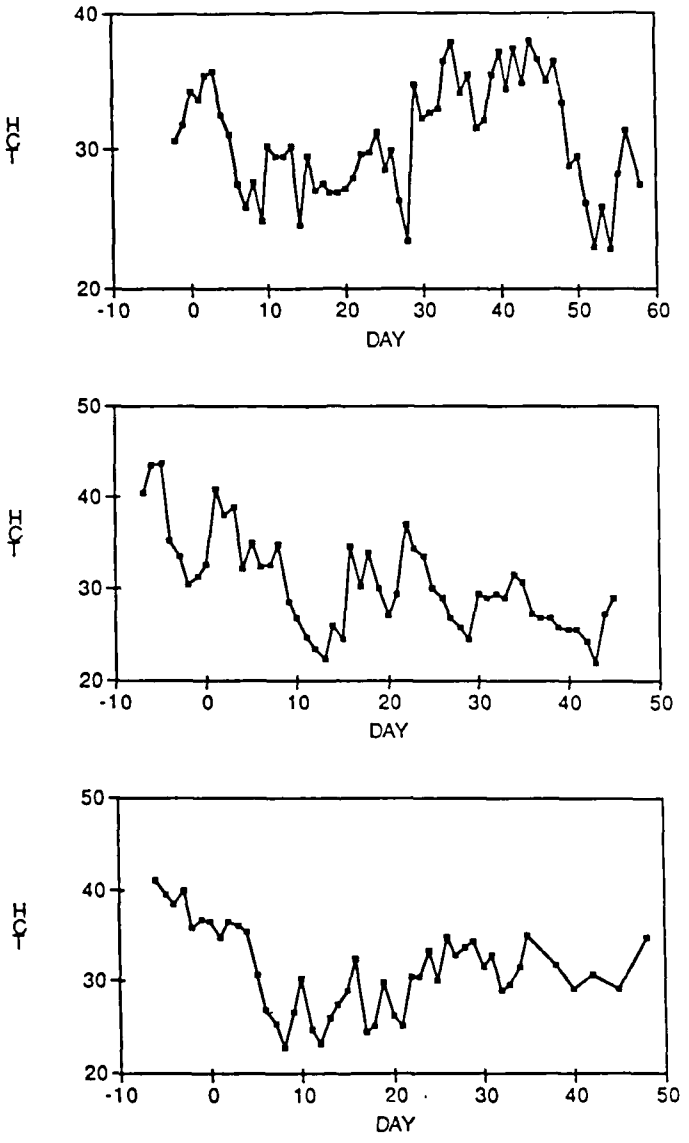

Figure 18. Serial hematocrit determinations. Top: Subject CML-7 who received erythrocyte transfusions on day7,9,14-16,21-23,25-28,30-32,36,37,39,42,44,54. Center: Subject CML-8 who received erythrocyte transfusions on day 5,13,15,21,29,43. Bottom: Subject AML-4 who received erythrocyte transfusions on day 8,12,13,17,21.

Figure 17 shows that the erythrocytes transfused from the blood bank to be of a relatively constant size, corresponding to established normals of 80-100 fl for the MCV. Serial MPV studies tended to show more variability than the MCV following transfusion of blood bank platelets, although the vast majority had an MPV within a normal size range (Figure 19). Splenic sequestration and hemostatic challenges may be the major determinants of the MPV fluctuations.

Subject NRB-2 clearly demonstrates the ablility of the spleen to remove platelets tagged by antibody (Figure 20). This patient developed a platelet antibody shortly after bone marrow transplantation and despite numerous platelet transfusions, the platelet count failed to rise above extremely thrombocytopenic levels. Platelet antibodies were not noted amongst the remaining transplant study population.

Several groups have shown that platelet size varies inversely with platelet age (Corash et al., 1978; Karpatkin, 1969A) while others have denied this relationship (Boneu et al., 1973; Paulus et al., 1975; Penington et al., 1976A & B). In this study Subject LEU-1 following chemotherapy for acute myelogenous leukemia, showed a steady decrease in the MPV while the platelet count steadily increased. In order to support the theory that young platelets are large, the MPV would be expected to remain elevated during the period of rapid increase in platelet count since the majority of circulating platelets would be young, but the opposite was found to occur. The finding of a decrease in the MPV as the platelet count increased, collaborates the findings of Bailey et al. (1982), Bessman (1982), and Levin et al. (1983) that platelet age is not the sole determinant of platelet size.

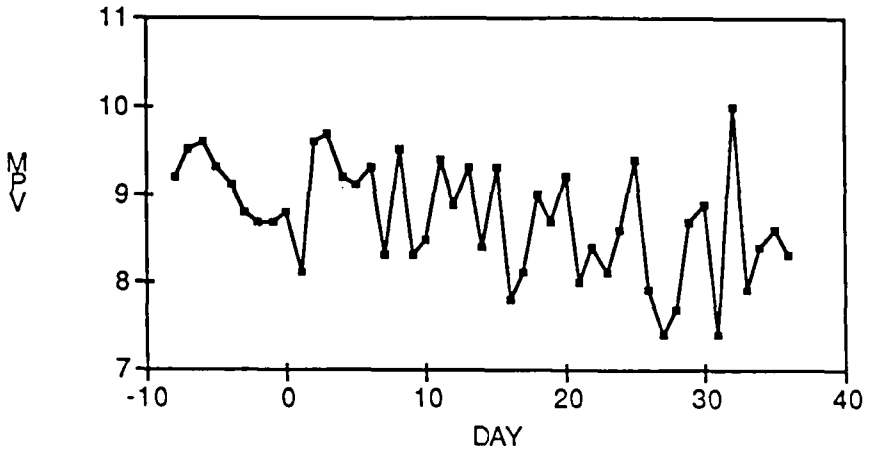

Figure 19. Subject ALL-9. Variation in the MPV following platelet transfusions on day 1-7,9,11-20,22,23,26,27,29-32,35.

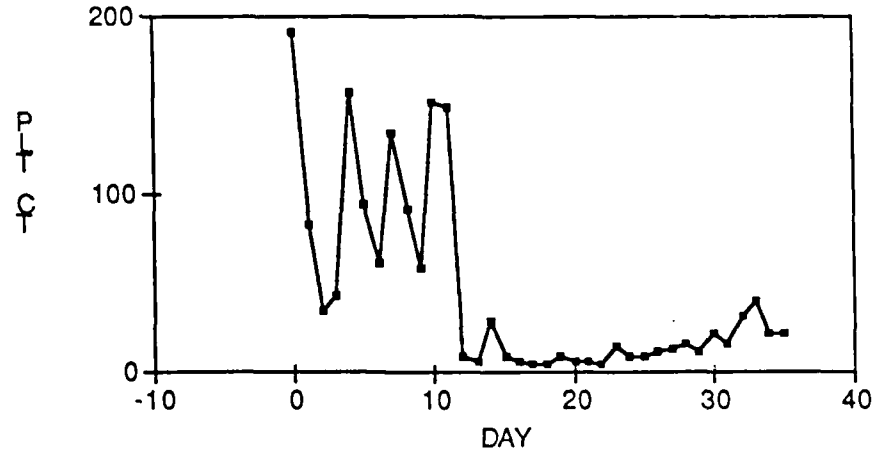

Figure 20. Subject NRB-2 serial platelet count. Platelets were transfused on day 2,4,7,10,12-35.

The daily determination of the MPV and platelet count of bone marrow transplant recipients was found to be of little diagnostic value as an indicator for a future rise in the platelet count. Only 2 of 31 subjects evaluated showed any sign of following a model for a rise in the MPV as being an indicator for a future increase in the platelet count. In addition, the interpretation of daily results is difficult due to the sustained low platelet counts many of these patients sustain in conjunction with the large number of platelet transfusions required.

Serial analyses of the platelet count and the MPV of newly diagnosed leukemic patients may be of benefit to the attending practitioner who has the decision to make as to whether to transfuse the patient with platelets during a thrombocytopenic period, or whether the transfusion of platelets can be delayed in hope that the platelet count will begin to rise. This decision may play a critical role in the patients future care, particularly if the patient is a potential candidate for bone marrow transplantation, since there is a potential for the development of a platelet antibody each time platelets are transfused.

APPENDIX

Patient Data

Abbreviations:

WBC= white blood cell count ($x10^9/L$)

HCT= hematocrit (%)

MCV= mean corpuscular volume (fl)

PLT CRT= platelet crit

PLT CT= platelet count ($x10^9/L$)

MPV= mean platelet volume (fl)

Transfusions:

a= platelets

b=erythrocytes

c=erythrocytes (leukocyte poor)

d= Plasma fresh frozen

e= cryoprecipitate

f= granulocyte/platelet pheresis

g= granulocyte pheresis

h= leukocyte concentrate

i= bone marrow

j= erythrocytes (deglycerized)

Subject LEU-1

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
1	6.4	35	103	.105	121	8.7	
2	6.0	32	103	.095	111	8.6	
3	4.7	32	103	.093	109	8.5	
4	2.7	32	102	.088	102	8.6	
5	1.2	31	102	.078	90	8.7	
6	1.0	30	102	.064	75	8.5	
7	1.2	28	103	.055	65	8.5	a
8	0.7	24	102	.099	114	8.7	b
9	0.3	29	98	.063	74	8.5	
10	0.6	28	99	.045	53	8.4	
11	0.7	27	99	.033	40	8.3	
12	0.6	26	99	.020	24	8.4	b
13	0.3	30	98	.011	15	7.3	a
14	0.6	27	97	.055	61	9.0	b
15	0.7	33	95	.014	14	10.0	a
16	1.0	32	95	.012	15	8.3	a
17	0.9	32	95	.015	15	9.7	a
18	0.6	26	94	.010	13	7.4	a,b
19	0.4	30	93	.009	11	7.8	a
20	0.3	28	95	.010	12	8.0	a
21	0.4	28	91	.014	12	11.4	a,b
22	1.5	28	90	.032	28	11.3	b
23	2.0	27	89	.042	36	11.8	
24	3.2	26	89	.077	56	13.7	b
25	4.9	32	89	.143	108	13.2	
26	7.9	33	89	.207	168	12.3	
27	11.6	35	89	.275	235	11.7	
28	8.9	35	90	.361	334	10.8	
29	9.1	36	90	.400	392	10.2	
30	8.8	37	90	.429	429	10.0	

Subject LEU-2

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
1	29.5	29	92	.040	42	9.6	a
2	8.9	26	91	.029	30	9.8	b
3	2.1	30	91	.060	64	9.4	
4	1.3	33	90	.042	43	9.8	
5	1.1	32	91	.037	38	9.7	a
6	0.7	27	90	.016	18	8.8	b
7	0.5	29	90	.058	73	7.9	
8	0.4	33	88	.035	40	8.7	
9	0.5	31	89	.025	30	8.2	
10	0.7	31	89	.019	22	8.8	
11	1.1	28	89	.013	14	9.1	a
12	0.7	27	89	.042	48	8.7	
13	0.7	26	88	.027	33	8.2	b
14	0.6	33	89	.019	23	8.2	
15	0.6	32	90	.013	16	8.4	a
16	0.6	31	89	.028	30	9.2	
17	0.5	31	89	.016	19	8.5	a
18	0.4	29	88	.043	47	9.1	
19	0.3	28	89	.030	34	8.7	
20	0.2	30	88	.028	30	9.3	
21	0.1	26	89	.018	21	8.7	a,b
22	0.2	29	88	.066	65	10.2	
23	0.2	29	87	.071	66	10.7	
24	0.5	30	89	.082	72	11.4	
25							
26	0.4	27	88	.066	64	10.4	
27	0.4	27	88	.050	51	9.9	b
28	0.3	30	87	.048	43	11.3	
29	0.4	31	88	.045	41	11.0	
30	0.5	28	88	.028	26	10.6	
31	0.5	30	88	.032	31	10.4	
32	0.5	30	88	.013	16	8.1	a
33	0.6	30	86	.032	31	10.3	
34	0.8	28	87	.022	23	9.6	
35	1.0	30	87	.020	20	10.1	
36	0.9	29	87	.013	16	8.0	a
37	1.1	30	87	.052	57	9.2	
38	1.2	31	86	.044	46	9.5	
39	1.4	32	86	.031	31	10.1	
40	1.4	30	87	.028	30	9.4	
41	1.6	30	86	.024	27	8.8	
42	2.1	31	87	.019	19	10.0	
43	1.9	29	87	.013	16	7.9	a

Subject LEU-3

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
1	3.9	30	101	.283	399	7.1	
2	4.1	31	100	.294	4.8	7.2	
3	4.2	29	100	.229	322	7.1	
4	3.8	28	100	.213	292	7.3	
5	2.4	24	100	.170	233	7.3	
6	1.0	25	100	.155	209	7.4	
7	0.7	25	100	.124	168	7.4	
8	0.2	22	99	.080	106	7.5	b
9	0.3	32	96	.057	78	7.3	b
10	0.2	33	95	.045	63	7.2	
11	0.1	32	95	.029	38	7.6	
12	0.2	29	95	.020	23	8.8	a
13	0.4	27	95	.010	13	7.5	a
14	0.2	27	94	.023	26	8.9	
15	0.2	29	94	.015	19	8.0	
16	0.2	27	94	.030	37	8.1	a
17	0.2	34	91	.014	16	9.0	b
18	0.2	34	91	.013	19	6.7	a
19	0.3	32	91	.014	18	7.8	a
20	0.2	31	91	.020	25	8.0	
21	0.4	30	91	.019	24	8.1	
22	0.5	31	90	.025	38	6.7	
23	0.7	31	91	.032	42	7.5	
24	1.0	33	91	.058	78	7.4	
25	1.8	32	91	.111	154	7.2	
26	2.4	31	90	.122	180	6.8	
27	3.3	30	91	.174	256	6.8	

Subject LEU-4

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
1	5.0	37	88	.006	9	6.9	a
2	5.1	34	90	.011	15	7.5	a
3	3.7	34	87	.019	22	8.7	
4	2.1	37	88	.011	13	8.3	a
5	1.2	38	88	.042	45	9.3	
6	1.3	37	88	.020	21	9.7	
7	0.5	37	86	.016	22	7.3	
8	0.7	37	88	.012	16	7.6	a
9	0.4	29	87	.064	70	9.1	b
10	0.5	38	85	.035	39	8.9	b
11	0.3	35	87	.012	17	7.3	
12	0.4	35	87	.012	17	7.1	a
13	0.2	31	86	.023	27	8.4	
14	0.3	30	86	.011	13	8.2	a
15	0.4	27	86	.044	45	9.7	
16	0.6	30	84	.285	223	12.8	
17	0.4	30	86	.149	132	11.3	a
18	0.7	29	85	.210	189	11.1	
19	0.6	28	86	.074	73	10.1	
20	0.4	25	85	.037	41	9.8	
21	0.3	23	86	.036	58	6.3	a,b
22	0.3	24	86	.149	151	9.9	a,b
23	0.2	29	84	.022	34	6.6	a
24	0.3	27	85	.018	20	9.1	a
25							
26	0.2	31	86	.011	16	6.6	a,g
27	0.2	29	87	.020	26	7.8	a,b,f,g
28	0.2	30	87	.010	15	6.4	b,f
29	0.3	35	86	.023	32	7.3	a,f,g

Subject ALL-1

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
-5	4.5	33	92	.149	169	8.8	
-4	4.5	18	91	.158	180	8.8	d
-3	3.4	16	91	.154	175	8.8	b
-2	7.9	25	94	.169	196	8.6	b
-1	2.9	32	96	.160	191	8.4	b
0	0.7	31	92	.113	138	8.2	i
1	0.3	25	90	.059	76	7.7	
2	0.0	26	90	.044	60	7.4	b
3	0.0	32	90	.037	47	7.9	b
4	0.0	33	88	.053	68	7.8	a
5	0.0	31	89	.024	29	8.2	
6	0.0	16	88	.024	37	7.2	a
7	0.1	26	82	.033	39	8.4	b
8	0.1	35	88	.026	33	8.0	
9	0.3	31	88	.058	62	9.4	a
10	0.2	27	88	.060	66	9.1	a,b
11	0.6	33	89	.054	89	8.0	a
12	0.9	36	88	.081	97	8.4	
13	1.5	33	89	.064	77	8.3	a
14	1.9	31	89	.067	76	8.8	
15	2.8	31	88	.048	55	8.8	a
16	3.0	28	89	.069	77	9.0	
17	3.7	26	89	.076	79	9.6	a
18	6.5	28	89	.064	67	9.6	a
19	8.9	28	90	.091	96	9.5	
20	9.0	28	90	.046	52	8.8	
21	9.5	28	91	.041	47	8.7	a
22	9.2	25	91	.068	73	9.3	b
23	9.8	39	92	.051	54	9.5	
24	10.1	38	92	.050	51	9.8	
25	8.8	36	92	.033	37	9.0	
26	9.0	34	91	.031	32	9.7	
27	10.0	36	91	.032	36	9.0	a
28	11.0	38	92	.038	39	9.7	
29	10.0	33	94	.033	34	9.8	
30	12.8	37	94	.043	43	10.0	a
31	12.5	33	94	.078	85	9.2	a
32	13.3	35	94	.082	86	9.5	
33	10.5	32	95	.059	63	9.4	
34	10.6	32	96	.054	56	9.6	
35	12.5	34	95	.045	53	8.5	

Subject ALL-2

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-5	3.5	27	96	.076	83	9.1	
-4	2.8	32	92	.067	79	8.5	b
-3	1.6	30	92	.052	64	8.1	
-2	1.1	28	92	.043	53	8.1	
-1	0.7	28	93	.043	55	7.9	
0	0.3	25	93	.039	48	8.2	b,i
1	0.2	30	92	.015	24	6.2	a
2	0.0	30	91	.040	46	8.7	a
3	0.0	32	91	.112	113	9.9	
4	0.0	35	92	.082	84	9.8	
5	0.0	22	91	.055	63	8.8	a,b
6	0.0	28	92	.058	62	9.4	
7	0.0	26	91	.029	35	8.3	
8	0.0	21	91	.017	18	9.7	a,b
9	0.0	32	90	.095	107	8.9	a,b
10	0.0	27	91	.145	161	9.0	
11							
12	0.1	27	90	.059	70	8.4	
13	0.1	24	90	.025	28	8.9	b
14	0.2	29	90	.043	47	9.2	a
15	0.7	30	90	.014	15	9.4	a
16	0.9	26	89	.064	65	9.8	b
17	1.2	32	90	.034	32	10.6	a
18	0.9	30	89	.031	33	9.4	a
19	1.0	28	89	.031	35	8.8	a
20	1.0	27	89	.010	13	7.8	a,b
21	1.5	30	89	.017	21	8.2	a
22	1.6	31	89	.036	37	9.8	
23	1.5	33	86	.073	82	8.9	a
24	2.1	32	87	.045	48	9.3	
25	1.1	27	88	.025	29	8.5	a,b
26	1.8	30	89	.009	12	7.9	a
27	1.9	28	89	.006	10	6.3	a
28	2.4	20	90	.015	15	9.8	a,b
29	2.8	26	89	.039	37	10.6	a,b
30	3.1	31	89	.029	27	10.6	a
31	2.4	31	88	.067	70	9.6	
32	2.9	31	87	.049	49	10.0	
33	2.7	29	88	.086	84	10.2	a
34	1.7	26	89	.074	71	10.4	
35	1.6	32	89	.052	51	10.1	
36	1.1	28	88	.032	32	10.1	a
37	0.6	24	88	.033	37	9.0	a,b
38	0.9	35	87	.012	13	9.4	a,b
39	0.9	34	88	.016	18	8.9	a
40	1.1	35	88	.019	19	10.2	a
41	0.8	31	87	.010	13	7.9	a

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
42	0.8	29	88	.013	15	8.4	a
43	1.2	30	88	.102	91	11.2	
44	1.4	30	88	.067	63	10.6	a
45	1.5	30	89	.067	62	10.8	
46	1.6	31	89	.051	46	11.1	a
47	1.6	30	90	.073	85	8.6	
48	1.7	31	89	.049	55	8.9	
49	1.8	30	90	.039	46	8.4	
50	2.1	30	90	.026	34	7.6	a
51	1.9	28	90	.024	27	9.0	

Subject ALL-3

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-8	0.2	26	91	.222	231	9.6	
-7	0.7	27	91	.226	240	9.4	
-6	0.6	27	90	.191	208	9.2	
-5	0.2	27	90	.157	172	9.1	
-4	0.1	27	90	.122	139	8.8	
-3	0.0	26	90	.083	92	9.0	
-2	0.0	25	90	.062	69	9.7	
-1	0.0	22	90	.027	30	9.1	b
0	0.0	28	89	.017	19	9.2	a,i
1	0.0	27	89	.113	110	10.3	b
2	0.0	34	90	.087	84	10.3	
3	0.0	32	90	.072	69	10.5	
4	0.0	31	89	.043	44	9.8	b
5	0.0	30	89	.076	90	8.4	
6	0.0	31	89	.045	53	8.5	a,b,h
7	0.3	38	89	.209	205	10.2	
8	0.1	37	89	.130	140	9.3	f
9	0.4	36	90	.132	136	9.7	a,f
10	1.2	31	90	.192	194	9.9	b,f
11	1.4	32	89	.103	108	9.5	b,f
12	1.3	35	89	.117	116	10.1	a,b,f,g
13	1.0	33	87	.125	124	10.1	a,f
14	3.4	31	87	.080	81	9.9	f
15	3.0	30	88	.099	89	11.1	a,b,f
16	2.3	31	86	.100	104	9.6	a,f
17	2.1	31	86	.043	36	11.9	f
18	1.5	30	86	.033	39	8.4	a,f
19	2.3	28	85	.046	45	10.2	f
20	4.3	27	85	.043	48	8.9	a,b,g
21	3.9	34	87	.061	61	10.0	a
22	4.5	35	86	.039	48	8.2	
23	5.6	31	85	.087	105	8.3	a
24	6.0	29	86	.056	63	8.9	
25	6.2	32	87	.084	92	9.1	a
26	8.1	28	87	.033	37	9.0	a,c
27	7.5	28	88	.066	74	8.9	a
28	9.2	28	88	.164	173	9.5	a
29	10.4	32	87	.110	124	8.9	a
30	8.8	37	87	.101	131	7.7	a,b
31	10.0	30	86	.098	127	7.7	a
32	9.1	30	86	.070	86	8.1	a
33	12.0	30	87	.071	85	8.3	a
34	10.8	26	87	.095	108	8.8	
35	15.9	22	87	.080	89	9.0	a,b
36	13.0	27	89	.135	157	8.6	a
37	13.7	25	90	.117	138	8.5	

Subject ALL-4

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-5	4.3	36	93	.275	339	8.1	
-4	8.2	33	93	.384	468	8.2	
-3	9.0	31	92	.265	323	8.2	
-2	5.0	31	92	.238	290	8.2	
-1	2.8	32	93	.250	305	8.2	
0	1.4	31	93	.193	248	7.8	i
1	0.8	29	91	.122	155	7.9	
2	0.4	28	91	.099	116	8.5	
3	0.2	28	92	.064	79	8.1	
4	0.0	28	93	.042	52	8.0	
5	0.0	28	93	.025	28	9.0	a
6	0.0	29	92	.057	63	9.0	
7	0.0	27	92	.030	33	9.1	
8	0.1	27	91	.065	65	10.0	a
9	0.1	25	92	.043	46	9.4	b
10	0.1	29	88	.029	33	8.9	a
11	0.4	28	88	.032	37	8.6	a
12	0.7	27	88	.045	48	9.3	a
13	1.4	25	88	.036	38	9.4	a
14	2.0	21	88	.053	56	9.4	a,b
15	2.9	27	89	.037	39	9.4	
16	4.0	30	89	.059	52	11.4	a
17	2.4	29	88	.046	48	9.5	a
18	3.0	25	88	.046	49	9.3	a
19	2.5	24	88	.044	44	10.1	b
20	2.8	29	90	.032	34	9.5	a
21	2.9	28	88	.038	32	11.9	a
22	3.8	28	87	.025	26	9.5	a
23	4.7	27	88	.030	34	8.7	
24	5.0	29	88	.036	31	11.5	a
25	6.1	27	87	.031	36	8.6	a
26	5.9	26	86	.035	34	10.4	a
27	5.0	26	86	.040	40	9.9	a,b
28	10.2	32	87	.062	60	10.3	
29	10.7	31	87	.046	46	10.0	
30	12.4	31	86	.057	53	10.8	
31	13.2	32	86	.048	42	11.4	a
32	13.7	31	87	.061	61	10.0	
33	11.9	31	87	.059	58	10.1	
34	11.8	29	86	.045	49	9.2	

Subject ALL-5

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-5	6.1	44	95	.178	171	10.4	
-4	7.3	36	95	.167	169	9.9	
-3	5.7	36	95	.136	139	9.8	
-2	3.3	33	94	.106	113	9.4	
-1	2.8	37	95	.120	128	9.4	
0	0.5	32	94	.071	82	8.6	i
1	0.5	29	94	.041	44	9.3	
2	0.0	32	93	.026	30	8.5	a
3	0.0	31	92	.053	55	9.6	
4	0.0	30	93	.030	33	9.1	
5	0.0	29	93	.015	14	10.6	a
6	0.0	30	93	.045	46	9.7	
7	0.0	26	94	.022	29	7.7	a,b
8	0.1	31	92	.019	21	9.2	
9	0.1	28	91	.007	10	7.1	a
10	0.2	27	92	.035	34	10.3	a
11	0.4	27	91	.039	40	9.7	a
12	0.6	27	91	.045	44	10.3	
13	1.0	26	92	.051	54	9.5	a
14	1.8	24	92	.042	42	9.9	a
15	1.8	25	90	.043	48	8.9	a
16	2.5	25	91	.053	46	11.5	a,b
17	3.5	28	90	.040	38	10.6	a
18	4.5	28	89	.054	46	11.8	
19	4.6	30	90	.050	42	12.0	a
20	4.4	28	90	.066	67	9.9	
21	4.2	28	89	.044	48	9.2	a
22	4.9	28	88	.101	111	9.1	a
23	6.1	26	88	.073	66	11.0	a
24	7.0	27	88	.099	98	10.1	
25	7.3	26	88	.074	67	11.0	a
26	7.2	27	89	.081	82	9.9	
27	9.1	29	89	.074	71	10.5	
28	11.3	32	90	.071	61	11.7	
29	13.3	34	91	.074	59	12.5	
30	15.8	32	90	.084	65	13.0	a
31	14.5	32	90	.067	61	11.0	
32	16.4	33	91	.078	74	10.5	
33	18.6	32	92	.104	86	12.1	
34	18.1	33	93	.082	75	10.9	
35	19.4	36	94	.098	80	12.2	
36	16.5	34	94	.070	58	12.0	
37	13.8	34	96	.068	58	11.8	
38	13.7	37	96	.083	70	11.8	
39	11.8	36	95	.059	57	10.4	
40	10.9	37	96	.050	51	9.9	
41	9.4	35	97	.068	59	11.5	
43	9.4	33	97	.075	62	12.1	

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
43	8.6	33	97	.072	62	11.7	
44	7.5	32	97	.059	51	11.5	
45	7.8	32	97	.049	51	9.6	
46	7.8	33	98	.064	69	9.3	
47	8.3	34	97	.063	61	10.3	
48	9.2	32	97	.044	46	9.5	
49	10.0	33	97	.063	54	11.6	
50	7.5	31	97	.060	58	10.4	
51	7.8	29	98	.066	52	12.6	
52	7.4	29	99	.066	63	10.5	
53	7.9	31	99	.063	54	11.7	
54	8.0	31	98	.081	68	11.9	
55							
56	8.6	34	100	.052	45	11.5	
57	8.5	34	101	.036	34	10.7	
58	7.6	33	101	.059	49	12.0	

Subject ALL-6

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-7	5.4	31	96	.263	356	7.4	
-6	2.4	29	95	.217	285	7.6	
-5	2.4	30	96	.190	250	7.6	
-4	3.7	30	96	.174	212	8.2	
-3	3.2	30	95	.111	142	7.8	
-2	2.0	30	95	.078	105	7.4	
-1	0.1	28	96	.045	65	6.9	b
0	0.0	32	93	.013	21	6.2	a,i
1	0.3	35	93	.085	89	9.5	
2	0.0	39	92	.044	45	9.9	
3	0.1	32	93	.008	11	6.9	a
4	0.0	30	92	.020	23	8.5	a
5	0.0	26	92	.019	20	9.7	a,b
6	0.0	31	92	.027	30	8.9	a
7	0.0	30	92	.054	63	8.6	a
8	0.0	28	92	.054	56	9.6	a,b
9	0.0	25	92	.065	68	9.6	a,b
10	0.0	30	91	.024	27	9.1	a
11	0.0	28	92	.056	54	10.4	
12	0.0	26	91	.057	56	10.1	a
13	0.0	23	91	.069	70	9.8	a,b
14	0.0	28	90	.072	70	10.3	a
15	0.0	25	89	.076	64	11.8	
16	0.0	24	89	.103	113	9.1	a,d
17	0.0	24	88	.059	57	10.4	a,b,d
18	0.0	29	87	.110	106	10.4	
19	0.0	28	88	.098	84	11.7	a
20	0.1	26	87	.057	48	11.9	a
21	0.0	23	87	.057	57	10.0	a,b,e
22	0.0	26	87	.199	201	9.9	a
23	0.1	25	87	.191	203	9.4	a,b
24	0.1	26	87	.164	161	10.2	a
25	0.1	33	88	.106	93	11.4	b,d,e
26	0.0	32	88	.130	135	9.6	a
27	0.1	28	88	.132	129	10.2	a
28	0.1	28	89	.140	141	8.9	
29	0.1	28	88	.146	147	9.9	a
30	0.2	26	87	.140	130	10.8	a
31	0.1	27	87	.129	123	10.5	b
32	0.3	26	87	.066	58	11.3	a
33	0.4	31	86	.148	142	10.4	
34	0.8	29	87	.102	93	11.0	f,g
35	1.3	27	86	.110	109	10.1	
36	0.8	28	85	.172	140	12.3	f,g
37	1.0	24	87	.264	232	11.4	
38	0.9	25	88	.207	177	11.7	b,f,g
39	1.0	28	83	.290	234	12.4	
40	1.0	28	86	.027	28	9.8	a,b,f,g

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
41	0.9	24	85	.205	167	12.3	
42	1.5	31	84	.017	42	4.2	a,f,g
43	3.2	28	83	.225	183	12.3	a,g
44	8.4	26	83	.080	102	7.8	f
45	9.2	30	86	.031	37	8.3	a,f
46	5.5	37	87	.042	45	9.3	a,b,e,f
47	2.2	35	86	.060	52	11.6	b,d,f
48	2.0	30	85	.078	59	13.3	a,b,d
49	1.4	40	87	.025	28	8.8	a,b,d,f
50	0.8	37	86	.009	14	6.6	a
51	0.5	37	85	.049	51	9.7	b

Subject ALL-7

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-2	0.5	31	91	.095	108	8.8	
-1	0.2	31	91	.057	67	8.5	
0	0.0	29	90	.025	32	7.8	a,i
1	0.0	29	90	.047	57	8.3	a
2	0.0	28	90	.106	105	10.1	
3	0.0	27	90	.083	83	10.0	
4	0.0	33	88	.053	55	9.6	a
5	0.0	26	89	.127	125	10.2	
6	0.0	24	88	.086	94	9.2	b
7	0.0	28	87	.047	53	8.9	
8	0.0	29	89	.077	78	9.9	a
9	0.0	29	88	.047	46	10.2	
10	0.0	28	88	.024	27	9.1	a
11	0.0	26	88	.072	72	10.0	
12	0.0	26	86	.066	62	10.7	
13	0.0	24	86	.026	29	8.8	a,b
14	0.0	33	86	.083	76	10.9	b
15	0.0	33	86	.056	46	12.1	a,h
16	0.1	30	87	.092	86	10.7	f
17	0.1	29	86	.103	98	10.5	f
18	0.8	26	86	.118	107	11.0	
19	0.2	24	86	.109	105	10.4	f,g
20	0.1	26	85	.056	67	8.4	b,
21	0.2	26	85	.070	67	10.4	
22	0.3	25	85	.047	37	12.7	b,f,g
23	0.4	26	85	.028	31	9.1	a,g
24	0.6	23	86	.039	41	9.4	b
25	0.5	29	86	.029	32	9.0	a,f,h
26	0.9	28	86	.018	18	9.8	a,b
27	2.0	36	87	.027	31	8.7	a,b,d
28	1.7	32	88	.022	26	8.5	a,b
29	0.8	31	87	.019	22	8.6	a
30	0.9	27	86	.021	23	9.1	a,b,d
31	0.9	35	88	.011	12	9.4	a,b,d
32	0.6	33	88	.074	92	8.0	a
33	0.6	30	87	.036	44	8.1	a
34	0.8	30	88	.040	49	8.2	a
35	1.0	32	88	.033	39	8.4	a,b
36	1.1	32	87	.048	57	8.5	a
37	1.7	32	88	.078	95	8.2	a,b
38	1.1	32	88	.074	80	9.3	a
39	1.1	34	87	.079	82	9.6	a
40	1.5	31	86	.108	121	8.9	a
41	1.4	29	86	.080	87	9.2	a
42	1.9	28	87	.090	102	8.9	
43	1.8	26	87	.095	114	8.3	a
44	1.6	28	85	.094	108	8.7	a,b
45	1.4	27	88	.089	106	8.4	a

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
46	1.5	27	87	.090	101	8.9	b
47	1.3	34	87	.046	52	8.9	a
48	1.0	31	87	.062	67	9.3	
49	1.1	30	87	.035	38	9.2	a
50	0.9	28	87	.063	69	9.1	
51	1.1	25	88	.089	82	10.9	a
52	1.4	36	87	.063	69	9.2	a,b
53	1.3	35	87	.082	98	8.4	a
54	1.5	32	87	.101	129	7.8	a
55	1.3	31	87	.065	81	8.0	a
56	1.1	29	87	.078	101	7.7	
57	1.1	28	87	.052	70	7.5	
58	1.6	30	86	.038	50	7.5	
59	1.4	27	87	.019	25	7.7	
60	1.4	28	87	.058	67	8.6	a
61	1.2	25	87	.039	47	8.2	
62	1.6	26	87	.033	40	8.3	
63	1.6	27	88	.023	25	9.3	a
64	1.3	25	87	.050	55	9.1	
65	1.3	25	88	.037	39	9.4	
66	1.0	23	87	.024	29	8.4	b
67	0.7	32	87	.016	20	8.0	a
68	1.0	34	86	.043	53	8.1	
69	1.1	32	86	.026	33	7.9	
70	0.9	32	87	.023	25	9.3	
71	1.0	33	87	.016	20	8.0	
72	1.0	32	86	.053	55	9.6	a
73	1.3	30	87	.033	33	10.0	
74	1.5	30	87	.023	23	10.1	
75	1.6	30	87	.020	23	8.7	a
76	1.4	29	86	.059	65	9.1	
77	2.1	30	86	.063	42	8.5	
78	2.1	28	87	.029	31	9.4	a
79	2.2	27	87	.067	74	9.0	

Subject ALL-8

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-6	3.4	27	93	.114	146	7.8	
-5	2.3	29	93	.095	110	8.6	
-4	0.9	27	92	.064	77	8.3	b
-3	0.2	32	91	.044	50	8.8	
-2	0.0	34	90	.031	38	8.2	
-1	0.0	33	89	.018	24	7.6	a
0	0.0	31	90	.031	33	9.5	i
1	0.0	29	91	.020	22	9.3	a
2	0.0	29	91	.074	80	9.2	
3	0.0	28	91	.058	59	9.9	
4	0.0	27	92	.042	44	9.5	
5	0.0	25	90	.023	24	9.5	a,b
6	0.0	26	90	.056	60	9.3	b
7	0.0	29	91	.035	39	9.1	
8	0.0	27	91	.069	71	9.7	a
9	0.0	24	91	.053	55	9.7	a,b
10	0.0	28	90	.036	39	9.3	
11	0.0	27	89	.045	46	9.8	a
12	0.0	27	89	.023	24	9.6	a
13	0.0	28	89	.034	39	8.8	
14	0.0	27	88	.025	27	9.2	a
15	0.0	20	89	.065	74	8.8	a,b
16	0.0	31	88	.119	129	9.2	a
17	0.0	27	89	.083	73	11.4	a
18	0.0	29	88	.135	135	10.0	a
19	0.0	27	88	.068	69	9.8	a
20	0.0	27	87	.085	83	10.2	a
21	0.0	24	88	.032	32	9.9	a,b
22	0.0	40	89	.044	46	9.5	a
23	0.0	32	88	.057	58	9.9	a
24	0.0	26	89	.079	84	9.4	a
25	0.0	28	87	.074	75	9.9	a
26	0.0	26	88	.094	98	9.6	
27	0.0	25	88	.054	54	10.0	a,b
28	0.1	29	88	.038	39	9.8	a
29	0.4	23	88	.049	55	9.0	a,b
30	0.4	30	87	.081	86	9.4	a,d
31	0.5	24	87	.062	62	10.0	b
32	0.4	31	87	.024	25	9.7	a,d
33	0.2	29	87	.043	47	9.2	a,b,d
34	0.7	29	88	.035	34	10.3	a,d
35	0.9	27	87	.058	61	9.5	a,d
36	1.0	28	88	.009	11	8.0	a
37	1.2	28	87	.034	37	9.2	
38	1.6	25	86	.019	20	9.6	a
39	2.1	28	88	.042	49	8.5	c
40	1.6	25	87	.039	44	8.9	a
41	1.6	23	87	.005	7	7.3	a,c

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
42	1.8	25	88	.035	38	9.1	a,c
43	3.6	26	89	.061	69	8.8	a,c
44	3.1	33	89	.033	41	8.1	a,c,d
45	4.3	33	90	.042	50	8.4	a,c,d
46	3.6	35	88	.036	41	8.9	a,c
47	5.9	38	89	.053	60	8.8	a,c
48	5.8	39	88	.027	30	8.9	a
49	5.7	40	89	.041	49	8.4	a,c,e
50	5.2	39	89	.036	40	9.0	a,c
51	4.2	37	89	.034	39	8.8	a
52	3.1	39	88	.027	33	8.2	a,c
53	1.6	39	88	.038	43	8.8	a
54	2.4	38	86	.070	79	8.9	a
55	3.4	40	85	.052	55	9.4	a
56	4.0	42	85	.036	36	10.1	a,e
57	4.5	34	86	.074	73	10.2	a,c,e
58	6.7	33	86	.068	65	10.4	a,c
59	6.5	36	87	.027	25	10.9	a
60	5.6	34	88	.056	51	10.9	a,c
61	4.0	35	87	.076	71	10.7	a

Subject ALL-9

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-8	5.3	33	93	.193	210	9.2	
-7	7.1	31	94	.185	195	9.5	
-6	10.9	32	94	.191	199	9.6	
-5	6.3	32	94	.175	188	9.3	
-4	3.4	32	94	.147	162	9.1	
-3	2.5	31	94	.125	142	8.8	
-2	2.0	32	94	.097	112	8.7	
-1	0.9	30	93	.069	79	8.7	
0	0.1	32	93	.049	56	8.8	i
1	0.0	32	94	.023	28	8.1	a
2	0.0	32	93	.024	25	9.6	a
3	0.0	27	92	.013	13	9.7	a
4	0.0	24	92	.009	10	9.2	a,b
5	0.0	34	91	.050	55	9.1	a
6	0.0	30	88	.033	45	9.3	a
7	0.0	29	90	.020	24	8.3	a
8	0.0	31	90	.024	25	9.5	
9	0.1	29	90	.012	14	8.3	a
10	0.1	27	90	.014	17	8.5	
11	0.2	26	88	.033	35	9.4	a,b
12	0.7	33	87	.023	26	8.9	a,b
13	1.0	32	88	.034	37	9.3	a
14	1.0	32	88	.013	16	8.4	a
15	0.7	32	87	.020	21	9.3	a
16	0.5	31	87	.023	30	7.8	a
17	0.7	31	86	.035	43	8.1	a
18	0.7	30	86	.018	20	9.0	a
19	0.7	29	85	.028	32	8.7	a
20	0.5	27	86	.048	52	9.2	a
21	0.4	28	86	.035	44	8.0	
22	0.6	26	86	.055	65	8.4	a
23	1.0	27	85	.039	48	8.1	a
24	0.8	24	85	.028	33	8.6	
25	0.9	23	84	.016	17	9.4	
26	1.0	22	84	.013	16	7.9	a,b
27	0.7	28	87	.010	13	7.4	a
28	1.1	30	87	.009	12	7.7	
29	1.4	27	86	.008	9	8.7	a
30	1.2	27	87	.014	16	8.9	a
31	1.2	24	86	.012	16	7.4	a,b
32	1.3	33	86	.018	18	10.0	a
33	1.5	34	86	.046	58	7.9	
34	2.1	34	84	.030	36	8.4	
35	1.8	33	85	.018	21	8.6	a
36	1.4	32	85	.034	41	8.3	

Subject AML-1

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
-3	3.4	27	93	.181	229	7.9	
-2	2.7	26	92	.162	208	7.8	
-1	1.5	27	93	.153	199	7.7	b
0	0.5	32	90	.121	149	8.1	i
1	0.4	34	89	.094	124	7.6	
2	0.2	35	90	.064	82	7.8	
3	0.1	34	89	.040	51	7.9	
4	0.0	32	90	.035	40	8.7	
5	0.0	30	89	.012	15	7.9	a
6	0.0	26	90	.086	95	9.1	a,b
7	0.0	29	90	.114	133	8.6	a,g,h
8	0.1	31	90	.153	189	8.1	f
9	0.1	29	90	.079	94	8.4	g
10	0.1	32	90	.134	131	10.2	f
11	0.1	30	90	.088	94	9.4	g
12	0.0	30	89	.066	67	9.9	f
13	0.2	29	89	.027	26	10.2	
14	0.2	28	89	.041	33	12.5	a,b
15	0.1	24	89	.025	21	11.8	a
16	0.1	30	88	.078	87	9.0	a
17	0.0	29	89	.043	45	9.6	
18	0.1	28	88	.106	112	9.5	
19	0.2	28	88	.055	56	9.8	
20	0.7	28	88	.028	32	8.8	a
21	0.2	26	88	.068	79	8.6	a
22	0.3	24	88	.055	65	8.5	b
23	0.9	33	86	.023	27	8.4	a
24	0.8	29	86	.064	78	8.2	
25	1.4	29	87	.011	12	8.9	a
26	0.8	25	87	.061	85	7.2	a
27	1.3	27	86	.023	28	8.3	a
28	0.9	23	85	.045	54	8.4	b
29	1.2	31	84	.022	24	9.1	a
30	1.3	29	84	.086	95	9.0	
31	1.9	34	84	.058	56	10.3	a
32	1.0	31	85	.063	69	9.1	
33	1.9	29	83	.023	21	11.0	
34	2.0	30	84	.007	8	8.7	a
36	1.9	28	84	.078	68	8.9	a,b
36	2.3	38	85	.051	55	9.3	
37	2.6	36	85	.026	31	8.4	a
38	1.8	36	85	.064	70	9.1	
39	2.5	38	85	.040	43	9.2	
40	1.7	38	84	.030	34	9.1	
41	1.8	38	83	.023	28	10.3	a
42	1.5	35	84	.049	49	9.9	
43	1.5	34	84	.035	36	9.7	
44	1.5	31	83	.027	31	8.5	a

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
45	1.7	30	83	.019	21	9.0	a
46	1.5	27	84	.030	32	9.4	
47	2.0	27	84	.015	20	7.4	a
48	1.7	25	84	.049	42	11.6	a
49	2.0	25	84	.083	74	11.2	
50	2.4	32	87	.074	69	10.7	a,b
51	1.2	31	88	.087	81	10.7	a
52	1.8	31	88	.070	65	10.7	
53	2.2	33	89	.057	46	12.4	
54	2.7	34	90	.044	36	12.2	

Subject AML-2

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-1	4.3	31	107	.087	110	7.9	
0	0.5	29	106	.063	83	7.6	i
1	0.3	32	105	.052	69	7.5	
2	0.1	31	104	.046	60	7.7	a
3	0.0	33	104	.089	114	7.8	
4	0.0	27	103	.044	59	7.4	
5	0.0	32	103	.022	30	7.4	a
6	0.1	22	102	.070	84	8.3	b
7	0.0	32	97	.100	110	9.1	a
8	0.0	31	95	.078	93	8.4	
9	0.0	27	96	.035	42	8.3	
10	0.0	28	96	.027	34	7.9	a
11	0.0	28	95	.030	38	7.8	
12	0.0	28	94	.025	31	8.0	a
13	0.0	26	94	.065	78	8.3	b
14	0.0	32	94	.049	59	8.3	
15	0.0	31	94	.011	13	8.4	a,b
16	0.0	32	93	.070	80	8.7	a,b
17	0.0	28	92	.057	63	9.0	
18	0.0	28	92	.037	42	8.9	a
19	1.5	26	90	.081	85	9.5	b,f
20	1.3	34	88	.070	68	10.3	f
21	0.2	31	88	.063	59	10.6	f
22	1.3	33	87	.084	78	10.8	a
23	1.4	32	87	.103	106	9.7	
24	2.1	27	88	.041	42	9.8	a
25	3.1	27	87	.078	90	8.7	
26	3.4	26	87	.023	27	8.7	b
27	3.9	30	87	.041	52	7.8	a
28	5.5	30	87	.011	14	7.7	a,b
29	4.7	28	87	.056	61	9.2	a
30	5.1	27	86	.025	27	9.3	a
31	4.9	26	88	.011	15	7.2	a
32	3.2	26	87	.008	10	7.8	a
33	2.6	23	87	.026	30	8.6	
34	2.3	22	86	.005	9	6.1	a,b
35	1.7	31	83	.045	52	8.6	a
36	2.2	27	83	.039	48	8.1	
37	3.8	27	83	.018	18	9.8	a
38	1.7	24	82	.044	50	8.8	b
39	2.9	32	84	.015	16	9.5	a
40	4.2	31	84	.045	48	9.4	
41	4.4	32	85	.016	15	10.5	
42	3.8	27	85	.058	68	8.5	a
43	5.5	29	86	.011	16	7.1	a
44	4.7	28	86	.032	38	8.3	
45	4.8	27	85	.010	12	8.0	a

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
46	4.4	26	86	.040	47	8.6	
47	5.1	24	86	.013	14	9.2	a,b
48	6.1	31	85	.031	37	8.5	
49	8.2	29	86	.013	15	8.9	
50	6.6	27	85	.020	26	7.6	a
51	5.4	22	84	.017	22	7.7	b
52	5.5	34	85	.010	12	8.6	a,b
53	4.8	34	87	.038	42	9.0	
54	4.7	37	87	.013	14	9.1	
55	4.9	32	86	.077	73	10.5	a
56	3.9	30	88	.098	98	10.0	a
57	3.8	29	87	.064	62	10.3	
58	3.6	29	89	.038	37	10.3	
59	4.1	27	89	.024	21	11.4	a
60	4.3	27	89	.048	55	8.7	
61	4.6	25	90	.031	36	8.6	b
62	5.3	35	88	.073	84	8.7	a
63	6.5	36	88	.041	45	9.1	
64	6.6	32	88	.090	94	9.6	a
65	6.9	32	88	.056	62	9.1	
66	7.7	30	88	.036	38	9.5	
67	8.8	28	89	.022	23	9.6	a
68	7.2	26	90	.092	90	10.2	
69	6.4	24	90	.057	56	10.2	b
70	8.0	36	88	.031	31	10.0	a
71	5.6	32	88	.045	49	9.2	
72	4.6	30	88	.031	33	9.5	a
73	5.9	31	88	.051	56	9.1	

Subject AML-3

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-6	7.4	34	99	.117	148	7.9	
-5	4.0	33	98	.120	146	8.2	
-4							
-3	4.3	30	101	.109	136	8.0	
-2	0.8	28	99	.094	120	7.8	
-1	0.2	28	99	.073	91	8.0	
0	0.1	30	98	.059	72	8.2	i
1	0.1	35	95	.061	69	8.8	
2	0.0	35	96	.049	57	8.6	
3	0.0	33	96	.022	27	8.2	a
4	0.0	31	95	.047	57	8.3	
5	0.0	29	95	.031	38	8.1	a
6	0.0	26	96	.073	84	8.7	
7							a
8	0.0	24	94	.062	76	8.2	
9	0.0	24	95	.030	36	8.2	b
10	0.0	28	94	.023	26	8.8	a
11	0.0	28	93	.039	43	9.0	
12	0.0	28	94	.031	36	8.6	a
13	0.0	26	93	.057	59	9.6	
14	0.0	26	93	.045	47	9.5	
15	0.0	26	92	.062	92	9.6	a
16	0.0	25	92	.057	58	9.8	
17	0.0	23	91	.022	22	9.9	a,b,d
18	0.0	18	92	.056	60	9.4	b
19	0.0	28	91	.039	42	9.4	a,f
20	0.0	29	91	.050	51	9.8	f
21	0.0	27	90	.052	42	12.5	a,f
22	0.4	29	89	.082	66	12.4	b,d,f
23	0.5	32	88	.067	57	11.7	a,b,d,f
24	0.7	32	89	.063	60	10.5	a,b,d,f
25	0.7	27	89	.070	68	10.3	a,d
26	1.1	29	90	.059	61	9.6	b,d,f
27	1.6	32	89	.068	71	9.6	a,b,d,f
28	2.4	31	88	.035	36	9.8	a,d,f
29	3.2	29	87	.023	29	8.1	a,d,f
30	2.1	35	88	.050	58	8.6	a,b,d
31	4.6	26	87	.045	56	8.0	a,b,d
32	2.4	29	88	.047	53	8.8	a,b,d
33	2.4	32	88	.025	27	9.4	a
34	2.1	32	87	.057	65	8.8	a,c
35	1.5	35	88	.070	71	9.8	a,d
36	1.3	35	88	.040	44	9.1	a,c
37	0.9	36	89	.029	36	8.0	a,c
38	0.7	38	89	.043	45	9.5	a,c
39	0.8	34	88	.046	46	9.9	a,c
40	1.3	33	87	.065	74	8.8	a
41	2.0	32	88	.063	71	8.9	a,c,d

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
42	2.0	34	88	.050	60	8.4	a,c
43	2.2	34	88	.081	93	8.7	a
44	1.6	32	88	.048	51	9.5	a
45	2.4	31	88	.067	73	9.2	a,c,d
46	1.6	29	87	.056	61	9.1	a
47	2.2	29	87	.069	78	8.8	a
48	1.9	28	88	.067	68	9.8	a,c
49	3.5	31	88	.065	65	10.0	a
50	3.0	29	88	.061	65	9.4	a,c,d
51	4.4	36	87	.084	90	9.3	a
52	4.6	32	87	.081	90	9.0	a,c
53	3.7	33	88	.068	69	9.9	a
54	3.1	32	87	.069	73	9.4	a,c
55	3.5	37	87	.087	90	9.7	a
56	3.0	37	86	.088	99	8.9	a
57	2.9	34	87	.107	111	9.6	a
58	4.0	34	87	.110	116	9.5	a
59	4.7	33	87	.105	109	9.6	a
60	5.3	32	86	.108	105	10.3	a
61	5.1	30	87	.113	119	9.5	a,c
62	7.0	35	87	.117	122	9.6	a
63	5.3	32	87	.126	130	9.7	
64	7.0	26	88	.130	133	9.8	a
65	6.7	30	88	.094	98	9.6	
66	6.6	25	89	.095	104	9.1	a,c
67	7.5	30	87	.084	93	9.0	a

Subject AML-4

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-6	6.9	41	102	.179	211	8.5	
-5	11.6	39	104	.202	224	9.0	
-4	10.8	38	104	.187	208	9.0	
-3	7.5	40	104	.172	191	9.0	
-2	4.2	36	104	.146	164	8.9	
-1	4.3	37	104	.135	153	8.8	
0	2.0	36	103	.124	143	8.7	i
1	0.3	35	103	.102	122	8.4	
2	0.0	36	102	.085	103	8.3	
3	0.0	36	100	.058	69	8.4	
4	0.0	35	101	.042	49	8.6	
5	0.0	31	100	.020	25	7.9	a
6	0.0	27	99	.030	36	8.2	
7	0.0	25	98	.018	22	8.0	
8	0.0	23	98	.040	47	8.5	a,b
9	0.0	27	96	.028	33	8.4	a
10	0.0	30	96	.028	32	8.8	
11	0.0	25	96	.026	29	9.0	
12	0.0	23	96	.035	45	7.8	a,b
13	0.0	26	96	.028	35	8.1	b
14	0.1	28	94	.037	45	8.2	a
15	0.1	29	94	.029	34	8.4	a
16	0.2	32	94	.027	30	8.9	a
17	0.2	24	94	.043	48	8.9	b
18	0.2	25	93	.044	50	8.8	a
19	0.7	30	93	.025	29	8.5	a
20	0.4	26	93	.029	35	8.3	
21	0.6	25	93	.033	39	8.5	a,b
22	0.8	30	90	.036	38	9.5	a
23	1.2	31	89	.031	33	9.3	a
24	2.3	33	88	.019	20	9.3	a
25	2.5	30	90	.039	42	9.4	a
26	3.3	35	90	.054	61	8.8	
27	2.6	33	90	.029	34	8.6	
28	3.0	34	91	.033	35	9.4	a
29	2.3	34	91	.050	47	10.6	a
30	2.2	32	92	.067	60	11.1	a
31	3.3	33	92	.066	61	10.9	a
32	5.3	29	92	.119	119	10.0	a
33	3.9	30	93	.132	133	9.9	a
34	3.6	32	92	.129	130	9.9	
35	3.7	35	94	.090	85	10.6	
38	4.2	32	93	.060	64	9.3	
40	2.9	29	94	.066	75	8.8	
42	2.6	31	93	.097	118	8.2	
45	3.3	29	94	.111	143	7.7	
48	4.0	35	95	.190	237	8.0	

Subject AML-5

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
0	0.3	28	99	.076	85	8.9	i
1	0.0	36	96	.067	81	8.3	
2	0.0	35	96	.047	57	8.2	
3	0.0	36	96	.035	42	8.3	a
4	0.0	34	95	.054	66	8.2	
5	0.0	31	94	.043	51	8.4	
6	0.0	32	94	.028	36	7.7	a
7	0.0	35	94	.043	49	8.8	
8	0.0	27	94	.036	43	8.4	a
9	0.0	32	93	.061	69	8.8	
10	0.0	28	86	.041	49	8.4	
11	0.0	27	93	.039	46	8.5	a
12	0.0	25	94	.078	91	8.6	
13	0.0	29	92	.013	12	10.6	a,b
14	0.0	35	90	.062	69	9.0	b,d
15	0.0	35	89	.039	42	9.2	a,d,e
16	0.0	28	89	.072	83	8.7	a,c
17	0.0	32	87	.044	49	9.0	a
18	0.0	31	87	.049	56	8.8	a,b
19	0.0	34	87	.031	39	7.9	a,b
20	0.0	36	87	.050	61	8.2	a,b
21	0.0	39	88	.052	59	8.8	a,d,e
22	0.1	32	87	.050	57	8.8	a
23	0.4	32	87	.036	48	7.5	a,b
24	0.9	40	88	.020	23	8.9	a
25	0.8	34	87	.059	70	8.4	a,b
26	0.9	39	86	.030	37	8.0	a
27	1.0	38	86	.028	34	8.2	a,b
28	0.7	36	85	.060	72	8.3	a,b
29	0.9	35	87	.068	83	8.2	a,b,d
30	1.0	35	86	.082	90	9.1	a,b
31	0.9	34	92	.073	82	8.9	a,d

Subject AML-6

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-5	2.5	28	101	.049	60	8.2	
-4	3.9	26	104	.041	49	8.4	
-3	4.0	24	103	.030	36	8.2	b
-2	2.6	29	98	.020	24	8.5	a
-1	0.8	29	98	.050	58	8.6	
0	0.0	29	97	.043	50	8.6	i
1	0.0	28	99	.028	33	8.5	a
2	0.0	27	98	.046	56	8.3	
3	0.0	15	97	.037	49	7.6	a
4	0.0	27	98	.058	75	7.7	
5	0.1	26	98	040	53	7.5	a
6	0.0	25	99	.066	85	7.8	
7	0.1	25	100	.047	62	7.6	
8	0.1	23	100	.035	47	7.4	a,b
9	0.1	23	97	.044	56	7.9	b
10	0.6	29	96	.030	39	7.7	a
11	0.6	28	96	.050	62	8.1	a
12	1.0	27	96	.046	56	8.3	a
13	1.4	25	96	.035	42	8.3	a
14	1.7	23	97	.025	31	8.2	b
15	2.2	27	95	.023	27	8.6	a
16	2.1	30	96	.025	28	8.9	
17	2.2	27	96	.023	26	8.8	a
18	2.0	26	96	.033	36	9.2	a
19	2.0	26	97	.019	20	9.4	
20	2.9	26	97	.028	31	9.0	a
21	2.6	26	97	.022	27	8.2	a
22	2.5	26	97	.028	33	8.5	a,b
23	2.4	31	96	.025	30	8.3	a
24	3.4	31	95	.035	42	8.3	a
25	2.2	30	96	.041	47	8.8	a
26	2.4	30	96	.069	76	9.1	a
27	3.3	30	96	.081	88	9.2	a
28	4.5	30	96	.093	98	9.5	
29	3.7	30	97	.061	61	10.0	
30	4.7	31	97	.053	57	9.3	
31	6.1	30	98	.045	47	9.5	a
32	5.4	30	98	.063	64	9.8	a
33							
34	4.8	32	99	.068	72	9.5	
35	3.8	30	98	.036	40	8.9	
36	4.0	32	100	.055	56	9.8	
37	3.6	30	99	.038	36	10.5	a
38	3.6	28	100	.061	61	10.0	
39	2.8	28	100	.049	52	9.4	
40	3.0	31	100	.053	54	9.8	
41	3.2	30	98	.049	47	10.5	
42	2.7	31	99	.043	44	9.7	

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
43	3.1	31	100	.036	33	10.8	
44	2.9	30	100	.034	35	9.6	
45	2.9	29	100	.035	35	9.9	
46	3.3	30	102	.036	34	10.5	
47	3.2	30	101	.038	39	9.7	
48	3.3	31	103	.039	41	9.5	
49	4.6	30	103	.040	41	9.8	
50	4.8	33	103	.048	48	10.1	a
51	4.0	29	102	.045	49	9.2	
52	3.4	25	102	.046	43	10.6	a
53	3.9	29	99	.057	55	10.3	b
54	2.7	28	100	.044	39	11.2	
55	3.3	28	99	.046	40	11.5	a
56	4.1	29	100	.050	49	10.2	
57	3.4	30	99	.038	41	9.4	a
58	2.7	29	99	.068	68	10.0	
59	2.1	30	99	.028	42	6.8	
60	2.3	33	100	.047	47	10.1	
61	2.1	32	100	.040	36	11.0	a
62	2.3	30	100	.046	49	9.5	
63	2.3	31	100	.042	41	10.2	

Subject CML-1

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-6	25.2	32	115	.302	336	9.0	
-5	25.0	32	114	.338	363	9.3	
-4	19.3	31	113	.270	276	9.8	
-3	11.2	29	114	.242	237	10.2	
-2	5.4	28	113	.242	255	9.5	
-1	2.9	26	113	.218	232	9.4	
0	1.8	26	113	.203	221	9.2	i
1	0.9	30	109	.187	203	9.2	
2	0.5	30	109	.149	173	8.6	
3	0.3	31	109	.135	159	8.5	
4	0.2	30	108	.092	107	8.6	
5		30	107	.068	78	8.7	
6	0.0	26	107	.024	29	8.6	a
7	0.0	26	107	.018	19	9.3	a
8	0.0	24	105	.027	30	9.1	a,b
9	0.0	23	103	.013	16	7.9	a,b
10	0.0	24	100	.023	29	7.9	a,b
11	0.0	25	97	.026	27	9.6	a
12	0.0	26	96	.025	29	8.6	a
13	0.0	27	95	.015	18	8.5	b
14	0.0	25	96	.013	16	8.4	a
15	0.0	27	96	.017	17	9.8	a,b
16	0.0	26	94	.018	19	9.4	b
17	0.1	28	95	.026	26	10.0	a
18	0.0	26	94	.023	25	9.1	a
19	0.0	25	94	.023	25	9.2	a,b
20	0.0	27	93	.033	37	8.8	a
21	0.0	22	95	.028	29	9.6	a
22	0.1	27	92	.030	35	8.6	a,b
23	0.2	26	92	.033	38	8.6	a
24	0.2	26	92	.029	35	8.4	a
25	0.2	24	91	.035	41	8.6	a,b
26	0.4	27	92	.039	47	8.4	a,b
27	0.2	28	91	.038	46	8.3	b
28	0.3	28	90	.035	38	9.1	a
29	0.4	27	90	.036	43	8.4	a
30	0.2	29	90	.028	33	8.5	
31	0.3	24	89	.029	35	8.4	a,b
32	0.2	25	88	.029	32	9.2	b
33	0.5	29	87	.024	24	9.8	a
34	0.7	28	88	.031	36	8.6	a
35	0.9	26	88	.033	38	8.7	a
36	0.7	26	88	.031	37	8.5	a
37	0.8	24	88	.027	33	8.3	b
38	0.7	27	87	.035	42	8.3	a
39	0.6	27	88	.025	28	8.9	a
40	0.9	27	87	.032	41	7.9	a
41	0.9	28	88	.027	32	8.4	

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
42	1.3	27	89	.028	35	7.9	a
43	1.3	26	88	.029	37	7.8	a
44	1.2	26	89	.027	33	8.1	a
45	1.2	25	89	.022	27	8.2	b
46	1.7	31	89	.033	38	8.6	
47	1.7	31	90	.036	43	8.3	
48	2.2	36	90	.043	51	8.5	
49	2.2	35	90	.029	38	7.7	
50	1.9	33	89	.026	28	9.2	
51	1.7	31	90	.031	32	9.8	
52	1.5	30	90	.039	43	9.1	
53	1.9	31	90	.033	35	9.4	
54	2.1	33	90	.035	42	8.3	
55	1.7	31	90	.035	40	8.8	
56	1.6	31	91	.034	39	8.8	a
57	1.5	31	89	.029	34	8.6	
58	1.4	32	90	.029	35	8.4	
59	1.2	30	89	.019	21	9.1	a
60	2.2	33	89	.036	37	9.7	
61	1.4	30	90	.025	25	9.9	
62	1.5	30	90	.028	29	9.8	
63	2.3	32	91	.024	22	11.0	
64	3.0	32	91	.052	53	9.8	a
65	3.1	31	89	.038	42	9.1	
66	3.1	32	90	.030	34	8.9	
67	2.8	30	89	.023	24	9.6	
68	1.2	28	89	.022	23	9.7	
69	1.5	30	90	.021	24	8.6	

Subject CML-2

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-5	16.0	33	104	.365	392	9.3	
-4	4.6	32	103	.285	300	9.5	
-3	1.2	29	102	.243	258	9.4	
-2	1.3	27	103	.224	233	9.6	
-1	0.8	26	103	.185	199	9.3	b
0	0.4	30	99	.166	171	9.7	i
1	0.2	35	96	.139	143	9.7	
2	0.4	36	95	.111	111	10.0	
3	0.3	36	95	.071	72	9.8	
4	0.3	35	95	.043	43	9.9	
5	0.1	34	93	.019	21	8.9	a
6	0.0	30	93	.031	37	8.3	
7	0.0	31	93	.024	30	8.0	a
8	0.0	27	92	.036	44	8.1	
9	0.0	26	92	.016	20	8.0	a,b
10	0.1	28	91	.068	77	8.8	
11	0.0	26	89	.025	29	8.7	a
12	0.0	24	88	.038	43	8.9	b
13	0.1	26	87	.022	25	8.8	
14	0.1	25	89	.012	13	9.1	a,b
15	0.2	26	87	.051	57	9.0	a
16	0.2	25	88	.061	68	9.0	b
17	0.4	28	87	.037	44	8.5	a
18	0.4	29	88	.028	32	8.8	a
19	0.5	27	87	.035	38	9.3	
20	0.3	31	87	.033	36	9.2	a
21	0.8	25	87	.039	39	10.1	
22	0.8	26	87	.035	33	10.6	
23	0.7	21	88	.051	59	8.7	a,b
24	0.8	24	87	.041	45	9.0	b
25	0.8	25	88	.060	64	9.4	a
26	0.7	24	88	.047	48	9.7	a
27	0.8	22	89	.055	58	9.3	
28	1.2	26	88	.047	48	9.8	b
29	1.2	26	88	.035	37	9.4	
30	1.2	24	87	.063	67	9.4	a
31	1.4	25	88	.059	63	9.4	
32	1.5	26	88	.059	58	10.1	
33	2.4	25	88	.059	56	10.6	
34	2.9	26	89	.073	67	10.9	

Subject CML-3

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-5	6.3	40	106	.343	373	9.2	
-4	3.9	38	106	.302	332	9.1	
-3	3.2	35	106	.257	279	9.2	
-2	2.6	37	106	.214	230	9.3	
-1	0.8	37	106	.175	192	9.1	
0	0.5	44	103	.141	164	8.6	i
1	0.2	42	103	.096	107	9.0	
2	0.3	40	102	.051	60	8.5	
3	0.2	39	102	.030	35	8.7	a
4	0.0	36	101	.046	61	7.6	
5	0.0	34	101	.029	33	8.8	a
6	0.0	36	103	.044	57	7.7	
7	0.0	31	101	.019	22	8.6	a
8	0.0	28	100	.035	46	7.6	
9	0.0	27	101	.052	68	7.7	a
10	0.0	26	101	.035	45	7.8	a,b
11	0.0	35	97	.035	45	7.8	
12	0.0	33	97	.021	23	9.1	a
13	0.0	32	97	.037	44	8.4	
14	0.0	30	97	.018	20	9.0	a
15	0.0	31	96	.038	50	7.7	
16	0.0	27	95	.016	21	7.6	a
17	0.0	26	94	.021	29	7.2	
18	0.0	18	95	.012	16	7.5	a,b
19	0.0	20	93	.030	39	7.6	a,b
20	0.0	24	93	.025	31	8.2	a
21	0.1	21	91	.090	101	8.9	b
22	0.2	28	91	.053	63	8.4	
23	0.2	26	90	.038	46	8.3	a,b
24	0.2	22	90	.023	28	8.1	a,b
25	0.1	27	90	.031	39	8.0	
26	0.3	26	90	.030	35	9.0	a
27	0.2	26	91	.014	16	8.8	
28	0.3	24	91	.034	41	8.4	b
29	0.3	23	90	.022	25	8.6	a
30	0.5	26	89	.014	16	8.8	b
31	0.9	26	89	.018	20	8.8	
32	1.0	25	90	.026	29	9.1	a
33	1.3	26	90	.025	27	9.3	
34	1.5	25	90	.047	50	9.4	a,b
35	1.3	25	89	.037	39	9.6	
36	2.2	28	90	.041	45	9.2	a
37							
38	2.6	28	91	.042	44	9.5	
39	3.9	28	91	.041	43	9.6	
40	4.6	27	90	.063	72	8.8	a
41	3.6	27	90	.058	63	9.2	
42	4.0	28	91	.055	60	9.2	

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
43	4.2	28	90	.048	53	9.0	
44	4.3	28	92	.043	50	8.7	
45	4.2	27	90	.044	47	9.3	
46	5.3	29	91	.047	51	9.3	
47	5.6	27	91	.043	49	8.7	
48	5.3	28	91	.060	64	9.4	
49	3.9	25	91	.048	52	9.3	
50	4.0	25	90	.044	48	9.2	b

Subject CML-4

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-7	7.7	30	85	.653	634	10.3	
-6	8.9	29	84	.566	572	9.9	
-5	4.2	26	84	.469	474	9.9	
-4	2.7	28	84	.449	436	10.3	
-3	1.7	29	84	.400	396	10.1	
-2	2.1	33	84	.504	504	10.0	
-1	0.9	33	84	.501	482	10.4	
0	0.4	34	83	.368	372	9.9	i
1	0.3	38	83	.322	310	10.4	
2	0.3	38	84	.247	227	10.9	
3	0.3	37	84	.181	162	11.2	
4	0.2	35	83	.091	88	10.2	
5	0.2	33	83	.055	58	9.5	
6	0.0	32	83	.058	56	10.3	a
7	0.0	26	82	.031	33	9.4	a
8	0.0	25	83	.046	48	9.3	a,b
9	0.0	25	82	.019	24	7.9	a,c
10	0.0	28	82	.031	35	9.0	a
11	0.0	27	82	.032	34	9.4	
12	0.0	27	82	.036	41	8.9	a
13	0.0	21	81	.006	8	7.4	a,b
14	0.0	26	83	.029	36	8.1	a
15	0.0	23	83	.025	24	10.3	b
16							a
17	0.0	22	81	.040	48	8.4	b
18	0.1	22	83	.049	59	8.3	a
19	0.1	24	84	.030	33	9.0	a,b
20	0.0	24	86	.045	48	9.4	a,b
21	0.2	25	87	.037	42	8.9	a
22	0.2	24	86	.055	64	8.6	a,b
23	0.5	28	86	.048	57	8.5	a
24	0.5	27	86	.058	72	8.0	a
25	0.7	28	85	.076	89	8.5	a
26	0.9	24	86	.073	82	8.9	b
27	0.7	24	85	.033	34	9.7	
28	0.9	27	85	.043	50	8.6	a,b
29	0.8	28	83	.064	69	9.3	a
30	0.7	26	84	.023	29	8.1	a
31	0.9	27	84	.023	27	8.7	a
32	0.8	23	83	.032	32	9.9	a,b
33	1.3	22	83	.015	19	7.8	a,b
34	1.7	30	84	.006	10	6.0	a
35	1.8	24	83	.009	12	7.2	a
36	2.2	30	84	.013	17	7.4	a,b
37	2.8	27	84	.013	16	7.9	a
38	2.4	24	83	.015	16	9.1	a,b
39	2.3	26	86	.016	19	8.3	a,b
40	1.8	27	85	.017	21	8.1	a

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
41	1.8	29	84	.008	11	7.1	a
42	1.8	28	85	.025	25	9.4	a
43	1.6	26	85	.041	41	9.9	a
44	1.3	22	84	.046	50	9.2	b
45	1.6	27	86	.029	30	9.5	
46	1.6	26	86	.036	38	9.4	a
47	1.4	29	86	.019	22	8.5	a,b
48	1.5	31	88	.022	21	10.6	a
49	1.1	27	87	.027	25	10.7	
50	1.1	28	86	.039	41	9.4	a
51							a
52	1.5	26	87	.030	31	9.7	b
53	2.9	32	88	.030	30	10.1	a
54	2.2	31	88	.037	39	9.4	
55	1.9	28	88	.034	34	10.0	
56	1.3	30	88	.040	43	9.4	a
57	1.2	27	87	.033	33	9.9	a
58	1.3	28	88	.040	44	9.2	
59	1.3	27	88	.023	26	8.7	
60	2.2	27	89	.027	27	10.0	a
61	1.5	25	89	.022	21	10.6	
62	1.4	27	90	.032	33	9.6	b
63	1.5	34	90	.021	21	10.2	a,b

Subject CML-5

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
-3	7.2	30	112	.540	628	8.6	
-2	3.2	31	112	.571	672	8.5	
-1	2.1	28	111	.457	538	8.5	
0	0.8	25	112	.415	482	8.6	b,i
1	1.6	30	106	.296	352	8.4	
2	0.3	29	105	.244	290	8.4	
3	0.0	33	106	.166	195	8.5	
4	0.0	27	104	.118	136	8.7	a,b
5	0.0	25	106	.056	64	8.7	a
6	0.0	35	97	.041	47	8.8	
7							a
8	0.1	32	97	.013	16	8.1	a
9	0.1	29	97	.053	56	9.4	a
10	0.0	40	97	.011	13	8.4	a
11	0.0	25	97	.008	12	6.3	a
12		29	92	.022	27	8.3	a,b
13	0.5	27	86	.005	7	7.0	a,b
14	0.5	32	86	.008	13	6.3	a
15	0.2	28	87	.004	6	5.9	a
16	0.5	28	88	.002	3	5.0	a,b
17	0.5	27	87	.004	7	5.9	a

Subject CML-6

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-7	37	33	88	.427	480	8.9	
-6	48	33	88	.436	501	8.7	
-5							
-4	9.3	30	88	.322	343	9.4	
-3	2.1	29	88	.320	333	9.6	
-2	1.1	30	87	.262	285	9.2	
-1	1.4	31	87	.247	277	8.9	
0	0.8	28	87	.188	214	8.8	i
1	0.5	31	87	.142	167	8.5	
2	0.2	31	87	.114	133	8.6	
3	0.1	29	87	.074	90	8.2	
4	0.1	25	87	.045	53	8.4	a,b
5	0.1	34	87	.037	45	8.3	b
6	0.0	32	88	.020	21	9.7	a
7	0.0	32	88	.030	32	9.3	
8	0.0	28	88	.044	49	9.0	a,b
9	0.0	29	87	.034	41	8.3	a,b
10	0.0	29	86	.047	51	9.3	
11	0.0	28	88	.042	44	9.6	a
12	0.0	24	88	.026	28	9.4	b
13	0.0	28	88	.025	24	10.6	a,b
14	0.0	26	88	.044	42	10.4	a
15	0.0	25	88	.029	29	10.0	a,b
16	0.0	29	87	.043	38	11.2	a
17	0.0	32	87	.030	27	11.1	a
18	0.0	29	87	.032	31	10.3	a
19	0.0	29	87	.031	27	11.5	a
20	0.0	26	87	.036	35	10.4	
21	0.0	26	87	.011	12	9.2	a
22	0.0	26	86	.050	53	9.4	a
23	0.0	25	86	.051	54	9.5	b
24	0.0	28	87	.040	40	10.0	a,b
25	0.0	31	86	.044	45	9.7	a
26	0.0	32	86	.028	30	9.3	a
27	0.0	26	87	.025	29	8.5	a
28	0.0	29	86	.046	53	8.6	a
29	0.0	24	85	.043	45	9.5	b
30	0.0	31	87	.041	48	8.5	a
31	0.2	28	86	.034	39	8.6	a
32	0.6	30	86	.033	36	9.1	
33	0.6	31	86	.056	64	8.7	a
34	0.9	31	87	.058	64	9.0	
35	0.7	28	87	.037	39	9.4	
36	0.8	28	86	.067	71	9.4	a
37	1.4	28	85	.060	61	9.9	
38	1.4	25	86	.067	66	10.1	a
39	1.4	27	86	.088	89	9.9	
40	2.2	26	88	.098	99	9.9	

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
41	2.6	29	88	.140	132	10.6	
42	3.2	30	88	.163	157	10.4	
43	3.8	28	88	.149	152	9.8	
44	4.4	27	88	.197	182	10.8	
45	3.8	26	87	.197	193	10.2	
46	6.0	28	87	.166	175	9.5	

Subject CML-7

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-2	1.6	30	100	.185	250	7.4	
-1	1.2	32	99	.143	193	7.4	
0	0.5	34	99	.112	150	7.5	i
1	0.4	34	97	.065	86	7.6	
2	0.3	35	97	.053	66	8.0	
3	0.3	36	97	.036	41	8.7	a
4	0.2	32	97	.038	44	8.7	
5	0.2	31	97	.021	27	7.7	a
6	0.2	28	96	.040	45	8.9	
7	0.0	26	96	.048	52	9.2	a,b
8	0.0	28	93	.055	60	9.1	a
9	0.0	25	95	.043	53	8.2	a,b
10	0.0	30	92	.034	40	8.5	a
11	0.0	29	92	.027	31	8.6	a
12	0.0	29	91	.036	41	8.7	
13	0.0	30	91	.019	21	8.9	a
14	0.0	24	91	.058	61	9.5	a,b
15	0.0	29	89	.047	53	8.9	b,f,g
16	0.0	27	88	.068	66	10.3	b,f
17	0.1	28	90	.070	65	10.8	f
18	0.1	27	89	.040	39	10.4	f
19	0.1	27	90	.071	57	12.5	a,f
20	0.1	27	88	.078	66	11.8	a,b,f
21	0.1	28	89	.048	43	11.2	a,b
22	0.2	30	88	.040	43	9.3	a,b,d
23	0.3	30	87	.034	38	9.0	b,f
24	1.2	31	87	.049	46	10.7	f
25	1.0	28	86	.040	41	9.7	a,b,f
26	1.7	30	86	.055	51	10.7	a,b,d,f
27	2.2	26	89	.090	96	9.4	a,b,d,e,f
28	3.2	23	88	.081	86	9.4	a,b,d,e
29	4.3	35	88	.083	81	10.2	a,b
30	3.7	32	88	.090	87	10.4	a,b,d
31	4.1	33	89	.075	77	9.8	a,b,d,e
32	3.0	33	89	.063	63	10.0	a,b
33	3.4	36	89	.069	67	10.3	a
34	2.7	38	90	.047	44	10.6	a
35	2.6	34	89	.046	47	9.8	a
36	2.5	35	90	.068	64	10.7	a,b
37	1.4	32	88	.068	71	9.6	a.b
38	2.2	32	89	.081	77	10.5	a
39	1.7	35	89	.054	52	10.4	a,b
40	1.7	37	88	.056	58	9.7	a
41	1.7	34	87	.040	41	9.7	a
42	1.3	37	87	.049	47	10.4	b
43	1.2	35	87	.049	49	10.1	a
44	1.1	38	89	.035	35	10.1	a,b

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS
45	0.9	36	88	.050	49	10.3	a
46	0.7	35	88	.051	52	9.8	a
47	0.6	36	88	.042	44	9.5	a
48	0.4	33	87	.033	34	9.6	a
49	0.5	29	88	.037	39	9.4	a
50	0.6	29	88	.044	47	9.4	a
51	0.2	26	88	.030	29	10.5	a
52	0.4	23	88	.035	39	9.1	a
53	0.5	26	88	.040	46	8.8	a
54	0.4	23	88	.029	27	10.8	a,b
55	0.5	28	88	.036	36	10.1	a

Subject CML-8

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-7	21.5	40	108	.367	459	8.0	
-6	28.9	43	109	.491	578	8.5	
-5	17.2	44	109	.421	490	8.6	
-4	6.2	35	108	.199	221	9.0	
-2	1.6	33	108	.144	164	8.8	
-2	0.8	30	107	.128	144	8.9	
-1	0.4	31	107	.199	134	8.9	
0	0.4	33	106	.105	123	8.5	i
1	0.4	41	105	.100	114	8.8	
2	0.2	38	104	.069	79	8.8	
3	0.1	39	104	.042	48	8.8	
4	0.1	32	103	.050	55	9.0	a
5	0.0	35	98	.068	77	8.8	a,b
6	0.0	32	98	.036	38	9.5	a
7	0.0	33	99	.055	57	9.6	a
8	0.0	35	99	.046	46	9.9	a
9	0.0	28	100	.023	27	8.6	a
10	0.0	27	101	.032	33	9.6	a
11	0.0	25	98	.036	39	9.2	a
12	0.0	24	100	.032	34	9.3	a
13	0.0	22	99	.028	32	8.7	b
14	0.1	26	95	.034	40	8.6	a
15	0.0	24	94	.033	36	9.2	a,b
16	0.2	35	94	.044	46	9.5	
17	0.2	30	92	.041	43	9.6	a
18	0.5	34	93	.056	62	9.1	a
19	0.2	30	92	.039	45	8.7	a
20	0.4	27	92	.041	45	9.0	a
21	0.3	29	91	.044	46	9.5	b
22	0.6	37	89	.044	48	9.2	a
23	0.6	34	88	.049	55	9.0	
24	0.8	34	88	.044	50	8.8	a
25	0.9	30	89	.057	66	8.7	
26	1.1	29	88	.034	39	8.8	a
27	1.0	27	90	.039	49	7.9	
28	1.0	26	90	.029	37	7.8	a
29	1.0	25	89	.045	49	9.1	a,b
30	1.5	29	88	.052	56	9.2	
31	0.9	29	88	.021	24	8.8	a
32	0.8	29	90	.031	37	8.4	
33	0.9	29	88	.030	33	9.0	a
34	1.1	32	89	.032	35	9.1	
35	1.1	31	89	.023	23	10.1	a
36	1.1	27	90	.037	42	8.8	
37	1.1	27	92	.031	35	8.8	
38	1.6	27	90	.022	27	8.3	a
39	1.8	26	89	.020	23	8.5	
40	2.1	26	90	.021	22	9.7	

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
41	2.9	26	90	.026	30	8.6	
42	3.1	24	90	.024	31	7.6	
43	3.0	22	92	.024	29	8.2	b
44	3.7	37	91	.030	36	8.2	
45	3.0	29	91	.036	44	8.2	

Subject AA-1

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-6	1.6	27	86	.042	50	8.3	a
-5	1.0	26	87	.029	35	8.3	
-4	0.6	26	86	.074	83	8.9	
-3	0.2	24	86	.094	101	9.3	a,b
-2	0.2	29	86	.069	75	9.2	
-1	0.1	29	86	.065	60	10.2	
0	0.0	27	86	.086	100	8.6	a,i
1	0.1	31	85	.082	93	8.8	
2	0.2	32	86	.064	77	8.3	
3	0.2	30	85	.047	52	9.1	a
4	0.2	30	86	.050	62	8.1	
5	0.1	29	85	.037	46	8.1	
6	0.1	28	86	.043	53	8.1	a
7	0.0	26	85	.033	42	7.8	
8	0.0	24	85	.055	57	9.7	a,b
9	0.0	29	86	.014	16	8.9	a
10	0.0	27	85	.044	47	9.3	a
11	0.0	25	85	.051	57	9.0	a
12	0.0	37	84	.046	54	8.6	
13	0.0	23	84	.056	60	9.4	a,b
14	0.0	32	85	.074	78	9.5	
15	0.0	30	84	.036	38	9.5	a
16	0.0	26	84	.047	52	9.1	a
17	0.0	24	85	.053	58	9.1	a,b
18	0.0	31	84	.069	78	8.8	a
19	0.0	29	84	.060	68	8.8	a
20	0.0	29	85	.068	79	8.6	
21	0.0	26	83	.051	57	8.9	a
22	0.0	26	83	.071	77	9.2	a
23	0.2	22	84	.076	84	9.1	a,b
24	0.8	30	84	.084	96	8.7	
25	0.9	29	83	.060	70	8.6	
26	0.7	29	83	.051	61	8.3	
27	0.8	30	84	.036	45	7.9	
28	0.7	31	84	.028	31	8.9	a
29	0.5	28	82	.041	46	8.9	
30	0.5	25	83	.020	21	9.3	a
31	0.5	25	84	.051	55	9.3	b
32	0.7	31	85	.039	43	9.0	a
33	1.0	31	84	.076	81	9.4	
34	1.4	31	85	.060	63	9.6	a
35	1.9	30	84	.070	71	9.8	
36	2.9	32	84	.064	72	8.9	a
37	2.6	29	83	.078	84	9.3	
38	4.4	34	83	.069	84	8.2	
40	3.5	31	84	.066	79	8.3	
41	2.9	30	84	.068	85	8.0	

Subject AA-2

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS.
-1	0.0	30	84	.045	56	8.1	a,b
0	0.0	28	84	.080	89	9.0	i
1	0.3	28	84	.090	107	8.4	a
2	0.3	31	84	.070	86	8.1	
3	0.0	29	86	.063	75	8.4	a
5	0.0	28	85	.052	63	8.2	
5	0.0	27	84	.037	46	8.1	
6	0.0	26	85	.016	20	7.8	a
7	0.0	26	85	.051	55	9.3	
8	0.0	23	84	.040	46	8.7	b
9	0.0	28	85	.043	49	8.8	a
10	0.0	29	86	.027	32	8.5	a
11	0.0	27	86	.048	55	8.7	
12	0.0	27	85	.034	40	8.4	
13	0.0	26	85	.045	53	8.4	a
14	0.0	26	85	.027	33	8.1	a
15	0.0	26	84	.043	47	9.1	b
16	0.0	25	85	.023	25	9.4	a
17	0.0	25	85	.052	60	8.7	
18	0.0	29	85	.018	21	8.5	a
19	0.0	22	84	.015	17	8.7	a,b
20	0.0	27	86	.036	41	8.9	a,b
21	0.0	31	87	.036	43	8.4	a
22	0.0	30	86	.049	56	8.8	a
23	0.0	33	85	.047	54	8.7	
24	0.0	29	85	.053	60	8.9	a
25	0.0	29	85	.053	57	9.3	a
26	0.0	29	86	.038	41	9.2	a
27	0.2	34	87	.044	50	8.8	a,b,f
28	0.3	32	86	.058	59	9.8	
29	0.2	27	86	.048	50	9.7	f
30	0.2	32	86	.044	45	9.7	f
31	2.1	32	85	.025	26	9.6	a,f,g
32	5.5	30	86	.019	25	7.4	a
33	5.1	30	86	.018	24	7.3	a,d
34	1.7	30	86	.005	8	6.5	a
35	2.2	25	86	.008	11	7.3	a,b
36	3.1	35	85	.018	18	9.9	a,b
37	3.1	33	85	.013	16	8.1	a
38	2.3	32	85	.013	18	7.3	a
39	3.1	32	85	.027	30	9.0	a
40	2.9	31	85	.009	12	7.5	a
41	4.0	31	85	.021	23	9.1	a
42	3.4	28	85	.026	29	9.0	a
43	4.2	27	84	.072	80	9.0	a
44	3.8	26	84	.095	104	9.1	a
45	4.6	22	82	.099	101	9.8	a,b
46	4.7	19	84	.036	38	9.4	a,b,d

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS.
47	5.0	30	88	.041	41	10.1	a,b
48	5.6	31	88	.073	82	8.9	a,b
49	6.0	29	87	.066	75	8.8	
50	5.1	32	85	.074	76	9.7	a,b
51	5.3	29	86	.087	88	9.9	
52	5.8	33	87	.030	30	9.9	a,b
53	6.8	34	88	.086	87	9.9	a
54	8.2	33	88	.084	76	11.1	a
55	8.2	33	88	.087	64	13.6	a
56	7.6	33	88	.091	84	10.8	
57	7.0	31	89	.093	83	11.2	a,b
58	7.0	34	89	.144	144	10.0	a,b
59	6.6	35	89	.054	52	10.4	a
60	6.0	35	89	.052	54	9.7	
61	7.9	24	89	.123	113	10.9	a,b,c,d
62	7.9	30	89	.073	72	10.2	a,b,d
63	8.2	30	89	.122	127	9.6	a
64	6.1	29	87	.080	78	10.3	
65	6.5	29	89	.069	72	9.6	a
66	7.3	27	90	.083	91	9.1	a
67	8.1	30	89	.092	96	9.6	a
68	8.5	28	90	.079	84	9.4	
69	8.4	30	90	.040	43	9.4	a
70	11.1	34	90	.072	73	9.9	
71	10.1	32	90	.051	49	10.4	a
72	10.5	35	90	.071	83	8.5	
73	14.8	35	90	.055	58	9.5	
74	14.1	35	90	.036	38	9.4	
75	11.6	33	90	.023	23	10.2	
76	12.8	34	91	.029	30	9.7	
77	12.4	32	90	.023	22	10.5	a
78	11.9	32	91	.056	52	10.8	
79	1.6	32	90	.028	29	9.5	a
80	11.7	32	90	.090	88	10.2	
81	11.6	32	91	.048	45	10.7	
82	13.5	32	90	.026	27	9.8	a
83	12.3	26	88	.050	55	9.0	b
84	11.2	34	88	.024	26	9.2	a,b
85	11.9	34	87	.059	54	11.0	a
86	10.4	33	88	.053	54	9.8	
87	9.2	31	88	.039	40	9.8	a
88	6.0	28	86	.034	37	9.1	
89	9.9	35	86	.030	36	8.2	
90	7.5	33	88	.056	54	10.4	

Subject AA-3

DAY	WBC	HCT	MCV	PLTCRIT	PLTCT	MPV	TRANS
-5	2.7	24	93	.045	40	11.2	
-4	1.9	22	93	.034	30	11.2	a
-3							b
-2	1.1	26	91	.043	48	9.0	
-1	0.6	25	91	.035	38	9.1	
0	0.1	24	91	.037	42	8.9	a,i
1	0.2	34	89	.047	50	9.4	
2	0.3	31	89	.034	39	8.7	
3	0.2	31	90	.026	30	8.8	
4	0.2	30	88	.060	62	9.7	a
5	0.1	28	88	.046	49	9.4	a
6	0.1	25	87	.067	70	9.5	
7	0.1	27	89	.056	60	9.3	
8	0.1	25	86	.039	42	9.4	a
9	0.1	23	85	.049	55	8.9	b
10	0.0	30	86	.035	38	9.3	a
11	0.1	29	87	.061	63	9.7	
12	0.2	30	86	.049	51	9.6	a
13	0.4	29	86	.085	97	8.8	
14	0.8	29	86	.065	75	8.6	
15	0.9	27	85	.059	67	8.8	a
16	1.8	27	86	.051	58	8.8	
17	1.4	25	84	.025	25	9.8	a
18	2.1	26	86	.066	73	9.1	
19	0.8	22	85	.066	26	8.2	a
20	1.2	24	85	.075	77	9.8	b
21	1.1	30	87	.048	49	9.8	
22	2.6	31	88	.053	56	9.4	
23	3.0	31	88	.053	54	9.1	
24	5.0	34	89	.049	49	9.9	
25	5.1	30	88	.048	49	8.5	
26	6.8	33	90	.042	47	8.1	
27	6.0	33	90	.038	42	9.0	
28							
29							
30	5.2	31	90	.029	34	8.5	
31	5.5	30	91	.031	32	9.7	
32	3.9	28	91	.027	34	7.9	
33	3.6	28	91	.029	34	8.4	
34	2.7	26	91	.027	29	9.3	
35	2.5	30	91	.029	30	9.6	
36	2.7	29	93	.035	40	8.7	
37	3.2	28	91	.034	45	7.5	

Subject AA-4

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
-3	0.4	36	92	.032	36	9.0	a
-2	0.0	36	91	.052	57	9.1	
-1	0.0	37	91	.035	39	8.9	a
0	0.0	34	90	.058	70	8.3	i
1	0.0	40	90	.051	64	8.0	
2	0.3	38	90	.032	42	7.5	a
3	0.1	39	90	.058	74	7.8	
4	0.0	40	89	.046	60	7.6	a
5	0.0	37	90	.095	116	8.2	
6	0.0	35	90	.064	76	8.4	
7	0.0	33	89	.041	49	8.4	
8	0.0	30	90	.022	26	8.3	
9	0.0	26	90	.055	60	9.2	a,b
10	0.0	32	89	.049	50	9.9	a
11	0.0	30	88	.064	72	8.9	
12	0.0	30	88	.029	34	8.6	
13	0.0	25	89	.027	33	8.2	a
14	0.0	29	88	.030	38	7.9	b
15	0.0	26	89	.038	45	8.5	a
16	0.0	39	89	.037	36	10.3	a
17	0.0	25	88	.066	70	9.4	f
18	0.4	22	88	.066	73	9.0	a,f
19	0.7	15	87	.066	71	9.3	b
20	0.7	25	87	.017	16	10.5	a,b
21	0.8	30	88	.077	78	9.9	a,d,e
22	1.2	30	89	.125	130	9.6	a,b
23	1.3	30	89	.145	169	8.6	
24	1.5	30	88	.066	69	9.5	a
25	1.6	27	88	.060	65	9.3	
26	1.0	25	87	.015	15	10.1	a
27	0.7	23	87	.028	37	7.7	a,b
28	0.9	25	86	.044	45	9.7	a
29	0.9	23	87	.063	72	8.8	b
30	1.3	38	87	.015	17	8.6	a
31	1.1	33	86	.050	55	9.1	a,d,e
32	0.9	31	86	.032	33	9.6	a
33	0.5	25	87	.064	74	8.6	a,b,d,e
34	0.5	28	88	.072	80	8.4	a
35	0.7	29	87	.063	64	9.8	
36	0.8	26	87	.074	77	9.6	a
37	0.6	27	86	.121	139	8.7	
38	1.4	25	86	.042	48	8.7	b,d,e
39	0.9	27	86	.096	105	9.1	a,b,d
40	1.0	29	87	.039	40	9.8	a
41	1.2	28	87	.101	93	10.9	a,b
42	1.1	26	87	.098	83	11.8	d,e
43	1.1	26	87	.082	76	10.8	a

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
44	1.3	23	86	.097	100	9.7	a,b,d,e
45	1.6	28	87	.097	101	9.6	a
46	2.0	26	87	.068	62	11.0	a
47	1.7	29	87	.098	89	11.0	a
48	1.6	27	87	.098	93	10.5	b
49	1.5	29	88	.074	76	9.8	a,b,d,e
50	1.4	29	88	.136	140	9.7	a
51	1.5	28	89	.126	138	9.1	
52	1.6	27	88	.080	89	9.0	a,b,d,e
53	1.3	31	87	.075	88	8.5	a,b
54	1.4	32	88	.135	147	9.2	a
55	1.9	32	87	.107	115	9.3	a
56	2.4	29	87	.122	127	9.6	
57	1.6	26	86	.062	67	9.3	a,b
58	1.9	28	86	.099	108	9.2	a
59	1.5	28	85	.083	91	9.1	a
60	2.1	25	85	.093	103	9.0	a,b
61	2.4	31	86	.109	130	8.4	
62	2.2	28	86	.119	132	9.0	a
63	1.6	25	86	.067	74	9.0	a,b
64	1.8	28	86	.098	103	9.5	
65	2.0	26	86	.051	59	8.6	
66	1.8	22	86	.068	73	9.3	a,b
67	1.6	28	87	.037	37	9.9	a
68	1.7	27	86	.060	71	8.4	
69	1.4	30	86	.027	35	7.8	a,b
70	1.2	28	87	.034	40	8.6	
71	1.0	27	87	.019	19	9.8	a,d
72	1.8	22	88	.051	57	9.0	a,b,d
73	2.5	28	87	.060	67	9.0	a
74	2.1	27	88	.082	83	9.9	a,d
75	1.6	27	87	.098	93	10.5	b
76	1.6	31	86	.071	63	11.2	
77	1.9	31	86	.084	82	10.2	a
78	2.5	32	86	.086	84	10.2	a
79	2.1	29	85	.051	52	9.9	a
80	3.6	29	84	.079	85	9.3	
81	2.4	26	85	.052	59	8.8	a
82	1.7	23	84	.068	78	8.7	b
83	2.7	29	85	.038	42	9.1	
84	2.3	25	84	.020	21	9.3	a,b
85	1.8	24	85	.063	75	8.4	b
86	2.6	32	86	.040	50	7.9	
87	1.9	30	86	.022	24	9.1	a
88	1.4	28	85	.039	41	9.6	
89	0.8	23	36	.018	22	8.4	
90	0.8	21	86	.039	46	8.5	a,b

Subject AA-5

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
-4	3.8	34	87	.126	139	9.1	b
-3	3.5	33	87	.092	35	9.7	
-2	1.1	33	87	.066	68	9.7	a
-1	0.3	33	87	.036	39	9.3	
0	0.2	29	86	.012	16	7.8	i
1	0.3	35	88	.104	128	8.1	a
2	0.2	31	87	.070	88	7.9	
3	0.1	30	88	.051	67	7.6	
4	0.1	30	88	.039	53	7.4	a
5	0.0	14	88	.023	31	6.7	
6	0.0	26	86	.012	16	7.3	b
7	0.0	31	87	.055	58	9.4	a
8	0.0	31	88	.026	26	9.9	
9	0.1	29	88	.006	6	9.3	a
10	0.0	28	88	.019	23	8.2	
11	0.0	27	88	.026	29	8.9	a
12	0.0	38	88	.010	14	7.0	a
13	0.0	31	88	.016	16	7.8	a
14	0.0	25	88	.068	85	8.0	
15	0.0	15	86	.060	71	8.4	a,b
16	0.0	31	89	.114	137	8.3	a,b
17	0.0	23	87	.087	105	8.3	a,b
18	0.0	30	88	.075	85	8.4	c
19	0.0	31	89	.007	10	7.3	a,b
20	0.0	19	89	.105	112	9.4	a,b,d

Subject NRB-1

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
-4	1.7	23	85	.216	327	6.6	
-3	1.4	23	86	.214	320	6.7	b
-2	1.3	35	87	.187	287	6.5	
-1	0.6	34	87	.180	272	6.6	
0	0.4	32	87	.138	206	6.7	i
1	0.2	29	86	.097	142	6.8	
2	0.0	29	86	.075	115	6.5	
3	0.0	26	86	.046	69	6.7	
4	0.0	24	85	.026	36	7.1	a
5	0.0	23	85	.132	159	8.3	b
6	0.0	30	87	.095	104	9.1	
7	0.0	29	85	.061	66	9.2	b
8	0.0	29	85	.121	121	10.0	a
9	0.0	24	85	.086	96	9.0	a,b
10	0.1	30	84	.085	97	8.8	b
11	1.0	29	83	.149	180	8.3	a
12	0.6	41	85	.104	128	8.1	b
13	1.1	40	85	.052	63	8.2	
14	1.2	38	86	.028	32	8.7	
15	1.4	36	84	.104	129	8.1	a
16	1.2	35	86	.077	89	8.7	
17	1.2	25	85	.052	68	7.6	
18	1.0	30	84	.032	42	7.6	a
19	0.8	28	86	.113	120	9.4	
20	0.8	27	84	.059	62	9.5	
21	1.0	34	86	.034	39	8.8	a,b
22	0.7	31	86	.169	214	7.9	
23	0.8	30	86	.141	183	7.7	
24	0.9	30	85	.106	140	7.6	
25	0.8	27	85	.060	84	7.2	
26	0.8	32	84	.033	48	6.8	b
27	0.6	32	84	.044	60	7.4	a
28	0.2	30	86	.030	41	7.3	
29	0.8	29	87	.123	131	9.4	a
30	0.9	27	86	.086	91	9.4	
31	0.9	25	86	.060	65	9.3	b
32	1.1	36	85	.044	48	9.1	b
33	0.4	34	85	.022	24	9.2	b
34	0.2	30	86	.047	53	8.8	
35	0.8	27	85	.022	24	9.2	a,i
36	0.5	24	84	.038	48	7.9	b
37	0.5	33	85	.017	19	9.1	a
38	0.5	32	87	.070	82	8.5	
39	0.6	31	85	.067	29	9.2	
40	0.7	28	85	.150	170	8.8	a
41	0.9	27	86	.072	78	9.2	
42	0.8	27	85	.032	33	9.7	a
43	0.2	22	85	.097	105	9.2	a,b

DAY	WBC	HCT	MCV	PLTCRT	PLTCT	MPV	TRANS
44	0.4	29	85	.017	15	11.2	a
45	1.4	25	86	.010	13	7.9	a
46	1.1	33	87	.098	111	8.8	b
47	1.9	31	86	.029	30	9.6	a
48	1.7	28	88	.102	110	9.3	
49	1.7	27	88	.038	37	10.3	a
50	1.8	25	86	.032	33	9.8	a
51	1.9	34	88	.019	22	8.6	a,c
52	1.6	31	86	.176	183	9.6	a
53	1.7	30	87	.125	126	9.9	
54	1.5	29	87	.091	91	10.0	
55	1.3	29	88	.059	60	9.8	a
56	0.8	26	87	.122	140	8.7	
57	1.0	25	86	.078	85	9.2	
58	1.8	26	87	.056	59	9.5	
59	1.6	24	87	.031	34	9.1	a
60	1.0	32	87	.192	223	8.6	a,b
61	1.6	28	87	.065	68	9.6	
62	1.4	27	88	.091	101	9.0	a
63	1.5	26	87	.069	77	8.9	
64	1.7	28	86	.042	48	8.7	
65	1.4	21	87	.115	122	9.4	a,b
66	0.9	27	88	.058	62	9.4	
67	1.0	23	88	.057	64	8.9	a,b
68	0.8	30	89	.153	166	9.2	a
69	1.4	32	89	.232	258	9.0	a,e
70	1.5	20	90	.158	176	9.0	
71	1.6	25	89	.092	103	8.9	
72	1.8	26	90	.055	61	9.0	a
73	1.2	22	88	.045	49	9.1	b
74	1.9	28	87	.074	88	8.4	a
75	1.9	36	88	.129	150	8.6	b
76	1.8	36	87	.095	106	9.0	
77	1.5	32	86	.069	78	8.8	a

Subject NRB-2

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
0	0.5	36	89	.131	192	6.8	i
1	0.2	31	88	.061	83	7.4	
2	0.2	31	88	.026	35	7.4	a
3	0.0	28	88	.040	43	9.2	
4	0.0	25	87	.140	157	8.9	a
5	0.0	23	88	.085	94	9.0	b
6	0.0	31	87	.056	62	9.0	
7	0.0	24	87	.127	135	9.4	a
8	0.0	19	88	.087	92	9.5	b
9	0.1	27	88	.053	59	8.9	
10	0.5	26	87	.126	152	8.3	a
11	0.3	27	87	.113	149	7.6	
12	0.7	24	86	.006	8	7.1	a,b
13	0.2	26	87	.004	6	7.3	a
14	0.3	23	87	.027	28	9.6	a,b
15	0.2	27	88	.006	9	6.9	a
16	0.4	21	87	.004	6	6.5	a,b
17	1.2	24	86	.002	4	5.6	a
18	0.9	24	87	.002	4	5.9	a,b
19	1.3	27	86	.005	8	5.9	a,b
20	0.9	36	89	.003	5	6.0	a,b
21	0.8	35	89	.002	5	4.9	a,b
22	0.9	33	88	.002	4	4.7	a,b
23	1.2	30	87	.013	15	8.7	a,b
24	2.1	33	85	.006	8	7.1	a,b
25	1.7	30	87	.005	9	6.0	a,b,d
26	1.7	33	87	.007	11	6.3	a,b
27	1.0	29	85	.011	13	8.5	a,b,d,e
28	1.2	27	86	.014	16	8.6	a,b
29	2.5	28	86	.008	11	7.2	a,b
30	0.9	32	86	.015	21	7.3	a,b,d,e
31	1.3	38	91	.011	16	7.1	a,b,d,e
32	1.2	41	89	.023	31	7.3	a,b,d,e
33	1.1	34	90	.033	40	8.3	a,b,d,e
34	2.8	34	88	.016	22	7.4	a,e
35	3.5	28	86	.018	22	8.1	a,b

Subject MPS-1

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
1	1.7	25	84	.111	117	9.5	i
2	1.0	29	85	.088	95	9.3	
3	0.3	28	84	.067	73	9.2	
4	0.0	27	85	.050	53	9.4	
5	0.0	26	85	.030	33	9.0	a
6	0.0	24	84	.054	56	9.7	
7	0.0	24	83	.045	51	8.9	
8	0.0	24	83	.030	33	9.1	a
9	0.0	21	83	.057	69	8.3	
10	0.0	30	85	.022	27	8.1	a,b
11	0.0	27	85	.041	49	8.4	
12	0.0	27	84	.031	38	8.2	a
13	0.1	27	84	.073	78	9.3	
14	0.1	30	84	.044	50	8.8	a
15	0.2	22	84	.093	106	8.8	b
16	0.3	32	86	.065	76	8.6	
17	0.7	32	86	.056	67	8.4	
18	0.7	31	85	.046	56	8.2	
19	0.7	32	86	.032	41	7.7	
20	0.6	29	85	.037	46	8.1	a
21	0.7	30	86	.049	58	8.5	
22	0.7	30	88	.045	54	8.3	
23	0.8	31	87	.038	46	8.2	
24	0.9	29	88	.028	34	8.3	
25	1.0	27	86	.023	30	7.7	
26	1.0	25	88	.062	77	8.1	a
27	0.8	26	88	.048	61	7.8	
28	0.9	24	87	.033	44	7.6	
29	1.0	28	86	.028	36	7.9	
30	1.3	26	88	.022	29	7.5	
31	1.6	28	87	.021	25	8.3	a
32	1.9	30	87	.096	132	7.3	a
33	3.2	33	87	.092	126	7.3	
34	3.4	35	87	.109	149	7.3	
35	3.9	33	88	.097	139	7.0	
36	5.0	34	86	.099	127	7.8	
37	4.8	25	86	.081	101	8.0	
38	2.3	19	88	.024	32	7.4	
39	3.9	28	89	.037	49	7.5	b
40	2.2	24	89	.038	52	7.4	
41	1.8	28	88	.047	60	7.8	
42	2.1	25	89	.042	55	7.7	
43	1.8	25	88	.041	60	6.9	
44	1.4	26	89	.047	63	7.4	
45	3.0	28	90	.046	59	7.8	
46	3.1	26	90	.045	59	7.7	
47	3.2	25	90	.040	52	7.6	
48	4.8	28	92	.045	59	7.7	

DAY	WBC	HCT	MCV	PLT CRT	PLT CT	MPV	TRANS
49	5.4	27	93	.050	67	7.5	
50	6.0	26	94	.047	60	7.9	
51	5.6	24	94	.063	74	8.5	
52	4.8	26	95	.051	61	8.4	
53	5.5	28	95	.065	79	8.2	
54	4.7	27	96	.060	79	7.6	
55							
56	2.7	27	96	.071	95	7.5	
57							
58	2.5	28	96	.074	91	8.1	
59							
60	3.4	30	96	.076	97	7.8	
61							
62	3.2	27	95	.057	75	7.6	
63							
64	3.0	26	95	.057	71	8.0	
65							
66	4.2	28	95	.057	69	8.2	d
67							d
68	2.8	26	95	.057	71	8.0	
69							
70	2.3	24	93	.051	62	8.2	
71							
72	2.4	27	93	.054	67	8.0	
73							
74							
75							
76	3.4	27	93	.067	80	8.4	
77							
78	3.4	28	93	.065	80	8.1	
79							
80	6.7	30	92	.071	84	8.5	
81							
82	3.3	30	93	.049	62	7.9	
83							
84	3.0	27	92	.058	66	8.8	
85							
86	2.7	26	91	.050	62	8.1	
87	3.1	24	91	.048	61	7.9	
88							
89	3.6	26	89	.051	61	8.3	
90	2.9	21	89	.048	58	8.3	b
91	3.2	30	89	.057	69	8.3	
92	2.6	30	89	.060	70	8.6	

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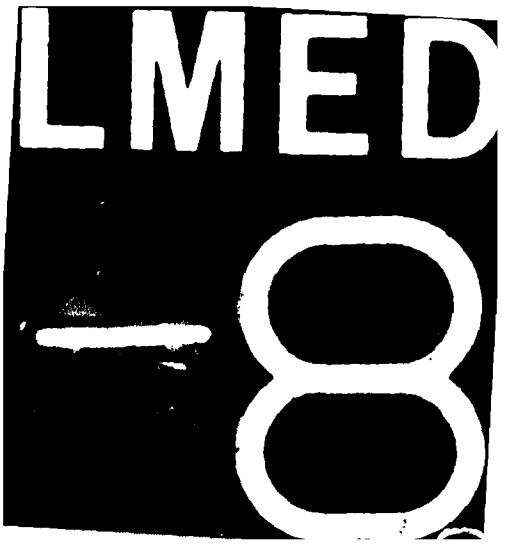
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