

White Blood Counts In Apparently Healthy Sudanese Blood Donors in Gezira State (Sudan)

AA Abbas^{1*}, A.KH Khalil¹, H Yasir², S Fadlallah³ and O Huwaida⁴

¹Department of Basic medical science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia.

²Sennar University, Sudan.

³Taif University, Kingdom of Saudi Arabia.

⁴Ministry of Health, Sudan.

Accepted 13th November, 2015

Objective: To detect white blood counts in apparently healthy male blood donors, to establish safety for both donor and recipient and also to transfuse safe blood and blood products. To perform white blood count for donors using automated machine (Blood cell counter).

Material and Methods: Venous blood samples were taken from 500 apparently healthy male donors and the white blood count was measured using an automated cell counter (sysmex KN21), accompanied by peripheral blood films were assessed to detect any abnormalities and for differential count.

Results: The study revealed that the mean values of white blood cells were found to be 5.696 ± 1.7989 with minimum count $2.1 \times 10^9 /L$ and maximum count $14.1 \times 10^9 /L$, a considerable numbers of donors were leucopenic, 77 cases ranged from 2.1 to 3.9 which represents 15.4% and a few numbers with leucocytosis, 5 cases ranged from 11.1 to 14.1 which represents 1%, dominated by neutrophils which suggestive of pyogenic infection, high eosinophil percentage more than 4% observed in 121 donors (24.2%), the bulk of this eosinophilia probably reflects asymptomatic parasitism (e.g. schistosomiasis). Monocyte percentage more than 8 % observed in 6 donors (1.2%) and high lymphocyte percentage with the reactive forms of lymphocyte also detected most probably due to chronic infection.

Conclusion: The study revealed that significant numbers of donors with low & high white blood count, which indicate that they were not eligible for blood donation.

Key words: Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils.

INTRODUCTION

The modern transfusion medicine is concerned with proper selection and utilization of blood components. Safe and efficient blood transfusion practice depends on elimination of clerical errors within the laboratory. Consideration also given to the patient's clinical history, particularly with respect to previous transfusion, pregnancy and drugs and a satisfactory pre-transfusion testing to ensure donor-recipient compatibility is essential. About 5% of the general population donates blood. Almost all donations are from volunteers. The first step in the donation process, registration, makes a record of the donor who can be contacted in the future, if necessary. The information requested include, name, sex, date of birth, telephone number, the donor must also sign consent. Very little whole blood is used, this enables each product to be stored under ideal conditions, prolonging its life and making available the appropriate product for a particular clinical

situation to allow proper selection and utilization of blood components. The blood components, red cells, platelets, granulocytes, fresh frozen plasma and cryoprecipitate are made directly from a unit of whole blood. The major goal of transfusion medicine practice has been to reduce the risk of transfusion transmitted infection to as low level as possible. In order to approach the desired level of zero risk of transfusion of allogeneic blood multiple layers of safety are needed (Dodd 2002). Methods used in attempting to maximize safety from donated allogeneic units, include donor selection criteria, donor medical history, the confidential unit exclusion (CUE) option, donor deferral registries, laboratory testing of donated units and modification of the blood units after collection either by leucocyte removal or physicochemical procedures for pathogen inactivation.

All blood donors are asked about their medical history to help determine if they can safely donate blood without experiencing any negative health effects (Dodd 2002).

White blood cells

The name "White blood cell" derives from the fact that after centrifugation of a blood sample, the white cells are found in the *Buffy coat*, a thin layer of nucleated cells between the sedimented red blood cells and the blood plasma, which is typically white in color. The scientific term leukocyte directly reflects this description, derived from Greek *leukos* - white, and *kytos* - cell (Bain BJ -1996). White blood cells, or leukocytes, are cells of the immune system defending the body against both infectious disease and foreign materials. Several different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system (Bain BJ -1996). There are several different types of white blood cells. They all have many things in common, but are all different. One primary technique to classify them is to look for the presence of granules, which allows the differentiation of cells into the categories granulocytes and a granulocytes (Amador 1975).

Granulocytes (polymorph nuclear leucocytes)

Leukocytes characterized by the presence of different staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes, which primarily act in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils, which are named according to their staining properties (Amador 1975).

A granulocytes (mononuclear leucocytes)

Leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes. The cells include lymphocytes, monocytes, and macrophages (Amador 1975).

Leucopoiesis

The blood granulocytes are formed in the bone marrow from a common precursor cell (stem cells). In the granulopoietic series progenitor cells, myeloblasts, promyelocytes form a proliferative or mitotic pool of cells while the metamyelocyte, band and segmented granulocytes make up a post mitotic maturation compartment (Amador 1975). The bone marrow normally contains more myeloid cells than erythroid cells in the ratio of 2:1 to 12:1 the largest proportion being neutrophil and metamyelocytes. In the stable or normal state, the bone marrow storage compartment contains 10 -15 times the number of granulocyte found in the peripheral blood (Dacie 2006). Following their release from the bone marrow granulocyte spend only 6 – 10 hours in the circulation before moving into the tissue where they perform their phagocytic function. It has been estimated that they spend an average 5 – 6 days in the tissues before they are destroyed during defensive actions or as the result of senescence.

Control of leucopoiesis

The granulocytes series arises from bone marrow progenitors cells which are increasingly specialized. Many growth factors are involved in this maturation process, including interleukin-1 (IL-1), IL-3, IL-5, IL-6, IL-11, granulocyte-macrophage-colony-stimulating factor (GM-CSF), G-CSF and monocyte CSF. The growth factor stimulates proliferation and differentiation and also affects the function of the mature cells on which they act (e.g. phagocytosis, superoxide generation and cytotoxicity in the case of neutrophils phagocytosis, cytotoxicity and production of other cytokines by monocytes. Increased granulocytes and monocytes production in response to an infection is induced by increased productions of growth factors from stromal cells and T- Lymphocytes stimulated by endotoxin, IL – 1 or tumor necrosis factor TNF (Daniel Catovsky - 2005).

Neutrophils

This cell has a characteristic dense nucleus consisting of between two to five lobes and pale cytoplasm with an irregular outline containing many fine pink – blue (azurophilic) or grey – blue granules (Daniel Catovsky - 2005). The granules divided into:

- A. Primary: This appears on the promyelocytes stage.
- B. Secondary which appear on myelocytes stage and predominate in the mature neutrophil.

Both types of granules are lysosomal in origin, the primary contain myeloperoxidase, acid phosphates and other acid hydrolyses, the secondary contains collagenase, lactoferrin and lysozyme, the life span of neutrophil in the blood is only about 10 hours and days in the spleen and other tissue (Daniel Catovsky - 2005).

Neutrophil precursors

The earliest recognizable precursor is the myeloblast a cell of variable size, which has a large nucleus with fine chromatin and usually 2-5 nucleoli. The cytoplasm is basophilic and no cytoplasmic granules are present, the bone marrow contains up to 4% of myeloblast. Myeloblast gives rise by cell division to promyelocytes which are slightly larger cells and have developed primary granules in the cytoplasm. These cells then produced myelocytes which have specific or secondary granules, the nuclear chromatin is now more condensed and nucleoli are not visible (A.V. Hoffbrand – 2005). The myelocytes give rise by cell division to metamyelocytes, non-dividing cells, which have an indented or horseshoe shaped nucleus a cytoplasm filled with primary and secondary granules. Neutrophil forms between metamyelocytes and fully mature neutrophil are termed band, stab, or juvenile. These cells may occur in normal peripheral blood. They do not contain the clear fine filamentous distinction between nuclear lobe, which is seen in mature neutrophil (A.V. Hoffbrand – 2005).

Eosinophils

These cells are similar to neutrophil, except that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than 3 nuclear lobes. Eosinophil myelocytes can be recognized, but earlier stages are indistinguishable from neutrophil precursors. The blood transit

time for eosinophils is longer than for neutrophils. They enter inflammatory exudates and have a special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation (A.V. Hoffbrand – 2005).

Basophils

These cells are only occasionally seen in normal peripheral blood. Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing inflammation. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules which hide it. They are characterized by their large blue granules (A.V. Hoffbrand – 2005).

Monocytes

These are usually larger than other peripheral blood leucocytes and possess a large central oval or indented nucleus with clumped chromatin. The abundant cytoplasm stains blue and contain many fine vacuoles, giving a ground – glass appearance. The monocytes precursors in the marrow (monoblast, promonocytes) are difficult to distinguish from myeloblast and monocytes. Monocytes spend only a short time in the marrow and after circulating for 20 to 40 hours, leave the blood to enter the tissue where they mature and carry out the principle function. Their extravascular lifespan after their transformation to macrophages may be also long as several months or even years (A.V. Hoffbrand – 2005).

Lymphocytes

Lymphocytes are immunologically competent cells that assist the phagocytes in defense of the body against infection and other foreign invasion. In postnatal life, the bone marrow and thymus are the primary lymphoid organs in which lymphocytes develop. The secondary lymphoid organs in which specific immune responses are generated are the lymph nodes, spleen and lymphoid tissues of the alimentary and respiratory tracts (Daniel Catovsky - 2005). By their appearance under light microscope there are two broad categories of lymphocytes namely the large granular lymphocytes. Most but not all large granular lymphocytes are more commonly known as natural killer cells. The small lymphocytes are the T- cells and B-cells. The formation of lymphocytes is known as lymphopoiesis, B- cells mature in the bone marrow and circulate in the peripheral blood until they undergo recognition of antigen. While T- lymphocytes migrated to the thymus where they differentiate into mature T cells during the passage from the cortex to the medulla. They live weeks to several years, which are very long compared to other leukocytes. T&B cells are the major cellular component of the adaptive immune response. The T cell is involved in cell mediated immunity whereas B cells are responsible for humoral immunity (Daniel Catovsky - 2005).

The function of the T&B cell is to recognize the specific antigen, during processes known as antigen presentation. The B cell responds to pathogens by producing large quantity of antibodies which then neutralize foreign objectives (Bacteria, Viruses). In response to pathogens some T cells called helper T cells produce cytokine that direct the immune response while other T cells called cytotoxic T cell produce toxic granules that induce the death of pathogen infected cells (Daniel Catovsky - 2005). Natural killer cells (NK) are cytotoxic CD 8+ cells that lack the T- cell receptor (TCR) and also they are a part of innate immune system and play a major role in defending the

host from both tumor and virally infected cells. N.K distinguishes infected cells and tumors from the normal and infected cells by recognizing alteration in the level of a surface molecule called major histocompatibility complex class I. They are large cells with cytoplasmic granules and typically express surface molecules CD16 (Fc receptor), CD56 and CD57. NK cells are activated in response to family of cytokines called interferons. Activated NK cells release cytotoxic granules (cell killing) which then destroy the altered cells. They were named (NK cell) because of the initial notion that they don't require prior activation in order to kill cells, which are missing Major Histocompatibility Complex I (MHC I) (Daniel Catovsky - 2005).

MATERIAL AND METHODS

Study area

The study was carried out in the Central blood bank, Wad Medanni teaching hospital. +Wad Medanni is the capital of Gezira state; it is considered one of the largest states in Sudan with an area of 35.304 km and a population of 4 million. The Central Blood Bank provides blood donation services to 4 governmental hospitals and other special hospitals in Wad medanni. About 1600 to 1700 donors attend the central blood bank monthly. Different types of blood components (whole blood, packed red cells, platelets, fresh frozen plasma) are prepared from whole blood using large refrigerated centrifuges. All donors are selected according to the accepted criteria for donation, including age, weight, physical and medical examination and screening for viral infections (hepatitis B, C and HIV) and the test for syphilis. A haemoglobin level assessment is performed by copper sulphate method and donors are reported as fit for donation if a drop of blood sinks in a copper sulphate solution, of a certain specific gravity.

Study population

Apparently healthy male Blood donors attending the Central Blood Bank (500 donors).

Selection criteria

Donors were selected according to the accepted criteria for donation.

- Age between 18- 60 years.
- Weight: 50 Kg (110 pounds) and more.
- Haemoglobin : 12.5 g/dl.- 17.5 g/dl

Donors were selected with clinical examination (abdominal, cardiopulmonary), pulse and blood pressure were measured, VDRL, hepatitis B,C and HIV were screened.

Exclusion criteria

- All donors should be clinically in a good health, subject with any disease symptoms and signs should be excluded.
- Any person taking medications.

Study design

Descriptive, prospective cross sectional study was conducted in wad Medanni central blood bank, during the period from 16\03\2009 to 26\12\2009.

METHODS

Sample collection

A total of 500 apparently healthy adult male donors were screened for White blood cell count. This analysis was conducted at the Wad Medanni central blood bank, department of pathology (medical laboratory) and the central laboratory of the Wad Medanni teaching hospital. Venous Blood samples were taken from an antecubital vein by a 5ml syringe. The site of collection was cleaned using 70% alcohol and left to dry. An elastic tourniquet was applied if needed to the arm. 2.5 ml of blood was taken in a container with 0.05ml (K2 EDTA) as an anticoagulant with a concentration of 1.5- 2.2 mg/ml and then the sample gently mixed. The blood samples were tested within 2 hours of sample collection using an automated blood cell counter (sysmex KN21 analyzer) with a flow cytometry using a laser light to perform white blood count. It is calibrated by a standardized commercially prepared calibrator (Dacie 2006).

Making a blood film

Manual spreading of blood films using frosted glass slides were performed. The frosted glass slides were clean and free of grease. A drop of blood was placed near one end of the slide and spreader was applied at an angle of 45, in front of the drop of blood, making a thin blood film using a cover glass as spreader and allowed to dry. Then they were labeled with the donor number and date of sample collection. The films were then fixed in absolute methanol for 10-20 minutes. The films were placed horizontally on the staining rack and flooded with Leishman's stain and left for 4 minutes. A double volume buffer was added with gentle blowing over the surface without touching the film surface. The films were left for another 8 minutes and then washed off with buffered distilled water. The back of the slide was cleaned using cotton dipped in alcohol and then left to dry (Dacie 2006).

Examination of the blood films

The identification of the specimen was checked and matched with the white blood cells report. The films were examined macroscopically to confirm adequate spreading followed by microscopic examination. A low power field (10 objectives) to assess the quality of the stain and (40 objectives) to determine the suitable area for blood film examination (Dacie 2006). The white blood cells report was examined and an assessment of their number, size, morphology and presence of aggregate were also evaluated.

The aim of this study was to detect the white blood cells count in 500 apparently healthy male donors selected according to the accepted criteria for donation including age, weight, physical and medical examination. All donors were subjected to screening for viral infections (hepatitis B, C and HIV) and the test for syphilis. The White blood cell count was estimated using an automated cell counter (sysmex KN 21).

Statistical analysis

The results were analyzed using statistical software package of social sciences (SPSS) version 17 and descriptive data were expressed as means.

Ethical clearance

Ethical clearance was obtained from the University of Gezira ethical committee and blood bank authority. Verbal informed consent was obtained from all donors.

RESULTS

Table (1) showed the white blood cells count mean value was found to be 5.64 ± 1.7 standard deviation with a minimum value 2.1 and maximum value 14.1, with 77 cases ranged from 2.1 to 3.9, 372 cases ranged from 4 to 8, 46 cases ranged from 8.1 to 11 and 5 cases ranged from 11.1 to 14.1. The differential white blood count was performed manually based on counting 100 cells and expressed as a percentage. These manual differential white blood cells were correlated with the automated differential white blood cells.

Table (2, 3, 4, 5 and 6) showed: The neutrophil count mean value was found to be 54.43 ± 11.576 standard deviation with a minimum value 12% and maximum value 80%, the lymphocyte count mean value was found to be 41.20 ± 11.497 standard deviations with a minimum value 16% and maximum value 88%. The eosinophil count mean value was found to be 3.18 ± 2.269 standard deviation with a minimum value 1% and maximum value 16%, the monocyte count mean value was found to be 2.07 ± 1.496 standard deviation with a minimum value 1% and maximum value 10% and the basophil count mean value was found to be 1.22 ± 0.441 standard deviation with a minimum value 1% and maximum value 2%.

Microscopic examination

Hypersegmented neutrophils (1-2) observed in 15 cases (3%) with lymphocytes predominance in 159 cases (31.8%) and reactive lymphocytes (2-10) in 105 cases (21%), eosinophil count more than 4% detected in 72 cases (14 %) and 15 cases (3%) with monocyte count more than 8%. No lymphoblast, myeloblast, promyelocytes, myelocytes, metamyelocytes detected in the blood films.

DISCUSSION

The mean values of white blood cells were found to be 5.696 ± 1.7989 with minimum count $2.1 \times 10^9 /L$ and maximum count $14.1 \times 10^9 /L$ and 77 donors (15.4%) presented with a white blood count less than $4 \times 10^9 /L$ (leucopenic donors). White blood count more than $11 \times 10^9 /L$ was observed in 5 donors (1%) dominated by neutrophils which suggestive of pyogenic infection. The neutrophil percentage less than 50 % observed in 165 donors (33%) and 231 donors (46.2%) and neutrophil percentage more than 70 % observed in 26 donors (5.2%) with lymphocyte percentage more than 40 % observed accompanied by reactive lymphocytes most probably due to chronic infection (Wilson -1991). The eosinophils percentage of more than 4% observed in 121 donors (24.2%), the bulk of this eosinophilia probably reflects asymptomatic parasitism (e.g. schistosomiasis). Monocyte percentage more than 8 % observed in 6 donors (1.2%) (Wilson -1991).

CONCLUSION & RECOMMENDATIONS

1. The study revealed that significant number of donors with low & high white blood count.
2. Although incidental findings such as CLL, CML or pancytopenia were not detected, some donors with

such condition may have been missed without performing FBC.

3. The blood transfusion services in Sudan should follow the standard international guidelines and the donor questionnaire form should be properly completed.
4. Donors with abnormal peripheral blood picture and abnormal serological tests should be deferred from donating and referred to a physician for further examination and management.

REFERENCES

- Amador E. Health and normality. JAMA 1975; 232: 953–5.
A.V. Hoffbrand (Essential haematology) , Fifth edition 2005.
Bain BJ. Ethnic and sex differences in the total and differential white cell count and platelet count. J Clinical Pathology 1996;49:664-6.
Dacie J.V Lewis, SM. Practical haematology Tenth edition 2006. London.
Daniel Catovsky , A Victor Hoffbrand , Edward GD Tuddenham , Anthony R Green Postgraduate Hematology, Fifth edition 2005
Dodd, RY, Notari, EP, Stramer, SL. Current prevalence and incidence of infectious disease markers and estimated window period risk in the American Red cross blood donor population. Transfusion 2002.
Wilson (1991) Harrison's Medicine, McGraw, p. 360-1