A POSTGRADUATE THESIS

ON

NADIR PLATELET COUNTS IN PATIENTS ON DOXORUBICIN AND CYCLOPHOSPHAMIDE (AC); AND CYCLOPHOSPHAMIDE, DOXORUBICIN, VINCRISTINE AND PREDNISONE (CHOP) FOR BREAST CANCER AND NON-HODGKIN'S LYMPHOMA RESPECTIVELY.

BY

DR. YOHANNIE B. MLOMBE
(BSc. Med. Sci. St Andrews, Scotland. M.B.B.S. UCL/UNIMA)

P. 0. BOX 48021 00100
NAIROBI.

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DECLARATION

This dissertation is my original work and has not to my knowledge been presented for a degree in any other university.

Signed_____ Date 23/5/2007

DR. YOHANNIE B. MLOMBE
(BSc. Med. Sci., M.B.B.S.)

This dissertation has been submitted for examination with our approval as university supervisors.

Signed_____ Date 16/11/07

PROF N.A. OTHIENO ABINYA
ASSOCIATE PROFFESOR OF MEDICINE,
DEPT OF CLINICAL MEDICINE AND THERAPEUTICS, HAEMATOLOGY AND ONCOLOGY SECTION,
SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES-UON

Signed_____ Date 16-11-07

DR F ABDALLAH
LECTURER, DEPT OF HAEMATOLOGY AND BLOOD TRANSFUSION,
SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES-UON

Signed_____ Date 27.11.07

DR MARK JOSHI
SENIOR LECTURER AND CLINICAL EPIDEMIOLOGIST, DEPT OF CLINICAL MEDICINE AND THERAPEUTICS;
DIRECTOR, CLINICAL EPIDEMIOLOGY UNIT;
SCHOOL OF MEDICINE; COLLEGE OF HEALTH SCIENCES-UON

Signed_____ Date 27/11/2007

DR CATHERINE N NYONGESA
CONSULTANT RADIATION ONCOLOGIST;
CANCER TREATMENT CENTER; KENYATTA NATIONAL HOSPITAL

DEDICATION

To my lovely and caring wife, Gina; to my thoughtful and patient mother; and to the memory of my hardworking and steadfast late father.

TITLE:

NADIR PLATELET COUNTS IN PATIENTS ON DOXORUBICIN AND CYCLOPHOSPHAMIDE (AC); AND CYCLOPHOSPHAMIDE, DOXORUBICIN, VINCRISTINE AND PREDNISONE (CHOP) FOR BREAST CANCER AND NON-HODGKIN'S LYMPHOMA RESPECTIVELY.

INVESTIGATOR:

DR. YOHANNIE B. MLOMBE
(BSc. Med. Sci. St Andrews, Scotland. M.B.B.S. UCL/UNIMA)

SUPERVISORS:

PROF N.A. OTHIENO ABINYA
ASSOCIATE PROFFESOR OF MEDICINE,
DEPT OF CLINICAL MEDICINE AND THERAPEUTICS, HAEMATOLOGY AND ONCOLOGY SECTION,
SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES-UON.

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SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES-UON

DR MARK JOSHI
SENIOR LECTURER AND CLINICAL EPIDEMIOLOGIST, DEPT OF CLINICAL MEDICINE AND THERAPEUTICS;
DIRECTOR, CLINICAL EPIDEMIOLOGY UNIT;
SCHOOL OF MEDICINE; COLLEGE OF HEALTH SCIENCES-UON

DR CATHERINE N NYONGESA
CONSULTANT RADIATION ONCOLOGIST;
CANCER TREATMENT CENTER; KENYATTA NATIONAL HOSPITAL

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LIST OF ABBREVIATIONS

AC	Doxorubicin and Cyclophosphamide
AC-21	3-weekly pulses of AC
AJCC	American Joint Committee On Cancer
ANC	Absolute neutrophil count
BMA	Bone Marrow Aspirate
BSA	Body surface area
CA	California
CAP	College Of American Pathologists
CHOP	Cyclophosphamide, Doxorubicin Vincristine & Prednisone
CHOP-21	3-weekly pulses of CHOP
CLIA	Clinical Laboratory Improvement Amendments
DNA	deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediamine Tetraacetic Acid
Epo	Erythropoietin
EQAS	External Quality Assessment
ER	Estrogen Receptor
FBC	Full Blood Count
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
Hb	Haemoglobin
HGF	Hematopoietic Growth Factor
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSC	Hematopoietic Stem Cell
IEQAS(H)	International External Quality Assessment Scheme For General Haematology
IL	Interleukin
ITP	Idiopathic Thrombocytopenic Purpura
IV	Intravenous
KNH	Kenyatta National hospital
LDH	Lactase Dehydrogenase
MAPSS	Multi-Angle Polarized Scatter Separation
MGDF	Megakaryocyte growth and development factor

NHL	Non Hodgkin's Lymphoma
NJ	New Jersey
PBF	peripheral blood film
PEG-rHUMGDF	Pegylated recombinant human megakaryocyte growth and development factor
PI	Principal Investigator
PLT	Platelet
PO	per oral (by mouth)
PS	Perfomance Status
rHTPO	Recombinant human thrombopoietin
RNA	Ribonucleic Acid
SPSS	Statistical Package for the Social Sciences
TLC	Total lymphocyte count
TP II	Topoisomerase II
TPO	Thrombopoietin
UK	United Kingdom
UKNEQAS	UK National External Quality Assessment Scheme
WBC	White Blood Cells
WHO	World Health Organization

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ABSTRACT

Background: Patients on chemotherapy suffer severe myelosuppression leading to poorer treatment outcome locally compared to economically advantaged countries. Effective and safe use of chemotherapy demands that febrile neutropaenia and haemarrhage, which are the most important consequences of chemotherapy-induced myelosuppression in the acute stage, should be well managed; and formulation of meaningful local guidelines to achieve timely interventions requires analysis of nadir counts. A clearer pattern of nadir neutrophil counts has been better described locally than that of nadir platelet counts. With this in mind, we set out to study the effects of using doxorubicin and cyclophosphamide (AC) for breast cancer and cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) for non-hodgkin's lymphoma (NHL) on the platelet counts of our patients.

Objectives: To determine the nadir platelet counts on day ten to fourteen of the first two cycles, and their relationship to the risk of haemarrhage in patients on standard treatment for breast cancer and non-Hodgkin's lymphoma (NHL) with doxorubicin and cyclophosphamide (AC) and cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) protocols respectively.

Methods: A prospective descriptive study using a real life usual practice scenario carried out at Kenyatta National Hospital (KNH) in Nairobi, Kenya. Samples of three milliliters of venous blood were taken from study patients on

day 1 of the first three cycles and between day 10 and day 14 of the first two cycles and were analysed for full blood counts. Study patients were also evaluated for episodes of bleeding and for factors known to be associated with haemarrhage in chemotherapy induced thrombocytopaenia.

Results: Seventy eight patients were analysed, of whom 61 (78.2%) had breast cancer and 17 (21.8%) had NHL. The mean platelet count for the 78 patients dropped by *142.31x10^9/L* in cycle 1 from *335.50 (SD 98.4) x10^9/L* to a nadir of *193.19 (SD 73.7)x10^9/L* then rose by *128.22 x10^9/L* to *321.41 (SD 104.6)x10^9/L* by day 22. In cycle 2 the mean platelet count for the 78 patients dropped by *185.54x10^9/L* to a nadir of *135.87 (SD 75.97)x10^9/L* then rose by *171.28x10^9/L* to *307.15 (SD 113.97)x10^9/L* by day 43. This trend represents a 42.4% drop in cycle 1 and a 57.7% drop in cycle 2. Low nadir counts were associated with old age (≥60 years) and low baseline platelet counts in both cycles as well as low baseline total lymphocyte counts in cycle 1. In terms of nadir platelet count depths, high values were associated with high baseline platelet counts in both cycles and doxorubicin and cyclophosphamide (AC) therapy for breast cancer in cycle 2. Severe thrombocytopaenia occurred only in the second cycle and only in 3 (3.8%) patients (two patients had grade 3 thrombocytopaenia and one patient had grade 4 thrombocytopaenia). Only one patient had a minor bleeding episode (grade 1) which was not attributable to platelets.

Conclusion: There is a consistent but clinically insignificant drop in platelet counts in patients on doxorubicin and cyclophosphamide (AC) and

cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) for breast cancer and non-Hodgkin's lymphoma (NHL) respectively; with a tendency towards an accumulative effect. Old age and low baseline platelet counts were associated with low nadir platelet counts in both cycles; and AC therapy for breast cancer was associated with deeper platelet nadir drops from baseline.

1. INTRODUCTION

Myelotoxicity is a major side effect of most anticancer drugs. The local experience is that patients who suffer severe bone marrow toxicity have poorer outcomes compared to economically advantaged countries [1]. The kinetics of cytopenias depends on the life-span of the cells involved with neutropaenia occurring first, followed by thrombocytopaenia and lastly severe anaemia. Safe and effective use of chemotherapy requires good management of the effects of myelosuppression which, in the acute stage, involves management of febrile neutropaenia and haemarrhage. The local pattern and guidelines of how best to manage febrile neutropaenia in chemotherapy-induced myelosuppression in solid tumors and lymphoma are more comprehensive than the local pattern and guidelines of how best to manage thrombocytopaenia in these patients [1,2-3].

Platelet transfusion therapy is an integral part of modern oncological practice and is used to either treat haemarrhage associated with chemotherapy-induced thrombocytopaenia or more commonly, to prevent haemarrhage in such patients. However, platelet transfusion therapy is not without its drawbacks as it can be complicated by transfusion transmitted infections, allergic reactions and refractoriness due to alloimmunisation and unavailability in most centers. Despite reports indicating that growth factors such as Interleukin-11 (rHuIL-11) and thrombopoietin (rHuTPO) are capable of increasing platelet count, reducing platelet nadir, or decreasing the duration of platelet transfusions [4-7], optimization of platelet therapy remains the standard practice in the optimal management of chemotherapy induced thrombocytopaenia. Unfortunately, while it is agreed that the there is a relationship between platelet counts and the risk of bleeding in chemotherapy-induced thrombocytopaenia [8-11], guidelines

as to the optimization of platelet transfusions in solid tumor and lymphoma patients with chemotherapy-induced thrombocytopaenia vary widely [8,11-17] to such an extent that clinical research in this area remains desirable [11].

The concept of nadir counts is important in the practice of oncology; knowledge of their trend helps to warn of life threatening myelosuppression. They also help assess the adequacy of chemotherapy dosing; and different drugs and protocols are associated with different blood cell count nadir patterns [18]. Determination of nadir counts is therefore highly desirable in the supportive care of patients on chemotherapy. Nadir platelet counts can provide guidance as to how best to give platelet transfusions to ensure that their drawbacks are minimized without risking the serious consequences of haemarrhage in chemotherapy-induced thrombocytopaenia.

Breast cancer and Non-Hodgkin's lymphoma are very common malignancies at Kenyatta National Hospital as reported by L. Mungania [19]. Doxorubicin and cyclophosphamide (AC) is the standard chemotherapeutic protocol for breast cancer; and cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) is the standard chemotherapeutic protocol for non-Hodgkin's lymphoma at Kenyatta National Hospital.

This study was designed to evaluate the degree and pattern of chemotherapy induced thrombocytopaenia and its associated factors of clinical importance in patients with breast cancer and Non-Hodgkin's lymphoma who are on treatment using Cyclophosphamide and Doxorubicin (AC) and Cyclophosphamide, Doxorubicin, Vincristine and Prednisone (CHOP) regimen respectively.

2. BACKGROUND INFORMATION

2.1. MYELOTOXICITY

Toxicity to the normal tissues of the body is one of the two main obstacles to the clinical efficacy of chemotherapy; the other one being the development of cellular drug resistance.

The cell cycle is central to the mechanisms of action of chemotherapy agents. In every population of cells, there are 3 sub-populations: (i) cells that continuously proliferate going from mitosis in one cycle to the next; (ii) terminally differentiated cells that irrevocably leave the growth cycle and are destined to live without dividing again; (iii) non-dividing cells or non-cycle cells that do not divide but can reenter the cell cycle if an important stimulus is applied [20-21]. The cell cycle is divided into four phases. During the M phase, the replicated chromosomes are separated and packaged into two new nuclei by mitosis and the cytoplasm is divided between two daughter cells by cytokinesis. The other three phases comprise of the interphase: G1 (gap1) which is a period of growth during which the cell determines its readiness to commit to DNA synthesis, S phase (DNA synthesis) during which period the genetic material is replicated and no other replication is permitted thereafter; and the G2 (gap 2) phase where the fidelity of DNA replication is determined and errors corrected [21]. Growth in size occurs throughout the cell cycle at a steady pace but S phase and mitosis occur in discrete periods. All cycling cells go through the four different phases G1, S, G2 and mitosis. Any population of cells can increase in number by any one of three mechanisms: (a) shortening of the cell cycle which results in more cells

being produced per unit time; (b) decreasing the rate of cell death; and (c) moving G_0 cells into the cell cycle. All three mechanisms operate in normal and abnormal growth [20,22-23] and are important in determining the aggressiveness of tumors. The cell cycle transitions between G1 and S and between G2 and M are tightly regulated to ensure that cells are prepared to divide and minimize errors in replication. These check points are manned by cyclin dependent kinases and cyclins. The checkpoint regulating the transition from G1 to S is frequently disrupted in cancer [21-22]. Regardless of the pathogenesis of the tumor, most have mechanisms to bypass the G1-S checkpoint, avoid activation of cell suicide pathways and propagate cells with damaged DNA [20-21].

The Hematopoietic Stem Cell (HSC) is a pluripotent stem cell characterized by an extensive proliferation and differentiation capacity, with the ability to self renew on a population basis [24] and it gives rise to all cellular components of blood (figures 1 & 2). Under the influence of haematopoietic growth factors (HGF), the stem cell divides and differentiates via progenitor cell into various mature cell types [24-25]. This process of formation and production of peripheral blood cells is known as haematopoiesis. Under physiological conditions, it is a tightly regulated highly efficient system exquisitely responsive to functional demands. The most active compartment of the marrow cellular proliferation and differentiation are generally the lineage committed progenitor subsets. True stem cells are extremely immature progenitor cells in the marrow and are usually in a quiescent state. The control of haematopoiesis involves a complex interaction among haematopoietic elements, bone marrow stromal cells (non-haematopoietic tissue immediately abutting haematopoietic tissue, also called

the haematopoietic microenvironment), and soluble cytokines and haematopoietic growth factors (HGF's) [26].

Figure 1: Haematopoietic lineage

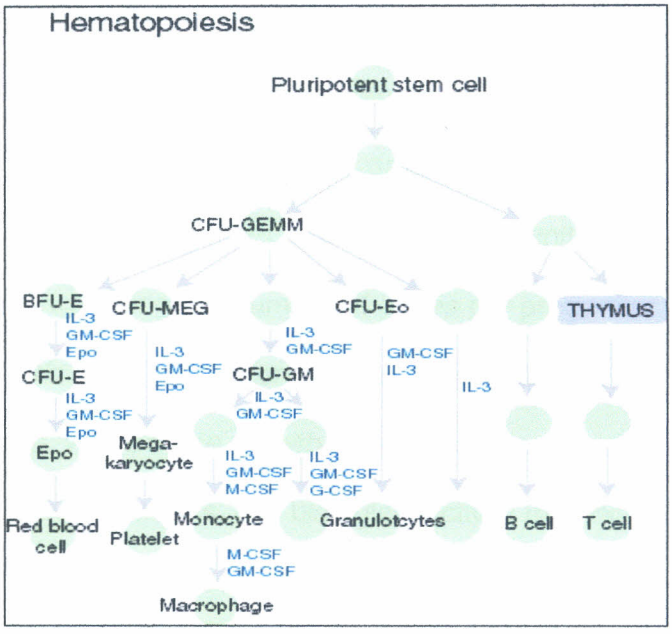
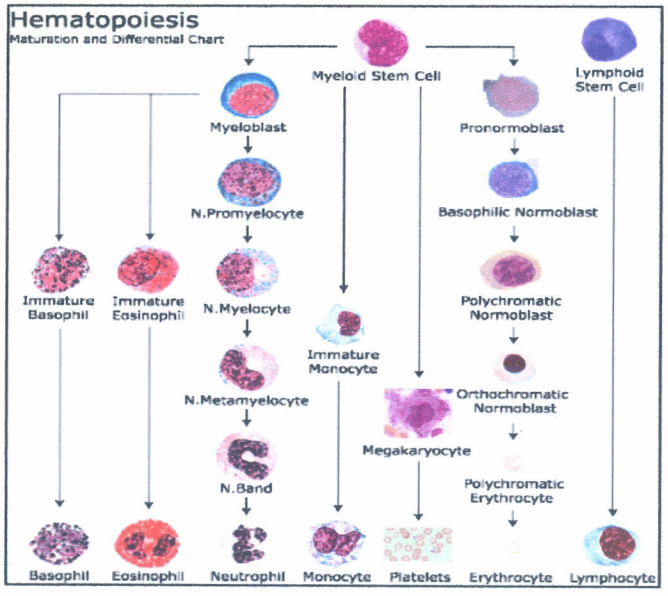


Figure 2: Haematopoiesis Cell Maturation



Thrombopoiesis involves the production of platelets from megakaryocytes. Megakaryocyte development can be conceptually divided into three stages: the development of early stage progenitor cells, subsequent expansion and differentiation into promegakaryoblasts, and mature megakaryocytes [27]. Many cytokines and hematopoietic growth factors influence megakaryocytopoiesis by increasing the number of committed progenitor cells, decreasing cycling time, and increasing the number of cycles per progenitor. Early-acting cytokines include stem cell factor (kit ligand), IL-3, IL-6, IL-11, GM-CSF, and erythropoietin (Epo). While these growth factors may increase lineage-specific differentiation of primitive elements, this biological activity may not translate directly into the ability to increase platelet production, a role that the more specific actions of TPO may support optimally. The liver and kidneys produce thrombopoietin constitutively [28-31] and release it into the circulation. Platelets have receptors for thrombopoietin and remove it from plasma [32-33]. In states of thrombocytopaenia, little of the released thrombopoietin is metabolized, and the resulting high plasma thrombopoietin concentrations stimulate the hypoplastic marrow. In addition, marrow stroma produces thrombopoietin during thrombocytopaenia [30]. The high plasma thrombopoietin concentrations help restore megakaryocyte and platelet production. In contrast, during thrombocytosis, the high numbers of platelets remove most of the thrombopoietin from the circulation, and marrow stromal-cell production nearly ceases; as a result, little thrombopoietin is left to act on the numerous megakaryocytes in the marrow, allowing it to return to steady-state platelet production. When cytotoxic therapy is administered, before the platelet count starts to fall, the rate of platelet production drops precipitously as the majority of megakaryocytes and their precursors are destroyed. However,

because the existing platelets continue to bind thrombopoietin, the increase in the plasma thrombopoietin concentration is delayed until thrombocytopaenia intervenes, many days later. Only when thrombocytopaenia is severe do plasma thrombopoietin concentrations and platelet production increase. Although TPO, the c-Mpl ligand, is the primary endogenous regulator of thrombopoiesis, clinical studies have been conducted on a number of early-acting cytokines to fully evaluate their thrombopoietic potential [4]. In particular, preclinical studies had indicated that IL-1, IL-3, IL-6, GM-CSF, and IL-11 could stimulate megakaryocyte growth and platelet production [4,34].

Anti-neoplastic drugs are of two general categories: (i) those that act upon the cell throughout its cycle i.e. phase non-specific, these can either be cell cycle specific (they can kill dividing cells at any point in the cell cycle e.g. alkylating agents, platinum compounds, cell-signaling inhibitors, trastuzumab – figure 3) or cell cycle non-specific (they can kill nondividing cells e.g. steroid hormones, antitumor antibiotics except bleomycin); (ii) those that act preferentially during one or more of the non-resting phases i.e. phase specific (i.e they are all cell cycle specific – figure 3). Phase specific drugs include those that interfere with DNA synthesis (specific to the S phase), those that block protein synthesis (mainly S and G2 specific) and those that inhibit microtubule assembly (M phase specific). For cycle specific agents, recovery occurs rapidly within 7 to 14 days whereas; non-cycle specific agents cause a much more delayed nadir in 4 to 5 weeks [35-36].

Figure 3: Action Sites For Cell Cycle Specific Agents.

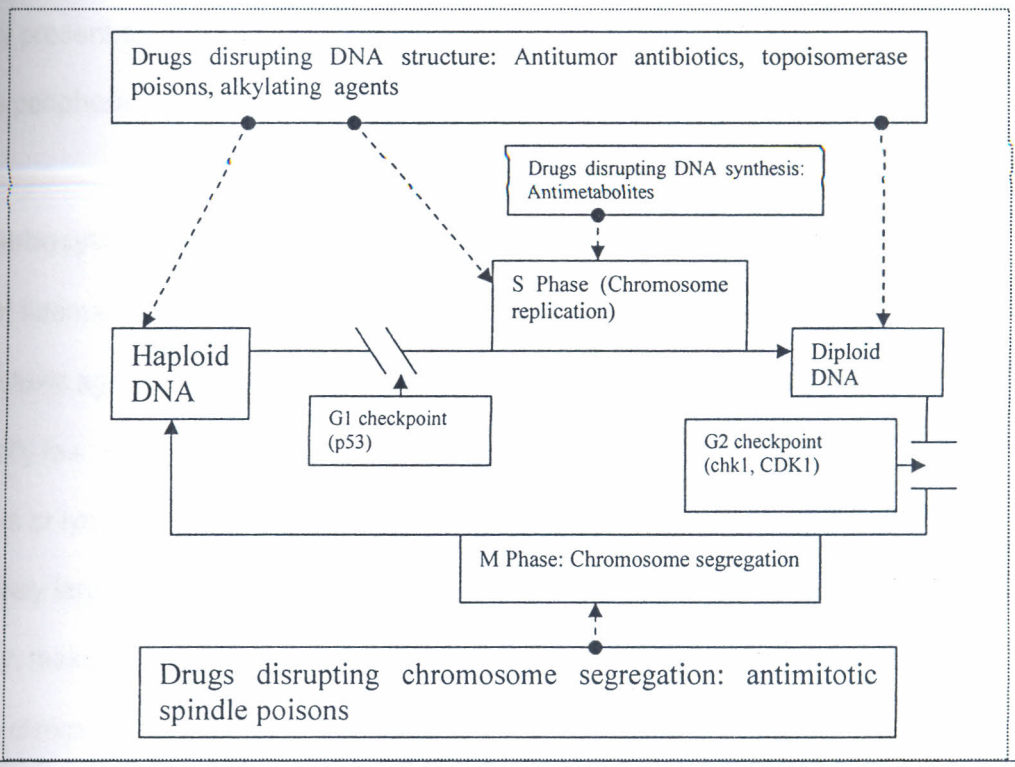


Fig. 3: Action sites for cell cycle specific agents include phase-specific agents which can act in e.g. S (antimetabolites) and M (spindle poisons) phases; and phase-nonspecific agents which can kill cells throughout the cycle but cause arrest of cell cycle progression at "checkpoints." The G1 checkpoint is mediated through p53 acting on CDKs 4,6, and 2, and the G2 checkpoint is mediated in part by the *chk1* kinase acting on CDK1.

Bone marrow toxicity is caused by cytotoxic drugs mainly, if not entirely, by either causing damage to the mitotic/stem cell compartment of the marrow or by slowing cell division by; (a) interfering with purine or pyrimidine biosynthesis, (b) blocking DNA strand duplication, (c) disrupting the microtubules mitotic spindles, (d) interfering with RNA formation, translation, and transcription processes central to protein synthesis [36-37]. This results in decreased production of blood cells, through reduction of rapidly growing progenitor stem cells in the marrow. Bone marrow suppression is dose related and is dependent on continued administration of the drug [36-38] and, in addition, the pharmacological action of a drug, the route and

schedule of administration, drug metabolism and the pattern of cell sensitivity influence bone marrow damage [35-36]. The bone marrow failure caused by cytotoxic therapy is probably the most common form of temporary marrow failure states [23,36]. It may present as pancytopaenia with anaemia, leukopaenia and thrombocytopaenia in the peripheral blood due to deficient hematopoesis.

Thrombocytopaenia when it occurs in solid tumours or lymphoma, increases the risk of haemarrhage, necessitates platelet transfusions and limits the doses of myelotoxic agents [8-10,12,39-41]. Several studies have suggested that there are typically low numbers of platelet transfusions per cycle among patients with solid tumors or lymphoma as compared to patients with leukaemia. However, the relatively large size of the total patient population with solid tumours or lymphoma locally, makes management of their episodes of thrombocytopaenia a significant issue clinically and economically and therefore a subject worthy of study [42]. Reported conditions associated with major bleeding in patients with solid tumours or ymphoma and chemotherapy-induced thrombocytopaenia include necrotic metastatic lesions, tumour vascular invasion, coagulopathies and severe infection [8-,12-13,39]; poor performance status; low baseline platelet, lymphocyte and absolute neutrophil counts on day 1 of chemotherapy [40].

.2. MANAGEMENT OF CHEMOTHERAPY–INDUCED THROMBOCYTOPAENIA

Platelet transfusions, at the time of writing this thesis, are the most effective means of controlling bleeding and the acute risks of severe thrombocytopaenia associated with the treatment of haematologic malignancies and solid tumors [16]. However, there are a number of issues related to platelet transfusion therapy

that support the need for optimization of platelet therapy; if not the need for strategies to reduce or eliminate platelet transfusions. These include availability, cost, refractoriness, transfusion reactions and disease transmission [16,43-44].

Availability is a problem because blood products, including platelets, are a limited resource; however, while packed red blood cells can be stored for 42 days, platelets must be discarded after only 5 days. This short shelf life contributes to a chronic restriction of the supply of usable platelets [45].

Cost is an issue because, although platelets are harvested from voluntary donors, platelet transfusions are known to be among the most expensive supportive measures in the field of haematology and oncology [46]. In one study, platelet transfusion costs ranged from $389 (merged units of random platelet donors) to $661 (for a specially processed single donor apheresis) [16,47]. Refractoriness to platelet transfusions is associated with significantly greater inpatient costs and length of stay [48]. Efforts have been made to control costs and increase efficiency of transfusion practices by utilising lower-dose single-donor platelet transfusions, but this practice may actually increase overall hospital transfusion costs [49].

Refractoriness is identified in 15% to 40% of patients who receive multiple platelet transfusions over time [16].

Reactions may be due to recipient HLA antibodies reacting with donor leukocytes or to cytokines in the donor plasma [50-51]. Approximately 5% to 30% of platelet transfusions are associated with a reaction, usually of the febrile,

nonhaemolytic type [16]. Steps to decrease the incidence of reactions include leukocyte depletion, platelet washing, or infusion of platelets stored for 3 days or less [52-53].

Transfusion-associated infections are another risk of platelet administration. Septic reactions are the most common serious risk of platelet transfusion. Compared with platelet concentrates, the use of single-donor platelets can significantly reduce the incidence of these reactions [54].

Better alternatives to platelet transfusion remain illusive. Chemotherapy dose reduction is a possible solution to the issue of platelet transfusion complications; the rationale is that dose reduction would lead to less incidence and severity of thrombocytopaenia thereby reducing the need for platelet transfusions. However, dose reductions may reduce antitumor effect [41].

Platelet growth factors are another possible solution to the issue of platelet transfusion complications but despite extensive investigations, early-acting cytokines (IL-1, IL3, IL-6, Granulocyte-Macrophage Colony Stimulating Factor - GM-CSF), and recombinant human interleukin-11 (rHuIL-11) have not provided clinicians with an optimal or reliable thrombopoietic agent that can be viewed as fully safe and effective. In human studies, administration of IL-1ß increased platelet counts [5]. Unfortunately, IL-1 has significant proinflammatory properties, inducing fever, hypotension, fluid retention, and supraventricular arrhythmias, all of which combine to eliminate its therapeutic utility as a thrombopoietic agent [55-56].

The activity of IL-3, as yet, has not been confirmed in appropriately powered

human clinical trials. Clinical trials of recombinant IL-6 have reported side effects of fever, headache, myalgias, hyperbilirubinemia, rapid development of anaemia, and fatigue with only modest thrombopoietic activity [57-59].

IL-11 is a protein product of bone marrow stromal cells [60]. Recombinant human interleukin-11 (rHuIL-11, Oprelvekin, Neumega®) was the first commercially available thrombocytopoietic cytokine[4]. It has been licensed for the prevention of severe thrombocytopaenia and the reduction of the need for platelet transfusion following myelosuppressive chemotherapy in patients with nonmyeloid malignancies who are at high risk of severe thrombocytopaenia. However, rHuIL-11 has significant side effects and demonstrates only modest efficacy.

Thrombopoietin, the c-Mpl (the cellular homologue of the murine myeloproliferative leukemia retrovirus oncogene, v-Mpl) ligand, as stated earlier, is the primary endogenous regulator of thrombopoiesis. Pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) was a variant form of human thrombopoietin developed to target c-Mpl. Unfortunately, the molecule proved immunogenic when administered via a subcutaneous route of administration. A subset of both cancer patients and normal platelet-donor volunteers developed neutralizing antibodies following treatment with PEG-rHuMGDF. Consequently, the molecule was withdrawn from clinical development in 1998 [61].

Despite the somewhat naïve hopes that primitive-acting cytokines might be effective therapy for chemotherapy-induced thrombocytopaenia, several candidate molecules in this regard have failed to fulfill this promise. MPL-

targeted therapy still offers some hope for the future and further research will undoubtedly continue in this area. In the meantime, the standard therapy for chemotherapy–induced thrombocytopaenia remains platelet transfusions and because they have drawbacks, ***debate*** continues on optimization of platelet transfusion therapy in terms of prophylactic use versus therapeutic use; and if prophylactic use then at what threshold?

In solid tumor or lymphoma patients with chemotherapy-induced thrombocytopaenia, controversies about appropriate platelet thresholds for prophylaxis have been particularly intense [11-16,62-69]. Based on retrospective studies Belt et al. [8] and Goldberg et al. [12] proposed a prophylactic threshold of less than $10\times10^9/L$ while Fanning et al. [13] suggested that a lower threshold of less than $5\times10^9/L$ would be acceptable. Hence generally, recommendations have varied from a prophylactic threshold of less than $20{,}000x10^9/L$ [11] to a threshold of less than $10{,}000x10^9/L$ [11,15,67] to a threshold of less than $5{,}000x10^9/L$ [13] to considerations of only performing therapeutic platelet transfusions [17]. Of note is the fact that the American Society of Clinical Oncology has pointed out that clinical studies in this area are highly desirable [11].

2.3. NADIR PERIPHERAL BLOOD CELL COUNTS AND CYTOPENIAS

"Nadir count" implies the lowest count. The concept implies that chemotherapy causes a drop in peripheral blood cell numbers which then rise as the marrow recovers. Recovery results in part from the fact that most haematopoietic stem cells are in a resting state in G_0 phase which protects them from excessive damage induced by common chemotherapeutic treatments [23-25]. The depletion of the other dividing cells in the marrow by these drugs, however, stimulates the

protected stem cell to re-enter the cell cycle and eventually repopulate the bone marrow [20-21,23]. The storage compartment of the bone marrow can supply mature cells to the peripheral blood for 8-10 days after the pool of primitive haematopoietic progenitor cells cease the normally active process of division and maturation that replenishes the pool of the latter more differentiated committed progenitor cells [23]. Following injury to the bone marrow either by chemicals, radiation or infection, the kinetics of cytopenia induction reflects the lifespan of the cells in peripheral blood. On average, neutrophils have a half-life of 6-8 hours, platelets of 7-10 days, and erythrocytes of 120 days. The life span of lymphocytes is difficult to determine. Unlike the other cells, lymphocytes (up to about 70%) circulate back from tissues into blood and B and T-cells do so at different rates and they spend different amounts of time within the tissues. It is believed that both B and T-cells have long life spans and that they can last the lifetime of an individual. Suppression of the peripheral blood cells is therefore generally noted a week or so later after a toxic insult to the bone marrow. In previously untreated patients, several of the most commonly used cytotoxic agents when administered cause leukopaenia and thrombocytopaenia by day 9 or 10 after treatment. Nadir counts are reached in 14 -18 days and recovery is evident by day 21 and is complete by day 28 [22-23] . The different life spans of the cell lines do not imply that those with longer life spans are not affected by chemotherapy. These cells must all be replaced on a daily basis in order to maintain optimal numbers. And when the bone marrow is unable to replace them, all the cells lines will begin to drop in numbers. The nadir depth of any cell line is therefore mainly an interaction of the duration of bone marrow suppression and the life span of the cells involved; in that given enough period of bone marrow (and all lymphoid tissue) suppression, all cell lines would

eventually reduce to zero. Neutrophil counts are the first to show a drop because of their very short life span compared to the other cell lines. They thus provide a good idea as to when the cytopenic effect of chemotherapy exactly starts. If the marrow recovers within a week, then platelets would be the next cell line to show a significant drop in numbers. If the suppression went on for 120 days then haemoglobin would also show a substantial drop in numbers. But a week is enough for there to be a slight but clinically insignificant (to not less than 8 g/dL) drop in haemoglobin levels. Lymphocytes would hardly show a drop with a myelosuppression lasting a week or so.

The relationship between platelet count and the risk of bleeding was first described in patients with leukemia in a landmark study by Gaydos and Freireich (the pioneer of platelet transfusions in the 1960s) et al in 1962 [70]. However Gaydos and his colleagues did not demonstrate a clear threshold where haemarrhage occurred. In spite of this fact, Gaydos's study is often quoted as a justification for a threshold of $20x10^9/L$ for platelet transfusions. In solid tumor and lymphoma patients with chemotherapy-induced thrombocytopaenia, the relationship between platelet count and haemarrhage was demonstrated first by Belt et al in 1978 [8] then later by Dutcher et al [9] in 1984. The rate of haemarrhagic complications increases as the platelet nadir increases and no clear threshold is evident; similar to what happens in leukemia. In these patients, when they are without any other risk factors for bleeding, major haemarrhage has been shown to be rare at platelet counts above $10\times10^9/L$ [8-9,12-13,39] in the few studies reported so far.

As regards which factors affect nadir platelet counts, essentially some of the

factors which predispose thrombocytopenic patients to bleeding also have been said to be related to the pattern of platelet nadir counts. These include low baseline platelet counts, low baseline total lymphocyte counts (TLC), and low baseline absolute neutrophil counts (ANC) [40]. Old age has also been shown to be an important factor which is associated with low nadir counts. Older individuals have been found to be at an increased risk for myelotoxicity because of a reduction in haemopoietic reserve [71-74]. Current available data [75-77] suggest that aged bone marrow may compensate for the reduced ability of haematopoietic precursors to proliferate with the use of a higher number of such precursors leading to an excess of kinetically active myeloid progenitors and their dividing progeny, this in turn causes enhanced susceptibility to chemotherapy-induced damage. Since chemotherapy mainly hits cycling early and intermediate haemopoietic precursors, and spares most of the quiescent stem cells, bone marrow of aged individuals may offer more cellular targets to cytotoxic drugs due to its increased content of active progenitors. The age-associated expansion of defective stem cells, leading on one hand to a reduced ability to repopulate the intermediate precursor cell compartments, and on the other hand to the accumulation of kinetically active myeloid progenitors more sensitive to chemotherapy-induced damages, may explain the poor tolerance of aged bone marrow to cytotoxic drugs. It has also been suggested that the reduced haematopoietic reserve in the older individuals might be related to a reduced endogenous production of cytokines but there is no evidence that the production of cytokines that stimulate haemopoiesis is significantly altered in the elderly population [78-79]. Age-related changes of the haematopoietic microenvironment might also determine a reduced ability of this tissue to support the proliferation and the survival of haematopoietic stem and progenitor

cells under condition of stress [80-81].

The possibility that patients may actually have raised platelet counts when put on chemotherapy has been raised [1]. This could be as a result of secondary or reactive platelet production whose causes include acute and chronic infection, inflammatory disorders, chronic iron deficiency, tissue damage from trauma or surgery, drugs (e.g. steroids, vincristine), splenectomy and hyposplenism, malignancy per se (Hodgkin's Disease, solid tumors), and rebound from chemotherapy. These factors cause reactive increases in platelet counts mainly through stimulation of cytokines. This problem (reactive thrombocytosis) is often transient and clinically insignificant [82]. Otherwise Gemcitabine is perhaps the only cancer therapy agent which has been specifically reported to cause both thrombocytopaenia and thrombocytosis [83]. Another explanation for the elevated platelet counts could have been a technical problem. Automated platelet counting methods may give falsely low or falsely high values even when the machine is functioning properly. However, this problem often occurs at low platelet counts [84] and/or in some known instances of spurious elevation of platelet counts [85-87]. And then, again, malfunction of the automated counter at the time of the study could not be ruled out [1].

2.4. MANAGEMENT OF BREAST CANCER

Breast cancer and non-Hodgkin's lymphoma are common malignancies locally, ranking second and fourth respectively at KNH as was reported by Mungania [19]. These malignancies are both potentially chemo-curable since they have high dose response curves. However, high doses of chemotherapy enhance tissue

toxicity creating a major limitation in the treatment of these malignancies. Consequently, mitigation of bone marrow toxicity is likely to improve the outcome of these malignancies.

Breast Cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast. Epithelial malignancies of the breast account for 1/3 of all cancers in women. Human breast cancer is a clonal disease and may exist for a long period as either a non-invasive or invasive but non-metastatic disease. Breast cancer is a hormone dependant disease, with female: male ratio of 150:1 [88]. Breast cancer is commonly treated by various combination of surgery, radiation therapy, chemotherapy and hormone therapy. Prognosis and selection of therapy may be influenced by age and menopausal status of the patient, stage of disease, histological and nuclear grade of the primary tumor, estrogen (ER) and progesterone (PR) receptor status, measures of proliferative capacity and Her2 /neu gene amplification [88-89].

Cellular Classification and Staging Of Breast Cancer: Breast cancer can be classified as ductal, lobular, nipple or undifferentiated each with its own sub-classes. Infiltrative or invasive ductal cancer is the most common histological type of breast cancer, comprising 70-80% of all [88]. The American Joint Committee on Cancer (AJCC) staging system provides for grouping patients with respect to prognosis through the TNM Classification (appendix III). Therapeutic decisions are formulated in part according to staging categories but primarily according to tumor size, lymph node status, estrogen and progesterone receptor levels in the tumor tissue, menopausal status and the general health of the a patient. Histological sub-classifications are of prognostic importance with

mucinous, medullary and lobular sub-types having a favorable outcome [89-91].

Breast Cancer Treatment Options: Stages I, II, IIIA Breast Cancer often requires a multi modality approach to treatment. Irrespective of the eventual procedure selected, the diagnostic biopsy and surgical procedure to be used as the primary treatment should be performed as two separate procedures. Estrogen recepter (ER) and progesterone receptor (PR) status should be determined for the primary tumor. Other pathologic characteristics that may be of value include: grade, proliferative activity, Her2/neu status [89,92]. Options for surgical management of the primary tumor include: breast conserving surgery plus radiation therapy, mastectomy plus reconstruction and mastectomy alone. All histological types of invasive breast cancer may be treated with breast conserving surgery plus radiation [93]. Adjuvant systemic therapy include hormonal therapy with tamoxifen for node positive, ER positive, premenopausal patients; cytotoxic drugs for node negative patients with large tumors or other adverse prognostic factors. For postmenopausal women, tamoxifen and aromatase inhibitors such as exemestane and anastrozole are used. Commonly used combination regimens include:- AC (Doxorubicin and Cyclophosphamide); Doxorubicin and docetaxel; Doxorubicin and paclitaxel; CAF (Cyclophosphamide, Doxorubicin, 5 flourouracil); CMF (Cyclophosphamide, methotrexate, 5 flourouracil); and AC-Pax (Cyclophosphamide, Doxorubicin, Paclitaxel) [93-95].

2.5. MANAGEMENT OF NON-HODGKIN'S LYMPHOMA

Non-Hodgkin's Lymphomas (NHL) are a heterogeneous group of

Lymphoproliferative malignancies with differing pattern of behavior and responses to treatment [96]. NHL usually originates in lymphoid tissues and can spread to other organs. However unlike Hodgkin's disease NHL is less predictable and has a greater predilection to disseminate to extra nodal sites. The prognosis depends on histological type stage and treatment [97]. NHL can be divided into 2 prognostic groups, the indolent Lymphoma and the aggressive Lymphomas. Indolent NHL types have a relatively good prognosis, with median survival as long as 10 years, but they are usually incurable in advanced clinical stages [96-99]. The aggressive and highly aggressive types of NHL have a shorter natural history but a significant number (30% - 60%) of these patients can be cured with intensive combination chemotherapy regimens. In general with modern treatment of patients with NHL, overall survival at 5yrs is approximately 50% - 60% [96-97].

Cellular Classification And Staging For NHL: WHO modification of the REAL classification recognizes 3 major categories of lymphoid malignancies based on morphology and cell lineage. These include B cell neoplasms, T cell / Natural killer cell neoplasms and Hodgkin's Lymphoma. Both Lymphoma and Lymphoid Leukemias are included because both solid and circulating phases are present in many lymphoid neoplasms and distinction between them is artificial. Staging is important in selecting treatment for patients with NHL [97-98]. The Ann Arbor staging system is commonly used for patients with NHL. In this system, stages I to IV adult NHL can be sub-classified into A and B categories: B for those with well-defined generalized symptoms and A for those without (appendix II).

Treatment Options Of NHL: Treatment of NHL depends on the histological type

and stage. Although localized presentations are uncommon in NHL, the goal of treatment should be cure. Long term disease controls with radiation can be achieved in a significant number of patients with indolent stage I or II NHL. Rarely, when radiation therapy is contra-indicated, chemotherapy can be employed for symptomatic patients [99-101]. Traditionally radiation therapy had been the primary treatment of patients with Stage I or contiguous Stage II aggressive NHL. However, disease free survival using radiation therapy alone is 60 - 70% at 5 yrs. The success of combination chemotherapy in early stage disease has led to combinations of chemotherapy and radiation therapy or to the use of chemotherapy alone [100-102]. Surgery has an important role in the diagnosis and treatment of primary extra-nodal aggressive lymphomas without other site involvement. Due to their unpredictable pattern of relapse, chemotherapy is often used as the primary treatment modality [100-101]. For Indolent, non - contiguous NHL, standard treatment options include; deferred therapy with careful observation for asymptomatic patients; purine nucleoside analogues (fludarabine and 2chlorodeoxyadenosine); oral alkylating agents with or without steroids e.g. cyclophosphamide or chlorambucil; combination chemotherapy alone (CVP[cyclophosphamide, vincristine, prednisone], CHOP[cyclophosphamide, vincristine, doxorubicin, prednisone], FND[Fludarabine, Mitoxantrone, dexamethasone]); Rituximab + combined chemotherapy; intravenous chemotherapy and total body irradiation followed by autologous or allogeneic bone marrow or peripheral stem cell transplant; chemotherapy alone vs. anti-idiotype vaccine + chemotherapy [99-101].

2.6. CHOP And AC Protocol Drugs

Cyclophosphamide is an alkylating agent which, as a class of drugs, were among the first to be identified as chemotherapeutic agents, and are still important components of modern chemotherapeutic regimens. They posses an important property of disassociating into a positive electrophylic alkyl group, capable of attacking negatively charged electron rich nucleophilic sites adding alkyl groups at oxygen, nitrogen, phosphorus or sulphur atoms. It is their ability to form a variety of DNA adducts that sufficiently alter DNA structure and function or both so as to have cytotoxic effect that makes them useful chemotherapeutic agents [103]. Alkylating agents work by three different mechanisms all of which achieve the same end result - disruption of DNA function and cell death. In the first mechanism an alkylating agent attaches alkyl groups to DNA bases (figure 4). This alteration results in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases. Alkylated bases prevent DNA synthesis and RNA transcription from the affected DNA. A second mechanism by which alkylating agents cause DNA damage is the formation of cross-bridges, bonds between atoms in the DNA (figure 5). In this process, two bases are linked together by an alkylating agent that has two DNA binding sites. Bridges can be formed within a single molecule of DNA or a cross-bridge may connect two different DNA molecules. Cross-linking prevents DNA from being separated for synthesis or transcription. The third mechanism of action of alkylating agents is the induction of mispairing of the nucleotides leading to mutations. In a normal DNA double helix, A always pairs with T and G always pairs with C. Alkylated G bases may erroneously pair with Ts (figure 6). If this altered pairing is not corrected it may lead to a permanent mutation. Cyclophosphamide

undergoes several metabolic changes before generating the major reactive metabolite phosphoramide mustard (figure 7). Acrolein, one of the reactive metabolites, reacts by depleting cellular glutathione and causing DNA alkylation and is notorious for causing haemarrhagic cystitis and predisposes to transitional cell carcinoma [36,103]. Cyclophosphamide plays a major role in treatment of breast cancer and other neoplastic diseases and is used in various combinations for treatment of lymphoid malignancies. It produces significant leukopaenia, immunosuppresion and mild thrombocytopaenia. Other complications include sterility in men, ammenorrhea in women, and can cause the syndrome of inappropriate antidiuretic hormone synthesis leading to hyponatremia, seizures and death. It has also been noted to be leukemogenic and can cause an acute cardiac necrosis [36,38,103-104].

Figure 4: Alkylating agent mechanism 1

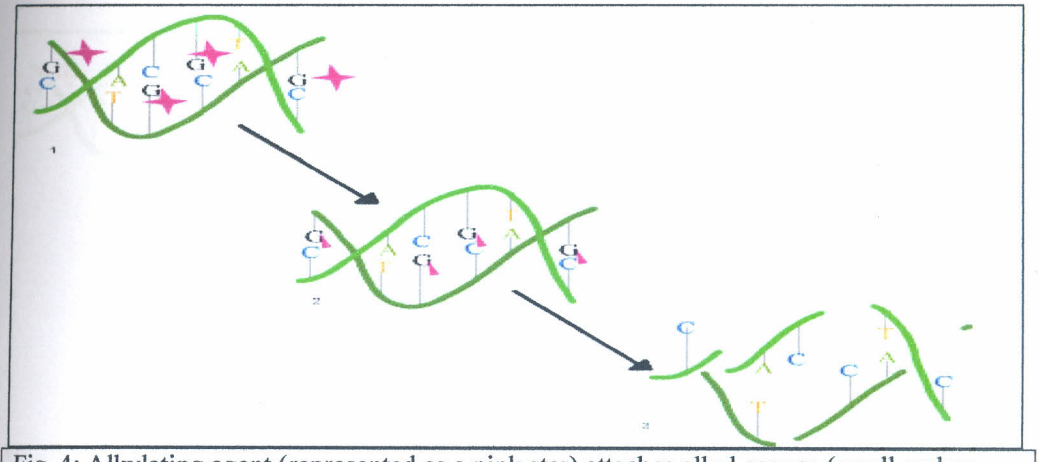


Fig. 4: Alkylating agent (represented as a pink star) attaches alkyl groups (small carbon compounds-depicted as pink triangles) to DNA bases. This alteration results in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases. Alkylated bases prevent DNA synthesis and RNA transcription from the affected DNA

Figure 5: Alkylating agent mechanism 2

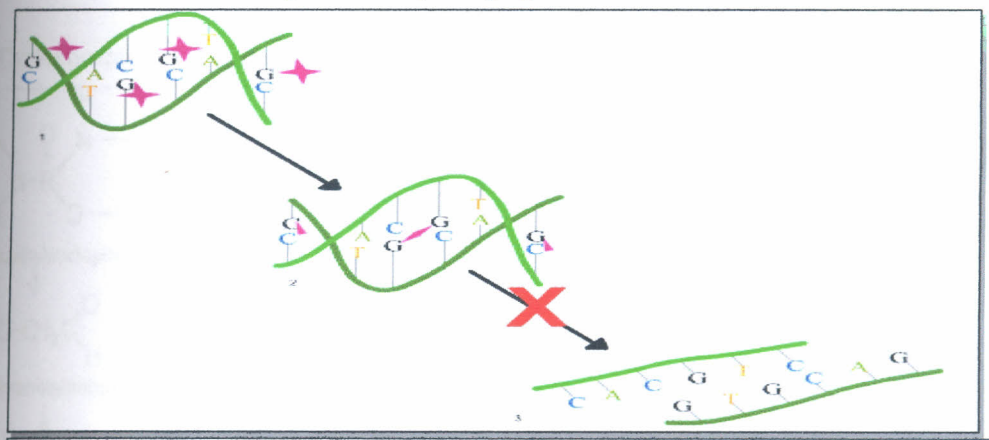


Fig. 5: Formation of cross-bridges, bonds between atoms in the DNA (pink linkages); two bases are linked together by an alkylating agent that has two DNA binding sites. Bridges can be formed within a single molecule of DNA or a cross-bridge may connect two different DNA molecules. Cross-linking prevents DNA from being separated for synthesis or transcription

Figure 6: Alkylating agent mechanism 3

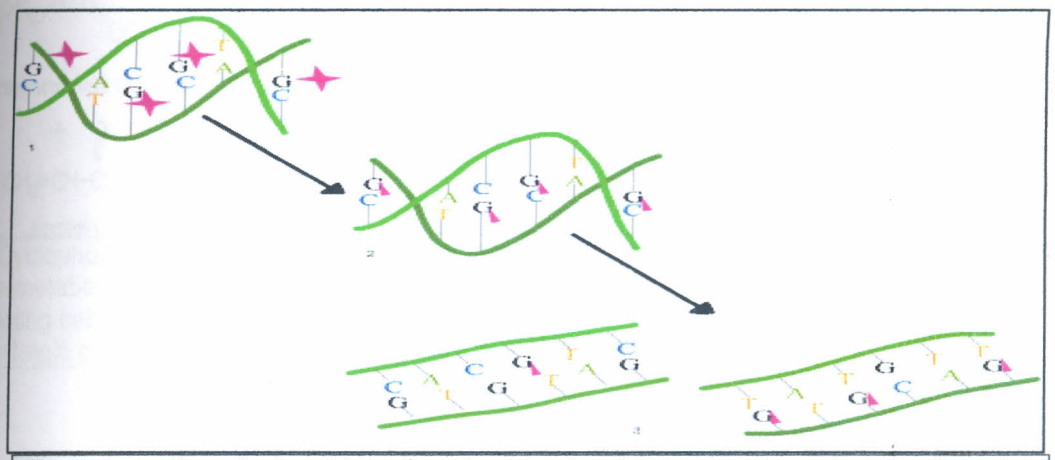


Fig. 6: Induction of mispairing of nucleotides leading to mutations. In a normal DNA double helix, A always pairs with T and G always pairs with C. Alkylated G bases may erroneously pair with Ts. If this altered pairing is not corrected it may lead to a permanent mutation

Figure 7: Cyclophosphamide metabolism

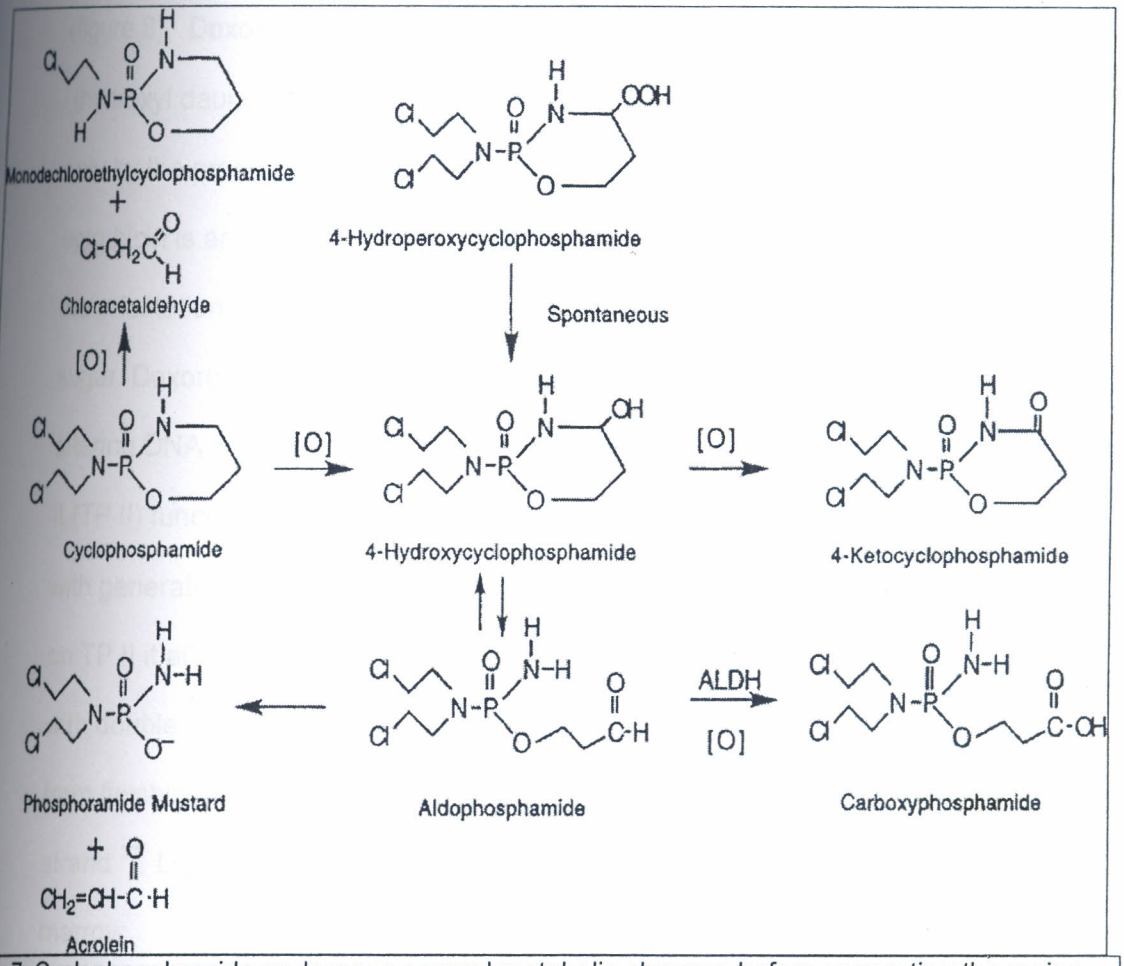


Fig. 7: Cyclophosphamide undergoes several metabolic changes before generating the major reactive metabolite phosphoramide mustard. Acrolein, one of the reactive metabolites, reacts by depleting cellular glutathione and causing DNA alkylation and is notorious for causing haemarrhagic cystitis and predisposes to transitional cell carcinoma

Doxorubicin (Hydroxydaunorubicin, Adriamycin®) This is an antitumor antibiotic (an anthracycline) isolated from a mutant strand of the fungus streptomyces, which demonstrates greater activity in treatment of solid tumors including breast cancer and malignant lymphoma [36-37]. Doxorubicin is the most widely active and frequently used antineoplastic agent. All anthracyclines share a quinone-containing rigid planar aromatic ring structure bound by a glycosidic bond to an amino sugar, daunosamine

(figure 8). Doxorubicin (hydroxyl daunorubicin) differs from daunorubicin only by the presence of a C-14 hydroxyl group, and epirubicin is an epimer of doxorubicin differing only in the orientation of the C-4 hydroxyl group on the sugar. Doxorubicin intercalates into DNA, thereby altering DNA structure replication, and topoisomerase II (TP II) function. It also undergoes redox cycling with generation of free radicals. Through its effects on TP II it allows formation of the protein associated with double strand breaks, but prevents the enzyme from finishing its cycle with relegation of broken strand [37]. Doxorubicin has a high incidence of bone marrow suppression, which manifests itself mainly as a neutropaenia that is most severe 10—14 days after treatment and lasts about 7 days. A white cell count as low as $1.0x10^9/L$ is to be expected. This neutropaenia together with the mucositis that may occur are dose limiting. Alopecia is a universal finding, which causes significant patient distress [36-38]. Other adverse effects include, cardiac toxicity, which is a function of the total dose, and may present as cardiomyopathy induced chronic heart failure. An acute cardiotoxicity unrelated to cumulative dose may occur, this presents as conduction defects and pump failure [36-38].

Figure 8: Anthracycline structure

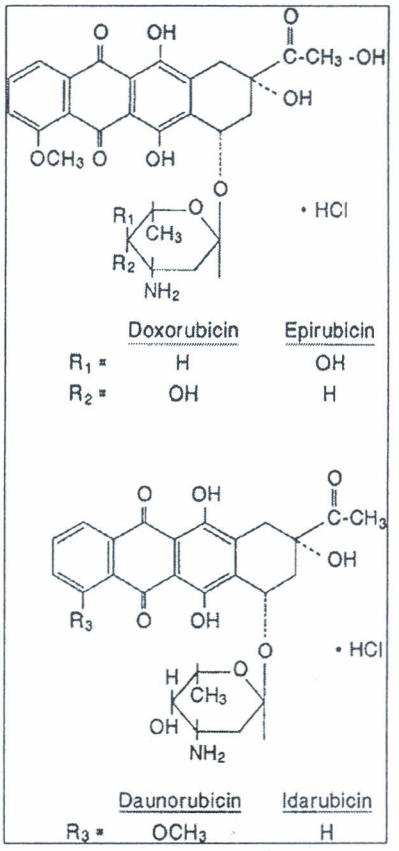


Fig. 8: All anthracyclines share a quinone-containing rigid planar aromatic ring structure bound by a glycosidic bond to an amino sugar, daunosamine

Vincristine (Oncovin®): This is a vinca-alkaloid, which is an essential part of combination chemotherapy regimens for acute lymphoblastic leukemia and plays an important role in combined chemotherapy for both Hodgkin's and non-Hodgkin's lymphoma and other solid tumors [105]. The vinca alkaloids are dimeric molecules composed of two multiringed units (figure 9): an indole nucleus (catharanthine) and a dihydroindole nucleus (vindoline). Vinca alkaloids cause mitotic arrest by binding to specific sites on tubulin hence preventing polymerization of tubulin. This disrupts the formation of microtubules. In vivo, they induce cytolytic effects on non-proliferating cells of the G1 phase in a drug concentration and duration of exposure dependent fashion [105]. Peripheral neurotoxicity, resulting from axonal degeneration is the most frequent dose limiting effect. Vincristine has not been shown to have significant bone marrow suppression [36,38,105]. In fact, vincristine has been used to treat idiopathic thrombocytopenic purpura (ITP) and hence it may have a small protective effect against chemotherapy induced thrombocytopaenia; and like other vinca alkaloids, it has been known to cause thrombocytosis [106-107] - an effect which has been put to clinical use in platelet transfusions [108]. Some of the mechanisms by which vincristine has been shown to cause increases in platelet counts include immunosuppression [109] and inhibition of microtubule-dependent events required for macrocyte-monocyte phagocytic function [110-111]. The effect is likely to be small though in CHOP-21 because for a good effect vincristine needs to be given weekly [112-114].

Figure 9: Structure of the vindoline and catharanthine rings

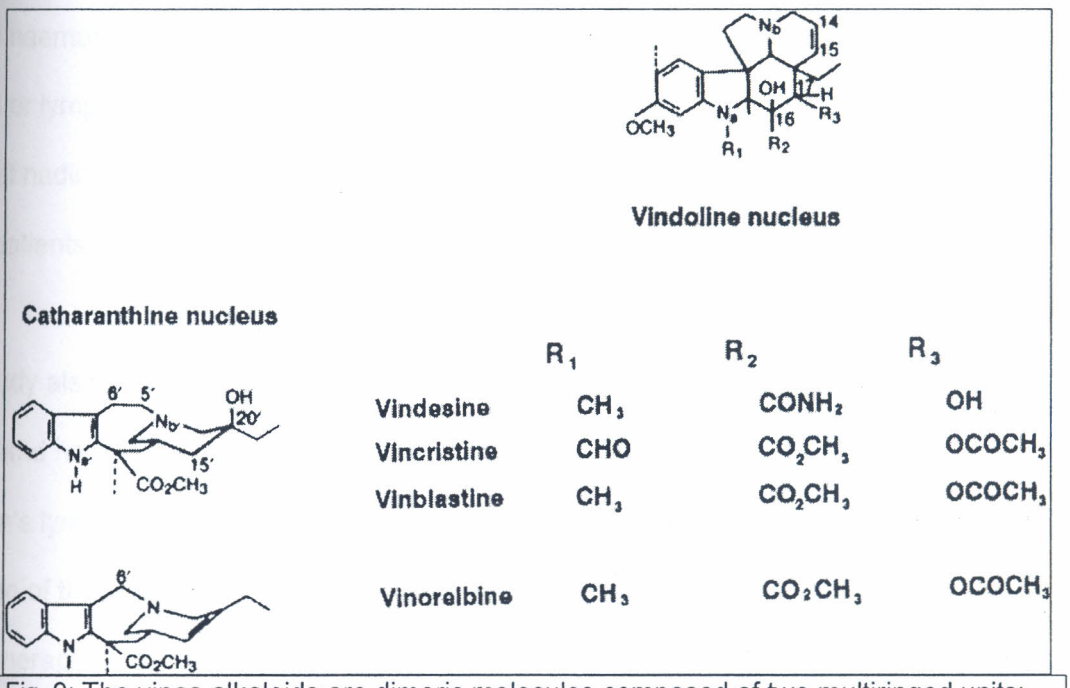


Fig. 9: The vinca alkaloids are dimeric molecules composed of two multiringed units: an indole nucleus (catharanthine) and a dihydroindole nucleus (vindoline)

Prednisone: Glucocorticoids suppress mitosis in lymphocytes and fibroblasts and appear to inhibit transcription. This so called lympholytic effect is employed in the chemotherapy of the lymphocytic leukaemias and lymphoma where they induce apoptosis. There is no reported direct effect on bone marrow. Prednisone is also used to treat immune thrombocytopaenia.

3. RATIONALE

Chemotherapy is one of the major causes of thrombocytopaenia which can lead to severe haemarrhage. In order to provide optimal supportive care to patients with solid tumors or lymphoma who are on chemotherapy, there was need to locally establish the depth of nadir platelet counts and their relationship, if any, to the risk of bleeding in these patients.

This study also sought to analyse factors contributing to the depth of nadir platelet counts and haemarrhage in patients on chemotherapy for breast cancer and Non-Hodgkin's lymphoma. Identification of these factors may be of assistance in the selection of those patients who would benefit from a conservative management of chemotherapy induced thrombocytopaenia.

An earlier study at KNH [1] had raised the possibility that chemotherapy in lymphoma and olid tumors may induce thrombocytosis at times. These findings which are inconsistent ith current literature required further study; specifically to address the possibility of ethodology limitations as regards the platelet count component of the earlier study.

4. RESEARCH QUESTION

What is the pattern and relationship to haemarrhage of chemotherapy-induced thrombocytopaenia in lymphoma and solid tumors?

5. STUDY OBJECTIVES

5.1. PRIMARY OBJECTIVE

The primary objective was to establish the platelet count nadirs on day 10 to 14 of the first two cycles of chemotherapy.

5.2. SECONDARY OBJECTIVES

The secondary objectives were to describe (1) the pattern and magnitude of change in platelet counts (2) factors associated with nadir platelet depths and (3) factors associated with haemarrhage.

6. MATERIALS AND METHODS

6.1. STUDY DESIGN

Prospective descriptive study, using a real life usual practice scenario.

6.2. STUDY AREA SITES

The study was conducted at the Haematology and Oncology outpatient Clinic, the Cancer Treatment Center and Medical Wards of KENYATTA NATIONAL HOSPITAL (KNH). Kenyatta National Hospital serves as the main tertiary referral and university teaching hospital in Kenya. It is the main public hospital in Kenya which offers specialist oncology care.

6.3. SELECTION OF PATIENTS

Patients were included if they were aged 13 years and above; had a histological diagnosis of breast cancer or NHL and were staged and eligible for a study chemotherapy protocol for (1) adjuvant breast cancer chemotherapy, or (2) advanced breast cancer disease, or (3) indolent advanced stage (III and IV) NHL, or (4) indolent early stage I and II NHL with B-symptoms, or (5) aggressive and highly aggressive NHL in all stages other than stage IA. Patients were recruited only after they had given an informed written consent or after a parent or an accompanying relative/guardian had given an informed written consent in cases where patients were below the age of consent [English and Kiswahili consent explanation and forms are in appendices V(A) to V(D)].

To exclude confounding factors, patients were excluded if they had clinical or biochemical evidence of organ dysfunction not explained by the disease process e.g. renal insufficiency (calculated GFR below 50 mls/min - a level which is generally considered to represent renal dysfunction across all age groups as well as being a cut-off point for withholding of or adjusting the doses of AC and CHOP protocols) or liver dysfunction (transaminases above five times normal); an ECOG performance score of 3 and above (ECOG performance scoring system appears in appendix I); contraindications to any of the component drug comprising the study protocol; ELISA positivity for HIV Ab upon routine work up; baseline thrombocytopaenia of less than $100x10^9$/L or concurrent idiopathic thrombocytopenic purpura. Also excluded were patients who did not give or could not give an informed written consent.

6.4. SAMPLE SIZE AND SAMPLING

Sample size was calculated using the sample size formula for a prevalence study for a single proportion [115], $n=Z^2P(1-P)/d^2$; where **n** is the sample size, **Z** represents confidence interval, **P** is prevalence and **d** is the level of precision. We used a confidence interval of 95% (**Z**=1.96) and a level of precision (**d**) of 0.05. **P** was determined using estimates from previous published studies which had analyzed incidences of our clinical outcome of interest (haemarrhage) in lymphoma and solid tumor patients; but with regard for feasibility of collecting the required number of samples in the time available - bearing in mind the fact that we did not necessarily set out to determine prevalence of haemarrhage in our study population.

The Incidence of haemarrhagic complications in patients with solid tumors was

firstly studied by Belt et al.[8] in a cohort of 718 patients receiving myelosuppressive chemotherapeutic agents and 75 patients (10.4%) experienced one or more episodes of haemarrhage. However bleeding was due to tumor invasion in 25 patients (33.3%), to disseminated intravascular coagulation in 7 patients (9.3%), and was unrelated to malignant neoplasms or drug treatment in 6 patients (8%). Thirty-seven patients (49.3% of those who bled or **5.2%** of the total number of patients) had haemarrhages associated with drug-induced thrombocytopaenia. All other published studies reviewed for the purposes of this study reported incidences lower than 5.2% [9-10,13] except for the 1994 study of Goldberg et al. [12] which had a relatively high rate of minor bleeding episodes at 18.7%. A rate of 18.7% would have required a minimum of 234 patients and a pilot analysis of patient numbers in the study area sites had indicated that recruiting 234 patients would have taken close to two years (21 months), so the rate of 18.7% was treated as an outlier. It is however important to note that the 1994 study of Goldberg et al reported a rate of 4.9% for major bleeding which is less than 5.2%.

Assuming the prevalence (**P**) of haemarrhage secondary to chemotherapy induced thrombocytopaenia to be 5.2% (i.e. 0.052), a minimum sample size of 76 was obtained. Seventy-eight patients were recruited through consecutive sampling over a period of eight months from mid September 2006 to mid May 2007.

6.5. SCREENING AND RECRUITMENT PROCEDURE

The study was approved by the KNH Research and Ethics Committee before any patients were enrolled. The principal investigator with the help of a study assistant, screened and recruited study cases both in the wards and outpatient clinics using a screening and recruitment proforma. The records and charts of all new patients

presenting to the outpatient clinics or admitted for workup were reviewed. All records with histologically diagnosed and staged breast cancer or non-Hodgkin's lymphoma were selected. Non-staged patients were followed up and selected if and when they got staged during the study period. After baseline workup and assessment of eligibility for chemotherapy by the usual care team, patients for whom one of the study protocols was recommended by the attending oncologist and who had satisfied the inclusion criteria and were outside the exclusion criteria, were recruited. Baseline workup by the usual care team included full blood counts (FBC), renal and liver function tests, HIV testing (with pre and post test counseling), and any other investigation deemed necessary by the attending doctor e.g. staging investigations. Assessment of eligibility for chemotherapy by the usual care team included evaluation for clinical evidence of cardiac disease which would make anthracycline based drugs contraindicated, physical examination to exclude any obvious infection, and perfomance assessment to establish the ECOG performance score.

Study Chemotherapy Protocols: For breast cancer cases, chemotherapy protocol of relevance to the study was 3 weekly cycles of intravenous Cyclophosphamide *600 mg/m^2* day 1 and Doxorubicin *60 mg/m^2* day 1. For NHL cases, chemotherapy protocol of relevance to the study was 3 weekly cycles of intravenous Cyclophosphamide *750mg/m^2* day 1, Doxorubicin *50mg/ m^2* day 1 and Vincristine *1.4mg/ m^2* day 1 and oral Prednisone *60mg/ m^2* days 1 to 5.

6.6. STUDY PROCEDURE

Chemotherapy was given by the usual care team. The usual practice was to check

blood counts for all patients on the above protocols at least weekly. In consultation with the attending oncologist, the principal investigator (PI) arranged for three milliliters of venous blood to be taken from every study patient for complete blood counts on day 1 of the first three cycles and between day 10 and day 14 (inclusive of both days) of the first two cycles; using an Ethylenediamine Tetraacetic Acid (EDTA) whole blood tube. A peripheral blood smear was also prepared at the time of venipuncture. After mixing the tube ten times by gentle inversion, a specimen was either maintained at room temperature up to a maximum of 24 hours or was refrigerated up to a maximum of 48 hours within which period it was analysed. The PI facilitated analysis of the samples at KNH haematology laboratory which uses a CELL DYN® 3200 automated haematology analyser (Abbott Diagnostics, Santa Clara CA U.S.A.). The PI then ensured that results were placed in the patient's file and were also verbally communicated to the usual care team when clinically significant. Quality assurance measures were observed to ensure accurate platelet count results. The KNH haematology laboratory is subjected to external and internal quality assessment measures. Automated haematology analysers may sometimes, even when functioning properly, give falsely high platelet counts by counting other elements such as bacteria and cytoplasmic fragments as platelets or may give falsely low counts when platelets stick together. For this reason, the blood smears prepared at the time of venipuncture were subjected to examination by the study designated haematologist to obtain an approximate quantity of platelets and thereby validate the automated analyser results. This was done by averaging ten high-power fields microscopically and then multiplying by 15,000 to arrive at a platelet count in 1,000 per microliter.

Haematology Laboratory Quality Assessment: The KNH haematology laboratory

is part of International External Quality Assessment Scheme (IEQAS) [116] which is a WHO EQAS (World Health Organisation External Quality Assessment Scheme) for haematology distributed to 80 laboratories in 58 WHO member states by the WHO Collaborating Centre based within the United Kingdom (UK) National External Quality Assessment Scheme (UKNEQAS) located at Watford General Hospital, UK. The scheme assesses the ability of the laboratory to correctly quantify the haemoglobin level and the number of white blood cells (WBC) and platelets. In addition, blood film slides are also provided to either determine the percentage of reticulocytes, differential and cell morphology or to identify blood parasite species. The CELL DYN® 3200 automated analyser is a medium throughput optical flow cytometer that applies the principles of multidimensional light scatter (the MAPSS™) for generation of blood count data. The instrument uses a helium neon gas laser which emits red light (633nm). In terms of Internal Quality Control, the KNH haematology laboratory conforms to College Of American Pathologists (CAP) requirements [117] and Clinical Laboratory Improvement Amendments (CLIA) regulations [118] in its use of the CELL-DYN 3200 analyser. At the beginning of the day, the machine primes itself when it is switched on. At the end of priming it displays a range of internal results which the technician checks to verify validity. An enzymatic cleaner is then run through the analyser followed by a daily control. Full quality control using control cells is done on a regular basis.

Assessment For Haemarrhage And Its Associated Factors: As per routine, patients on chemotherapy were evaluated at least on a weekly basis for development of new symptoms and signs and resolution of those present at commencement of chemotherapy. The principal investigator facilitated weekly review of both study in-patients and out-patients. This included an interview and a

sical examination. Significant features which were looked for included the mber and severity of episodes of haemarrhage and occurrence of known tures associated with haemorrhage in chemotherapy induced thrombocytopaenia [12,13,39] which included features of infection such as fever, mucositis etc. Out-tients were advised to report if they developed a problem before the subsequent heduled visit and were encouraged to contact the PI through their medical care eam if they got admitted to KNH within the study period. Bleeding episodes were to e dichotomised into minor episodes (WHO Grades 1 or 2 – appendix IV [119-120] – ncluding petechiae, ecchymoses, superficial bleeding of gums, microscopic ematuria, blood-tinged sputum, mild epistaxis, and vaginal bleeding not requiring ed cell transfusion) or major episodes (WHO Grades 3 or 4, including fatal haemarrhage at any site or epistaxis, vaginal bleeding, or major organ haemarrhage equiring red cell transfusion)

6.7. ETHICAL CONSIDERATIONS

The study was conducted in accordance with the Declaration of Helsinki on "Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects" [121] and permission to carry out the study was obtained from the KNH Ethics and research committee. Informed written voluntary consent was sought from the patient or the parent/guardian for those patients less than 18 years of age. Those who declined consent were ***not*** discriminated against. Study patients received standard routine care and usual care and evaluation procedures were facilitated. Confidentiality was maintained.

6.8. DATA COLLECTION

A data collection sheet consisting of a screening proforma and recruitment proforma was used to collect data (appendix VI). The primary endpoint was nadir platelet counts. Intended secondary endpoints included: number and severity of bleeding episodes, diagnosis/regimen, baseline absolute neutrophil counts (ANC) and baseline total lymphocyte count (TLC), and baseline platelet (PLT) counts.

6.9. DATA ANALYSIS

Descriptive statistics (mean, median, percentiles) were used for platelet counts and for frequency of secondary endpoints. These have been presented as tables, bar charts, and boxplots. Homogeneity of variance was tested using the Levene statistic and distribution was checked using one-sample Kolmogorov-Smirnov test. Median nadir platelet counts and median nadir platelet count depths between the two cycles were compared using the Wilcoxon signed ranks test for related samples. The relationships between secondary endpoints (which were a combination of interval and nominal variables) and nadir counts as well as the depths of nadir platelet counts were performed using t-test and One-Way ANOVA to compare means; and the Mann-Whitnney U (Wilcoxon Rank Sum W) and Kruskal Wallis tests to compare medians. Post hoc analysis was done using Tukey HSD test. P value was considered significant at less than .05 for all tests.

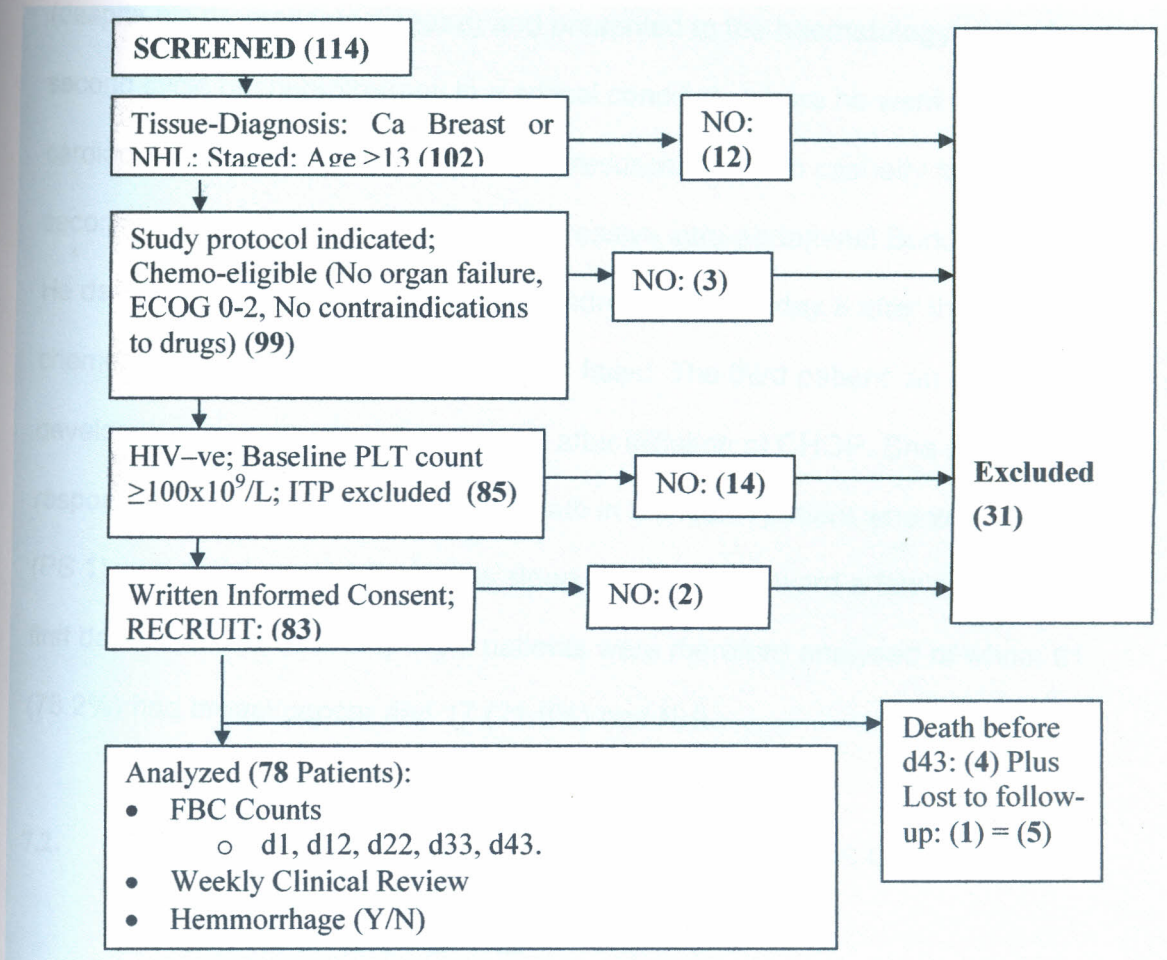
All data processing and analysis was done using the statistical package SPSS (11.5.1 for Windows; SPSS Inc., Chicago, Illinois, USA).

7. RESULTS

7.1. SCREENING AND RECRUITMENT

A total of 114 patients were screened (figure 10). Thirty-one patients were excluded – 12 of them did not have tissue diagnosis (11 suspected NHL and 1 suspected breast cancer); three were started on alternative regimens by their attending physicians (2 NHL put on MECOD-A and 1 breast cancer put on Docetaxel/Adriamycin); 14 were HIV positive (13 NHL and 1 breast cancer); and two withheld consent (they were unwilling to indicate yes or no on the consent form; both NHL).

Figure 10: SCREENING AND RECRUITMENT FLOW CHART



Thus 83 patients were recruited into the study. However, of the recruited patients, one (NHL) was lost to follow-up after receiving the first cycle of chemotherapy and 4 died before day 43 of chemotherapy (i.e. before day 1 of third cycle). All the patients who died were NHL patients from the medical wards; three had a performance scale (PS) of 2 and one had a PS of 1. Of the three patients with a PS of 2, one (a young man in his 30s) had a NHL-associated superior vena cava syndrome which responded to CHOP soon after day 1 of treatment; then a few days later the patient's symptoms returned. The patient was not keen on further investigations to confirm possible resistance to CHOP. He asked for discharge from the ward (despite his progressive disease) and presented to the haematology clinic, for his second cycle of chemotherapy in a critical condition, where he went into cardiopulmonary arrest and attempts to resuscitate him in casualty failed. The second patient (a teenager) had an aggressive intra-abdominal Burkitt's Lymphoma. He developed features of tumor lysis syndrome around day 8 after the initiation of chemotherapy. Efforts to resuscitate him failed. The third patient, an elderly lady, developed severe sepsis around day 6 after initiation of CHOP. She failed to respond to antibiotics. The cause of death in the fourth patient who was clinically fit (PS 1) was not clear. He died in his sleep at night in the ward a few days after his first dose of CHOP. Seventy eight patients were therefore analysed of whom 61 (78.2%) had breast cancer and 17 (21.8%) had NHL.

7.2. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS.

Forty-nine patients (62.8%) were recruited from the Cancer Treatment Center, 23 patients (29.5%) were recruited from the Haematology Clinic and 6 patients (7.7%) were recruited from the Medical Wards.

In compiling our baseline demographic and clinical data we were guided by WHO recommendations on the "*minimum sets of patient and tumour characteristics necessary for identification of the patient population under study in describing results of cancer treatment*" [119].

Overall the mean age of patients was 45.2 (SD 12.7) years and median was 44 years with minimum and maximum ages of 14 and 73 years respectively. The majority of patients were in the 35-64 year age group.

The mean age for breast cancer patients was 46.1 (SD 11.1) years, median was 45 years with a range of 25 – 73 years. The majority of Breast cancer patients were in the 35-54 year age group. There were no breast cancer patients in the 14-24 year age group.

The mean age for NHL patients was 42.3 (SD 17.3) years, median was 40 years with a range of 14 – 67 years. NHL patients were more spread out across the age groups than the Breast Cancer patients and most of them were in the 14-24 and 55-64 year age groups.

Overall 12 patients (15.4%) were male and 66 patients (84.6%) were female. The male to female ratio was 1:5.5 . Among the 61 Breast Cancer patients, one (1.6%) was male (F:M ratio 60:1); and for the 17 NHL patients 11 (64.7%) were male (M:F ratio about 2:1).

The majority of the patients came from Central (37%) and Eastern (24%) provinces as is expected for Kenyatta National Hospital; no patient was from North Eastern Province. Among the NHL patients, in addition to North Eastern Province, there

were no patients from Coast and Nairobi provinces. Additionally, Eastern (29%) and Nyanza (24%) Provinces had more patients each than Central Province (18%). Western Province also had relatively more patients (12%) than the overall patient representation (5%). For the Breast Cancer patients, patient representation was similar to the overall pattern.

The majority of the patients were in stage II disease (50%) and had ECOG performance score of 1 (84.6%). All analyzed patients received the first two cycles of their scheduled chemotherapy. The patient demographic and clinical characteristics are summarized in table 1.

Table 1: Summary Of Patient Demographic And Clinical Characteristics

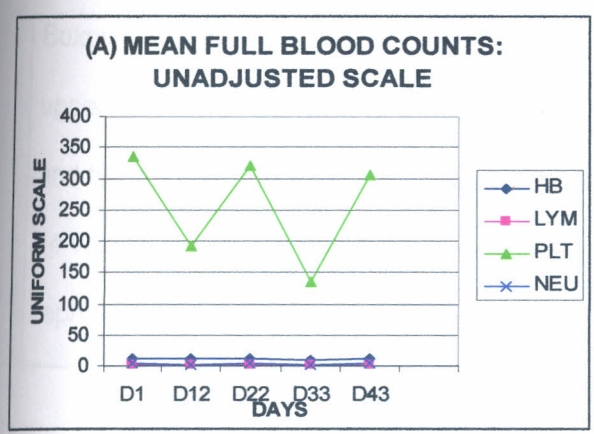
Characteristic		Overall		Breast Cancer		NHL	
Sex	Male	12(15.4%)	100%	1(.6%)	100%	11(64.7%)	100%
	Female	66(84.6%)		60(98.4%)		6(35.3%)	
Age (years)	Mean(SD)	45.2(12.7)		46.1(11.1)		42.3(17.3)	
	Median(Range)	44(14-73)		45(25-73)		40(14-67)	
Home Province	Central	37%	100%	43%	100%	18%	100%
	Eastern	24%		23%		29%	
	Rift Valley	12%		10%		18%	
	Nyanza	10%		7%		24%	
	Nairobi	8%		10%		0%	
	Western	5%		3%		12%	
	Coast	4%		5%		0%	
	North Eastern	0%		0%		0%	
Disease Stage	0	1(1%)	100%	1(1.6%)	100%	N/A	100%
	I	3(4%)		3(4.9%)		0(0%)	
	II	39(50%)		36(59%)		3(17.4%)	
	III	18(23%)		17(27.9%)		1(5.9%)	
	IV	17(22%)		4(6.6%)		13(76.5%)	
ECOG Perfomance Score	0	1(1.3%)	100%	0	100%	1(5.9%)	100%
	1	66(84.6%)		58(95.1%)		8(47.1%)	
	2	11(14.1%)		3(4.9%)		8(47.1%)	
BSA[$m^{(2)}$]	Mean(SD)	1.66(0.21)		1.65(0.19)		1.68(0.28)	
	Median(Range)	1.68(1-2.18)		1.68(1-2)		1.67(1.13-2.18)	

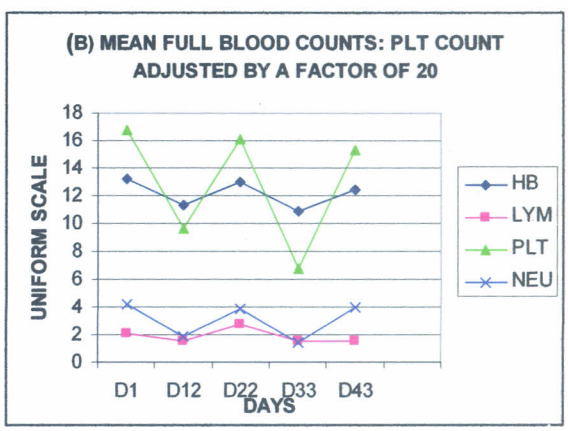
7.3. FULL BLOOD COUNT RESULTS

7.3.1. COMPARATIVE TRENDS

The trend in mean values for haemoglobin levels, total lymphocyte counts, absolute neutrophil counts and platelet counts are shown in figure 11. These values have different scales so that when projected on a uniform scale (part A of figure 11), only the platelet count line graph is appreciable compared to the small scaled haemoglobin, total lymphocyte and absolute neutrophil values. In part B of figure 11 the platelet count values have been scaled down by a factor of 20 which has brought the platelet count line graph down to the range of the other three parameters. In this way it is easy to appreciate the oscillatory nature of the variations in the haemoglobin, absolute neutrophil counts and platelet count levels with a mean change of about 2g/dL in haemoglobin levels from baseline to nadir and that the nadir haemoglobin levels on average did not go below 8g/dL which is a clinically significant trigger for blood transfusion in patients on chemotherapy for breast cancer and lymphoma. It is also important to note from part B of figure 11 that there was on average a $2x10^9$/L drop in absolute neutrophil count values from baseline values to average nadir counts of below $2x10^9$/L. This represents significant neutropaenia. The line graph for total lymphocyte count is almost a flat line implying little change in values.

Figure 11: Full Blood Count Mean Values On A Uniform Scale

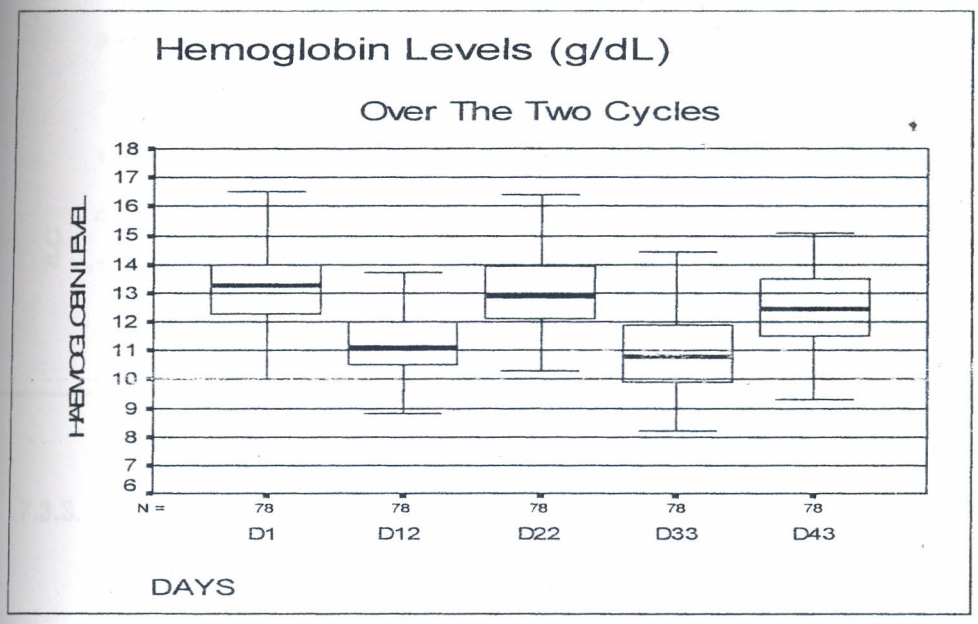




7.3.2. HAEMOGLOBIN AND TOTAL LYMPHOCYTE COUNT LEVELS

Analysis of haemoglobin (figure 12) and total lymphocyte count (figure 13) levels showed that the mean haemoglobin level dropped by 1.83 g/dL from 13.17(SD1.4) g/dL to 11.34(SD1.39) g/dL from day 1 to day 12 in cycle 1 then rose by 1.64 g/dL to 12.98 (SD 1.3) g/dL by day 22. In cycle 2 it dropped by 2.05 g/dL to 10.94 (SD 1.42) g/dL around day 33 then rose by 1.49 g/dL to 12.42 (SD 1.34) g/dL by day 43.

Figure 12: Haemoglobin Level Parameters

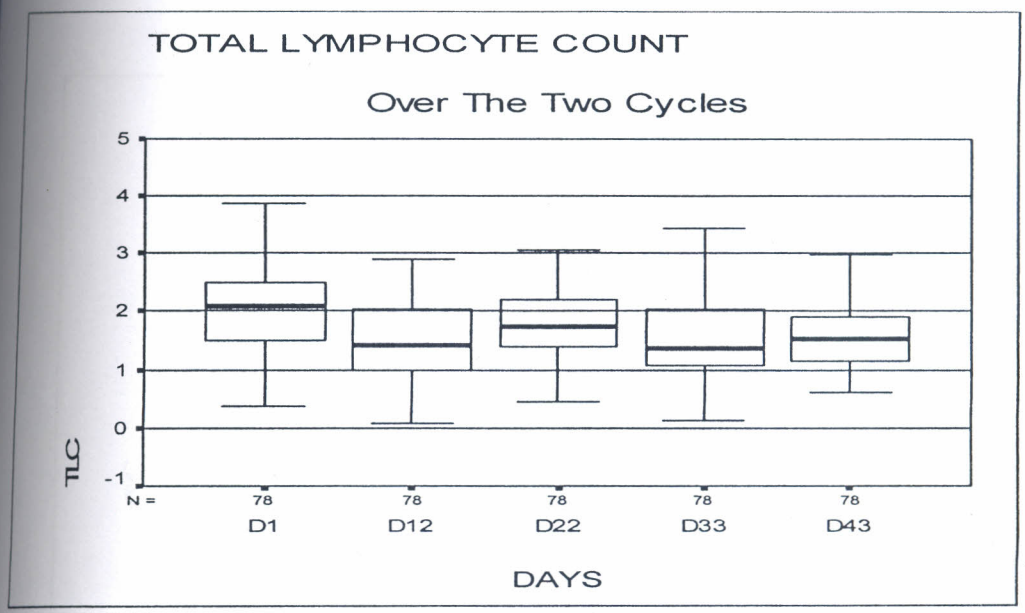


Boxplot for haemoglobin levels: whiskers represent maximum and minimum values, upper and lower borders of each box represent 75th and 25th percentiles respectively and the middle line in the box represent the median value. D12 represents day 10 14 values in cycle 1 and D33 represents day 10-14 values in cycle 2. D22 and D43 represent day 1 of 2nd and 3rd cycles respectively

The mean total lymphocyte count (TLC) dropped by *0.50x10^9/L* from *2.06 (SD 0.88)*

$x10^9/L$ to *1.56 (SD 0.8)* $x10^9/L$ in cycle 1 between day 1 and day 12, then rose by *1.17* $x10^9/L$ by day 22. In cycle 2 it dropped by *1.15* $x10^9/L$ to 1.58 (SD *0.73)* $x10^9/L$ by day 33 then rose by *0.02* $x10^9/L$ to *1.6 (SD 0.64)* $x10^9/L$ by day 43. As per the study design, the haemoglobin level and the total lymphocyte count changes were not analyzed statistically but we noted, as expected, that they were clinically insignificant as alluded to above.

Figure 13: Total Lymphocyte Count Parameters

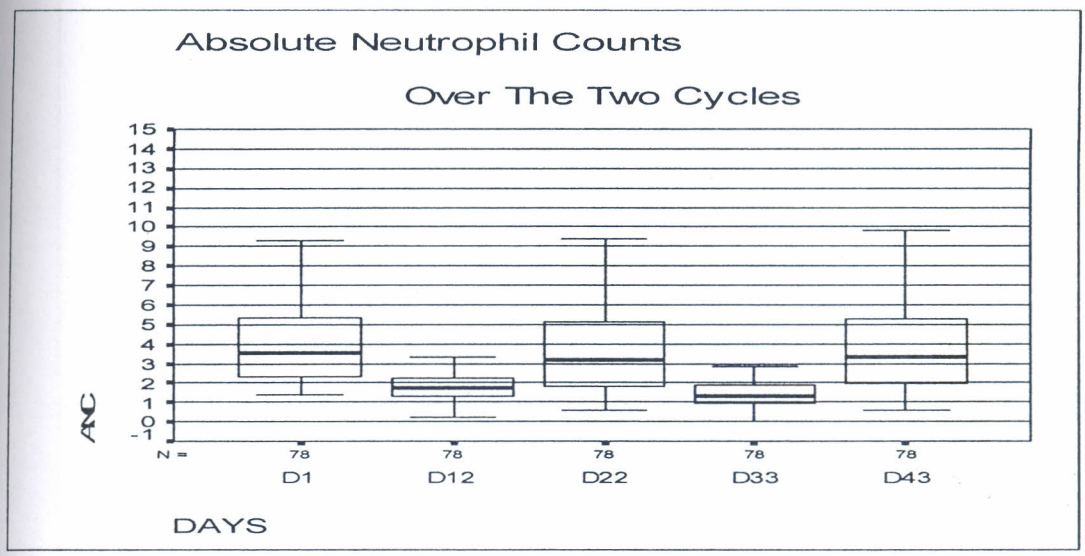


7.3.3. ABSOLUTE NEUTROPHIL COUNTS (ANC)

The mean absolute neutrophil counts (figure 14) in cycle 1 dropped by *2.3* $x10^9/L$ *[from 4.18 (SD 2.6)* $x10^9/L$ *to 1.88 (SD 1.0)* $x10^9/L$] from day 1 to day 12, then rose by *1.99* $x10^9/L$ to *3.87 (SD 2.95)* $x10^9/L$ by day 22. In cycle 2 it dropped by *2.41* $x10^9/L$ to *1.46 (SD 0.84)* $x10^9/L$ on day 33 then rose by *2.53* $x10^9/L$ to *3.99 (SD 2.96)* $x10^9/L$ by day 43. In cycle1, 9 patients had ANC values of less than $1x10^9/L$ (grade 3 and 4 neutropaenia), the lowest count was 0.21 $x10^9/L$ and the highest

count was 6.27x10^9/L. In cycle 2, 20 patients had ANC values of less than 1x10^9/L, the lowest count was 0.04x10^9/L and the highest was 4.9x10^9/L. As per the study design, these changes were not analyzed statistically but we noted - as expected – that they represented clinically significant incidences of severe neutropaenia as alluded to above.

Figure 14: Absolute Neutrophil Count Parameters



7.3.4. PLATELET (PLT) COUNTS

7.3.4.1. Nadir Counts, Trend & Nadir Depths

The mean platelet count dropped by ***142.31*** *x10^9/L* (depth) in cycle 1 from *335.50 (SD 98.4) x10^9/L* to ***193.19 (SD 73.7)*** *x10^9/L* (nadir count) between day 1 and day 12 then rose by *128.22 x10^9/L* to *321.41 (SD 104.6) x10^9/L* by day 22. In cycle 2 the mean platelet count dropped by ***185.54*** *x10^9/L* (depth) to ***135.87 (SD 75.97)*** *x10^9/L* (nadir count) by day 33 then rose by *171.28*

Figure 16: Platelet Count Parameters

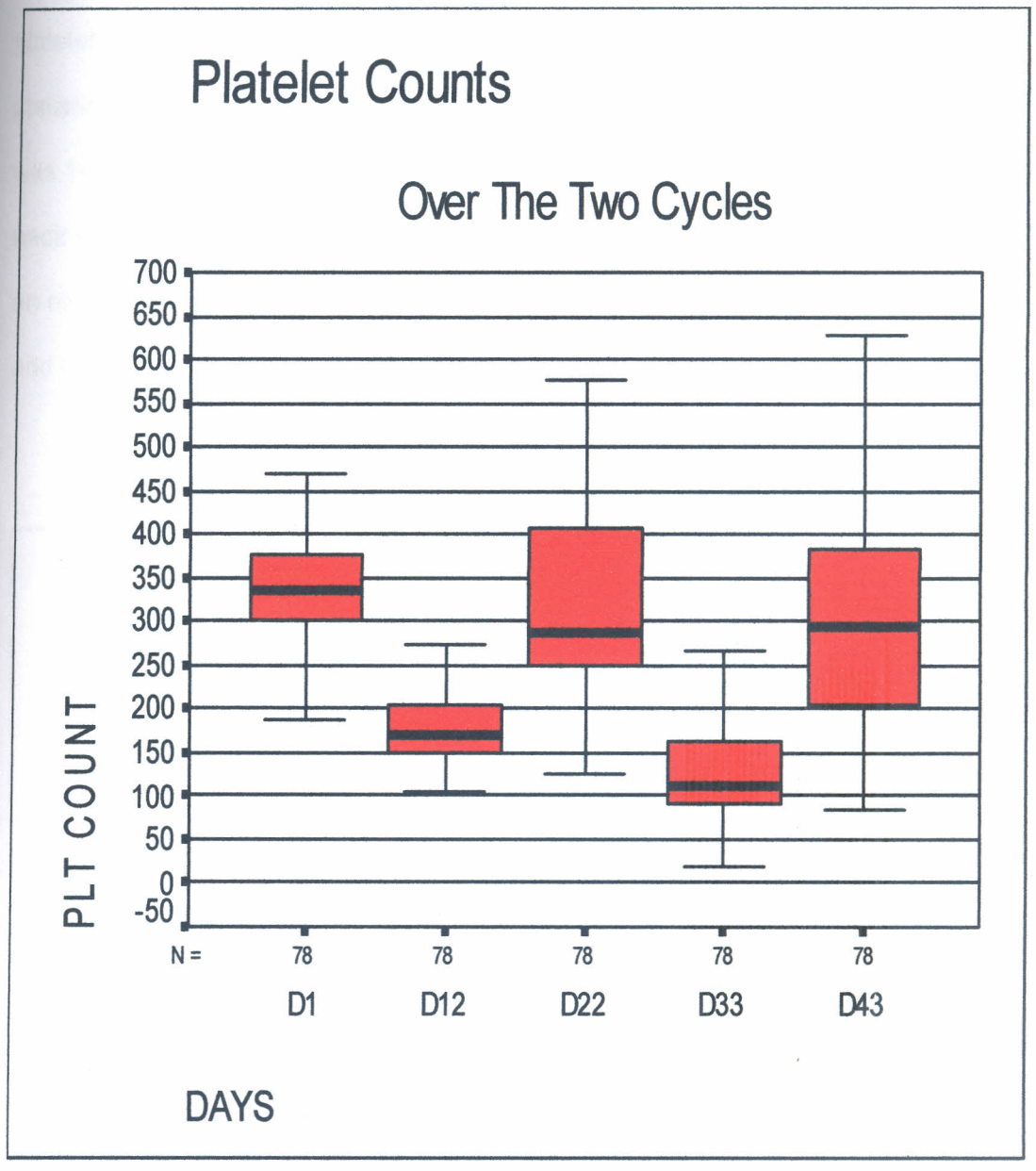


Fig. 16: Boxplot for platelet counts: whiskers represent maximum and minimum values, upper and lower borders of each box represent 75th and 25th percentiles respectively and the middle line in the box represent the median value. D12 represents day 10-14 values in cycle 1 and D33 represents day 10-14 values in cycle 2. D22 and D43 represent day 1 of 2nd and 3rd cycles respectively

x10^9/L to *307.15 (SD 113.97) x10^9/L* by day 43. This represented **42.4%** drop in cycle 1 and **57.7%** drop in cycle 2. The lower nadir count in cycle 2 compared to cycle 1 was statistically significant (p<0005) but the higher platelet count nadir depth in cycle 2 compared to cycle 1 did not reach statistical significance (p=0.062). The lowest platelet count nadir in cycle 1 was 104 x10^9/L and the highest nadir was 453 x10^9/L. In cycle 2 the lowest nadir was 20 x10^9/L and the highest nadir was 360 x10^9/L. Figure 15 shows an oscillatory nature (red line) of the mean platelet count pattern whose trend and parameters are depicted in figure 16.

Figure 15: Oscillatory pattern of platelet counts

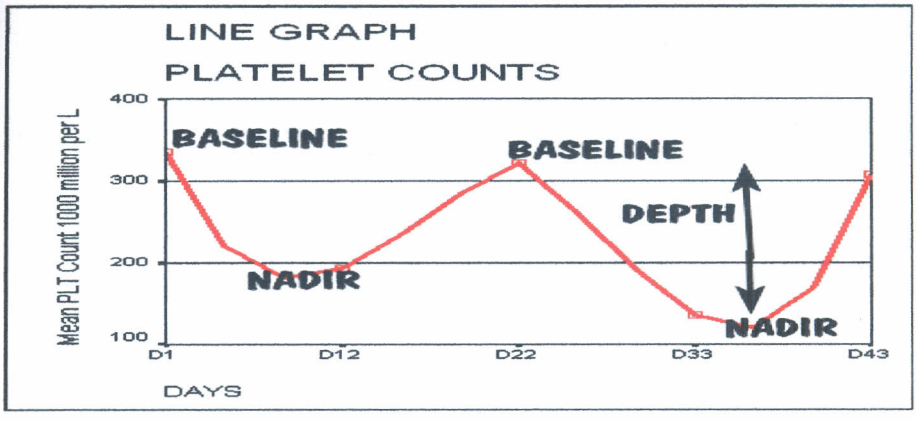


Figure 16: Platelet Count Parameters

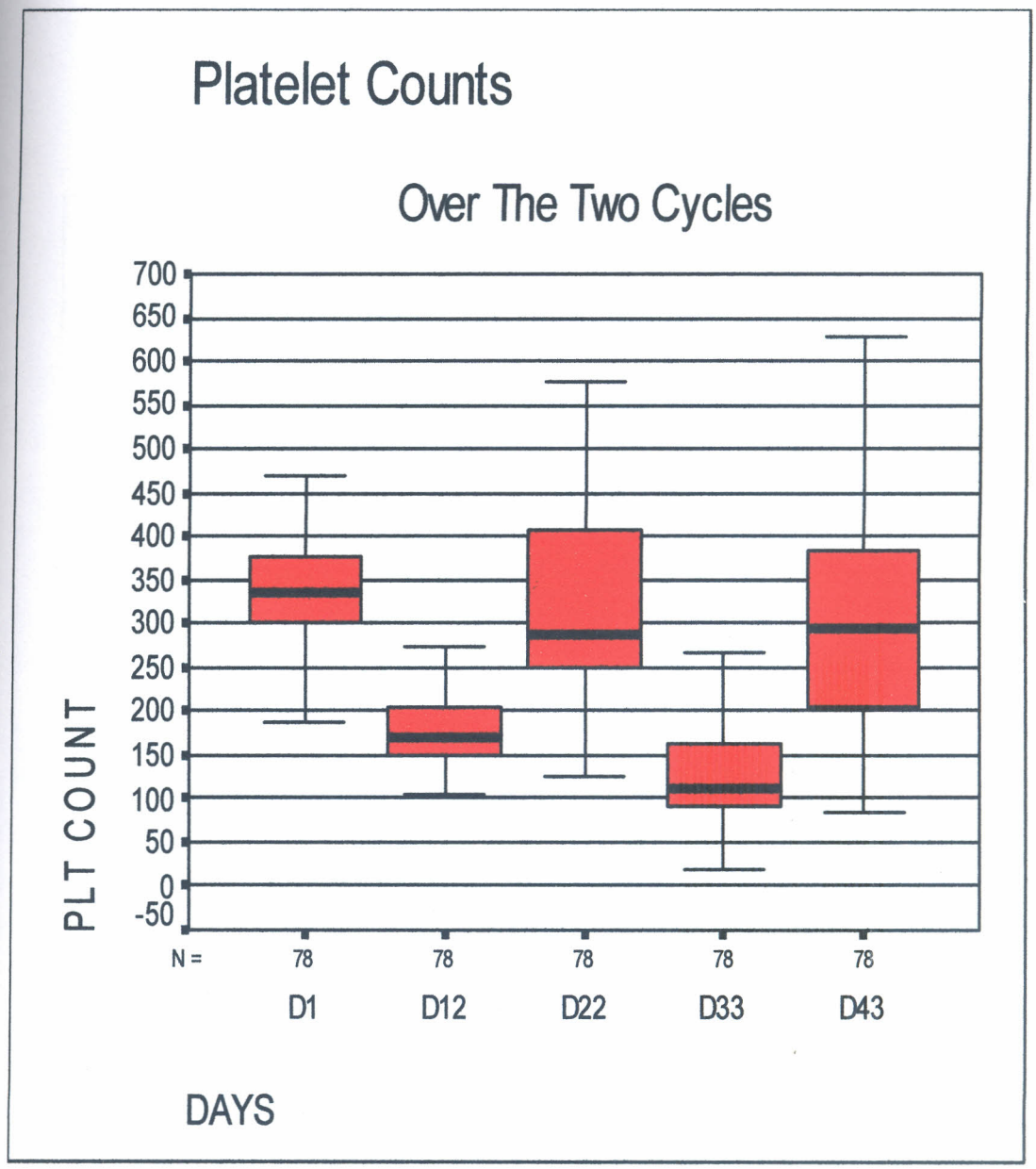


Fig. 16: Boxplot for platelet counts: whiskers represent maximum and minimum values, upper and lower borders of each box represent 75th and 25th percentiles respectively and the middle line in the box represent the median value. D12 represents day 10-14 values in cycle 1 and D33 represents day 10-14 values in cycle 2. D22 and D43 represent day 1 of 2nd and 3rd cycles respectively

The frequencies (by percentage) of the platelet count depths ranges for cycle 1 and cycle 2 are graphically depicted in figures 17-18.

Figure 17: Depths Of Platelet Nadirs in Cycle 1

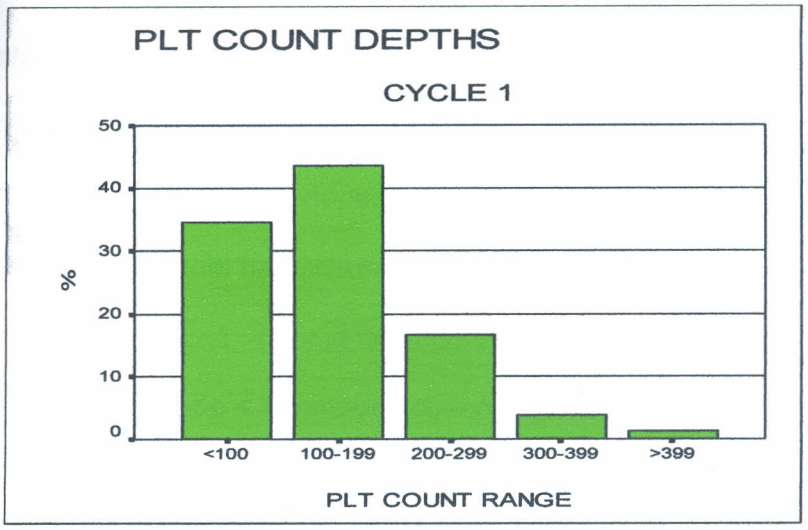
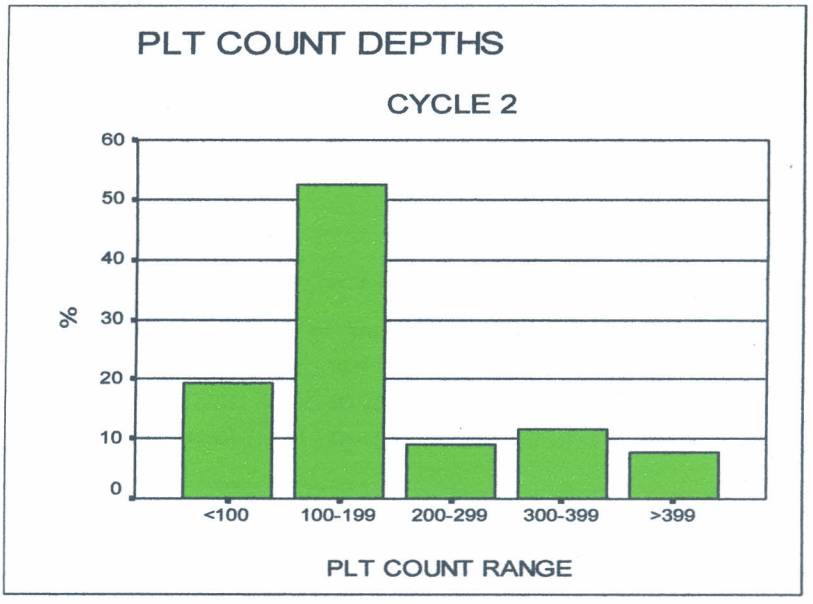


Figure 18: Depths of platelet count nadirs in cycle 2



7.3.4.2. GRADES OF THROMBOCYTOPAENIA

By day 12, 58 patients (74.4%) had normal platelet counts and 20 patients (25.6%) had grade 0 thrombocytopaenia. In contrast, by day 33 (table 2), 22 patients (28.2%) had normal platelet counts and 27 patients (34.6%) had grade 0 thrombocytopaenia; 18 patients (23.1%) and 8 patients (10.3%) had grade 1 and 2 thrombocytopaenia respectively; and severe thrombocytopaenia had occurred in 3 patients (3.8%) - two had grade 3 *thrombocytopaenia (nadir platelet counts of 28.2 x 10^9/L and 32.3 x10^9/L),* and one had grade 4 thrombocytopaenia (platelet nadir count of 20 x 10^9/L).

Table 2: Grades Of Thrombocytopaenia at Day 33 by WHO Criteria

		No Of Patients	Percent
Grade	0	27	34.6
	1	18	23.1
	2	8	10.3
	3	2	2.6
	4	1	1.3
Normal PLT count		22	28.2
	Total	78	100.0

Table 2: WHO Criteria *(appendix IV)*[119-120]: Platelets count (x109/L) ≥100=grade 0; 75-99=grade 1; 50-74=grade 2; 25-49=grade 3 and <25=grade 4. For purposes of the above table, grade 0 implies counts ≥100 and less than 150; values from ≥150 to upper limit of normal were categorized as "normal platelet (PLT) count". Grade 3 and 4 was considered "severe thrombocytopaenia".

The patient with a nadir of 20 x 10^9/L had NHL and was aged 60, her therapy was interrupted twice because of leukopaenia, her baseline PLT count in cycle 1 was 141 x10^9/L with a baseline absolute neutrophil count of 2.11 x 10^9/L and she had stage II disease. The patient with a nadir of 28.2 x 10^9/L

had stage IIIB breast cancer, was aged 66 and had a baseline PLT count of 244x10^9/L in cycle 1. The third patient who had a nadir of 32.3 x10^9/L had stage IIA breast cancer, was aged 56 and had a baseline PLT count of 243 in cycle 1. None of these three patients had any episodes of bleeding.

There was less incidence of thrombocytopaenia compared to neutropaenia in the study in both cycles and thrombocytopaenia was mostly associated with worse grades of neutropaenia (tables 3-4).

Table 3: Nadir Thrombocytopaenia Against Nadir Neutropaenia in Cycle 1

		Grade Of Thrombocytopaenia Day 12		
		0	Normal PLT Count	Totals
Grade Of Neutropaenia Day 12	0	1	21	22
	1	12	17	29
	2	5	14	19
	3	1	3	4
	4	1	3	4
Totals		20	58	78

Table 3: WHO Criteria *(appendix IV)*[119-120]:
(a) grades of thrombocytopaenia - platelets count (x10^9/L) ≥100=grade 0; 75-99=grade 1; 50-74=grade 2; 25-49=grade 3 and <25=grade 4. For purposes of the above table, grade 0 implies counts ≥100 and <150; counts from ≥150 to upper limit of normal werecategorized as "normal platelet (PLT) count". Grade 3 and 4 was considered "severe thrombocytopaenia".
(b) *Grades of neutropaenia* - Granulocytes (x10^9/L) ≥2.0=grade 0; 1.5-1.9=grade 1; 1.0-1.4=grade 2; 0.5-0.9=grade 3; <0.5=grade 4.
This table shows that apart from one patient who had grade 0 thrombocytopaenia and grade 0 neutropaenia, all the patients had worse grade of neutropaenia compared to thrombocytopaenia in cycle 1.

Table 4: Nadir Thrombocytopaenia Against Nadir Neutropaenia in cycle 2

		Grade Of Thrombocytopaenia Day 33						
		0	1	2	***3***	***4***	Normal PLT count	Totals
Grade Of Neutropaenia Day 33	0	0	0	1	0	0	11	12
	1	10	1	0	0	0	7	18
	2	13	13	0	0	0	3	29
	3	***1***	***3***	***7***	***1***	***0***	***1***	13
	4	***3***	***1***	***0***	***1***	***1***	***0***	6
Totals		27	18	8	2	1	22	78

Table 4: WHO Criteria *(appendix IV)*[119-120]:
(a) grades of thrombocytopaenia - platelet count ($x10^9$/L) ≥100=grade 0; 75-99=grade 1; 50-74=grade 2; 25-49=grade 3 and <25=grade 4. For purposes of the above table, grade 0 implies counts ≥100 and <150; counts from ≥150 to upper limit of normal werecategorized as "normal platelet (PLT) count". Grade 3 and 4 was considered "severe thrombocytopaenia".
(b) *Grades of neutropaenia* - Granulocytes ($x10^9$/L) ≥2.0=grade 0; 1.5-1.9=grade 1; 1.0-1.4=grade 2; 0.5-0.9=grade 3; <0.5=grade 4.
This table shows that apart from one patient who had worse degree of thrombocytopaenia than neutropaenia, all the patients had either equal or worse grade of neutropaenia compared to thrombocytopaenia. There were more thrombocytopenic patients in cycle 2 than in cycle 1.

7.3.4.3. FACTORS RELATED TO NADIR COUNTS AND NADIR DEPTHS

To analyze the relationship between nadir counts and relevant factors, median nadir platelet counts were used for comparisons in both cycles because platelet nadir counts in both cycles had a non-normal distribution. Low nadir platelet counts were associated with (a) old age (≥60 years) in both cycles, p=0.025 in cycle 1 and p=009 in cycle 2; (b) low baseline platelet counts in both cycles, p=0.021 cycle 1 and p=0.029 in cycle 2; and (c) low baseline total lymphocyte counts (TLC) in cycle 1, p=0.020 (table 5).

Table 5: Factors Related To Platelet Nadir Counts

Independent Variable	Category	Cycle 1 Median(Range) x10^9/L	Cycle 1 P Value	Cycle 2 Median(Range) x10^9/L	Cycle 2 P Value
Age Groups in Years	>=60	147.5(130-205)	***p=0.025***	79.6(20-200)	***p=009***
	<60	169.5(104-453)		117(28-360)	
Baseline total lymphocyte count (TLC) x10^9/L	<1.3	152(130-255)	***p=0.020***	117(20-346)	p=0.502
	1.3-1.9	170(135-359)		106(32-357)	
	2-2.9	166(104-421)		113(28-360)	
	3-3.5	319(163-453)		128(59-169)	
	>3.5	192(169-215)		95.4(82-109)	
Baseline platelet (PLT) count x10^9/L	100-149	138.5(130-170)	***p=0.021***	77(61-154)	***p=0.029***
	150-249	158.5(133-229)		90.6(56-177)	
	250-349	161.5(112-359)		127(32-346)	
	350-449	176(104-421)		101.5(20-357)	
	>449	179(146-453)		119.37(56-360)	

Table 5: Median nadir platelet counts were used for comparisons because platelet nadir counts in both cycles had a non-normal distribution. This table shows that low nadir counts were associated with (a) old age (≥60) in both cycles, (b) low baseline total lymphocyte count in cycle 1, and (c) low baseline platelet counts in both cycles.

As regards platelet nadir depths, the values in cycle 1 had a normal distribution whereas the values in cycle 2 had a non-normal distribution. Therefore, ***mean*** platelet nadir depth values were compared in cycle 1 and ***median*** platelet nadir depth values were compared in cycle 2. High nadir depths were associated with (a) AC therapy for breast cancer in cycle 2, p=0.024; and (b) high baseline platelet (PLT) counts in both cycles, p<0005 in both cycles. Patients in the 14-24 year and 55-64 year age groups had low platelet nadir depths in cycle 1, p=0.025 (Table 6).

Table 6: Factors Related To Platelet Nadir Depths

Independent Variable	Category	Cycle 1		Cycle 2	
		Mean(SD) $x10^9/L$	P Value	Median(Range) $x10^9/L$	P Value
Age Groups in Years	14-24	42.75(41.65)	***p=0.025***	63.0(-89 to 436)	p=0.728
	25-34	193.78(104.42)		132.0(85-322)	
	35-44	168.33(125.19)		160.0(45-426)	
	45-54	148.29(82.87)		156.0(-17 to 393)	
	55-64	76.71(96.68)		174.0(17-464)	
	65-74	149.29(57.5)		159.0(64-467)	
Diagnosis/ Therapy	NHL/ CHOP	153.24(142.16)	p=0.639	113.0(-89 to 467)	***p=0.024***
	Breast Ca/ AC	139.26(97.31)		160.0(-17 to 467)	
Baseline platelet (PLT) Counts $x10^9/L$	100-149	-4.75(26.35)	***p<0005***	60.0(-17 to 64)	***p<0005***
	150-249	47(42.67)		149.3(66-174)	
	250-349	132.38(76.86)		156.0(-89 to 284)	
	350-449	172.17(92.28)		313.5(63-403)	
	>449	272.56(137.59)		392.7(152-467)	

Table 6: The nadir depth values in cycle 1 had a normal distribution whereas the values in cycle 2 had a non-normal distribution. Therefore, **mean** platelet nadir depth values were compared in cycle 1 and **median** platelet nadir depth values were compared in cycle 2. This table shows that (a) patients in the 14-24 and 55-64 age groups had lower platelet nadir depth values compared to patients in the rest of the age groups in cycle 1, (b) AC therapy for breast cancer had deeper platelet nadir count drops than CHOP for NHL in cycle 2; and (c) high baseline platelet counts were associated with deeper platelet nadir drops in both cycles.

7.3.4.4. INCREASE IN PLATELET COUNTS

Seven patients had higher platelet counts on days 10-14 after chemotherapy than at the beginning of chemotherapy, 5 of them had an increase in platelet counts in the first cycle and 2 had an increase in the second cycle. All of the patients, except one, were breast cancer patients on AC. However, the increase did not reach the level of thrombocytosis i.e. the increase was within

the normal range for platelet counts. In all the seven instances, the platelets continued to rise up to the beginning of the next cycle.

7.4. HAEMARRHAGE, THERAPY INTERRUPTIONS AND SUPPORTIVE CARE

One NHL patient (1.3%) had minor bleeding (grade 1; from mucous membranes). He had an ECOG PS of 2 (ECOG scale is in appendix I) and central nervous system (CNS) involvement for which he was receiving intrathecal methotrexate. He had a nadir platelet count of 303 on day 12 and 177 on day 33. His bleeding episode was attributable to methotrexate associated mucositis. Otherwise the rest of the patients reported no bleeding and had no features of bleeding on physical examination. No therapy was interrupted on the account of thrombocytopaenia. These findings were consistent with the resultant mean and median levels of nadir platelet counts in the study. There were two therapy interruptions both of them owing to leukopaenia of less than 2×10^9/L. One of these patients (from the cancer treatment center) was subsequently admitted to the medical ward with febrile gastroenteritis. No study patient received platelet transfusions. One patient received whole blood transfusion prior to the beginning of cycle 1. She had presented with a haemoglobin level of 8.49 g/dL, white blood counts (WBC) and platelet (PLT) counts were normal.

8. DISCUSSION

We set out to determine the nadir platelet counts on day 10-14 of the first two cycles of AC for breast cancer patients and the first two cycles of CHOP for patients with non-Hodgkin's lymphoma; and to describe factors which are associated with these counts as well as factors which are associated with episodes of haemarrhage in patients on these chemotherapy protocols. Our patients were a fair representation of the population of study. Demographic characteristics were similar to those from the study by Waweru[1] which had comprised 71% breast cancer patients (78.2% in this study) and 29% NHL patients (21.8% in this study). The provincial distribution of patients most likely reflects the catchment area pattern for KNH. However, we noted that Nyanza province increased in prominence as regards non-HIV associated NHL compared to breast cancer suggesting that there are provincial factors underlying the incidence of these cancers – an association of NHL with malaria being a possibility in this case. Although we did not set out to study this finding, we note that it is an important finding which can not be ignored.

The relatively large percentage of breast cancer patients in this study compared to non-Hodgkin's lymphoma patients reflects the findings of Mungania [19] where breast cancer was more common than non-Hodgkin's lymphoma while, of course, both were among the commonest cancers in Kenyatta National Hospital. The age distribution for NHL was more spread out than that of breast cancer reflecting the fact that NHL is a diverse group of conditions affecting all age groups.

The majority of those studied were relatively fit patients seen in the outpatient clinic setting with ECOG performance score of 1 (84.6%) and stage II disease (50%). On average there was more stage IV disease and more ECOG PS 2 scores among non-

odgkin's lymphoma patients than among breast cancer patients. Still overall, study atients were without any other obvious causes of myelosuppression or bleeding pisodes apart from the chemotherapy which they were receiving.

Mean haemoglobin levels showed a slight drop which was followed by a subsequent rise in both cycles. The relatively insignificant effect of chemotherapy on haemoglobin level is due to the fact that red blood cells have a long half life (120 days) [22-23]. Although a low pre-treatment haemoglobin level has been shown to negatively influence outcome in the management of a number of cancers such as cancer of the cervix [122], bladder [123] and head and neck [124-125]; there is no evidence for significant influence of haemoglobin levels on the clinical response to chemotherapy in breast cancer and non-Hodgkin's lymphoma and, in any case, correction of anaemia as warranted clinically is all that is required whenever it occurs during chemotherapy [126]. Neutropaenia was common in this study and this is consistent with other studies [1,2-3].

There was an oscillatory pattern to platelet counts in this study in that there was a consistent drop in platelet counts when patients were put on AC for Breast Cancer and CHOP for NHL; and these drops were followed by consistent rises in platelet counts to near baseline levels. There was evidence of an accumulative effect because nadir platelet counts were lower in cycle 2 than in cycle 1. This trend is inconsistent with the findings of Waweru[1] and it is now apparent that the lack of a pattern and the tendency towards thrombocytosis - in that study - was as a result of methodological limitations in terms of malfunction of the platelet counting aspect of the automated counter. The CELL DYN® 3200 machine used in this study also malfunctioned at times during this study and at such times the platelet counts were counted manually. Automated platelet counting methods may also give falsely low or falsely high values even when the

machine is functioning properly [84-87].

In both cycles old age (≥60 years) and low baseline platelet count levels were associated with low nadir platelet counts; and additionally, in cycle 1, low baseline total lymphocyte counts were also associated with low nadir platelet counts. These findings are not unique to this study. Older individuals have been found to be at an increased risk for myelotoxicity because of a reduction in hemopoietic reserve [73-74]. It is also logical that low platelet count nadirs should develop in patients who start chemotherapy with low baseline platelet counts. On the other hand the fact that low baseline total lymphocyte counts were associated with low nadir counts in cycle 1 may just mean that low baseline total lymphocyte counts act as surrogate markers of a weaker hematopoietic system rather than that they are a direct contributor of low platelet count nadirs. JY Blay et al demonstrated that patients with low baseline platelet counts ($<150\ x10^9/L$) and low baseline total lymphocyte counts ($<0.7\ x10^9/L$) were more prone to developing thrombocytopaenia [40].

Part of the reason why the American Society of Clinical Oncology recommended that clinical studies should be done in the area of chemotherapy induced thrombocytopaenia in patients with solid tumors and lymphoma [11], is because the studies so far available in this area are retrospective and few in number. One notable study is that of A. Wunderlich et al [127] where they compared 2- and 3-weekly CHOP (CHOP-14 and CHOP 21 respectively) and cyclophosphamide, vincristine, etoposide and prednisolone (CHOEP-14 and CHOEP-21 respectively) regimens in terms of practical application and hematotoxicity. Of relevance to our study is the CHOP-21 arm of that study which showed that (1) generally there was an oscillatory pattern to platelet count nadirs in CHOP-21 similar to our findings with the exception that Wunderlich et al

not demonstrate an accumulative effect; and (2) there were more pronounced cyclic dir platelet patterns in elderly patients with lower nadir values and they surmised that is indicated a larger chemosensitivity and a more intense activation of regulatory ocesses.

n terms of nadir platelet count depths, high values were associated with high baseline platelet counts ($p<0005$ in both cycles); and breast cancer patients had higher values than NHL patients ($p=0.024$) in cycle 2. Patients in the 14-24 and 55-64 year age groups had lower depth values compared to patients in the rest of the age groups ($p=0.025$) in cycle 1. The finding that higher baseline PLT counts were related to high nadir depths suggests that a fixed proportion (rather than a fixed number) of platelets is affected when patients are given CHOP for NHL and AC for breast cancer so that higher starting PLT count values lead to larger quantities of adversely affected platelets. This makes logical sense in that "the higher one is from the ground, the deeper one would fall should something go wrong, for instance", to use an analogy. In terms of thrombopoiesis, larger quantities of platelets would consume larger quantities of thrombopoietin [32-33] leading to low levels of circulating thrombopoietin and therefore a weaker stimulatory effect as platelets suddenly begin to drop, allowing for a deeper drop before the bone marrow is activated enough to restrain the drop. The platelet nadir depths were not statistically significant between elderly and young patients, in contrast, 14-24 and 55-64 year age groups had statistically significant lower nadir depths than the rest of the age groups. There was therefore a discordance between nadir depths and nadir counts in terms of age in that deeper drops in platelet counts from baseline levels did not necessarily translate into lower nadir depths. This can only mean that a lower baseline platelet count was a stronger predictor of a low nadir count than the value of the drop from baseline. Also of note is the fact that the majority of NHL patients

were in the 14-24 and 55-64 year age groups – the same age groups which had lower nadir depths than the rest of the age groups. This was therefore more likely because of a mild protective effect against thrombocytopaenia of CHOP in NHL patients, than an effect of age per se. This was clearer in cycle 2 where breast cancer patients had deeper nadirs than NHL patients. As pointed out earlier, vincristine and prednisone have been used to treat thrombocytopaenia and their ability to raise platelet counts may moderate CHOP induced thrombocytopaenia compared to AC alone.

In keeping with other local studies, there was no clinically significant episodes of bleeding in the study patients and severe thrombocytopaenia (grades 3 and 4) was rare [1,3]. Studies elsewhere (mainly retrospective) have also largely reported low rates of thrombocytopaenia and haemarrhage [8-10,12-13,40]. Many of these studies have looked at a wide range of solid cancers such as ovarian cancer, pelvic sarcoma, cervical cancer, breast cancer, melanoma, primary brain cancer, hypernephroma etc. In addition, the patients studied have often been on a wide variety of chemotherapy regimens, most of them intense ones. Those patients who have shown tendency to bleed have often had other confounding problems such as poor performance status, necrotic malignant lesions, coagulopathies, bone marrow infiltration etc – many of which we excluded from our study. We dwelt on hematopoietic growth factors earlier on in the "background" section mainly to dismiss them as an ideal alternative to platelet transfusion in terms of effectiveness but their other important drawback in terms of local use is their high cost [128]. The finding of no clinically significant episode of bleeding in this study while being a "disappointing" negative finding, is good for our practice and our patients in that for this group of patients there should be no pressure to use thrombopoietic growth factors; and even our platelet transfusion use should be minimal in this relatively common group of conditions locally.

In this study, as in all the reported local studies, there was significant neutropaenia; and thrombocytopaenia where it occurred, almost always was accompanied by neutropaenia[3].

The few incidences which occurred in this study of increased platelet counts after chemotherapy could be as a result of secondary or reactive platelet production as outlined earlier. In our patients breast cancer and NHL per se or rebound platelet increase from chemotherapy could have been the cause(s) of the observed platelet increases. Ideally, knowledge of when the increase occurred during a cycle would help in unraveling the nature of these platelet count increases but this was not possible because, by design, the study only did one full blood count test in the period between the first and last days of each cycle.

9. LIMITATIONS

By design, the study had excluded very sick patients (PS 3 and above) and HIV positive patients in order to exclude confounders. These two groups of patients, however, constitute a significant population of patients locally who are likely to either bleed at platelet count levels where our study patients could not bleed; or to develop severe thrombocytopaenia as a combined effect of chemotherapy and other factors. The findings of this study should therefore be applied with care in these patient populations.

Only two treatment courses were evaluated in this study. The outcome after 6-8 courses of chemotherapy might have been different especially as there was a hint of an accumulative effect on the nadir platelet counts.

Nadir counts as a measure of hematological toxicity are specific to chemotherapy regimens. The findings of this study can only be applied to breast cancer patients on AC-21 and NHL patients on CHOP-21.

The platelet counting machine at the KNH Haematology laboratory caused some problems during the study period. Some samples had to be subjected to manual verification, this may have improved the accuracy of the results at the expense of precision. However, manual verification remains the "gold standard" of platelet counting procedures.

10.CONCLUSION

There is a consistent but clinically insignificant drop in platelet counts in patients on doxorubicin and cyclophosphamide (AC) and cyclophosphamide, doxorubicin, vincristine & prednisone (CHOP) for breast cancer and non-Hodgkin's lymphoma (NHL) respectively; with a tendency towards an accumulative effect with further administration of chemotherapy. Old age and low baseline platelet counts were associated with low nadir platelet counts in both cycles; and low baseline total lymphocyte counts were associated with low nadir platelet counts in cycle 1. AC therapy for breast cancer and high baseline platelet counts were associated with deeper platelet nadir drops from baseline.

11. RECOMENDATIONS

In patients receiving doxorubicin & cyclophosphamide (AC) for breast cancer and cyclophosphamide, doxorubicin, vincristine & prednisone (CHOP) for non-Hodgkin's lymphoma (NHL), the main hematological toxicity to watch out for remains neutropaenia. However, this study suggests that old patients, breast cancer patients and those who start chemotherapy with relatively low platelet levels need careful monitoring of their platelet counts.

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13 APPENDICES

Appendix I: Performance Status Scale by the Eastern Co-operative Oncology Group (ECOG)

SCALE	DESCRIPTION
0	Fully active, able to carry out all pre - disease performance without restrictions, no special care or complaints, asymptomatic disease
1.	Active, or has normal activity but with effort, restricted in physically strenuous activity, requires no special care, has minor sign/symptoms of disease.
2.	Ambulatory but disabled, unable to work but may be up and about more than 50% of waking hours. Able to live at home, with need for some assistance, may be able to care for most needs or may require frequent medical care and considerable assistance. The disease may be progressing.
3.	Severely disabled or completely disabled, confined to bed or chair more than 50% of waking hours, hospitalization may be necessary. Requires specialized medical care and assistance, there may no self-care with total confinement to bed.
4.	Moribund, fatal processes progressing rapidly.
5	Dead

Appendix II: Staging Non - Hodgkin's Lymphoma

The B designation symptoms:	Unexplained loss of more than 10% of body weight in the last 6 months before diagnosis.
	Unexplained fever with temperatures above 38 degrees C.
	Drenching night sweats.
Stage I	Involvement of a single lymph node region (I) or
	localized involvement of a single extra-lymphatic organ or site (IE).
Stage II	Involvement of 2 or more lymph node regions on the same side of the diaphragm (II) or
	localized involvement of a single associated extra-lymphatic organ or site and its regional lymph nodes with or without other lymph node regions on the same side of the diaphragm (IIE). [*The number of lymph node regions involved may be indicated by a subscript (e.g., II 3)*]
Stage III	Involvement of lymph node regions on both sides of the diaphragm (III) that may also be accompanied by localized involvement of an extra-lymphatic organ or site (IIIE), by involvement of the spleen (IIIS), or both (IIIS+E).
Stage IV	Disseminated (multi-focal) involvement of 1 or more extra-lymphatic sites with or without associated lymph node involvement or
	isolated extra-lymphatic organ involvement with distant (non-regional) nodal involvement
	The designation "E" is used when extra-nodal lymphoid malignancies arise in tissues separate from, but near, the major lymphatic aggregates
	Stage IV refers to disease that is diffusely spread throughout an extra-nodal site, such as the liver
	If pathologic proof of involvement of 1 or more extra-lymphatic sites has been documented, the symbol for the site of involvement, followed by a plus sign (+), is listed.*

*Sites are identified by the following notation:			
N = Nodes H = Liver	L = Lung M = Bone Marrow	S = Spleen P = Pleura	0 = Bone D = Skin

Appendix III: Staging in Breast Cancer

TNM DEFINITIONS		
Primary Tumor (T)	TX	Primary tumor cannot be assessed
	TO	No evidence of primary tumor
	Tis	Carcinoma in situ; intraductal carcinoma, lobular carcinoma in Situ or Paget's disease of the nipple with no associated tumor
	Note: Paget's disease associated with a tumor is classified according to the size of the tumor.	
	T1	Tumor 2.0 cm or less in greatest dimension
	T1 mic	micro-invasion 0.1 cm or less in greatest dimension
	T1a	Tumor more than 0.1cm but not more than 0.5cm in greatest dimension.
	T1 b	Tumor more than 0.5cm but not more than 1 .Ocm in greatest dimension.
	T1 c	Tumor more than 1.0cm but not more than 2.0cm in greatest dimension
	T2	Tumor more than 2.0cm but not more than 5.0cm in greatest dimension
	T3	Tumor more than 5.0cm in greatest dimension
	T4	Tumor of any size with direct extension to (a) chest wall or (b) skin
	T4a	Extension to chest wall
	T4b	Edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast.
	T4c	Both of the above (T4a and T4b).
	T4d	Inflammatory Carcinoma

Regional Lymph nodes (N):	NX	Regional Lymph nodes cannot be assessed (e.g. previously removed).
	NO	No regional lymph node metastasis.
	N1	Metastasis to movable ipsilateral axillary lymph node (s).
	N2	Metastasis to ipsilateral axillary lymph node (s) fixed to each other or to other structures
	N3	Metastasis to ipsilateral internal mammary lymph node(s).
Pathologic Classification (PN)	pNX	Regional lymph nodes cannot be assessed (not removed for pathologic study or previously removed)
	pNO	No regional lymph node metastasis
	pN1	Metastasis to movable ipsilateral axillary lymph node(s)
	pN1a	Metastasis in 1 to 3 lymph nodes, any more than 0.2cm and all less than 2.0cm in greatest dimension.
	pN1 bii	Metastasis to 4 or more lymph nodes, any more than 0.2cm and all less that 2.0cm in greatest dimension
	pN1biii	Extension of tumor beyond the capsule of a lymph node metastasis less than 2.0cm in greatest dimension
	pN1 biv	Metastasis to a lymph node 2.0cm or more in greatest dimension.
	pN2	Metastasis to ipsilateral axillary lymph node(s) fixed to each other or to other structures
	pN3	Metastasis to ipsilateral internal mammary lymph node(s)
Distant Metastasis (M)	MX	Presence of distant metastasis cannot be assessed
	MO	No distant metastasis
	MI	Distant metastasis present (includes metastasis to ipsilateral supraclavicular lymph nodes)

AJCC STAGE GROUPINGS	
Stage 0	Tis, NO, MO
Stage 1	T1,*NO,MO
	*T1 includes T1 mic
Stage IIA	TO, N1, MO T1*, N1**, MO T2, NO, MO
	*T1 includes T1mic
	**the prognosis of patients with pN1a disease is similar to that of patients with pNO disease
Stage IIB	T2, N1, M0
	T3, N0, M0
Stage IIIA	TO, N2, MO T1 ,*N2, MO T2, N2, MO T3, N1, MO T3, N2, MO
	*T1 includes T1 mic
Stage IIIB	T4, Any N, MO
	Any T, N3, MO
Stage IV	Any T, Any N, M1

Appendix IV: Who Recommendations For Grading Of Acute And Subacute Hematologic Toxicity (Adults)

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Haemoglobin (g/dL)	≥11.0	9.5-10.9	8.0-9.4	6.5-7.9	<6.5
Leukocytes ($x10^9$/L)	≥4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
Granulocytes ($x10^9$/L)	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
Platelets ($x10^9$/L)	≥100	75-99	50-74	25-49	<25
Haemarrhage	≥none	petechiae	mild blood loss	gross blood loss	debilitating blood loss

Appendix V(A): Information For Volunteer Patients

Background: During your visit to hospital, you were found to have cancer and to require treatment with cancer drugs. Now you are asked to be in a study for people receiving cancer drugs. The study is called "**Nadir Platelet Counts In Patients On Doxorubicin & Cyclophosphamide (AC) And Cyclophosphamide, Doxorubicin, Vincristine & Prednisone (CHOP) For Breast Cancer And Non-Hodgkin's Lymphoma Respectively**". This study is trying to find ways to minimize the side effects of cancer drugs on patients. This study is being done by the School Of Medicine in the University Of Nairobi.Your doctor, and this information sheet, will tell you about the study. This will help you decide if you want to be in the study.

***Effects of cancer drugs on platelets*:** Cancer drugs work by killing cancer cells that are multiplying. In the process of doing so these drugs also kill other important normal multiplying cells such as bone marrow cells which produce blood cells. This leads to a temporary but sometimes dangerous reduction in the number of blood cells such as platelets. Platelets help to prevent bleeding.

The purpose of the study: The overall goal of the study is to find out the extent to which platelet numbers are reduced by cancer drugs. A total of 100 patients receiving cancer drugs at Kenyatta National Hospital will be in the study.
The results will help doctors in future to better decide, in advance, what measures would best minimize the effects of reduced platelet numbers on patients receiving cancer drugs.

What happens if you join the study: If you join the study, members of the study team will collect information from your hospital file in your first and second treatment cycles. Members of the study team will also ask questions about your health and examine you in order to identify any treatment related complications in the same two cycles. No member of the study team will interfere with your medical care. You shall receive only standard medical care as ordered by your attending clinician. Nothing will be experimental. No necessary treatment shall be withheld from you and no unnecessary expense shall be incurred on your part due to the study. Please also understand that the study team is not liable for expenses incurred during your treatment or admission. Personal information divulged shall remain confidential.
Close follow up during the study period will be beneficial for you since complications will be detected early.
You will continue to receive regular care from your attending clinician after the study has ended.

Leaving the study: You may leave the study at any time. This will not affect your future medical care.

This information sheet discloses your cancer diagnosis. If you wish to keep this sheet, it is better to keep it in a place where only persons who know you have cancer can find it.

Appendix V(B): Consent to participate in the study

I have been told about the study. I have been given the following information:
It is a study to find out the extent to which platelet numbers are reduced by cancer drugs. The results will help doctors in future to better decide, in advance, what measures would best minimize the effects of reduced platelet numbers on patients receiving cancer drugs.
The study team will not interfere with the care given by my attending clinician. The study team will collect information during the first two cycles of my treatment from my hospital file, by talking to me and/or by examining me. My attending clinician will continue to give me medical care after the study has ended.
All information that I give on this study will be kept private. Only staff directly involved in the study will know what I say.
I have read the above information, or it has been read to me. I have had the chance to ask questions. All my questions have been answered. In case I need more information I can ask any study doctor.
In case I have questions about the study, I can contact Dr. YB Mlombe in person through KNH or Internal Medicine Department, University Of Nairobi; or by phone at 0720-467127.
If I have questions or concerns regarding being harmed by the study, I know I can contact Prof K Bhatt, Chairperson of the Kenyatta National Hospital Ethics and Research Committee.Tel. 0722771603.

I agree to take part in this study. I understand that I have the right to leave the study at any time without affecting my future health care.
☐ **YES** ☐ **NO**

Participant's signature:_____Date:_____

I certify that I gave all information regarding the study and _____ appears to understand the objectives and procedures of the study. I will ensure that all terms of this consent form will be respected.
Principal Investigator's or designee's signature: _____Date:_____

Witness (mandatory if the patient cannot read):
I certify that _____has received all information regarding the study. He/she apparently understood the information and freely gave his/her consent.
Witness signature:_____ Date:_____

Legal guardian (mandatory if the patient is under the legal age for consent)
I certify that _____ has received all information regarding the study. He/she apparently understood the information and freely gave his/her consent.
Legal guardian's signature: _____ Date:_____

KIPENGELE V(C): MAELEZO KUHUSU WAGONJWA WA KUJITOLEA

Msingi: Wakati ulipokuja hospitali, iligunduliwa yakua una ugonjwa wa saratani na unahitajik kupata matibabu ukitumia vidonge vya kutibu ugonjwa wa saratani. Sasa unaombwa kujihusisha kwenye utafiti wa watu wanaotumia vidonge vya kutibu saratani. Utafiti unaitwa "***Nadir Platelet Counts In Patients On Doxorubicin & Cyclophosphamide (AC) And Cyclophosphamide, Doxorubicin, Vincristine & Prednisone (CHOP) For Breast Cancer And Non-Hodgkin's Lymphoma Respectively***." Utafity huu unajaribu kutafuta njia za kuweza kupunguza athari zinazotokana na utumizi wa vidonge vya saratani kwa wagonjwa. Utafiti huu unafanywa na Chuo Kikuu cha Nairobi Kitivo cha Madawa. Daktari pamoja na stakabadhi hii ya maelezo watakufahamisha juu ya utafiti huu. Hii itakuezesh kuamua kama unataka kuweko katika utafiti huu.

Athari ya vidonge vya saratani katika chembe za damu (platelets): Vidonge vya saratani hufanya kazi kwa kuua viinin vya saratani vinavyotapakaa katika damu. Katika taratibu yakufanya hivyo, vidonge hivi pia huua chembechembe zingine muhimu zinazotapakaa, kama vile kiini cha damu kinachopatikana ndani ya mfupa (bone marrow) ambacho huzalisha chembechembe za damu. Hii huleta upungufu kwa muda au huleta hatari ya kupungua kwa idai ya chembechembe za damu (platelets). Chembechembe hizi huzuia kuvuja kwa damu.

Madhumuni ya utafiti: Lengo la utafiti huu ni kutafuta idadi gani ya chembechembe (platelets) inayopunguzwa kwa utumizi wa vidonge vya saratani. Idadi ya wagonjwa mia mora (100) wanaopata vidonge vya kuzuia saratani katika hospitali kuu ya Kenyatta watakuwa katika utafiti huu. Matokeo haya yatawasaidia madaktari siku za baadae kutoa uamuzi bora, kimbele, na ni hatua gani muhimu zitakazopunguza athari ya upungufu wa idadi ya chembechembe za damu kwa wagonjwa wanaotumia vidonge vya kuzuia saratani.

Nini matokeo yake ukijiunga na urafiti huu: Kujiunga na utafiti huu, wattalamu wa utafiti watapata habari zako kutoka kwenye stakabadhi zako za hospitali kwa ajili ya matibabu yako ya kwanza na ya pili. Wataalamu wa utafiti watakuuliza maswali kuhusu hali yako ya afya na watakuchunguza kwa ajili ya kutaka kugundua athari zozote zengine zilizotokana na matibabu ya kwanza na ya pili. Hakuna mtaalamyu yeyote katika utafiti huu atakayeingilia katika huduma yako ya afya. Utapata huduma za matibabu ya kawaida kama alivyoagiza mtaalamu wako. Hakuna majaribio. Hakuna huduma ya lazima itayozuiliwa kwako na pia gharama za ziada kwa ajili ya utafiti. Tafadhali elewa ya kwamba gharama si juu ya wataalamu wa utafiti huu pamoja na kulazwa. Habari za kibinafsi zitakazotolewa zitabakia kuwa ni za siri. Kufuatilia kwa karibu wakati wa utafiti utakuwa na faida kwako kwani athari zinaweza kugunduliwa mapema. Utaendelea kupata huduma za kawaida kutoka kwa mtaalamu wako baada ya utafiti kumalizika.

Kujiondoa katika utafiti: Unaweza kujiondoa kwenye utafiti wakati wowote. Hii haitaathiri huduama zako za matibabu za baadae.

Stakabadhi hii inaonyesha uvumbuzi wa hali yako ya saratani. Ikiwa unataka kuhifadhi stakabadhi hii, utahitajika kuiweka mahali ambapo wale watu wanaojua ya kwamba unao ugonjwa wa saratani wanaweza kuipata.

KIPENGELE V(D): IDHINI YA KUJIHUSISHA NA UTAFITI

Nimeelezewa kuhusu utafiti huu. Nimeelezewa yafuatayo: Ni utafiti wa kudundua ni kiwango gani cha chembechembe (platelets) hupunguzwa na matumizi ya vidonge vya saratani. Matokeo yatawawezesha madaktari kutoa maamuzi bora siku za baadae, ni hatua gani za muhimu ambazo zitakazowezesha kupunguza athari ya kupungua kwa idadi ya chembechembe za damu kwa wagonjwa wanaotumia vidonge vya saratani. Wataalamu wa utafiti hawataingilia katika huduma za matibabu ninazopata kutoka kwa mtaalamu wangu.

Wataalamu wa utafiti huu watapata habari zangu kutoka kwa stakabadhi zilizoko hospitalini kwa ajili ya matibabu yangu ya kwanza na ya pili, kwa kuzugumza na mimi na kunifanyia uchunguzi. Mtaalamyu ataendelea kunihudumia baada ya utafiti kumalizika.

Habari zote nitakazotoa katika utafiti huu zitahifadhiwa kwa siri. Wataalamu wanaohusika moja kwa moja na utafiti huu watajua nitakayoyasema.

Nimesoma maelezo yalioko hapo juu au nimesomewa. Nimekuwa na nafasi ya kuuliza maswali. Maswali yangu yote yalijibiwa. Ikiwa nitahitaji maelezo zaidi, nitauuliza dakatari yeyote katika utafiti huu.

Nikiwa nitakuwa na maswali kuhusu utafiti huu, nitawasiliana na Dr. YB Mlombe kupitia hospitali ya Kenyatta au Department of Internal Medicine, Chuo Kikuu cha Nairobi, nambari ya simu ya mkono (mobile phone) 0720-467127.

Ikiwa nitakuwa na maswali kuhusu kuathiriwa kutokana na utafiti huu, najua nitawasililan na Prof. Bhatt, Mwenyekiti wa Kenyatta National Hospital, Ethics and Research Committee, simu ya mkono (mobile phone) 0722-771603.

Nimekubali kujihusisha na utafiti huu. Ninaelewa ya kwamba nina haki ya kujiondoa kwenye utafiti huu wakati wowote bila ya kudhuru huduma zangu za baadae.

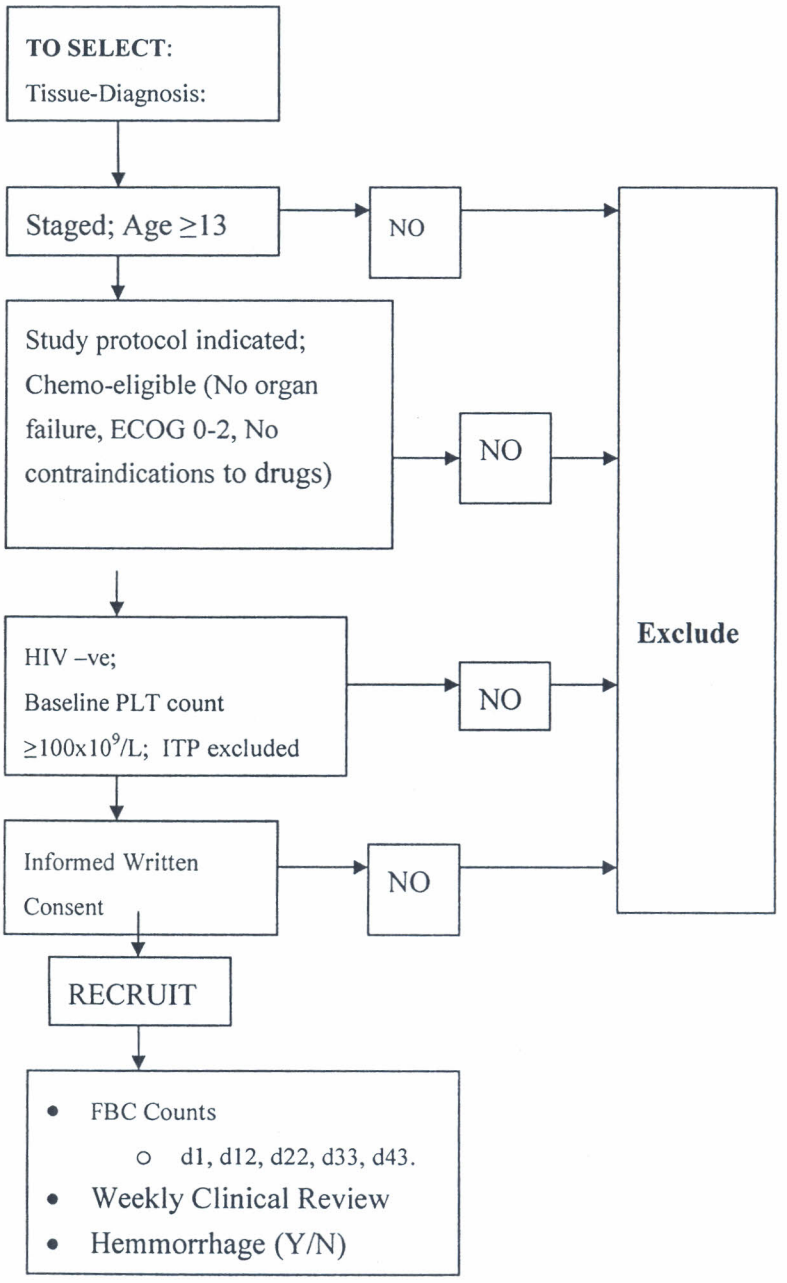
Ndio ☐ La ☐
Sahihi ya mhusika:..... Tarehe:.....

Ninasahihisha ya kuwa nimetoa habari zote kuhusu utafiti huu na ..... na pia kuelewa na malengo na taratibu za utafiti. Nitahakikisha masharti yote yalioko katika hii stakabadhi ya idhini yataheshimiwa.
Sahihi ya mkaguzi mkuu:..... Tarehe:.....

Shahidi (lazima ikiwa mgonjwa hawezi kusoma):
Ninasahihisha ya kwamba ..... nimepata maelezo yote kuhusu utafiti huu. (Bwana, Bi) alielewa maelezo na akatoa idhini yaki.
Sahihi ya shahidi:..... Tarehe.....

Mtunzi kisheria (legal guradian) (lazima ikiwa mgonjwa yu chini ya umri wa kutoa idhini)
Ninasahihisha ya kwamba ..... nimepata maelezo yote kuhusu utafiti huu. (Bwana, Bi) alielewa maelezo na akatoa idhini yaki.
Sahihi ya mtunzi kisheria:..... .. Tarehe:.....

Appendix VI(A): Screening And Recruitment Flow Chart



Appendix VI(B): Data Collection Form

UniqueNo	Age

Sex	Residence

Height	Weight	BSA

Performance Status	Histological Diagnosis	Disease Stage	Regimen

Date Day 1	Date Day 22	Date day 43

Cyclophosphamide Dose d1	Cyclophosphamide Dose d22	Adriamycin Dose d1	Adriamycin Dose d22

Vincristine Dose d1	Vincristine Dose d22	Prednisone Dose d1	Prednisone Dose d22

Body Temp d1	Body Temp D12	Body Temp d22	Body Temp d33	Body Temp d43

Other Features Of Infection d1	Other Features Of Infection d12	Other Features Of Infection d22

Other Features Of Infection d33	Other Features Of Infection d43

Minor Bleeding Episodes d1-d14	Minor Bleeding Episodes d15-d21

Minor Bleeding Episodes d22-33	Minor Bleeding Episodes d34-43

Major Bleeding Episodes d1-d14	Major Bleeding Episodes d15-21

Major Bleeding Episodes d22-33	Major Bleeding Episodes d34-43

Date FBC d1	Hb	WBC	ANC	TLC	PLT	Platelet Manual Count

Date FBC d12	HB	WBC	ANC	TLC	PLT	Platelet Manual Count

Date FBC d22	HB	WBC	ANC	TLC	PLT	Platelet Manual Count

Date FBC d33	HB	WBC	ANC	TLC	PLT	Platelet Manual Count

Date FBC d43	HB	WBC	ANC	TLC	PLT	Platelet Manual Count

Special events/comments: