A POSTGRADUATE THESIS

ON

NADIR PERIPHERAL BLOOD CELL COUNTS IN PATIENTS ON TREATMENT FOR NON-HODGKIN'S LYMPHOMA AND BREAST CANCER WITH CYCLOPHOSPHAMIDE, DOXORUBICIN, VINCRISTINE & PREDNISONE (CHOP) AND DOXORUBICIN & CYCLOPHOSPHAMIDE (AC) RESPECTIVELY.

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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

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LIST OF ABBREVIATIONS

1) AC-Doxorubicin and Cyclophosphamide
2) ANC-Absolute neutrophil count
3) BMA- Bone Marrow Aspirate
4) CHOP- Cyclophosphamide, Doxorubicin Vincristine & Prednisone
5) CSF- Colony stimulating factor
- G-CSF-Granulocyte Colony stimulating factor
- GM-CSF- Granulocyte Macrophage Colony stimulating factor
- M-CSF-Macrophage Colony stimulating factor

6) DNA- deoxyribonucleic acid
7) ECOG- Eastern Cooperative Oncology Group
8) ER- Estrogen Receptor
9) Hb- Hemoglobin
10) HGF-Hematopoietic Growth Factor
11) HLA-Human Leukocyte Antigen
12) HSC-Hematopoietic Stem Cell
13) KEMRI-Kenya Medical Research Institute
14) KNH- Kenyatta National hospital
15) LDH-Lactase Dehydrogenase
16) NHL-Non Hodgkin's Lymphoma
17) PR-Progesterone Receptor
18) RNA-Ribonucleic Acid
19) TPII-Topoisomerase II
20) TIBC-Total Iron Binding Capacity
21) WBC-White Blood Cells
22) WHO-World Health Organization

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ABSTRACT

Poor hematological status and development of sepsis due to temporary bone marrow failure are major contributing factors to treatment schedule interruptions and hence poor outcome in our set up. With this in mind, we set out in this study to find out, the effects of treatment using CHOP for NHL and AC for breast cancer on the peripheral blood cells of our patients.

Objectives; To determine the complete blood counts on day ten to fourteen of the first two cycles and day twenty one of the first cycle, and subsequently to determine the peripheral blood nadirs on day ten to fourteen of the two cycles.

Design and Setting: A prospective descriptive study using a real life usual practice scenario at Kenyatta National Hospital.

Results: Following the administration of the first cycle of chemotherapy on day 10 to 14, the median WBC reached a nadir that was 45% and 40% lower than the baseline median WBC count for the breast cancer and NHL treatment groups respectively.

Upon recovery of the bone marrow on day 21, the median WBC rose by 30% from the nadir obtained on day10 to 14 in both treatment groups. This was followed 10 to 14 days after the administration of the second cycle, by a median nadir count that was 40% lower than the day 21 median count.

The ANC followed a similar trend. The median ANC reached a nadir that was 60% and 70% lower than the baseline median ANC for breast cancer and NHL treatment groups respectively, 10 to 14 days after the first cycle of chemotherapy was administered.

Subsequently on day 21, the median ANC rose by 60% and 55% from the nadir median ANC for breast cancer and NHL treatment groups respectively. After the administration of the second cycle, the median ANC reached a nadir that was 60% lower than the median ANC on day 21.

The median WBC nadir was lower in second cycle for both groups. The individual case nadir was lower in the first cycle for NHL and second cycle for breast cancer treatment group. For the ANC, the lowest median nadir was in cycle two in both groups and the individual case nadir was lowest in cycle one. Platelets did not show any pattern in trend during the phases of the cycle. The hemoglobin levels did not change significantly either.

Conclusion: Our patients seem to suffer severe bone marrow toxicity. Sixty two percent of treatment schedule interruptions were due to neutropenia, 31% due to anemia and only 7% due to low platelets. Febrile neutropenia was a rare occurrence though fatal when it occurred.

1. INTRODUCTION

Malignancies are increasingly becoming major causes of morbidity and mortality in all age groups. On the other hand, more and more research papers are publishing reports on improved management, newer treatment modalities, and an improvement in the survival rates in western countries, for patients with certain malignancies. Consequently, even in cancers that were previously thought to be incurable, newer generation drugs and more drug combinations have been documented to be effective, albeit for a while.

The major side effect of most anticancer drugs is myelosuppression. This often limits the doses of the drugs and reduces the frequency of administration. With many cancers, the outcome would be better if drug doses could be increased.

Hemorrhagic deaths may occur in these patients due to thrombocytopenia. One approach has been to prevent hemorrhage by platelet transfusion. However, transfusion transmitted infections, allergic reactions and refractoriness due to allo-immunization and unavailability in most centers have limited their usefulness to a certain extent.

Septic deaths still occur due to neutropenia despite great advances in antimicrobial therapy. Transfusion of granulocytes has practically no role in the management of febrile neutropenia. Their short half-life in circulation, mechanical fragility and the tendency to cause the clinical syndrome of pulmonary compromise, has limited their use.

Anemia occurs in these patients due to hemorrhage, temporary reduction of erythroid precursors, and chemotherapy associated renal toxicity causing a reduction in the production of erythropoetin. Blood transfusion has been an effective way to restore the hemoglobin level without delaying treatment significantly. However in this era of fatal and incurable blood borne diseases, great caution is required in using blood or blood products.

For these reasons, specific recombinant reconstituted stimulators of peripheral blood cell production have been isolated for use, with significant clinical implications. In developed countries, the use of these factors has facilitated optimization of drug dosages and reduction of treatment interruptions hence improving the outcome in their patients. In our local set up, their use has been greatly hampered by cost.

This study was designed to define the degree of treatment related bone marrow suppression, using nadir neutrophil counts of patients with two very common malignancies in our setup i.e. Non-Hodgkin's lymphoma (NHL) and breast cancer using Cyclophosphamide, Doxorubicin, Vincristine and Prednisone (CHOP) and Cyclophosphamide and Doxorubicin (AC) regimen respectively.

2. BACKGROUND INFORMATION

2.1 HEMATOPOIESIS

All cellular components of blood are derived from pluripotent stem cells in the bone marrow and other hematopoietic centers in the body. Under the influence of hematopoietic growth factors (HGF), the stem cell divides and differentiates via progenitor cell into various mature cell types (1, 2). This process of formation and production of peripheral blood cells is known as hematopoiesis. Under physiological conditions, it is a tightly regulated highly efficient system exquisitely responsive to functional demands. Maintenance of a normal number of blood cells requires continuous production, to replace the aging or damaged cells in circulation (2, 3).

Turnover of differentiated hematopoietic cells in a 70 kilogram adult is over half a trillion cells per day. These include 200 billion erythrocytes and 70 billion leucocytes. This is supported by the hematopoietic stem cell, which forms approximately 0.001% of all bone marrow nucleated cell (4). 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. This protects them from excessive damage induced by common chemotherapeutic treatments (1, 2, 4).

The bone marrow's storage compartment can supply mature cells to the peripheral blood for 8-10 days, after the pool of primitive hematopoietic progenitor cells, cease the normally active process of division and maturation that replenishes the pool of the latter more differentiated committed progenitor cells (4). Following injury to the bone marrow either by chemicals, radiation or infection, the kinetics of cytopenias 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. Lymphopoiesis on the other hand, is a very inefficient

process with only 5% of the generated cells surviving to be ejected out of the marrow or thymus into the periphery (3, 4).

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 leukopenia and thrombocytopenia 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 (3, 4).

Control of production is largely by negative feedback. When demand for production of cells of a particular lineage increases or peripheral levels of the cells falls, stimulatory cytokines (HGF's) are released which initiate the generation of new cells of the particular lineage (1-4).

2.1.1 The Hematopoietic Stem Cell (HSC)

The HSC is a cell characterized by extensive proliferation and differentiation capacity, with the ability to self renew on a population basis (2). They express a variety of cell surface proteins that have the ability to rapidly home to the marrow after an intravenous injection. Human stem cells lack markers of lineage commitment. The most primitive cells are characterized by low-level expression of relatively large numbers cytokine receptors. However, these cells express a variety of adhesion proteins, presumed to be involved in marrow homing namely alpha-4, alpha-5, alpha-6, L- selectin and platelet endothelial cell adhesion molecule (1, 2, 4).

The stem cell also possesses a functional plasticity in response to cytokines as it transits the cell cycle. The earlier progenitor cells are multipotent but as division and differentiation proceeds, later progenitor are formed that are committed to three, two or one cell lines (3, 4).

Clinically, stem cells have the following implications;

A number of human tumors have been found to be characterized by the presence of a specific translocation e.g. Philadelphia chromosome and chronic myelogenous leukemia. Studies have supported the hypothesis that most tumors result from clonal expansion of a single malignant cell (4).

A variety of germ line mutations results in development of a spectrum of hematological diseases e.g. sickle cell anemia and thalassemia. Specific pattern of gene expression in these inherited hematological diseases, provide a clinical setting in which transfer of genes to somatic cell is likely to correct the underlying defect. The introduction of intact globin genes or genes encoding enzymes critical for cellular immune function e.g. alanine deaminase into HSCs should permit the development of functionally normal erythrocytes, T cells and B cells.

Transplantation of allogeneic HSCs from HLA haplotype matched donors remains treatment of choice for patients with genetic disorders of hematopoiesis.

hematopoietic progenitor cell transplant has been used to treat several Hematological malignancies and is currently being evaluated as a means of delivering dose intensive therapy to patients with a variety of solid tumors (4).

The non-hematopoietic tissue immediately abutting hematopoietic tissue forms the hematopoietic microenvironment. This influences hematopoiesis, and appears capable of supporting most primitive stem cells and controlling their proliferation and self-renewal.

The crucial micro environmental interaction between bone marrow hematopoietic cells and, micro vascular endothelial cells and connective tissue stromal cells can be disrupted either by primary tumor in hematological diseases intrinsic to the marrow or by metastatic spread of tumor to the marrow from neoplasms of other organs. Associated symptomatology or radiological abnormalities are commonly lacking initially (1-4).

2.1.2 The Cell Cycle

The growth of tissues and organs may occur by an increase in number of cells, an increase of size of cells or both. In homosapiens, growth in cell number is by far the most important component in development. From a single fertilized egg, an adult

grows to an average of 10^{15} cells, on the other hand, the increase in size of the cells from newborn to adult human is only threefold to fourfold. Upon maturity, the number of cells remains essentially constant but cell division continues at a brisk rate to replace approximately 10^{12} cells that die daily in organs like gastrointestinal tract, skin and bone marrow. If the number of cells that are produced exceeds the number of cells that die there in growth or hypertrophy, if the produced cells are fewer than the dead cells, atrophy occurs (5, 6).

In every population of cells, there are 3 sub-populations:
Cells that continuously proliferate going from mitosis in one cycle to the next.
Terminally differentiated cells that irrevocably leave the growth cycle and are destined to live without dividing again.
Non-dividing cells or non-cycle cells that do not divide but can re-enter the cell cycle if an important stimulus is applied (5, 6).
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 the 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 this period the genetic material is replicated and no other replication is permitted there after. In G2 (gap 2) the fidelity of DNA replication is determined and errors corrected (6).

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.
Bone marrow stem cells are in Go phase (resting) and are capable of reproducing themselves and producing the different lineages of hematopoietic cells when recalled to the cycle. Fortunately, in the Go phase they are protected from most chemotherapeutic agents used to treat cancer. However, the depletion of the other dividing cells in the marrow by these drugs stimulates the protected stem cell to re-enter the cell cycle and eventually repopulate the bone marrow (4, 5, 6).

The mechanism of cell growth is substantially the same in all dividing cells. The process ensures that the cell accurately duplicates its contents especially in chromosomes. Any population of cells can increase in number by any one of three mechanisms:

Shortening of the cell cycle resulting in more cells being produced per limit time, decreasing the rate of cell death and moving Go cells into the cell cycle.

All three mechanisms operate in normal and abnormal growth (3, 4, 5).

In tumors, all three mechanisms are important in determining the aggressiveness of tumors. This is best characterized by its doubling time, which in the real measure of aggressiveness of a tumor and a better index of aggressiveness.

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 (3, 6).

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 (3, 5, 6).

2.1.3 Effects of Cytotoxic Drugs

The major side effect of most cytotoxic and immunotherapeutic agents is myelosuppression. The pharmacological action of a drug, the route and schedule of administration, drug metabolism and the pattern of cell sensitivity may influence the pattern of marrow damage (7, 8).

Anti neoplastic drugs are of two general categories.

Those that act upon the cell throughout its cycle i.e. phase non-specific.

Those that act preferentially during one or more of the non-resting phases i.e. phase specific.

Drugs that interfere with DNA synthesis will be specific to the S phase, those that block protein synthesis mainly to S and G2 and those that inhibit microtubule

assembly to M phase. For cycle specific agents, recovery occurs rapidly within seven to fourteen days whereas; non-cycle specific agents cause a much more delayed nadir in four to five weeks (7, 8).

Cytotoxic drugs act mainly if not entirely by either; damaging the mitotic or stem cell compartment of the marrow or, slowing cell division by interfering with purine or pyrimidine biosynthesis, blocking DNA strand duplication disrupting the microtubules mitotic spindles and interfering with RNA formation, translation, and transcription processes central to protein synthesis. (8, 10)

Common cytotoxic agent almost invariably affect bone marrow function by, causing decreased production of blood cells, through reduction of rapidly growing progenitor stem cells in the marrow. Consequently, bone marrow suppression is dose related and is dependent on continued administration of the drug (7-10).

Doxorubicin

This is an antitumor antibiotic isolated from a mutant strand of the fungus streptomyces, which demonstrates greater activity in treatment of solid tumors including breast cancer and malignant lymphoma (8, 10).

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 (10).

Doxorubicin has a high incidence of bone marrow suppression, which manifests itself mainly as a neutropenia that is most severe 10—14 days after treatment and lasts about seven days. A white cell count as low as $1.0x10^9/l$ is to be expected. This neutropenia together with the mucositis that may occur are dose limiting. Alopecia is a universal finding, which causes significant patient distress (8-10).

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 (8-10).

Vincristine

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 (11).

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 (11).

Peripheral neurotoxicity, resulting from axonal degeneration is the most frequent dose limiting effect. Vincristine has not been shown to have significant bone marrow suppression (8, 9, 11).

Cyclophosphamide

Alkylating agents were among the first to be identified chemotherapeutic agents, and are still important components of modern chemotherapeutic regimens. They posses an important property of associating 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 (12).

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 hemorrhagic cystitis and predisposes to transitional cell carcinoma (8, 12).

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 leukopenia, immunosuppresion and only mild thrombocytopenia. Other complications include sterility in men, ammenorrhea in women, and it 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 (8, 9, 12, 13).

Prednisone

Glucocorticoids suppress mitosis in lymphocytes and fibroblast 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. However Cushing's syndrome, inadvertent adrenal suppression on withdrawal of high dose glucocorticoid or infections common in immunosuppression can be significant complications (9).

2.2 BONE MARROW FAILURE.

Cytotoxic therapy for malignancy is probably the most common cause of temporary marrow failure states (4, 8). This may present as pancytopenia with anemia, leukopenia and thrombocytopenia in the peripheral blood due to deficient hematopoesis. This should be distinguished from destruction of erythrocytes in hemolytic anemia, platelets in idiopathic thrombocytopenic purpura or hypersplenism and granulocytes in immunological leukopenias (4, 7, 8).

As mentioned earlier, kinetics of cytopenia induction following marrow injury reflects the life span of the cells peripherally. The first lineage to drop is the neurophils with a half-life of 6-8 hours, followed by platelets with a half-life of 7-10 days. Anemia in the absence of bleeding develops over a long time, the half-life of erythrocytes being 120 days (4, 7).

2.2.1 Neutropenia.

This is defined as an absolute Neutrophil count of less than $2.0x10^9/l$. The consequences of reduced or absent neutrophils are dramatic in grade 3 * Neutropenia, with increased susceptibility to infection. In grade 4 neutropenia control of endogenous microbial flora is impaired and opportunistic infections set

in. Below $0.2x10^9$ /l, the inflammatory response of the body breaks down (14-16). (appendix 1)

Cancer chemotherapy causes an acute neutropenia (within 6-14 days) after conventional doses of anthracyclines but alkylating agents differ in timing of cytopenias. Compared to the chronic neutropenias, acute neutropenia is more likely to be associated with increased risk of infection. There is a variable delay before deleterious effects on neutrophil cell production are reflected. The length of the delay is related to the size of the marrow neutrophil reserve before administration of the agent and the rate of consumption thereafter (8, 9, 14).

Neutropenia in the absence of cytotoxic therapy in a patient with Hodgkin's disease, or non Hodgkin's lymphoma, suggests marrow invasion with tumor or fibrosis (15, 16).

Febrile neutropenia is defined as a temperature greater or equal to 38.5 ^{0} C on three readings, or temperature greater or equal to 38 ^{0}C, but less than 38.5^o C in a neutropenic patient (14-16).

Fever with neutropenia, is an important end point in patients receiving chemotherapy for cancer. In 1965, Bodey et al (17) showed a direct correlation between duration of granulocytopenia and risk of infection. In patients whose absolute neutrophil counts were below $1.0x10^9$/L for one week; the chance that they would develop infection was more than 50%. However, as the duration of granulocytopenia increased, the risk approached 100%.

Transfusion of granulocytes has no role in the management of febrile neutropenia. Their exceedingly short half-life, mechanical fragility and clinical syndrome of leucostasis associated with their use have limited their usefulness (14). See Appendix 1

2.2.2 Thrombocytopenia

Dangerous degrees of thrombocytopenia do not frequently complicate management of patients with solid tumors receiving cytotoxic chemotherapy

unless the bone marrow is infiltrated (18). Anecdotally though, thrombocytopenia has been reported to complicate and hamper solid tumor treatment in our set up. However, when it occurs, thrombocytopenia increases the risk of hemorrhage, necessitates platelets transfusions and limits the doses of myelotoxic agents (18, 19).

Platelet transfusions can prevent bleeding. However, transfusion related infections, allergic complications and refractoriness due to alloimmunization reduce their usefulness to some extent (19-21).

2.2.3 Anemia.

Anemia in patients with cancer can be mild to severe and may be attributable to many causes. The incidence and magnitude of anemia also increases as the disease progresses (22).

Replacement of marrow hematopoietic elements by tumor is not essential for development of anemia even in patients with widely metastatic cancer (12, 23).

Malignancy associated anemia is designated anemia of chronic illness only if; the cellular pattern of the marrow is normal, the serum iron and TIBC are low, the iron content of the marrow is normal or increased and the ferritin levels are elevated.

Other causes of anemia in malignancy may include active hemolysis, uncontrolled bleeding, nutritional deficiency or marrow replacement and these must be ruled out (22-24).

2.3 ROLE OF HEMATOPOIETIC GROWTH FACTORS Colony Stimulating Factors (CSF)

2.3.1 Granulocyte-CSF (G- CSF)

This is one of the hematopoietic factors, which stimulates the proliferation, differentiation and enhances survival of neutrophil granulocytes (3, 25, 26).

G-CSF has been shown to also modulate the function of mature neutrophils, including super oxide release, degranulation, and chemotaxis, expression of adhesion molecules and phagocytosis as well as bactericidal activity. Recently, it has been shown to influence cytokine and cytokine antagonist release (3, 25, 26).

G-CSF is produced by hematopoietic cells, monocytes, macrophages and lymphocytes, upon activation by lipopolysaccharide, Tumor necrosis factor, Interferon alpha, Interleukin-4 and Interleukin –7 (25).

Other cells e.g. fibroblasts, endotheliocytes, astrocytes and bone marrow stromal cells can produce G- CSF after activation by lipopolysaccharide, Interleukin -1 and Tumor necrosis factor alpha (3, 25).

G- CSF acts on a relatively mature progenitor cell population that is primarily committed to neutrophilic differentiation and has been reported to increase the number of hematopoietic progenitor cells in circulation by ten fold (25-27).

Two forms of recombinants G- CSF, the pegulated and non pegulated are available commercially and are in clinical use for treatment of neutropenia, chemotherapy induced myelosupression, recovery for aplasia following bone marrow transplant and mobilization of CD 34+ progenitor cells in peripheral circulation (26-29).

In one study, Brunchud et al (30) in 1987 showed that in 12 patients with small cell lung cancer given recombinant human G CSF the number of peripheral neutrophils increased rapidly to a maximum of $100 \times 10^9/l$ at a dose of 10ug/kg

/day. These neutrophil were shown to be functionally normal in their tests of motility and bactericidal activity.

Later in 1993, Trillet (31) and associates showed that G- CSF reduces the infectious complications of cytotoxic chemotherapy.

2.3.2 Granulocyte/Macrophage CSF (GM- CSF)

Stimulates the growth of granulocyte and monocyte colonies as well as the early growth of erythroid and megakaryocytic progenitor cells, modulates the function of mature granulocytes and macrophages and has a stem cell mobilizing effect. Both G-CSF and GM-CSF increase the neutrophil count through amplification in the bone marrow maturation compartment but kinetic studies show that, the action of G-CSF is faster because it shortens time to release of newly formed neutrophils from five to one day (26, 28, 32, 33, 34).

M-CSF mainly stimulates the production of macrophages and activates the function of mature cells (3, 27.)

2.3.3 Erythropoietin.

This is a glycoprotein formed primarily by the kidney that triggers erythropoiesis, i.e. a process by which new red blood cells are produced (35).

In health, the body is able to maintain the balance between erythropoietin production and hemoglobin levels. However condition like renal dysfunction, inflammation, infection, tumor growth, cancer chemotherapy and bone marrow transplant inhibit erythropoietin production or impairs its function. This results in insufficient number of red cells and hence low hemoglobin levels and anemia (35-37).

Several recombinant human erythropoietin formulations are available for prevention or treatment of anemia related to erythropoietin deficiency (28, 36).

2.3.4 Thrombopoietin.

The use of platelet transfusion to prevent bleeding in thrombocytopenic patients has been complicated by allergic complications and refractoriness due to allo-immunization (18 - 20).

A search for a specific stimulator of platelet production has yielded thrombopoietin and other related megakaryocytic growth and development factors (MGDF) (35, 36, 37).

In 1995, Kuter and Rosenbug (38) demonstrated that in prolonged thrombocytopenia caused by infection or chemotherapeutic agents, thrombopoetin had a circulating concentration that varied inversely and proportionally to total platelet counts.

Thrombopoetin, interleukin 11 and other cytokines have several actions during megakaryocyte development. They cause increase in size and number of megakaryocytes and stimulate their expression of platelet specific markers CD 41 and CD 61 (37-40).

In addition, thrombopoetin in synergy with erythropoietin stimulates growth of erythroid progenitor cells and with stem cell factor (SCF) and interleukin 3 stimulates proliferation and prolongs survival of hematopoietic stem cells and all types of blood cell progenitors (37-41).

Two recombinant MGDF's have undergone extensive clinical testing. These include recombinant human thrombopoetin and pegulated recombinant human MGDF. Both are potent stimulators of platelet production in humans and decrease the extent of thrombocytopenia associated with non-myeloablative chemotherapy and may reduce the need for platelet transfusions (37, 39, 40).

2.4 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 (42).

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 (43).

NHL can be divided into two prognostic groups, the indolent lymphoma and the aggressive lymphomas. Indolent NHL types have a relatively good prognosis, with median survival as long as 10years, but they are usually incurable in advanced clinical stages (42, 43, 44).

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 five years is approximately 50% - 60% (42, 43).

2.4.1 Cellular Classification and Staging

WHO modification of the REAL classification recognizes three 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 a treatment for patients with NHL (43, 44).

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. (See appendix II)

Other factors like age, performance status, tumor size, LDH values and number of extra-nodal sites though not included in the above staging system are important for the staging and prognosis of patients with NHL. To identify subgroups of patients most likely to relapse, an international index was compiled. The model identifies 5 significant risk factors prognostic of real survival (45). Appendix II

2.4.2 Treatment Options

Treatment of NHL depends on the histological type and stage. Although localized presentations are uncommon in NHL, the goal of treatment should be cure in those shown to have truly localized disease after staging. Long term disease controls within radiation fields can be achieved in a significant number of patients with indolent stage I or II NHL by radiation. Rarely, when radiation therapy is contra-indicated, chemotherapy can be employed for symptomatic patients (46, 47, 48).

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 to 70% at five years. 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 (47, 48).

Surgery has an important role in the diagnosis and treatment of primary extra-nodal aggressive lymphomas without other size involvement. Due to their unpredictable pattern of relapse, chemotherapy is often used as the primary treatment modality (47, 48).

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. (Under evaluation)
- Chemotherapy alone vs. anti-idiotype vaccine + chemotherapy (phase III trials) (46-48).

2.5 BREAST CANCER

Breast Cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast. Epithelial malignancies of the breast account for a third 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(49). 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 (49-50).

2.5.1. Cellular Classification and Staging

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 breast cancer histological type, comprising 70%-80% of all (49).

The American Joint Committee or Cancer (AJCC) staging system provides for grouping patients with respect to prognosis through the TNM* Classification. Therapeutic decisions as 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 (51 52). * (appendix III)

2.5.2. 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 reception (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, HER 2 / neu status (50, 53).

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 (54).

Adjuvant systemic therapy, include hormonal therapy with tamoxifen, and chemotherapy ovarian ablation or chemotherapy alone. Chemotherapy is recommended for node (+), receptor (-), high-risk node (-) receptor (-) patients and those whose tumors have progressed on hormone therapy .The overall results of available evidence suggest that the addition of chemotherapy to tamoxifen in postmenopausal women with ER – Positive disease results in a small but significant survival advantage (54, 55).

Commonly used combination regimens include.

- AC –Doxorubicin and Cyclophosphamide
- Docetaxel and Doxorubicin.
- CAF – Cyclophosphamide, Doxorubicin, 5 flourouracil.
- CMF- Cyclophosphamide, methotrexate, 5 flourouracil.
- Doxorubicin and paclitaxel.
- AC-Pax_ Cyclophosphamide, Doxorubicin, Paclitaxel
- AC-Tax_ Cyclophosphamide, Doxorubicin, Taclipaxel((54-56)).

3. RATIONALE

Breast cancer and non-Hodgkin's lymphoma are common malignancies in our set-up, ranking second and fourth respectively at KNH as was reported by Mungania (57). Both malignancies are potentially chemo-curable since they have high dose response curves. However, high doses of chemotherapy enhance tissue toxicity. Consequently, bone marrow toxicity is a major limitation in the treatment of these malignancies.

Circulating neutrophil counts have traditionally been one of the clinical criteria used to determine whether a patient has recovered sufficiently from a previous cycle of chemotherapy, and is ready to tolerate the next planned cycle. The highest reduction in neutrophil count has been reported to occur either in the first or second cycle (8, 9).

Despite reports of improved outcome and better prognosis in patients with cancer from developed countries (44-46), we continue to note poor treatment outcomes in our set up. Contributing factors include; late stage of disease at diagnosis, delay in commencing treatment and treatment schedule interruptions (personal communication).

Poor hematological status and development of sepsis due to temporary bone marrow failure are major contributing factors to these treatment schedule interruptions (58). Supportive care during and after chemotherapy is essential. Measures include nutritional support, rehydration, prevention of infection by reverse barrier nursing and/ or prophylactic antibiotics use, and blood or platelet transfusions as required. Currently use of HGFs has brought a new ray of hope (28, 29, 31). However their use has to be justified in view of the prohibitive cost.

There is therefore a need to research into treatment related complications, as a means to reduce these interruptions especially in this era of hematopoietic growth factors and to optimize on treatment using aggressive regimen in an attempt to improve on outcome in our patients.

3.1 Research question

What is the treatment related peripheral blood cell count nadirs in patients with NHL and Breast cancer treated with CHOP and AC respectively?

4.0 OBJECTIVES

4.1 Broad objective:

To determine nadir peripheral cell counts in patients on standard treatment for breast cancer and NHL.

4.2 Specific Objectives:

4.2.1 Determine the complete blood counts between day 10 and 14 after administration of the first and second cycle of chemotherapy and day 21 of the first cycle.

4.2.2 Describe the pattern and magnitude of change in the peripheral blood cells in the two cycles.

4.2.3 Determine the peripheral blood cell nadirs on day 10 to 14 of each of the two cycles.

5. RESEARCH PLAN

5.1 Study Design

This was a prospective descriptive study, using a real life usual practice scenario.

5.2 Study Areas

The study was conducted at the Hematology and Oncology outpatient Clinic, radiotherapy clinic, and Medical Wards of Kenyatta National Hospital.

S

5.3 Study subjects

These were patients aged 13 years and above with histological diagnosis of breast cancer or NHL, staged and eligible for chemotherapy.

5.4 Study duration

The study was carried out over ten months between February 2004 and December 2004.

5.5 Sample size and sampling

Assuming the prevalence of neutropenia to be 6.5% and taking a confidence interval of 95% and a level of precision of 0.05, using the formula;

$$N = \frac{Z^2 P (1-P)}{(0.05)^2}$$

a minimum sample size of 93 was obtained. However, a total of 100 patients were recruited through consecutive sampling. (59)

6.0 METHODOLOGY

6.1 Screening

The principal investigator screened and recruited study cases both in the wards and the outpatient clinics using a standardized screening and recruitment proforma.

In the outpatient clinics, the records and charts of all new patients presenting to the clinics with breast cancer or NHL during the study period was scrutinized. Chemo-naive cases with histologically diagnosed and staged breast cancer or non-Hodgkin's lymphoma were selected. The principal investigator facilitated the staging process for the non-staged cases and routine work up for all cases.

All records of confirmed cases with breast cancer and NHL admitted for workup were reviewed and chemo-naive cases with histologically diagnosed and staged breast cancer or non-Hodgkin's lymphoma were selected. Staging (for the non-staged patients), baseline workup for renal and liver functions and any other investigation deemed necessary by the attending doctor were facilitated. As part of the usual care all patients were pre and post counseled for HIV testing as recommended by the attendant oncologist.

The patients were also evaluated for clinical evidence of cardiac disease which would make anthracycline based drugs contraindicated.

At this point in liaison with the oncologist on duty, the most appropriate regimen for the patient was selected and instituted. The cases for whom the AC- 50/500 regimen was recommended, were recruited.

As part of staging for NHL, a unilateral bone marrow aspirate (2cm deep) was taken and presented to the study-designated hematologist for reporting to standardize the results.

6.2 Assessment of eligibility for chemotherapy

A physical examination was performed on all cases for whom chemotherapy was indicated and the study protocol recommended. This was to exclude any obvious infection.

The ECOG performance scale, as a possible predictor of prognosis was assessed and cases with ECOG 4 and above were excluded. Appendix1

Two milliliters of venous blood for baseline complete blood count was taken for all patients and analyzed using a coulter counter module 4.(see 6.2.1) Those with low hemoglobin levels (< 10g/dl) received a top up transfusion. For patients with low white cell count (ANC < $1.0x10^9$/L), chemotherapy was withheld until the cell count was suitable and antibiotics were prescribed as deemed necessary in conduction with the attending doctor. Suitability of counts for chemotherapy was defined as hemoglobin levels ≥10g/dl), ANC ≥ $1.5x10^9$/L and platelets above 100 $x10^9$/L.

Three mililitres of venous blood was also obtained for analysis of baseline renal and liver functions. Those with significant renal i.e. GFR below 50 mls/ min or liver dysfunction i.e. Transaminases above five times normal not explained by the disease process were excluded.

6.2.1 Peripheral blood analysis using the coulter counter module 4

The coulter counter is a machine designed to perform a multi parameter analysis of whole blood samples using two dilutions. A dilution of 1:125 is used for WBC analysis and 1:6250 is used for both erythrocytes and platelet analysis.

The coulter method of counting and sizing is based on detection and measurement of changes in electrical resistance produced by a particle (in this case the blood cell) suspended in a conductive liquid traversing a small aperture.

An electrical pulse suitable for counting and sizing results from the passage of each cell past an electrode submerged on each side of the aperture. The number of impulses generated represents the cell count and the amplitude of the electrical impulse represents the cell volume.

The coulter counts particles whose volume is more than thirty six femtolitres in a higher dilution of 1:6250 as red cells and particles with a volume of between two and twenty femtolitres in the same dilution as platelets. The hemoglobin level is evaluated by the photocurrent generated by the circuitry.

The total white cell count is the number of particles whose volume is between thirty five and four hundred and fifty femtolitres after lyses of the erythrocytes using one of the reagents in a solution whose dilution is 1:125. Neutrophils are counted as particles that are between one hundred and four hundred and fifty femtolitres in volume. Consequently the lymphocytes are obtained by subtracting the number of particles that are between one hundred and four hundred and fifty femtolitres from the total number of particles between thirty five and four hundred and fifty femtolitres.

The electronic counting cycle consists of three-4 seconds count periods separated by half a second delays. The microprocessor then compares the three counts and performs a statistical test of precision and corrects for coincidence error. The final count is then computed into the relevant units of expression for the parameter and printed using an in built electronic printer.

6.3 Recruitment

6.3.1 Inclusion criteria

Patients with breast cancer were included if they were to receive chemotherapy for adjuvant therapy or metastatic disease.

NHL cases were included if they had indolent advanced stage (III and IV) disease, early stage I and II with B- symptoms or if they had aggressive and highly aggressive all stages other than stage IA who voluntarily signed an informed written consent. (Appendix III)

6.3.2 Exclusion Criteria

Patients were excluded if they had had prior chemotherapy or radiotherapy for same disease or had evidence of either clinical or chemical evidence of organ failure as explained above.

Also excluded were patients with an ECOG performance score of 4 and above and those who had contraindications to any of the component drug comprising the study protocol.

6.3.3 Ethical Considerations

Permission to carry out the study was granted by the KNH Ethics and research committee.

Voluntary consent was sought from patient and the parent or guardian for those patients less than 18 years of age.

Usual care and evaluation procedures were facilitated in these patients.

Results of the investigation were communicated to the health care providers to facilitate improved care of the patient.

Those who declined consent were not discriminated upon.

The principal investigator ensured continued care after the five-week period by handing over to the usual care teams in the relevant clinics.

6.4 Study Chemotherapy protocol

With the help of an enlisted research assistant (MO) NHL cases were given chemotherapy as 3 weekly cycles of:

Cyclophosphamide	(IV)	750mg/m^2	day1
Doxorubicin	(IV)	50mg/ m^2	day1
Vincristine	(IV)	1.4mg/ m^2	day1
Prednisone	(PO)	60mg/ m^2	day 1 to 5

And breast cancer cases also received chemotherapy as 3 weekly cycles of:

Cyclophosphamide	(IV)	500 mg/ m^2	day1
Doxorubicin	(IV)	50 mg/ m^2	day1

Blood counts for all patients were repeated between day 10 and 14 and day 21 of the first and second cycle. The primary physician was alerted of any complications that occurred to facilitate prompt treatment.

6.4.1 Study Procedure

Intravenous chemotherapy was administered on day one of a twenty-one cycle and the oral prednisone was given daily for five days. Between day 10 and 14 after chemotherapy of two consecutive cycles and on day 21after first cycle, 2 mls of blood were drawn and this was processed on a coulter counter module 4.(see 6.2.1) A PBF was also prepared and platelet counted using the counting chamber if the counts fell below $100x10^9/L$.

Patients were also evaluated for fever or any other signs of infection on day 10- 14 and were advised to report if they developed a problem before the next scheduled visit.

6.5 Data Collection

A standardized data collection sheet consisting of a screening proforma and recruitment proforma was used to collect the data. (Appendix IV)

6.7 Data Analysis

Descriptive statistics (mean, mode, median, standard deviation) were used, and presented as tables, pie charts, bar graphs, and frequency diagrams. The Mann-Whitnney U-Wilcoxon Rank Sum W test was used for continuous data in subgroup analysis. For categorical data the Pearson's correlation coefficient and the Mantel-Haenszel test for linear association were used and a P value of less than 0.05 was considered significant. To calculate the percentage changes during the various stages of the cycles the median count was used due the large variance obtained with the mean counts. However similar values were obtained for percentage changes using the mean percentage count of individual cases.

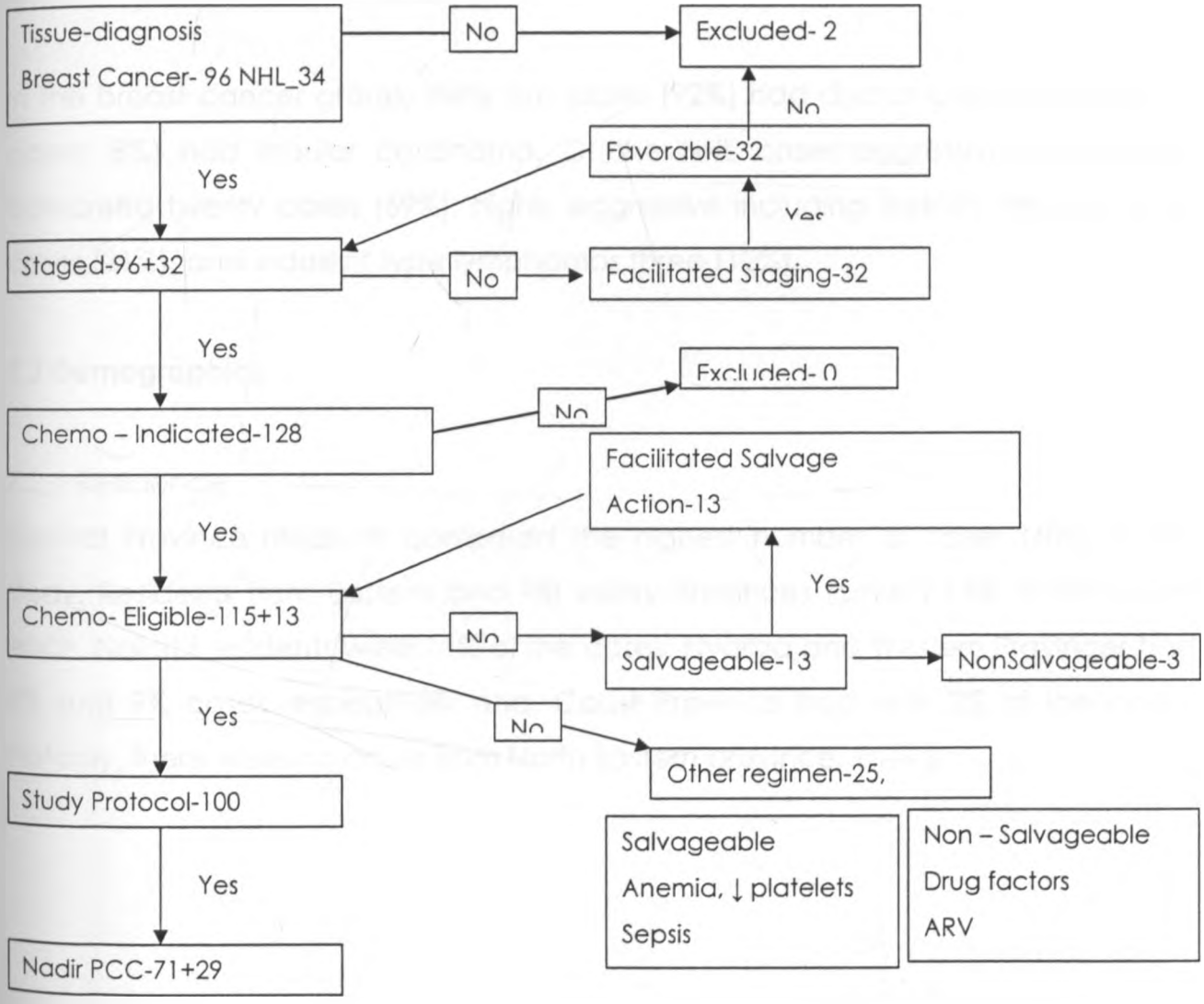
7.0 RESULTS

7.1 Screening and Recruitment

A total of one hundred and thirty cases with either breast cancer or NHL were screened during the study period. Of these cases, ninety-six were screened at the cancer treatment center and thirty-four from the Hematology-Oncology clinic and/or medical wards. Of the130 cases screened, 96 had the diagnosis of breast cancer and the remaining 34 Non-Hodgkin's lymphoma. (NHL)

Thirty cases were excluded in total. Twenty-five of them had breast cancer. For eighteen of the cases with breast cancer, the attending oncologist recommended alternative regimen and the other seven were scheduled to begin with radiotherapy followed by chemotherapy. (Figure 1)

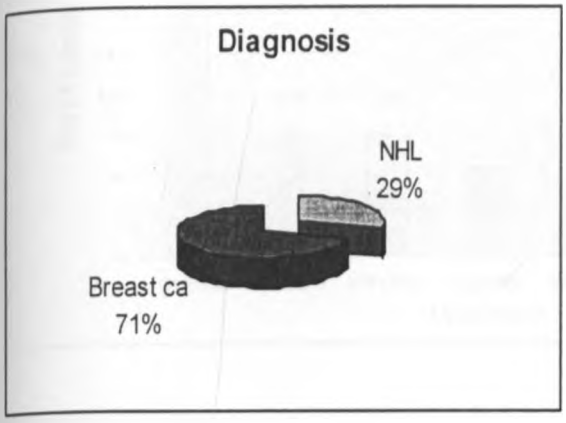
Figure 1: Screening and Recruitment flow chart



The remaining five with NHL either had poor functional scores or did not have a documented tissue diagnosis.

One hundred cases with tissue diagnoses were recruited of whom 71(71%) had breast cancer and 29(29%) had Non Hodgkin's Lymphoma (NHL). (Figure 2)

Figure 2; Percentage of cases in each Diagnosis group



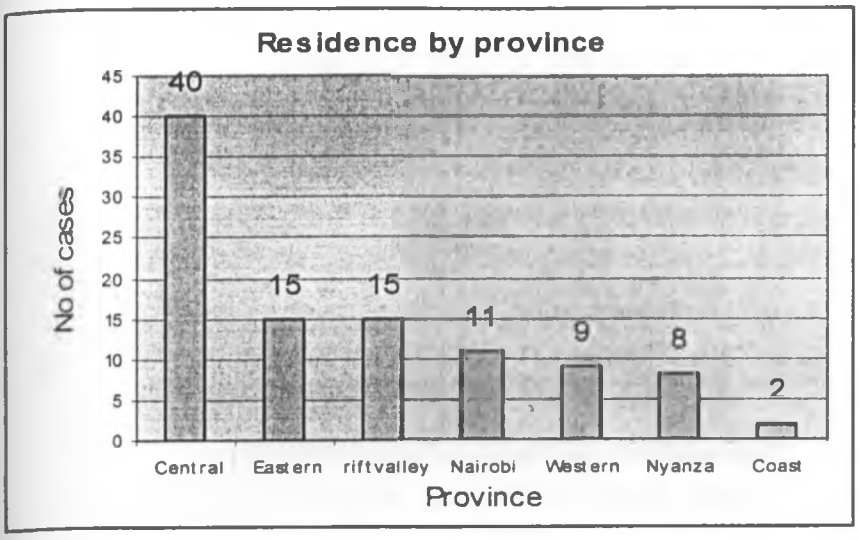
In the breast cancer group, thirty five cases (92%) had ductal carcinoma and six cases (8%) had lobular carcinoma. Of the NHL cases aggressive phenotypes comprised twenty cases (69%), highly aggressive including Burkitt's lymphoma six cases (21%), and indolent type lymphomas three (10%).

7.2 Demographics

7.2.1 Residence

Central Province residents comprised the highest number of cases (40%) in the study. Residents from Eastern and Rift valley Provinces formed 15% of the cases each. Nairobi residents were 11% of the cases, Nyanza and Western Provinces had 8% and 9% cases respectively and, Coast Province had only 2% of the cases. Notably, there were no cases from North Eastern province. (Figure 3)

Figure 3: Residence by Province



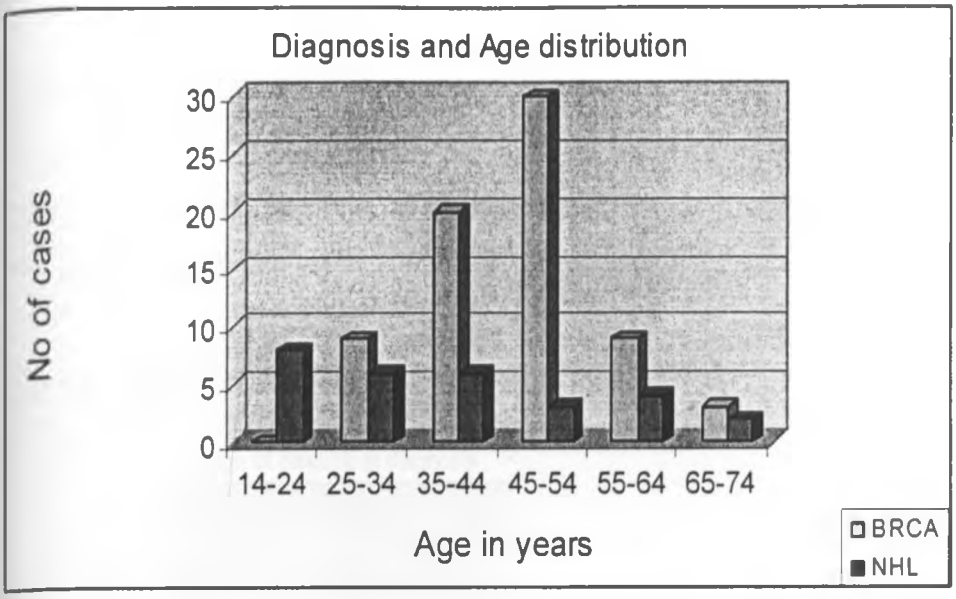
7.2.2 Age and gender distribution

Overall, 83(83%) of the cases were female and 17(17%) males. The male to female ratio for this study was 1:4.3. The youngest recruited case was fourteen years and the oldest was sixty six years with a median age of 45 years. (Table1).

Of the breast cancer group, only two cases (2.8%) of the group were males, while in the NHL group, 17(58.6%) were males, with a M to F ratio of1.4 to1.

For the breast cancer group, the youngest case was 24 and the oldest 66 years. The median age was 46 years. The peak age of occurrence was 45-54 years with the majority of the cases being in the 35-55 age groups. (Figure 4)

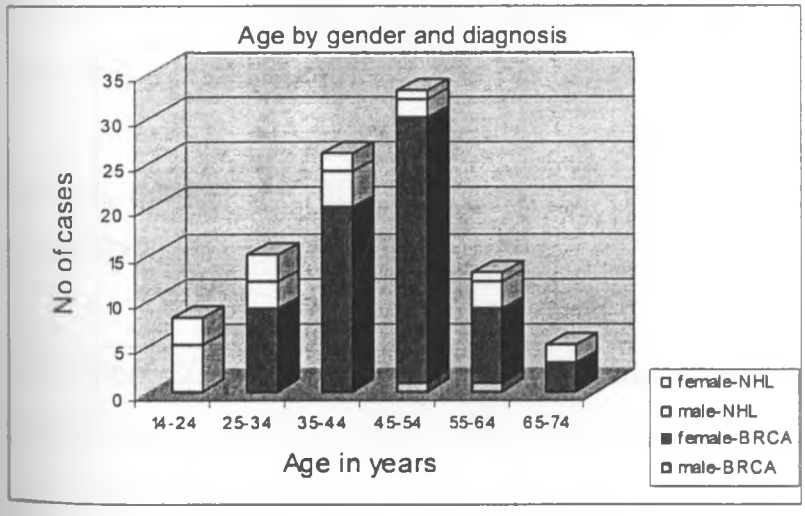
Figure 4: Age Distribution according to tumor types



The youngest case was 14 and the oldest 65 years with a median age of 36 years for NHL treatment group.

The NHL cases were distributed across all the age groups but the majority of the cases were in the age range of 14 -44 years. (Figure 3 & 4)

Figure 5: Age distribution by Gender and Diagnosis

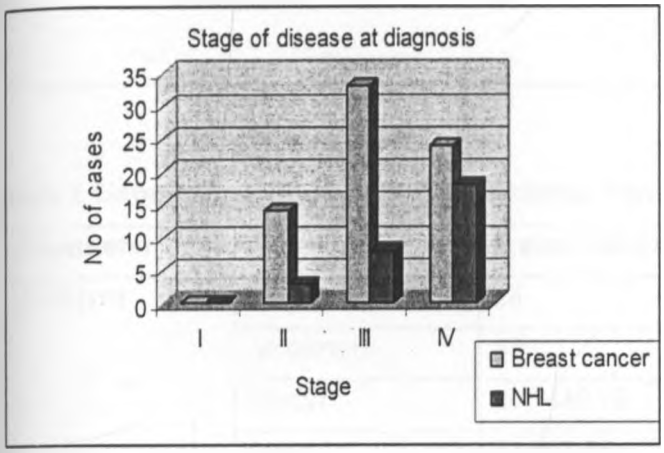


7.3 Clinical status

7.3.1 Stage of Disease

In the breast cancer group, 57(80%) of the cases had stages III and IV disease, with most of them in stage III. In the NHL group, of the 26(90%) who had stage III and IV disease, 18(62%) had stage IV disease. ((Figure 6 & Table 1))

Figure 6: Stage of Disease at Diagnosis



In the breast cancer group, 46cases, (65%) had evidence of disease in at least two of the sampled lymph nodes. However, 10 cases (14%) did not have lymph node sampling done.

For NHL cases, only 8(27.6%) had evidence of bone marrow disease on cytology and 10(37.5%) were positive for HIV.

Fifty three (80%) of the breast cancer cases were in ECOG I and II with the majority 38(54%) being in the latter score. For NHL cases, 26(90%) were in ECOG II and III with 17(58.6%) in ECOG III. (Figure 7 & Table 1)

Figure 7: Performance Score at Diagnosis

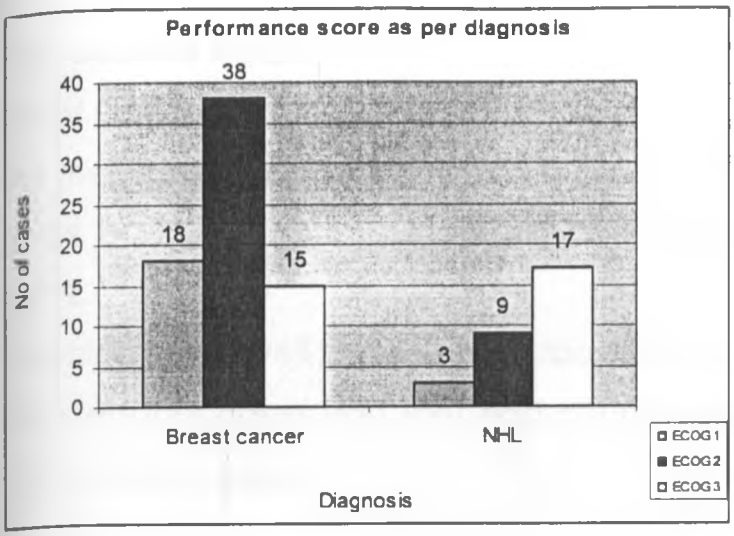


Table 1 Summary of Demographic and clinical Parameters

Parameter		Breast Cancer		Non-Hodgkin's Lymphoma	
AGE(yrs)	Minimum	26		14	
	Maximum	66		65	
	Mean	46.14±8.72		36±17.036	
	Male	54.5±7.78		35.4±16.48	
	Female	45.9±8.68		38.42±18.38	
	Median	46		36	
BSA	Minimum	1.1		1	
	Maximum	2.1		1.84	
	Mean	1.7±0.2		1.6±0.2	
	Median	1.7		1.61	
Gender (%)	Male	2.8		58.6	
	Female	97.2		41.4	
	Ratio	1:35.5		1.4:1	
Performance Score n=	ECOG 1	18(25.4%)		3(10.3%)	
	ECOG 2	38(53.5%)	53(75%)	9(31%)	26(90%)
	ECOG 3	15(21.1%)		17(58.6%)	
Stage n=	II	14(19.7%)		3(10.3%)	
	III	33(46.5%)	57(80%)	8(27.6%)	26(90%)
	IV	24(33.8%)		18(62.1%)	

7.4 Peripheral White Blood Cells Profile

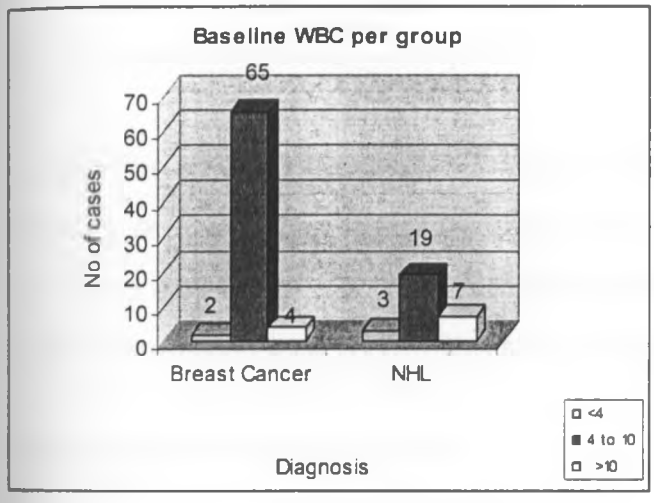
7.4.1 Baseline WBC

Breast Cancer

The mean baseline total WBC was 6.7± 2.4x10^9/L with a median of 6.3x10^9/L and a range of 3.6 x10^9/L to 19.5 x10^9/L. The interquartile range (IQR) was 5.35 to 7.45 x10^9/L.

Sixty nine cases (96%) had counts above the lower limit of normal of 4x10^9/L. These included four cases (6%) that had counts above the upper limit of normal of 10 x10^9/L. (Figure 8 & Table 2)

Figure 8: Baseline WBC



Categories of WBC
Low- <4 x10^9/L
Normal 4-10 x10^9/L
High- >10 x10^9/L

NHL

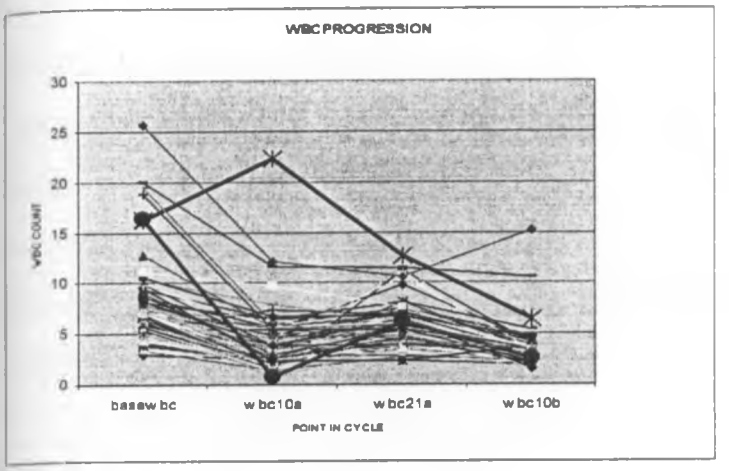
The mean baseline total WBC for this group was 8.5±5.9x10^9/L with a median of 6.3 x10^9/L. The range was from 3.04 to 25.6 x10^9/L with an IQR of 5.58 to 7.65 x10^9/L.

Twenty six cases (90%) had WBC counts above the lower limit of normal (4 x10^9/L). Included in this group were seven cases (24%) that had counts above 10 x10^9/L. (Figure 8 & Table 2)

The highest white cell count stood at 25.6 x10^9/l and the lowest was 3 x10^9/l with a mean of 7.2 ± 3.7 x10^9/l at baseline overall. (Table 2)

During the course of the study, the trend of WBC progression for most of the cases demonstrated a drop 10 to 14 days after administration of therapy, a rise 21 days after and a further drop on day 10 to 14 after the second chemotherapy dose. (Figure 9)

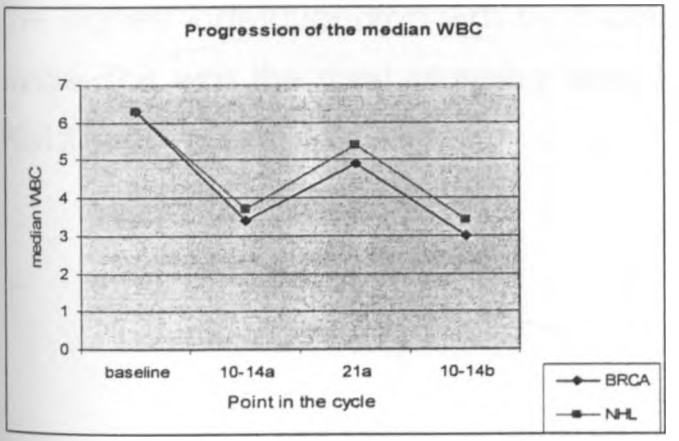
Figure 9: Individual WBC Trends in both cycles



Base_wbc= baseline WBC
Wbc_10a-= WBC Day 10-14 cycle I
Wbc_21 = WBC Day 21
Wbc_10b = WBC Day 10-14 cycle II

Progression of the median WBC in both treatment groups, highlighted the fact that majority of the cases followed this trend in both groups. However, the P value for the trend could not be determined because only two points in each phase of the cycle were available as per the study design. (Figure 10)

Figure 10: Trend in the median WBC



Note; see key on fig 9

7.4.2. First cycle decline

Breast cancer

After administering first cycle chemotherapy, the total WBC dropped to a mean of 3.69±1.79 x10^9/L on day 10 to 14 of the first cycle. The range was 0.7 to 10.9 x10^9/L with an IQR of 2.43 to 4.83 x10^9/L. (Table 2)

From the baseline median of 6.3 x10^9/L the median dropped to 3.4 x10^9/L. This was the highest drop for this treatment group with a mean drop of 3.0±2.0 x10^9/L. (Table 2 and 3). The median WBC on day 10 to 14 of the first cycle dropped from the baseline by 45%.

The highest individual WBC drop in this treatment group was by a count of 13.4 x10^9/L. (Table 3)

NHL

The mean WBC on day 10 to 14 of the first cycle for the NHL group was 4.7±4.3 x10^9/L. The range was from 0.7 to 22.3 x10^9/L, the IQR was 2.5 to 5.47 x10^9/L with a median of 3.7 x10^9/L. From a median baseline WBC of 6.3 x10^9/L, the median dropped to 3.7 x10^9/L. This was the highest drop of the 2 cycles with a mean drop of 3.9±4.4 x10^9/L. This represented a 40% drop in the median WBC on day 10 to 14 from baseline. (Table 2 & 3)

The highest individual drop was by a count of 15.6 x10^9/L and it occurred in this cycle. This was the most alarming drop from a baseline of 16.4 to 0.8 x10^9/L, a 95.1% drop. (Table 3)

Table 2: **Peripheral Blood Cell Profiles**

		BREAST CANCER				NON-HODGKIN'S LYMPHOMA			
		mean	Median (%Δ)	Min	max	Mean	Median (%Δ)	min	max
HB g/dL	Baseline	13.145±1.324	13.2	9.5	16.6	12.364±1.86	12.0	9.76	16.6
	10-14a	12.396±1.504	12.5	8.8	16.6	11.707±1.903	11.5	8	15.3
	21a	12.551±1.819	12.6	12.6	15.4	12.079±2.105	12.3	5.3	15.3
	10-14b	12.296±1.350	12.2	9.2	15.3	12.155±1.927	11.9	7.8	17.2
WBC x10⁹/L	Baseline	6.7±2.428	6.3	3.6	19.5	8.546±5.586	6.3	3.04	25.6
	10-14a	3.696±1.795	3.4 (45%)	1.4	10.9	4.691±4.338	3.7 (40%)	0.7	22.3
	21a	5.504±2.070	4.9 (30%)	2.26	11.6	5.98±2.422	5.4 (30%)	2.67	12.6
		From base	4.9 (20%)				5.4 (14%)		
	10-14b	3.049±1.186	3 (40%)	1.23	10.6	3.861±2.808	3.4 (40%)	1.29	15.3
ANC x10⁹/L	Baseline	3.754±1.867	3.4	1.38	13.0	4.808±3.643	3.6	1.7	15.4
	10-14a	1.305±0.739	1.2 (65%)	0.62	4	1.453±1.777	1.1 (70%)	0.06	9.8
	21a	3.191±1.920	2.8 (60%)	0.69	9.72	2.827±1.348	2.5 (60%)	0.83	6.7
		From base	2.8 (20%)				2.5 (30%)		
	10-14b	1.094±0.541	1.0 (60%)	0.26	4.6	1.344±1.317	1.0 (60%)	0.32	7.8
RBC x10⁹/L	Baseline	4.704±0.469	4.76	3.36	5.68	4.396±0.892	4.33	2.46	7.04
	10-14a	4.499±0.525	4.44	3.2	5.69	4.244±0.684	4.04	3.04	5.49
	21a	4.575±0.516	4.59	3.52	5.69	4.470±0.710	4.36	3.07	6.33
	10-14b	4.427±0.476	4.410	3.16	5.590	4.404±0.880	4.39	2.56	7.53
PLT x10⁹/L	Baseline	329±89	328	109	512	302±142	289	58	733
	10-14a	354±126	335	112	816	356±126	355	61	601
	21a	362±134	350	139	897	344±145	310	100	693
	10-14b	307±118	306	85	768	317±126	296	115	779

NB: For the WBC and ANC the percentage changes in the median from the previous point are indicated next to the median value hence the label (%Δ). The figures outside the brackets represent absolute value

7.4.3. Recovery

Breast cancer

By the third week, the median WBC increased to 4.9 $x10^9$/L. The mean WBC on day 21 was 5.5±2.1 $x10^9$/L with a range of 2.26 to 11.6 $x10^9$/L and an IQR of 4.25 to 6.29 $x10^9$/L at this point.

From a median WBC of 3.7 $x10^9$/L on day 10 to 14 of the first cycle, the median WBC rose to 4.9 $x10^9$/L by day 21. The mean increase was 1.8±2.3 $x10^9$/L. This translated into 30% restitution in the median WBC by the time of the next cycle. However, from the median baseline WBC of 6.3 $x10^9$/L, a WBC of 4.9 $x10^9$/L by day 21 represented a 20% drop in the median from the baseline. (Table 2 and 3)

The highest individual recovery by day 21was with a count of 9.6 $x10^9$/L. (Table 3)

NHL

The mean WBC on day 21 in this group was 6.0±2.4 $x10^9$/L. The median was 5.4 $x10^9$/L with a range of 2.67 to 12.6 $x10^9$/L and an IQR of 4.37 to 7.08 $x10^9$/L.

From a median WBC of 3.7 $x10^9$/L by day 10 to 14 of the first cycle, the median WBC rose to 5.4 $x10^9$/L on day 21. The mean increase was 1.29±2.79 $x10^9$/L. This represented 30% restitution in the median WBC in this group, before the next chemotherapy cycle. On the other hand, this represented a 14% drop in the median from the baseline WBC count. (Table 2 & 3)

The highest individual increase in WBC by day 21 was by a count of 5.4 $x10^9$/L. (Table 3)

7.4.4. Second cycle decline

Breast cancer

After the second cycle chemotherapy was given, the WBC again dropped to a mean of 3.1±1.2 $x10^9$/L with a range of 1.23 to 10.6 $x10^9$/L, and an IQR of 2.39 to 3.58 $x10^9$/L on day 10 to 14 of the second cycle. The median reduced to 3 $x10^9$/L with a mean reduction of 2.5±2.1 $x10^9$/L. This represented a drop of about 40% from

the median WBC on day 21 of 4.9 x10^9/L, which was essentially the day one of the 2nd cycle.

The highest individual decrease by day 10 to 14 of the second cycle was by a count of 9.9 x10^9/L.

Table 3: Absolute Changes in the Peripheral Blood Parameters in both cycles of Therapy

		BREAST CANCER				NON-HODGKIN'S LYMPHOMA			
	Days	mean	Median (%Δ)	min	max	mean	Median (%Δ)	min	max
HB g/dL	1-14	-0.749±1.334	-0.6	-4.4	4.9	-0.658±1.471	-0.4	-4.3	2.0
	14-21	0.155±1.377	0.1	-9	3.1	0.372±1.811	0.2	-5.6	4.6
	21-31	-0.254±1.682	-0.3	-3.7	10.2	0.076±1.320	0	-2.2	4.8
	Base-21	0.296± 0.830	0.1	-1.8	3.1	-0.285±2.379	0.2	-8.9	2.9
WBC	Base-10a	-3.024±1.986	-2.7	-13.4	2	-3.855±4.395	-3.1	-15.6	6.1
x10^9/L	10a-21a	1.808±2.255	1.4	9.6	-2	1.289±2.793	1.6	-5.4	9
	21a-10b	-2.455± 2.06	-2.2	-9.9	1.4	-2.119±2.013	-2.3	-6.2	4.6
	Base-21a	-1.216± 2.86	-1.5	-15.0	6.7	-2.566±4.313	-1.4	-0.5	2.1
ANC	Base-10a	-2.44 ±1.506	-2.1	-9	-0.09	-3.355±3.063	-2.3	-11.7	-0.4
x10^9/L	10a-21a	1.88±1.896	1.5	8.32	-1.4	1.374±1.972	1.4	6.0	-5.2
	21a-10b	-2.097± 1.83	-1.7	-8.28	1.25	-1.482±1.563	-1.5	-5.7	3.2
	Base-21a	-0.563± 2.33	-0.8	-10.4	6.73	-1.981±3.645	-1.1	-10.8	3.7
RBC	1-14	-0.205±0.508	-0.230	-1.6	1.14	-0.152±0.571	-0.03	-1.6	0.7
X10^6/L	14-21	0.076 ±0.306	0.03	-0.64	0.99	0.226±0.550	0.11	-0.6	1.5
	21-31	-0.148± 0.44	-0.140	-1.61	1.360	0.066±0.447	-0.04	-1.1	1.2
	Base-21	-0.129 ±0.49	-0.120	-1.28	1.150	0.074±0.794	0.17	-1.9	1.7
PLT	1-14	25± 144	35	-321	513	55±189	69	-500	447
x10^9/L	14-21	9± 94	3	-212	244	-12±112	-6	-298	260
	21-31	-56± 131	-14	-441	191	-28±156	-13	-474	370
	Base-21	33 ±134.	20	-228	594	43±205	25	-564	422

-ve denotes a decrease

Figures in brackets represent percentage change.

NHL

In this group, the mean WBC on day 10 to 14 of the second cycle was 3.9±2.8 $x10^9$/L with a median of 3.4 $x10^9$/L. The range was 1.29 to 15.3 $x10^9$/L and the IQR was 2.5 to 3.9 $x10^9$/L. This had reduced from a median WBC on day 21 of 5.4 $x10^9$/L. The mean decrease was 2.1±2.0 $x10^9$/L. This represented an approximately 40% drop in the median at this point. (Table 2 and 3) The highest individual decrease in WBC on day 10 to 14 of the second cycle was by a count of 6.2 $x10^9$/L in this treatment group. (Table 3)

7.4.5. Nadirs

Breast cancer

The lowest median WBC on day 10 to 14 after chemotherapy (nadir) for breast cancer was 3 $x10^9$/L and for NHL was 3.4 $x10^9$/L. Both of these occurred in the second cycle and the ranges were 1.23 to 10.6 $x10^9$/L with a mean of 3.1±1.2 $x10^9$/L and. 1.29 to 15.3 $x10^9$/L with a mean of 3.9±2.8 $x10^9$/L for breast cancer and NHL groups respectively.

The lowest individual nadirs were 0.7 $x10^9$/L (700cell/ml) for NHL and 1.23 $x10^9$/L (1230cells/ml) for breast cancer and which occurred in the first and second cycles respectively.

The corresponding median nadir for first cycle was 3.4 $x10^9$/L and the corresponding individual nadir was 1.4 $x10^9$/L in the same cycle for breast cancer. (Table 2, 3 and 4)

The corresponding median nadir for NHL in the first cycle was 3.7 $x10^9$/L and the corresponding lowest individual nadir was 1.29 $x10^9$/L in the second cycle.

7.5. Peripheral Absolute Neutrophil Counts-(ANC) profile

7.5.1 Baseline

Breast Cancer

The mean baseline ANC was 3.8± 1.9 x10^9/L with a median of 3.4 x109/L and a range of 1.38 to 13.0 x10^9/L. The IQR for the baseline ANC was a 2.6 to 4.1 x10^9/L

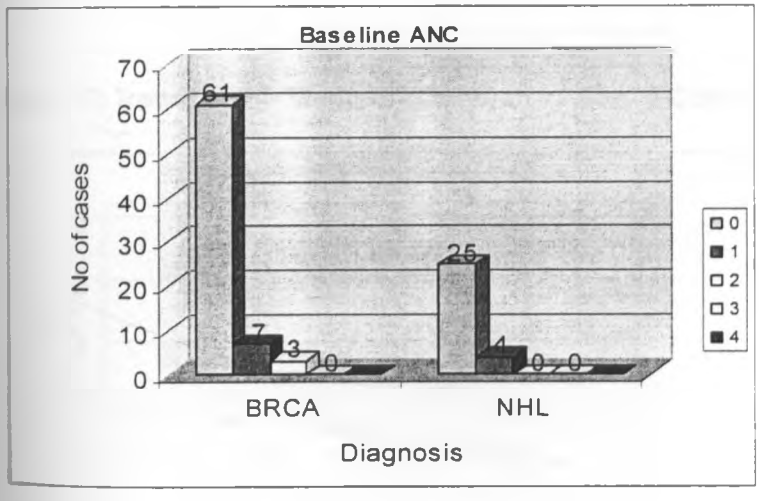
Sixty one cases (86%) in this group had counts above the normal cut off of 2x10^9/L. (i.e. grade 0 neutropenia (Appendix 1). The remaining 10 cases (14%) had grade 1 neutropenia at baseline (Figure 11 and Table 2).

NHL

The mean baseline ANC for this group was 4.8±3.6 x10^9/L with a median of 3.6 x10^9/L. The range was from 1.7 to 15.4 x10^9/L. The IQR for this treatment group was 2.4 to 4.9 x10^9/L.

Twenty five (86%) NHL cases, had grade 0 neutropenia. The remaining four cases (14%), had grade 1 neutropenia at baseline. (Table 2 and Fig 11)

Figure 11 Baseline ANC



Key

Grades of neutropenia

Grade 0_ >2x10^9/l

Grade1_1.5-2x10^9/l

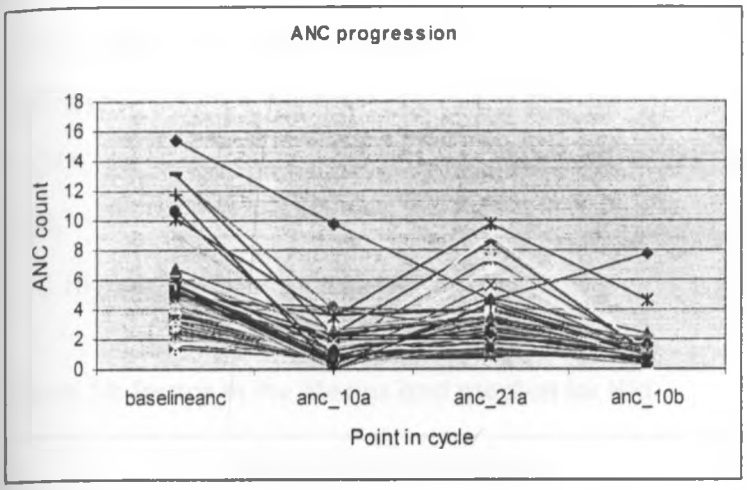
Grade2_1-1.5x10^9/l

Grade3_0.5-1x10^9/

Grade 4_<0.5 x10^9/l

The general trend of ANC progression for each individual case demonstrated a drop 10-14 days after administration of therapy a rise 21days after and another drop on day 10-14 after the second chemotherapy course for most of the cases.(Figure 12)

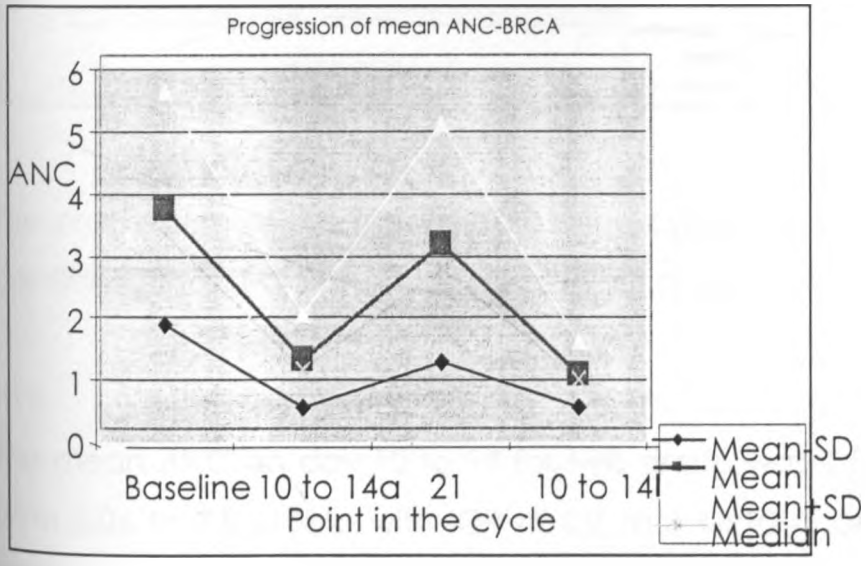
Figure 12: Individual ANC Trends



NB: see key in fig 9

The progression of the means and their lower and upper limits across the cycles for the breast cancer group we supports the fact that this was the trend for majority of the cases. (Figure 13)

Figure 13: Trends in the Mean and median for Breast Cancer



7.5.2. First cycle decline

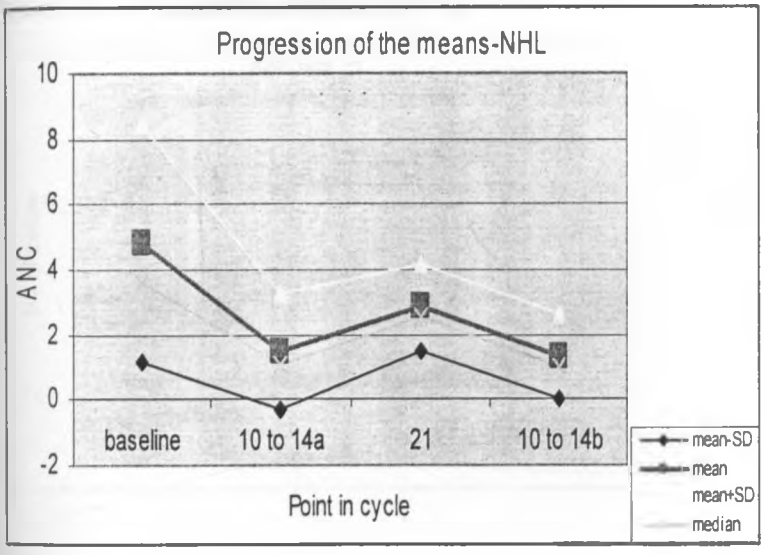
Breast cancer

After administering chemotherapy, the ANC dropped to a mean of 1.3±0.7 x10^9/L with a median of 1.2 x10^9/L by day 10 to 14 of the first cycle. The range was 0.062-4.0 x10^9/L with an IQR of 0.86 to 1.43 x10^9/L.

From the baseline median of 3.4 x10^9/L the median drop to 1.2 x10^9/L. This represented the highest drop for this treatment group with a mean drop of 2.4±1.5 x10^9/L. (Table 2 & 3). The percentage drop in the median ANC from the baseline was 60%.

The highest individual drop in this cycle was by a count of 9.0 x10^9/L.

Figure 14: Trends in the Means and median for NHL



The progression of the means across the cycles for the NHL treatment group also confirmed that majority of the cases in this group followed the same trend. (Figure 14)

NHL

The mean ANC on day 10 to 14 for NHL group was 1.5±1.8 x10^9/L. The range was from 0.06 to 9.8 x10^9/L, with IQR of 0.7 to 1.4 x10^9/L and a median of 1.1 x10^9/L. From a median baseline ANC of 3.6 x10^9/L, the median ANC dropped to 1.1 x10^9/L. This was the highest drop of the 2 cycles with a mean drop of 3.4±3.0 x10^9/L and

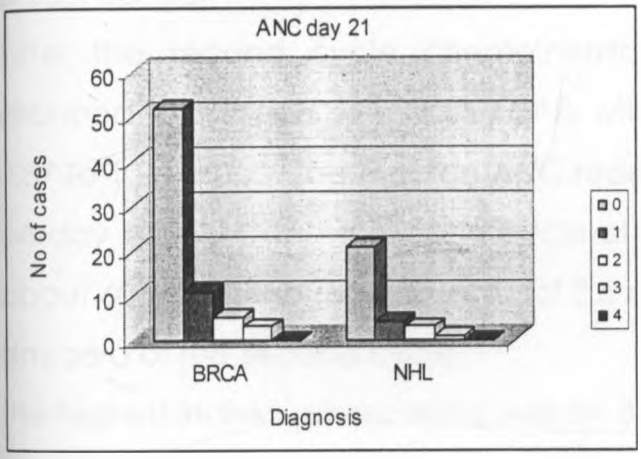
this represented a 70% drop in the median ANC from baseline by day 10 to 14 in this group. (Table 2 and 3)

The highest and most alarming individual drop was by a count of 11.7 $x10^9$/L a 99.5% drop from 11.8 $x10^9$/L at baseline to 0.06 $x10^9$/L and it occurred in the same cycle. (Table 3)

7.5.3 Recovery

For both groups, by day 21 (recovery phase) 52 case (73%) of breast cancer and 21 cases (72%) of NHL that had returned to grade 0 neutropenia. The distribution of cases by grades of neutropenia was also similar to the distribution at baseline. (Figure 11 and 15)

Figure 15: ANC Distribution by day 21



Breast cancer

By the third week the median ANC rose to 2.8$x10^9$/L. The mean ANC on day 21 was 3.2±1.9 $x10^9$/L with a range of 0.69 to 9.7 $x10^9$/L, and an IQR of 2 to 3.6 $x10^9$/L.

From a median ANC on day 10 to 14 of first cycle of 1.2 $x10^9$/L, the median ANC on day 21 rose to 2.8 $x10^9$/L. The mean restitution was 1.8±1.9 $x10^9$/L, which translated into 60% restitution in the median ANC by the time of the next cycle. However, from the median baseline ANC of 3.8 $x10^9$/L, the ANC on day 21 of 2.8 $x10^9$/L represented a 20% drop in the median from the baseline. (Table 2)

The highest individual restitution was by a count of 8.32 $x10^9$/L. (Table 3)

NHL

The mean ANC on day 21 in this group was 2.8±1.3 $x10^9$/L. The median was 2.5 $x10^9$/L with a range of 0.83 to 6.7 $x10^9$/L and IQR of 2.0 to 3.5 $x10^9$/L.

From a median ANC on day 10 to 14 of 1.1 $x10^9$/L the median ANC rose to 2.5$x10^9$/L. The mean recovery was 1.4±2.0 $x10^9$/L, which represented 55% restitution in the median ANC in this group by the next chemotherapy cycle. On the other hand, this represented a 30% drop from the baseline median ANC. (Table 2)

The highest individual restitution was by a count of 6.0 $x10^9$/L. (Table 3)

7.5.4 Second cycle decline

Breast cancer

After the second cycle chemotherapy was given, the median ANC again dropped to a mean of 1.1±0.5 $x10^9$/L with a range of 0.26 to 4.6 $x10^9$/L and IQR of 0.87 to 1.26 $x10^9$/L. The median ANC reduced to 1 $x10^9$/L with a mean drop in ANC on day 10 to 14 of the second cycle of 2.1±1.8 $x10^9$/L. This represented a drop of about 60% from the median ANC of 2.5 $x10^9$/L on day 21, which was essentially the day zero of the second cycle.

The highest individual decrease was by 8.3 $x10^9$/L in the second cycle.

NHL

In this group the mean ANC on day 10 to 14 of the second cycle was 1.3±1.3 $x10^9$/L with a range of 0.32-7.8 $x10^9$/L and an IQR of 0.9 to 1.29 $x10^9$/L. The median had reduced from 2.5 $x10^9$/L on day 21 to 1 $x10^9$/L with a mean drop in ANC on day 10 to 14 of cycle two of 1.48±1.56$x10^9$/L. This represented an approximately 60% drop in the median ANC. (Table 2.)

The highest individual decrease was by a count of 5.7 $x10^9$/L.

7.5.5. Nadirs

Breast cancer

The lowest median ANC for breast cancer (nadir) was 1 x10⁹/L. This occurred in the second cycle. The range was 0.26 to 4.6 x10⁹/L with an IQR of 0.87 to 1.26 x10⁹/L and a mean of 1.1±0.5-x109/L.The nadir for first cycle was higher at 1.2 x10⁹/L.

The individual case nadir was 0.062 x10⁹/L (62cells/ml), which occurred, in the first cycle. (Table 2) The individual case nadir of second cycle was higher at 0.26 x10⁹/L.

Majority of the cases on day 10 to 14 of both cycles had developed grade 3 or 4 neutropenia whereas most of the cases on day 21 had reverted back to grade 0 or 1 neutropenia. (Table 4) Only 3 (4%)of the cases developed grade IV neutropenia. 28 cases (39%) developed grade III or IV neutropenia in cycle 1 and 34 cases (48%) in cycle 2. (Table 4)

Table 4; ANC progression by grades of neutropenia

	BREAST CANCER								NON HODGKIN'S LYMPHOMA							
GRADE	Baseline		Day 10-14 cycle I		Day-21		Day10-14 cycle II		Baseline		Day10-14 cycle I		Day-21		Day10-14 cycle II	
	n	%	n	%	n	%	n	%	n	%	N	%	n	%	N	%
0	61	86	10	14	52	73	2	3	25	86	3	10	21	72	3	10
1	7	10	7	10	11	16	5	7	4	14	3	10	4	14	2	7
2	3	4	26	37	5	7	30	42	0	0	12	42	3	10	10	35
3	0	0	25	35	3	4	31	44	0	0	5	17	1	4	13	45
4	0	0	3	4	0	0	3	4	0	0	6	21	0	0	1	3

NHL

The lowest (nadir) median ANC in this treatment group was 1 x10⁹/L, which occurred, in the 2nd cycle. The mean nadir was 1.3±1.3 x10⁹/L with a range of 0.32 to 7.8 x10⁹/L and an IQR of 0.96 to 1.29 x10⁹/L. The individual case nadir was 0.06 x10⁹/L (60 cells /ml), which also occurred, in the first cycle. (Table 2) The individual

case nadir of second cycle was higher at 0.32 x10⁹/L. In the first cycle, six cases (21%) developed grade IV neutropenia and only 1 case (3%) in second cycle. However 11 cases (38%) in first cycle and 14 cases (48%) in second cycle developed grade III and IV neutropenia respectively. (See Table 4)

7.5.6 Summary

There was a 40% drop in total WBC counts from the baseline following chemotherapy in the NHL treatment group and 45% in the breast cancer treatment group by day ten to fourteen of the first cycle. At recovery there was a rise by 30%, from the day ten to fourteen counts in both groups, followed by another 40% drop from the counts on day 21 after the second cycle was given. The IQR showed that majority of the cases had counts below 4 x10⁹/L on day ten to fourteen of both cycles and above 4 x10⁹/L on day 21. (Table 5)

Table 5: Summary of Median Changes, Percentage Changes, and Nadirs

	BREAST CANCER						NON_HODGKIN'S LYMPHOMA					
Day	Median x10⁹/L	IQR x10⁹/L	Max Δ x10⁹/L	%Δ	Nadirs x10⁹/L		Median x10⁹/L	IQR x10⁹/L	Max Δ x10⁹/L	%Δ	Nadirsx10⁹/L	
					group	case					group	case
WBC												
10-14a	3.4	2.43-4.38	↓ 3.4	↓ 45%	34	1.4	3.7	2.5-5.47	↓15.6	↓40	3.7	0.7
21	4.9	4.25-6.29	↑ 9.6	↑ 30%			5.4	4.37-7.1	↑5.4	↑30		
10-14b	3.0	2.39-3.58	↓ 9.9	↓ 40%	3	1.23	3.4	2.5-3.9	↓ 9.9	↓40	3.4	1.29
ANC												
10-14a	1.2	0.86-1.43	↓ 9	↓60%	1.2	0.062	1.1	0.7-1.4	↓1.7	↓70	1.1	0.06
21	2.8	2.0-3.6	↑8.32	↑60%			2.5	2.0-3.5	↑ 6	↑55		
10-14b	1	0.87-1.26	↓8.3	↓65%	1	0.26	1	0.9-1.29	↓5.7	↓60	1	0.32

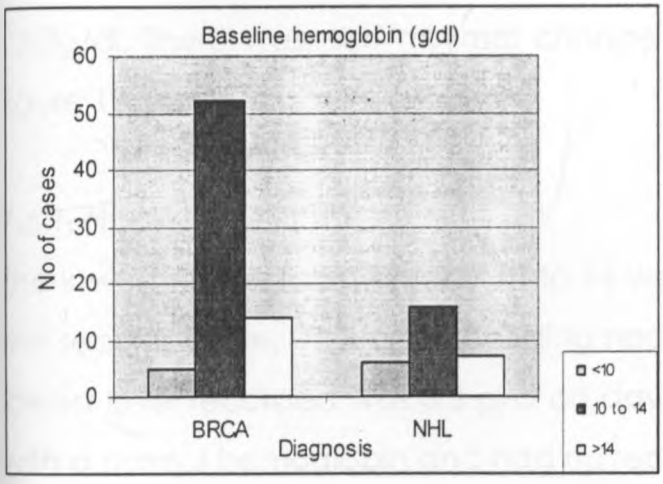
The ANC followed a similar trend with a 60% reduction for breast cancer treatment group and 70% drop for NHL from the baseline ANC count in cycle one, followed by 60% and 55% restitution respectively on day 21 and another 60% drop from the count on day 21 in both groups by day ten to fourteen of second cycle. Similarly

the IQR showed that majority of the cases had grade 2 and above neutropenia on day ten to fourteen of both cycles and grade 0 or 1 neutropenia on day 21.
The median WBC nadir was lower in second cycle for both groups. The individual case nadir was lower in the first cycle for NHL and second cycle for breast cancer treatment group. For the ANC, the lowest median nadir was in cycle two in both groups and the individual case nadir was lowest in cycle one. (Table 5)

7.6 Hemoglobin (Hb) Profile

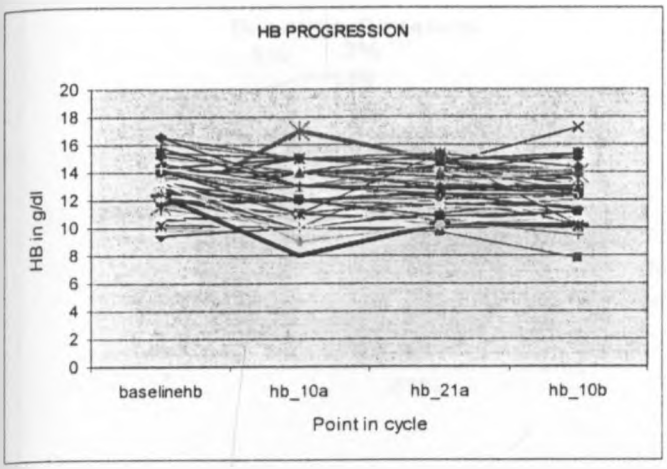
Only 11% of the cases had hemoglobin levels below 10g/dl at baseline. However, all these cases had levels above 9g/dl and were transfused before chemotherapy but no repeat blood counts were done till at day 10-14.

Figure 16: Baseline Hemoglobin



The mean hemoglobin at baseline was 13.2±1.3g/dl with a median of 12.6g/dl for breast cancer.

Figure 17: Hemoglobin Trends



There was minimal change in the course of cycles as depicted in figure 15. See table 2 For NHL, the mean hemoglobin at baseline was 12.4±1.9g/dl with a median of 12.3g/dl. There was also minimal change in the course of cycles as depicted in figure 17. (Table 2)

7.6.1. Nadir

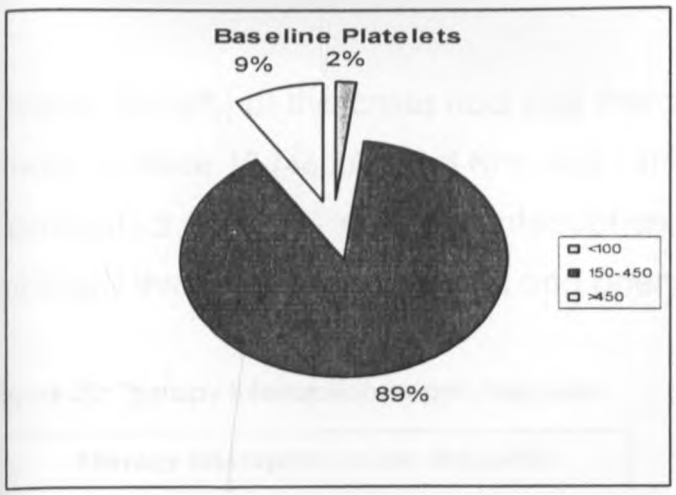
The lowest hemoglobin on day 10 to 14 was 7.8 g/dl in NHL group that occurred in the second cycle. The corresponding nadir for first cycle was 8 g/dl. However, the lowest level recorded was 5.3 g/dl on day 21 in a case with NHL, who had started with a normal hemoglobin and had no reported incidence of bleeding.

For breast cancer group the lowest on day 10 to 14 was 8.8 g/dl in cycle 1 and the corresponding hemoglobin for second cycle was 9.2 g/dl.

7.7 Platelet Progression

At baseline only 2 percent of the cases had platelets below 100 $x10^9/l$, 9 percent had counts above 450 $x10^9/l$ and 89 percent had counts within normal range of 150-450 $x10^9/l$. (Figure 18)

Figure 18: Baseline Platelets

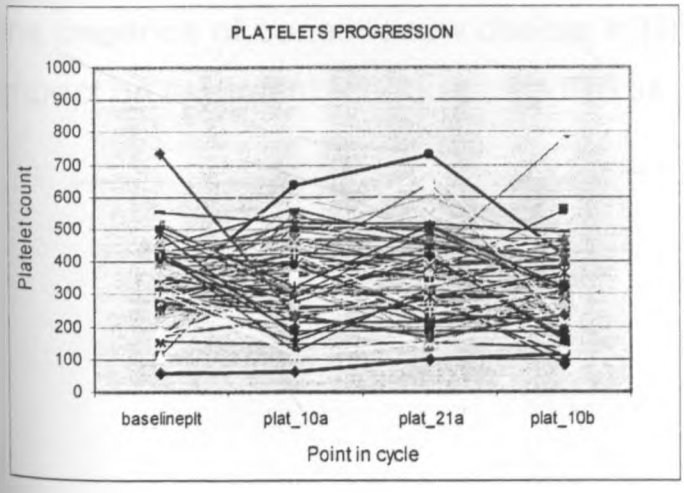


At baseline the lowest platelet count was 109 x10^9/l and the highest 512 x10^9/l. The lowest count on day 10-14 of cycle one was 112 x10^9/l in a breast cancer case and for cycle two was 85 x10^9/l.

After chemotherapy there was no predictable pattern of change in the platelet count. However some patients had platelet increases of up to 600 to 700 x10^9/l. (Figure 19)

For the NHL group, the lowest platelet count was 58 and the highest 733 at baseline. The nadir for day 10 to 14 of first cycle was 61 x10^9/l and 115 x10^9/l for day 10 to 14 of second cycle

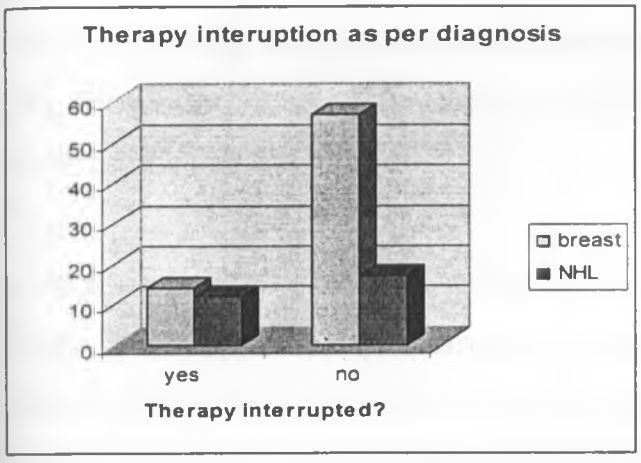
Figure 19: Platelet Trends



8.0 INTERRUPTION OF THERAPY

Overall, 26(26%) of the cases had their therapy interrupted during the course of the study, of these 12 (46.2%) had NHL and 14(53.8%) had breast cancer. Neutropenia contributed to 61.5% of these interruptions. (Fig 20) Other reasons for interruption included thrombocytopenia 7.7% and anemia 30.8%.

Figure 20: Therapy interruption as per diagnosis



Among the 10 cases (35%) with NHL who had HIV disease, five (50%) had their therapy interrupted due to neutropenia. However the presence of HIV infection did not significantly impact on treatment schedule interruption. p=0.56

The presence of bone marrow disease in (8)27.6% of NHL cases did not significantly impact on treatment schedule also. P=0.56

9.0 DISCUSSION

During the ten-month study period, 96 cases with high risk and /or metastatic breast disease were screened. This can be extrapolated to, roughly 10 cases of high risk and metastatic disease per month. This suggests that breast cancer is fairly common in our set up. Breast cancer is a common malignancy, ranking second to cervical cancer in women as seen at KNH (57).

The median age for breast cancer cases was 46 years and the peak age of occurrence was 45 to 54 years. This figure correlates with the median age of 41 years found in a study of nadir neutrophils counts in breast cancer treated with AC (58). However, this is 10 years younger than the peak age of occurrence in Caucasians (60, 61).

At diagnosis, 80% of breast cancer cases had Stage III and IV and 75% were in either in ECOG 2 or 3 performance score. All these seem to support the distinct peculiarities about breast cancer in Africa, which has been reported to occur predominantly in pre-menopausal women, is diagnosed in stage III and IV and is of higher histological grade than that reported in females with the disease in developed countries (60-63).

In the same study period, 34 cases of highly aggressive, aggressive and indolent stage 2 and 3 NHL were screened. This figure is about one and half times the number extrapolated from a retrospective study by Othieno Abinya and colleagues on NHL between 1990 and 2000 at KNH. In the study, they found 207 case records of NHL in ten years that can be extrapolated to about 20 cases per year (64).

As a whole, NHL affects more males than females. In this study, the M: F ratio was 1.4 to 1. This compares with what is established. However, this figure is about half of what was reflected in earlier studies (65, 66). In those studies, a M: F ratio of 2.5:1 was reported which compared well with a ratio of 2.3 to 1 found earlier by Kasili and Bowry in 1977 on a study on patterns and pathology of malignant lymphomas

(66). This difference could be attributed to the smaller number of cases with NHL in this study compared to those the earlier studies (64- 66).

The median age was 36 years and NHL cases were distributed right across the age groups. The documented peak age is 55 -65 years. However, specific histological sub types tend to cluster around certain age groups and especially the aggressive and highly aggressive subtypes cluster in the younger population.

With the more than 20 different subtypes grouped broadly into the three subgroups due to lack of access to immunohistochemical testing facilities, the age scatter and mean age below 40 years is expected. This was similarly reflected in earlier quoted study in which 52.9% of the cases were below 40 years and only 18% of the cases were above 60 years (89). Kasili and Bowry (66) had also reported a mean age of 41 for NHL in 1977.

Both the total WBC counts and neutrophil counts dropped significantly following chemotherapy in both cycles. Our discussion however will focus on neutrophils which naturally are used to monitor the toxicity of chemotherapy and the readiness of a patient for the next course of treatment.
In this study, neutrophil counts were shown to be markedly reduced on day 10- 14 (by 60-65%) when we expected maximum effect of the topoisomerase II inhibitor doxorubicin and alkylating agent cyclophosphamide on the bone marrow. By day 21, more than 70% of the cases had restored their counts to normal levels. However, on the whole the counts did not return to original baseline levels dropping by 20% from their baseline levels as reflected by the change in median counts between the baseline and day 21. Although this is the expected trend that has been shown in some other studies (8, 58, 67), this study is unique in that the average percentage changes were determined and group nadirs in addition to individual case nadirs were determined. Individual case drops were variable and ranged from 6.4% to 99.5%. The mean changes in the groups had a wide variance therefore the median counts were used to describe the group data.

There appear to be no historical reports on percentage median changes locally or internationally. This finding, compared to single point data and individual case nadir only, is probably more useful in projecting and planning treatment especially in relation to prophylactic hematopoietic growth factors.

In a study of nadir neutrophil counts in 18 cases with breast cancer grade III or IV neutropenia developed in 17 out of 35 (48.6%) treatments on AC 50/500 by the fifth cycle of treatment (58). In this study, out of 142 treatments in the breast cancer group on AC-50/500, grade III and IV neutropenia developed in 62 treatments (43.7%) by the second cycle. (Table 4)

Despite the higher number of courses in that earlier study, these figures are relatively comparable at this point. However it is important to highlight that in the earlier study no pattern in severity of neutropenia was reported for the five courses of treatment, neither was there a report of the same in the NASBP-B22 and B25 trials (58, 67). This differs from this study in that there seem to emerge a pattern. In this study we noted that there was an increase by 10% in the number of cases with grade III or IV neutropenia in the second cycle. We also noted that the percentage change in the median ANC remained constant in both cycles. There was also a 20% decrease in the median ANC from the baseline value on day 21. All these three points suggest that, subsequent courses would be starting with progressively lower absolute neutrophil counts and hence would have lower nadirs. Therefore by the fourth or fifth course, grade III or IV neutropenia, would develop in a higher percentage of treatments than quoted in preceding studies (58, 67). However a longer follow up would have helped us clarify the issue.

In the real life usual practice scenario our oncologists seemed to prefer starting our patients on the lower dose AC-50/500. Subsequently though our dosages were lower, grade IV neutropenia occurred in 4.2%. (6 of 142 treatments) In the NASBP-B22 trial (67), grade IV neutropenia occurred in 6.5% of treatments at 60/600 Taking into account that the average number of treatment courses were four for the NASBP-B22 trial and two for our trial and our doses were lower, then for the same number of courses our figure might have been higher. This seems to be supported by the figure reported in the earlier quoted local study, of 16.7% at 50/500 for an

average of 4.4 treatments (58). However in this study, there did not seem to be an increase in cases with grade IV neutropenia in cycle two.

This seems to suggest that the effect of these agents on the marrow is severer in our patients than in the NASBP B-22trial group. The possible contributing factors include poor nutritional status, poor hygiene and presence of recurrent infections and infestations (57, 58). This probably reflects also in the poorer functional scores in our patients. The other possible contributing factors are a slow process in establishing diagnosis or a more aggressive disease course supported by the late stage of disease at diagnosis (60, 62, 63,)

Hardly any studies have looked at the nadir counts in NHL. Given that nearly the same proportions were in grade III and IV neutropenia in cycle one and two in both treatment groups and the percentage median changes were similar in both treatment groups, therefore we could extend the same arguments to this group and generalize the applicability of this data to both groups.

Although, only 16% of the cases had therapy interrupted for neutropenia in cycle two, we would expect this number to gradually increase with subsequent cycles. From this study, in a period of three weeks after commencing therapy, in order to avoid interrupting their therapy 16 cases out of 100(16%) would have benefited from prophylactic hematopoietic growth factor support to maintain the relative dose intensity of doxorubicin and cyclophosphamide, without interrupting their treatment schedule.

Febrile neutropenia occurred in only two cases both of whom died. Prognostic factors that determine a patient's risk of developing febrile neutropenia or the risk of fatal outcome from neutropenia remains poorly understood (68, 69,). Therefore, the only defense we have is prevention.

Bone marrow disease did not significantly impact on therapy interruption as would be expected though the number of cases was small. Only eight cases (27.6%)

among those with NHL had bone marrow disease. Githang'a and Dave (70) reporting on evaluation of bone marrow aspirates (BMA) found that 45% of NHL cases had evidence of disease infiltration. However, this being predominantly a pediatric population, we may not directly extrapolate this to apply to the adult population. We may therefore require a similar study in adults to determine the effectiveness of BMAs in evaluating for disease infiltration with NHL. Never the less, our figure would probably have been higher if the gold standard of diagnosis, trephine biopsy had been done. However, due to logistic problems only bone marrow aspirates were done.

There was no significant change in the progression of red blood cells and hemoglobin levels during the six weeks in a broad sense. However, 30.8% of the interruptions to the treatment schedule were due to anemia.

Platelets were spuriously high during the study period. This had been observed at the same time by others at KNH even in non-study patients (Othieno Abinya- personal communication). This raises concern about existing platelets assaying methods at the institution. However, it is not clear whether this affects the counts in other non-cancer cases. To advance a theory that may explain the isolated platelet interference, we need to look into the coulter method of cell count, which depends on the size (volume of the cell) and the number of impulses generated. (See Section 6.2.1) The platelet count is derived from the number of particles with a volume of between two and twenty femtolitres in whole blood whereas, red cell are particles with a volume of thirty six femtolitres and above. Microcytic red cells and other particles, causes interference in platelet counts in the upper limits. Since both malignancies and chemotherapy are associated with increased red cell fragility and a risk of intravascular hemolysis may be, our coulter counters also include the particles so created in the platelet counts giving us spuriously high counts.

10.0 CONCLUSION

Severe neutropenia complicates the use doxorubicin and cyclophosphamide in our set up. Grade 3 or 4 neutropenia occurred in 43.7% of 200 treatments by the second cycle, and 62% of the 26 cases whose therapy was interrupted in the second cycle were due to neutropenia. This figure is higher than those quoted from other studies considering this study was up to second cycle. Though there is not much historical data in this regard, local data and data from the NASBP trials suggest that our patients seemed to suffer a severer bone marrow toxicity effects.

Hemoglobin levels did not show a significant change during the cycle although 30.8% of those whose treatment schedule was interrupted required blood transfusion.

Platelet counts have been reported to also drop with progressive courses. However, in this study there did not emerge any pattern of progression. On the contrary the platelets were spuriously high with no predictable pattern and with no explanation.

Febrile neutropenia did not occur frequently despite the relatively high number of cases with grade III to IV neutropenia. This only occurred in 2% of the cases with 100% fatality. It is therefore still difficult to predict which neutropenic patient will develop fever and infection, but it is important to prevent death due to treatment related complications.

11.0 RECOMMENDATIONS

Strict monitoring of blood counts during cytotoxic therapy must be adhered to and steps taken to avoid severe neutropenia and to prevent infections which can prove fatal in those who develop neutropenia, using any available and affordable means.

We need to re-evaluate our current platelet assaying method to avoid misleading counts which might cost lives especially in chemotherapy treated cases. However, in cases this is not a laboratory error, a study to follow up the platelet response and its determinants in chemotherapy is called for to resolve this issue.

Several of our patients required transfusion after just one course of chemotherapy, and their treatment schedule was delayed. We need to be more aggressive in our blood donor drives to enhance availability of blood and blood products in our blood banks, or to seek alternative ways of boosting our patient's hemoglobin levels e.g. erythropoietin use.

11.1 Study logistic constraints

Bone marrow aspirates were done instead of the recommended trephine biopsies due to logistic problems. This may have led to an under estimation of the bone marrow disease incidence in the NHL group.

We were not able to get blood for transfusion in time at the study center due to logistics causing untimely treatment schedule delays.

The study took ten months instead of the proposed six months due to the breakdown of the coulter counter machine.

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13.0 APPENDICES

Appendix I A. GRADES OF NEUTROPAENIA (WHO)

Grade 0	$>2.0 \times 10^9$ /L
Grade 1	$1.5 - 2.0 \times 10^9$ /L
Grade 2	$1.0 - 1.49 \times 10^9$ /L
Grade 3	$0.5 - 0.99 \times 10^9$ /L
Grade 4	$<0.5 \times 10^9$ /L

Appendix I B. 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 is given to patients with any of the following 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 (1) 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). Note: 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 O = Bone D = Skin

Other prognostic factors – NHL

Serum LDH (Normal vs Elevated)

Performance Status (0 or 1 vs 2 – 4)

Stage (I or II vs III or IV)

Extra-nodal site involvement (0 or 1 vs 2 – 4)

Patients with 2 or more risk factors have less than a 50% chance of relapse free and overall survival at 5 years. (27, 28)

Appendix III: Staging in Breast Cancer

TNM Definitions

Primary Tumor (T)

TX: Primary tumor cannot be assessed

T0: 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

T1mic: micro-invasion 0.1cm or less in greatest dimension

T1a: Tumor more than 0.1cm but not more than 0.5cm in greatest dimension.

T1b: Tumor more than 0.5cm but not more than 1.0cm in greatest dimension.

T1c: 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).

N0: 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).

pN0: 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.

pN1bii: 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.

pN1biv: 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.

M0: No distant metastasis

M1: Distant metastasis present (includes metastasis to ipsilateral supraclavicular lymph nodes).

AJCC stage groupings

Stage 0

Tis, N0, M0

Stage 1

T1, *N0, M0

*T1 includes T1mic

Stage IIA

T0, N1, M0 T1, *N1, ** M0 72, N0, M0

*T1 includes T1mic **the prognosis of patients with pN1a disease is similar to that of patients with pN0 disease.

Stage IIB

T2, N1, M0

T3, N0, M0

Stage IIIA

T0, N2, M0 T1,*N2, M0 T2, M0 T3, N1, M0 T3, N2, M0

*T1 includes T1mic

Stage IIIB

T4, Any N, M0

Any T, N3, M0

Stage IV

Any T, Any N, M1

Appendix IV: Consent forms.

Consent explanation

We are conducting a study to evaluate the effects of the treatment used to treat your condition on your blood cells. This will help us to make treatment plans that will minimize interruptions of treatment in future and improve chances of cure. We want you to understand that;

Only Standard procedures will be carried on you.

The treatment you shall receive is standard and nothing is experimental.

Other necessary treatments shall not be withheld from you due to the study.

No unnecessary expense shall be incurred on your part.

The investigator is not liable for expense incurred during 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.

Consent form

I ---------- has been explained to, the purpose and the conditions of my involvement in this study. I agree to the above and give consent to be included in the study/ I agree to the above and give consent for ---------- ---------- to be included in the study

Name _____ Witness_____

Signature _____ Signature._____

Date _____ Date _____

Appendix V: PRO – FORMA

Demographic Data

Name _____ Age ☐ Sex ☐

Residence_____ Height ☐ Weight ☐

Body Surface Area ☐ Performance Scale ☐

Diagnosis; I. Ca Breast ☐ II. NHL ☐

Stage I ☐ Stage II ☐ age III ☐ age IV ☐

Regimen I. AC ☐ II. CHOP ☐

Dosage

Cyclophosphamide	
Adriamycin	
Vincristine	
Prednisone	

Cycle of Treatment

One ☐ two ☐ three ☐

Therapy Interrupted? Yes No

1. If Yes, why

Anemia ☐ Sepsis ☐ Bleeding ☐

Other (specify) ---------

Hemograms

	Hb	Wbc	ANC	MCHC	MCV	MCH	Platelet
Baseline							
Day 10-14							
Day 21							
Day 10-14							
Day 21							

1. Hb(g/dl) (i) >14 2. WBCx10^9/l >10 3. ANC x10^9 /l >2000

(ii) 11 – 14 4 – 10 1500 - 2000

(iii) 9 – 10 <4 1000 - 1500

(iv) <9 500 – 1000

(v) <500

4. MCH, MCV, MCH Platelet 10^9 /l (I) >450

Increased (i) (II) 100 – 450

Normal (ii) (III) 20 – 100

Decreased (iii)

(IV)< 20

HIV status Positive negative

(ii) If positive is the patient on ARVs? Yes no