CYTOPENIA FOLLOWING IMATINIB TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) IN KENYA: A STUDY AT GIPAP CLINIC, NAIROBI HOSPITAL

A dissertation submitted for part fulfilment of the requirements for the degree of Fellowship Medical Oncology, University of Nairobi

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DECLARATION

I certify that this dissertation is my own original work and has not been presented for a degree elsewhere. I agree to adhere to this protocol as outlined and that the study will be conducted according to guidelines of Good Clinical Practice and local ethical and legal requirements.

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DEDICATION

I dedicate this work to my family and to the Chronic Myeloid Leukemia patients, without whom, its completion would not have been possible.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
AP	-	Accelerated Phase
ATP	-	Adenosine Triphosphate
BCR-ABL	-	Breakpoint Cluster Region – Abelson
BMA	-	Bone Marrow Aspirate
BMI	-	Body Mass Index
BP	-	Blastic Phase
CBC	-	Complete Blood Count
CHR	-	Complete Hematologic Response
CML	-	Chronic Myeloid Leukemia
CP	-	Chronic Phase
CTCAE	-	Common Terminology Criteria for Adverse Events
CyR	-	Cytogenetic Response
DNA	-	Deoxyribonucleic Acid
ERC	-	Ethics and Review Committee
GIPAP	-	Glivec International Patient Assistance Program
HSCT	-	Hematopoietic Stem Cell Transplant
IRIS	-	International Randomized Study of Interferon and STI571
LAP	-	Leucocyte Alkaline Phosphatase
LLN	-	Lower Limit of Normal
MRD	-	Minimal Residual Disease
RNA	-	Ribonucleic Acid
NCDs	-	Non – Communicable Diseases
OR	-	Odds Ratio
PBF	-	Peripheral Blood Film
PI	-	Principal Investigator
RT – PCR	-	Real Time Polymerase Chain Reaction
SEER	-	Surveillance, Epidemiology and End Results
TKI	-	Tyrosine Kinase Inhibitor
WHO	-	World Health Organization

ABSTRACT

Background: Imatinib mesylate is the preferred initial treatment for all phases of Philadelphia positive CML. During treatment, patients may develop cytopenia, namely anemia, neutropenia or thrombocytopenia either as monocytopenia, bicytopenia or pancytopenia. The cytopenia is often due to toxicity but may also arise from disease transformation or resistance to therapy.

Objective: The study aimed to determine the type, grade, time to development, and duration of the cytopenia. In addition, the risk factors associated with cytopenia including sociodemographic characteristics, clinical characteristics and baseline laboratory characteristics were studied.

Methods: This was a retrospective case-control study at the GIPAP clinic at the Nairobi Hospital. It was carried out on all adult patients aged 18 years and above seen in the GIPAP clinic from 2007-2015 on follow-up for at least 36 months. The study population were patients diagnosed with CML, who developed cytopenia within 12 months of initiating imatinib mesylate. Baseline socio – demographic data, clinical data, hematologic data and molecular data were retrieved from charts of both cases and controls and entered into a predesigned study proforma.

Analysis: For descriptive analysis, measures of central tendency were used (mean, median, mode, standard deviation and variance). For differences between groups, chi-square tests, t-tests and ANOVA were used. To identify relationships, binary logistic regressions were employed. Univariate and multivariate analyses were done to identify independent predictors of cytopenia. Odds ratios (OR) were presented including the 95% confidence intervals and respective p values.

Results: The results indicate that monocytopenia is the most common type of cytopenia at 63.6%. Anaemia was the most common type of mocytopenia at 34% whereas anaemia plus neutropenia was the most common bicytopenia at 12.7%. Pancytopenia was seen in only 5 out of the 94 patients. One half of the patients with any kind of cytopenia were at grade 2. The cytopenia developed within three months of initiating Imatinib and had resolved by 12

months since initiation of Imatinib for anaemia and thrombocytopenia, and by month 24 for neutropenia. Baseline characteristics, time duration to diagnosis of CML, spleen size, presence of B symptoms and level of BCR-ABL1 were not found to be associated with development of cytopenia. A baseline thrombocytopenia and thrombocytosis, baseline neutropenia and a baseline anaemia were associated with increased odds of having cytopenia.

Recommendations: the outcome of this study recommends that physicians should be more alert during hematologic monitoring of these patients. Our results also suggests that physicians to continue imatinib at 400 mg/day or at lower doses while supporting patients during the myelosuppression period due to the good recovery of cell counts during follow-up.

CHAPTER 1: BACKGROUND

Non-Communicable Diseases (NCDs), including cancer are an emerging health problem in sub Saharan Africa. WHO projects that cancers will precede all other causes of mortality in the world by the year 2030. WHO also estimates that in 2015 alone, 8.8 million people worldwide died from a cancer. Chronic Myeloid Leukemia (CML), a well-known myeloproliferative neoplasm has been in existence for close to two centuries.

The disease affects even children but is predominant in older people. While there is paucity of accurate data on leukemia from resource limited countries, data available from Globocan 2018 reports that the global incidence and mortality of all leukemia in 2018 is 437,033 and 309,006 respectively (1). The United States SEER database estimates an incidence of about 8430 patients with CML in 2018, with 1090 expected deaths. In addition, there are 67.6% of CML patients that were diagnosed from 2008 to 2014 and survived the disease by end of 5 years (2). Reports from UK's Hematological Malignancy Research Network is 0.9 persons per 100,000 in the population while survival at 5 years is 78.9% (3).

In Africa, a much lower prevalence was reported in Globocan 2012 with a 5-year prevalence of 10,739 for all leukemia. Only about 10678 cases of all leukemia are diagnosed in Africa annually, with a mortality of 9483. In Kenya, the 5-year prevalence of all leukemia is 3845 with an annual incidence in 2018 of 1699 and mortality of 1311 (4). The number of new cases of leukemia in the country is expected to double by the year 2035. The Globocan dataset is not segregated by type of leukemia. However, since CML constitutes 15-20% of all leukemia, it remains a significant global health burden.

In the developed world, CML affects the elderly with majority aged 64 years at diagnosis (2). In addition, fewer women than men are affected with more women surviving the disease compared to the men, who are likely to die from the disease. CML affects whites and non-Hispanics compared to blacks (2). Mortality rates from CML are higher among older adults, or those between 75 and 84 years of age. Mortality rates for CML have been on the decline on average 1.5% each year from 2005 to 2014 (2).

In Kenya, majority of patients are aged 42 years at diagnosis. The males are predominantly affected than females with a ratio of 3:2 at the GIPAP clinic. Further, within the country, there is no ethnic predilection for the disease. Survival rates in Kenya are most likely

impacted by poverty which result in lack of diagnostics and impaired access to treatment services.

CML is an acquired rather than a hereditary abnormality. The factors that initiate its development are unknown. Only ionizing radiation has been linked to its development based on the observation that it is more common in persons surviving atomic bombings. Other possible causes include cigarette smoking (5) as well as obesity or high BMI (6, 7). Conversely, other research work on smoking as a cause of CML failed to establish a link (8). A multivariable analysis of CML patients reported that being female, being highly educated and living a very physically active lifestyle reduces risk of developing CML. In the same study, smoking of too many cigarettes and having a high BMI raised the probability of getting CML. The dietary habits of the participants was not found to increase the risk of developing CML (9).

A study conducted in Kenya's CML clinic at the Nairobi Hospital on the association between occurrence of CML and patients' occupations, and also on personal and family history of cancer reported a chance of familial association with other cancers. There was no occupational association found except for three workmates who developed myeloid leukemia (10).

CHAPTER 2: LITERATURE REVIEW

2.1: INTRODUCTION

2.1.1 DEFINITION

CML is due to a clonal disorder that cause granulocyte cell line proliferation (11). It develops from a transformed pluripotent hematopoietic stem cell.

At diagnosis, all patients have the typical shortened chromosome 22 (12-13).

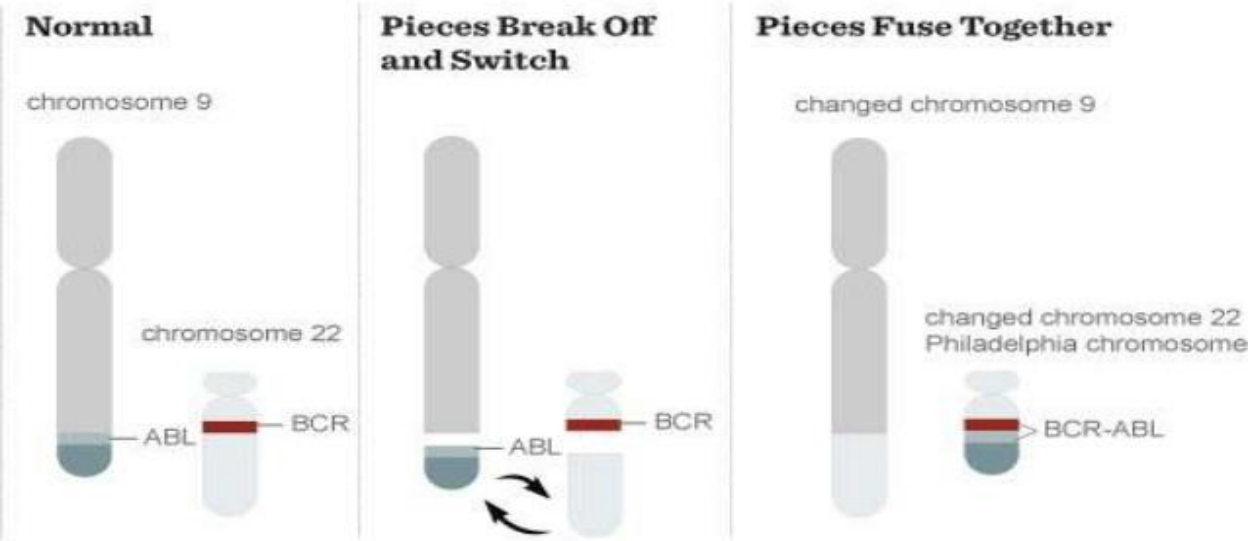
The fusion protein resulting from this translocation, BCR-ABL1, is a tyrosine kinase which acts independent of any stimulation and results in Chronic Myeloid Leukemia developing (14).

2.1.2 PATHOGENESIS OF CML

CML develops following a translocation that occurs reciprocally between two somatic chromosomes, t (9:22) as shown in figure 1 (15).

At molecular level, a gene from chromosome 22, specifically its long arm, known as BCR is fused with a gene located on chromosome 9's long arm, known as ABL1. The resulting fused gene forms a BCR–ABL1 chimeric transcript. This protein is a tyrosine kinase that functions independent from the normal signaling pathways (16, 17). Its activity leads to development of malignancy through uncontrolled proliferation of granulocytes, slowed or absent cell death and abnormal cell to cell adhesion. Unlike acute leukemia, their maturation process remains normal and thus the disease is not characterized by an increased blast cell count unless in situations where acquisition of additional mutations result in progression to advanced disease (18, 19).

Figure 1: Fusion between BCR and ABL1 on chromosome 9 and 22



The BCR-ABL fusion transcript is present in all patients diagnosed with the disease. Its absence excludes CML. However, the formation of the BCR-ABL protein does not on its own cause CML and many healthy individuals have been found to have it at low levels in the marrow. Further, the BCR points of breakpoints may occur between exons 12 to 16 resulting in major breakpoint cluster region (M-BCR) or m-BCR (minor). The points of breakage of ABL1 may also vary. They may occur between exons 1a and 1b, before exon 1b, or after exon 1a (20, 21)

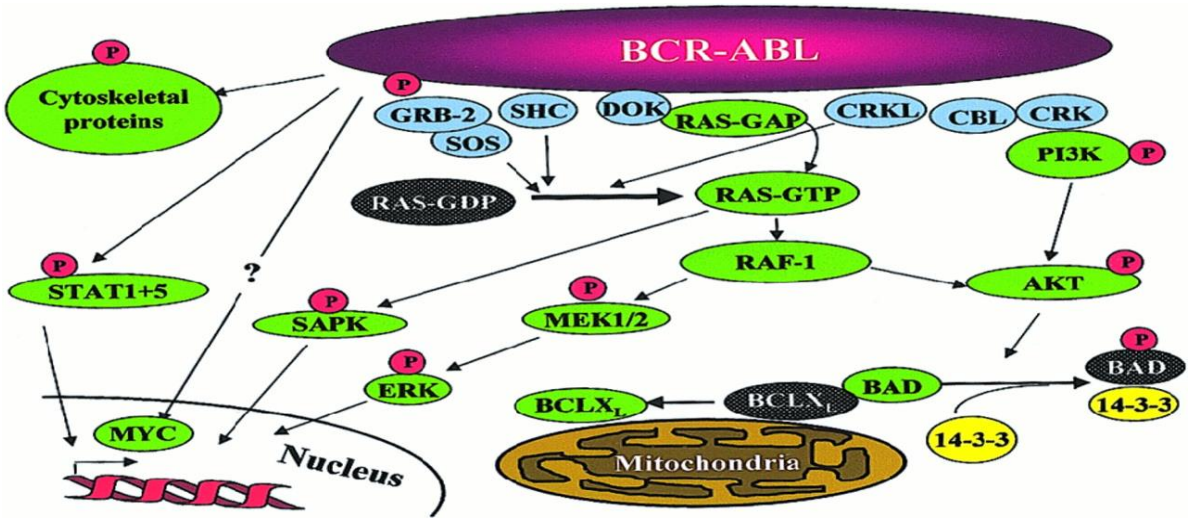
Properties of BCR-ABL Myeloid Cells

Myeloid cells containing BCR-ABL fusion protein do not adhere effectively to each other, to the surrounding matrix cells, bone marrow microenvironment cells. This promotes uncontrolled cell proliferation (22).

Secondly, BCR-ABL1 transcripts promote reduced apoptosis through different mechanisms such as by increasing bcl-2 and inhibiting Bad which are anti-apoptotic and pro-apoptotic proteins respectively. They may also inhibit caspases which are key in the apoptosis pathway by blocking cytochrome C release from the mitochondria (22).

Finally, BCR-ABL tyrosine kinase increases unregulated proliferation of myeloid cells by activating MYC, PI3K, MAP and the Ras pathways as shown in figure 2 (22).

Figure 2: Signaling pathways that promote proliferation of myeloid cells



2.1.3: DIAGNOSIS OF CML

Complete Blood Count

This test may reveal elevated total white cell blood count, elevated neutrophils, increased basophils and eosinophils, low, normal or increased platelets, anemia that is normocytic normochromic though at times maybe microcytic and low or absent staining of leukocyte alkaline phosphatase (LAP). LAP is usually reduced or absent due to decreased myeloid cell death. This increased longevity leads to loss of important enzymes such as LAP but also to an increase in the circulation of blasts and other myeloid precursors and nucleated red blood cells.

Bone Marrow Analysis

Both the aspirate and trephine show hypercellularity due to uncontrolled expansion of both mature and precursor myeloid cell lines, megakaryocytes and red cells. The bone marrow may also show minimal fibrosis, or even hypocellularity as the disease advances.

Cytogenetic Studies

These studies reveal the Philadelphia chromosome in marrow samples. However caution is advised because this shortened chromosome 22 may also be seen in de novo or evolved acute leukemia and the size of the resulting protein differs in these different types of acute leukemia. Cytogenetic studies have been surpassed by QT-PCR.

Quantitative RT – PCR

This test is very sensitive and is used for diagnosis as well as for monitoring for remission after therapy initiation. The recommended frequency of monitoring for remission by all international guidelines is every three months. Standardization of the test has been achieved to ensure reproducibility and accuracy. However the test is often unavailable or too expensive in developing countries resulting in poor compliance with guideline recommendations for treatment monitoring.

2.2: CAUSES OF CYTOPENIA IN CML PATIENTS

2.2.1: Presentation of patients in Advanced – Phase CML

The disease typically presents in three phases. Most patients are diagnosed with chronic phase CML (CP). If no effective treatment is administered, patients inevitably progress to an advanced disease either accelerated phase (AP) or blastic phase (BP). The three different phases of CML, how to make a diagnosis and their symptoms are depicted in Table 1.

Patients may present directly in blastic phase at diagnosis whereas others progress to blastic phase just after treatment onset.

Ahmed R in 2009 reported the pattern at first presentation as chronic phase (77.8%), accelerated phase (15.5%) and blastic phase (6.7%) in a study in Pakistan (23).

A study in India reported that out of 68 patients seen at first presentation, 39 (57%) presented in CML-CP, 10 in accelerated phase (15%) and 19 in blastic phase (28%). On examination, massive splenomegaly was found in 62% of patients in accelerated and blastic phase as compared to 38% patients in CP (24).

Another study from the developing country reported that at presentation, 240 (87.3%) were in CML-CP, 22 (8%) in CML - AP and 13 (4.7%) in CML-BP (25).

Table 1: Different Phases at CML Presentation

CML Phase	Definition
CML-CP	WHO defines this phase as blood or marrow blasts less than 10%. Most CML patients, that is, approximately 90%, present to their doctor in CML- CP. The symptoms during this period are non-specific and slowly progressive over a period of 5 years (12). This progression often occurs in the face of no treatment. Signs and symptoms easy fatigability, loss of appetite, fevers, sweating, loss of weight malaise, abdominal swelling, pain or early satiety. Patients may at times be asymptomatic with the diagnosis being made on a routine CBC. The chronic phase tends to be responsive to treatment with an event free survival of more than 80% (26, 27).

CML-AP	WHO 2016 defines CML-AP 10-19% blood or marrow blasts. In addition, these patients have very high WBC, basophilia of ≥ 20%, low or very high platelet count despite therapy. The spleen is also massive and the marrow fibrosed despite treatment. This phase develops due to acquisition of additional mutations by the BCR-ABL positive cells (28, 29). Due to the poor response to treatment, these patients need higher doses of therapy (12). The median survival of this phase is approximately 5 years.
CML-BP	WHO 2016 defines this phase as blasts of ≥ 20% or extramedullary blast proliferation (30). These patients also have cytopenia and basophilia. Patients have bleeding tendencies including petechial hemorrhages, bruisability, nose bleeding, heavy menses, bone pain and fevers. The bleeding and infectious complications are due to its close resemblance to an acute leukemic process, with inability of bone marrow cells to mature. This phase is unresponsive to treatment and the median survival is weeks to 6 months (31).

Molecular basis of transformation to advanced phase CML

Progression of CML-CP is accompanied by myelofibrosis resulting in bone marrow failure, cytopenia and a poor prognosis (32). The time interval to transformation to advanced disease is varies with each patient. Twenty five percent (25%) of CML –CP do not progress for up to 5 years, 5% do not progress for 10 years, whereas for others, progression is an immediate occurrence just after initiation of treatment. During progression to accelerated or blastic phase, several nonrandom, secondary chromosomal alterations take place (33). The possible processes that result in transformation to advanced CML include further phosphorylation of BCR-ABL. Further, this protein interacts with xeroderma pigmentosum group B protein which reduces its catalytic function and results in impaired DNA repair processes (34). In addition, the BCR-ABL is anti-apoptotic and this potentially promotes inaccurate DNA repair resulting in the new DNA alterations in myeloid cells positive for BCR-ABL and eventually to progression to advanced phase CML. Excessive proliferation which is a key characteristic of CML is also a risk factor for additional DNA mutations. Common chromosomal mutations that develop in advanced CML include the presence of an additional Philadelphia chromosome, the occurrence of an isochromosome i (17q) which causes TP53 loss. The TP53 gene loss in turn causes impaired DNA damage responses (35, 36). In addition, there is development of trisomy 8 where the MYC gene resides. The MYC gene tends to be overexpressed in blastic phase CML and is a possible driver of progression to advanced disease (37). However, trisomy 8 is present in patients with cytogenetic remission from CML as well (38). Other chromosomal alterations such as trisomy of chromosome 19, 21, and 17, and chromosome 7 deletion, translocation t (3; 21), t (7; 11), complex karyotypes may also drive progression through their effect on transcription factors that cause cell proliferation and differentiation (38, 39). Literature on disease progression to advanced CML indicates that CP-CML patients have less mutations while those with advanced disease have almost 20 times the number of alterations as those in CP. This data lends credence that multiple gene alterations accumulate during CML progression to advanced CML.

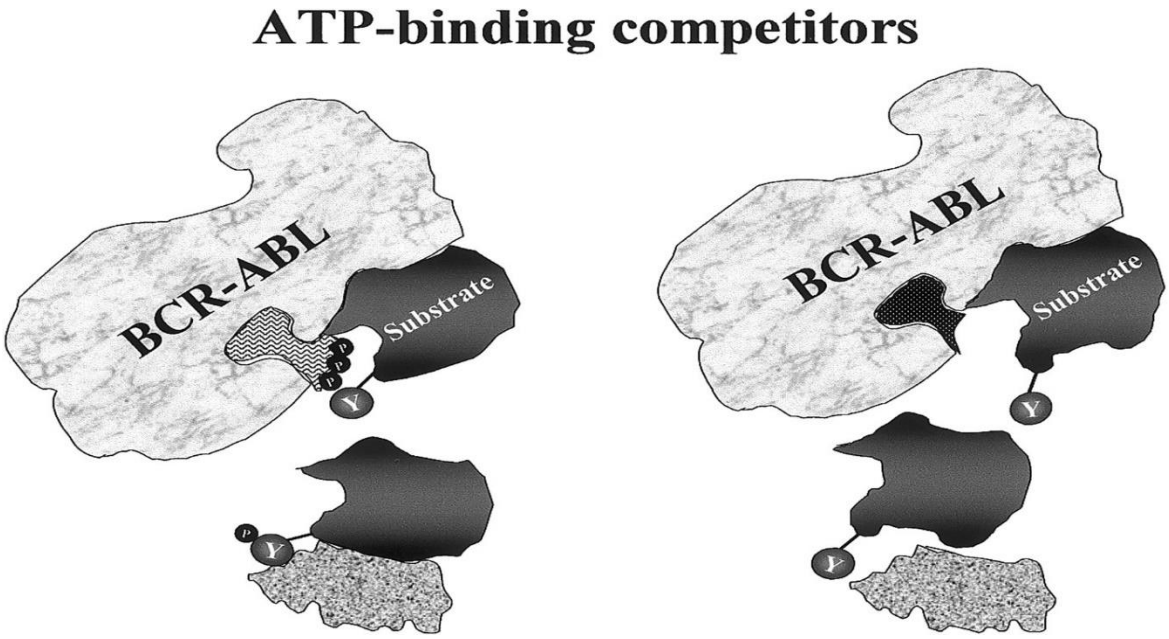
Treatment with imatinib has shown more success in chronic than in advanced CML up to 40% of CML AP and 20% of CML-BP surviving the disease when Hematopoietic Stem Cell

Transplant (HSCT) is not conducted (40, 41). Treatment of advanced CML is also associated with higher relapses even when remission is attained and reduction in overall survival of these patients. Patients with advanced CML also tend to have increased baseline BCR-ABL1 messenger ribonucleic acid (mRNA) and protein, up to 3-fold compared to CML-CP and this results in signaling pathway stimulation, changes in clonality, independence from growth factor influence, and reduced or absent apoptosis. These changes in turn result in increased speed of tumor development and progression of CML (42, 43).

2.2.2: Treatment Related Toxicity

Tyrosine Kinase Inhibitors (TKIs) block activity of adenosine triphosphate (ATP) competitively as shown in figure 3 (44). These drugs are the first choice for both chronic and advanced disease. However, they tend to less efficacious for the advanced disease, often requiring higher doses or need for HSCT.
Blockade of BCR-ABL transcript in turn block signaling pathways involved in proliferation and stimulates apoptosis and cellular adhesion(18).

Figure 3: How TKIs act to block proliferation



Imatinib, the first TKI to be discovered, is the preferred drug for both chronic and advanced disease. (45). The International Randomized Study of Interferon and STI571 (IRIS) study showed that imatinib at 400 mg once daily was more effective and had much fewer adverse effects in newly diagnosed patients with chronic disease. Further, among the CML-CP patients on imatinib, more than 80% were alive at 10 years, almost half remained compliant to their treatment, and more than 80% had a complete cytogenetic response while the progression free rate was 97% (46). Studies using second generation TKIs have yielded similar benefits.

Other drugs that have been employed in the treatment of CML include hydroxyurea, busulfan, and omacetaxine.

In addition, HSCT remains an option for treatment all CML patients not achieving remission or not responding to TKIs.

Imatinib adverse events

CML patients often tolerate imatinib treatment well with only grade I-II adverse drug events being the most frequent. Most of these toxicities occur early after initiation of imatinib. The toxicities are often easily manageable and potentially reversible either with dose reduction or temporary drug discontinuation.
The degree of adverse events are also determined by phase of CML under treatment as well by the dose administered with higher doses of drugs resulting in more severe adverse drug events. These adverse drug events include bone marrow toxicities like anemia, neutropenia and thrombocytopenia. These commonly occur in the initial 12 months of treatment. Non-hematological toxicities that are frequent include fluid retention/edema, gastrointestinal toxicities such as diarrhea, nausea, vomiting, abdominal discomfort, and cutaneous reactions such as rash. All these tend to be transient and resolve following dose reduction or temporary discontinuation of therapy.

Hematological toxicity

Bone marrow adverse events tend to occur early following initiation of imatinib therapy, usually in the first year of treatment (47). More severe bone marrow toxicity occur with advanced phase CML either CML-AP or CML –BP. More than 95% of patients with cytopenia due to imatinib toxicity don't need discontinuation of imatinib due to the cytopenia.

On the other hand, patients who respond to imatinib for prolonged durations rarely develop cytopenia. However, mild anemia has been observed relatively commonly, but with no definite explanation of this phenomenon. Persistent or chronic cytopenia in patients responding to imatinib affect the level of has been reported to affect life quality.
It is not known why some patients develop myelosuppression on imatinib whereas others do not. Possible explanation could be that imatinib blocks c-kit, a protein involved in early stages of formation of blood cells. The myelosuppression may also result from the active blockade of normal blood cell progenitors by TKIs. Other possible causes of myelosuppression include bone marrow hypoplasia, myelofibrosis, transformation to advanced CML, development of myelodysplatic syndrome, low baseline hemoglobin and

other cell counts, cytopenia from prior IFNa or busulphan use and stem cell pool reduction from TKI use. Regular monitoring of hematologic picture using bone marrow analysis may be helpful in determining the cause of cytopenia.
Myelosuppression following therapy with imatinib may have a negative effect on overall response because therapy is often withheld during bone marrow recovery or a reduction in dose is made.

Sneed, in their study of over 100 CML patients on imatinib, reported, based on the American NCI criteria for toxicity, that neutropenia more than or equal to grade 3 developed in 64 patients (45%), and low platelet count of grade 3 or more developed in 31 patients (22%). Cytopenia worse than grade 3 resulted in significantly reduced rates of major or complete cytogenetic responses. This was more so for persistent myelosuppression beyond two weeks of its development.

In yet another multivariable analysis, baseline low platelet count, prolonged myelosuppression and need for imatinib dose reductions significantly affected cytogenetic responses (47).

All grades of anemia was present after about 6 months in about half of patients initiated on treatment with imatinib. However, the anemia was mild with WHO grade 3–4 occurring only 3–8% of patients. In this population, the cytopenia tended to be of short duration (48, 49).

The development of new onset anemia, thrombocytopenia and anemia after 5 years of follow-up was rare at 4%, 9% and 17% respectively in the IRIS trial. (50). These cytopenia diminished over time during follow-up. Grade 3 or 4 myelosuppression was infrequent after the initial two years of therapy.

In a systematic review, the incidence of neutropenia, anemia, thrombocytopenia, was reported as 9.3-14.9, 8-19.8, 13.8-15.1%, respectively (51).
A study conducted in Hyderabad, India among 683 CML patients aged between 21-75 years, treated with imatinib showed that 46, 25, and 37 treated patients had grade 2 anemia, neutropenia and thrombocytopenia respectively. Among them, 18 and 13 were reported as bicytopenia and pancytopenia respectively. Persistent cytopenia was reported in approximately 60 of the patients. Evaluation of their marrow demonstrated hypoplasia, persistent disease in marrow, megaloblastosis and disease progression as the causes of persistent cytopenia. (52).

Research from Africa, specifically from Cote d'Ivoire over a six year study period, reported that a majority of patients with mortality was related to severe myelosuppression with grade 3 -4 cytopenia developing in 40%–60% of advanced CML patients (27).

2.2.3 Tyrosine Kinase Inhibitor Resistance

Failure to achieve the expected response to initial tyrosine kinase therapy is often due to resistance to the drugs.

Resistance may be primary. This develops in patients who have never demonstrated a response nor used TKI therapy and is defined as failing to attain, within three to six months of treatment, a complete hematologic response (CHR) or any cytogenetic response (CyR). It is also defined by non-achievement of a major cytogenetic response (MCyR) by twelve months or of a complete cytogenetic response (CCyR) by eighteen months (53). Most patients will achieve hematologic response primarily after initiation of imatinib but may fail to achieve cytogenetic response primarily as has been shown in 20% of chronic patients.

Less than optimal response to treatment is not attaining a cytogenetic response by three months of TKI treatment or a major cytogenetic response by six months or an incomplete cytogenetic response by twelve months or no major molecular response at eighteen months of treatment (53).

Secondary or acquired resistance occurs in patients who have used and attained response initially. It is identified by hematological or cytogenetic relapse, or the worsening of the disease at any time during treatment in a patient who had the expected response. (54).

Resistance to TKI treatment has been shown to be common in advanced disease (55).

Resistance is caused by mutations in the binding domain of BCR-ABL which block the binding contact between TKI and BCR-ABL1 by altering the amino acids that confer the correct conformation to BCR-ABL that enables it ordinarily to contact the TKI (56-58). Some mutations may occur without previous use of TKIs and this is the main cause of primary resistance leading to worsening of disease early in the course treatment with TKIs or non-durability of the initial response.

Very rarely, gene amplification or BCR-ABL protein amplification maybe the cause of primary or acquired resistance. Mutations of this type tend to be found in secondary resistance and in advanced disease (59 - 61).

Studies on influence of high levels of BCR-ABL1on resistance to therapy are in existence but have not yielded consistent results. One study suggested that there was a shorter time to development of BCR-ABL1 mutations in presence of high BCR-ABL1 transcript levels while another human model suggested that high BCR-ABL1 expression was significantly linked to better response to TKI therapy.

Finally, resistance to TKI therapy may occur due to poor compliance with treatment, drug-food interactions, drug to drug interactions and due self-discontinuation of therapy (62).

CHAPTER 3: STUDY JUSTIFICATION AND OBJECTIVES

3.1 JUSTIFICATION

Cytopenia occurs frequently in CML patients on treatment with imatinib and tends to be an early event, occurring within 12 months of imatinib initiation and is likely with advanced disease. Conversely, patients responsive to imatinib for prolonged durations rarely develop cytopenia with only sporadic cases reported in the real-life setting. It is not clear, in our setting, why some patients develop cytopenia and others do not. The type, grade and time course of cytopenia in these patients is not known. Despite this, cytopenia has been reported to be an independent adverse predictor of failure to achieve remission. Furthermore, patients who develop cytopenia tend to receive lower doses of imatinib or to stop treatment. The adjustment in imatinib dose will in turn influence attainment of remission.

Novartis Pharmaceutical and Max Foundation have collaborated to ensure the supply of imatinib in developing countries for all patients with CML. Patients pay for the diagnostic CBC, bone marrow trephine and biopsy as well as for the BCR-ABL1 test, and thereafter, are provided with imatinib at no cost. The patients are expected to pay for monitoring tests such as quarterly complete blood count and BCR-ABL1. Currently, the GIPAP clinic has 1200 patients at the Nairobi Hospital since 2007. An average of 150 patients attend the clinic bi-weekly. The clinic is centralized and receive patients from all over the country. The age range of patients seen in the clinic is six years to 75 years. The males that attend the clinic are in similar proportion to the females, majority initiate treatment with hydroxyurea and almost 90% present to the GIPAP clinic in chronic phase of the illness. Patients who initiate treatment are compliant with rates of approximately 80% (63).

Other services offered in the clinic include clinical assessment, treatment with imatinib as the initial therapy, and the provision of second line therapy.

Anecdotal reports from the clinic indicates that a substantial proportions of patients develop cytopenia in the course of treatment with Imatinib. The cytopenia has neither been characterized nor are the risk factors for its development studied. Despite this, treatment decisions such as dose adjustments and/or discontinuation of therapy are often made in such patients. These decisions have an impact on the outcomes of the affected patients.

3.2: RESEARCH QUESTION

What are the types, grades, time to development, duration and risk factors of cytopenia seen in CML patients following initiation of imatinib therapy?

3.3. STUDY OBJECTIVES

3.3.1: Primary Objectives

To determine the type, grade, time course and risk factors of cytopenia following the initiation of imatinib in CML patients attending GIPAP clinic at the Nairobi Hospital

3.3.2: Specific objectives

1. Determine types and grades of cytopenia at the GIPAP clinic
2. Determine the time to development and duration of cytopenia at the GIPAP clinic
3. Determine the odds that baseline demographic characteristics are associated with cytopenia at the GIPAP clinic
4. Assess the odds that baseline clinical characteristics are associated with cytopenia at the GIPAP clinic
5. Document the odds that baseline CBC and BCR-ABL1 levels are associated with cytopenia at the GIPAP clinic

CHAPTER 4: RESEARCH METHODOLOGY

4.1 Study Sites

The study was conducted at the Nairobi Hospital Glivec International Patient Assistance Program (GIPAP) clinic. This study site was selected because it has sufficient number of patients currently enrolled and active in care.

4.2 Study Design

This was a retrospective case-control study

4.3. Study Population

This study enrolled adults ≥ 18 years in age initiated on imatinib 400 mg since 2008 presenting with cytopenia, and followed up for at least 36 months.

Inclusion Criteria of Cases

1. All adults ≥ 18 years, attending the clinic for at least 24 months
2. CML disease on treatment with imatinib
3. All patients with cytopenia of grade 2 and above

Exclusion Criteria of Cases

1. Patients on myelosuppressive medications
2. Patients with co-existing benign or malignant myelosuppressive diseases
3. Attending clinic for less than 36 months

Inclusion Criteria of Controls

1. Adults ≥ 18 years, attending the clinic for at least 24 months with normal blood counts
2. CML disease on treatment using imatinib

Exclusion Criteria of Controls

1. Patients with medication or co-existing illness that may result in anemia, neutropenia or thrombocytopenia
2. Attending clinic for less than 36 months

4.4 Sample Size Determination (64)

$n = (Z_{\alpha/2}+Z_{\beta})^2 * (p_1 (1-p_1) + p_2 (1-p_2)) / (p_1-p_2)$

$Z_{\alpha/2}$ at confidence level of 95% is 1.96

Z_{β} at 80% power is 0.84

p_1 and p_2 are the expected sample proportions of the two groups

Estimated required sample size:

p1 = 76

p2 = 76

p1 + p2 = 152

4.5 Sampling Technique

The sampling included all patients' charts diagnosed with CML, obtained from the CML clinic. The cases were all patients with cytopenia who met the inclusion criteria whereas the controls were those without cytopenia, matched for age and sex. Because this was a dynamic population, a control was sampled each time a case was found (case-based sampling), matching cases and controls on calendar time. Data collection from the population under study was distributed equally over 3 months.

4.6 Data Collection

After obtaining ethical approval from the KNH/UON ERC, data on sociodemographic, clinical and laboratory characteristics were extracted from the patients' files. A coded questionnaire was used as the study instrument to abstract the information (Appendix 1). The Principal Investigator reviewed the data to ensure completeness and accuracy. Data was entered into an electronic database. Patient's names were left out to ensure confidentiality. Data was extracted from 2007 to 2015. Only the principal investigator and the study assistant had access to the files for the purposes of this study. The medical study assistant was a trained and certified nurse with experience in conducting research. In addition, to improve their competency in conducting this particular research, they were trained on communication skills, data collection, data analysis, and data verification.

4.7 Data Management and Statistical Analysis

Data was transferred into a Microsoft Excel worksheet, and imported into the statistical analysis software for data management and analysis. Continuous data was presented using means and respective standard deviations (SD). Counts and corresponding percentages were used for categorical variables such as gender of participants and cytopenia group. Bivariate comparisons such as comparisons of by cytopenia versus no cytopenia was done using chi square or fishers' exact tests for categorical variables as deemed appropriate. Univariable logistic regression analysis was employed for demographic, clinical and laboratory variables associated with cytopenia. The odds ratio (OR) and 95% Confidence Intervals was also reported. Stata package, version 15.1 was used during statistical analysis. There were some (50 out of 201 records) BCR-ABL at baseline values that were missing. To mitigate for this during the regression modeling, a category for missing data was created to ensure that the multivariable model includes all the observations as available for all the covariates. Statistical tests were evaluated for significance at the 5% level ($p<0.05$). Tables, bar charts, pie charts and line graphs were used to display results.

4.8 Variables and Definitions

Baseline Characteristics

This included age of participants which was categorized into 18-35, 36-50 and > 50 years, sex (male vs. females), marital status (categorized into married, single, divorced, separated), level of education (categorized into primary, secondary and tertiary), occupation (employed, not employed) as documented in the patients' charts at time of diagnosis of CML

Clinical Characteristics

Disease duration prior to diagnosis (early versus late), determined by presence of symptoms and diagnosis less than or more than 12 months after onset of symptoms. In the USA, 50% of CML are diagnosed early in the asymptomatic stage, as an incidental diagnosis due to elevated WBC. Delayed diagnosis of ≥ 12 months of CML may be associated with Hb < 10g/dl and may be used as a proxy measure of likelihood to transform to advanced CML.

Spleen size at diagnosis was measured by craniocaudal length and was defined as normal spleen size (< 11cm), moderately enlarged spleen (11-20 cm) or a massively enlarged spleen (>20 cm), persisting or increasing in size after diagnosis.

Presence of B symptoms was a proxy measure of delayed diagnosis mainly loss of weight of 10 kg or more in the preceding 6 months, fevers, and night sweats.

Time course of cytopenia was measured time to development of cytopenia in months as less than 3 months, 3-6 months and 6-12 months, as well as persistence of cytopenia, which looked at duration of cytopenia on follow-up CBC over a 36 month period. Difficult to control cytopenia may be a marker of transformation or resistance to imatinib in addition to being a marker of poor survival.

Hematological Characteristics

This was determined from the complete blood count.

The type and degree of severity of cytopenia was graded as per National Cancer Institute Common Terminology Criteria for Adverse Events v.3 in table 2 (72).

Table 2: Grading of Cytopenia

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Anemia in gram per dL	Lower limit of normal to 10	Less than 10-8	Less than 8-6.5	Less than 6.5	Death
Neutropenia ANC/mm^3	Lower limit of normal to 1500	Less than 1500-1000	Less than 1000-500	Less than 500	Death
Thrombocytopenia /mm^3	Lower limit of normal to 75 000	75 000 - 50 000	50 000 – 25 000	Less than 25 000	Death

LLN – Lower Limit of Normal

Monocytopenia was defined as a reduction in only one parameter from the full blood count. Bicytopenia was defined as reduction in two parameters from the full blood count. Pancytopenia was defined as reduction or absent platelets, red cells and white blood cells.

Baseline BCR-ABL1

Diagnostic molecular reports carried out on peripheral blood or marrow using RT-PCR respectively reviewed and categorized as 0-25%, 26-75%, 76-125% and more than 125%.

4.9 Ethical considerations

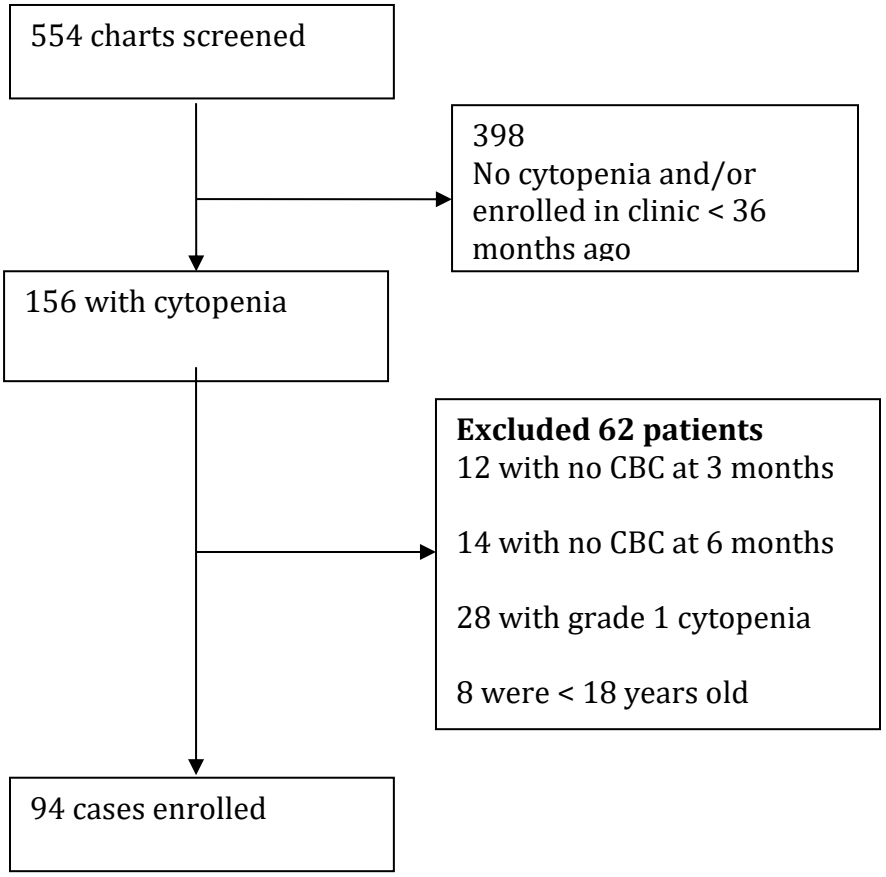
Permission to carry out the research was obtained from the KNH/UON Ethics and Research Committee. Patient identifiers such as names were not collected. All completed questionnaires were stored in lockable drawers. Data was stored in a password protected computer. The study was a minimal risk study since there was no direct patient involvement but a retrospective review of patient files. For confidentiality, the patients' charts were used only within the confines of the records department and only the investigators study assistants had access to the charts for the purposes of this study.

CHAPTER 5: RESULTS

5.1 Flow Chart of Screening and Recruitment of Cases

During the period between March and June 2018, a total of 554 patient files at the CML clinic were reviewed consecutively for eligibility of cases. One hundred and fifty six (156) patients were found to have cytopenia. To remove confounders, we excluded 62 patients. Ninety four patients were recruited into the study.

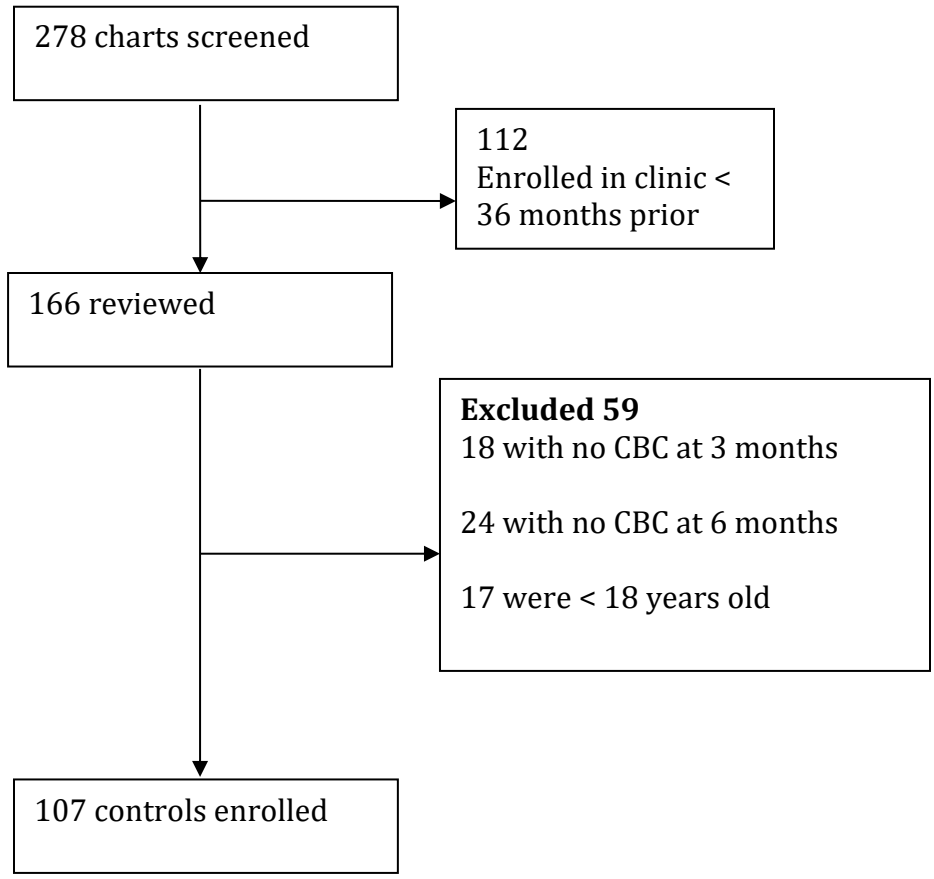
Figure 4: Flow Chart of Screening and Recruitment of Cases



5.2 Flow Chart of Screening and Recruitment of Controls

During the period between March and June 2018, a total of 278 patient files at the CML clinic were screened for eligibility. One hundred and sixty-six (166) qualified. To remove confounders we excluded 59 patients. A total of 107 patients met the inclusion criteria as CML patients with normal complete blood counts and were recruited as controls.

Figure 5: Flow Chart of Screening and Recruitment of Controls



5.3 Descriptive Analysis

Socio-Demographic Characteristics

The sample size of participants included in the analysis were 201, with the majority being males, 104 (51.7%) and most of the patients aged between 36 and 50 years, 85 (42.3%). Most of the participants were married, 147 (73.1%), employed, 77 (38.3%) and had attained secondary level of education, 96 (47.8%) as shown in table 3.

Type and Grade of Cytopenia

Sixty three percent (63 %) of the participants had monocytopenia with the predominant type being anemia at 32 (34%). The anemia was mostly of grade 2 in 50 % of the patients as shown in table 4.

Among patients with bicytopenia, the most common type was anemia plus neutropenia at 12.7%.

Clinical Characteristics

More than half of the participants had late time duration to diagnosis, 142 (70.6%). Forty percent (40 %) of the participants had ≤3 months from imatinib initiation to development of cytopenia, 38 (40%). One hundred and sixty one (80%) had moderate splenomegaly of 11-20 cm spleen size while 78.6% presented with presence of B symptoms at diagnosis as depicted in table 5.

Laboratory Characteristics

Most of the participants had a low baseline BCR-ABL at 0-25%, 72 (35.8%), normal baseline platelets at 150-450 (x 10^9)131 (65.5%), high baseline neutrophils at 7.6-100 (x 10^9), 90 (45.7%) and a baseline hemoglobin >10 g/dL, 104 (52.3%) as depicted in table 6.

Duration of Cytopenia

The anemia, thrombocytopenia and neutropenia had good recovery to normal by month 12 of treatment while neutropenia recovered by month 24 as depicted in table 7 and figures 8, 9, 10.

Figure 6: Distribution by Gender

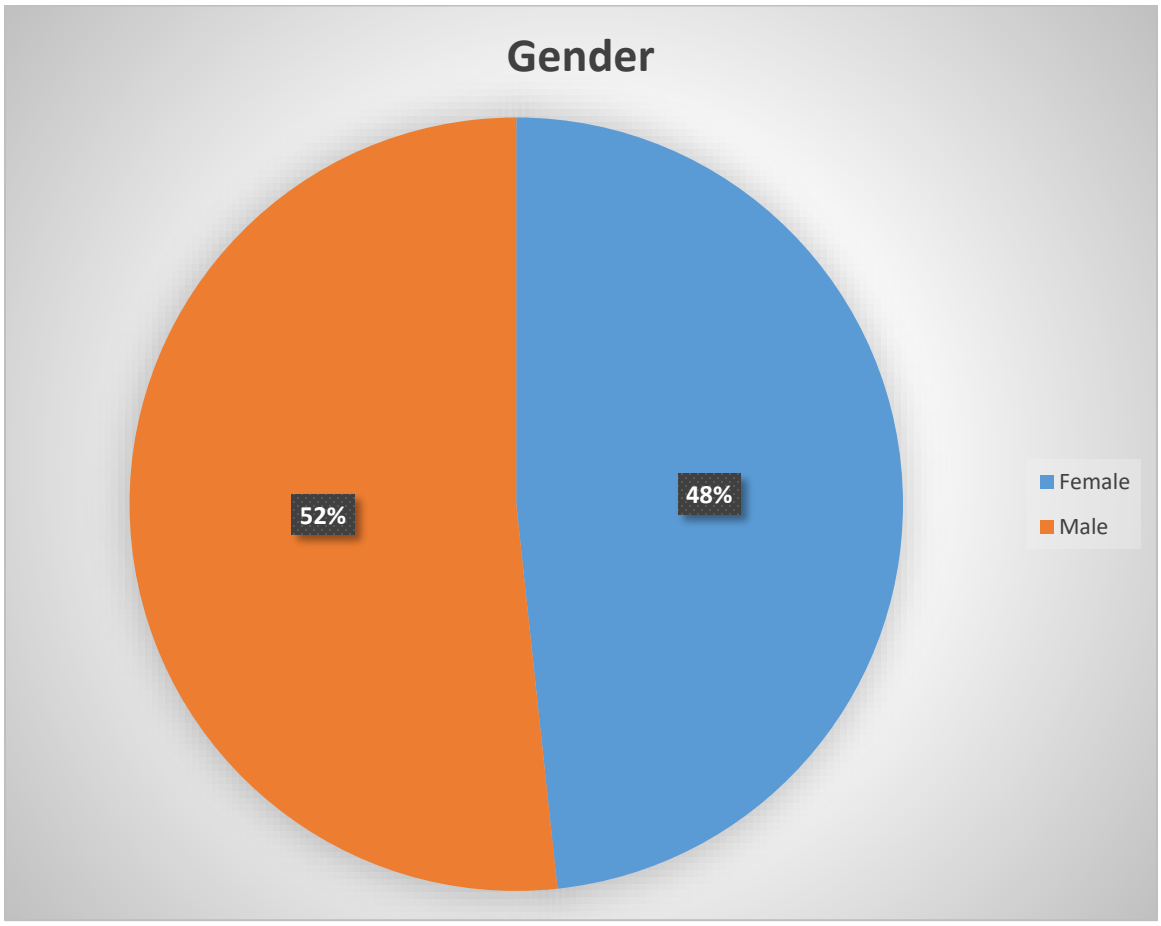


Figure 7: Distribution by Age of the Study Participants

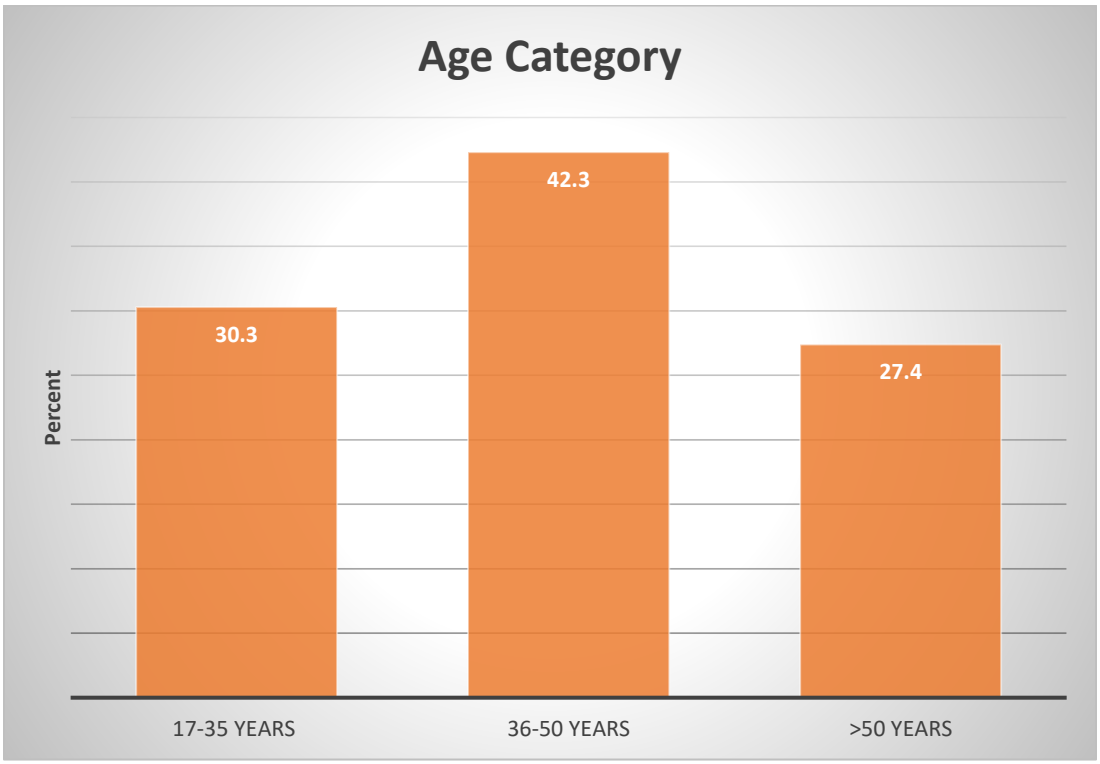


Table 3: Descriptive Analysis of Socio-demographic Characteristics

Variable	n (%)
Gender	
Female	97 (48.3)
Male	104 (51.7)
Age category	
17-35 years	61 (30.3)
36-50 years	85 (42.3)
>50 years	55 (27.4)
Marital status	
Divorced	2 (1)
Married	147 (73.1)
Single	51 (25.4)
Widowed	1 (0.5)
Occupation	
Employed	77 (38.3)
Retired	9 (4.5)
Self-employed	75 (37.3)
Student	9 (4.5)
Unemployed	31 (15.4)
Education	
Primary	11 (5.5)
Secondary	96 (47.8)
Tertiary	94 (46.8)

Table 4: Descriptive Analysis of Type and Grade of Cytopenia as per the CTCAE

Variable	**Grade 2**	**Grade 3**	**Grade 4**	**Total n (%)**
Anaemia (g/dL)	8 - 10	6.5 - 8	<6.5	**32 (34)**
	17	**13**	**2**	
Neutropenia	≥1000-1500	500-1000	<500	**26 (27.6)**
	10	**14**	**2**	
Thrombocytopenia	50000-75000	25000-50000	<25000	**8 (8)**
	5	**2**	**1**	
Anaemia + neutropenia				**12 (12.7)**
Neutropenia + thrombocytopenia				**8 (8)**
Anaemia + thrombocytopenia				**3 (3)**
Pancytopenia				**5 (5)**
Total				**94**

Table 5: Descriptive Analysis of Clinical Characteristics

Variable	n (%)
Time duration to diagnosis of CML	
Early	59 (29.4)
Late	142 (70.6)
Time from imatinib initiation to development of Cytopenia	
≤three months	38 (40)
Three to six months	33 (34.7)
Six to twelve months	24 (25.3)
Size of spleen	
11-20 cm	161 (80.1)
>20 cm	5 (2.5)
Normal	35 (17.4)
B symptoms present at diagnosis	
No	43 (21.4)
Yes	158 (78.6)

Table 6: Descriptive Analysis of Laboratory Characteristics

Variable	n (%)
BCR-ABL1 level (%)	
0-25	72 (35.8)
26-75	56 (27.9)
76-125	19 (9.5)
>125	4 (2)
Missing	50 (24.9)
Baseline platelets (x 10^9)	
<150	22 (11)
150-450	131 (65.5)
451-999	42 (21)
1000+	5 (2.5)
Baseline neutrophils (x 10^9)	
<1.5	22 (11.2)
1.5-7.5	41 (20.8)
7.6-100	90 (45.7)
>100	44 (22.3)
Baseline HB (g/dL)	
<6.5	3 (1.5)
6.5-7.9	19 (9.5)
8-10	73 (36.7)
>10	104 (52.3)

Table 7: Descriptive Analysis, Summary of Laboratory Values over Time

		Baseline		12 month		24 month		36 month	
		Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Anaemia									
Monocytopenia grade									
	2	11.1 (1.89)	11.5 (9.6-12.3)	13.1 (1.84)	13.6 (12.2-13.9)	13.2 (1.78)	12.7 (11.8-13.6)	12.6 (1.63)	12.9 (11.6-13.4)
	3	8.7 (2.88)	7.4 (7.1-7.8)	12.5 (2.05)	12.5 (11.2-13.9)	11.7 (2.67)	14.75 (12.05-15.9)	13.5 (1.09)	13.3 (9.3-15.1)
	4	4.4 (2.03)	4.44 (3-5.87)	14.4 (0.14)	14.4 (14.3-14.5)	14.3 (0.99)	14.3 (13.6-15)	13.5 (2.26)	13.5 (11.9-15.1)
Total		9.7 (2.92)	9.4 (7.36-12.05)	12.9 (1.89)	13.4 (11.95-14)	13.2 (1.92)	13.1 (11.8-15)	13.7 (1.02)	12.95 (11.2-13.8)
Neutropenia									
Monocytopenia grade									
	2	53.1 (79.22)	18.5 (1.37-68.43)	3.1 (9.46)	1.12 (0.9-1.36)	1.8 (0.88)	1.4 (1.2-2.17)	1.8 (0.79)	1.8 (1.23-2)
	3	35.3 (45.53)	21.1 (1.64-68.9)	0.9 (0.19)	0.9 (0.78-1.01)	1.9 (1.37)	2.12 (0.78-3.07)	1.9 (0.37)	1.83 (1.63-2.2)
Total		50.4 (74.59)	18.5 (1.37-68.43)	2.8 (8.7)	1.07 (0.9-1.31)	1.8 (0.94)	1.4 (1.2-2.82)	1.8 (0.74)	1.8 (1.3-2)
Thrombocytopenia									
Monocytopenia grade									
	1	154 (.)	154 (154-154)	124 (.)	124 (124-124)	118 (.)	118 (118-118)	92 (.)	92 (92-92)
	2	341.2 (409.33)	187 (96-301)	138.4 (81.58)	143 (69-208)	169.8 (60.25)	182 (120-193)	188.4 (84.64)	161 (146-165)
	3	144.3 (139.65)	144.25 (45.5-243)	116 (72.12)	116 (65-167)	132 (132.94)	132 (38-226)	241 (.)	241 (241-241)
Total		268.6 (329.53)	170.5 (81.5-272)	131 (68.24)	133.5 (67-187.5)	153.9 (71.42)	151 (110-209.5)	182.1 (82.1)	161 (132-241)

Figure 8: Median Hemoglobin Trends over a 36 Month Time Period

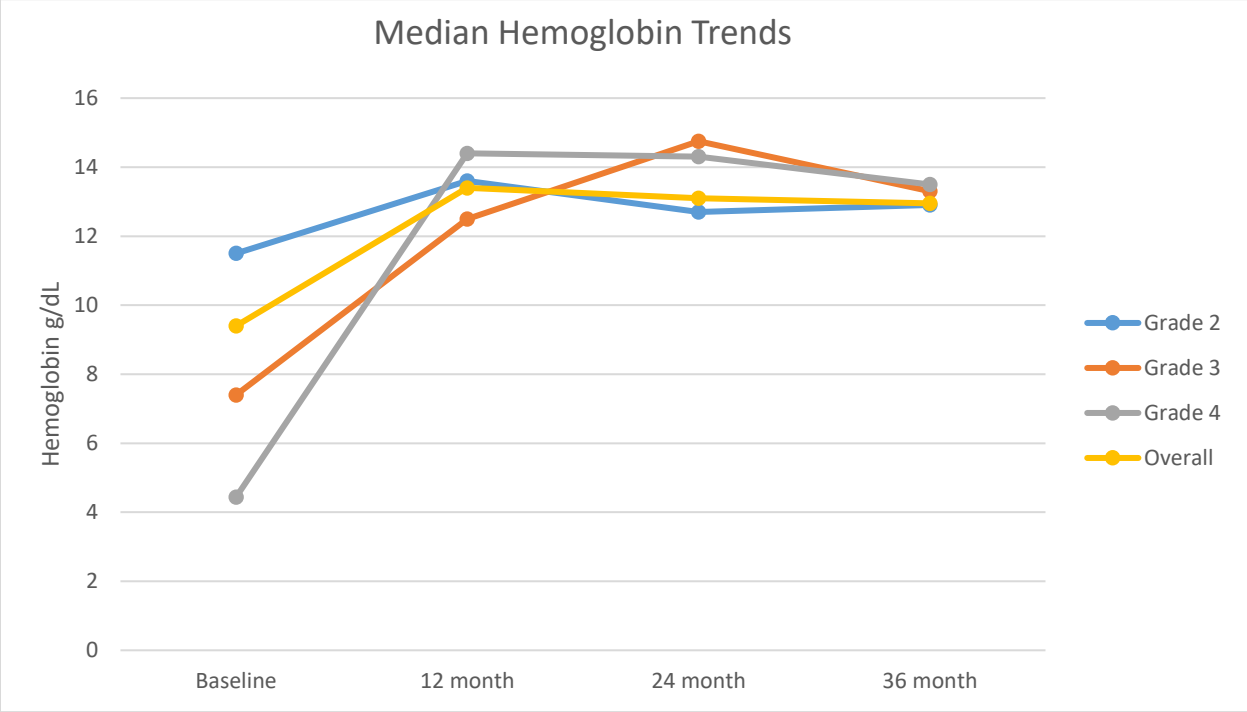


Figure 9: Median Neutrophil Trends over a 36 Month Time Period

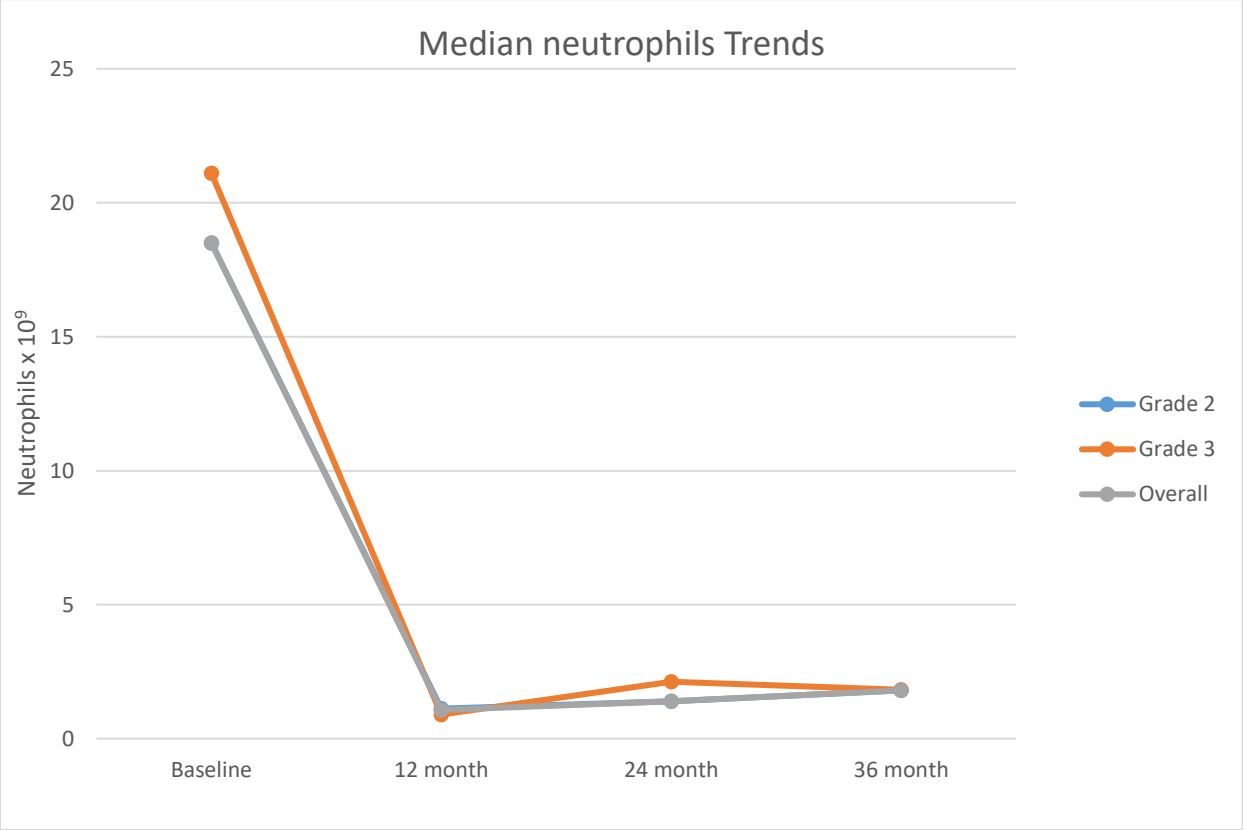
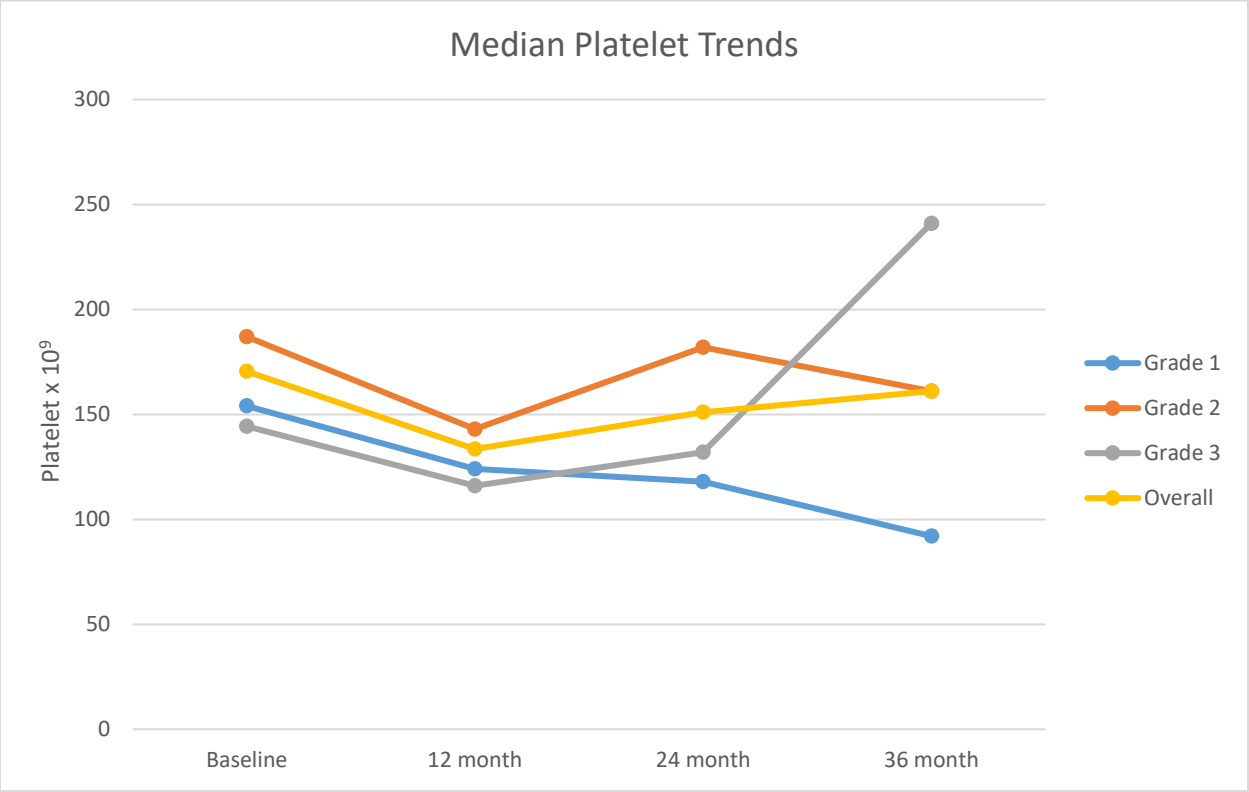


Figure 10: Median Platelet Values over a 36 Month Time Period



6.4 Bivariate Analysis

Social and Demographic Characteristics of the Study Participants

The proportion of females with no cytopenia was higher than those with cytopenia, however without any statistical significance, 54 (50.5%) vs. 43 (45.7%), p=0.504. There was no statistical difference among participants with no cytopenia and cytopenia with respect to age, marital status, occupation and education, all p values > 0.05, as shown in table 8.

Clinical Characteristics of the Study Participants

There was no statistical difference among participants with no cytopenia and cytopenia in relation to time duration to diagnosis, spleen size and presence of B symptoms, all p values > 0.05 as shown in table 9.

Laboratory Characteristics of the Study Participants

There was a significantly lower proportion of the participants with 0-25% baseline BCR-ABL1 among no cytopenia group relative to cytopenia group, 37 (34.6%) vs. 35 (37.2%), p=0.034. The proportion of participants with normal baseline platelets, 150-450 x 10^9 was significantly different among no cytopenia and cytopenia group, 80 (75.5%) vs. 51 (54.3%), p<0.001. There was a significantly greater proportion of the participants with elevated baseline neutrophils of 7.6-100 x 10^9 s among no cytopenia group than those among the cytopenia group, 58 (56.3%) vs. 32 (34%), p<0.001. More so, the proportion of participants with baseline haemoglobin >10 g/dL was significantly greater among no cytopenia group than among the cytopenia, 57 (54.3%) vs. 47 (50%), p<0.001 as shown in table 10.

Table 8: Bivariate Analysis of Socio – Demographic Characteristics

Variable	No Cytopenia n (%)	Cytopenia n (%)	P value
Gender			
Female	54 (50.5)	43 (45.7)	0.504
Male	53 (49.5)	51 (54.3)	
Age category			
17-35 years	31 (29)	30 (31.9)	0.498
36-50 years	43 (40.2)	42 (44.7)	
>50 years	33 (30.8)	22 (23.4)	
Marital status			
Divorced	2 (1.9)	0 (0)	0.321
Married	80 (74.8)	67 (71.3)	
Single	24 (22.4)	27 (28.7)	
Widowed	1 (0.9)	0 (0)	
Occupation			
Employed	34 (31.8)	43 (45.7)	0.069
Retired	3 (2.8)	6 (6.4)	
Self-employed	49 (45.8)	26 (27.7)	
Student	4 (3.7)	5 (5.3)	
Unemployed	17 (15.9)	14 (14.9)	
Education			
Primary	7 (6.5)	4 (4.3)	0.333
Secondary	55 (51.4)	41 (43.6)	
Tertiary	45 (42.1)	49 (52.1)	

Table 9: Bivariate Analysis of Clinical Characteristics

Bivariate analysis	No cytopenia	Cytopenia	P value
Time duration to diagnosis			
Early	36 (33.6)	23 (24.5)	0.154
Late	71 (66.4)	71 (75.5)	
Spleen size			
11-20 cm	83 (77.6)	78 (83)	0.632
>20 cm	3 (2.8)	2 (2.1)	
Normal	21 (19.6)	14 (14.9)	
B symptoms present at diagnosis			
No	26 (24.3)	17 (18.1)	0.284
Yes	81 (75.7)	77 (81.9)	

Table 10: Bivariate Analysis of Laboratory Characteristics

Variable	No Cytopenia n (%)	Cytopenia n (%)	P value
BCR-ABL at baseline (%)			
0-25%	37 (34.6)	35 (37.2)	0.034*
26-75%	25 (23.4)	31 (33)	
76-125%	13 (12.1)	6 (6.4)	
>125%	0 (0)	4 (4.3)	
Missing	32 (29.9)	18 (19.1)	
Baseline platelets (x 10^9)			
<150	4 (3.8)	18 (19.1)	<0.001*
150-450	80 (75.5)	51 (54.3)	
451-999	22 (20.8)	20 (21.3)	
1000+	0 (0)	5 (5.3)	
Baseline neutrophils (x 10^9)			
<1.5	4 (3.9)	18 (19.1)	<0.001*
1.5-7.5	27 (26.2)	14 (14.9)	
7.6-100	58 (56.3)	32 (34)	
>100	14 (13.6)	30 (31.9)	
Baseline HB (g/dL)			
<6.5	1 (1)	2 (2.1)	<0.001*
6.5-7.9	0 (0)	19 (20.2)	
8-10	47 (44.8)	26 (27.7)	
>10	57 (54.3)	47 50)	

* Statistically significant

5.5 Logistic Regression Analysis

Sociodemographic characteristics were not significantly associated with the development of cytopenia as shown in table 11. Respondents who had a baseline platelet count > 450 x 10^9/L and a baseline count less than 150 x 10^9/L were significantly associated with increased odds of suffering from cytopenia in both univariable and multivariable analysis, compared to those with normal platelets. The participants had an increase in the odds of having cytopenia for baseline neutrophils below 1.5 x 10^9/L in both univariable and multivariable analysis as shown in table 12. Those with neutrophils counts above 100 x 10^9/L only demonstrated an increase in the odds of developing cytopenia in the univariable analysis. Similarly, there was an increase in the odds of having cytopenia for participants with baseline haemoglobin <7.9g/dL) in both the univariable and multivariable analysis. There was no significant association with level of BCR-ABL1 count and cytopenia as shown in table 13.

Table 11: Logistic Regression Analysis, Socio-Demographic Characteristics

	Univariable OR (95% CI)	P value	Multivariable OR (95% CI)	P value
Age Category				
17-35 years	0.991 (0.513-1.913)	0.978	1.153 (0.507-2.62)	0.734
36-50 years	Reference		Reference	
>50 years	0.683 (0.343-1.357)	0.276	0.815 (0.308-2.15)	0.681
Gender				
Female	0.828 (0.475-1.442)	0.504	1.468 (0.589-3.65)	0.41
Male	Reference		Reference	
Marital Status				
Married	Reference		Reference	
Single/divorced/widow	1.194 (0.639-2.229)	0.578	0.965 (0.39-2.385)	0.938
Employment Status				
Not Employed	0.824 (0.402-1.689)	0.597	0.56 (0.195-1.611)	0.282
Employed	Reference		Reference	
Self Employed	0.42 (0.218-0.807)	0.009	0.36 (0.13-1.003)	0.051
Education Level				
Primary/Secondary	0.667 (0.382-1.164)	0.154	0.615 (0.25-1.511)	0.289
Tertiary	Reference		Reference	

Table 12: Logistic Regression Analysis, Clinical Characteristics

	Univariable		Multivariable	
	Odds Ratio (95% CI)	P value	Odds Ratio (95% CI)	P value
Time duration to diagnosis				
Early	0.639 (0.344-1.185)	0.155	0.681 (0.154-3.016)	0.613
Late	Reference		Reference	
Spleen size				
Abnormal	Reference		Reference	
Normal	0.717 (0.341-1.505)	0.379	0.469 (0.122-1.794)	0.268
B symptoms				
No	0.688 (0.346-1.366)	0.285	1.368 (0.328-5.707)	0.667
Yes	Reference		Reference	

Table 13: Logistic Regression Analysis, Baseline Laboratory Characteristics

	Univariable		Multivariable	
	OR (95% CI)	P value	OR (95% CI)	P value
Baseline BCR-ABL1 (%)				
0-25	Reference		Reference	
26-75	1.311 (0.65-2.642)	0.449	1.059 (0.421-2.662)	0.904
>75	0.813 (0.316-2.092)	0.668	0.688 (0.192-2.463)	0.565
Missing	0.595 (0.284-1.246)	0.168	0.316 (0.111-0.905)	0.032
Baseline Neutrophils $x10^9$				
<1.5	8.679 (2.459-30.63)	0.001	17.571 (3.909-78.987)	<0.001
1.5-7.5	Reference		Reference	
7.6-100	1.064 (0.489-2.313)	0.875	1.087 (0.398-2.971)	0.87
>100	4.133 (1.672-10.22)	0.002	2.776 (0.798-9.653)	0.108
Baseline Hb (g/dL)				
<7.9	25.47 (3.302-196.4)	0.002	32.231 (3.502-296.653)	0.002
8-10	0.671 (0.363-1.241)	0.204	0.598 (0.269-1.327)	0.206
>10	Reference		Reference	
Baseline platelets x 10^9				
<150	7.059 (2.26-22.05)	0.001	17.036 (4.079-71.157)	<0.001
150-450	Reference		Reference	
451+	1.783 (0.91-3.491)	0.092	2.771 (1.083-7.088)	0.033

CHAPTER 6: DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

6.1. DISCUSSION

The study, conducted among 201 CML patients, consisted of 94 cases and 107 controls. Despite the occurrence of cytopenia among patients on Imatinib, available studies in sub – Saharan Africa in these patients are few. Possible explanations for the development of cytopenia include disease progression, resistance to TKIs, or adverse events due to Tyrosine Kinase Inhibitor (TKI) therapy. This study looked at the latter. The results indicate that monocytopenia is more common than bicytopenia and pancytopenia. Anaemia was the most common type of monocytopenia whereas anemia plus neutropenia was the most common bicytopenia. Pancytopenia was seen in only 5 of the 94 patients. Majority of the patients with any kind of cytopenia were at graded at 2. The cytopenia developed within three months of initiating imatinib and had resolved by 12 months since initiation of imatinib for anemia and thrombocytopenia, and by month 24 for neutropenia. Baseline characteristics, time duration to diagnosis of CML, spleen size, presence of B symptoms and level of BCR-ABL1 was not found to be associated with development of cytopenia. A baseline thrombocytopenia and thrombocytosis, baseline neutropenia and a baseline anemia were associated with an increased odds of having cytopenia.

Analysis of the 201 patients revealed that cases and the controls were well matched in age and sex. The number of females and males enrolled in the study was equal at 97 and 104 respectively (p value 0.736), with good distribution between the cases and the controls. Data reported previously from studies done in the same population reports males are predominantly affected at a ratio of 3:2 among CML patients. The similar number of males and females in this study was to achieve a sex matched study population.

Forty two percent (42%) of the patients were aged between 36-50 years, 30% between 17-35 years and 27% more than 50 years. The distribution of the age groups among cases and controls were similar. The young age of patients in this study is similar to that reported for this same patient population in literature. However, this median age is much lower than what is reported in the western literature. SEER and MRC data have reported patients with CML to have a median age of 66 years (2).

The younger age at development could be as a consequence of Kenya's young population bulge (65) as well as due to a lower life expectancy, in contrast to the more developed nations. Young patients have better bone marrow reserve which may influence grade and duration of cytopenia.

Majority of the patients were married (73%). This is much higher than the marital status of the Kenya population of 54.6% as reported by respondents during the Kenya Demographic Health Survey 2014 (65). However, the marital status between the cases and controls in our study was similar.

Seventy five percent (75%) of the patients were employed with most being in self-employment. Nine were retired and nine others were students. There was similarity in employment status between the groups. This high employment status is a representative of the employment status in the country, which stands at 60% employment for women and 80% employment for men (65). Employment may be a positive factor because employed patients can afford transport to the facility as well as monitoring tests but may also limit ability of the employed patient to attend the CML clinic.

Literacy levels were high among the patients with 46% having attained tertiary education and 47% having attained secondary education. Data from the Kenya Demographic Health survey indicates that 88% of women and 92% of men are literate (65). There was similarity in education level between the groups. Literate patients have a better understanding of the complications of their treatment and how to mitigate.

Among the 94 patients with cytopenia, 66 (70%) had a monocytopenia, 23 (24%) a bicytopenia and 5% a pancytopenia. Anaemia was the most common among patients with monocytopenia at 34 %, followed by neutropenia at 27.6 %, and thrombocytopenia at 8 %. Anaemia plus neutropenia was the most common type of bicytopenia.

We report similar data to that from India who also reported anemia as common, seen in 46 (35%) patients. Thrombocytopenia occurred in 34 (25%) and neutropenia in 24 patients (17%). Bicytopenia was reported in 18 patients. This study included grade 2 to grade 4 cytopenia in the analysis and was conducted in a low resource setting similar to our study (52).

Our data is also similar to data from a study on adverse events that follow imatinib treatment. Anaemia was the most frequent myelotoxicity and occurred in 129 (65%) participants, followed by neutropenia in 57 (28%) patients and thrombocytopenia in 34 (17%) patients (66).

In another trial, during the first 12 months of treatment, grade 3 and 4 toxicity was noted among the 532 study participants. Neutropenia developed in 33%, thrombocytopenia in 18% and anemia in 6% (48) of the patients .

Imatinib administered to patients with early chronic disease led to grade 3-4 low neutrophils (14-19%), low platelets (8-10%) and low hemoglobin (3-4%) in research conducted by Druker BJ et al (50). This data differs from our data because of the lower levels of cytopenia reported. A study conducted in Indonesia likewise reported lower levels of cytopenia where anemia was the most common type of cytopenia at 20%, thrombocytopenia was second most common cytopenia at (14%), and neutropenia was low at (4%) (67). A study from Iraqi also documented low levels of cytopenia. In this study, 14% of patients with CML cases developed anemia. Further 10% and 4% of patients in the study developed neutropenia and thrombocytopenia respectively (68).

In the IRIS trial, imatinib administered to chronic patients led to grade 3 or 4 low hemoglobin (3%), low neutrophils (14.3%) and low platelets (8%) (46).

Low levels of cytopenia reported in several studies may have been due to inclusion of only grade 3 and 4 cytopenia in the analysis. Early diagnosis of CML may also result in lower levels of hematological toxicity while late diagnosis may result in higher levels of hematological toxicity. In our study, many patients presented with advanced disease.

Our study reported that among the 34% of patients with anemia, 18.1%, 13.8% and 27.6% had grade 2, grade 3 and grade 4 anemia respectively. Among the 27.6 % of patients with neutropenia, 14. 8% patients had grade 3, 10.6 % grade 2 and 2.1% patients had grade 4 neutropenia. For thrombocytopenia, grade 2 was the most common grade.

A study carried out in India reported high proportions of grade 2 cytopenia at 18.5%, 8.8% and 10.3% for anemia, neutropenia and thrombocytopenia respectively, followed by grade 3 at 11.8%, 6.6% and 7.4% for anemia, neutropenia and thrombocytopenia respectively.

Grade 4 cytopenia was at 3.7%, 3.1% and 7.4% for anemia, neutropenia and thrombocytopenia respectively (52).

A second study, conducted in India, reported similar grades of cytopenia as our study. Among the 27% of patients in this study with neutropenia, 10% had grade 1-2, 11% of patients developed grade 3 and 6% of patients developed grade 4 neutropenia whereas among the 15% of patients who had thrombocytopenia, 9% developed grade 3 and 6% developed grade 4 thrombocytopenia. Anaemia occurred in only 10% of the patients (69).

Yet another study that only looked at grade 3 and 4 toxicity reported that among the patients that developed neutropenia, 11% got grade 3 and 5% got grade 4 neutropenia while 7% had low platelets (70). This data was also similar to our data.

Zhou reported from his study, reported that grade 3 to 4 neutropenia, grade 3 to 4 anemia, and grade 3 to 4 thrombocytopenia occurred in 21.8%, 17.8%, 5.9% of patients with CML-CP, respectively (71).

A randomized phase III study of chronic patients reported higher values of grade 3 cytopenia than of grade 4 cytopenia among patients diagnosed early and treated with imatinib (46).

Kantarjian reported higher grades of cytopenia in late CP-CML with 35%, 20% and 7% developing neutropenia, thrombocytopenia and anemia respectively of grade 3 to 4 (70). Advanced CML as well as high imatinib doses of 600 mg were associated with higher proportion of cytopenia (30).

Majority of patients who develop cytopenia following diagnosis and treatment for CML develop mild grades of cytopenia, usually grade 1 to 2. Fewer patients develop grade 3 to 4 cytopenia and these are the patients who require closer follow-up and interventions to manage the cytopenia.

There was recovery of anemia within 12 months of initiation of treatment. Grade 2 and 3 neutropenia improved to grade 1 by month 12 and to normal by month 24.

Four patients with grade 2 and 3 thrombocytopenia also recovered normal platelet counts by 24 and 36 months respectively.

Similarly, from other studies, grade 3 and grade 4 cytopenia resolved soon after treatment interruption and did not recur with resumption of treatment (72).

In another trial, myelosuppression as a toxicity developed early in the treatment course with imatinib therapy but had resolved in the majority of patients after the first 12 months of follow-up (73).

Hochhaus et al also reported that myelotoxicity from imatinib was generally an early event. Their patient population developed myelotoxicity in the initial first year of treatment with imatinib than after one year (48).

The recovery of the cell counts within 12 months of initiating treatment could be a reflection of bone marrow normalization and regeneration (74). However, some isolated cases of recurrent myelosuppression requiring extended treatment interruptions have been reported and maybe a consequence of advanced disease (69). Conversely, the cytopenia may recover within days after its development as has been documented prior where the median duration of thrombocytopenia and neutropenia was less than 3 weeks (72).

The duration of cytopenia may be influenced by duration of CML prior to diagnosis. A study reported patients with long duration of CML disease prior to diagnosis tend to have advanced disease. In the event of myelotoxicity development, the duration of cytopenia tends to be more prolonged especially with higher grades of cytopenia. (75).

Cytopenia developed within 3 months of imatinib initiation in 40% of our patients, within 3-6 months in 34.7% of our patients and 6-12months in 25% of our patients.

Myelosuppression may develop at any time point during treatment of CML but is common in the initial 2-4 weeks of therapy especially for advanced disease (45).

The development of severe cytopenia rapidly in patients with a myeloid bulge shortly after TKI initiation is a phenomenon that is still under study. The elevation of leucocytes in this patients probably reflects the high disease burden at diagnosis. During treatment with TKI therapy, the malignant clone is eliminated but there is an associated delay in the formation of the normal marrow stem cell and precursors and subsequently, poor marrow and blood repopulation.

Once normal blood cell formation resumes, the myelosuppression that has been induced by TKI treatment resolves and the risk of developing myelosuppression later in the course of treatment reduces.

Likewise, the blood and marrow laboratory results often normalize (48).
Studies have reported onset of cytopenia between 3-5 weeks of imatinib therapy. Grade 1 and 2 hematological toxicity tend to favor early CP while grade 3-4 was frequent in late CP (76).
Clinical characteristics that predispose to a higher risk of myelotoxicity have been reported and include prolonged period of illness, prior drug induced cytopenia, low baseline cytopenia, and high baseline marrow blasts. Patients should be screened for these risk factors at baseline and thereafter monitored closely early after imatinib initiation to enable prompt identification and interventions if cytopenia develops.

In our study, social and demographic characteristics such as age, sex, marital status, occupation and education level were not associated with higher odds of developing cytopenia. However, in a study conducted in Iraqi, females on imatinib had a predilection for anemia than males (68). This higher likelihood is probably due to the lower level of hemoglobin found in females at baseline compared to males. It is not known but it is possible that females experience a higher myelosuppressive effect from imatinib than males. Outside of the nature of CML, anemia may also be related to other comorbidities which might be confounding factors in the analysis of outcomes.

Age was not a factor in predicting odds of cytopenia in this study. This has been corroborated by a study that followed up patients for 67 months and reported similar baseline spleen size, baseline blood cell counts, baseline blast counts, and sokal risk score for different age groups (77). Baseline cytopenia, if it were present, may be a predictor of development of cytopenia following imatinib therapy.

Mild to moderate splenomegaly up to 20 cm or just above or at the umbilicus was found in 80% of the patients, 17.4% had normal spleen and 2.5 % had a massive spleen either more than 20 cm or below the umbilicus.
Spleen size is a strong independent pre-treatment risk factor and is used to prognosticate the response to treatment by the Sokal and Hasford Score.

Splenomegaly maybe a marker of advanced disease and is known to be significantly linked to poor outcomes (78). In our study, increased spleen size did not significantly increase the odds of developing cytopenia.

Among the 201 patients studied, 142 (70.6%) had severe symptoms that developed within months of presenting to the hospital, and 59 patients (29.4%) had non-specific and non-severe symptoms that were insidious in onset for a year or more. Presence of B symptoms was used as a proxy measure of delayed diagnosis and was defined as an unintentional loss of weight in the preceding 6 months of more than 10 kg, fevers, and night sweats. B symptoms were present in 78.6% of patients and absent in 21.4%. The incidence of neutropenia and thrombocytopenia as well as its severity is higher in CP-CML presenting late after initiation of patients on 400 mg daily. Research has shown that cytopenia does occur in almost 40% with late diagnosis compared to cytopenia proportion of 26% in patients diagnosed with early CML-CP. This difference was significant and suggests that patients with a late diagnosis are at risk and should be monitored closely at onset of treatment and cytopenia managed promptly once it develops.

At diagnosis, up to 50% of patients in the well to do countries are diagnosed incidentally after performing a routine blood count. On the other hand, in developing countries, patients present late or following the onset of symptoms. The most common clinical presentation of patients with CML was found to be splenomegaly (72.5%), followed by fatigue (53.7%), hepatomegaly (33.33%), bleeding dysfunction (14.5%), fever (24.6%) and night sweats (5%). However, patients still attain good response to treatment with cytopenia developing in 14% (79).
In our study, late presentation to the hospital with a diagnosis of CML did not significantly increase the increased odds of developing cytopenia.

Sixty eight percent (68%) of the 201 patients in our study had a baseline neutrophil count above the upper limit of normal (>7500/uL), 20.8% had neutrophils within the normal and 11.2 % had neutrophil counts less than 1500/uL. In addition, among the 201 patients, 104 (52.3%) had a Hb more than 10g/dL, 73 (36.7%) between 8-10g/dL, 9.5% between 6.5-7.9 while three patients had a Hb of < 6.5 g /dL. Majority, that is, 65.5% had normal platelet

count, 23.5% had platelet counts above 50,000/uL and 11% had platelets less than 150,000/uL.

Baseline anemia at diagnosis is known to accompany a higher baseline white blood cell counts, more frequent splenomegaly, and more CML related deaths (80) . A study among 527 patients in Nigeria reported that patients with anemia had intermediate risk that affected their outcomes (81) .
In our study, a baseline neutrophil count below 1500/uL and a baseline hemoglobin level < 7.9 g/dL significantly increased the odds of developing cytopenia. This is possibly due to bone marrow dysfunction or fibrosis which is part and parcel of the disease process. Such patients should be closely monitored with additional bone marrow assays to assess progress.
Both thrombocytosis and thrombocytopenia were associated with increased odds of developing cytopenia. This too, could be a factor of bone marrow dysfunction and requires close follow-up. In addition, cytopenia at baseline and persisting after onset of treatment may reflect disease progression or resistance to treatment. These patients require close follow-up and monitoring, both through bone marrow aspirate and trephines but also close monitoring of their molecular response rates.

Among the 156 patients with BCR-ABL1 results, 35.8 % had levels of 0-25%, 27.9% had levels of 26-75%, 9.5% had levels of 76-125% and four patients had levels more than 125%.

A few studies have reported that higher BCR-ABL1 levels are associated with poor outcomes. One study demonstrated that patients who experienced severe and very severe myelotoxicity had a significantly higher BCR-ABL1 value after conducting FISH studies. Higher BCR-ABL of FISH testing may suggest that a certain predominant malignant hematopoietic clone is present (82).

Another study found that suboptimal responders or patients with imatinib resistance had a higher median amount of BCR-ABL at diagnosis (104.154) compared to patients obtaining a sufficient response (53.478) despite them having similar WBC counts. The increased

amounts of BCR-ABL transcripts could be a marker of the presence of an aggressive leukemic clone among the myeloid cell line. In our study, level of baseline BCR-ABL1 did not have any impact on hematological toxicity.

Treatment with imatinib works much better in chronic disease than in transformed disease with poor survival outcomes in accelerated phase and in blastic phase when Hematopoietic Stem Cell Transplant (HSCT) is not done (40, 41). Treatment of progressed disease is also associated higher relapse rates. Patients with advanced phase CML tend to have increased baseline BCR-ABL1 messenger ribonucleic acid (mRNA) and protein, up to 3-fold, compared to CML-CP, which tend to be associated with increased signaling through various pathways , changes in clonality of the malignant cells, growth factor independence of the malignant cells and blockade in apoptosis, resulting in increased speed of leukemia development (42) (43) .

6.2 LIMITATIONS

The study was a retrospective study and thus has several limitations as might be expected. There was missing data that could have affected the outcomes of our study. The study is limited to grade 2 and above of cytopenia and therefore the data can only be applied to patients with grade 2-4 cytopenia. The sokal score was of interest to us but could not be calculated due to lack of bone marrow blast counts. It was therefore not possible to risk score the patients fully and correlate prognostic score with cytopenia development. The sample of patients was not large and this may make it difficult to infer results to the general population. On the other hand, our findings are similar to those of studies conducted both in Africa and in the developing world.

6.3 RECOMMENDATIONS

This study recommends that physicians should have a high index of suspicion to recognize patients at risk of developing cytopenia. This include patients with low baseline cytopenia as well as patients with baseline thrombocytosis. Our results also suggests that physicians to consider continuing imatinib at normal doses or even at lower doses but in the meantime, provide adequate blood and growth factor support during seasons of myelotoxicity.

6.4 SUMMARY

Patients who develop imatinib induced cytopenia have baseline cytopenia and thrombocytosis. BCR-ABL1 level does not help to predict odds, nor does the clinical characteristics. Monocytopenia is common, mainly anemia, followed by neutropenia and is mostly grade 2. The cytopenia developed within 3 months of initiating imatinib but this event reverses soon, with a majority recovering their blood counts within a year. Further, a low baseline cytopenia and baseline thrombocytosis is associated with increased odds of developing cytopenia.

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CHAPTER 8: APPENDICES

Appendix I - study proforma

Study No. ☐☐☐☐

Date: ☐☐ ☐☐ ☐☐☐☐ IP No. ☐☐☐☐☐☐☐

DOB (month, year) ☐☐ ☐☐☐☐ ge (years) ☐☐☐

DEMOGRAPHICS

1. Gender 1 = Male 2 = Female ☐

2. Marital status ☐

1=Single 2=Married 3=Divorced 4=Widowed 5=Separated

3. Usual residence …….

4. Usual occupation

   1 = self-employed ☐nployed 3 = unemployed 4 = retired

   5 = training/student

5. Level of education ☐

   1 = None 2 = Primary school 3 = Secondary school 4= Tertiary

Type of cytopenia ☐

Monocytopenia ☐

1 = anaemia 2 = neutropenia 3 = thrombocytopenia

Bicytopenia ☐

1 = anaemia + neutropenia 2= anaemia + thrombocytopenia 3 = neutropenia + thrombocytopenia

Pancytopenia ☐

1 = Yes 2 = No

Grade of cytopenia ☐

1=Grade 1 2= Grade 2 3=Grade 3 4=Grade 4 5=Grade 5

6. Spleen size (cm ☐

1 = Normal 2 = 11 - 20 cm 3 = ≥ 20 cm

7. B symptoms Y ☐ N ☐

8. Time duration to diagnosis ☐

1 = early/asymptomatic 2 = late/symptomatic

9. Time from imatinib initiation to development of cytopenia (in months) ☐

1 = < 3 months 2 = 3-6 months 3 = 6-12 months

10. Transient vs. Persistent cytopenia among cases

	wbc	neut	lymph	baso	rbc	hb	platelets		
Baseline									
3 months									
6 months									
12 months									
18 months									
24 months									
36 months									

11. Complete blood count at baseline for the controls

	wbc	neut	lymph	baso	rbc	hb	platelets			
Baseline										

12. Baseline PBF

12. Baseline BMA

1 = CML - CP 2 = CML - AP 3 = CML – BP 4 = Hypoplastic 5 = Bone marrow fibrosis

13. Molecular characteristics

a) BCR –ABL (%) or

14. Sokal score

CYTOPENIA FOLLOWING IMATINIB TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) IN KENYA: A STUDY AT GIPAP CLINIC, NAIROBI HOSPITAL

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June 13, 2018

Dr. Angela Awino McLigeyo
Principal Investigator(Fellowship in Medical Oncology)
Dept.of Clinical Medicine and Therapeutics, UON
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. McLigeyo

RESEARCH PROPOSAL – CYTOPENIA FOLLOWING IMATINIB TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) IN KENYA (P126/03/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is from 13th June 2018 – 12th June 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chairperson, KNH-UON ERC
The Assistant Director, Health Information, KNH
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