HAEMATOLOGICAL PARAMETERS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS AT KENYATTA NATIONAL HOSPITAL, NAIROBI

DR. JACQUELINE WANGECHI NJOROGE, M.B.Ch.B,
RESIDENT, MMed - INTERNAL MEDICINE.

A DISSERTATION SUBMITTED IN PART FULFILLMENT OF THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI.

2016

STUDENT'S DECLARATION

I declare that this dissertation entitled **"**Haematological parameters in systemic lupus erythematosus patients at Kenyatta National Hospital, Nairobi" is my original work and as far as I am aware it has not been submitted either wholly or in part to this or any other university for the award of any degree or diploma.

Signed: ..... Date: …….

DR JACQUELINE WANGECHI NJOROGE
Resident MMed, Internal Medicine,
Principal Investigator.
H58/68399/2011

SUPERVISORS' DECLARATION

This dissertation is submitted with our approval.

1. **PROF. OMONDI OYOO, MBChB, MMED (Med), FRCP (Edin), FACR.**
   Associate Professor, Department of Clinical Medicine and Therapeutics, University of Nairobi.

   Signed..... Date: ……

2. **PROF. GRACE.W. KITONYI: MBChB , FRCPath, PGD – RM.**
   Associate Professor of Hematology and Blood Transfusion, Department of Human Pathology, University of Nairobi.

   Signed: ..... Date: ……

3. **DR ANNE K. BARASA, MBChB, MMED (Path),**
   Lecturer, Department of Human Pathology,
   University of Nairobi
   Signed: ..... Date: ……..

4. **DR ANDREW ODHIAMBO: MBChB, MMED (Med)**
   Tutorial Fellow, Department of Clinical Medicine and Therapeutics
   University of Nairobi.

   Signed: ..... Date: ……..

DEDICATION

I dedicate this book to my young family, my husband Joshua, children Angel and Adrian for their overwhelming support and being a source of inspiration for me during the course of my studies.

ACKNOWLEDGEMENTS

First I give thanks to God for His grace and strength that enabled me carry out this study.

Secondly, I offer my sincere gratitude to the following people who made this study possible:

- My supervisors: Prof. Oyoo, Prof. Kitonyi, Dr Odhiambo, Dr Barasa for their guidance and supervision.
- My mentor Prof Mcligeyo for his guidance and support.
- My fellow registrars for their support and encouragement.
- The staff of Kenyatta National Hospital Rheumatology, Renal clinic and haematology laboratory.
- The staff of Kenyatta National Hospital medical records department.
- The staff at Lancet laboratory.
- Exchange programme students from University of Texas Medical Board for their assistance in data entry.
- All the patients who agreed to participate in the study.
- Mr. Philip Ayieko for his help with data analysis.
- My family for their encouragement and prayers during my masters program.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

FIGURES

TABLES

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
APL	Antiphospolipid Syndrome
ACD	Anaemia of Chronic Disease
AIHA	Autoimmune Hemolytic Anaemia
CBC	Complete Blood Count
CI	Confidence Interval
CNS	Central Nervous System
CT	Computed Tomograph
CTCAE	Graded using Common Terminology Criteria for Adverse Events
DMDS	Disease Modifying Drugs
ECLAM	European Consesus Lupus Activity Measurement Score
EDTA	Ethylene Diamine Tetraacetic Acid
ESR	Erythrocyte Sedimentation Rate
fL	Fentolitre
g/dL	Grams per decilitre
Hb	Hemoglobin
Hct	Hematocrit
IDA	Iron Deficiency
IgG	Immunoglobulin g
IgM	Immunoglobulin m
IFN	Interferon
ITP	Immune Thrombocytopenic Purpura
KNH	Kenyatta National Hospital
LDH	Lactate Dehydrogenase
mL	Millilitre
NEQAS	National External Quality Assessment Service
NSAIDS	Non Steroidal Anti-inflammatory Drugs
OR	Odds Ratio
PI	Principal Investigator
PBF	Peripheral Blood Film
pg	Picogram
RBC	Red blood cells
Rh	Rhesus
SLE	Systemic Lupus Erythematosus
SLICC	Systemic Lupus International Collaborating Clinics Classification Criteria's

SPSS	Statistical Package for Social Sciences
TNF	Tumor Necrosis Factor
TNF TRAIL	TNF Related Apoptosis Inducing Ligand
TTP	Thrombotic Thrombocytopenic Purpura
UON	University Of Nairobi
uL	Microlitre
Vit B12	Vitamin B12
WBC	White blood cells
WHO	World Health Organization

ABSTRACT

Background

Systemic lupus erythematosus (SLE) is an autoimmune disorder that results in multi-systemic inflammatory damage. It's often severe and can affect virtually all organs including the hematologic system. Its aetiology is still poorly understood

Haematological abnormalities are common among patients with SLE. The most frequent haematological abnormalities include anaemia, leucopenia and thrombocytopenia. These abnormalities are markers of disease activity and have been found to be independent determinants of mortality therefore understanding their prevalence is important in patient evaluation.

While these abnormalities have been widely studied in other parts of the world, no study has been conducted on Kenyan patients afflicted by SLE thus there exist a gap regarding haematological parameters in SLE patients and hence the need for this study. We performed this study to understand haematological parameters in a tertiary hospital in Nairobi, Kenya.

Objective

The main objective of this study was to determine the prevalence of haematological abnormalities, among SLE patients on follow up at Rheumatology and Renal Outpatient clinics at Kenyatta National Hospital. Specifically, the study aimed to describe the prevalence of anaemia, leucopenia, leucocytosis, thrombocytopenia and thrombocytosis and to identify patient factors associated with these abnormalities.

Methods

A cross-sectional descriptive study was carried out on SLE patients attending the Rheumatology and Renal outpatient clinics at KNH. Seventy one consecutive SLE patients were screened for eligibility between 5th March 2015 and 5th of June 2015. Of these sixty five were recruited and enrolled into the study. Clinical and social demographic data was captured and recorded in a pre-designed questionnaire. Subsequently, four millilitres of blood was collected for measurement of a complete blood count, reticulocyte count, erythrocyte sedimentation rate and peripheral blood film examination. The tests were undertaken at the KNH Department of Human Pathology, unit of Haematology and Blood Transfusion using a

CELL-DYN 3700 automated blood counter. ESR interpretation was undertaken at the same laboratory by the Wintrobe method and a PBF was reported after staining with maygrunwald / giemsa stain by direct visualization on a microscope at various powers of magnification by hematologists who were supervisors for this study and the PI

Results

Sixty five eligible SLE patients were recruited into the study. The mean (SD) age was 36. 5(± 12) years. There were 3 (5%) males and 62 (95%) females. Forty nine (75%) patients had at least one abnormality. The abnormalities involved all the three cell lines. The prevalence of abnormalities were; anaemia 43%, leucopenia 26% and thrombocytopenia 20%. Disease duration less than one year was significantly associated with anaemia, p=0.035, OR = 3.5 (95% CI 0.9-15.1).

Conclusion

Haematological abnormalities are the second most common manifestation of the disease after arthritis and arthralgia among SLE patients on follow up at Kenyatta National Hospital Rheumatology and Renal clinic. Though majority of these abnormalities were mild to moderate, the proportions of anaemia, leucopenia and thrombocytopenia were substantially high. There was a significant association between anaemia and duration of disease.

Recommendations

I. A larger longitudinal study to correlate thrombocytopenia and leucopenia with demographics and drugs. . This may require a multicenter approach to avail sufficient number of patients.
II. A study to correlate these haematological abnormalities with disease activity in patients with SLE which may be useful as surrogate markers of disease activity in the resource constrained settings.
III. Long term follow up of subgroup of patients who had thrombocytopenia to determine long term outcome

1.0 INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder that results in multi-systemic inflammatory damage. It's often severe and can affect virtually all organs including the hematologic system. Its aetiology is still poorly understood.

It occurs worldwide but it's more prevalent among black Americans and Hispanics (1).Different prevalence rates have been reported ranging from 20 to 150 cases per 100,000 (2). The epidemiology of SLE in Africa is largely undetermined. There has been increasing case report of SLE among black Africans in Kenya and South Africa (3-5). The prevalence of SLE locally is largely undetermined. Oyoo et al in 2004 reported a prevalence of 1.56% in Kenya(6). However larger epidemiological studies are needed to determine the prevalence of disease in Kenya.

The clinical course of SLE is variable and may be characterized by periods of remissions and chronic or acute relapses. The disease is more common in women of childbearing age(67).

Haematological abnormalities have been noted to be among the commonest in systemic lupus erythematosus (SLE) patients in several studies (7, 14, 15, and 16). This is attributed to blood and blood vessels together containing more diverse number of antigens than any other organ in the body and in SLE auto antibodies are known to develop against any antigen or tissue. Hemolytic anaemia, leucopenia, lymphopenia, and thrombocytopenia are part of the diagnostic criteria for SLE according to American College of Rheumatology criteria (ACR) 1997 (8) and the more recently validated Systemic Lupus International Collaborating Clinics Classification Criteria (SLICC) 2012 for Systemic Lupus Erythematosus (9).

Most of the hematologic parameters are markers of disease activity based on the various validated tools for measuring disease activity (10). Some patients present with hematologic abnormality as their initial and only manifestation and without a high index of suspicion the diagnosis of SLE can be missed. The haematological changes, though very commonly seen, are not properly evaluated or estimated and are not given enough representation in the American College of Rheumatology (ACR) criteria for diagnosis of SLE. (11-13)

Different studies report different prevalence rates. In a study conducted in Saudi Arabia by Al Arfaj AS et al (14) haematological disorders were reported in 82.7% of patients with SLE. Agrawal SR et al in Central India in 2012 reported hematologic manifestation in SLE in

72.4% of patient while Houman et al 2004 in Tunisia reported a prevalence rate of 81%(15,16).

The most common haematological abnormalities include anaemia, leucopenia and thrombocytopenia. They commonly result from an immune mediated bone marrow failure, excessive peripheral cell destruction or certain drugs and infections (17). SLE patients with anaemia of chronic disease (ACD) had a significantly higher disease activity. (18) Interestingly the severity of correlates with disease activity only among iron deficient patients and not among those with ACD and autoimmune hemolytic anaemia (AHA) (18). Thrombocytopenia early in the course of SLE is indicative of more severe and active disease. Severe thrombocytopenia is an independent predictor of damage accrual. It is also an independent predictor of mortality. Patients with thrombocytopenia need close monitoring for possible undesirable outcomes (19-20). Leucopenia is also common in SLE and usually reflects disease activity (20-21)

1.1 LITERATURE REVIEW

Systemic Lupus Erythematosus (SLE) is a multisystemic autoimmune disease whose reported prevalence in different parts of the world ranges from 20-150 cases per 100,000 (2). It has been reported to occur infrequently among blacks in Africa, however there has been increasing case reports in Kenya and South Africa.(3-5)

The most common pattern of presentation is a mixture of constitutional complaints with skin, musculoskeletal, mild hematologic and serologic involvement (22). However, some patients have predominately hematologic, renal, or central nervous system manifestations. The pattern that dominates during the first few years of illness tends to prevail subsequently (23-24). SLE can present with haematological manifestations alone or along with features of other system involvement. With a low index of clinical suspicion or inadequate follow up the diagnosis may be delayed or missed at the time of presentation, in those with haematological abnormalities as the initial manifestation.(6)

1.2 HAEMATOLOGICAL ABNORMALITIES

Haematological disorders have been reported among the commonest in SLE in several studies, for example in a study conducted in Saudi Arabia, Bennett et al found a prevalence of 82.7%(13). The major manifestations are , leucopenia, and thrombocytopenia. These vary widely among patients, and they are listed as the most common manifestations of SLE in the SLICC criteria for SLE classification which includes hemolytic with reticulocytosis, leucopenia (<4.0x109/L) or lymphopenia (<1.0x109/L), or thrombocytopenia (<100x109/L) in the absence of other known causes eg drugs, portal hypertension (9)

1.2.1 ANAEMIA

Anaemia is a common haematological abnormality in SLE that is defined as hemoglobin levels of < 12g/dL for women and <13.5 g/dL for men. It affects most patients at some time in the course of their disease. Multiple mechanisms contribute to the development of ,including inflammation, renal insufficiency, blood loss, dietary insufficiency, medications, haemolysis, infection, hypersplenism, myelofibrosis, myelodysplasia, and aplastic that is suspected to have an autoimmune pathogenesis (20,21,25-30). In a study by Voulgarelis M et al comprising 132 anaemic patients with SLE, anaemia of chronic disease was found in

37.1% of the cases, iron deficiency anaemia in 35%, autoimmune hemolytic anaemia in 14.4% and other causes of anaemia in 12.9% of the patients (29).

1.2.1.1 Anaemia of chronic inflammation

A frequent cause of anaemia in SLE is suppressed erythropoiesis from chronic inflammation, this form of anaemia is usually normocytic and normochromic with a relatively low reticulocyte count (21). Although serum iron levels may be reduced, bone marrow iron stores are adequate and the serum ferritin concentration is elevated. The major mediator of the chronic inflammation is hepcidin, a central regulator of iron homeostasis that inhibits release of iron from macrophages and iron absorption in the small intestine. This results in iron-limited haematopoiesis.

As in other chronic illnesses, serum erythropoietin levels may be inappropriately low for the degree of anaemia. However, some of the apparent reduction in serum erythropoietin may be spurious; auto antibodies to erythropoietin may interfere with commercial laboratory testing (31). Low levels of erythropoietin due to chronic inflammation or renal insufficiency and presence of anti-erythropoietin auto antibodies which are associated with European Consensus Lupus Activity Measurement (ECLAM) high score are found in some patients (10)

1.2.1.2 Renal insufficiency

SLE is associated with renal insufficiency. An inappropriately low level of erythropoietin is a hallmark of due to renal insufficiency. The primary cause of anaemia in this setting is typically deficient production of erythropoietin by the diseased kidneys

1.2.1.3 Iron deficiency

It is defined by serum ferritin below20 μg/dl. Iron deficiency anaemia may reflect acute or chronic blood loss from the gastrointestinal tract, usually secondary to medications (nonsteroidal anti-inflammatory drugs or steroids), or may be due to excessive menstrual bleeding. Long-term anaemia of chronic inflammation can also lead to iron deficiency, since, as mentioned earlier, hepcidin, the key inducer of the of chronic inflammation, inhibits iron absorption from the gastrointestinal tract.

Pulmonary hemorrhage is a rare cause of anaemia in SLE. Not all patients have haemoptysis. Other symptoms of alveolar hemorrhage are dyspnoea and cough. The presence of alveolar infiltrates on a chest radiograph or ground-glass opacities on chest CT are suggestive of alveolar hemorrhage.

1.2.1.4 Pure Red cell aplasia

Pure red cell aplasia, probably due to antibodies directed against either erythropoietin or bone marrow erythroblasts, has been observed in SLE, although it is rare. (27, 28, 32) This form of anaemia usually responds to steroids, although cyclophosphamide and cyclosporine have been successfully employed.

Even rarer are isolated case reports of aplastic anaemia, presumably mediated by auto antibodies against bone marrow precursors. (33-35)

In addition, bone marrow suppression can also be induced by medications, including antimalarials and immunosuppressive drugs used in SLE.

1.2.1.5Autoimmune hemolytic anaemia

Overt autoimmune hemolytic anaemia (AIHA), characterized by an elevated reticulocyte count, low serum haptoglobin levels, increased indirect bilirubin concentration, and a positive direct Coombs' test, has been noted in up to 10 percent of patients with SLE(20,21,26,30,37).The presence of hemolytic anaemia may be associated with other manifestations of severe disease such as renal disease, seizures, and serositis (37).

Other patients have a positive Coombs' test without evidence of overt haemolysis. The presence of both immunoglobulin and complement on the red cell is usually associated with some degree of haemolysis, while the presence of complement alone (e.g., C3 and/or C4) is often not associated with haemolysis (20, 21, 25, 26). The antibodies are "warm," IgG, and are directed against Rh determinants. IgM mediated cold agglutinin haemolysis is uncommon.

1.21.1.6 Microangiopathic hemolytic anaemia

Lupus has also been associated with a thrombotic microangiopathic hemolytic anaemia (TMA) (37) as manifested by a peripheral blood smear showing schistocytes and elevated serum levels of lactate dehydrogenase (LDH) and bilirubin. Many affected patients also have

thrombocytopenia, kidney involvement, fever, and neurologic symptoms. This pentad of features is compatible with a diagnosis of thrombotic thrombocytopenic purpura (TTP). However, the pathogenesis of thrombotic microangiopathy in these patients is likely heterogeneous, as it may reflect vasculitis or antiphospholipid syndrome as well (38, 39).

Other patients with microangiopathic red cell destruction do not have fever or neurologic disease, producing a pattern of hemolytic-uremic syndrome. The pathogenesis of this syndrome is not completely understood.

The presence of aPL in SLE patients with severe hemolytic anaemia, renal dysfunction, and central nervous system involvement has also been reported (40)

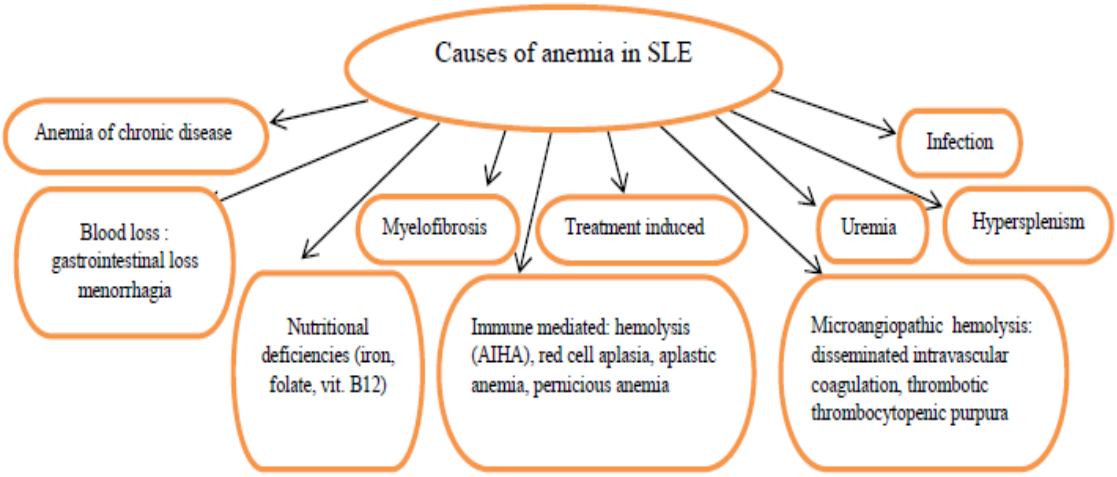


Fig. (1). Causes of anaemia in SLE. (17)

1.2.1.7 Pancytopenia

Refers to a reduction in all the cell three lines i.e. red cells, leucocytes and platelets. Although peripheral destruction of red cells, leukocytes, and platelets may occur together and lead to clinically significant pancytopenia, depression of all three cell lines also suggests bone marrow failure, as is the case in aplastic anaemia. Thus, bone marrow examination is the most important diagnostic test to perform.

Causes of marrow failure include drugs and coincidental diseases including: the acute leukemias, large granular lymphocyte leukemia, the myelodysplastic syndromes, marrow replacement by fibrosis or tumor, severe megaloblastic anaemia, paroxysmal nocturnal hemoglobinuria (PNH), and overwhelming infection. In addition, unexplained cytopenias can be associated with bone marrow necrosis, dysplasia, and distortion of the bone marrow architecture as was shown by M. Voulgarelis et al (30) in 2006.

Among patients with SLE an unusual cause of pancytopenia is the macrophage activation syndrome. The demonstration of hemophagocytosis in the bone marrow or in material obtained from peripheral lymph nodes is a characteristic finding (62).

1.2.2 LEUCOPENIA

Leucopenia is defined as a white cell count (WBC) count less than 4×10^9/ L. However in Africans the lower limit of normal WBC is lower 2.6×10^9/ L (41). Leucopenia is common in SLE and usually reflects disease activity. A study by Schur P H et al found the prevalence of lymphopenia in SLE ranges from 20 to 81% and its degree may correlate with disease activity (26). A white blood cell count of less than 4500/microL has been noted in approximately 50 percent of patients, especially those with active disease (20, 21), while lymphocytopenia occurs in approximately 20 percent (20). In comparison, a white blood cell count below 4×10^9/ L (an American College of Rheumatology criterion for SLE) occurs in only 15 to 20 percent of patients (20). Neutropenia, lymphocytopenia, and decreased circulating eosinophils and basophils may all contribute to leucopenia.

Reduced surface expression of complement regulatory proteins CD55 and CD59 has been found in leucopenic patients with SLE. Deficiency of these proteins may make these cells susceptible to complement-mediated lysis. There is increasing evidence that endogenous production of type 1 interferons (IFNs) is implicated in the pathogenesis of neutropenia and lymphopenia in SLE.

A study by Ronnblom L et al (41) showed that elevated serum levels of IFN-α in SLE correlates inversely with leucocyte numbers.

Immunosuppressive agents like azathioprine or cyclophosphamide have the potential to worsen leucopenia via bone marrow suppression, which is less common.

1.2.2.1Neutropenia

Neutropenia is defined as neutrophil count of less than 2.5 X 10^9/ L in Caucasians and studies have shown lower levels in Blacks 1.3 X 10^9/ L (41). It is a common feature of SLE, with a prevalence rate of 50 to 60 %(68). Clinically, increased susceptibility to infections is a major cause of morbidity and mortality in patients with SLE (69, 70). In this regard, not only treatment with steroids and/or immunosuppressive drugs but also neutropenia is responsible for the increased incidence of infections. (71-74).

Neutropenia in SLE is multifactorial; it may be mediated by anti-neutrophil antibodies. A study by Wataru M et al (43) showed increased levels of TNF-related apoptosis-inducing ligand (TRAIL) in SLE may contribute to neutropenia through excessive neutrophil apoptosis mediated neutropenia. Other possible causes include immune mechanisms, medications (e.g., cyclophasphomide or azathioprine), bone marrow dysfunction, or hypersplenism (20, 21, 44, 45). Clinical features that may be associated with moderate to severe neutropenia (absolute neutrophils <1 X 10^9/ L) include infection, anaemia, thrombocytopenia, and a history of neuropsychiatric involvement (45).

Functional defects of neutrophils have also been noted. They are thought to be induced by immune abnormalities (e.g., immune complexes, inhibition of complement-derived chemotactic factors) and/or medications (e.g., glucocorticoids) (46, 47)

1.2.2.2 Lymphocytopenia

Lymphocytopenia refers to lymphocytes count less than 1.5 x 10^9/ L. Lymphocytopenia involving suppressor T cells, has been observed in 20 to 75 percent of patients, particularly during active disease (20, 25, 26, 48, 49). This finding is strongly associated with IgM, cold reactive, complement fixing, and presumably cytotoxic antilymphocyte antibodies; such antibodies were noted in 26 of 29 patients with SLE and the antibody titer correlated directly with the degree of lymphopenia (50).

Another potential mechanism of lymphocytopenia is increased apoptosis as reflected by increased expression of Fas antigen on T cells (51).

1.2.2.3 Decreased eosinophils and basophils

Steroid therapy may result in low absolute eosinophil and monocyte counts (52). The number of basophils may also be decreased in SLE, particularly during active disease (53). Basophil degranulation with release of platelet activating factor and other mediators may play a role in immune complex deposition and vascular permeability

1.2.3 LEUCOCYTOSIS

This is defined as leucocytes more than 10.2 and 11 x 10^9/ L in Blacks and Caucasians respectively (41). Leucocytosis (mostly granulocytes) can occur in SLE. When present, it is usually due to infection or the use of high doses of glucocorticoids (54), but may occur during acute exacerbations of SLE. A shift of granulocytes to more immature forms (a "left" shift) suggests infection.

1.2.4 THROMBOCYTOPENIA

This is defined as platelet count less than 100 X 10^9/ L in Blacks and 150 X 10^9/ L in Caucasians (66). It has a reported prevalence ranging from 7 to 30% in large series of patients with SLE. Mild thrombocytopenia (platelet counts between 100 X 10^9/ L and 150 X 10^9/ L) has been noted in 25 to 50 percent of patients; while counts of less than 50 X 10^9/ L occur in only 10 percent (20, 21, 24, 44). There are several potential causes of thrombocytopenia in patients with SLE. Immune mediated platelet destruction is most often the cause, but platelet consumption may also occur in association with microangiopathic hemolytic anaemia or be due to impaired platelet production as a result of the use of cytotoxic, immunosuppressive, or other drugs

The major mechanism is immunoglobulin binding to platelets followed by phagocytosis in the spleen, as in idiopathic thrombocytopenic purpura (ITP) (55). Membrane glycoproteins (GP) are most often the target of such antibodies (e.g., GP IIb/IIIa) but anti-HLA specificity also occurs (56).

Antigen-dependent B cell development in lymphoid tissues is influenced by binding of CD40 on B cells to CD40-ligand on activated T cells. The finding of autoantibodies to CD40-ligand in patients with SLE, APS, and ITP, but not in the serum of healthy blood donors suggests

that interference with T cell and B cell interaction may play a role in the development of thrombocytopenia (57)

Other important mechanisms in selected patients include bone marrow suppression by immunosuppressive drugs (other than corticosteroids) and increased consumption due to a thrombotic microangiopathy (thrombotic thrombocytopenic purpura [TTP]. Antiphospholipid syndrome and antibodies that block the thrombopoietin receptor on megakaryocytes or their precursors have also been shown to cause thrombocytopenia in SLE (36).

ITP may be the first sign of SLE, followed by other symptoms as long as many years later. Wang GJ et al reported that 5-15% of patients with ITP fulfill the criteria for the diagnosis of SLE at the time of presentation and approximately 3.6% patients with SLE developed ITP over 4 years (58). It has been estimated that 3 to 15 percent of patients with apparently isolated ITP go on to develop SLE (59). Evans syndrome (i.e., both autoimmune thrombocytopenia and autoimmune hemolytic anaemia) also may precede the onset of SLE.
A retrospective study of 126 SLE patients study by Nossent JC et al [20] found thrombocytopenia was an independent risk factor for increased mortality in SLE in late-onset thrombocytopenia and was associated with an increased mortality. In a more recent retrospective study by Ziakas PD et al (60) of 632 patients with SLE, the authors found that the prevalence of thrombocytopenia was 58% at the time of diagnosis. There was an apparent association between thrombocytopenia and disease activity, increased mortality and hypocomplementemia. It was also associated with significant organ damage, such as heart, kidneys and the CNS, however severe bleeding was only experienced by minority of patients. Thrombocytopenia in SLE can also be a complication of immunosuppressant therapy such as azathioprine and it is rarely caused by antimalarials such as hydroxychoroquine.

1.2.5 THROMBOCYTOSIS

Thrombocytosis is defined as platelets > 300 x 10^9/ L and >400 x 10^9/ L in Blacks and Caucasians respectively (66). It's a less frequent finding in SLE, usually a reactive thrombocytosis but may also be associated with active disease (61). Castellino et al (61) found a prevalence of thrombocytosis defined as platelet ≥400 x 10^9 / L of 3.7%. Three of these patients had one or more of the following features on peripheral blood smear: Howell-Jolly bodies, spherocytes, and/or target cells. Ultrasound, CT, and liver-spleen scintigraphy

failed to demonstrate a spleen. All three patients had aPL (61). These observations suggest that autosplenectomy may occur in patients with SLE, perhaps mediated by aPL.

1.2.6 ERYTHROCYTE SEDIMENTATION RATE

Acute phase reactants, such as the erythrocyte sedimentation rate (ESR) and serum C-reactive protein levels are less reliable markers of disease activity in lupus than in many other inflammatory conditions, including rheumatoid arthritis and polymyalgia rheumatica (63). In a study by Vila et al (64) elevated ESR was associated with disease activity and accumulated damage.

2.0 STUDY JUSTIFICATION

Haematological complications are common manifestation of SLE. They are easily diagnosed on a complete blood count. All of them though challenging have an effective treatment. Leucopenia and thrombocytopenia are markers of disease activity. High disease activity is associated with increased morbidity and poor prognosis. Thrombocytopenia is an independent determinant of mortality. Drugs used in management for example Cyclophosphomide and azathioprine may impact unfavourably on haematological parameters.

There is paucity of local data on haematological profiles in SLE patients both locally and in Africa as a whole. Almost all existing data is from Europe, America and Asia. This data may not be generalizable due to racial differences between these populations. There is thus need to identify the kind of haematological abnormalities present in our local population. The objective of this study was to identify the prevalence of various cellular haematological abnormalities and form a basis for future studies into each of the documented abnormalities.

The study also provides an objective overview of the abnormal hematologic parameters in this population and may help come up with a policy of testing for these haematological parameters as a guide to diagnosis and for appropriate patient management.

2.1 RESEARCH QUESTION

What are the haematological abnormalities among SLE patients on follow-up at Kenyatta National Hospital Rheumatology and Renal Outpatient Clinics?

2.2 OBJECTIVES

2.2.1 Broad Objective

To determine the prevalence of haematological abnormalities in SLE patients on follow-up at KNH Rheumatology and Renal outpatient clinics.

2.2.2 Specific Objectives

1. To determine the prevalence of anaemia in SLE patients at KNH.
2. To determine the prevalence of quantitative leucocytes abnormalities i.e. leucopenia and leucocytosis in SLE patients at KNH.
3. To determine the prevalence of quantitative platelet abnormalities i.e thrombocytopenia and thrombocytosis in SLE patients at KNH

3.0 MATERIALS AND METHODS

3.1 Study design

A cross sectional descriptive hospital based study.

3.2 Study site

The designated area of the study was the Rheumatology and Renal outpatient clinics of Kenyatta National Hospital, a teaching and referral hospital in Nairobi, Kenya. As of September 2013 there were 66 SLE patients on regular follow up. The Rheumatology clinic runs every Thursday from 2.00 PM till about 5.00 PM and Renal clinic on Friday 8.00 AM to 1.00 PM.

3.3 Study population

The study population was SLE patients satisfying ACR criteria as diagnosed by a rheumatologist on follow-up at KNH Rheumatology and Renal Clinic.

3.4 Inclusion criteria

All SLE patients older than 13 years and who gave informed written consent and assent for those below 18 years were recruited into the study.

3.5 Exclusion criteria

There were no criteria for exclusion of patients diagnosed to have SLE.

3.6 Sampling procedure and sample size calculation

3.6.1 Sample size calculation

The sample size was calculated using the finite population correction formula as shown below.

Table 1: Minimum sample size required for the study variables, *n*

Study Variable	P	n	$n=\frac{NZ^2 \times p(1-P)}{d^2(N-1)+Z^2p(1-p)}$
Hematologic manifestations	82%	52	
Anaemia	62.9%	56	
Leucopenia	15.7%	51	
Thrombocytopenia	39.8%	57	
Thus, the minimum sample size ***n*** necessary is **57**			**N=** Size of target population**= 66** **p =** prevalence from other studies**(** Sasidharan in India 2011) **Z=** Statistic for a level of confidence(1.96) **d**= Precision (0.05) **n**=minimum sample size required (57)
Haematological Manifestations of SLE at Initial Presentation:Is It Underestimated? **P. K. Sasidharan,M. Bindya, and K. G. Sajeeth Kumar North Karela India 2011(6)**			
Shiruli et al 2013 Cardiovascular Risk factors in patients with SLE at KNH (64)			

3.7 Sampling procedure

All consecutive SLE patients seen during the study period between, 5th March 2015 and 5th June 2015 and who met the inclusion criteria were recruited into the study. Seventy one patients were assessed for eligibility, 66 fulfilled the inclusion criteria. One patient declined to give consent and was excluded. Sixty five patients were included in the study.

3.8 Clinical methods

The following steps were followed for consecutive SLE patients attending Rheumatology and Renal outpatient clinics between the period of 5th March and 5th June 2015.

i) A screening proforma was administered by the PI, (**Appendix 8)**.
ii) Those meeting the inclusion criteria were recruited into the study. The recruited SLE patients were given a study registration number which was recorded in the patient's hospital file to avoid double registration in the subsequent visits.
iii) The PI administered the study questionnaire, (**Appendix 9)** on each of the recruited patients. History included: socio-demographic data, current medication, history suggestive of anaemia, bleeding tendencies and frequent infections. The SLE clinical files

were perused to corroborate some aspects of the history specifically diagnostic criteria and current medication.

iv) The PI conducted a physical examination of patient that focussed features of SLE for example malar rash, dicoid rash, photosensitivity rash and oral ulcer. Patients were also examined for features and possible etiology of anaemia, for example pallor, jaundice, glossitis, koilonychia and any stigmata of bleeding disorder, for example petechiea and purpura.

v) The PI then used a sterile needle and syringe to collect 4mls of blood aseptically from the antecubital fossa. The blood was put into a sterile EDTA vacutainer and taken to the laboratory within 4 hours to avoid degenerative changes for measurement of CBC, ESR, reticulocyte count and PBF preparation.

3.9 Laboratory methods

The CBC was done at Kenyatta National Hospital's haematology department laboratory using the department's automated CELL-DYN 3700 analyzer. This was done within twenty-four hours of specimen collection. . ESR interpretation was undertaken at the same laboratory by the Wintrobe method. The PBF was reported in the same laboratory after staining with MAYGRUNWALD / GIEMSA stains by direct visualization on a microscope at various powers of magnification by haematologists who are supervisors for this study and the PI.

3.10 Quality Assurance

The standard operating procedures in all aspects of this study was adhered to at all times and the recommended procedure for specimen collection was adhered to at all times. This included proper phlebotomy site cleaning and the use of appropriate vacutainers. Proper labeling of the specimens and storage was adhered to at all times to minimize pre-analytical sources of errors. The CELL-DYN 3700 analyzer was calibrated according to manufacturers and Kenya Bureau of Standard recommendations because KNH as an institution is ISO certified. The KNH haematology laboratory runs daily internal quality control on all tests. KNH haematology laboratory also runs external quality controls. It participates in the WHO National External Quality Assessment Service, (NEQAS). Every tenth sample was sent to Lancet laboratory in Nairobi Kenya for counterchecking.

3.11 STUDY VARIABLES

3.11.1Dependent study variables

Hematology laboratory reffence ranges were used to define study variables

1. Anaemia: Hb level <12.0 g/dL, (in females) and < 13.5 g/dL, (in males).
2. Leucopenia – Leucocytes count $< 4 \times 10^9$/L
3. Lymphocytopenia – Lymphocyte count $< 1.5 \times 10^9$/L
4. Neutropenia- Neutrophil count $< 1 \times 10^9$/L
5. Leucocytosis – Leucocytes count $> 11 \times 10^9$/L.
6. Thrombocytopenia- Platelet count $< 150 \times 10^9$/L.
7. Thrombocytosis - platelet count $>400 \times 10^9$/l
8. ESR Normal range 0-20 mm/hr (females) 0-9mm/hr(males)

3.11.2 Independent study variables

The independent study variables included age, gender, disease duration from diagnosis and treatment modality which was categorized as NSAID, steroids, antimalarials and other disease modifying drugs (DMD)

3.12 DATA MANAGEMENT

3.12.1Data acquisition

Data was acquired through a detailed interview and the patients' files and filled into a predesigned study questionnaire, **(Appendix IV).**

3.12.2 Data Privacy

Standards to protect personal data were followed. Data collection instruments had minimum possible subject identifiers; only the first name and a serial number were entered in the study questionnaire and specimen labels.

3.12.3 Data Storage

The filled questionnaire and laboratory results forms, (data forms) were verified for completeness by the principal investigator. The data forms were kept in a secure lockable cabinet only accessible by the PI and the statistician.

The data was entered electronically using the Statistical Package for Social Sciences (SPSS) version 21, (SPSS Inc., Chicago, IL, USA). This electronic data did not bear patients' names or unique identifiers; a serial number was used instead.

Upon completion of entry, the hard copy forms were used to clean and verify correctness of the entered data and then stored safely in the lockable cabinet.

The electronic file was backed up in three compact discs and stored offsite.

3.13 Statistical Analysis

Statistical analysis was done using SPSS version 21. Analysis included descriptive statistics such as means, medians and standard deviation for continuous variables and frequency distributions for categorical variables, with their corresponding 95% confidence intervals (CI). Comparisons for continuous data was made using the t-test, and of categorical data using the chi-square test.

Prevalence of study variables, (e.g. anaemia, leucopenia and thrombocytopenia) was calculated as the propotiorn of subjects having the variable divided by the total number of subjects. Precision was indicated by 95% confidence interval (CI) limits. A p value ≤ 0.05 was considered significant. The final results are presented below in the form of tables, charts and graphs.

3.14 ETHICAL CONSIDERATIONS

The study was undertaken after approval by the department of internal medicine, university of Nairobi and the KNH - UON scientific and ethical research committee.

Patients eligible to participate in the study were included only after providing consent/assent following the process as outline.

The patients were informed that the project involves local research, the purpose of the research and the procedures of the study with full details of all the tests to be done. They were assured that participation was voluntary and no medical attention was to be denied should they decline to participate. They were also informed of the medical benefits and also physical and psychological harms to their satisfaction prior to being included in the study.

The PI assured them of full and free access to their results and therapeutic interventions were recommended where need arose, according to the accepted standards of practice.

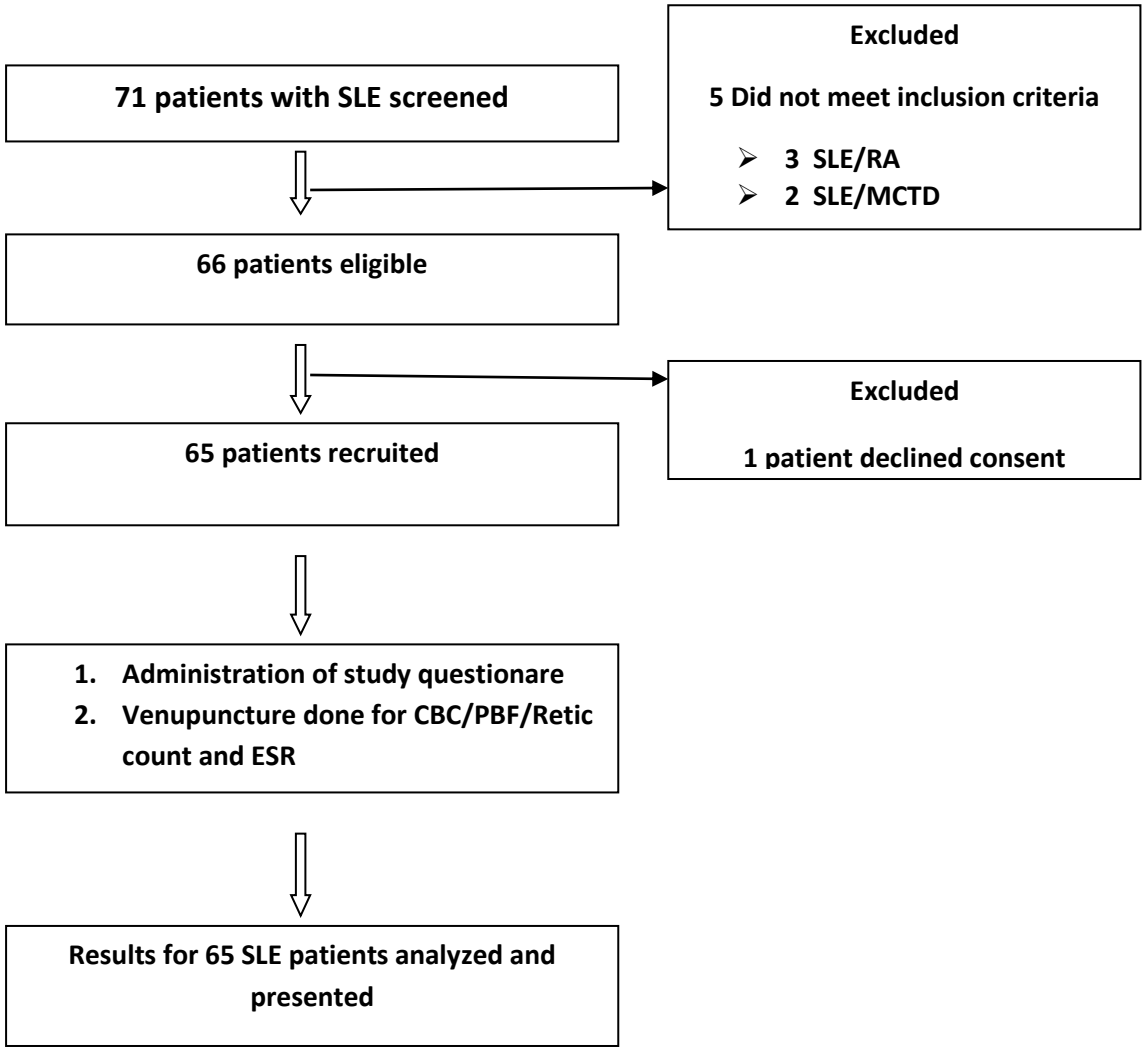
Confidentiality was strictly maintained and all data was stored securely, only revealed upon a need to know basis and all costs regarding investigations in this study was borne by the principal investigator. Following the full explanation and acceptance by the patient of the above, they were requested to sign the consent or assent form (Appendix). All patients recruited in this study underwent the standard care as offered at KNH –Rheumatology and Renal clinics. Only specimen needed for the study, (4 mls of venous blood) were obtained from the patient and study results were communicated to the physician attending to the patient.

4.0 RESULTS

In a period of 4 months (March 2015 to June 2015) 71 patients with SLE were identified, of these 66 met the ACR criteria for SLE and were recruited to the study. Three patients had SLE and Rheumatoid Arthritis (RA) while two had SLE with mixed connective tissue disease (MCTD) and were excluded. One patient was eligible but refused to give consent to have blood tests done. Final analysis included 65 patients.

Figure 2: The recruitment process

Figure 2: Patients flow chart



Baseline characteristics of the study population

Majority of patient recruited were females with a male to female ratio of 1:21.The mean age was 36.49 ±12.2 years. The youngest was 18.0 years while the oldest was 62.0 years. Mean age at diagnosis was 33 ±12.1 years and the median age was 35 years. The median duration of illness was 36 months. Majority of the patients at 55% were in the reproductive age group between 21 and 40 years. Most patients had attained post primary education at 78.5%. Majority of the patients had some form of occupation 61.5% .Most patients recruited were from rural setting at 56.9% while 43.1% were from Nairobi and its environs.

A summary of the patients' baseline characteristics of the study population are as shown in table 2.

Table 2: Baseline characteristics of the study population

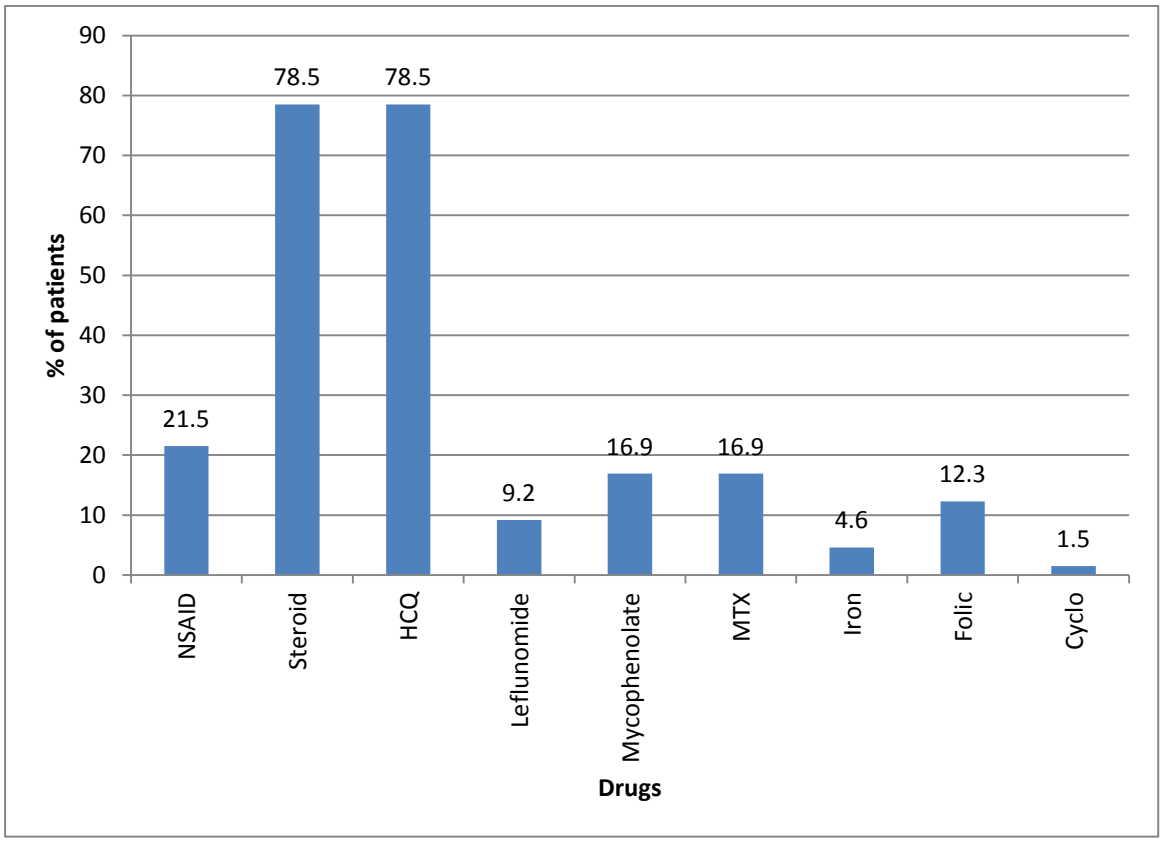
Characteristic	Frequency(%)
Sex	
Male	3 (5%)
Female	62 (95%)
Age(years)	
Mean(SD)	36. 5 (± 12)
Range	18-62
Median	35
Age distribution(years)	
>20	7 (11%)
21-40	36 (55%)
>41	22 (34%)
Age at diagnosis	
Mean(SD)	33 (±12)
Duration of disease in months	
Median(IQR)	36 (12-60)
Level of education	
Primary	14 (21.5%)
Secondary	36 (55.4%)
Tertiary	15 (23.1%)
Occupation	
Employed	15(23.0%)
Self employed	25(38.5%)
None	25(38.5%)
Residence	
Urban	28(43.1%)
Rural	37(56.9%)

Medications taken by study participants

The most commonly used disease modifying agents were steroids and hydroxychloroquine at 78.5%. A few patients at 9.2% were on leflunomide, 10.5% on mycophenolate and 10.5% on methotrexate as shown in the table above. None of the patients were on biologics. 21.5 % of patients were on NSAIDS for pain management. Only a minority of the patients 4.6% were on iron and 12.3% on folic acid. Folic acid was predominantly co- prescribed with methotrexate.

Figure 3 summarizes the distribution of medication the study population was taking at the time of the study

Figure 3: Medications taken by study participants



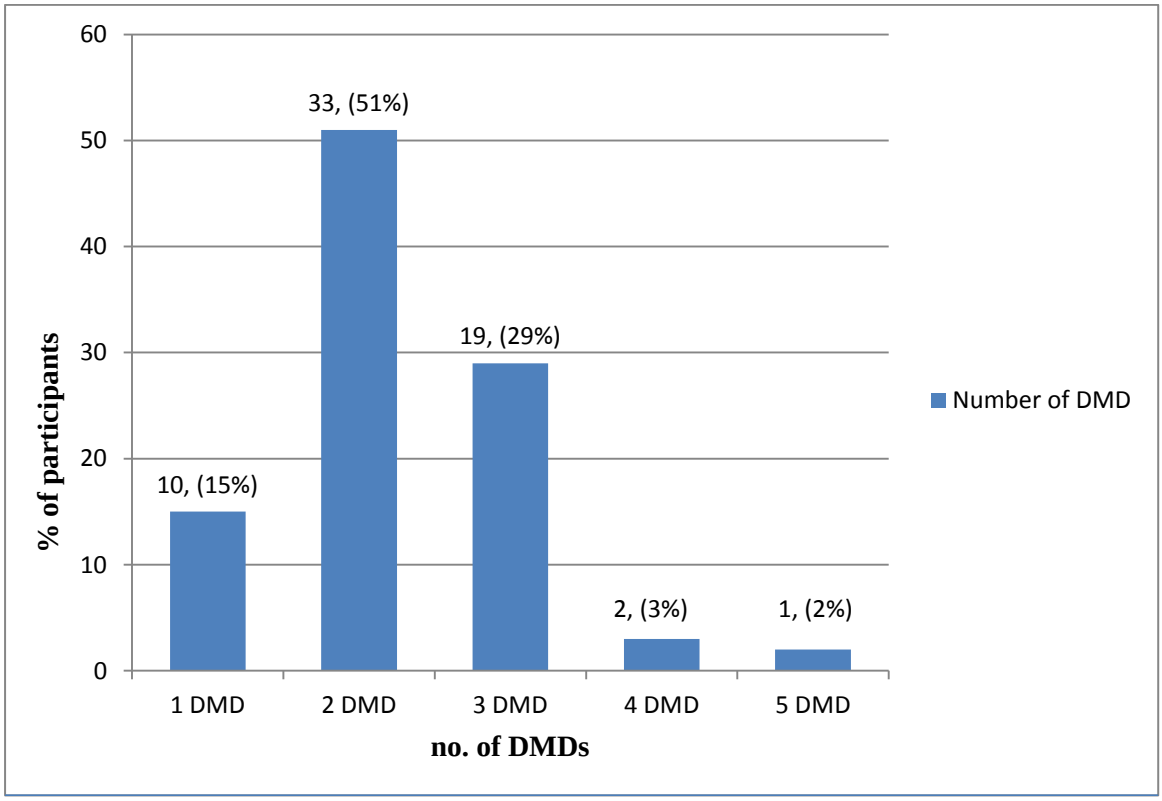
NSAID (Non Steroidal Anti-inflammatory drugs), HCQ (Hydroxychoroquine), MTX(methotrexate), Cyclo(Cyclophosphomide).

Number of disease modifying drugs taken by each study participant

The drugs were used singly or in combination. Majority of the patients 55(85%) were on combination therapy to achieve maximum therapeutic effect. Most of the patients were on two drugs at 33(51%) while only 1(2%) patient was on five drugs.

Figure 4 shows the number of disease modifying drugs that each study participant was taking.

Figure 4: Number of disease modifying drugs taken by each study participant



DMD (Disease Modifying Drugs)

HAEMATOLOGICAL PARAMETERS

The mean hemoglobin was 12g/dl with a range of 5.4-17.9g/dl. The mean WBC was 6.2 x 10^9/L with a range of 1.1-17.1X 10^9/L The mean platelet count was 263.8 X 10^9/L with a range of 28-521 X 10^9/L. The mean ESR was 30mmhr with a range of 1-122mm.

Table 3 summarizes results of haematological parameters in the study population.

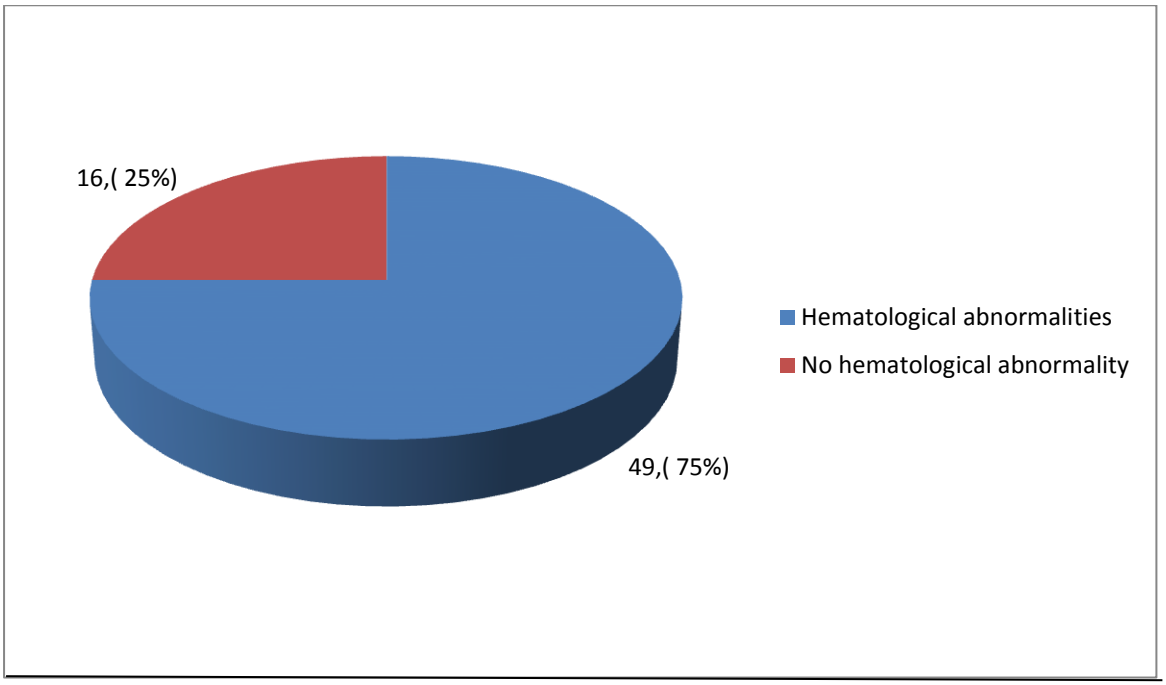
Table 3: Haematological parameters of study participants (n=65)

Parameter	**Median(Range)**	**Mean±SD**	**Ref range(Male)**	**Ref range(female)**
RBC(x10^{12}/L)	4.5 (1.9-5.9)	4.3 (0.8)	4-6	3.5-6.5
Hemoglobin(g/dl)	12.4 (5.4-17.9)	12 (2.6)	13.5-18	12-15
WBC (x 10^9 /L)	5 (1.1-17.1)	6.2 (3.3)	4-11	4-11
Neutrophil (x 10^9/L)	2.8 (0.1-14.8)	3.7 (2.7)	2.0-7.5	2.0-7.5
Lymphocytes (x 10^9/L)	1.6 (0.3-6.4)	1.8 (1.1)	1.5-4.0	1.5-4.0
Platelets (x 10^9 /L)	266 (28-521)	263.8 (107)	150-400	150-400
ESR(mmhr)	30 (1-122)	38.2 (28)	0-9	0-20

PREVALENCE OF HAEMATOLOGICAL ABNORMALITIES

Forty nine (49) study participants (75%; CI-63.1%-85.2%) had some haematological abnormality as shown in Figure 5.

Figure 5: Prevalence of haematological abnormalities, n=65

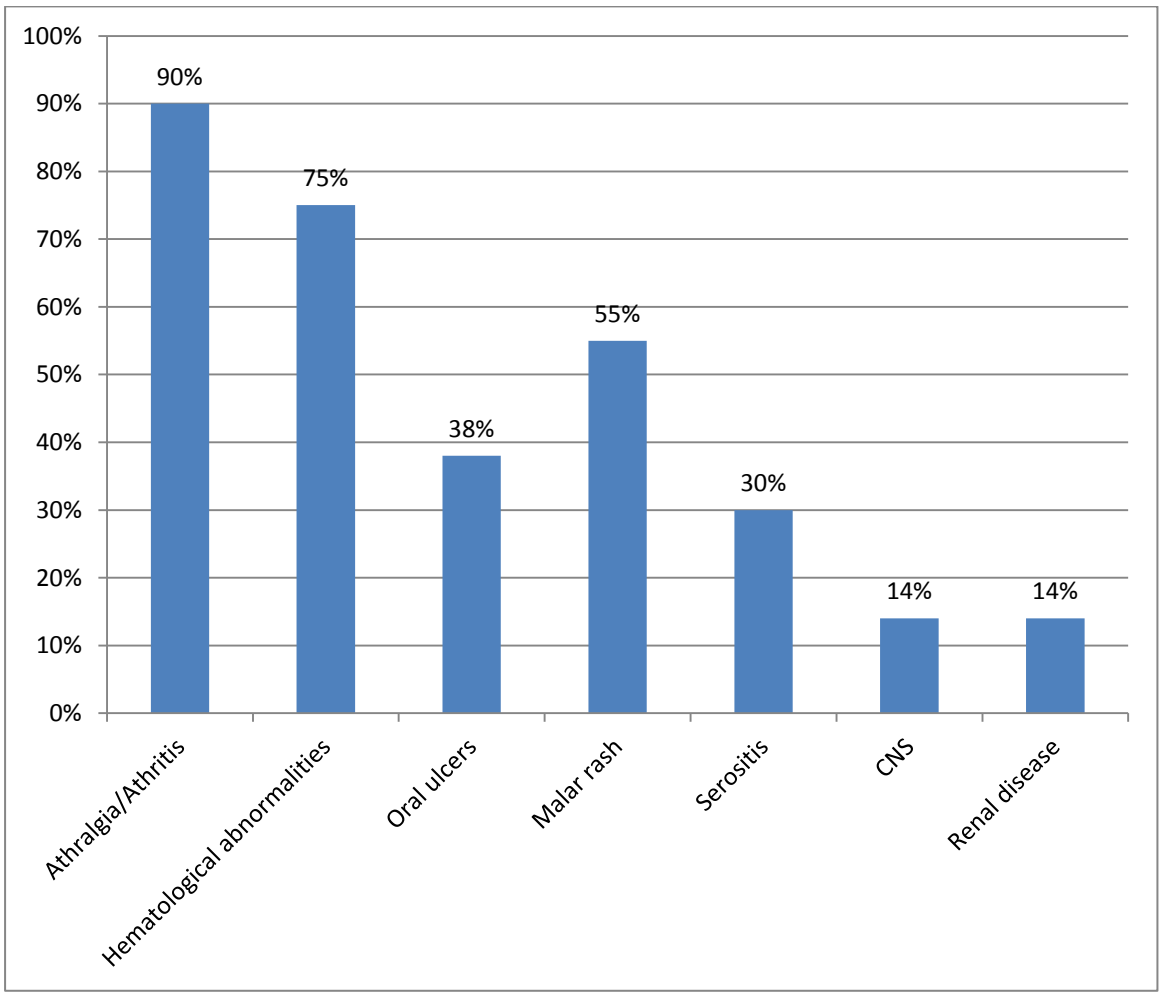


Distribution of clinical features of SLE in the study population

A comparison of haematological abnormalities with other clinical features revealed that athralgia/arthritis was the most common presentation at 90% followed closely by haematological abnormalities at 75%. Only 15% of patients presented with CNS and renal disease.

Figure 6 shows the distribution of some clinical features in the study population.

Figure 6: Distribution of clinical features of SLE in the study population n=65



CNS (Central Nervous System)

Prevalence of various haematological abnormalities

The prevalence of anaemia was 43.1 %(95% CI 30.7-55.4 %) in the study population. 26.2% (95% CI 15.2-37.1%) of patients had leucopenia mainly lymphocytopenia (44.6%). Nine 9.2 % (95% CI 2-16.5%) participants had leucocytosis mainly neutrophilia. Minority of the patients had platelet abnormalities with 20% (95% CI 10-30%) having thrombocytopenia and 12.3 %(95% CI 4.1-20.5) having thrombocytosis.

Table 4 summarizes the different types of haematological abnormalities in participants

Table 4: Prevalence of various haematological abnormalities amongst study participants, n=65

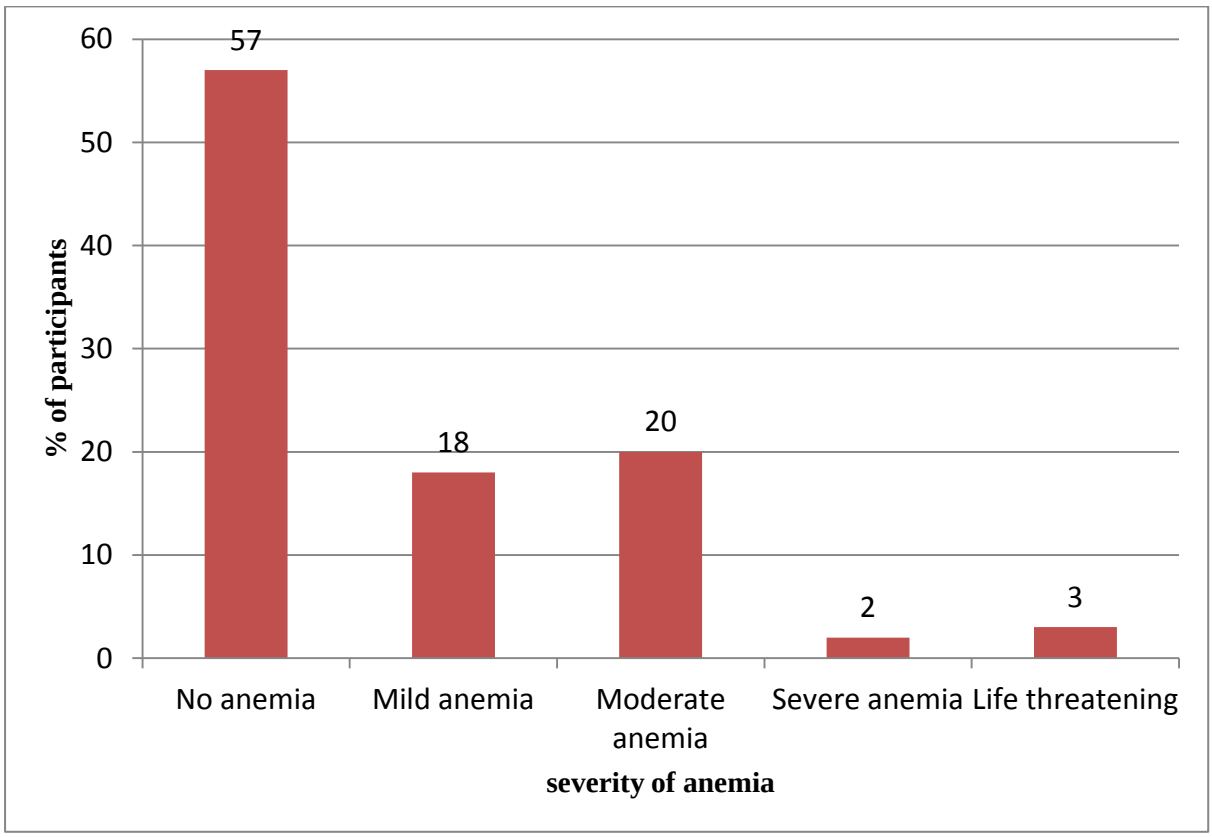
Abnormality	Frequency	Percentage	95% CI
Anaemia	28	43.1%	30.7-55.4
Leucopenia	17	26.2%	15.2-37.1
❖ Neutropenia	18	27.7%	17.3-40.2
❖ Lymphocytopenia	29	44.6%	32.2-57.5
Leucocytosis	6	9.2%	2-16.5
❖ Neutrophilia	6	9.2%	2-16.5
❖ Lymphocytosis	3	4.6%	112.9
Thrombocytopenia	13	20%	10-30
Thrombocytosis	8	12.3%	4.1-20.5

Severity of anaemia

Majority of the participants 12(18%) and 13(20%) had mild to moderate anaemia respectively while 2(3%) had life threatening anaemia

Figure 7 shows the severity of anaemia in study population

Figure 7: Severity of anaemia in the study participants (n=65)

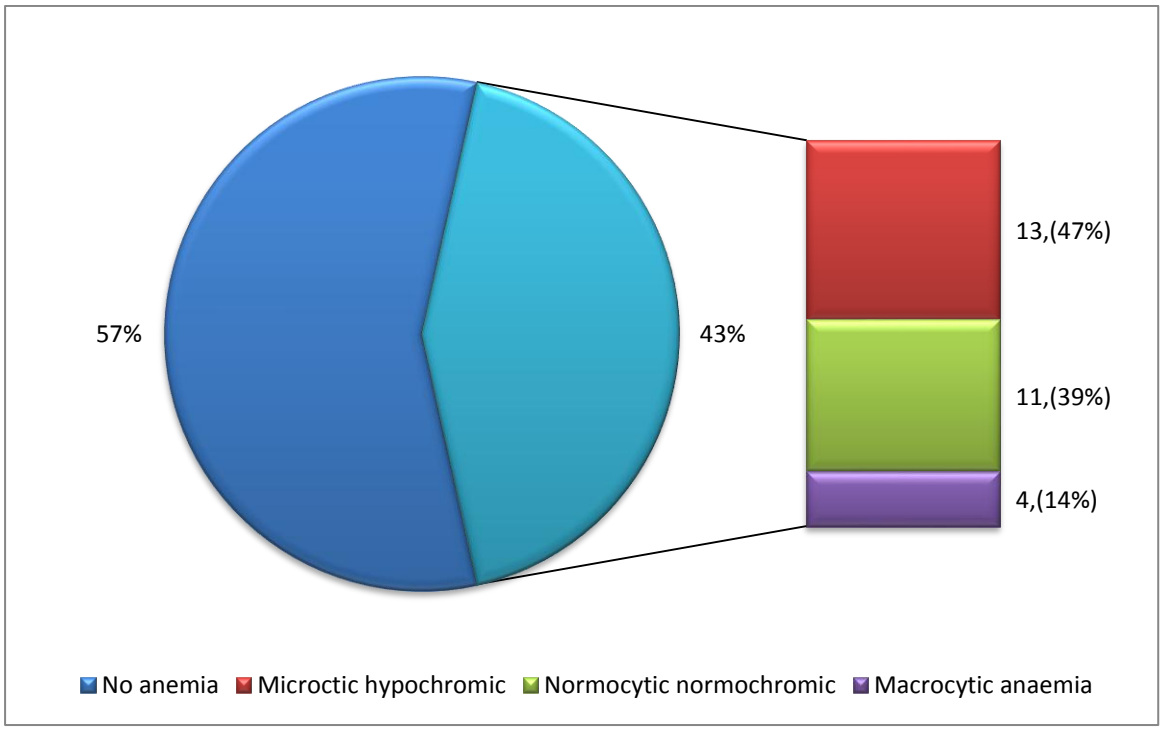


Type of anaemia

Of the 28(43.1%) of the patients who had anaemia majority had a microcytic hypochromic anaemia 13 (47%) while 11(39%) had a normocytic normochromic anaemia Four participants 4(14%) had macrocytic anaemia.

Figure 8 below shows the distribution of types of anaemia in the study population.

Figure 8: Type of anaemia in study participants' n=65

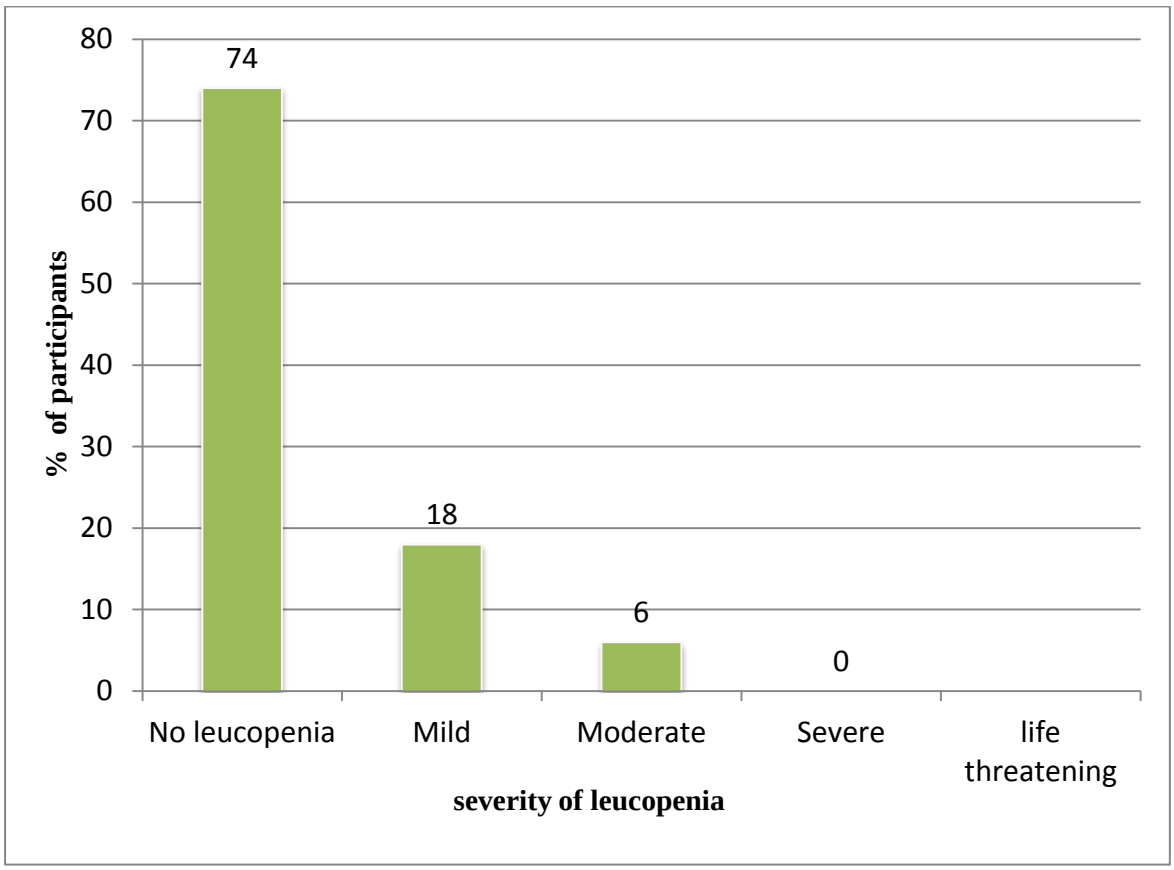


Severity of leucopenia

Majority of the participants had mild leucopenia 12 (18%) while only 4(6%) had moderate leucopenia.

Figure 9 shows severity of leucopenia

Figure 9: Severity of leucopenia in the study participants (n=65)

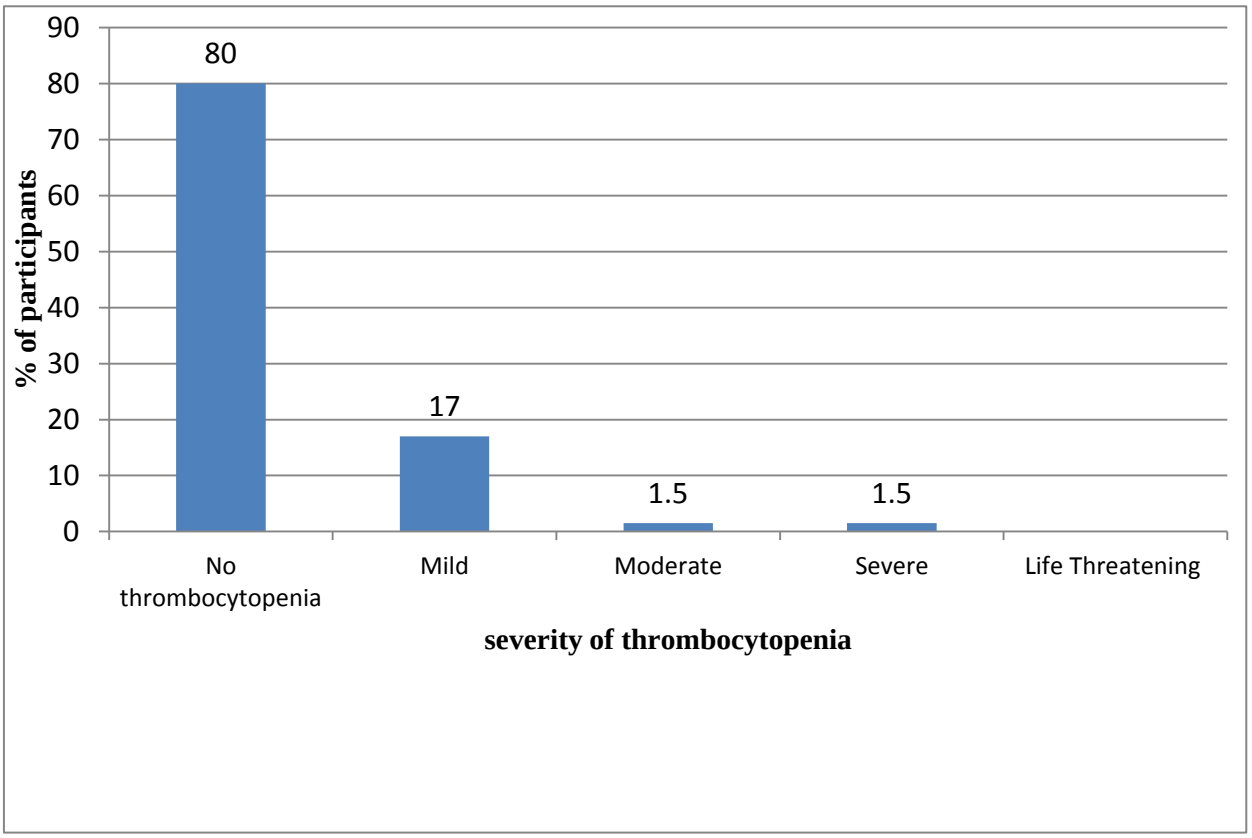


Severity of thrombocytopenia

Majority of the participants 11(17%) had mild thrombocytopenia. Only 1(1.5 %) of the participant had severe thrombocytopenia. None of the participants had life threatening thrombocytopenia

Figure 10 shows severity of thrombocytopenia in the study population

Figure 10: Severity of thrombocytopenia in the study participant



Anaemia association with selected patient characteristics

There was a statistically significant increased risk of being anaemic for the patients who had the disease for less than one year (p=0.035), OR = 3.5 (95% CI 0.9-15.1). There was no significant association of anaemia with the patient's sex or treatment regimen as shown by the p values which were >0.05.

Table 5 shows association of anaemia with patient characteristics, n=65

Table 5: Anaemia association with selected patient factors

Characteristic	**Anaemic(n=28)**	**Not anaemic (n=37**	**P value**
Sex Male Female	 2(7.1) 26(92.9)	 1(2.7) 36(97.3)	 0.573
Duration of illness 1-11 months 12-59 months 60 months and above	 10(35.7) 10(35.7) 8(28.6)	 5(13.5) 22(59.5) 10(27.0)	 0.035 0.058 0.89
NSAID Yes No	 5(17.9) 23(82.1)	 9(24.3) 28(75.7)	 0.53
Steroid Yes No	 22(78.6) 6(21.4)	 29(78.4) 8(21.6)	 0.985
Hydroxychloroquine Yes No	 23(82.1) 5(17.9)	 28(75.7) 9(24.3)	 0.53
Other DMD Yes No	 16(57.1) 12(42.9)	 22(59.5) 15(40.5)	 0.851

Leucopenia association with selected patient characteristics

There was no significant association between leucopenia and other patients' factors as shown below by the p values >0.05.

Table 6 below shows association of leucopenia with selected patient factors

Table 6: Association of leucopenia with some participant characteristics, n=65

Characteristic	Leucopenic(n=17)	Not Leucopenic (n=48)	P value
Sex Male Female	 0(0.0) 17(100.0)	 3(6.3) 45(93.8)	 0.561
Duration of illness 1-11 months 12-59 months 60 months and above	 3(17.6) 10(58.8) 4(23.5)	 12(25.0) 22(45.8) 14(29.2)	 0.536 0.357 0.655
NSAID Yes No	 3(17.6) 14(82.4)	 11(22.9) 37(77.1)	 0.650
Steroid Yes No	 11(64.7) 6(35.3)	 40(83.3) 8(16.7)	 0.108
Hydroxychloroquine Yes No	 15(88.2) 2(11.8)	 36(75.0) 12(25.0)	 0.254
Other DMD Yes No	 8(47.1) 9(52.9)	 30(62.5) 18(37.5)	 0.267

Thrombocytopenia association with selected patient characteristics

Thrombocytopenia was not significantly associated with sex, duration of illness or treatment.

Table 7 shows co-relation of thrombocytopenia with patient characteristics

Table 7: Thrombocytopenia association with selected characteristics, n=65

Characteristics	**Thrombocytopenic (n=13)**	**Not Thrombocytopenic(n=52)**	**P value**
Sex Male Female	0(0.0) 13(100.0)	3(5.8) 49(94.2)	1.000
Duration of illness 1-11 months 12-59 months 60 months and above	2(15.4) 8(61.5) 3(23.1)	13(25.0) 24(46.2) 15(28.8)	0.462 0.321 0.678
NSAID Yes No	1(7.7) 12(92.3)	13(25.0) 39(75.0)	0.175
Steroid Yes No	9(69.2) 4(30.8)	42(80.8) 10(19.2)	0.365
Hydroxychloroquine Yes No	8(61.5) 5(38.5)	43(82.7) 9(17.3)	0.097
Other DMD Yes No	8(61.5) 5(38.5)	30(57.7) 22(42.3)	0.801

5.0 DISCUSSION

The total number of patients identified with SLE for a period of 4 months was 71 of whom 65 met the ACR criteria for SLE. This number is almost similar to that seen by Shiruli et al (65) and Odhiambo et al (75)

The mean age of study population was 36.4years and the mean age at diagnosis was 33 years. The male to female ratio was 1:21.These findings are similar to that of other studies done both locally and in the African continent (65, 75).

Many of the participants in this study were drawn from wider catchment area of KNH with Nairobi contributing more than a third of the total population (43.1%) while most (56.9%) of the participants came from a rural setting.

The most common drug used in lupus treatment is HCQ (78). Majority of the patients (78.5%) were on hydroxychloroquione and a similar proportion (78.5%) were on steroid therapy. Preference for use of HCQ can be attributed to its affordability and its proven benefits in SLE (79). Active disease is treated with steroids and therefore majority of our patients probably had active disease

5.1 Haematological abnormalities

In this study haematological abnormalities were the second most common manifestations (75%) of SLE after athralgia and arthritis. Anaemia was the most common abnormality present in 43% of patients followed by leucopenia (26%) and thrombocytopenia 20%. Severe haematological involvement has been associated with significant CNS and renal disease (88) and this raises a concern of possible severe disease in our SLE population.

These figures are comparable to what has been found in other studies conducted in other parts of the world. In Nigeria Houman et al (16) found a prevalence rate of 81%. Several studies done in India by Sasidharan et a l and Agrawal et al found prevalence rate of 82% and 72.4% respectively(11,12). In two multicenter French and Turkey studies that looked at childhood onset lupus they found the most common initial manifestation of SLE was haematological disorder (80, 81). Western literature also indicates that haematological abnormalities are common presentations of SLE (82). These findings support our observation

and emphasizes that haematological abnormalities are a common manifestation of SLE patients.

5.2 Anaemia

In this study anaemia was present in 43.1% of the patients. Although the mean hemoglobin was 12g/dl, and the median was 12.4g/dl, the hemoglobin range was 5.4 - 17.9g/d. The aetiology of anaemia in SLE is usually heterogeneous and may result from immune and non immune mechanisms. Some of the possible causes of anaemia in our patients are anaemia of chronic disease (ACD), iron deficiency anaemia (IDA), autoimmune hemolytic anaemia (AIHA), and drug induced myelotoxicity. Other rare causes eg aplastic anaemia and myelofibosis may also have contributed to anaemia in our SLE population.ACD in our population may have been due to chronic inflammation and renal disease while IDA may have been due to menorrhagia as most of our participants were young females in the reproductive age group, gastrointestinal bleeding due to the frequent use of NSAIDS and steroids, nutritional and possibly due to hookworm infestations.

The prevalence of anaemia in this study is lower than that found by Sasidharan et al (11) in India. They found an anaemia prevalence of 62%. This could be attributed to co-existing high prevalence of anaemia in India (approximately 50%) in the rural areas as compared to Kenya's prevalence of 38%, (WHO global data base on anaemia burden (83). The reason given for the high anaemia burden among Indians are nutritional related being predominantly vegetarian society with limited nutritional iron sources and chronic blood loss from hookworm infestations in rural areas as well as presence of thalassaemia syndromes (84). This study population was predominantly urban. Other possible cause could be due to the proportion of patients on DMARDS in the two study populations. Additionally study focussed on a highly preselected population which was being followed up in a tertiary setting with improved care and ability to access quality health care.

Anaemia in SLE is largely multifactorial but morphologically most of the study population had microcytic hypochromic anaemia. Microcytic anaemia is usually due to either iron deficiency anaemia (IDA) or less commonly anaemia of chronic disease (ACD).These findings differ from other studies in other centers where normocytic normochronic anaemia

has been found to be most common(11,15). The high prevalence of microcytic anaemia can be explained by increased number of patients on steroids and NSAIDS, inadequate control of disease and possibly menstrual loss due to our study population consisting predominantly of young females in the reproductive age group.

There was a significant association of anaemia and disease duration with patients who had the disease less than one year more likely to be anemic. This population probably had active disease. Newly diagnosed SLE patients are normally started on disease modifying drugs which are then titrated over months. The disease response and therefore treatment to target may take a long time from the time of diagnosis of disease. This study did not demonstrate any association between anaemia and NSAID /Steroid use. This could be explained by regular prescription of proton pump inhibitors among KNH patients on long term NSAID and steroid, which prevents gastrointestinal blood loss.

Despite the high prevalence of moderate anaemia (20%) in our study population only a small proportion of patients were on treatment with hematinics, such as iron (4.3%) and folic acid (12.2%) indicating that anaemia in this group was largely untreated. Folic acid was co-prescribed with methotrexate. None of the patients was on erythropoesis stimulating agents. Unfortunately serum ferritin, folate and vitamin B 12 levels were not checked to further characterize anaemia.

5.3 White cell abnormalities

The mean white cell count in the study population was 6.2 x 10^9/L a median of 5 and a range 1.1-17.7 x 109/l. However in Africans a lower limit of normal WBC of 2.6 X 10^9/ L has been described (41).

5.3.1 Leucopenia

The prevalence of leucopenia in this study population was 26.2% which was mainly due to lymphopenia and neutropenia. Majority of the participants had mild leucopenia at 20%. Immune destruction of antibody coated WBC and active disease may have contributed to leucopenia in our population. Several studies have shown leucopenia is associated with active disease and steroid therapy (20, 21). Neutropenia in our population was largely multifactorial; it may have been due to immune mediated mechanism by anti-neutrophil antibodies, medications (e.g. azathioprine), bone marrow dysfunction, or Hypersplenism. Several studies have demonstrated these possible mechanisms (20, 21, 44, 45, and 48)

The leucopenia was more pronounced than in the Indian study by Sasidharan et al (11). Sasidharan's study found a leucopenia prevalence of 15.7% while Agrawal et al found a prevalence of 18.4% (15). This difference in leucopenia could be attributable to the racial differences between the two populations. Black Africans have been found to have a slightly lower WBC count than other races (41). We found neutropenia in 27.7% of patients, which was slightly lower than what has been described in other studies. Budman DR et al (48) described a prevalence rate of 50 to 60 %(68). We attributed our lower prevalence rate to the small number of patients who were on azathioprine at 23.1 % and cyclophosphomide at 1.5%.

Lymphocytopenia was present in 44.6% of our study population. We attributed these to possible active disease and possibly due to large proportion of patients on steroids. The prevalence of lymphocytopenia in SLE has been shown to be variable ranging from 20 to 81% and its degree correlates with disease activity (26). Most recently a study by Agrawal et al(4) found a prevalence rate of 48.3% which is almost similar to our finding.

5.3.2 Leucocytosis

Leucocytosis was present in 9.2% of study population, majorly driven by neutrophilia. We attributed this to the high proportion of patients who were on steroids. Other possible explanation is the patients may have had active infection (54).

5.4 Platelet abnormalities

The mean platelet cell count in the study population was 263.8x 10^9/L,a median of 266 X 10^9/L and a range 28-521 X 10^9/ L. These figures are much higher than in normal healthy adults. Mukibi et al (85) found a mean platelet count of 200 x 10^9/ L in healthy Kenyan adults. A more recent study by Rajab et al (86) on haematological parameters in healthy Kenyan blood donors found a mean platelet count of 241.2+- 86.6 X 10^9/ L with median value of 235.1x 10^9/ L. It can therefore be inferred from previous studies that patients with SLE have slightly higher platelet counts. This could be a general reactive response to chronic inflammation in our SLE population.

5.4.1 Thrombocytopenia

Several mechanism may have contributed to thrombocytopenia in our population among them immune destruction, drugs, infections and possibly bone marrow suppression. . Thrombocytopenia in our study population was most of the time mild and benign and not associated with any overt bleeding. These patients did not require any specific treatment. Nevertheless since thrombocytopenia is an independent risk factor for mortality (20, 60), the sub-group of patients with thrombocytopenia will require more aggressive management and more frequent follow up.

In their Indian study, Sasidharan et al found a thrombocytopenia prevalence of 39.8% in SLE patients (11).The prevalence of thrombocytopenia in this study was 20% which was significantly lower. This difference could be partly due to the fact that their study looked at thrombocytopenia as an initial presentation of SLE while in this study platelets counts were measured among participating patients at different times in the course of their illness. In addition majority of our patients were already on treatment and had achieved some control of the disease. Agrawal et al in their study found a lower prevalence of thrombocytopenia of 14.9% (15).However it is notable that in Agrawal's study, thrombocytopenia was defined as a platelet count below 100 $x10^9$/ L as opposed to this study where we defined thrombocytopenia as a platelet count below 150 x 10^9/ L

5.4.2Thrombocytosis

There were 8 cases (12.3%) of thrombocytosis of which 3 cases had confirmed APLAS. The other 5 cases had not been investigated for APLAS. A plausible explanation for this is a possible reactive thrombocytosis in our study population due to the high prevalence of microcytic hypochromic anaemia. Other possible causes described in literature are active disease and autosplenectomy (61).However none of the participants with thrombocytosis had features of hyposplenism in their blood films (such HJ bodies and target cells) and imaging to establish autosplenectomy was beyond the scope of the study.

Our prevalence of 12.3% was significantly higher than reported in other studies. Castellino et al (61) found a prevalence of 3.7% in Caucasians with SLE. These differences may be attributed to racial differences.

5.5 Erythrocyte sedimentation rate

The mean ESR 38.2mm/hr with a median of 30mm/hr and range of 1-122mm/hr. Majority of the patients (66%) had an elevated ESR. This may be an indicator that most of our patients had active disease as several studies have shown that elevated levels of ESR may be associated with disease activity and accumulated damage (64).

6.0 CONCLUSION

Haematological abnormalities are the second most common manifestation of the disease after arthritis and arthralgia among SLE patients on follow up at Kenyatta National Hospital Rheumatology and Renal clinic. Though majority of these abnormalities were mild to moderate and clinically asymptomatic, the proportions of anaemia, leucopenia and thrombocytopenia were substantially high. There was a significant association between anaemia and duration of disease.

6.1STUDY LIMITATIONS

I. Relative changes in the blood counts could have been missed as only a one time count was done in the study.
II. Recall bias was a major challenge encountered this may affect the reporting of duration of disease.

6.2 RECOMMENDATIONS

i. A larger longitudinal study to correlate thrombocytopenia and leucopenia with demographics and drugs. This may require a multicenter approach to avail sufficient number of patients.
ii. A study to correlate these haematological abnormalities with disease activity in patients with SLE, which may be useful as surrogate makers of disease activity in resource constrained settings.
iii. Long term follow up of subgroup of patients who had thrombocytopenia to determine the long term outcome.

REFERENCES

1. Arbucle MR, James JA, Dennis GJ et al. Rapid clinical progression to diagnosis among African American men with systemic lupus erythematosus Lupus.2003;12:99-106
2. Pons Estel GJ, Alarcon GS, Scofield L et al. Understanding the epidemiology and progression of systemic lupus erythematosus. Semin Arthritis Rheum 2010 39:257.
3. Dessein PH, Gledhill RF, Rossouw DS Systemic Lupus Erythematosus in Black South Africans. South Afri Med J.1985: 15(3) 387-389.
4. Adelowo OO, Oguntona SA Pattern of Systemic Lupus erythematosus among Nigerians. Clin Rheumatol 2009; 28: 699-703.
5. Ekwom PE. Systemic Lupus erythematosus (SLE) at the Kenyatta National Hospital. Clin Rheumatol 2013; 32(8): 1215-1217
6. O. Oyoo Rheumatic disorders in Kenya: Spectrum of disease Health Line Dec 2004.Vol 8 No 4
7. P. K. Sasidharan, M. Bindya, and K. G. Sajeeth Kumar Haematological Manifestations of SLE at Initial Presentation: Is It Underestimated? ISRN Hematol. 2012; 961872.
8. Hochberg MC. Updating the American College of Rheumatology revised criteria the classification of systemic lupus erythematosus.Arthritis Rheum 1997; 40(9): 1725
9. Michelle Petri, M.D. M.P.H.,[1] Ana-Maria Orbai, M.D.,[1] Graciela S. Alarcón. Et al Derivation and Validation of Systemic Lupus International Collaborating Clinics Classification Criteria for Systemic Lupus Erythematosus Arthritis Rheum. Aug 2012; 64(8): 2677–2686.
10. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum1996; 39:363-9.
11. P. K. Sasidharan, "SLE as a haematological disease," in Hematolgy Today, M. B. Agarwal, Ed., pp. 953–966, Vikas Publications, Mumbai, India, 2010.
12. S. Singh, L. Kumar, R. Khetarpal et al., "Clinical and immunological profile of SLE: some unusual features," Indian Pediatrics, vol. 34, no. 11, pp. 979–986, 1997.
13. J. C. Bennett, J. Claybrook, H. Kinsey, and H. L. Holley, "The clinical manifestations of systemic lupus erythematosus. A study of forty-five patients," Journal of Chronic Diseases, vol. 13, no. 5, pp. 411–425, 1961

14. Al Arfaj AS, Khalil N. Clinical and immunological manifestations in 624 SLE patients in Saudi Arabia. Lupus 2009; 18(5): 465-73.
15. Agrawal SR, Tiewsoh I, Rajput A, Jain A et al. A crossectional hospital based study of clinical and immunological profile of systemic lupus erythematosus patients from central rural India. Indian J Allergy Asthma Immunol 2013; 27:33-7
16. Houman MH, Smiti-Khanfir M, Ben Ghorbell I and M Miled.Systemic lupus erythematosus in Tunisia: demographic and clinical analysis of 100 patients. Lupus. 2004; 13:204–11
17. Bashal F. Haematological disorders in patients with systemic lupus erythematosus. Open Rheumatol J. 2013 Oct 18; 7:87-95
18. Michalis Voulgarelis, Styliani I G Kokori, John P A Ioannidis et al in systemic lupus erythematosus: aetiological profile and the role of erythropoietin Ann Rheum Dis 2000; 59:217–222
19. Fernandez M, Alarcón GS, Apte M, et al Systemic lupus erythematosus in a multiethnic US cohort: XLIII.The significance of thrombocytopenia as a prognostic factor Arthritis Rheum. 2007 Feb; 56(2):614-21.
20. Nossent JC, Swaak AJ Prevalence and significance of haematological abnormalities in patients with systemic lupus erythematosus. Q J Med. 1991 Jul; 80(291):605-12
21. Keeling DM, Isenberg DA. Haematological manifestations of systemic lupus erythematosus. Blood Rev 1993; 7; 199
22. Von Feldt JM. Systemic lupus erythematosus. Recognizing its various presentations. Postgrad Med 1995; 97:79, 83, 86 .
23. Estes D, Christian CL.The natural history of systemic lupus erythematosus by prospective analysis. Medicine (Baltimore) 1971; 50:85.
24. Fessler BJ, Boumpas DT.Severe major organ involvement in systemic lupus erythematosus. Diagnosis and management. Rheum Dis Clin North Am 1995; 21:81.
25. Laurence, J, Wong, JE, Nachman, R.The cellular hematology of systemic lupus erythematosus. In: Systemic Lupus Erythematosus, 2d Ed, Lahita, RG (Ed), Churchill Livingstone, New York 1992
26. Shoenfeld, Y, Ehrenfeld, M. Hematologic manifestations. In: The Clinical Management of Systemic Lupus Erythematosus, 2d Ed, Schur, PH (Ed), Lippincott, Philadelphia 1996.

27. Liu H, Ozaki K, Matsuzaki Y, et al. Suppression of haematopoiesis by IgG autoantibodies from patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 1995; 100:480.
28. Habib GS, Saliba WR, Froom P. Pure red cell aplasia and lupus. Semin Arthritis Rheum 2002; 31:279.
29. Voulgarelis M, Kokori SI, Ioannidis JP, et al. in systemic lupus erythematosus: aetiological profile and the role of erythropoietin. Ann Rheum Dis 2000; 59:217.
30. Giannouli S, Voulgarelis M, Ziakas PD, Tzioufas AG. in systemic lupus erythematosus: from pathophysiology to clinical assessment. Ann Rheum Dis 2006; 65:144.
31. Schett G, Firbas U, Füreder W, et al. Decreased serum erythropoietin and its relation to anti-erythropoietin antibodies in of systemic lupus erythematosus. Rheumatology (Oxford) 2001; 40:424.
32. Hara A, Wada T, Kitajima S, et al. Combined pure red cell aplasia and autoimmune hemolytic anaemia in systemic lupus erythematosus with anti-erythropoietin autoantibodies. Am J Hematol 2008; 83:750
33. Winkler A, Jackson RW, Kay DS, et al. High-dose intravenous Cyclophosphomide treatment of systemic lupus erythematosus-associated aplastic anaemia. Arthritis Rheum 1988; 31:693.
34. Brooks BJ Jr, Broxmeyer HE, Bryan CF, Leech SH. Serum inhibitor in systemic lupus erythematosus associated with aplastic anaemia. Arch Intern Med 1984; 144:1474.
35. Roffe C, Cahill MR, Samanta A, et al. Aplastic in systemic lupus erythematosus: a cellular immune mechanism? Br J Rheumatol 1991; 30:301
36. Nesher G, Hanna VE, Moore TL, et al. Thrombotic microangiographic hemolytic anaemia in systemic lupus erythematosus. Semin Arthritis Rheum 1994; 24:165.
37. Jeffries M, Hamadeh F, Aberle T, et al. Haemolytic in a multi-ethnic cohort of lupus patients: a clinical and serological perspective. Lupus 2008; 17:739.
38. Matsuyama T, Kuwana M, Matsumoto M, et al. Heterogeneous pathogenic processes of thrombotic microangiopathies in patients with connective tissue diseases. Thromb Haemost 2009; 102:371.
39. George JN, Vesely SK, James JA. Overlapping features of thrombotic thrombocytopenic purpura and systemic lupus erythematosus. South Med J 2007; 100:512

40. Sultan SM, Begum S, Isenberg DA. Prevalence, patterns of disease and outcome in patients with systemic lupus erythematosus who develop severe haematological problems. Rheumatology (Oxford) 2003; 42:230.
41. Anyaegbu CC, Okpala IE, Aken Ova YA et al Peripheral blood neutrophil count and candidacidal activity correlate with the clinical severity of sickle . Eur J Hematol 1998.60:267-268
42. Ronnblom L. Potential role of IFNa in adult lupus. Arthritis Res Ther 2010; 12(Suppl. 1): S3.
43. Wataru M, Masuki Y, Higashimoto I, *et al*. TNF-related apoptosis inducing ligand is involved in neutropenia of systemic lupus erythenatosus. Blood 2004; 104 (1) 184-91
44. Budman DR, Steinberg AD. Hematologic aspects of systemic lupus erythematosus. Current concepts. Ann Intern Med 1977; 86:220.
45. Martínez-Baños D, Crispín JC, Lazo-Langner A, Sánchez-Guerrero J. Moderate and severe neutropenia in patients with systemic lupus erythematosus. Rheumatology (Oxford) 2006; 45:994.
46. Perez HD, Lipton M, Goldstein IM. A specific inhibitor of complement (C5)-derived chemotactic activity in serum from patients with systemic lupus erythematosus. J Clin Invest 1978; 62:29.
47. Abramson SB, Given WP, Edelson HS, Weissmann G. Neutrophil aggregation induced by sera from patients with active systemic lupus erythematosus. Arthritis Rheum 1983; 26:630.
48. Rivero SJ, Díaz-Jouanen E, Alarcón-Segovia D. Lymphopenia in systemic lupus erythematosus. Clinical, diagnostic, and prognostic significance. Arthritis Rheum 1978; 21:295.
49. Vilá LM, Alarcón GS, McGwin G Jr, et al. Systemic lupus erythematosus in a multiethnic US cohort, XXXVII: association of lymphopenia with clinical manifestations, serologic abnormalities, disease activity, and damage accrual. Arthritis Rheum 2006; 55:799.
50. Winfield JB, Winchester RJ, Kunkel HG. Association of cold-reactive antilymphocyte antibodies with lymphopenia in systemic lupus erythematosus. Arthritis Rheum 1975; 18:587.
51. Amasaki Y, Kobayashi S, Takeda T, et al. Up-regulated expression of Fas antigen (CD95) by peripheral naive and memory T cell subsets in patients with systemic lupus

erythematosus (SLE): a possible mechanism for lymphopenia. Clin Exp Immunol 1995; 99:245.

52. Isenberg DA, Patterson KG, Todd-Pokropek A, et al. Haematological aspects of systemic lupus erythematosus: a reappraisal using automated methods. Acta Haematol 1982; 67:242.
53. Camussi G, Tetta C, Coda R, Benveniste J. Release of platelet-activating factor in human pathology. I. Evidence for the occurrence of basophil degranulation and release of platelet-activating factor in systemic lupus erythematosus. Lab Invest 1981; 44:241
54. Boumpas DT, Chrousos GP, Wilder RL, et al. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. Ann Intern Med 1993; 119:1198.
55. Pujol M, Ribera A, Vilardell M, et al. High prevalence of platelet autoantibodies in patients with systemic lupus erythematosus. Br J Haematol 1995; 89:137.
56. Michel M, Lee K, Piette JC, et al. Platelet autoantibodies and lupus-associated thrombocytopenia. Br J Haematol 2002; 119:354.
57. Nakamura M, Tanaka Y, Satoh T, et al. Autoantibody to CD40 ligand in systemic lupus erythematosus: association with thrombocytopenia but not thromboembolism. Rheumatology (Oxford) 2006; 45:150.
58. Wang GJ.et al Clinical features of haematological abnormality in SLE-related haematological disorders. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2002 Aug; 10(4): 359-61.
59. Karpatkin S. Autoimmune thrombocytopenic purpura. Blood 1980; 56:329.
60. Ziakas PD, Giannouli S, Zintzaras E, et al. Lupus thrombocytopenia: clinical implications and prognostic significance. Ann Rheum Dis 2005; 64:1366.
61. Castellino G, Govoni M, Prandini N, et al. Thrombocytosis in systemic lupus erythematosus: a possible clue to autosplenectomy? J Rheumatol 2007; 34:1497.
62. Lambotte O, Khellaf M, Harmouche H, et al. Characteristics and long-term outcome of 15 episodes of systemic lupus erythematosus-associated hemophagocytic syndrome. Medicine (Baltimore) 2006; 85:169.
63. Esdaile JM, Abrahamowicz M, Joseph L, et al. Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus. Why some tests fail. Arthritis Rheum 1996; 39:370.

64. Vilá LM, Alarcón GS, McGwin G Jr, et al. Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXIX. Elevation of erythrocyte sedimentation rate is associated with disease activity and damage accrual. J Rheumatol 2005; 32:2150.
65. Shiruli BC, Oyoo GO, Ogola EN et al Cardiovascular risk factors and carotid atherosclerosis in patients with systemic lupus erythematosus at Kenyatta National Hospital Afr J Rheumatol 2014; 2(2): 64-69
66. Essien EM, Usanga EA, Ayeni O. The normal platelet count and platelet factor 3 availability in some Nigerian population groups. Scandinavian Journal of Hematology. 1973; 10: 378-383
67. Cervera R, Khamashta MA, Font J, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore) 2003; 82:299
68. Budman DR, Steinberg AD. Hematologic aspects of systemic lupus erythematosus: current concepts.Ann Intern Med. 1977; 86:220-229.
69. Staples PJ, Gerding DN, Decker JL, Gordon RS Jr. Incidence of infection in systemic lupus erythematosus.Arthritis Rheum. 1974;17:1-10.
70. Ginzler E, Diamond H, Kaplan D, et al. Computeranalysis of factors influencing frequency of infection in systemic lupus erythematosus. ArthritisRheum. 1978;21:37-44.
71. Lee P, Urowitz MB, Bookman AA, et al. Systemic lupus erythematosus: a review of 110 cases withreference to nephritis, the nervous system, infections,aseptic necrosis and prognosis. Q J Med. 1977;46:1-32.
72. Zurier RB. Reduction of phagocytosis and lysosomalenzyme release from human leukocytes by serum from patients with systemic lupus erythematosus.Arthritis Rheum. 1976;19:73-78.
73. Landry M. Phagocyte function and cell-mediated immunity in systemic lupus erythematosus. Arch Dermatol. 1977; 113:147-154.
74. Yu CL, Chang KL, Chiu CC, et al. Defective phagocytosis, decreased tumour necrosis factor alpha production, and lymphocyte hyporesponsiveness predispose patients with systemic lupus erythematosus to infections. Scand J Rheumatol.1989;18:97-105.
75. Odhiambo J, Oyoo GO, Amayo E et al An evaluation of quality of life in ambulatory patients with systemic lupus erythematosus attending rheumatology clinic in Kenyatta National Hospital Afr J Rheumatol 2014; 2(1): 18-22.

76. Wadee S, Tikly M, Hopley M. Causes and predictors of death in South Africans with systemic lupus erythematosus. Rheumatology. 2007; 46: 1487-1491.
77. Manzi S. Epidemiology of systemic lupus erythematosus. Am J Manag Care. 2001 Oct. 7(16 Suppl):S474-9.
78. Alarcon GS, McGwin G Jr, Bastian HM. Systemic lupus erythematosus in three ethnic groups. VII [correction of VIII]. Predictors of early mortality in the LUMINA cohort. LUMINA Study Group. Arthritis Rheum. 2001; 45(2):191-202.
79. Nathalie Costedoat-Chalumeau, Bertrand Dunogue,Nathalie Morel et al Hydroxychloroquine :A multifaceted treatment in lupus. Lupus 2014,vol 43 (6):e167-e180)
80. B.Bader-Meunier,J. B. Armengaud, E. Haddad et al Initial presentation of childhood-onset systemic lupus erythematosus: a French multicenter study, Journal of Peadiatrics, vol 146, no 5, pp 648-653,2005.
81. Gokce et al, Haematological features in children with systemic erythematosus are they more common than appreciated? Pediatric Rheumatology, vol 9, 1, p 242,2011.
82. Hahn Bh et al., "Systemic lupus erythematosus," in Harrison's Principles of Internal Medicine, S. Anthony Fauci, L. Stephen Hauser, and L. Dan Longo, Eds.,McGraw-Hill, New York, NY,USA, 18 edition, 2012.
83. WHO Global Database on -The database on includes data by country on prevalence of and mean hemoglobin concentration – NFHS WHO Collaboarative report 2010 234-45.
84. Anita Nadkarni, Supriya Phanasgaonkar, Roshan Colah et al Genetic Testing. June 2008, 12(2): 177-180. doi:10.1089/gte.2007.0080.Prevalence and Molecular Characterization of α-Thalassemia Syndromes among Indians
85. Mukibi J.M, Okelo G.A, Kanja C Platelet in normal Kenyan adults. East African medical journal 03/1981; 58(2):136-9
86. Rajab J A, Muchina W P, Orinda DA et al. Blood Donor Haematology parameters in two regions of Kenya. East African Med Journal 2005;82:123-127
87. Common Terminology Criteria for Adverse Events v3.0 (CTCAE) Publish Date: August 9, 2006
88. Sultan SM, Begum S, Isenberg DA. Prevalence, patterns of disease and outcome in patients with systemic lupus erythematosus who develop severe haematological problems. Rheumatology (Oxford) 2003; 42:230-234.

APPENDICES

APPENDIX 1: STUDY EXPLANATION FORM

1. **Purpose of the study:**

My names are Dr. Jacqueline Wangechi Njoroge, a post graduate student at the University of Nairobi. I am undertaking a study to determine the blood parameters in SLE patients on follow up at Kenyatta National Hospital Rheumatology and Renal Clinic. Various blood abnormalities are common in SLE patients. They are related to the disease activity and the medication eg Immunosuppresives, steroid or NSAIDS being used. The study is being conducted at KNH, Rheumatology and Renal clinic with cooperation from the staff and permission from the hospital administration.

2. Procedures involved: Should you accept to join the study, you would be expected to:

i) Sign a consent form and participate in a survey that will take 10 to 15 minutes.
ii) Answer questions about your socio-demographic data, current medications and duration of disease from diagnosis. Your responses will be noted in writing on the study questionnaire.
iii) Undergo venepuncture for withdrawal of about 4mls of blood for tests. These tests will enable me to determine your full hemogram and peripheral blood film characteristics.

3. Your rights as a participant in this study:

i) Your participation in this research is voluntary.
ii) You will not be victimised if you refuse to participate in this study.
iii) If you choose to participate and not answer certain questions, you are free to do so.
iv) You are free to terminate the interview and withdraw from the study at any time.
v) You are free to ask questions before signing the consent form.
vi) All the results will remain confidential. Your individual responses will be stored in a locked place under my control and will only be seen by my statistician and me.

4. Risks to you as a participant in this study include:

- Minor discomfort or swelling at the venepuncture site. If any of these happens to you, contact Dr. Jacqueline Wangechi Njoroge 0722408915 for free examination and treatment.

5. Benefits to you as a participant in this study include:

i) Free evaluation of your full blood count and peripheral blood film free copy of your results will be availed to you on request. Any abnormalities noted will be communicated to your primary doctor for prompt intervention.

ii) The findings of this study will assist in formulating better patient care for you and other SLE patients.

If you have any question during the course of the study, you may contact the following:

1. DR JACQUELINE WANGECHI NJOROGE ,
   UNIVERSITY OF NAIROBI, DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS, Mobile: 0722-408915.
   Or
2. PROF M.L CHIDIA
   SECRETARY, KNH/UON ETHICAL REVIEW COMMITTEE
   TEL: 020-726300 Ext 44355
   P.O. Box 20723, Code 00202 Nairobi.
   Or
3. PROF OMONDI OYOO
   UNIVERSITY OF NAIROBI, DEPARTMENT OF CLINICAL MEDICINE AND THERAPUTICS
   Tel 020-27254552

If you agree to participate in the study, please sign the attached consent form. This consent form will not be linked to your answers.

APPENDIX 2: MAELEZO YA IDHINI

Kwa majina naitwa Dr Jacqueline Njoroge, mwanafunzi wa shahada ya uzamili katika Idara ya Magojwa ya Ndani (Internal Medicine) ya Chuo Kikuu cha Nairobi, nafanya utafiti kwa watu walio na ugojwa wa Chavi cha uso (SLE) naangalia mizingo ya damu ya wagojwa wanao fuatiliwa katika Hospitali kuu ya Kenyatta.

Nia ya utafiti

Utafiti huu si wa kupeana tiba lolote ila ni wa kuangalia mizingo ya damu ya wangojwa wanaoishi na ugojwa wa Chavi cha uso (SLE) katika hospitali kuu ya Kenyatta.

Taratibu

Kama unakubali kushiriki katika utafiti huu utaombwa:

1. Kujibu maswali kadhaa kuhusu ugonjwa wako
2. Kufanyiwa uchunguzi wa kimwili
3. Kutolewa mililita 4 za damu tupeleke kupima maabara

Hatari

Kwa kushiriki katika utafiti huu, mgojwa hatakuwa kwenye hatari yoyote ila atakuwa na maumivu madogo wakati wa kutoa damu.

Faida ya kushiriki:

1. Uchuguzi wote utafanywa bila malipo yoyote kutoka kwako. Mpelelezi mkuu ndiye atakayegharamia uchunguzi wa maabara
2. Matokeo ya uchaguzi huu yatafafanuliwa kwako na nakala iwekwe katika faili yako ya matibabu kwa ajili ya kutazamwa na daktari msingi katika kliniki.
3. Kwa wale watakao patikana na shida yoyote daktari wa kliniki ataelezwa ili aanze matibabu.

Usiri

Nakala yoyote itakayotokana na huu uchunguzi itahifadhiwa kwa usiri na kutumiwa kwa ajili ya utafiti huu tu.

Hitimisho

Kushiriki kwako katika utafiti huu ni kwa hiari yako na uko huru kutoka wakati wowote, katika kipindi hiki cha utafiti. Ukikataa kushiriki au utake kuondolewa kutokana na utafiti, haita adhiri kwa njia yoyote ubora wa matibabu yako.

Kwa maelezo au maswali yoyote kuhusu utafiti huu, unawza kuuliza:

Dr Jacqueline Njoroge
Mchunguzimkuu,
Nambari ya simu 0722408915
Idara ya Magojwa ya Ndani (Internal Medicine), Chuo kikuu Cha Nairobi

Prof Omondi Oyoo
Mtafiti Mshirika,
Nambari ya simu 020-27254552
Idara ya Magojwa ya Ndani (Internal Medicine), Chuo kikuu Cha Nairobi

Prof. M.L Chidia
KNH/UON –Ethic and Research Committee
Nambari ya simu 020 726300-9

APPENDIX 3: CONSENT

The purpose and procedure of this study entitled "Haematological Parameters in SLE patients on follow up at Kenyatta National Hospital, Nairobi" together with my rights, risks and benefits have been fully explained to me. I hereby give my written consent to allow myself to participate in the study.

NAME: ……..… SIGNATURE: …….

DATE: _____

WITNESS ……..…….. SIGNATURE: ……..……

DATE: _____

APPENDIX 4: STATEMENT OF ASSENT FOR MINORS (<18 YEARS)

The purposes of this study, procedure, benefits, risks and my rights have been fully explained to me. I hereby give my written assent to allow myself to participate in the study.

NAME:……..…… SIGNATURE:…..…..……

DATE: _____

WITNESS ……..… SIGNATURE:……..……

DATE: _____

APPENDIX 5: HIARI YA KUSHIRIKI NA SAHIHI

Baada ya kuelezwa na kuelewa kuhusu nia, taratibu, hatari na faida ya kushiriki kwa utafiti huu nanakumbali kushiriki kwa utafiti huu.

Jina…… …… Sahihi/ Kidole……

Tarehe……

Jina la shahidi……. Sahihi/ Kidole…….

Tarehe……

APPENDIX 6: HIARI YA KUSHIRIKI NA SAHIHI KWA WALIO NA UMRI MIAKA <18

Baada ya kuelezwa na kuelewa kuhusu nia, taratibu , hatari na faida ya kushiriki kwa utafiti huu ninakumbali kushiriki kwa utafiti huu.

Jina…… …… Sahihi/ Kidole……

Tarehe……

Jina la shahidi…… Sahihi/Kidole…….

Tarehe……

APPENDIX 7: INVESTIGATOR'S STATEMENT

I, the Principal Investigator, have fully educated the research participant on the purpose and implication of this study.

Signed: ..... **Date: _____**

APPENDIX 8: SCREENING QUESTIONNAIRE

Name: _____ Hospital No.:_____

Study Date: _____

1. CONSENT / ASSENT GIVEN:

YES ☐ **NO** ☐ **IF YES PROCEEDS TO 2.**

2. AGE OVER 13 YEARS:

YES ☐ **NO** ☐ **IF YES PROCEEDS TO 3.**

3. <u>FOR OFFICIAL USE ONLY</u>

RECRUITED?

YES ☐ **NO** ☐

Interviewers Name: _____

Signature:..... Date: _____

APPENDIX 9: STUDY QUESTIONNAIRE

HAEMATOLOGICAL PARAMETERS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AT KENYATTA NATIONAL HOSPITAL, NAIROBI

Study Date: _____ Study No.: _____ Hospital No.:_____

1. SOCIO-DEMOGRAPHIC DATA

Name: _____ Sex: _____

Residence: _____ Age in years: _____

Occupation: ☐ 1 = Employed 2= Self employed 3 = Retired 4 = Others

Education level: ☐ 1=Primary 2=High School 3=College/University 4=None

2. Clinical Details

Duration of disease from diagnosis ☐

Drug	Trade name	Commencement date	Current dose
NSAIDS			
Steroids			
Azathioprine			
Cyclophosphomide			
Mycophenolate			
Methotrexate			
Leflunomide			
Hydroxychloroquine			
Iron			
Folic			

Interviewers Name: _____

Signature: .....

Date: _____

APPENDIX 10: LABORATORY PARAMETERS

LAB TEST	**RESULTS**	**DATE**
RBC count, (x 10^{12} cells/L)		
Hb level (g/dL)		
HCT, (%)		
MCV (fL)		
MCH, (pg/cell)		
MCHC, (g/dL)		
Platelets, (x 10^9cells/L)		
WBC count, (x 10^9 cells/L)		
Neutrophils, (x 10^9 cells/L)		
Lymphocytes, (x 10^9 cells/L)		
Eosinophils, (x 10^9 cells/L)		
Basophils, (x 10^9 cells/L)		
Monocytes, (x 10^9 cells/L)		
ESR (mm/hr)		
Reticulocyte count		
PBF COMMENT		

Appendix 11: American College of Rheumatology Criteria for Classification of Systemic Lupus Erythematosus

1997 Update of the 1982 American College of Rheumatology revised criteria for classification of systemic lupus erythematosus

1. Malar Rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Nonerosive arthritis	Involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Pleuritis or pericarditis	1. Pleuritis--convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion ***OR*** 2. Pericarditis--documented by electrocardigram or rub or evidence of pericardial effusion
7. Renal disorder	1. Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed ***OR*** 2. Cellular casts--may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	1. Seizures--in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance ***OR*** 2. Psychosis--in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic	1. Hemolytic anaemia--with reticulocytosis

disorder	***OR*** 2. Leukopenia--< 4,000/mm^3 on ≥ 2 occasions ***OR*** 3. Lyphopenia--< 1,500/ mm^3 on ≥ 2 occasions ***OR*** 4. Thrombocytopenia--<100,000/ mm^3 in the absence of offending drugs
10. Immunologic disorder	1. Anti-DNA: antibody to native DNA in abnormal titer ***OR*** 2. Anti-Sm: presence of antibody to Sm nuclear antigen ***OR*** 3. Positive finding of antiphospholipid antibodies on: 1. an abnormal serum level of IgG or IgM anticardiolipin antibodies, 2. a positive test result for lupus anticoagulant using a standard method, or 3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Positive antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

The classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person is defined as having SLE if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.

From: Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997; 40:1725 (15).

Appendix 12: Grading of cytopenia using Common Terminology Criteria for Adverse Events v3.0 (CTCAE) Publish Date: August 9, 2006(89)

Grading of severity of anaemia

Hb	**Grade(g/dl**
No anaemia	>12
Mild	<12-10
Moderate	<10-8
Severe	<8-6.5
Life Threatening	<6.5

Grading of severity of leucopenia

Grade	**WBC (x 10^9 /L)**
No leucopenia	>4
Mild	<4-3
Moderate	<3-2
Severe	<2-1
Life threatening	<1

Grading of severity of thrombocytopenia

Grade	**Plt(x 10^9 /L)**
No Thrombocytopenia	150
Mild	<150-75
Moderate	<75-50
Severe	<50-25
Life threatening	<25

Appendix 13: Ethical Approval Letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/72

20th February, 2015

Dr. Jacqueline Wangechi Njoroge
Dept. of Clinical Medicine & Therapeutics
School of Medicine
University of Nairobi

Dear Dr. Njoroge

Research Proposal: Haematological parameters in systemic lupus erythromatosus patients at Kenyatta National Hospital, Nairobi (P733/12/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 20th February 2015 to 19th February 2016.

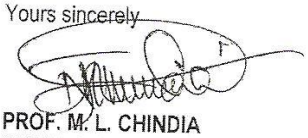
This approval is subject to compliance with the following requirements:

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 severe 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) 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*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
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 **www.erc.uonbi.ac.ke**

Protect to discover

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 Assistant Director, Health Information, KNH
The Chairperson, KNH/UON-ERC
The Dean, School of Medicine, UoN
The Chairman, Dept of Clinical Medicine & Therapeutics, UoN
Supervisors: Prof. Omondo Oyoo, Prof. Grace W. Kitonyi, Dr. Andrew Odhiambo, Dr. Anne K. Barasa

Appendix 14: KNH Approval Letter to Conduct Study

KENYATTA NATIONAL HOSPITAL
P. O. Box 20723, 00202 Nairobi

Tel: 2726300/2726450/2726550
Fax: 2725272
Email: knhadmin@knh.or.ke

Ref: KNH/SAD-MED/42B/VOL.I/89

Date: 6th March , 2015

Dr. Jacqueline Wangechi Njoroge
Department of Clinical Medicine & Therapeutics
School of Medicine
UNIVERSITY OF NAIROBI.

RE:APPROVAL TO CONDUCT A STUDY AT THE KNH MEDICINE DEPARTMENT

Following approval of your study by the KNH/UoN ERC and completion of the KNH study registration form, permission is hereby granted for you to collect data from the KNH Medicine Department to enable you complete your study on ***"Haematological parameters in systemic lupus erthromatosus patient in Medicine department"*** at *Kenyatta National Hospital, Nairobi County, Kenya.*

Kindly liaise with the Nursing Officer Incharge of Renal Unit and Nursing Officer incharge Clinic 23 (Haematology) for facilitation. By a copy of this letter, the Nursing incharge of Renal Unit and Clinic 23 (Haematology) is informed and requested to facilitate.

DR. ANN WAWERU
AD - MEDICINE

Copy to: Nursing Officer Incharge , Renal Unit
Nursing Officer Incharge, Clinic 23 (Cardiac)