HAEMATOLOGICAL ABNORMALITIES IN SOUTH AFRICANS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements of the degree of Master of Medicine (Internal Medicine)

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Declaration

I, Ivy Yaa Gyamaa Anafi, do hereby declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Ivy Yaa Gyamaa Anafi
Signed 6th April 2021.

Dedication

To my ever-loving family for their support and understanding.

James

Maxine

Laura

Karen

Abstract

Background: Little is known about haematological abnormalities in South Africans with systemic lupus erythematosus.

Method: A retrospective-nested case-control study of 240 SLE patients, comparing baseline haematology and clinical features in 200 known alive (AG) and 40 known deceased (DG) patients.

Results: Most patients were black (93.3%) and female (96.3%). Anaemia (70.8%) and lymphopaenia (58.8%) were the commonest haematological abnormalities. Compared to AG, DG had lower median haemoglobin (Hb) (9.5g/dl vs 10.8g/dl, $p<0.01$) and higher median red cell distribution width (RDW) (16.6fL vs 15.4fL, $p= 0.01$). Renal involvement was independently associated with anaemia and raised RDW >16fl (OR=2.52 and 1.85, respectively). Central nervous system involvement was associated with leucopaenia and neutropaenia (OR=2.61 and 3.26, respectively). Raised basophil count and RDW independently predicted death.

Conclusion: Haematological abnormalities at diagnosis were common in this predominantly black African cohort of SLE patients, often associated with major organ involvement. Increased basophil count and RDW at diagnosis predicted mortality.

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Abbreviations and acronyms

ACLA	Anticardiolipin antibodies
ACR	American College of Rheumatology
AG	Alive group
AIHA	Autoimmune haemolytic anaemia
ANA	Anti-nuclear antibody
AOCD	Anaemia of chronic disease
APS	Antiphospholipid syndrome
β2GP1	β 2 glycoprotein 1
BFU	Burst forming unit
C3	Complement component 3
C4	Complement component 4
CHBAH	Chris Hani Baragwanath Academic Hospital
CNS	Central nervous system
CRP	C-Reactive protein
DG	Deceased group
dsDNA	Double stranded DNA
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
Hb	Haemoglobin
HIV	Human Immunodeficiency syndrome

IC	Immune complex
ITP	Immune thrombocytopaenia
LN	Lupus nephritis
MCV	Mean cell volume
MPV	Mean platelet volume
NHLS	National Health Laboratory Service
NPSLE	Neuropsychiatric systemic lupus erythematosus
PRCA	Pure red cell aplasia
RDW	Red cell distribution width
SLICC	Systemic Lupus International Collaborating Clinics
TTP	Thrombotic thrombocytopaenic purpura
WCC	White cell count
vWF	Von Willebrand factor

1 INTRODUCTION

1. 1 Background to study

Systemic lupus erythematosus (SLE) is a multisystem immune-mediated inflammatory disorder that usually involves the skin, synovial joints and kidneys. The exact aetiopathogenesis of the disease is unknown but current evidence suggests an interaction among genetic, hormonal and environmental factors, that cause immune dysregulation and autoimmunity (Mok & Lau, 2003). Aberrant T and B cell function, complement deficiencies and abnormal cytokine function result in the characteristic immunologic abnormality of autoantibody production to various cell nucleus components, commonly referred to as antinuclear antibodies (ANA). Impaired clearance and phagocytosis of apoptotic cell debris and circulating immune complexes (ICs), lead to deposition of ICs in various organs. Loss of immune tolerance ultimately results in chronic inflammation and tissue damage (Tsokos, Lo, Reis, & Sullivan, 2016).

Haematological abnormalities are common in SLE. Their frequency and spectrum vary in different populations. Various researchers have investigated the association between haematological abnormalities and serological or clinical manifestations in SLE. For example, Thompson *et al* showed a significant association between leucopaenia, thrombocytopaenia and hypocomplementaemia with proteinuria, renal casts, anti-dsDNA and anti-Sm antibodies (Thompson, Juby, & Davis, 1993). Other studies have shown lymphopaenia to correlate with global disease activity and serves as a marker of disease relapse (Mirzayan, Schmidt, & Witte, 2000; Vilá et al., 2006). Severe neutropaenia increases the risk for infections (Fayyaz et al., 2015). Mody *et al* in Durban, South Africa, have shown that acute thrombocytopaenia in SLE is associated with an elevated risk of subsequent nephritis and mortality (Patel & Mody, 2014). However, not much is known about the total spectrum and wider implications of haematological abnormalities in SLE patients in sub-Saharan Africa.

1.2 Overview of Systemic Lupus Erythematosus

1.2.1 History of systemic lupus erythematosus

Recognition of SLE as a disease entity dates back to the tenth century (Smith & Cyr, 1988; Ugarte-Gil & Alarcón, 2016). Since then, there have been many developments that have led to an expanded understanding of the pathogenesis and improved management of the condition. During the early Classical period, SLE was viewed mainly as a cutaneous disease. The word 'lupus' which translates to wolf in Latin, was coined by an Italian physician, Rogerius, who in the thirteenth century described the erosive facial features as resembling the bite of a wolf (Mallavarapu & Grimsley, 2007). An ulcerating lesion in these patients was first described by Bateman and Willan, which was later recognised as lupus vulgaris. This lesion was subsequently explained by Kaposi to be due to tuberculosis which was highly prevalent at the time (Mallavarapu & Grimsley, 2007). In the 1850s, Biett and Cazenave developed the term 'lupus erythematosus' and the typical discoid lupus rash was described (Hahn & Wallace, 2002; Smith & Cyr, 1988). Ferdinand von Hebra described the butterfly rash in 1846. Kaposi brought to light the systemic nature of SLE in the Neoclassical era in 1872 as:

"…experience has shown that lupus erythematosus may be attended by altogether more severe pathological changes and even dangerous constitutional symptoms may be intimately associated with the process in question, and that death may result from conditions which must be considered to arise from the local malady" (Hochberg, 1991).

The lupus erythematosus (LE) cell was discovered by Hargraves *et al* in 1948. This marked the beginning of the Modern era of SLE. This discovery led to the link of immunology with the study of SLE. Antinuclear antibodies (ANA) and a false positive test for syphilis were recognised as being associated with SLE (Shore & Faricelli, 1977). Subsequently several autoantibodies have been described in clinical subsets of SLE. By studying animal models of the disease, the modern era of SLE has recognised that there is a genetic and familial predisposition to developing SLE (Alarcón-Segovia et al., 2005; Deng & Tsao, 2010).

1.2.2 Epidemiology of systemic lupus erythematosus

Systemic lupus erythematosus is described worldwide in all populations. Prevalence and incidence rates vary widely in different population groups. Highest rates are reported in North America with a prevalence of 241/100000 persons and incidence of 23.2/ 100000 person years (Rees, Doherty, Grainge, Lanyon, & Zhang, 2017). The reported rate is lowest in Africa and Ukraine with an incidence of 0.3/100000 person years (Rees et al., 2017). Differences in prevalence rates across continents may be attributable to underlying genetics of the population, differences in environmental exposure and underreporting of the disease in some areas. The disease shows a predilection for black females in the United States of America (USA) and runs a more aggressive course in this population (Rees et al., 2017). In South Africa, SLE has been shown to affect mainly young females of mixed or African ancestry (Tikly & Navarra, 2008).

1.2.3 Classification criteria for systemic lupus erythematosus

Classification criteria in rheumatology are primarily for research purposes but often used as guide to diagnosis in clinical practice. SLE classification criteria have been revised several times from the initial classification criteria in 1971 (Cohen, 1971). The criteria were revised by the American College of Rheumatology (ACR) in 1982 to include the ANA test, in which out of the eleven criteria, patients need to fulfil four to be classified as having SLE (Tan et al., 1982). Subsequently, presence of anti-phospholipid antibodies was included as an additional immunologic criterion in 1997 (Hochberg, 1997). As of 2012, the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria includes eleven clinical and six immunological criteria. Patients are diagnosed with SLE if they fulfil a minimum of four criteria, which must include a minimum of one clinical as well as one immunologic criterion. They also fulfil the classification criteria if they have lupus nephritis (LN) as the only clinical criterion with positive ANA or double stranded deoxy ribonucleic acid antibodies (anti-dsDNA) (Petri et al., 2012). More recently the European league against rheumatism (EULAR) with ACR have proposed a new classification criteria for SLE where all patients must have a positive ANA as an obligatory criteria, together with other weighted criteria (Aringer et al., 2019).

1.2.4 Clinical features of systemic lupus erythematosus

Clinical manifestations of SLE are diverse, most commonly presenting with constitutional symptoms of fatigue, fever, loss of appetite and also with skin and joint symptoms. Rarely, and especially in patients of African descent, nephritis can be the sole presenting feature (Tikly & Navarra, 2008). Other major organ involvement includes central nervous system presentations of psychosis or epilepsy and haematologic complications including immune thrombocytopaenia (ITP). The disease is typically characterised by phases of relapses and remissions.

The spectrum and severity of SLE varies in different populations. Photosensitivity is seen more in Caucasian patients whereas patients of African and Oriental descent have a higher risk of developing LN (Lewis & Jawad, 2017; Wadee, Tikly, & Hopley, 2007). Comorbidities exist commonly in SLE and are often associated with premature death. In Caucasian populations, coronary artery disease is common and infections, including tuberculosis are a major challenge in South Africans with SLE (Greenstein, 2016).

1.3 Haematological abnormalities

1.3.1 Overview

Haematological abnormalities are a common finding in SLE, often evident at initial presentation. It manifests as either cytopaenia or a coagulopathy. Sasidharan *et al* in North Kerala, India, showed 82% of SLE patients had some haematological abnormality at initial presentation (Sasidharan, Bindya, & Sajeeth Kumar, 2012). In South Africa, Wadee *et al* observed a haematological presentation was the third most common presentation after arthritis and malar rash in South Africans (Wadee et al., 2007).

Cytopaenia, defined as the reduction in the peripheral count of either erythrocytes, leucocytes and platelets, may be primarily due to the disease process itself, as a result of drugs used to treat SLE, or secondary to organ dysfunction such as chronic kidney disease. Cytopaenia in SLE are mostly asymptomatic but can present with severe illness as in the case of autoimmune haemolytic anaemia (AIHA), immune thrombocytopaenia (ITP) and thrombotic thrombocytopaenic purpura (TTP). The prevalence varies in different populations. Table 1.1 is a summary of the prevalence

of cytopaenias in SLE patients observed in different populations (Aleem, Al Arfaj, Khalil, & Alarfaj, 2014; Beyan, Beyan, & Turan, 2007; Nossent & Swaak, 1991).

Table 1.1 Prevalence of cytopaenias in systemic lupus erythematosus in different populations

Study	Netherlands Nossant et al (1991)	Turkey Beyan et al (2007)	Saudi Arabia Aleem et al (2014)
Sample size	126	115	624
Anaemia	13%	46%	63%
Lymphopaenia	20%	82%	40.3%
Neutropaenia	47%	20%	30%
Thrombocytopaenia	27%	40%	10.9%

Cytopaenias are components of the various classification criteria for SLE. These include leucopaenia, lymphopaenia and thrombocytopaenia (Petri et al., 2012; Tan et al., 1982). Patients with SLE may present clinically with other cytopaenias including anaemia and neutropaenia (Fayyaz et al., 2015).

1.3.2 Anaemia

The World Health Organisation (WHO) has defined anaemia as a state where the amount of erythrocytes or the oxygen carrying capacity falls below what is needed physiologically (WHO, 2015). Anaemia in adults is defined by haemoglobin level below 13g/dl in males, or below 12g/dl in non-pregnant females, and less than 11g/dl in pregnant females, based on a study in Gauteng province in South Africa (Lawrie et al., 2009).

Anaemia is common in patients with SLE due to multiple mechanisms. These include chronic inflammation, nutritional deficiencies, immune mediated as in autoimmune haemolysis, or autoimmune destruction of erythropoietic bone marrow cells, hypersplenism and renal insufficiency (Schur & Berliner, 2017).

1.3.2.1 Anaemia of Chronic Disorder

Anaemia of chronic disease (AOCD) occurs in the setting of systemic inflammation which causes decreased erythrocyte production and decreased erythrocyte survival (Weiss, Ganz, & Goodnough, 2019). It may co-exist with iron deficiency anaemia or other nutritional deficiencies.

In SLE, immune dysregulation causes a chronic inflammatory state, with associated release of cytokines including tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and interferon-gamma (IFN- ɤ). These cytokines suppress erythropoiesis to result in anaemia (Weiss et al., 2019).

Anaemia of chronic disease is a disorder of iron distribution. Hepcidin regulates iron homeostasis. It acts via ferroportin located on the duodenal enterocytes, to regulate the transfer of iron into plasma. Il-6, produced by inflammation, stimulates high levels of hepcidin, which cause inhibition of iron release from macrophages in the spleen and liver and also inhibits iron transport into plasma. Other inflammatory cytokines e.g. TNFα and IFN ɤ also cause bone marrow reprogramming. These cytokines have been shown to promote myelopoiesis and lymphopoiesis at the expense of erythropoiesis through the activation of transcription factor PU.1 and inhibition of transcription factor burst forming unit (BFU) (Orsini et al., 2019).

Inflammation causes suppression of erythropoietin and decreases erythropoietin receptors on erythroid progenitors, leading to erythropoietin resistance (Khalil et al., 2018). Presence of anti-epo antibodies in AOCD have been demonstrated (Voulgarelis et al., 2000). Inflammation also leads to increased destruction of erythrocytes by various mechanisms including macrophage activation causing haemophagocytosis with consequent decrease in the erythrocyte lifespan (Zoller et al., 2011).

Patients with AOCD mostly have a normocytic, normochromic anaemia accompanied by low transferrin saturation and high ferritin levels with a milieu of systemic inflammation. However, AOCD commonly coexists with iron deficiency anaemia and the diagnosis may be challenging.

1.3.2.2 Autoimmune haemolytic anaemia

The diagnosis of AIHA is made by establishing haemolysis and autoimmunity against erythrocytes. Haemolysis is associated with a low serum haptoglobin, increased lactate dehydrogenase, and indirect hyperbilirubinemia with reticulocytosis (Schur & Berliner, 2017). Autoimmunity against erythrocytes is established using direct antiglobulin test. Autoimmune haemolytic anaemia in SLE is mainly warm type IgG anti-erythrocyte antibodies mediated (Jeffries et al., 2008) and can be associated with antiphospholipid antibodies (Fong, Loizou, Boey, & Walport, 1992).

1.3.2.3 Pure red cell aplasia and aplastic anaemia

Pure red cell aplasia (PRCA) occurs in SLE albeit very rarely. There is severe anaemia and a low reticulocyte count. It occurs because of bone marrow suppression secondary to autoantibodies directed against red blood cell precursors (Habib, Saliba, & Froom, 2002).

Aplastic anaemia is bone marrow failure resulting in deficiency of all blood cell lines and is rarely seen in SLE. Both antibody mediated bone marrow suppression and a cell mediated bone marrow destruction have been shown to be possible mechanisms in SLE. (Bailey, Lilly, Bertoli, & Ball, 1989; Roffe, Cahill, Samanta, Bricknell, & Durrant, 1991).

1.3.3 Leucopaenia

Leucopaenia is defined according to the ACR criteria as a white cell count (WCC) $<4.0 \times 10^9$/L. documented on two or more occasions. Studies in the general population of Black South Africans show that the lower limit of the total WCC is lower than in Caucasians as shown by Lawrie et al in Gauteng, South Africa to be 3.9×10^9/L (Lawrie et al., 2009).

1.3.3.1 Lymphopaenia

Lymphopaenia is common amongst SLE patients and according to the ACR classification criteria for SLE, lymphopaenia is defined as an absolute lymphocyte cell count< 1.5×10^9/L. In Black South Africans, Lawrie et al have shown the lower limit of lymphopaenia to be 1.4 x10^9/L (Lawrie et al., 2009). Both T and B lymphocytes are affected, but T cells are more affected especially the CD4+ cells (Laurence, 1992). Pathogenesis of lymphopaenia is multifactorial including anti-lymphocyte autoantibodies that attach to lymphocytes, resulting in cell lysis (Massardo et al., 2009). Lymphopaenia in SLE may also be due to defects in apoptosis (Chen & Lin, 2011). This is because there is a reduction in surface expression of CD55 and CD59, which are complement regulatory proteins leading to increased lymphocyte susceptibility to complement mediated lysis (Ruiz-Argüelles & Llorente, 2007). Lymphopaenia often occurs with clinical disease activity. (Mirzayan et al., 2000). When absolute lymphocyte counts are very low the risk of infection is increased (Dias, Do Couto, Duarte, Inês, & Malcata, 2009).

1.3.3.2 Neutropaenia

Neutropaenia is defined as a neutrophil cell count <1.2x10^9/L by Lawrie *et al* in their study of FBC reference values in Gauteng South Africa (Lawrie et al., 2009). In sub-Saharan Africa, this may be compounded by the presence of benign ethnic neutropaenia (Haddy, Rana, & Castro, 1999). Multiple mechanisms for neutropaenia have been shown in SLE. These include the increased apoptosis of neutrophils and their precursors due to increased amounts of TNF related apoptosis inducing ligand (TRAIL) (Matsuyama et al., 2004), and presence of circulating antineutrophil antibodies (Starkebaum, Price, Lee, & Arend, 1978). Immunosuppressive treatments for SLE may cause drug-induced medullary hypoplasia and consequent neutropaenia. (Martinez-Banos, Crispin, Lazo-Langner, & Sánchez-Guerrero, 2006). Severe neutropaenia increases the risk for infections (Dias et al., 2009).

1.3.4 Thrombocytopaenia

Thrombocytopaenia in SLE is defined as platelet count <100x10^9/L, based on ACR classification criteria. Mechanisms of thrombocytopaenia in SLE include immune mediated destruction, decreased production, splenic sequestration and drug toxicity. Immune mediated thrombocytopenia is via two main mechanisms. These include the

presence of specific antibodies directed against GpIIb and GpIIIa proteins on the platelet cell membrane (Michel et al., 2002) and the presence of antibodies against thrombopoietin which regulates platelet production in the bone marrow (Ziakas, Papadaki, Psyllaki, & Voulgarelis, 2008). Antiphospholipid antibodies are also associated with thrombocytopaenia (Uthman, Godeau, Taher, & Khamashta, 2008).

Thrombocytopaenia can be of either an acute or chronic onset in SLE. Acute onset thrombocytopaenia is usually severe and related to disease activity. In a study by Patel *et al* in Durban, South Africa, acute thrombocytopaenia in SLE was shown to correlate with an increased risk of LN and mortality (Patel & Mody, 2014).

1.3.4.1 Immune thrombocytopaenia

Immune thrombocytopaenia (ITP) sometimes predates the diagnosis of SLE, or presents as an acute flare during the course of the disease (Velo-Garcia, Castro, & Isenberg, 2016). ITP commonly presents with very low platelet counts coupled with increased bone marrow production of megakaryocytes. Rarely, ITP can occur with Coombs positive AIHA, in the so-called Evan's syndrome (Deleze, Oria, & Alarcon-Segovia, 1988).

1.3.5 Microangiopathic haemolytic anaemia

Microangiopathic haemolytic anaemia (MAHA) is a cause of both anaemia and thrombocytopaenia in SLE. MAHA results from intravascular mechanical shearing of erythrocytes and haemolysis and is typically associated with schistocytes on peripheral blood smear. Underlying causes in SLE include thrombotic thrombocytopaenic purpura (TTP), disseminated intravascular coagulopathy (DIC), thrombotic microangiopathy (TMA) and catastrophic antiphospholipid syndrome.(Schur & Berliner, 2017).

1.3.5.1 Thrombotic thrombocytopaenic purpura

Thrombotic thrombocytopaenic purpura (TTP), rarely seen in SLE, presents with clinical features of fever, renal dysfunction, and mental confusion with thrombocytopaenia and microangiopathic haemolytic anaemia. ADAMTS 13 protease deficiency results in increase in large von Willebrand factor multimers that predispose to platelet aggregation and occlusive platelet thrombi. TTP may occur

prior to the diagnosis of SLE, or may manifest at diagnosis or later in the disease (Yuen et al., 2007).

1.3.6 Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio

Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are markers of disease activity in SLE. NLR and PLR are raised in patients with SLE than controls. Higher NLR levels are seen in patients with LN than those without nephritis (Wu, Chen, Yang, Chen, & Yang, 2016).

1.3.7 Mean platelet volume

Mean platelet volume (MPV) indicates the average size of platelets and reflects a balance between bone marrow production and peripheral destruction of platelets. It is a marker of inflammation in some systemic rheumatic diseases like ankylosing spondylitis and rheumatoid arthritis (Şahin, Yetişgin, Şahin, Durmaz, & Cengiz, 2016) .A lower MPV is seen with active SLE and it has a negative correlation with systemic lupus erythematosus disease activity score (SLEDAI) (Hartmann et al., 2018; Khan, Haider, Ayub, & Khan, 2017).

1.3.7 Red cell distribution width

Red cell distribution width (RDW) measures the range of variation of erythrocyte volume. The baseline RDW is a marker of disease flare and predicts therapeutic outcome (Zou et al., 2016).

1.3.8 Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is characterised by raised titres of antiphospholipid antibodies together with arterial or venous thrombosis and pregnancy related complications of recurrent abortions, stillbirths, pre-eclampsia and preterm delivery. Whilst primary APS occurs in isolation, secondary APS is often a feature of SLE. Cervera *et al* in a study of a 1000 SLE patients, showed that 20- 35% developed secondary APS and this affects mostly young females of reproductive age (Cervera et al., 2002). Tincani *et al* have described three different variations of APS manifestations in SLE patients (Tincani et al., 2009).

1. Patients with primary APS who evolve to develop SLE

2. Patients with SLE who subsequently manifest with secondary APS
3. Patients with SLE with positive antiphospholipid antibodies without clinical APS.

Antiphospholipid antibodies affect the coagulation cascade and inflammation by binding to platelets and endothelial cells, inducing a procoagulant state. Complement activation results in tissue factor activation and endothelial damage and subsequently, thrombosis. During pregnancy, APL antibodies cause dysfunction of the trophoblast and activates complement at the feto-maternal interphase. This results in impaired function of the placenta, resulting in poor pregnancy outcomes (Pierangeli et al., 2008).

Making a diagnosis of APS, requires having both clinical and laboratory findings based on the revised Sapporo classification criteria (Miyakis et al., 2006) (Appendix C). It can affect all organ systems and the manifestations are diverse including:

- Central nervous system: migraine, strokes, transient ischaemic attack, sagittal sinus thrombosis, transverse myelitis, and central retinal artery occlusion.
- Lungs: pulmonary embolism and pulmonary hypertension
- Cardiovascular system: deep vein thrombosis, peripheral artery occlusion, coronary artery disease.
- Renal: renal vein thrombosis, renal infarction, thrombotic microangiopathy
- Skin: Raynaud’s phenomenon, livedo reticularis
- Musculoskeletal system: avascular necrosis
- Reproductive system: recurrent early miscarriage, intrauterine foetal death, preeclampsia, intrauterine growth retardation.

1.4 Rational of study

Haematological abnormalities are common in SLE and contribute to morbidity and mortality. There is a paucity of data analysing the spectrum and frequency of these abnormalities in South Africa. Analysing the frequency and patterns of these abnormalities will lead to better prognostication and patient management.

1.5 Aims and objectives

The aim of the present study was to investigate haematological abnormalities in patients with SLE attending a tertiary hospital in South Africa.

Primary objective:

To determine the spectrum and frequency of haematological abnormalities in patients with SLE.

Secondary objectives:

1. To explore relationship of baseline haematological abnormalities with organ involvement and specific autoantibodies.

2. To explore the relationship of baseline haematological abnormalities with mortality.

The Human Research Ethics Committee, University of the Witwatersrand (Appendix D) and Chris Hani Baragwanath hospital (CHBAH) management approved the study.

2 METHODS

2.1 Study Design and Patients

A retrospective nested case-control study of 240 adult SLE patients, who attended the Lupus Clinic, Rheumatology Unit, (CHBAH), Soweto, South Africa between 1st January 2011 and 30th June 2018 was undertaken. We compared a convenient sample size of 200 alive patients (AG) with 40 known demised (DG) patients, matched only for gender and age ±3 years.

All patients met the 1997 ACR classification criteria for SLE (Hochberg, 1997) (Appendix B), were ≥16 years at diagnosis, and had at least six months follow-up at the Lupus Clinic. Patients with conditions known to be associated with haematological abnormalities, e.g., HIV and malignancies, and those on immunosuppressive therapy and oral corticosteroids prior to diagnosis of SLE were excluded.

Data were abstracted from clinical records and included demographics, disease duration and clinical features according to the 1997 ACR SLE classification criteria (Hochberg, 1997). Laboratory data were abstracted from clinical records and confirmed with National Health Laboratory Service (NHLS) Labtrak and DISA systems. These included haematological variables, serological tests (autoantibodies and complement) and serum creatinine. The full blood count (FBC) was recorded only at initial diagnosis. Serological tests were documented both at initial diagnosis and during the follow-up period.

2.2 Statistical methods

Data were collected on a datasheet (Appendix A) and transcribed onto an Excel spreadsheet. Parametric continuous variables were represented using means and standard deviations and non-parametric continuous variables with median and interquartile range (IQR). Categorical variables were also represented as percentages (%). Pearson's Chi-squared test was utilised to compare categorical variables between groups and where indicated, two-tailed Fisher's exact test. Student's T-test and Mann-Whitney test were utilised in comparing parametric and non-parametric continuous variables respectively, between groups. Haematological variables were explored in univariate logistic regression models and variables with p

value <0.2 were explored in the multivariate analysis. A p value <0.05 was regarded as significant (Daniel, 1988; Peduzzi, Concato, Kemper, Holford, & Feinstein, 1996). STATA version 14 software was used to perform statistical analysis.

3 RESULTS

3.1 Demographic and clinical characteristics

Most patients were female (96.3%) and of African ethnicity (93.8%) as shown in table 3.1. Mean (SD) age at presentation and duration of follow-up were 37.9 (11.6) years and 4.1 (2.2) years, respectively. Arthritis (66.7%) and discoid lupus (DLE) (50%) were the most prevalent clinical features and 43.8% of patients had renal disease. Two thirds of patients had one or more haematological abnormalities. All patients were ANA positive. The only significant difference between DG and AG was that oral ulcers were more frequent in AG (OR=0.32, *p*=0.02.

Table 3.1: Demographics and clinical features in 240 South Africans with systemic lupus erythematosus

Variable	**Overall (n=240)**	**Alive group (n=200)**	**Deceased group (n=40)**	**Odds ratio (95% CI)**	***P* value**
Female gender - n (%)	231 (96.3)	192 (96.0)	39 (97.5)	0.62 (0.01-4.82)	0.65
Ethnicity: African - n (%)	225 (93.8)	188 (94.0)	37 (92.5)	1.27 (0.22-5.03)	0.72
Age (years) - mean (SD)	37.9 (11.6)	38.3 (11.5)	35.9 (11.8)	-	0.24
Duration of follow-up (years) - mean (SD)	4.1 (2.2)	4.3 (2.2)	3.6 (2.2)	-	0.09
Malar rash- n (%)	86 (35.8)	75 (37.5)	11 (27.5)	0.63 (0.26-1.40)	0.28
Discoid rash - n (%)	120 (50.0)	99 (49.5)	21 (52.5)	1.13 (0.54-2.37)	0.86
Photosensitivity - n (%)	74 (30.8)	59 (29.5)	15 (37.5)	1.43 (0.65-3.06)	0.35
Oral ulcers - n (%)	67 (27.9)	62 (31.0)	5 (12.5)	0.32 (0.09-0.87)	0.02
Arthritis - n (%)	160 (66.7)	137 (68.5)	23 (57.5)	0.62 (0.29-1.34)	0.20
Serositis - n (%)	49 (20.4)	36 (18.0)	13 (32.5)	2.19 (0.94-4.90)	0.05
Renal disorder - n (%)	105 (43.8)	84 (42.0)	21 (52.5)	1.53 (0.73-3.20)	0.23
Neurologic disorder - n (%)	38 (15.8)	29 (14.5)	9 (22.5)	1.71 (0.65-4.17)	0.24
Haematologic disorder - n (%)	160 (66.7)	131 (65.5)	29 (72.5)	1.40 (0.63-3.27)	0.47
Immunologic disorder - n (%)	233 (97.1)	193 (96.5)	40 (100.0)	2.31 (0.29-8.45)	0.60
Antinuclear antibodies - n (%)	240 (100.0)	200 (100.0)	40 (100.0)	1	1

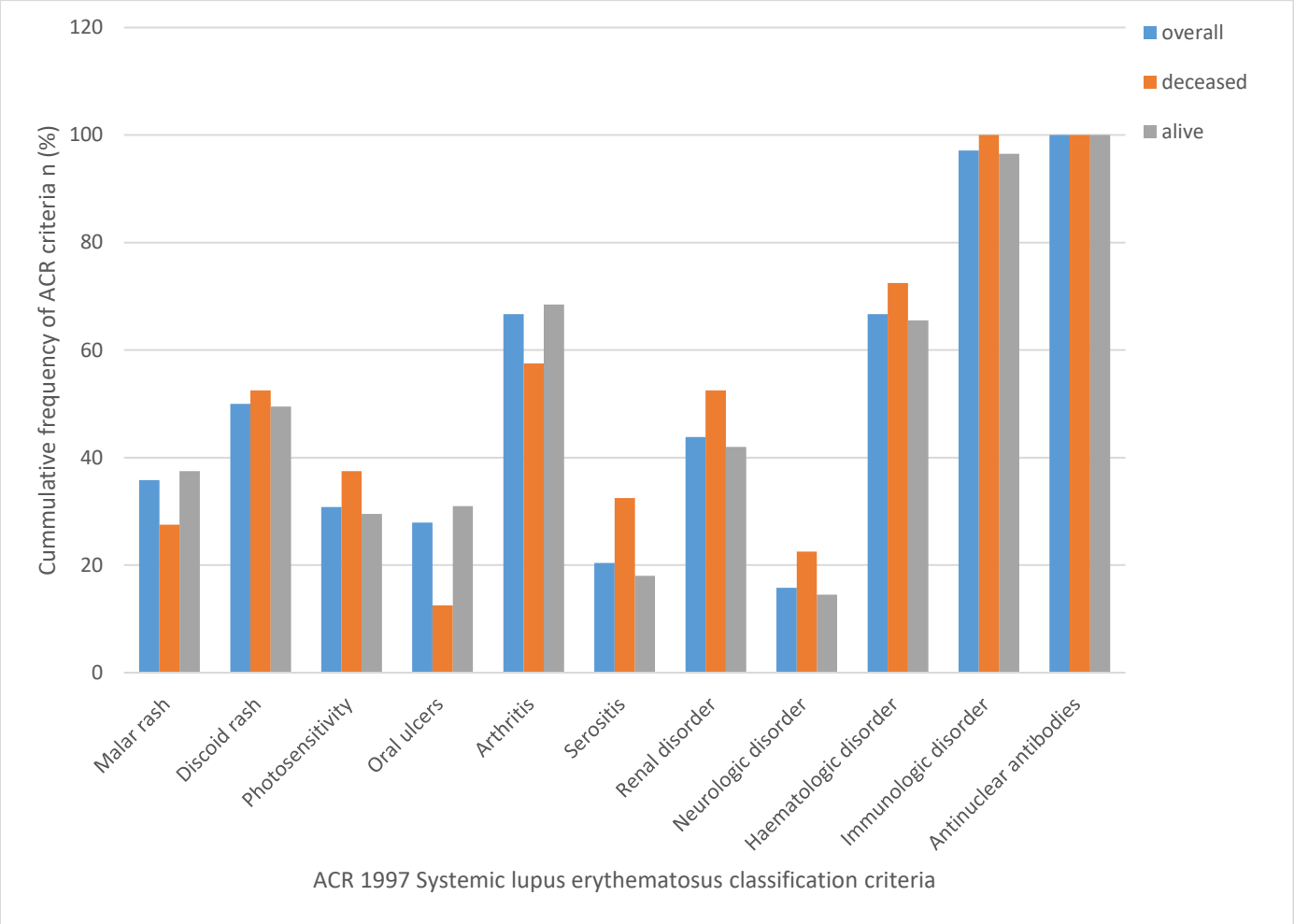

Figure 3.1: Cumulative frequencies of clinical and immunological features in 240 South African patients with systemic lupus erythematosus

3.2 Specific antinuclear antibodies and hypocomplementemia

As shown in Table 3.2, anti-ribonucleic protein (RNP) and anti-Smith (Sm) antibodies were the most common specific antinuclear antibodies, each occurring in almost three quarters of patients. 42% of patients had anti-double stranded DNA (dsDNA) antibodies and more than half had hypocomplementaemia. The frequencies of autoantibodies in AG and DG were not significantly different...

Table 3.2: Cumulative frequency of specific antinuclear antibodies and hypocomplementemia in 240 South Africans with systemic lupus erythematosus

	Total (n=240)	**Alive group (n=200)**	**Deceased group (n=40)**	**Odds ratio (95% CI)**	***P* value**
Anti-dsDNA - n (%)	101 (42.1)	82 (41.0)	19 (47.5)	1.30 (0.62-2.72)	0.49
Anti-Sm - n (%)	171 (71.25)	142 (71.0)	29 (72.5)	1.08 (0.48-2.55)	0.84
Anti-RNP - n (%)	178 (74.2)	149 (74.5)	29 (72.5)	0.90 (0.40-2.15)	0.79
Anti-Ro - n (%)	108 (45.0)	87 (43.5)	21 (52.5)	1.44 (0.69-3.01)	0.30
Anti-La - n (%)	55 (22.9)	44 (22.0)	11 (27.5)	1.34 (0.56-3.04)	0.45
C3/C4 hypocomplementemia - n (%)	141 (58.8)	119 (59.5)	22 (55.0)	0.83 (0.40-1.76)	0.60

dsDNA- double stranded DNA, Sm- smith, RNP-ribonucleic protein, C3-complement component 3, C4-complement component 4.

3.3 Haematologic abnormalities

Baseline full blood count (FBC) features are shown in Table 3.3. With respect to the red cell series, most patients had an anaemia (70.8%), with median haemoglobin significantly lower and median RDW significantly higher in DG than AG. With regards to the leucocyte series, just over a third of patients had a leucopaenia and 58.8% had a lymphopaenia. Median basophil count in the DG was significantly higher than in the AG. Thrombocytopaenia was found in 16% of patients. No significant differences in the frequency of thrombocytopaenia, absolute platelet count, MPV, NLR or PLR were observed between AG and DG.

Table 3.3: Baseline full blood count features in 240 South Africans with systemic lupus erythematosus

	Overall (n=240)	**Alive group (n=200)**	**Deceased group (n=40)**	**OR (95%CI)**	***P* value**
Red cells					
Haemoglobin (g/dl) – median (IQR)	10.6 (10.3-10.9)	10.8 (10.5-11.1)	9.5 (8.7-10.3)	**-**	<0.01
Anaemia - n (%)	170 (70.8)	139 (69.5)	31 (77.5)	1.95 (0.86-4.85)	0.09
MCV (fL) - median (IQR)	85.8 (84.8-86.7)	85.5 (84.5-86.5)	87.15 (84.2-90.2)	-	0.21
RDW (fL) - median (IQR)	15.6 (15.2-15.9)	15.4 (15.0-15.7)	16.6 (15.7-17.5)	**-**	0.01
Leukocytes					
Total leucocyte count ($X10^9$/L)- median (IQR)	5.4 (5.0-5.8)	5.3 (4.9-5.7)	6.2 (5.0-7.3)	-	0.08
Leucopaenia - n (%)	84 (35.0)	71 (35.5)	13 (32.5)	0.87 (0.39-1.89)	0.86
Neutrophil count ($X10^9$/L)- median (IQR)	3.5 (3.1-3.9)	3.4 (3.0-3.8)	4.1 (2.9-5.2)	-	0.19
Neutropaenia - n (%)	33 (13.8)	29 (14.5)	4 (10.0)	0.65 (0.16-2.04)	0.62

Lymphocyte count (x10^9/L)- median (IQR)	1.5 (1.4-1.6)	1.5 (1.4-1.6)	1.5 (1.2-1.9)	-	0.70
Lymphopaenia (%)	141 (58.8)	117 (58.5)	24 (60.0)	1.06 (0.51-2.29)	1.0
Basophil count (× 10^9/L)- median (IQR)	0.08 (0.07-0.09)	0.07(0.06-0.08)	0.13 (0.10-0.16)	-	<0.01
Eosinophil count (× 10^9/L)- median (IQR)	0.05 (0.04-0.06)	0.05 (0.04-0.06)	0.05 (0.03-0.06)	-	0.79
Monocyte count (× 10^9/L)- median (IQR)	0.3 (0.2-0.5)	0.3 (0.2-0.5)	0.32 (0.2-0.4)	-	0.78
Platelets					
Platelets (× 10^9/L)- median (IQR)	244 (227.8-261.2)	248 (229.5-266.2)	228 (185.4—270.3)	-	0.38
Thrombocytopaenia - n (%)	39 (16.25)	31 (15.5)	8 (20.0)	1.36 (0.49-3.39)	0.48
MPV (fL) - median (IQR)	10.4 (10.2-10.6)	10.4 (10.2=10.5)	10.5 (9.9-11.1)	-	0.52
Platelet to lymphocyte ratio- median (IQR)	244.5 (227.8-261.2)	247.9 (229.5-266.2)	227.9 (185.4-270.3)	-	0.38
Neutrophil to lymphocyte ratio - median (IQR)	3.51 (3.1-3.9)	3.4 (3.0-3.8)	4.1 (2.9-5.2)	-	0.19

MCV- mean cell volume, RDW- red cell distribution width, MPV- mean platelet volume.

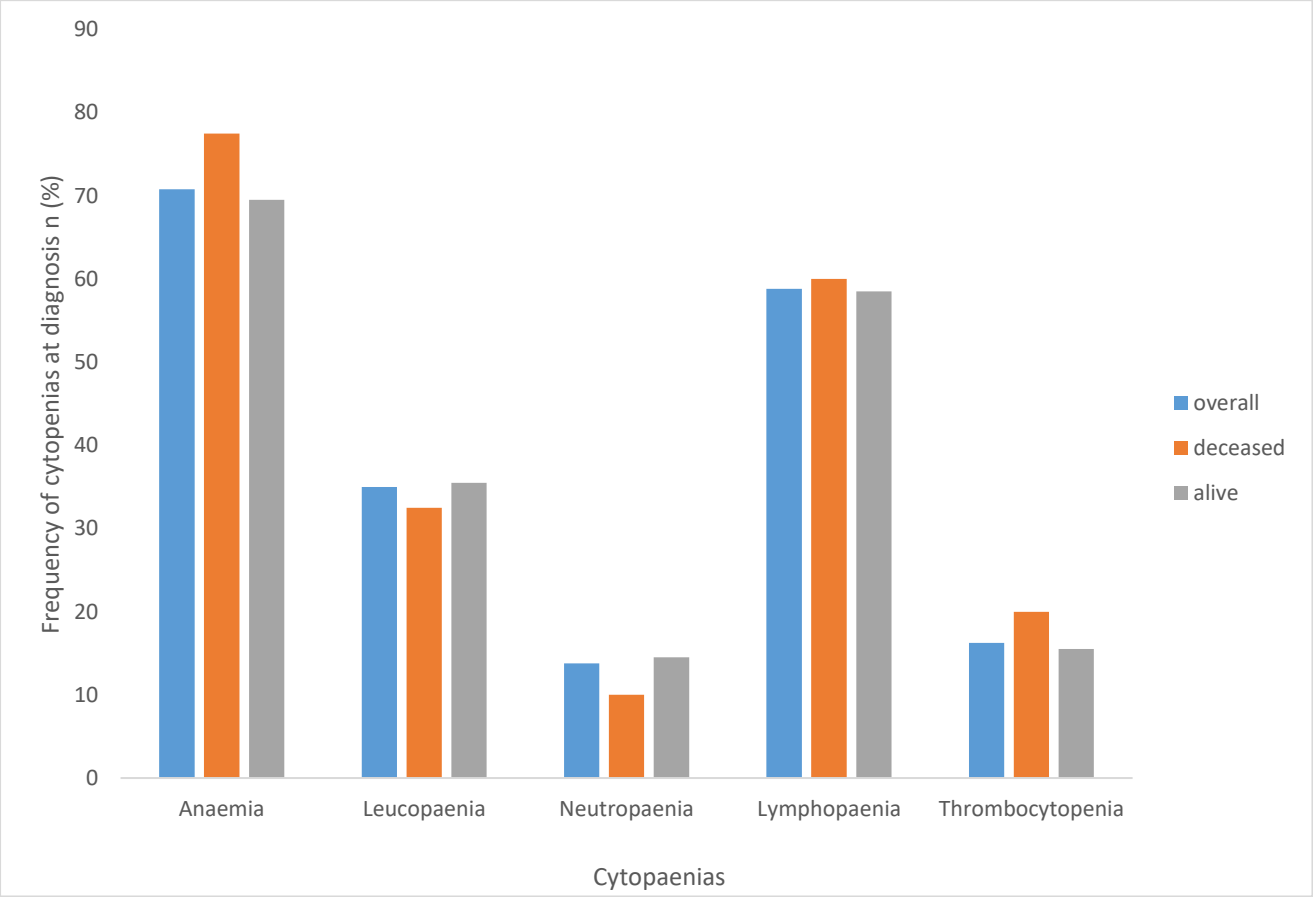

Figure 3.2: Frequency of cytopaenias at diagnosis in 240 South Africans with systemic lupus erythematosus

3.4 Autoimmune haemolytic anaemia, immune thrombocytopaenia and thro mbotic thrombocytopaenic purpura

Immune thrombocytopaenia (ITP) was the commonest immune mediated haematological event, occurring in just over 10% of patients and AIHA was observed more frequently in DG than AG, as shown in Table 3.4. 100 out of 147 patients who had a coombs test done had a positive test. However only 13 of these patients had clinically overt AIHA.

Table 3.4: Immune mediated haematological events in 240 South Africans with systemic lupus erythematosus

Haematological event	**Overall (n=240)**	**Alive group (n=200)**	**Deceased group (n=40)**	**Odds ratio (95% CI)**	***P* value**
AIHA - n (%)	13 (5.4)	6 (3.0)	7 (17.5)	6.86 (1.82-26.07)	<0.01
ITP - n (%)	26 (10.8)	20 (10.0)	6 (15.0)	1.59 (0.48-4.49)	0.35
Evan's syndrome - n (%)	10 (4.1)	7 (3.5)	3 (7.5)	2.24 (0.36-10.32)	0.38
TTP - n (%)	7 (2.9)	4 (2.0)	3 (7.5)	3.97 (0.55-24.34)	0.09

AIHA - autoimmune haemolytic anaemia, ITP - immune thrombocytopaenia, TTP - thrombotic thrombocytopaenic purpura

3.4 Antiphospholipid antibodies

As shown in Table 3.5, antiphospholipid antibodies were detected in about a quarter of patients, with the commonest being anti-cardiolipin antibody (ACLA) (26.3%), and least common being anti-beta2 glycoprotein-1 antibody (anti-β2GP1), (4%). Thirty six patients (15%) fulfilled the revised SAPPORO classification criteria for APS (Miyakis et al., 2006) of which 20 had pregnancy related events and 16 non pregnancy related thrombotic events. The commonest non-pregnancy related thrombotic event was deep vein thrombosis occurring in 13 (5.4%) patients. Other clinical manifestations of APS were stroke in two patients, renal vein thrombosis in one patient and dural vein thrombosis in one patient.

Table 3.5: Cumulative frequency of antiphospholipid antibodies and related clinical events in 240 South Africans with systemic lupus erythematosus

	Overall (n=240)	**Alive group (n=200)**	**Deceased group. (n=40)**	**Odds ratio. (95% CI)**	***P* value**
ACLA (Ig G or IgM) - n (%)	63 (26.3)	54 (27.0)	9 (22.5)	0.78	0.69
LA - n (%)	22 (9.2)	20 (10.0)	2 (5.0)	0.47	0.55
Anti-B2GP1 antibodies - n (%)	4 (1.7)	4 (2.0)	0 (0.0)	-	0.37
APS: pregnancy-related - n (%)	20 (8.3)	17 (8.5)	3 (7.5)	0.87	1.00
APS: thrombotic events - n (%)	16 (6.7)	16 (8.0)	0 (0.0)	-	0.06

ACLA– anticardiolipin antibodies, LA- lupus anticoagulant, B2GP1- beta 2 glycoprotein 1, APS antiphospholipid syndrome

3.5 Baseline inflammatory markers and Creatinine

As shown in Table 3.6, the mean ESR and creatinine at diagnosis was significantly higher in DG than AG and trend towards an increased mean CRP.

3.6 Other associated comorbidities

Severe infections requiring admission were seen in 14.2% of patients at diagnosis and occurred significantly more frequently in the DG than AG during the course of follow up at 37.5% and 9.5% respectively (OR=3.12, 95%CI=1.51- 6.47, p=0.002).

Table 3.6: Baseline inflammatory markers and Creatinine in 240 South Africans with systemic lupus erythematosus

	Overall (n=240)	**Alive group (n=200)**	**Deceased group (n=40)**	***P* value**
CRP (mg/L) - median (IQR)	7.0 (2.0-24.0)	6.0 (2.0-20.5)	12 (5.0-34.0)	0.08
ESR (mm/hr) - median (IQR)	35 (16.0-39.0)	31.5 (15.0-64.0)	59.0 (34.0-84.5)	<0.01
Creatinine (µmol/L- median (IQR))	74 (58.0-109.0)	71 (58.0-101.0)	89 (62.0-304.0)	<0.01

CRP- C reactive protein, ESR- erythrocyte sedimentation rate,

3.7 Association between baseline haematological parameters with clinical features and autoantibodies

As shown in Table 3.7 and 3.8, baseline univariate analysis showed median haemoglobin was significantly lower in patients with serositis, LN, hypocomplementaemia, and anti-Sm antibodies and higher in those with photosensitivity. Multivariate analysis showed that only LN and anti-Sm antibodies were independently associated with anaemia. A RDW >16 fL was independently associated with lupus nephritis. With respect to white cell series, low white cell count at baseline, had a positive association with CNS lupus, dsDNA and anti RNP

positivity in both univariate and multivariate analysis. Thrombocytopaenia was inversely associated with discoid lupus and arthritis on both univariate and multivariate analysis.

Table 3.7 Univariate associations of baseline haematological parameters with clinical features and autoantibodies in 240 South Africans with systemic lupus erythematosus

Clinical feature	**Present**	**Absent**	***P* value**
Discoid lupus	n=118	n=122	
Platelet count (x 10^9/L)	261 (178-343)	230 (120-302)	0.03
Platelet lymphocyte ratio	213.4 (137.8-310.4)	164.4 (89.0-66.0)	0.02
Photosensitivity	n=73	n=167	
Haemoglobin (g/dl)	11.2 (9.8-12.6)	10.4 (9.1-12.0)	0.01
Red cell distribution width (fL)	14.4 (13.6-15.7)	15.2 (13.8-17.2)	0.04
Oral ulcers	n=61	n=178	
Eosinophils (x 10^9/L)	0.04 (0.18-0.89)	0.02 (0.61-0.06)	0.03
Mean platelet volume (fL)	10.1 (9.4-10.8)	10.3 (9.8-11.2)	0.06
Arthritis	n=150	n=90	
Platelet count (x 10^9/L)	257 (180-352)	224 (120-297)	0.03
Serositis	n=42	n=198	
Haemoglobin (g/dl)	9.0 (6.9-10.4)	10.9 (9.6-12.3)	<0.0001
Red cell distribution width (fL)	15.3 (11.0-18.6)	14.6 (13.8-16.5)	0.10
Renal involvement	n=76	n=164	
Haemoglobin (g/dl)	10.0 (8.5-11.7)	11.1 (9.3-12.4)	0.005
Red cell distribution width (fL)	15.4 (14.0-17.3)	14.6 (13.7-16.2)	0.04
Neutrophil lymphocyte ratio	2.34 (1.66-4.11)	2.09 (1.35-2.84)	0.01
Central nervous system involvement	n=21	n=219	
Red cell distribution width (fL)	15.7 (14.2-17.5)	14.8 (13.8-16.7)	0.08
White cell count (x 10^9/L)	3.6 (2.4-4.9)	4.8 (3.4-6.7)	0.003
Neutrophils (x 10^9/L)	1.90 (1.4-2.8)	2.69 (1.9-4.5)	0.002
Eosinophils (x 10^9/L)	0.02 (0.00-0.03)	0.03 (0.01-0.08	0.02
Monocytes (x 10^9/L)	0.27 (0.17-0.32)	0.33 (0.23-0.46)	0.03
Platelets (x 10^9/L)	212 (94-277)	250 (173-326_	0.08
Anti-double stranded DNA antibodies	n=81	n=109	

White cell count (x 10^9/L)	4.2 (3.2-5.7)	5.0 (3.5-7.0)	0.02
Neutrophils (x 10^9/L)	2.40 (1.7-3.8)	2.8 (1.9-4.5)	0.05
Lymphocytes (x 10^9/L)	1.1 (0.8-1.7)	1.4 (0.9-2.0)	0.01
Monocytes (x 10^9/L)	0.28 (0.21-0.42)	0.34 (0.24-0.48)	0.01
Platelets (x 10^9/L)	262 (175-346)	236 (123-301	0.07
Platelet lymphocyte ratio	223.5 (185.6-352.3)	177.6 (86.2-253.2)	0.002
Anti-Sm antibodies	n=164	n=76	
Haemoglobin (g/dl)	10.5 (9.1-12.6)	10.8 (9.7-12.6)	0.04
White cell count (x 10^9/L)	4.6 (3.2-6.4)	5.3 (3.5-7.6)	0.10
Anti-RNP antibodies	n=172	n=68	
White cell count (x 10^9/L)	4.5 (3.2-6.4)	5.2 (3.9-7.1)	0.05
Neutrophils (x 10^9/L)	2.53 (1.78-3.94)	2.85 (2.14-4.73)	0.07
Anti-Ro antibodies	n=95	n=145	
Basophils (x 10^9/L)	0.07 (0.02-0.12)	0.03 (0.01-0.12)	0.06
Anti-La antibodies	n=51	n=189	
Lymphocytes (x 10^9/L)	1.65 (0.96-2.31)	1.15 (0.84-1.76)	0.006
Neutrophil lymphocyte ratio	1.85 (1.26-2.60)	2.17 (1.54-3.32)	0.009
Platelet lymphocyte ratio	160.3 (102.4-224.9)	201.8 (103.9-324.1)	0.06
C3/C4 hypocomplementaemia	n=25	n=115	
Haemoglobin (g/dl)	10.1 (9.1-11.7)	11.3 (9.5-17.5)	0.004
Mean cell volume (fL)	85.0 (79.0-90.0)	87.0 (82.0-91.8)	0.03
Red cell distribution width (fL)	15.3 (14.0-17.2)	14.4 (13.6-16.0)	0.005
Eosinophils (x 10^9/L)	0.02 (0.01-0.06)	0.4 (0.01-0.09)	0.005
Monocytes (x 10^9/L)	6.3 (0.22-0.42)	0.34 (0.24-0.49)	0.04
Anti-cardiolipin antibodies	n=53	n=163	
Haemoglobin (g/dl)	9.8 (8.8-11-8)	10.8 (9.3-12.3)	0.06
Monocytes (x 10^9/L)	0.28 (0.20-0.41)	0.32 (0.24-0.48)	0.01

All haematological values expressed as median (interquartile range)

Table 3.8 Multivariate logistic regression analysis of independent associations of hematological abnormalities with clinical features and autoantibodies in 240 South Africans with systemic lupus erythematosus

	OR (95% CI)	***P* value**
Anaemia		
Renal involvement	2.52 (1.19-5.34)	0.02
Anti-Sm antibodies	2.14 (1.10-4.16	0.02
Red cell distribution width >16 fL		
Renal involvement	1.85 (1.02-3.36)	0.04
Leucopaenia		
Central nervous system involvement	2.61 (1.04-6.57)	0.04
Anti-double stranded DNA antibodies	1.87 (1.04-3.38)	0.04
Anti-RNP antibodies	2.14 (1.12-4.24)	0.02
Neutropaenia		
Central nervous system involvement	3.26 (1.25-8.50)	0.02
Lymphopaenia		
Anti-La antibodies	0.48 (0.2-0.90)	0.02
Thrombocytopaenia		
Discoid lupus	0.40 (0.19-0.84)	0.02
Arthritis	0.43 (0.19-1.00)	0.05

Table 3.9 Multivariate logistic regression analysis of independent predictors of death in 240 South Africans with systemic lupus erythematosus

	OR (95% CI)	***P* value**
Death		
Basophil count	2039.2 (28.0-14836.9)	0.0005
Red cell distribution width	1.16 (1.01-1.33)	0.03
Oral ulcer	0.29 (0.10-0.88)	0.03
Infections	4.98 (2.06-12.04)	0.0004

As illustrated in table 3.9, patients that died had a higher basophil count and a high red cell distribution width. Severe infections requiring hospital admission was a predictor of mortality however the presence of oral ulcers was a protective feature against mortality.

4 DISCUSSION

In this study of 240 mainly black African females with SLE, laboratory haematological abnormalities were observed in the most patients at diagnosis. More than two thirds had an anaemia, over a third had leucopaenia, more than half had a lymphopaenia and thrombocytopaenia was found in about a sixth of patients. Several clinical haematological events occurred during the course of the disease, of which ITP seen in 10% of patients was the commonest, followed by AIHA, Evans syndrome and TTP. APS was seen in 15% of patients.

4.1 Red blood cells

The frequency of anaemia at diagnosis of 71% is higher than in other studies where range varied from 13 to 63% (Aleem et al., 2014; Beyan et al., 2007; Nossent & Swaak, 1991). This observation may be multifactorial. The higher frequency of lupus nephritis with accompanying anaemia of chronic disorder seen in this population is contributory. Although the median Hb was significantly lower in DG than AG, only raised RDW was an independent predictor of death. The correlation between a high RDW and disease activity and poorer therapeutic outcomes in SLE has been previously established (Zou et al., 2016) however its correlation with mortality in the context of SLE has not been reported previously. In other conditions like cardiovascular disease, cerebrovascular disease and breast cancer, increased RDW has in fact been shown to predict poor disease outcome (Li, Zhou, & Tang, 2017; Yao, Wang, Cai, Li, & Li, 2019). Autoimmune hemolytic anaemia which occurred in 5.4% of patients overall, carried a poor prognosis, occurring significantly more in the DG than AG. Studies done in other populations have shown prevalence of AIHA in SLE ranging between 8% and 28% (Beyan et al., 2007; Font et al., 2004) and this is associated with more severe manifestation of SLE (Jeffries et al., 2008).

4.2 White blood cells

White cell count abnormalities in SLE are not uncommon and studies looking at the prevalence of these abnormalities have had heterogenous results in different

population groups. Leucopaenia and lymphopaenia in SLE ranged between 22% to 41.8% and 15% to 82% respectively in a systematic literature review (Carli, Tani, Vagnani, Signorini, & Mosca, 2015). This current study showed a prevalence of 35% and 58.8% for leucopaenia and lymphopaenia respectively.

The baseline basophil count in the DG was significantly higher than in the AG and was an independent predictor of death. This relationship has not been previously established to this author's knowledge**.** Activation of basophils occurs during disease activity and this is associated with occurrence of LN (Charles, Hardwick, Daugas, Illei, & Rivera, 2010). This study showed a high number of patients with LN, which frequently leads to mortality (Ocampo-Piraquive, Nieto-Aristizábal, Cañas, & Tobón, 2018) and may explain the marked basophilia in the DG.

4.3 Platelets

Thrombocytopaenia is common in SLE. Its prevalence ranges from 10% to 40% as shown in studies from different populations (Fayyaz et al., 2015; Galanopoulos, Christoforidou, & Bezirgiannidou, 2017). It was seen in 16.25% of this population. There was no significant difference observed in baseline thrombocytopaenia, ITP or TTP between the AG and DG. Patel *et al* demonstrated that acute presentations of thrombocytopaenia during the course of SLE is associated with a high prevalence of LN and mortality (Patel & Mody, 2014).This study which only looked at thrombocytopaenia recorded at diagnosis did not reflect this view.

4.4 Antiphospholipid syndrome

Only 15% of patients had APS and no significant difference occurred between AG and DG. Other large cohort studies have estimated 20% to 50% of patients with SLE developing secondary APS (Cervera et al., 2002). The lower frequency observed may be due to under reporting of some pregnancy related events. It was observed that only miscarriages were documented in clinic notes. Preeclampsia, preterm deliveries and still births were not documented.

4.5 Association between haematological abnormalities and clinical features and specific autoantibodies

We observed several independent associations of haematological abnormalities with clinical and laboratory features in this study. Of note is the association of renal involvement with anaemia in multivariate analysis. Anaemia in SLE is associated with severe disease including lupus nephritis (Beyan et al., 2007; Samohvalov & Samohvalov, 2018). Lupus nephritis causes anaemia via various mechanisms including decreased erythropoietin levels and increased hepcidin, causing anaemia of chronic disorder. A lower baseline Hb level was associated with serositis, LN and anti SM antibodies in the univariate analysis. These views are supported by previous studies looking at the predictors of serositis in SLE patients (Ryu, Fu, & Petri, 2017), and studies looking at significance of anaemia in SLE (Samohvalov & Samohvalov, 2018) and the clinical association of anti SM in SLE (Arroyo-Ávila et al., 2015).

The association of leucopaenia and specifically neutropaenia with CNS lupus is noteworthy. Denburg *et al* suggested this when they showed there is a significant association between lymphocytic antibodies and neuropsychiatric lupus (Denburg, Behmann, Carbotte, & Denburg, 1994). In the univariate analysis, a low white cell count and lymphocyte count is significantly associated with presence of dsDNA and this supports the view in previous studies (Skare, Damin, & Hofius, 2015; Vilá et al., 2006). Leucopaenia and neutropaenia are shown to be associated with CNS lupus and there is a negative association between anti La and lymphopaenia in this study. These associations have not been previously established.

Thrombocytopaenia was shown to be inversely associated with the occurrence of discoid lupus and arthritis and photosensitivity was uncommon in patients with a low haemoglobin level. This specific negative association has not been previously published although Sasidharan *et al* in a previous study, showed an inverse association between haematological manifestation and presence of musculoskeletal features specifically arthritis (Sasidharan et al., 2012).

There have been conflicting conclusions with respect to the association between ds DNA and CNS lupus. Some studies support the view of a positive association (Steinman, 1979) and others do not (Muscal & Brey, 2010). With respect to anti RNP and CNS lupus, the anti RNP antibody is involved in the pathogenesis of neuropsychiatric SLE (NPSLE), however, its association with this clinical manifestation is unclear (Cozzani, Drosera, Gasparini, & Parodi, 2014). Some meta-analysis has shown that anti RNP is specifically associated with psychosis in NPSLE (Sciascia, Bertolaccini, Roccatello, Khamashta, & Sanna, 2014).

4.6 Predictors of mortality

Patients that died were more likely to have a high basophil count, a high red cell distribution width or had acquired a severe infection requiring hospital admission. The presence of oral ulcers was a protective feature against mortality. Infection is known to be an important predictor of mortality in SLE (Ocampo-Piraquive et al., 2018), however the association of mortality with basophilia and high RDW have not previously been established. Activation of basophils occurs during disease activity and this is associated with occurrence of LN (Charles, Hardwick, Daugas, Illei, & Rivera, 2010). This study showed a high number of patients with LN, which frequently leads to mortality (Ocampo-Piraquive, Nieto-Aristizábal, Cañas, & Tobón, 2018) and may explain the marked basophilia in the DG.

4.7 Characteristics of cohort

This general characteristics of the present cohort is consistent with previous studies, with the disease occurring mainly in women of child bearing age. (Hahn & Wallace, 2002). The most common clinical feature was arthritis. This is broadly consistent with previous studies done in South Africa (Budhoo, Mody, Patel, Dubula, & Mody, 2015; Wadee et al., 2007). Discoid lupus and LN were common features, each occurring in almost half of the patients. Anti-RNP and anti-Sm antibodies occurred in almost three-quarters of patients and were the most common specific autoantibodies. This differs slightly from findings by Tikly *et al* who showed anti RNP and anti-ds DNA to be the most frequently occurring autoantibodies in this population.(Tikly, Burgin, Mohanlal, Bellingan, & George, 1996).

5 LIMITATIONS OF STUDY

Like most retrospective studies, inaccuracies of data resulting in missing information, variation in documentation of clinical features by clinicians of variable experience, are shortcomings of the present study. Also, the relatively small sample size did not allow association analysis of hematological abnormalities with specific renal and NPSLE subgroups. Moreover, there was insufficient data to better characterize the anaemia, e.g., iron deficiency anaemia versus anaemia of chronic inflammation. The study was also undertaken in a single site in a public sector hospital.

Notwithstanding the above limitations, the present study provides new insights on the frequency and associations of baseline haematological abnormalities with major organ involvement, autoantibodies, and mortality in South Africans with SLE. Prospective studies in which the haematological abnormalities are better characterized are therefore needed.

6 CONCLUSION

The objective of the study was to explore the spectrum of haematological abnormalities in SLE patients who attended a tertiary lupus clinic in South Africa and to establish relationship between baseline haematological abnormalities with organ involvement, specific autoantibodies and mortality.

This study showed that haematological abnormalities are frequently seen at diagnosis of SLE and occurred in 66.7% of our patients. Notable among these were anaemia and lymphopenia which occurred in 70.8% and 58.8% of the population, respectively. Other abnormalities identified were neutropaenia, thrombocytopaenia, hypocomplementaemia, AIHA, TP, TTP and APLS.

Low levels of haemoglobin and higher RDW at diagnosis was seen significantly more commonly in the DG. AIHA was seen to occur significantly more commonly in the DG.

Patients having a low baseline Hb were more prone to developing lupus nephritis, and serositis and have anti-SM antibodies, but less likely to have photosensitivity. CNS lupus occurred more frequently in patients with dsDNA or anti-RNP and in patients with leucopaenia. Thrombocytopaenia was uncommon in patients with DLE or arthritis.

The FBC test which forms the basis for diagnosing these haematological abnormalities is one of the basic tests available at most levels of medical care in South Africa. A careful analysis of these parameters would lead to better management planning of SLE patients.

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8 APPENDICES

Appendix A: Data sheet

DATA SHEET

CASE NO:

1. DEMOGRAPHICS

Age: Gender: Ethnicity:

2. DISEASE DURATION

Date of first visit: Date of last follow up:

Duration of follow up (months):

3. ACR CLASSIFICATION CRITERIA AT DIAGNOSIS AND FOLLOW UP (CUMMULATIVE)

	At diagnosis	Follow up
Malar rash		
Discoid rash		
Photosensitivity		
Oral ulcers		
Arthritis		
Serositis		
Renal disorder		
Neurologic disorder		
Haematologic disorder		
Immunologic disorder		
Anti nuclear antibodies (ANA)		

4.HAEMATOLOGICAL FEATURES AT DIAGNOSIS AND AT FOLLOW UP

	At diagnosis	Follow up
Haemoglobin level (Hb)		
Red cell distribution width (RDW)		
Total white cell count (WCC)		
Neutrophils		
Lymphocytes		
Basophils		
Eosinophils		
Monocytes		
Platelet		
Mean platelet volume (MPV)		
C reactive protein (CRP)		
Erythrocyte sedimentation rate (ESR)		
Low C3/C4		
Anticardiolipin antibody (ACLA)		
Lupus anticoagulant (LA)		

B2 glycoprotein 1 (B2GP1)		
Idiopathic thrombocytopaenia (ITP)		
Autoimmune haemolytic anaemia (AIHA)		
Coombs antibody		

5.CLINICAL EVENTS.AT DIAGNOSIS AND FOLLOW UP

		At diagnosis	Follow up
Antiphospholipid syndrome	Pregnancy related		
	Non-pregnancy related (specify)		
Severe infection	Tuberculosis		
	Other acute infection requiring admission		
Organ dysfunction	Lupus nephritis		
	CNS lupus		
	Respiratory		
	Cardiac		

Appendix B: 1997 updated American College of Rheumatology classification criteria for systemic lupus erythematosus

Criteria	Definition
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. Serositis	a) Pleuritis—convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion *OR* b) Pericarditis—documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a) Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not performed *OR* b) Cellular casts—may be red cell, haemoglobin, granular,

	tubular, or mixed
8. Neurologic disorder	a) Seizures—in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance *OR* b) Psychosis—in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Haematologic disorder	a) Haemolytic anaemia—with reticulocytosis *OR* b) Leukopenia—less than 4,000/mm on 2 or more occasions *OR* c) Lymphopenia—less than 1,500/mm on 2 or more occasions *OR* d) Thrombocytopenia—less than 100,000/mm in the absence of offending drugs
10. Immunologic disorder	a) Anti-DNA: antibody to native DNA in abnormal titer *OR* b) Anti-Sm: presence of antibody to Sm nuclear antigen *OR* c) Positive finding of antiphospholipid antibodies on: • An abnormal serum level of IgG or IgM anticardiolipin antibodies • A positive test result for lupus anticoagulant using a standard method

	• A false positive test for at least 6 months confirmed by Treponema pallidum immobilisation or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with “drug-induced lupus” syndrome

* The proposed classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have systemic lupus erythematosus if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.

Appendix C: Revised Sapporo classification criteria for Anti- Phospholipid syndrome

Clinical criteria

The presence of either vascular thrombosis or pregnancy morbidity, is defined as follows:

Vascular thrombosis is defined as one or more episodes of venous, arterial, or small vessel thrombosis, with unequivocal imaging or histologic evidence of thrombosis in any tissue or organ. Superficial venous thrombosis does not satisfy the criteria for thrombosis for APS.

Pregnancy morbidity is defined as otherwise unexplained fetal death at ≥10 weeks gestation of a morphologically normal fetus, or one or more premature births before 34 weeks of gestation because of eclampsia, pre-eclampsia, or placental insufficiency*, or three or more embryonic (<10 week gestation) pregnancy losses unexplained by maternal or paternal chromosomal abnormalities or by maternal anatomic or hormonal causes.

*Accepted features of placental insufficiency include: i) abnormal or non-reassuring fetal surveillance tests ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia iii) oligohydramnios iv) postnatal birth weight less than the tenth percentile for the gestational age.

Laboratory criteria

The presence of aPL, on two or more occasions at least 12 weeks apart and no more than five years prior to clinical manifestations, as demonstrated by one or more of the following:

IgG and/or IgM aCL in moderate or high titer (>40 units GPL or MPL or >99th percentile for the testing laboratory)

Antibodies to β_2-glycoprotein I (anti-β_2GPI) of IgG or IgM isotype at a titer >99th percentile for the testing laboratory when tested according to recommended procedures

Lupus anticoagulant (LA) activity detected according to guidelines established by international society of Thrombosis and hemostasis.

Appendix D: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Ivy Anafi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180763

NAME: (Principal Investigator)	Dr Ivy Anafi
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Haematology abnormalities in South Africans with systematic lupus erythematosus
DATE CONSIDERED:	27/07/2018
DECISION:	Approved Unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Mohammed Tickly and Dr Kavita Makan
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	19/11/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore be due in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

01/12/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES