# HAEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN PATIENTS WITH SYSTEMIC SCLEROSIS ATTENDING A TERTIARY CARE HOSPITAL

**Vaishak Vinod**

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

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# DECLARATION

I, Vaishak Vinod declare that this research report is my own work. It is being submitted for the Degree of Master of 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.

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Vaishak Vinod

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Date

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Place

# DEDICATION

To my wonderful wife, Vidya, for her support and motivation.

For my beloved children, Riya and Dev.

# PRESENTATIONS ORIGINATING FROM THIS RESEARCH

## ***Poster presentation:***

Vinod V, Tikly M, Ickinger C. Baseline haematological abnormalities in systemic sclerosis and their association with organ involvement and mortality. South African Rheumatism and Arthritis Association (SARAA) congress, Durban, 2019.

# ABSTRACT

**Background**

To describe the frequency of full blood count and serological abnormalities at diagnosis of systemic sclerosis (SSc) and correlates with acute phase reactants, specific clinical features and mortality.

**Patients and Methods**

A retrospective case record review of SSc patients attending a tertiary rheumatology clinic from over a 17 year period. Parameters of the full blood count, inflammatory indices, serological markers and acute phase reactants were the focus of the study.

**Results**

Of the 135 patients, the majority were black females (92.6%) with diffuse cutaneous SSc (DcSSc). Anaemia was the commonest haematological abnormality (35%) followed by lymphopenia (29.4%) and thrombocytosis (16.9%).

**Haematological abnormalities at diagnosis and associations with clinical manifestations and mortality in patients with systemic sclerosis**

| HAEMATOLOGICAL PARAMETER | CLINICAL MANIFESTATIONS | | | |
|---|---|---|---|---|
| | PRESENT | ABSENT | OR (95% CI) | p-value |
| | PERICARDIAL EFFUSION | | | |
| Mean (SD) Hb (g/dL) | 11.5 (1.8) | 12.8 (1.7) | - | <0.01 |
| Anaemia (%) | 14/20 (70.0) | 31/109 (28.4) | 5.87 (2.07-16.66) | <0.01 |
| Mean (SD) RDW (%) | 16.7 (2.5) | 15.8 (1.9) | - | 0.14 |
| RDW >17 (%) | 6/12 (50) | 6/69 (8.7) | 6.30 (1.69-23.43) | <0.01 |
| | PULMONARY HYPERTENSION | | | |
| Mean (SD) RDW (%) | 16.8 (2.4) | 15.5 (1.7) | - | <0.01 |
| RDW >17 (%) | 10/28 (35.7) | 6/57 (10.5) | 4.72 (1.50-14.85) | 0.01 |
| | CARDIAC INVOLVEMENT | | | |
| Mean (SD) NLR (g/dL) | 3.7 (3.1) | 2.5 (1.8) | - | 0.02 |
| High NLR | 25/50 (50) | 15/52 (28.8) | 2.47 (1.09-5.58) | 0.04 |
| | DIGITAL LESIONS | | | |
| Mean (SD) NLR (g/dL) | 3.6 (3.0) | 2.5 (1.9) | - | 0.03 |
| High NLR | 24/53 (45.2) | 16/49 (32.7) | 1.71 (0.76-3.82) | 0.14 |
| | MORTALITY | | | |
| Mean (SD) NLR (g/dL) | 3.8 (3.2) | 2.6 (2.2) | - | 0.04 |
| High NLR | 18/33 (54.5) | 22/69 (31.9) | 2.56 (1.09-6.00) | 0.03 |

RDW - red cell distribution width; NLR – neutrophil lymphocyte ratio; Hb - haemoglobin

A red cell distribution width (RDW) >17% was associated with cardiopulmonary disease. Similiary, patients with cardiac disease and digital lesions had a higher mean neutrophil lymphocyte ratio (NLR). Furthermore, a NLR $\geq$ 3, was a predictor for mortality.

**Conclusion**

Red cell parameters such as anaemia and an increased RDW were common and associated mainly with cardiopulmonary disease and a high NLR with mortality.

# ACKNOWLEDGEMENTS

I would like to acknowledge and thank my supervisors, Dr Claudia Ickinger and Professor Mohammed Tikly, for their expertise and patience in guiding me through this journey. Without their support and encouragement, this would not have been possible.

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# ABBREVIATIONS

| | |
|---|---|
| ACA | Anti-centromere antibody |
| ACD | Anaemia of chronic disease |
| ACE-I | Angiotensin converting enzyme inhibitor |
| ACR | American College of Rheumatology |
| ARA | Anti-RNA polymerase antibody |
| ARB | Angiotensin receptor blocker |
| ATA | Anti-topoisomerase 1 antibody |
| CHBAH | Chris Hani Baragwanath Academic Hospital |
| CK | Creatine kinase |
| CRP | C-reactive protein |
| CT | Computed tomography |
| CTD | Connective tissues disease |
| DAS 28 | Disease activity score (28 Joint count) |
| DcSSc | Diffuse cutaneous systemic sclerosis |
| DLCO | Diffusing capacity for carbon monoxide |
| ESR | Erythrocyte sedimentation rate |
| EULAR | European League against Rheumatism |
| F/U | Follow up |
| FBC | Full blood count |
| FVC | Forced vital capacity |
| GAVE | Gastric antral vascular ectasia |
| GI | Gastrointestinal system |
| Hb | Haemoglobin |
| HCT | Haematocrit |
| HIV | Human immunodeficiency virus |
| HLA | Human leukocyte antigen |
| HRCT | High resolution computed tomography |

| | |
|---|---|
| IDA | Iron deficiency anaemia |
| IL-6 | Interleukin 6 |
| ILD | Interstitial lung disease |
| IQR | Interquartile range |
| LcSSc | Limited cutaneous systemic sclerosis |
| MCH | Mean cell haemoglobin |
| MCHC | Mean cell haemoglobin concentration |
| MCV | Mean cell volume |
| MPV | Mean platelet volume |
| mRSS | Modified Rodnan skin score |
| NHLS | National Health Laboratory Services |
| NLR | Neutrophil lymphocyte ratio |
| PAH | Pulmonary arterial hypertension |
| PSS | Progressive systemic sclerosis |
| RA | Rheumatoid arthritis |
| RDW | Red cell distribution width |
| RNA | Ribonucleic acid |
| RNAP | RNA polymerase enzyme |
| RP | Raynaud’s phenomenon |
| SD | Standard deviation |
| SIBO | Small intestinal bacterial overgrowth |
| SLE | Systemic lupus erythematosus |
| SRC | Scleroderma renal crisis |
| SSc | Systemic sclerosis |
| TB | Tuberculosis |
| TFR | Tendon friction rub |
| WCC | White cell count |
| WHO | World Health Organization |

# CHAPTER ONE: LITERATURE REVIEW

## 1.1 Introduction

Systemic sclerosis (SSc) or scleroderma is a systemic autoimmune condition that is characterised by immune dysregulation, generalised obliterative vasculopathy, fibrosis and antibody production with multi-organ involvement (1). The disease is characterised by the presence of skin fibrosis; the extent of skin involvement differentiates the disease into its two main subsets (2). Vascular endothelial injury by varied mechanisms and subsequent endothelial cell activation and dysfunction is thought to be the initial insult which culminates in organ fibrosis. The resulting organ dysfunction leads to clinical and biochemical abnormalities (1, 3).

## 1.2 History of systemic sclerosis

The first case suggestive of possible scleroderma was described by Cario Curizo in Italy in 1753. It was Fantonetti who used the term Scleroderma in 1836, 30 years later (4, 5). In its primitive descriptions it was thought to be a disease restricted to the skin with limited internal organ involvement. This concept was later modified based on autopsy case findings by Matsui who demonstrated significant organ involvement in patients who demised from this condition. This was validated by Robert Goetz, a German surgeon working in South Africa, who coined the term “progressive systemic sclerosis” (PSS). This was later changed to “systemic sclerosis” as not all patients showed disease progression (4, 5).

## 1.3 Epidemiology

The incidence and prevalence of the condition vary geographically and with ethnicity; the worldwide prevalence is between 150-300 cases per million. Reasons for regional variation are attributed to the exposure of specific environmental triggers as well as genetic variations (6). The disease displays a female predominance with a female to male ratio of 7:1 (7) in a local study and 4:1 in international literature (8). The mean age of disease onset is usually between 45 - 50 years (9). One prior local study showed a younger age of disease onset in black South African patients with a mean age of 36.1 years (10).

### 1.4 Aetiopathogenesis

The precise aetiopathogenesis of SSc is unknown. In genetically susceptible individuals, environmental stimulants seem to initiate autoantibody production and an intricate inflammatory cascade that results in disease initiation and propagation. Multiple human leukocyte antigen (HLA) associated and non-HLA associated loci are involved in the pathogenesis of SSc (11, 12). Choctaw Indians and African-Americans have a genetic predisposition that puts them at a higher risk of developing SSc (13). The African-American population is largely of West African origin and they are genetically notably different to the Sub-Saharan African population (10, 14). Various hydrocarbons and chlorinated solvents have also been implicated as triggers, however these only account for a minority of the cases. Silica dust was one of the first environmental triggers that was associated with the development of SSc (11). It is of importance in the South African setting as the gold mining industry and associated silica exposure was linked to the development of SSc in early studies (15, 16).

As depicted in figure 1 below, vascular injury is thought to be the most likely primary event in disease development. An initial incident facilitates endothelial injury with subsequent endothelial cell activation resulting in release of cytokines and chemokines. A cascade of events subsequently results in the transformation of fibroblasts to myofibroblasts. The end product is one of excessive extracellular matrix formation and deposition which culminates in obliterative vasculopathy and organ fibrosis with dysfunction (1, 11, 17).

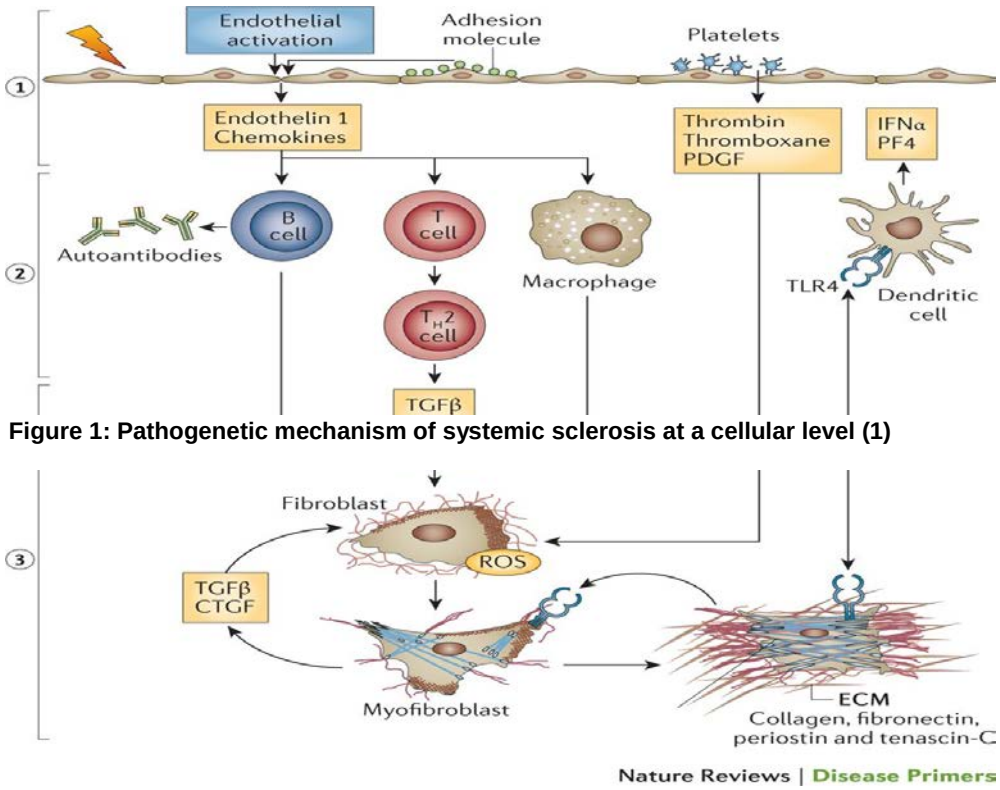



**Figure 1: Pathogenetic mechanism of systemic sclerosis at a cellular level (1)**

**Figure 1: Pathogenetic mechanism of systemic sclerosis at a cellular level**

### 1.5 Autoantibodies in systemic sclerosis

Autoantibodies in SSc are directed against constituents within the cell nucleus, the nucleolus or some against cell surface receptors. They are understood to play an important role in disease pathogenesis. The autoantibody profiles serve as surrogate markers for diagnosis, classification and predictors of organ involvement in SSc. Up to 95% of patients have one of the major seven SSc associated serum autoantibodies. Importantly, an individual does not usually have more than one of the scleroderma associated autoantibodies and the antibody status does not usually change with time. The two most commonly encountered antibodies are the anti-topoisomerase (ATA) and anti-centromere antibodies (ACA); the less frequently occurring antinucleolar antibodies include anti-PM-Scl, anti-U3 ribonucleoprotein (RNP), anti-Th/To and a variety of anti-ribonucleic acid (RNA) polymerase antibodies (18).

Anti-topoisomerase 1 antibodies are usually found in patients with diffuse cutaneous systemic sclerosis (DcSSc). It occurs in up to 40% of patients with DcSSc and in less than 10% with limited cutaneous systemic sclerosis (LcSSc) (19). Anti-topoisomerase 1 antibody positivity increases the likelihood of the development of pulmonary fibrosis

and is associated with a higher mortality (18).

Anti-centromere antibodies are a group of approximately six antibodies that are directed at specific centromeric proteins. The presence of ACA has a high specificity for SSc. It is not usually found in healthy individuals or individuals with other auto-immune diseases and is more often associated with LcSSc (18). Anti-centromere antibody positivity is associated with calcinosis, digital lesions and a reduced incidence and severity of pulmonary fibrosis. The presence of ACA, in comparison to ATA in SSc, is associated with less aggressive disease and improved survival (19). There are three major classes of anti-RNA polymerase enzymes (RNAP); in particular classes I, II and III. Specific antibodies are directed at these RNA polymerases. Anti-RNA polymerase antibody (ARA) positivity increases the likelihood of the development of scleroderma renal crisis (20), especially antibodies directed at RNAP type III (21, 22).

**1.6 Classification criteria for systemic sclerosis and subset classification**

Systemic sclerosis displays marked heterogeneity and inter-patient variation with respect to autoantibody profile, clinical presentation, disease progression, treatment response and clinical outcomes. There are no diagnostic criteria as in most other autoimmune rheumatic diseases; classification criteria such as the 1980 American College of Rheumatology (ACR) (23) classification criteria or the more recent ACR/ European League Against Rheumatism (EULAR) 2013 classification (Figure 2) are used primarily for research purposes and to guide with diagnosis of the disease (24).

**2013 ACR / EULAR Criteria For The Classification Of Systemic Sclerosis (Scleroderma)***

| Item | Sub-items(s) | Weight/score † |
|---|---|---|
| Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints *(sufficient criterion)* | - | 9 |
| Skin thickening of the fingers *(only count the higher score)* | Puffy fingers | 2 |
| | Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints) | 4 |
| Fingertip lesions *(only count the higher score)* | Digital tip ulcers | 2 |
| | Fingertip pitting scars | 3 |
| Telangiectasia | - | 2 |
| Abnormal nailfold capillaries | - | 2 |
| Pulmonary arterial hypertension and/or interstitial lung disease *(maximum score is 2)* | Pulmonary arterial hypertension | 2 |
| | Interstitial lung disease | 2 |
| Raynaud's phenomenon | - | 3 |
| SSc-related autoantibodies (anticentromere, anti–topoisomerase I [anti–Scl-70], anti–RNA polymerase III) *(maximum score is 3)* | Anticentromere 3<br>Anti–topoisomerase I<br>Anti–RNA polymerase III | 3 |

* The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestations (e.g., nephrogenic sclerosing fibrosis, generalized morphea, eosinophilic fasciitis, scleredema diabeticorum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy).

† The total score is determined by adding the maximum weight (score) in each category.
**Patients with a total score of ≥ 9 are classified as having definite scleroderma.**

**Figure 2: 2013 ACR/EULAR Classification criteria for systemic sclerosis (24)**
https://www.rheumtutor.com/diseases/scleroderma/

The hallmark of the disease is dermal fibrosis; normal tissue structure is disrupted and replaced with connective tissue rich in collagen due to the above mentioned processes. Clinically the extent of dermal fibrosis is assessed by the modified Rodnan skin score (mRSS) which evaluates skin thickness at 17 anatomically defined areas of the body (25, 26). The mRSS at diagnosis has important implications for outcomes. An increase in skin thickening, both in terms of rate of progression and extent, is associated with internal organ involvement and increased mortality (27).

Clinically the patients are sub-classified as having either limited or diffuse cutaneous SSc based on the pattern of skin thickening. Patients with skin thickening limited to the distal extremities (i.e. below elbows and knees) and the face are described as having LcSSc. Patients with skin fibrosis extending proximal to the elbows and knees with or without truncal involvement are classified as DcSSc (26). Patients with DcSSc usually have a rapid rate of skin thickening with significant internal organ involvement early in the course of the disease (28). Patients with LcSSc do also develop organ involvement, with 10-15% having isolated pulmonary arterial hypertension (PAH), while renal involvement is rare (29). It is the extent of organ involvement that dictates morbidity and mortality in SSc and earlier diagnosis and intervention is associated

with better outcomes (28).

In South African and other African experiences, DcSSc is more prevalent than LcSSc which differs from global trends (7, 10, 30). This is in keeping with the patterns noted in black African-American patients with SSc. In Caucasian population groups LcSSc is the predominant subset (13).

### 1.7 Visceral involvement in systemic sclerosis

Other organ systems that are frequently involved include the gastrointestinal, cardiovascular, respiratory, musculoskeletal and peripheral vascular systems (31).

#### 1.7.1 Respiratory system

Pulmonary involvement can be divided into either direct or indirect involvement. The disease affects either the lung parenchyma or vasculature. The two most common pathologies are pulmonary fibrosis and PAH. Together these two entities account for up to 60% of deaths in SSc (32). Interstitial fibrosis is common in SSc and has histological and radiological features in keeping with non-specific interstitial pneumonia. Patients with interstitial lung disease (ILD) may present with progressive dyspnoea or a dry cough with clinical findings of fine bibasal late inspiratory crackles. This clinical presentation or typical findings on chest radiography and a restrictive pattern on lung function test serve as important screening tools for the diagnosis of ILD in SSc (5). The diagnosis is usually confirmed on high resolution computed tomography (HRCT) scans. Indirect involvement includes restriction of chest wall expansion secondary to taut skin, chest wall deformities and microaspiration from reflux (5, 32).

#### 1.7.2 Cardiac involvement

Cardiac involvement is more common than previously estimated with studies showing a prevalence of cardiac disease in up to 25% of patients with SSc, more so in the DcSSc subset (33). The existence of cardiac disease is sometimes underdiagnosed due to the subtle nature of signs and symptoms. Systemic sclerosis can affect the heart either directly or indirectly, the disease process affects almost all structures of the heart; myocardial fibrosis and pericardial disease are the most prominent findings in autopsy studies. Other manifestations include myositis, coronary artery disease and arrhythmias. The disease process may result in either systolic or diastolic cardiac dysfunction. The underlying pathophysiology is that of microvascular ischaemia with

inflammation and fibrosis. Indirect cardiac involvement can occur secondary to renal and pulmonary diseases such as scleroderma renal crisis and pulmonary hypertension, respectively (33, 34).

### 1.7.3 Gastrointestinal system

The gastrointestinal (GI) system is the most commonly affected organ system second to skin and occurs in up to 90% of the patients with SSc. Any site along the GIT may be affected but oesophageal involvement is the most common site of disease. Manifestations of oesophageal disease include dysphagia, nausea, vomiting and symptoms of reflux secondary to dysmotility in conjunction with dysfunction of the lower oesophageal sphincter. Gastric antral vascular ectasia (GAVE) is a unique cause of anaemia in SSc and occurs in approximately 15% of patients with SSc. The small and large bowel are also frequently affected. Small bowel involvement may result in bacterial overgrowth and nutritional (B12 and folate) deficiencies. Manifestations of colonic disease include constipation, diarrhoea and haematochezia from multiple pathologies. Gastrointestinal involvement translates to a poorer quality of life, reduced survival and increased mortality (35, 36).

### 1.7.4 Renal manifestations

A devastating renal manifestation in SSc is the scleroderma renal crisis (SRC) occurring in up to 5-10% of patients with SSc. It is more common in females with DcSSc (37). Haematological, immunological and inflammatory abnormalities at diagnosis that increase the likelihood of developing SRC include anaemia, thrombocytopenia, anti-RNA polymerase III antibodies and an elevated erythrocyte sedimentation rate (ESR) (38). Scleroderma renal crisis (SRC) has a 5-year mortality of 30-40%, however the number of mortalities have declined over the last 30 years with the use of angiotensin converting enzyme inhibitors (ACE-I) (39).

### 1.7.5 Musculoskeletal system and skin

Musculoskeletal manifestations are quite prevalent in SSc and have varied presentations. Skin and soft tissue abnormalities due to fibrotic and vascular pathology contribute towards morbidity due to hampered functionality. Raynaud's phenomenon (RP) and sclerodactyly are some of the additional features of this disease and occurs in greater than 95% of patients. Digital lesions secondary to complicated RP include digital ulcers, digital pits, digital gangrene and pulp atrophy.

Raynaud's phenomenon, digital lesions, calcinosis, telangiectasia are "skin" manifestations in SSc.

Arthralgia is a common presenting complaint. It may be multifactorial in nature with pathology involving the overlying skin, joint capsule, tendon or bone. Joint involvement ranges from non-erosive arthritis to deforming arthritis and resorption of distal phalanges, acro-osteolysis. Tendon involvement may result in tenosynovitis, tendon rupture and tendon friction rubs (TFR). The presence of a TFR may allude to active disease and there is a positive association with the development of proximal myopathy, lung fibrosis and SRC (40). The prevalence of joint contractures and tendon friction rubs may be as high as 31% and 11% respectively (41).

The pathogenesis of scleroderma associated myopathy may be inflammatory or non-inflammatory in nature. The non-inflammatory myopathies are not well understood and may be secondary to neurological abnormalities or drug related side effects (42).

### 1.8 Haematological, inflammatory and immunological parameters

#### 1.8.1 Parameters of the full blood count

The full blood count (FBC) is a routinely performed investigation. It is an inexpensive and rapid test which has the potential to provide a vast amount of information if explored in depth (43). Parameters on the FBC include the white cell count (WCC) and differential count, the haemoglobin (Hb), mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), haematocrit (HCT) and the platelet count. The novel indicators of disease such as red cell distribution width (RDW), mean platelet volume (MPV) and the neutrophil lymphocyte ratio (NLR) can be obtained directly or indirectly calculated from the respective parameters of the FBC and differential count. When these parameters are viewed in clinical context they may provide an extensive amount of information regarding disease processes (43-46).

#### 1.8.2 White cell indices

Haematological parameters in populations have shown to be varied across different ethnic groups. Healthy subjects of African ethnicity have been shown to have a lower total WCC and neutrophil count compared to Caucasian subjects (47). This has also been documented in the South African black population residing in the Witwatersrand

area by Tikly et al. Their study proposed that a WCC value extending up to $3.5x10^9$/L represented normality in this population (10). In addition, a lower absolute neutrophil count and higher lymphocyte counts were described in these patients. This study was based in the same drainage area as the present study. These findings were validated in later studies conducted by Lawrie et al in conjunction with the National Health Laboratory Services (NHLS). Ethnic and gender specific reference ranges have since been recommended for the South African black population (48).

The NLR is a novel marker that has attracted interest over the last few years. It is a ratio of the absolute neutrophil count to the absolute lymphocyte count. The major benefit of this test is that it can be easily calculated from the FBC and differential count. The NLR has been studied as a predictive and prognostic tool in diseases such as malignancies, acute infections, cardiovascular diseases and rheumatic diseases (49). The variation in the NLR, in particular, elevated levels may allude to underlying systemic inflammation.

### 1.8.3 Red cell parameters

The World Health Organization (WHO) defines anaemia as a haemoglobin level of <12.0g/dL in females and <13.0g/dL in males (50). There is a varied distribution amongst the different ethnic groups as is the case with the WCC. Lawrie et al showed that black females had lower haemoglobin values in comparison to a combined ethnic group (Asian, Coloured and Caucasian). It was suggested that a haemoglobin of <13.4g/dL for males and <11.6g/dL for females should be used as the cut-off points for the diagnosis of anaemia in the South African black population (48). Other local studies proposed that a haemoglobin of less than 13.8g/dL in males and <12.4g/dL in females should constitute anaemia (51). This is relevant for our study as it pertains to the population we are studying as well as the laboratory we utilize for the analysis of our blood tests.

The RDW is a numerical description of the range in variation in the red blood cell volume; a marker of anisocytosis. It is impacted mainly by abnormalities in the maturation process of erythrocytes or due to increased red cell turnover states (43). Disordered or ineffective erythropoiesis secondary to inflammatory cytokines acting along various stages of the erythroid cell maturation process is the main reason for elevated RDW in chronic inflammatory conditions. The consequences of nutritional

deficiencies (iron, vitamin B12, folate), haemolysis and bleeding may also be significant (52). An elevated RDW and its association with clinical manifestations and its utility as a prognostic marker has been a topic of interest recently in a wide range of rheumatological (46, 53) and non-rheumatological conditions (43, 52, 54, 55). An elevated RDW has also been shown to be an independent marker of cardiovascular disease (46, 54).

### 1.8.4 Platelet parameters

Platelets play a significant role in haemostasis, thrombosis, inflammation, tissue healing and form an integral link to the immune system (56). Thrombocytosis may occur as an acute phase response in inflammatory or infective states or in response to blood loss. Thrombocytosis in these settings of inflammation reflects bone marrow hyper-stimulation in response to inflammatory cytokines (57).

Platelet indices and their associations with organ involvement in various conditions have been a recent topic of interest. The MPV is a popular parameter undergoing scrutiny as it is thought to be a better marker of platelet function than the actual change in platelet number. The MPV describes the average platelet volume and is normally included in the FBC but is frequently neglected by the attending physician. Alterations in the platelet volume typically signify platelet activity. A decrease in the MPV reflects platelet activation as part of an acute inflammatory process and is often accompanied by a reactive thrombocytosis. This reduction in the MPV is considered to be an acute negative phase reactant (43).

### 1.8.5 The complement system

The complement systems are comprised of numerous integral proteins that form part of the innate immune system. They serve as a link between the innate and acquired immune system. There are three main complement systems: the classical, the alternate and the lectin pathways (58). Low complement levels represent activation of either the classical or alternate pathway or both. It also reflects disease activity in many rheumatic diseases, in particular systemic lupus erythematosus (SLE) (59).

### 1.8.6 Inflammatory markers

C-reactive protein (CRP) is an acute phase reactant, a pentraxin synthesised by hepatocytes. Its synthesis and release is mediated by interleukin 6 (IL-6). CRP is

utilised as a marker for inflammation across a broad range of infective and inflammatory conditions. This protein may also be locally synthesised in atherosclerotic lesions by lymphocytes (60).

Another frequently used marker in inflammatory disorders is the ESR. It is a very non-specific test and an elevated level is a surrogate marker of ongoing inflammation (61). The Westergren method is the gold standard technique for determining the ESR (62). This is the method utilized at the Chris Hani Baragwanath Academic Hospital (CHBAH) laboratory as per standardised international protocol. The test describes the rate at which red blood cells suspended in plasma settle in a vertical tube over a one-hour period. In pro-inflammatory states, due to elevated levels of agglomerins such as fibrinogen, the red blood cells undergo rouleaux formation resulting in a higher ESR level (61). The reference values for ESR are age and gender specific with higher cut-off values for ages above 50 and female gender. An ESR of greater than 30mm/hr is one of the variables used in the Valentini disease activity index for systemic sclerosis (63).

## 1.9 Haematological, immunological and serological markers in rheumatic diseases

### 1.9.1 Haematological parameters

White cell indices have not been studied extensively in SSc. A single case series described haematological abnormalities in 180 patients with SSc in Beirut, however this study showed no abnormalities of significance in the WCC (64). Leukopenia was found in a minority of the patients and was thought to be related to overlap diseases such as SLE. A finding of interest was the presence of leukocytosis in around 14% of their cohort and its link with myopathy (64).

The elevation in the NLR is driven by a combination of neutrophilia and lymphopenia which is common in auto-immune rheumatic diseases (65). An elevated NLR has been investigated as an alternate marker for monitoring disease activity in patients with rheumatoid arthritis (RA) (66). Patients with seropositive RA with active disease were found to have higher NLR values in comparison to the control group as well as patients in remission (66, 67), and the trends in the NLR have been shown to be of value in monitoring response to disease modifying agents in RA (68). In some studies, there was a significant correlation between the change in NLR and the 28-

joint disease activity score (DAS-28) and CRP values (67, 68). The NLR has been found to be elevated in patients with SLE. There is a trend towards higher values in patients with active disease, and more so in those patients with lupus nephritis (69). These findings highlight the ability of this easily accessible and inexpensive marker to identify ongoing inflammation.

The changes in the NLR in relation to disease activity and organ involvement have previously been investigated in SSc. As in other autoimmune rheumatic diseases, the NLR was found to be higher in patients with SSc in comparison to a control group. It has been hypothesized that the elevated NLR in SSc may reflect active macro- or microvascular disease (65, 70). In SSc it has been linked with the clinical manifestations of cardiac dysfunction, pulmonary hypertension, digital ulcers, renal disease and active lung disease in the form of ILD (70). A number of studies have found NLR values to be significantly higher in patients with SSc associated ILD (65, 70, 71). Some propose a NLR value of greater than 2.59 to be associated with pulmonary disease (71).

In 1968 Westerman et al reported the frequency of anaemia in SSc to be around 29 percent (72). Subsequently Frayha et al showed anaemia (25%) to be the most common haematological abnormality in 180 patients with SSc (64). There is limited data in international and local literature exploring this topic. The presence of anaemia in SSc has been linked with reduced survival rates (73).

The aetiology of anaemia in SSc is multifactorial; iron deficiency anaemia (IDA) was the commonest cause in the study conducted by Westerman (72). It is important to remember that IDA represents the end stage on the spectrum of iron deficiency. In SSc, IDA may occur as a result of chronic GI losses or intestinal malabsorption. A unique cause of anaemia in SSc is GAVE or ‘water melon stomach’ (74). Deficiency of cobalamin may occur from malabsorption due to small intestinal bacterial overgrowth (SIBO) (75). Furthermore, the chronic inflammatory nature of SSc results in impaired iron utilisation secondary to reticulo-endothelial iron blockade. This results in the entity of anaemia of chronic disease (ACD) which is characterised by a normochromic normocytic anaemia with elevated ferritin levels and reduced soluble transferrin receptor levels (76). Cases of microangiopathic haemolytic anaemias have been described in association with malignant hypertension in SSc but can also occur

in normotensive patients (77). Other rarer aetiologies of anaemia include auto-immune haemolytic anaemias, myelodysplastic syndromes and other haematological and non-haematological malignancies (64, 78).

It is imperative to note that anaemia may play a significant role in the elevated RDW and it becomes a confounding variable when interpreting the RDW abnormalities in SSc.

Recent studies have shown that an elevated RDW has been associated with poor outcomes in many disease states including autoimmune rheumatological diseases. Patients with RA and SLE were found to have higher baseline RDW values irrespective of haemoglobin levels. Furthermore in RA and SLE, a positive correlation was identified with an elevated RDW, raised inflammatory markers and disease activity (79, 80).

In SSc, an elevated RDW may allude to the presence of anaemia, widespread vasculopathy, ongoing inflammation and active disease (46). Mean RDW values were reported to be higher in the DcSSc group in comparison to limited cutaneous subset, as one would expect with more extensive disease. Elevated RDW was also discovered in advanced disease. This was a surprising finding as one would anticipate patients to be in the fibrotic phase of their disease, indicating possible ongoing subclinical inflammation. In recent studies, patients with cardiac dysfunction, PAH, renal disease and peripheral vascular disease were shown to have significantly higher mean RDW values compared to patients without these clinical manifestatios (81). As cardiopulmonary disease is associated with poorer outcomes in SSc, the presence of an elevated RDW should encourage one to actively investigate for it (46, 82).

Platelets seem to be in a persistently activated state in SSc primarily due to widespread endothelial injury and thus have been postulated to play a role in the aetiopathogenesis and progression of SSc (83). Platelet activation results in the release of countless inflammatory cytokines, vasoactive substances and pro-fibrotic mediators. Higher MPV values have been documented in patients with SSc, particularly in the presence of cardiovascular disease and digital lesions (84). Therefore, the MPV may be considered as a surrogate marker of ongoing macro- or

microvascular disease. In the same study a negative correlation was also demonstrated between mean MPV values and disease activity scores for SSc (84).

### 1.9.2 Serological and immunological parameters

The Canadian Scleroderma Research Group have shown that an elevated CRP correlates with disease activity, severity, reduced survival and adverse cardiopulmonary outcomes in SSc (85, 86). Patients with DcSSc have higher CRP levels in comparison to the LcSSc subset. A sustained rise in CRP is more prominent in early disease where it parallels the initial predominant inflammatory process. Advance disease is associated more with a fibrotic process with a more attenuated or smoldering inflammatory process (1, 85). Despite these findings, only a quarter of patients show an elevated CRP in early disease (85). Studies have shown that elevated ESR levels are mainly found in patients with pulmonary hypertension (87). Hypocomplementemia in the setting of SSc may be linked to inflammatory myositis and vasculopathy, in particular SRC (88, 89). Other possibilities to consider with complement abnormalities are overlap with other autoimmune rheumatic diseases.

## 1.10 Systemic sclerosis and mortality

Of the rheumatic diseases, SSc has one of the highest disease related mortality rates, with cardiopulmonary disease being the leading cause of death (90, 91). Over the last thirty years there has been an increment in the 10yr survival rate from 12% to 66% (39), this is attributed mainly to earlier diagnosis and improved treatment modalities. In the absence of overt clinical manifestations of active disease, abnormalities of immunological and serological markers may be the only clue to ongoing disease. Haematological and immunological abnormalities in relation to mortality in SSc have not been extensively studied. Recent local (90) and international (73) literature has described the presence of an elevated CRP, ESR and the presence of anaemia at diagnosis to be predictors of mortality. Identifying such markers in SSc and its association with organ involvement may provide an invaluable opportunity to intervene and improve patient outcomes.

## 1.11 Systemic sclerosis in an African and South African context

There is a paucity of data on SSc from the African continent, in particular sub-Saharan Africa. In the early South African studies from the Witwatersrand area, there was an emphasis on silica exposure and the development of progressive systemic

sclerosis (15, 16). A descriptive study of the clinical and laboratory manifestations of scleroderma in the black South African population was performed in 1999 at the CHBAH Rheumatology unit (10). Subsequently two more studies, one assessing ILD in SSc (7) and the other evaluating the predictors of mortality in SSc have been produced from the same unit (90).

### 1.12 Conclusion

Haematological and immunological abnormalities have not been well described in SSc as opposed to the other autoimmune rheumatic diseases such as SLE and RA. Most of the parameters alluded to in the above text have not to my knowledge been described in patients with SSc in Sub-Saharan Africa. Some of the novel markers of inflammation are easily accessible and inexpensive. It would be invaluable if these markers could be utilised as diagnostic or prognostic tools in resource constrained settings in Africa. The identification of relationships between haematological parameters and organ involvement in SSc may assist in earlier detection of organ dysfunction, prognosticate and possibly improve treatment strategies.

### 1.13 Aims and objectives

#### 1.13.1 Aims

The study aims to describe the haematological and immunological abnormalities and its associations with organ involvement and mortality in patients with SSc attending the CHBAH Rheumatology Connective Tissue Diseases (CTD) clinic.

#### 1.13.2 Objectives

1. To describe the frequency and pattern of haematological and serological findings in patients with SSc at the time of diagnosis (baseline) and at the last follow-up visit.
2. To evaluate the association between haematological parameters and clinical features, autoantibody profile, disease subset and acute phase reactants in SSc.
3. To determine the relationship between haematological parameters and major organ involvement present at diagnosis or developing during the course of the disease.
4. To identify haematological and serological predictors of mortality.

# CHAPTER TWO : PATIENTS AND METHODS

## 2.1 Study design and study population

The study is a retrospective record review conducted at a single South African centre. The records of patients with confirmed SSc, based on the 2013 ACR/EULAR classification criteria (24), attending the CHBAH Rheumatology CTD clinic were reviewed. Period from 01/01/2000 until 30/6/2017 formed part of the study. Patients aged 18 years or older with a minimum follow up of 6 months were recruited for the study.

Exclusion criteria included:

- Overlap with other auto immune rheumatic disease
- Infection with human immunodeficiency virus (HIV)
- Active infection at time of diagnosis or at last follow up visit
- Diagnosis of malignancy during the course of disease

Ethics approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (Protocol no. - M160678) (Appendix B).

## 2.2 Data collection and definitions

Data were retrieved from patient clinical records and data sheets (Appendix A) were used to capture the information. Anonymity was maintained by allocating patients study numbers and the data sheets were only accessible to the investigators. The relevant information captured included the following:

1. Date and age at diagnosis of SSc by a rheumatologist.
2. Demographics such as age, gender and ethnicity.
3. Disease duration - defined as from onset of first non-Raynaud's features till date of last rheumatological visit.
4. Subset classification as per LeRoy and Medgser classification criteria (92).
5. Toxin exposure – toxins such as tobacco exposure as well as any other relevant toxins, either environmental or occupation related.
6. Medications administered during the disease process. These included the commonly used immunomodulatory drugs, anti-platelet agents, GIT agents, analgesics and vasodilatory agents.
7. At the end of the study period the patients were divided into 3 groups:
    - Alive – patients that were known to be alive and following up at CHBAH

CTD clinic

- Demised – patient that were known to have died as per records
- Lost to follow up – patients that were not known to be alive or demised were classified as being lost to follow up

Symptoms, features of organ involvement and relevant investigations were obtained from patient records.

Definitions of relevant symptoms, signs and clinical entities were as follows:

- Raynaud's phenomenon – defined as the presence of two out of the three triphasic colour changes occurring in the digits in response to cold exposure.
- Upper GIT symptoms - included features related to reflux, GAVE or features suggestive of malabsorption or SIBO.
- Lower GIT symptoms included diarrhoea, bloating, constipation and faecal incontinence.
- Digital lesions included the presence of either digital pits, ulcers or gangrene.
- Nailfold changes – characteristic capillary abnormalities detected on capillaroscopy consisting of either dilated/giant capillaries, haemorrhages, avascularity or neoangiogenesis.
- Telangiectasia – macroscopically visible dilated skin vessels primarily occurring either on the face or hands.
- Calcinosis – subcutaneous calcification present either clinically or on radiological investigation.
- Joint contractures – contractures of joints either due to overlying skin disease or secondary to inflammatory articular or bony damage.
- Tendon fiction rubs – the presence of a leather crepitus on palpation or auscultation of a joint due to damage of tendon sheath and fascia.
- Proximal myopathy – symmetrical weakness of the proximal muscles of the upper or lower limbs.
- Interstitial lung disease (ILD) – Since all patients who were suspected of having ILD had a high resolution computed tomography (HRCT) scan done, the diagnosis of ILD was based on characteristic HRCT scan findings (ground glass opacification, fibrosis or honeycombing).

**Organ involvement**

Cumulative organ involvement (features present at diagnosis or during the course of disease) was assessed.

Muscle defined as having either one of the following:

- Clinical evidence of proximal myopathy with or without an elevation in the creatine kinase (CK)
- Myopathic pattern on electromyography
- Biopsy proven myositis

Gastrointestinal system involvement – included the presence of either one of the following:

- Upper GIT or lower GIT symptoms or signs on history, examination or endoscopy
- Radiology evidence of gastro-oesophageal reflux on barium swallow studies

Joint was defined by the presence of either one of the following:

- Documented arthritis by a member of the rheumatology unit at CHBAH
- The presence of joint contractures or tendon friction rubs

Cardiac was defined by the presence of either one of the following:

- Electrocardiographic abnormalities suggestive of conduction abnormalities
- Echocardiographic (transthoracic) findings of either
    - Left ventricular systolic (ejection fraction <50%) and or diastolic dysfunction
    - Pericardial effusions
    - Pulmonary hypertension (PAP >35mmHg)

Renal included the presence of either one of the following:

- Presence of proteinuria on urine dipsticks (1+ or more)
- Serum creatinine of >120umol/l
- Clinical diagnosis of SRC by a rheumatologist

Lung included the presence of either one of the following:

- Restrictive pulmonary function test
- Forced vital capacity (FVC) of <80% of predicted
- Diffusing capacity of lung for carbon monoxide (DLCO) <80% of predicted
- Features on HRCT scan suggestive of ILD (ground glass opacification, fibrosis or honeycombing)

### Laboratory investigations

The following blood investigations were recorded at diagnosis and at last follow up visit (where available). Results were obtained from the clinical records and any outstanding results were extracted from the NHLS records. Local reference ranges were used. The results of the following tests were collected:

**PARAMETERS OF FULL BLOOD COUNT**

- White cell count and differential count
- Haemoglobin (Hb)
- Mean cell volume (MCV)
- Red cell distribution width (RDW)
- Platelet count
- Mean platelet volume (MPV)

**INFLAMMATORY MARKERS**

- C-Reactive protein (CRP)
- Erythrocyte sedimentation rate (ESR)

**HAEMATINIC PROFILE**

- Iron studies
- Vitamin B12
- Red cell folate

**OTHER**

- Complement levels (c3/c4)
- Albumin
- Creatinine
- Autoantibody profile

The following parameters were defined as;

1. Leucopaenia - $<4x10^9$/L, Neutropenia - $<2.0x10^9$ /L, Lymphopenia - $<1.0x10^9$/L
2. Anaemia – Male <13g/dL, Female <12g/dL
3. High RDW - >17%
4. Thrombocytosis - $>400x10^9$/L, Thrombocytopenia - $<150x10^9$/L
5. High CRP - ≥ 8mg/L
6. High ESR - ≥28mm/hr
7. Hypoalbuminemia - <40g/L

### 2.3 Statistical Analysis

The data was captured into a Microsoft Excel spread sheet and categorised to create a database. Data analysis was performed using a statistical software package, MedCalc (Version 18). Continuous data was expressed as means (± standard deviation (SD)) or medians (interquartile range (IQR)), the latter being used when the SD is greater than the mean. Categorical data was expressed as percentages. Comparisons between groups were made using the Chi-squared test or Fisher's exact test for qualitative data, and the two-tailed unpaired Student's t-test for

quantitative data. The odds ratios were calculated using 2x2 contingency tables and the Spearman rank correlation test was used to determine the relationship between variables. A p-value of <0.05 was regarded as statistically significant.

# CHAPTER THREE: RESULTS

## 3.1 Study Cohort

Of the 210 records reviewed, 135 patients met the inclusion criteria for this study. As shown in Figure 3, a total of 75 patients were excluded for a variety of reasons. The majority of the excluded patients were either those who had active tuberculosis (TB) at the time of diagnosis or at last follow up visit or those that were diagnosed with HIV or malignancy at any stage of the disease. This was followed by patients who did not meet the minimum follow up period of 6 months. Patients who did not meet criteria for SSc and who had overlap with other autoimmune rheumatic diseases constituted the rest of the excluded group.

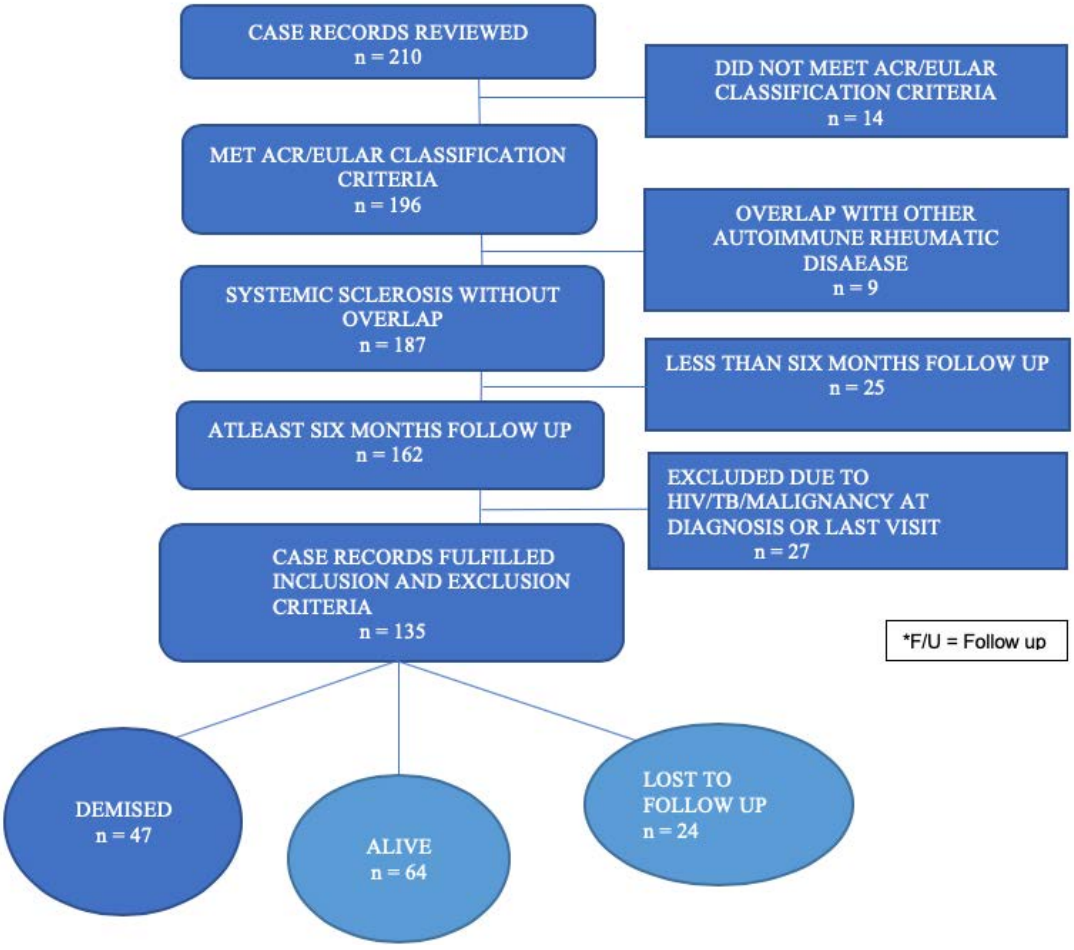



**Figure 3: Analysis of case records reviewed**

Of the patients who were included in the analysis only 64 patients (47.4%) were known to be alive and attending the CHBAH CTD clinic at the end of the study period. Twenty four (18%) patients who met the inclusion criteria for the study were lost to follow up and 47(34.8%) died (Table 2).

### 3.1.1 Demographics of study population

As shown in Table 1, the mean (SD) age and follow up at diagnosis were 42.9 (12.0) and 6.3 (6.1) years, respectively, with no difference between the subsets. Overall there was a female predominance (8.6:1) but significantly higher in the LcSSc subset (15.5:1). The majority were black Africans (92.6%) followed by Caucasians, Mixed race and Indian ethnicities. Diffuse cutaneous SSc was the predominant subset accounting for 76.6% of the patients. Only 20% were known smokers. All of the miners were men and all except one had DcSSc.

**Table 1: Sociodemographics of study cohort**

| SOCIODEMOGRAPHICS | Total (n=135) | DcSSc (n=102) | LcSSc (n=33) | p-value |
|---|---|---|---|---|
| Mean(SD) age at diagnosis in years | 42.9 (12.0) | 42.9 (12.0) | 42.8 (12.1) | 0.99 |
| Mean (SD) follow up in years | 6.3 (6.1) | 6.2 (5.8) | 6.8 (7.1) | 0.61 |
| Female:Male | 8.6:1 | 7.5:1 | 15.5:1 | 0.05 |
| Black ethnicity (%) | 125 (92.6) | 95 (93.1) | 30 (90.9) | 0.74 |
| Mining history (%) | 6 (4.4) | 5 (4.9) | 1 (3.0) | 0.98 |
| Smoker (%) | 27(20) | 17(16.7) | 10(30.3) | 0.15 |

SD – standard deviation; DcSSc – diffuse cutaneous systemic sclerosis ; LcSSc – limited cutaneous systemic sclerosis

### 3.2 Clinical manifestations

Most patients had the cardinal feature of Raynaud's phenomenon (98.5%) and just over half (50.4%) developed complications secondary to the digital ischaemia (Figure 4). These included digital pits (49.6%), followed by digital ulcers (23.7%) and a small proportion developed digital gangrene (9.6%). Digital lesions occurred equally in both subsets and there was no predilection for a particular disease subset. Less than 10% developed digital calcinosis cutis.

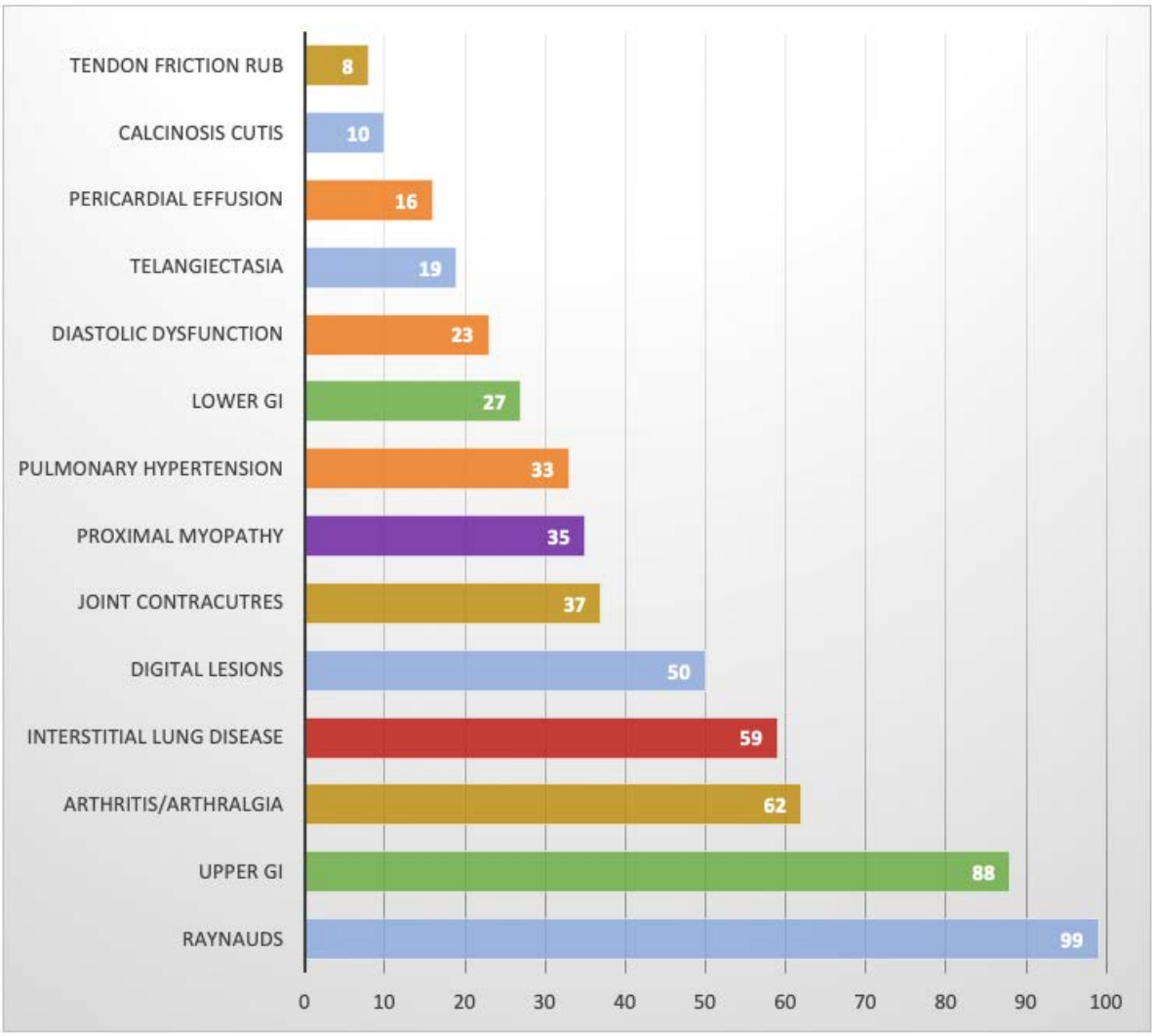



**Figure 4: Cumulative percentage frequency of main clinical features in 135 South Africans with systemic sclerosis (%)**

With respect to the disease subsets, patients with DcSSc had significantly higher frequency of ILD (OR=3.2) and proximal myopathy (OR=3.9) as shown in Table 2. Arthralgia with or without arthritis was a common symptom occurring in 62% of patients. Joint contractures were observed in 37% and tendon friction rubs were documented in 8.1%, and just over a third (34.8%) had a proximal myopathy.

As depicted in Figure 4 and Table 2, cardiopulmonary disease was a common clinical manifestation and was more prevalent in the DcSSc subset. Interstitial lung disease occurred in over 59% of patients and occurred more in the DcSSc subset in comparison to the LcSSc subset (OR=3.5). Pulmonary hypertension, mostly secondary to ILD, occurred in 32.8% and was the next most common cardiopulmonary manifestation. This was followed by diastolic dysfunction and pericardial effusions, 22.9% and 16.0%, respectively.

A large percentage of patients had upper GI symptoms at diagnosis and some developed symptoms during the course of the disease. The predominant complaint was that of reflux symptoms and this was confirmed on barium swallow studies. Only one patient was documented to have GAVE.

Renal dysfunction was noted in 8 (5.9%) patients and in four of the patients it was related to SRC .Two out of these four patients demised during the study period.

**Table 2: Organ involvement and mortality in 135 South African patients with systemic sclerosis**

| ORGAN INVOLVEMENT | Total (n=135) | DcSSc (n=102) | LcSSc (n=33) | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| SKIN | n (%) | n (%) | n (%) | | | |
| Raynaud's Phenomenon | 133 (98.5) | 101 (99) | 32 (97.0) | 3.16 | 0.19-5.19 | 0.40 |
| Digital pits | 67 (49.6) | 52 (60) | 15 (45.5) | 1.25 | 0.57-2.74 | 0.73 |
| Digital ulcers | 32 (23.7) | 26 (25.4) | 6 (18.2) | 1.48 | 0.55-4.00 | 0.59 |
| Digital gangrene | 13 (9.6) | 7 (6.9) | 6 (18.2) | 0.32 | 0.10-1.03 | 0.10 |
| Telangiectasia | 25 (18.5) | 17 (16.7) | 8 (24.2) | 0.63 | 0.24-1.62 | 0.47 |
| Calcinosis cutis | 13 (9.6) | 10 (9.8) | 3 (9.0) | 1.09 | 0.28-4.21 | 0.83 |
| MUSCULOSKELETAL SYSTEM | | | | | | |
| Arthralgia/Arthritis | 83 (61.5) | 65 (63.7) | 18 (54.5) | 1.46 | 0.66-3.24 | 0.46 |
| Tendon friction rubs | 11 (8.1) | 10 (9.8) | 1 (3.0) | 3.48 | 0.43-28.25 | 0.38 |
| Joint contractures | 50 (37.0) | 39 (38.2) | 11 (33.3) | 1.24 | 0.54-2.83 | 0.76 |
| Proximal myopathy | 47 (34.8) | 42 (41.2) | 5 (15.2) | 3.92 | 1.40-10.98 | 0.01 |
| CARDIOPULMONARY | | | | | | |
| Interstitial lung disease | 70/118 (59.3) | 59/88 (67.0) | 11/30 (36.7) | 3.51 | 1.50-8.35 | <0.01 |
| Pulmonary hypertension | 43/131 (32.8) | 33/98 (33.7) | 10 (30.3) | 1.17 | 0.50-2.74 | 0.89 |
| Pericardial effusions | 21/131 (16.0) | 17/98 (17.3) | 4/33 (12.1) | 1.52 | 0.47-4.90 | 0.66 |
| Diastolic dysfunction | 30/131 (22.9) | 25/98 (25.5) | 5/33 (15.2) | 1.92 | 0.67-5.50 | 0.32 |
| GASTROINTESTINAL SYSTEM | | | | | | |
| Upper GI | 119 (88.1) | 91 (89.2) | 28 (84.8) | 1.48 | 0.47-4.61 | 0.72 |
| Lower GI | 37 (2.4) | 32 (31.4) | 5 (15.2) | 2.56 | 0.91-7.24 | 0.11 |
| RENAL | 8 (5.9) | 6 (5.9) | 2(6.1) | 0.97 | 0.16-5.05 | 0.97 |
| MORTALITY | 47/111 (42.3) | 38/85 (44.7) | 9/26 (34.6) | 1.53 | 0.61-3.81 | 0.38 |

GI - gastrointestinal system;DcSSc - diffuse cutaneous systemic sclerosis; LcSSc - limited cutaneous systemic sclerosis

### 3.3 Medications

As shown in Table 3, the most commonly used drugs were proton pump inhibitors and calcium channel blockers. This was followed by angiotensin converting enzyme inhibitors/ angiotensin receptor blockers. Almost a third of patients were treated with corticosteroids in combination with immunomodulatory drugs including methotrexate, mycophenolate mofetil, azathioprine and intravenous cyclophosphamide, mainly for ILD and inflammatory myositis, and more frequently in the DcSSc subset as

expected. The vasodilatory prostacyclin, Iloprost, was used in just over 15% (12/68) of patients and exclusively for digital ischaemia.

**Table 3: Drugs used in the treatment of systemic sclerosis in 135 South African patients**

| MEDICATION | Total (%) (n=135) | DcSSc (%) (n=102) | LcSSc (%) (n=33) | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| ACE-I/ARB | 109 (80.7) | 85 (83.3) | 24 (72.7) | 1.88 | 0.74-4.73 | 0.20 |
| Calcium channel blocker | 126 (93.3) | 95 (93.1) | 31 (93.9) | 0.88 | 0.17-4.43 | 0.90 |
| Proton pump inhibitor | 129 (95.5) | 98 (96.1) | 31 (93.9) | 1.58 | 0.28-9.05 | 0.60 |
| Non-steroidal anti-inflammatories | 59 (43.7) | 44 (43.1) | 15 (45.5) | 0.91 | 0.41-2.00 | 0.80 |
| Iloprost | 15 (11.1) | 10 (9.8) | 5 (15.2) | 0.61 | 0.19-1.93 | 0.40 |
| Corticosteroids | 88 (65.2) | 76 (74.5) | 12 (36.4) | 5.12 | 2.21-11.82 | **<0.01** |
| Azathioprine | 20 (14.8) | 19 (18.6) | 1 (3.0) | 7.32 | 0.94-57.01 | **0.03** |
| Cyclophosphamide | 34 (25.2) | 31 (30.4) | 3 (9.1) | 4.37 | 1.24-15.39 | **0.01** |
| Mycophenolate mofetil | 17 (12.6) | 16 (15.7) | 1 (3.0) | 5.95 | 0.76-46.74 | 0.06 |
| Methotrexate | 44 (32.8) | 41 (40.2) | 3 (9.1) | 6.72 | 1.92-23.49 | **<0.01** |
| Rituximab | 2 (1.5) | 2 (2.0) | 0 (0) | - | - | 0.40 |

ACE-I - angiotensin converting enzyme inhibitor; ARB - angiotensin receptor blocker; DcSSc – diffuse cutaneous systemic sclerosis; LcSSc – limited cutaneous systemic sclerosis

## 3.4 Serological, immunological and inflammatory parameters

There was a trend towards higher rates of ANA positivity in the DcSSc subset as shown in Table 4. Similarly, there was also a trend towards higher ATA positivity in this subset. Just over 5% of the overall cohort had ACA.

**Table 4: Cumulative frequency of autoantibodies and frequencies of hypocomplementaemia and acute phase reactants at diagnosis in 135 systemic sclerosis patients**

| SEROLOGICAL, IMMUNOLOGICAL AND INFLAMMATORY MARKERS | Total (n=135) | DcSSc (n=102) | LcSSc (n=33) | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|
| ANA Positive (%) | 124 (92) | 96 (94.1) | 28 (84.8) | 2.86 | 0.81-10.1 | 0.09 |
| ATA Positive (%) | 29 (21.5) | 26 (25.5) | 3(9.1) | 3.42 | 0.96-12.15 | 0.08 |
| ACA Positive (%) | 8 (5.9) | 5 (4.9) | 3 (9.1) | 0.52 | 0.12-2.28 | 0.50 |
| Hypocomplementemia (%) | 24/108 (22.2) | 18/81 (22.2) | 6/27 (22.2) | 1.00 | 0.35-2.85 | 0.80 |
| Median CRP (mg/L) (IQR) | 6.0 (4.0 , 7.9) | 7.00 (4.0 , 9.0) | 4.50 (3.0 , 6.0) | | | 0.13 |
| High CRP (%) | 38/123 (30.9) | 32/95 (33.7) | 6/28 (21.4) | 1.86 | 0.69-5.05 | 0.32 |
| Median ESR (mm/hr) (IQR) | 23.0 (20.0 , 28.8) | 24.0 (20.6 , 30.4) | 20.5 (14.0 , 36.0) | | | 0.89 |
| High ESR (%) | 52/125 (41.6) | 40/93 (43) | 12/32 (37.5) | 1.26 | 0.55-2.87 | 0.73 |

ANA – antinuclear antibody; ATA – anti-topoisomerase antibody; ACA – anticentromere antibody; CRP – C-Reactive protein; ESR – erythrocyte sedimentation rate; DcSSc – diffuse cutaneous systemic sclerosis; LcSSc – limited cutaneous systemic sclerosis

A fifth of the patients had hypocomplementaemia, just under a third had a high CRP and 41% had a raised ESR at diagnosis. These finding were more common in the diffuse subset. Similiary, ATA positivity was also associated with a high CRP at diagnosis ($p<0.01$).

There was a significant inverse correlation between albumin and inflammatory markers, CRP ($p<0.01$) and ESR ($p<0.01$) at diagnosis (Figure 5 and 6). There was also a significant positive correlation between NLR and CRP ($r=0.41$, $p<0.01$, 95% CI 0.24-0.57) at diagnosis.

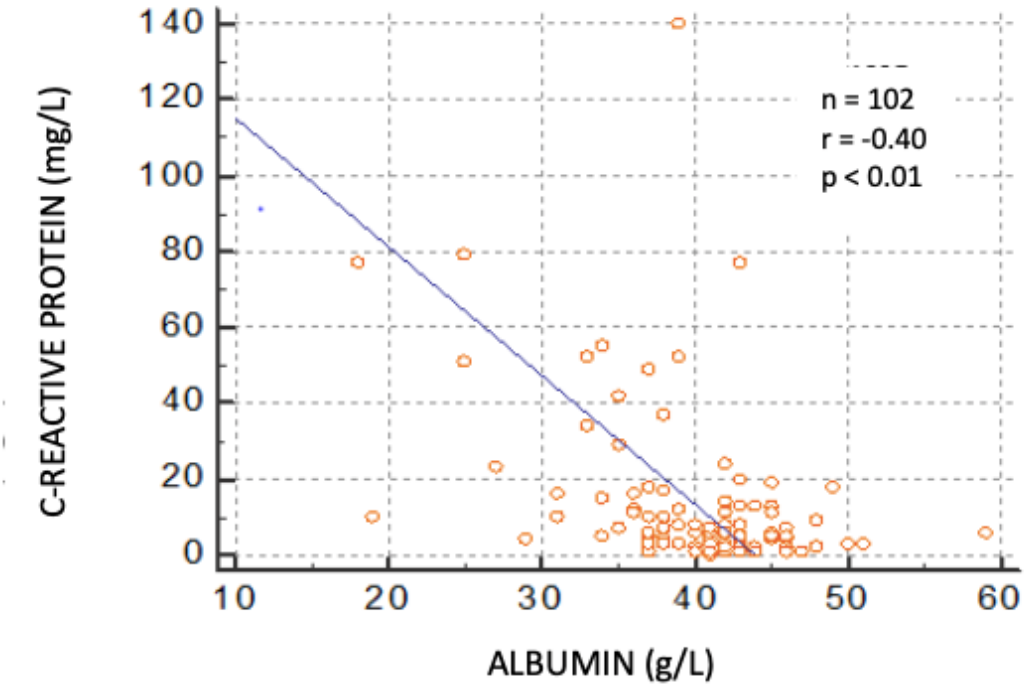



**Figure 5: Inverse correlation of C-reactive protein levels and albumin at diagnosis in systemic sclerosis patients**

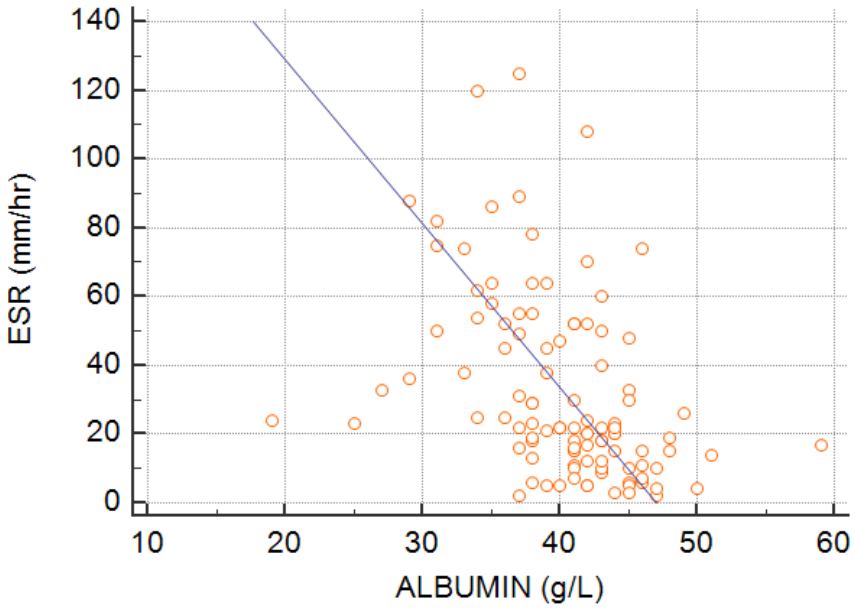



**Figure 6: Inverse correlation of erythrocyte sedimentation rate (ESR) and albumin at diagnosis in systemic sclerosis patients**

## 3.5 Haematological parameters

### 3.5.1 White cell parameters

At diagnosis, the mean (SD) total WCC was 7.3 x$10^9$/L (2.8) and significantly lower in the LcSSc subset compared to the DcSSc subset (Table 5). One tenth of patients had leucopaenia and significantly more in the LcSSc subset. Neutropaenia and lymphopaenia occurred in 3.9% and 29.4%, respectively, with no significant difference between the two subsets (Table 5).

**Table 5: White cell parameters at diagnosis in 135 systemic sclerosis patients**

| WHITE CELL PARAMETERS | Total (n=135) | DcSSc (n=102) | LcSSc (n=33) | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| Mean (SD) white cell count (x$10^9$/L) | 7.3 (2.8) (n=133) | 7.7 (2.9) | 6.2 (2.1) | | | <0.01 |
| Leucopaenia (%) | 13/133 (9.8) | 7/101 (6.9) | 6/32 (18.9) | 0.32 | 0.10-1.04 | 0.06 |
| Mean(SD) neutrophil count (x$10^9$/L) | 4.9 (2.4) | 5.0 (6.3) | 4.1 (1.9) | | | 0.13 |
| Neutropaenia (%) | 4/102 (3.9) | 3/76 (3.9) | 1/26 (3.8) | 1.03 | 0.10-10.33 | 0.73 |
| Mean (SD) lymphocyte count (10x$^9$/L) | 1.9 (0.8) | 2.0 (0.8) | 1.8 (0.7) | | | 0.20 |
| Lymphopaenia (%) | 30/102 (29.4) | 23/76 (30.3) | 7/26 (26.9) | 1.18 | 0.44-3.19 | 0.75 |
| Mean (SD) neutrophil lymphocyte ratio | 3.1 (2.6) | 3.1 (2.6) | 3.0 (2.5) (n=26) | | | 0.83 |

DcSSc – diffuse cutaneous systemic sclerosis; LcSSc – limited cutaneous systemic sclerosis

At diagnosis the overall mean (SD) NLR was 3.1 (2.6) and there was no significant

difference between the subsets (Table 5). There was an inverse correlation between NLR and albumin at diagnosis as depicted in Figure 7 below (p<0.01).

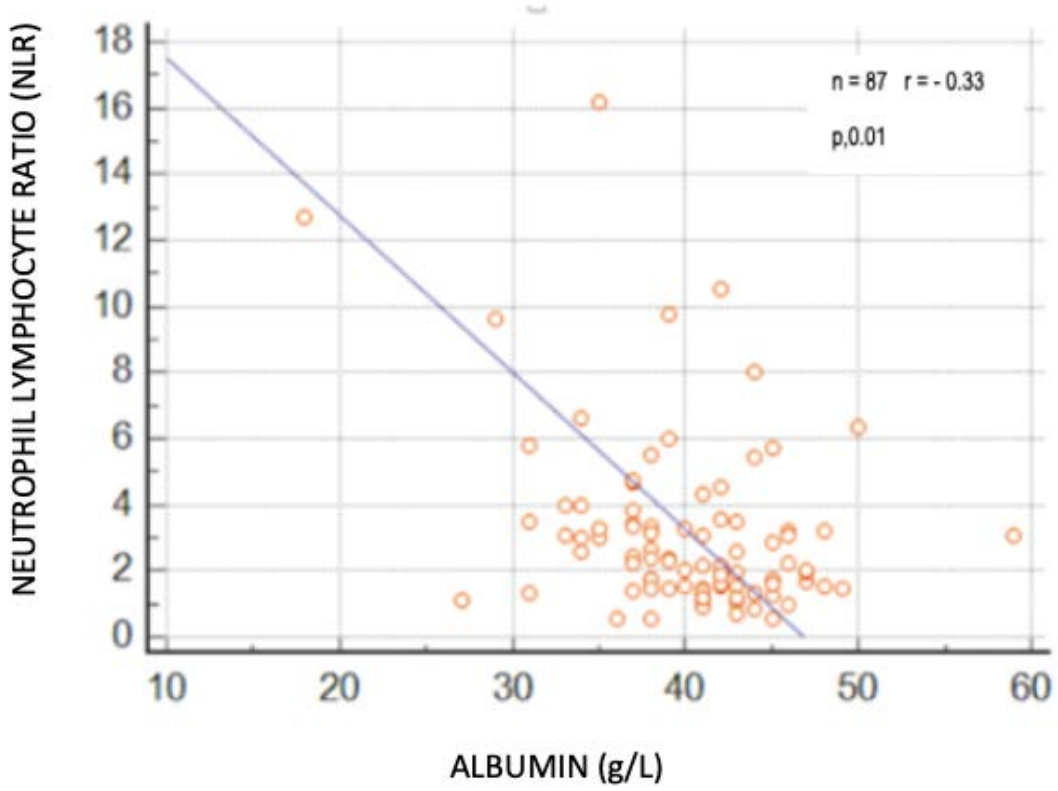



**Figure 7: Inverse correlation of serum albumin and neutrophil lymphocyte ratio (NLR) at diagnosis in patients with systemic sclerosis**

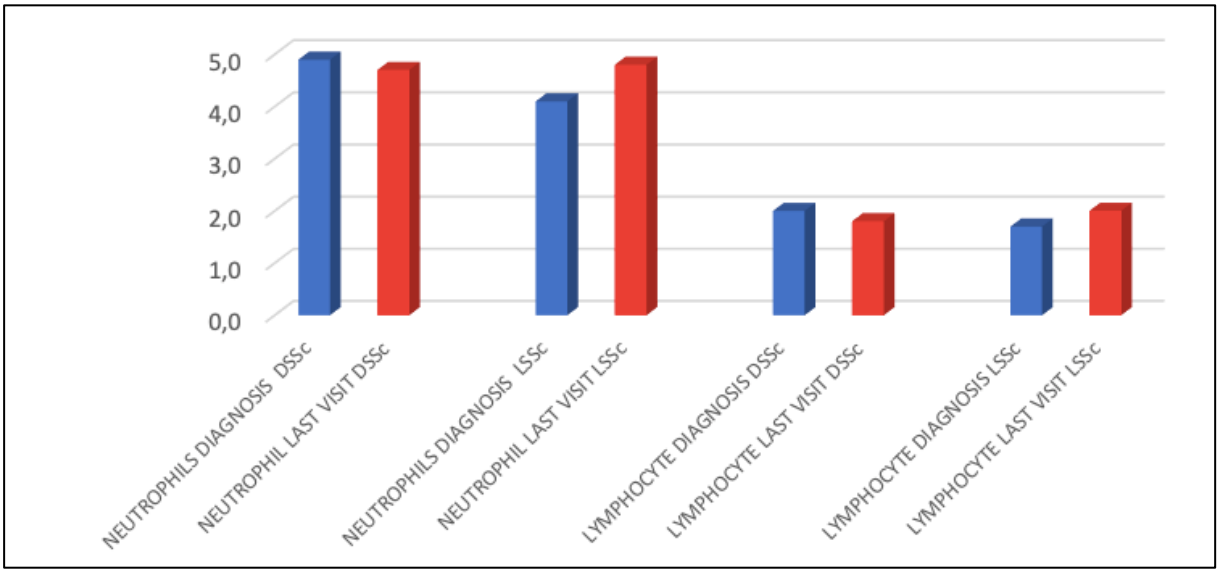
At last visit there was an increase in the frequency of lymphopaenia by 3.3% from diagnosis and this occured predominantly in the diffuse subset (p=0.02). In the limited subset the opposite finding was noted with a rise in the mean lymphocyte count and greater than 50% drop in the rate of lymphopaenia (Table 6).

**Table 6: White cell parameters at last visit in 135 systemic sclerosis patients**

| LAST VISIT | Total (n=132) | DcSSc (n=101) | LcSSc (n=31) | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| Mean (SD) white cell count (x$10^9$/L) | 7.0 (2.9) | 7.1 (3.1) | 6.8 (2.4) | | | 0.69 |
| Leucopaenia (%) | 15/135 (11.1) | 12/102 (11.8) | 3/33 (9.1) | 1.33 | 0.35-5.5 | 0.67 |
| Mean(SD) neutrophil count (x$10^9$/L) | 4.8 (2.4) | 4.7 (2.5) | 4.8 (2.3) | | | 0.86 |
| Neutropaenia (%) | 6/103 (5.8) | 4/78 (5.1) | 2/25 (8) | 0.62 | 0.11-3.62 | 1.00 |
| Mean (SD) lymphocyte count (10x$^9$/L) | 1.8 (0.9) | 1.7 (0.7) | 2.0 (0.7) | | | 0.17 |
| Lymphopaenia (%) | 31/95 (32.7) | 28/72 (38.9) | 3/23 (13.0) | 4.24 | 1.15-15.61 | 0.02 |
| Mean (SD) neutrophil lymphocyte ratio | 3.4 (3.0) | 3.6 (3.3) | 2.7 (1.5) | | | 0.24 |

DcSSc – diffuse cutaneous systemic sclerosis; LcSSc – limited cutaneous systemic sclerosis

At last visit the mean (SD) NLR in the DcSSc was higher than in the LcSSc subset, 3.6(3.3) compared to 2.7(1.5), however, not statistically significant. This was predominantly due to the worsening of lymphopaenia in the diffuse subset at last visit as demonstrated in Figure 8 below. In the LcSSc subset the opposite findings were noted.




DcSSc – diffuse cutaneous systemic sclerosis; LcSSc – limited cutaneous systemic sclerosis

**Figure 8: Comparison of neutrophil and lymphocyte count at diagnosis and last visit between the two disease subsets**

### 3.5.2 Red cell parameters

As shown in Table 7, the overall mean (SD) haemoglobin at diagnosis was 12.6g/dL (1.8), with no significant difference between the disease subsets. Over a third (35.3%) of patients were deemed to be anaemic at diagnosis. The majority had a normocytic anaemia (80.9%) and the remainder had a microcytic anaemia (19.1%).

**Table 7: Red cell indices at diagnosis in 135 patients with systemic sclerosis**

| RED CELL PARAMETERS | Total (n=135) | DcSSc (n=102) | LcSSc (n=33) | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| Mean (SD) Haemoglobin (g/dL) | 12.6 (1.8) | 12.5 (1.8) | 12.9 (1.7) | | | 0.30 |
| Anaemia (%) | 47 (35.3) | 37 (36.6) | 10 (31.3) | 1.27 | 0.45-2.98 | 0.70 |
| Normocytic anaemia (%) | 38/47 (80.9) | 31 (30.4) | 7 (21.2) | 1.62 | 0.64-4.13 | 0.37 |
| Microcytic anaemia (%) | 9/47 (19.1) | 6 (5.9) | 3 (9.1) | 0.61 | 0.14-2.59 | 0.69 |
| Mean (SD) RDW (%) | 15.8 (2.0) (n=88) | 16.1 (2.1) (n=69) | 14.9 (1.2) (n=19) | | | 0.02 |
| RDW >17 | 16/88 (18.2) | 15/69 (21.7) | 1/19 (5.3) | 5.00 | 0.62-40.56 | 0.09 |

DcSSc - diffuse cutaneous systemic sclerosis; LcSSc - limited cutaneous systemic sclerosis; RDW - red cell distribution width

There was a positive correlation between haemoglobin and albumin at diagnosis (p=0.002) as demonstrated in Figure 9 below.

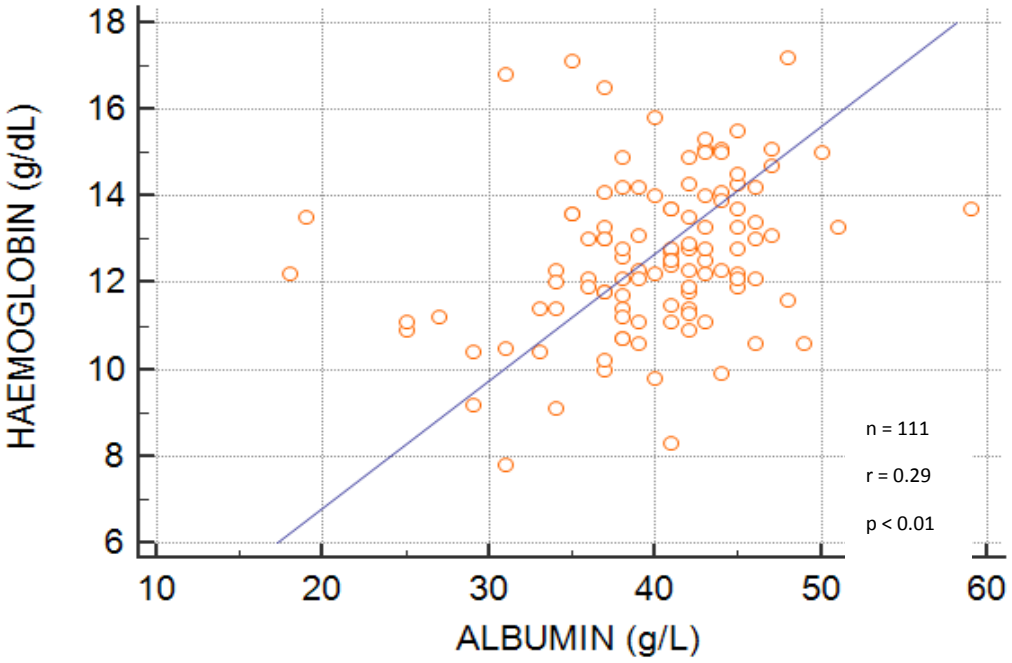



**Figure 9: Positive correlation of haemoglobin and serum albumin levels in systemic sclerosis patients**

The overall mean (SD) RDW was 15.8 (2.0), significantly higher in the DcSSc in comparison to the LcSSc subset as shown in Table 7. Patients with anaemia (47/135) had a significantly higher RDW compared to patients without anaemia (88/135) [17.2 (2.4) vs 15.2 (1.4) respectively, p<0.01] and similiarly there was an inverse correlation between the haemoglobin level and RDW at diagnosis as depicted in Figure 10 below.

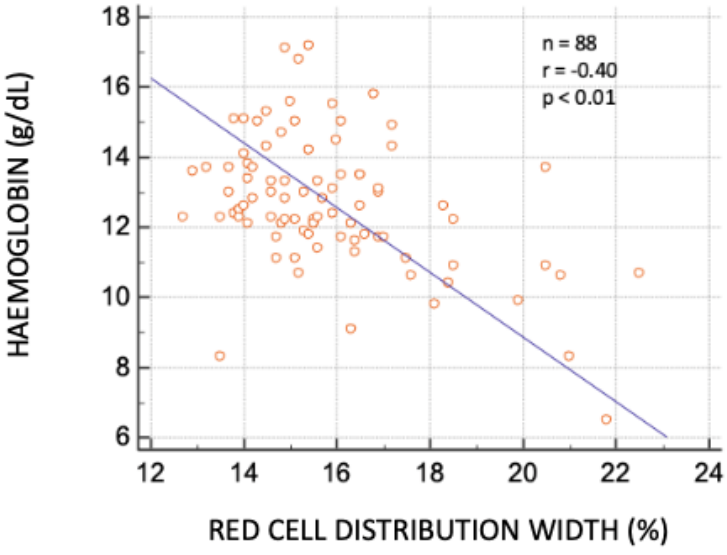
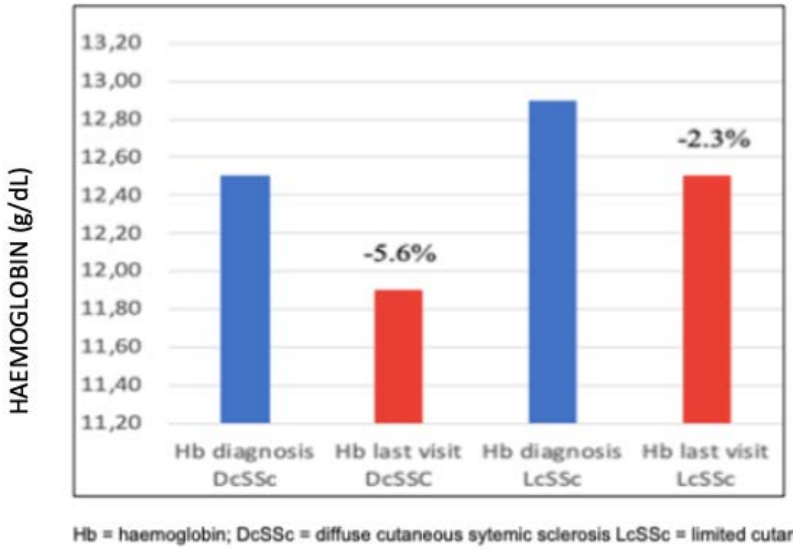



**Figure 10: Inverse correlation between haemoglobin and red cell distribution width at diagnosis in 135 patients with systemic sclerosis**

As illustrated in Figure 11, there was a drop in the overall mean haemoglobin by 0.6 g/dL (-5.6%) and 0.5g/dL (-2.3%) in the diffuse and limited subset, respectively, at last visit. Similiarly, more patients were also anaemic at last visit than at diagnosis (48.9%). Over half of the patients with DcSSc (53%) had anaemia as opposed to 39% of the LcSSc subset, a rise by 16.4% and 7.7%, respectively, from diagnosis. There was no significant difference in the trends from diagnosis with regards to the subtype of anaemia., with a predominance of normocytic anaemia followed by a microcytic picture.




Hb = haemoglobin; DcSSc = diffuse cutaneous sytemic sclerosis LcSSc = limited cutaneous systemic sclerosis

**Figure 11: Trends in haemoglobin levels at diagnosis and last visit in patients with diffuse and limited cutaneous systemic sclerosis**

### 3.5.3 Platelet parameters

As shown in Table 8 the mean (SD) platelet count was 315 (110) $x10^9$/L for the overall cohort, 3.2% of patients had thrombocytopaenia and 16.9% deemed to have thrombocytosis and there was no significant difference between the subsets. Patients with anaemia were more likely to have thrombocytosis than those without (12/47 vs 9/88) [OR (95%CI) = 3.01 (1.16-7.79), p=0.04].

The mean (SD) MPV at diagnosis for the entire cohort was 10.0 (1.6) with a trend towards a higher mean MPV in the LcSSc subset (p = 0.06).

**Table 8: Platelet parameters in South African patients with systemic sclerosis at diagnosis**

| PLATELET PARAMETERS | Total (n=124) | DcSSc (n=92) | LcSSc (n=32) | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|
| Platelet count (x10$^9$/L) | 315 (110) | 321 (115) | 299 (94) | | | 0.30 |
| MPV (fL) | 10 (1.6) (n=74) | 9.8 (1.5) (n=59) | 10.7 (1.8) (n=15) | | | 0.06 |
| Thrombocytopenia (%) | 4/124 (3.2) | 3/92 (3.0) | 1/32 (3.1) | 1.04 | 0.10-10.42 | 0.60 |
| Thrombocytosis (%) | 21/124 (16.9) | 16/92 (17.4) | 5/32 (15.6) | 1.14 | 0.38-3.40 | 0.80 |

MPV – mean platelet volume; fL – femtolitre ; DcSSc – diffuse cutaneous systemic sclerosis; LcSSc – limited cutaneous systemic sclerosis

Patients with hypoalbuminemia at diagnosis also had a lower MPV (9.5 fL vs 10.3 fL, p=0.03).

## 3.6 Association of haematological parameters with clinical features and serological markers

### 3.6.1 White cell parameters and organ involvement

Patient who developed cardiac disease and digital lesions had a higher NLR at diagnosis than those without (Table 9).

### 3.6.2 Red cell parameters and organ involvement

As shown in Table 9, patients who developed pericardial effusion had significantly lower baseline haemoglobin levels and similiarly the proportion of patients with anaemia at diagnosis compared to patients who did not develop pericardial effusion. Moreoever, the mean RDW was significantly higher in patients who developed a pericardial effusion compared to those who did not develop a pericardial effusion. Pulmonary hypertension was associated with a higher mean RDW at diagnosis and similiarly the proportion of patients with a RDW>17 was higher in the patients who developed pulmonary hypertension. Both inflammatory arthritis and proximal myopathy were associated with significantly higher platelet counts at diagnosis and a higher proportion having an absolute thrombocytosis.

**Table 9: Associations of haematological parameters at diagnosis with clinical manifestations in patients with systemic sclerosis**

| HAEMATOLOGICAL PARAMETER | CLINICAL MANIFESTATIONS | | | |
|---|---|---|---|---|
| | PRESENT | ABSENT | OR (95% CI) | p-value |
| | DIGITAL LESIONS | | | |
| Mean (SD) NLR | 3.6 (3.0) | 2.5 (1.9) | - | 0.03 |
| NLR > 3 (%) | 24/53 (45.2) | 16/48 (32.7) | 1.71 (0.76-3.82) | 0.14 |
| | CARDIAC INVOLVEMENT | | | |
| Mean (SD) NLR | 3.7 (3.1) | 2.5 (1.8) | - | 0.02 |
| NLR > 3 (%) | 25/50 (50) | 15/52 (28.8) | 2.47 (1.09-5.58) | 0.04 |
| | PERICARDIAL EFFUSION | | | |
| Mean (SD) Hb (g/dL) | 11.5 (1.8) | 12.8 (1.7) | - | <0.01 |
| Anaemia (%) | 14/20 (70.0) | 31/109 (28.4) | 5.87 (2.07-16.66) | <0.01 |
| | DIGITAL LESIONS | | | |
| Mean Hb (g/dL) | 12.1 (1.8) | 13.1 (1.7) | - | <0.01 |
| Anaemia (%) | 16/67 (23.9) | 31/66 (47.0) | 2.82 (1.35-5.92) | <0.01 |
| | PERICARDAL EFFUSION | | | |
| Mean (SD) RDW (%) | 16.7 (2.5) | 15.8 (1.9) | - | 0.14 |
| RDW >17 (%) | 6/12 (50) | 6/69 (8.7) | 6.30 (1.69-23.43) | <0.01 |
| | PULMONARY HYPERTENSION | | | |
| Mean (SD) RDW (%) | 16.8 (2.4) | 15.5 (1.7) | - | <0.01 |
| RDW >17 (%) | 10/28 (35.7) | 6/57 (10.5) | 4.72 (1.50-14.85) | 0.01 |
| | ARTHRALGIA/ARTHRITIS | | | |
| Mean (SD) Platelet count x109/L | 335 (109) | 286 (107) | - | 0.03 |
| Thrombocytosis (%) | 17/74 (23.0) | 4/50 (8.0) | 3.43 (1.08-10.90) | 0.02 |
| | PROXIMAL MYOPATHY | | | |
| Mean (SD) Platelet count x109/L | 345 (125) | 301 (99) | - | 0.04 |
| Thrombocytosis (%) | 11/41 (26.8) | 10/83 (12.5) | 2.68 (1.03-6.96) | 0.04 |

RDW - red cell distribution width; NLR – neutrophil lymphocyte ratio

### 3.6.3 Platelet parameters and organ involvement

Abnormalities of platelet indices at diagnosis and its associations with organ involvement included the cumulative development of arthritis (OR=3.4) and proximal myopathy (OR=2.7) in patients with thrombocytosis as shown in Table 9. There was a positive correlation between platelet count and RDW at diagnosis (p=0.06) in keeping with the inflammatory milieu.

### 3.6.4 Inflammatory markers and organ involvement

A high CRP at diagnosis was associated with pulmonary disease. Patients who had a cough, reduced DLCO and ILD had a significantly higher CPR as shown in Table 10. In patients with pulmonary hypertension the median CRP values were significantly higher than those without pulmonary hypertension (Table10).

**Table 10: Clinical features associated with high C-reactive protein levels at diagnosis in patients with systemic sclerosis**

| SEROLOGICAL PARAMETERS | CLINICAL MANIFESTATIONS | | | |
|---|---|---|---|---|
| | PRESENT | ABSENT | OR (95% CI) | p-value |
| | COUGH | | | |
| Median (IQR) CRP (mg/L) | 10.0 (4.0 , 19.3) | 5.0 (2.0 , 11.0) | - | 0.02 |
| High CRP (%) | 20/33 (60.6) | 30/89 (33.7) | 3.02 (1.33-6.90) | 0.01 |
| | REDUCED DLCO | | | |
| Median (IQR) CRP (mg/L) | 8.5 (4.0 , 18.0) | 3.0 (2.0 , 7.8) | - | <0.01 |
| High CRP (%) | 34/62 (54.8) | 13/51 (25,5) | 3.55 (1.59-7.93) | <0.01 |
| | INTERSTITIAL LUNG DISEASE | | | |
| Median (IQR) CRP (mg/L) | 8.0 (3.0 , 16.3) | 4.0 (1.5 , 9.0) | - | 0.02 |
| High CRP (%) | 33/65 (50.8) | 13/44 (29,5) | 2.46 (1.09-5.52) | 0.03 |
| | PULMONARY HYPERTENSION | | | |
| Median (IQR) CRP (mg/L) | 9.0 (4.0 , 17.3) | 4.0 (2.0 , 10.0) | - | <0.01 |
| High CRP (%) | 21/41 (51.2) | 28/79 (35,4) | 1.91 (0.89-4.11) | 0.07 |

CRP - C-reactive protein; DLCO -diffusing capacity for carbon monoxide

The mean albumin levels were significantly lower in patients with upper GI symptoms, arthralgia/arthritis, joint contractures and proximal myopathy and the proportion of hypoalbuminaemia was also higher in these groups as shown in Table 11 below.

**Table 11: Hypoalbuminaemia and associated clinical manifestations in patients with systemic sclerosis**

| SEROLOGICAL PARAMETERS | CLINICAL MANIFESTATION | | | |
|---|---|---|---|---|
| | PRESENT | ABSENT | OR (95% CI) | p-value |
| | UPPER GI | | | |
| Mean (SD) albumin (g/L) | 38.9 (6.1) | 42.3 (5.4) | - | <0.01 |
| Hypoalbuminaemia (%) | 38/77 (49.4) | 8/34 (23.5) | 3.17 (1.28-7.86) | 0.01 |
| | ARTHRALGIA/ARTHRITIS | | | |
| Mean (SD) albumin (g/L) | 39.7 (6.0) | 41.3 (6.6) | - | 0.3 |
| Hypoalbuminaemia (%) | 43/92 (46.7) | 3/19 (15.8) | 4.68 (1.28-17.2) | 0.02 |
| | JOINT CONTRACTURES | | | |
| Mean (SD) albumin (g/L) | 38.6 (6.4) | 40.2 (6.0) | - | 0.3 |
| Hypoalbuminaemia (%) | 13/20 (65) | 33/91 (36.3) | 3.26 (1.18-9.0) | 0.02 |
| | PROXIMAL MYOPATHY | | | |
| Mean (SD) albumin (g/L) | 38.2 (7.6) | 40.9 (4.8) | - | 0.02 |
| Hypoalbuminaemia (%) | 23/46 (50) | 23/71 (32.4) | 2.82 (1.27-6.28) | 0.02 |

Upper GI – upper gastrointestinal tract

### 3.7. Mortality and its associated haematological and immunological parameters

There were over a third (42.3%) of patients who demised from multiple pathologies (Table 2). The total WCC, neutrophil count and NLR were significantly higher in those that were known to have demised than the known alive. Similarly, when comparing the red cell count parameters at diagnosis between these two groups of patients, the only significant finding was that of higher mean haemoglobin levels in the patients that had demised (Table 12).

At last visit there was a similar trend with higher mean total WCC, neutrophil counts and NLR in the patients who had demised in comparison to the known alive group. The known demised group also had higher mean RDW values at last visit. In keeping with this, a RDW of greater than 17% at last visit, was significantly associated with mortality. Similarly, patients who demised also had higher median CRP levels and lower mean albumin levels at last visit (Table 12 and Figure 12).

**Table 12: Haematological and immunological parameters at diagnosis and at last visit of known alive and demised patients**

| HAEMATOLOGICAL AND SEROLOGICAL PARAMETERS | DEMISED (n=47) | ALIVE (n=64) | OR (95% CI) | p-value |
|---|---|---|---|---|
| | | DIAGNOSIS | | |
| Mean (SD) white cell count (x$10^9$/L) | 8.2 (3.0) | 6.7 (2.6) | | <0.01 |
| Mean (SD) neutrophil count (x$10^9$/L) | 5.6 (2.4) (n=33) | 4.3 (2.4) (n=51) | | 0.02 |
| Mean (SD) lymphocyte count (x$10^9$/L) | 1.9 (0.8) (n=33) | 2.0 (0.8) (n=51) | | 0.63 |
| Mean (SD) NLR | 3.8 (3.2) (n=33) | 2.6 (2.2) (n=51) | | <0.05 |
| NLR >3 (%) | 18/33 (54.5) | 22/69 (31.9) | 2.56 (1.09-6.00) | 0.03 |
| Mean (SD) haemoglobin (g/dL) | 13.1(1.6) | 12.3 (2.0) | | 0.02 |
| Anaemia (%) | 11/47 (23.4) | 36/86 (41.9) | 0.42 (0.19-0.94) | 0.04 |
| Mean (SD) RDW (%) | 15.7 (1.6) (n=27) | 15.9 (2.1) (n=49) | | 0.59 |
| RDW >17 (%) | 4/27 (14.8) | 12/61 (19.7) | 0.71 (0.21-2.44) | 0.77 |
| Mean (SD) platelet count (x$10^9$/L) | 304 (98) (n=41) | 323 (120) (n=60) | | 0.40 |
| Mean (SD) MPV (fL) | 9.7 (1.8) (n=22) | 10.1 (1.6) (n=42) | | 0.34 |
| Median (IQR) CRP (mg/L) | 7.0 (3.0 , 15.0) | 5.0 (3.0 , 10.0) | | 0.16 |
| Mean (SD) albumin (g/L) | 39.9 (6.6) | 40.8 (5.6) | | 0.50 |
| | | LAST VISIT | | |
| Mean (SD) white cell count (x$10^9$/L) | 7.9 (3.3) | 6.4 (2.6) | | <0.01 |
| Mean (SD) neutrophil count (x$10^9$/L) | 5.6 (2.7) (n=35) | 4.2 (2.3) (n=69) | | 0.01 |
| Mean (SD) ymphocyte count (x$10^9$/L) | 1.9 (1.1) (n=34) | 1.7 (0.8) (n=49) | | 0.39 |
| Mean (SD) NLR | 4.3 (4.0) (n=26) | 2.8 (2.3) (n=41) | | 0.06 |
| NLR >3 (%) | 15/26 (n=34) | 19/51 (37.3) | 2.30 (0.88-6.02) | 0.07 |
| Mean (SD) haemoglobin (g/dL) | 11.9 (2.5) | 12.2 (3.9) | | 0.37 |
| Anaemia (%) | 25/47 (53.2) | 41/86 (47.7) | 1.25 (0.67-0.54) | 0.59 |
| Mean (SD) RDW (%) | 17.1 (2.7) (n=35) | 15.9 (2.4) (n=63) | | <0.01 |
| RDW >17 (%) | 16/36 (44.4) | 13/79 (16.5) | 4.06 (1.67-9.85) | <0.01 |
| Mean (SD) platelet count (x$10^9$/L) | 312 (129) (n=44) | 302 (75) (n=55) | | 0.63 |
| Median (IQR) CRP (mg/L) | 16.0 (8.5 , 30 ) (n=44) | 3.5 (1.0 , 8.0) (n=62) | | <0.01 |
| Mean (SD) albumin (g/L) | 35 (6) (n=32) | 41 (5) (n=44) | | <0.01 |

NLR – neutrophil lymphocyte ratio; RDW – red cell distribution width; MPV – mean platelet volume CRP - C-reactive protein; RDW - red cell distribution width

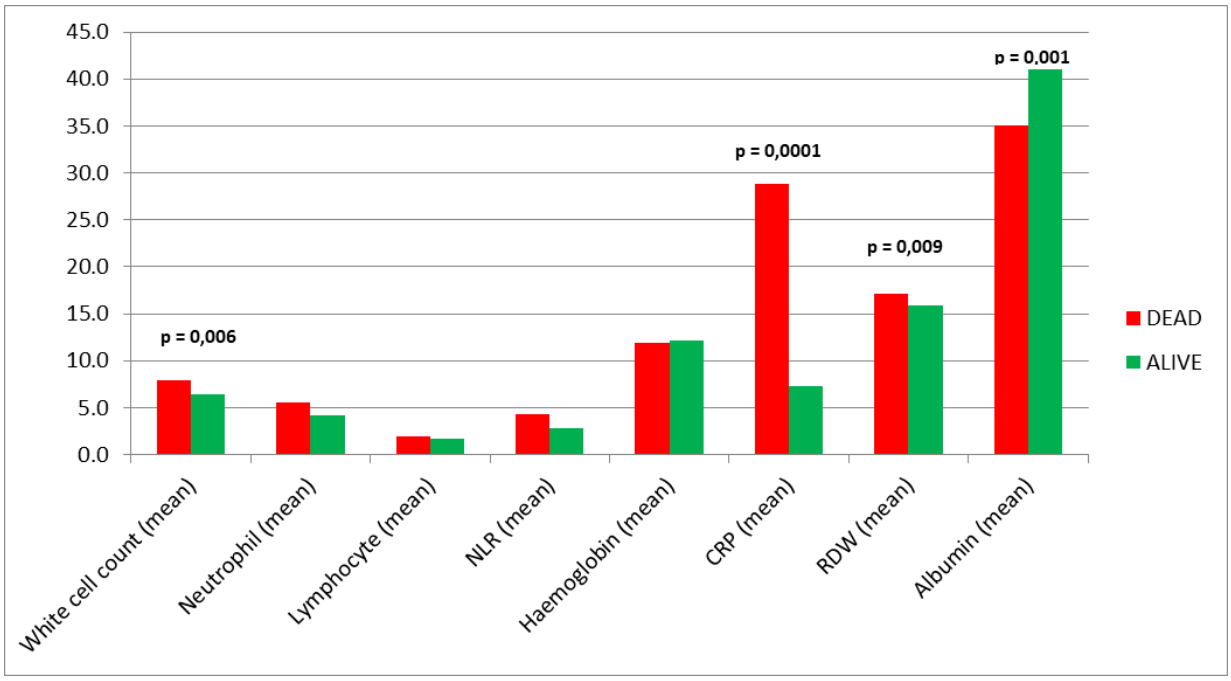



**Figure 12: Comparison of haematological and serological parameters in patients with systemic sclerosis at last visit between known alive and demised groups**

## 3.8 Clinical features associated with mortality

The development of digital lesions and pulmonary hypertension was associated with a greater than threefold increase in the likelihood of death (OR 3.6 and 3.3 respectively, p<0.01) as shown in Table 13.

**Table 13: Association between clinical manifestations and mortality**

| CLINICAL MANIFESTATIONS AND MORTALITY | NO (%) | P-VALUE | OR | 95% CI |
|---|---|---|---|---|
| Digital lesions | 33/47 (70.2) | **<0.01** | **3.6** | **1.7 – 7.6** |
| Pulmonary Hypertension | 23/46 (50) | **<0.01** | **3.3** | **1.5 – 7.0** |

# CHAPTER FOUR:

## 4.1 Discussion

In this study of predominantly black South African patients with mainly DcSSc, we found several haematological abnormalities at diagnosis of which the commonest were anaemia (35%) followed by lymphopaenia (29.4%) and thrombocytosis (16.9%). Moreover, we found numerous associations which were predictive of organ involvement and mortality. Some of these associations have been documented in other studies while some of the findings are novel.

Lymphopaenia was the predominant white cell abnormality and was followed by leucopaenia occurring in just under a tenth of patients and occurred at a higher frequency than found before (64, 93). Frayha et al, in their cohort of 180 patients with SSc found leucopaenia (5.5%) to be a feature of patients with overlap with SLE, unlike in our study where patients with overlap were excluded (64).

Several studies assessing NLR in SSc have shown higher values in patients with disease than those without (70, 71) as was the case in this study. The relationship of a high NLR in SSc patients with pulmonary hypertension, digital lesions, cardiac disease and ILD has been reported before (65, 70, 71). Recently, it has also been shown to be a marker of inflammation in SSc (94). In this present study, our findings were similar, with higher NLR at diagnosis in patients with cardiac disease and digital lesions with a trend towards higher values in those with pulmonary hypertension. Moreover, the relationship between inflammation and NLR was supported by the positive correlation between NLR and CRP, as well as an inverse correlation with albumin at diagnosis. We did not find a positive relationship with ILD.

A high NLR at diagnosis was also associated with mortality. Furthermore, patients who demised had higher NLR values both at diagnosis and at the last follow up visit and a NLR of > 3 at diagnosis was a positive predictive factor for death. The association of a high NLR with mortality has been described before in auto-immune rheumatic diseases (95) as well as non-rheumatic conditions (96), in particular, cardiovascular diseases (97) and malignancies (96). To the best of our knowledge,

this has not been reported before in SSc and highlights its utility as a prognostic marker.

The precise pathogenetic mechanism of an elevated NLR is not known at present, rather it reflects ongoing inflammation and may be a consequence thereof (70). This finding highlights the possibility of utilising the NLR as a surrogate marker for active inflammation in SSc (94), as is the case in other auto-immune rheumatic diseases such as SLE and RA (66, 69). There was a higher total mean WCC in the diffuse cutaneous subset for which the exact reason is not known, but perhaps like the NLR is a reflection of inflammation.

In this present study, over a third of the cohort were anaemic at diagnosis which was substantially higher than previous local and international studies (7, 64, 72). Westerman et al and Frayha and colleagues reported iron deficiency in as much as 50% and 33.3% of their cohort of 164 and 180 patients, respectively (64, 72). The majority of our patients had a normocytic anaemia. Haematinic profiles were not always done so we were not able to characterise the anaemia fully in some of the patients. The findings of a higher CRP and ESR values in these patients with a normocytic anaemia, as well as a low frequency of telangiectasia and GAVE, possibly suggest anaemia of chronic disease as the dominant cause of anaemia in our cohort. The direct relationship between haemoglobin levels and albumin as well as an inverse relationship with RDW suggest that the anaemia might be related to chronic inflammation.

We found that patients with anaemia at diagnosis were more likely to develop pericardial effusions and less likely to have digital lesions. They also had lower albumin levels and higher mean RDW values at diagnosis. In studies conducted in Thailand (73) and France (98), anaemia was shown to be an independent risk factor for mortality, however we did not find an association with death. All together these findings suggest, patients that are anaemic at diagnosis are more likely to have a more complicated course of disease.

The RDW is a marker of anisocytosis, the reasons for which are multifactorial in autoimmune rheumatic diseases such as SSc. In these conditions where inflammation is the dominant pathogenic mechanism in the early stages of disease,

the elevation in RDW is most likely related to interleukin and cytokine mediated negative effects on bone marrow function. These processes result in inadequate erythropoiesis with abnormal erythrocyte maturation, hence resulting in anisocytosis (43). In SSc there is a predominance of endothelial dysfunction with multiorgan involvement, severe oxidative stress and disseminated intravascular coagulation and collectively these factors contribute towards an elevated RDW (81).

Farkas and colleagues described higher RDW at diagnosis in patients with SSc and significantly higher values in the diffuse cutaneous subset in comparison to the limited cutaneous subset (46). This was confirmed in our study. Similarly, a high RDW was associated with cardiopulmonary disease, in keeping with the findings of Farkas et al (46). Zhao and colleagues described higher RDW values in patients with pulmonary hypertension in their cohort of 145 patients (81); we also found this in our study, moreover, RDW values of greater than 17% were linked to pulmonary hypertension and pericardial effusions.

A significant positive correlation between CRP and RDW as well as an inverse correlation with albumin was also demonstrated, which was in keeping with previous studies (46, 81). These findings reinforce the sentiments of an inflammatory process propagating the elevation of RDW in early disease. The absence of significant haematinic deficiencies, inferring from the fact that only a small proportion of patients had a microcytic anaemia as well as the absence of macrocytic anaemia, further supports the hypothesis suggested above. However, our sample size was small and full haematinic profiles were not always done.

Thrombocytosis was the predominant platelet abnormality at diagnosis with no significant difference between the disease subsets. Thrombocytosis was associated with the development of a proximal myopathy and arthritis, moreover, patients with these clinical manifestations had higher platelet counts as well.

The mechanism of thrombocytosis in these patients is most likely cytokine mediated bone marrow stimulation due to inflammation. This may result in increased intravascular platelet consumption secondary to chronic low grade disseminated coagulopathy. This phenomenon has been acknowledged in other auto-immune rheumatic diseases such as RA (99, 100). Our hypothesis is further supported by the

presence of higher RDW values in patients with thrombocytosis and a positive correlation between RDW with CRP at diagnosis.
In terms of inflammatory markers, a CRP level of ≥8 was considered to be high as per the Canadian Scleroderma research group (85). This value was used as the cut-off value in this study as well. In their cohort of 1043 patients, the Canadian Scleroderma research group, found that more than a quarter of their cohort had elevated CRP levels at diagnosis. Comparatively our cohort had over 40% with an elevated CRP at diagnosis.

High CRP at diagnosis was associated with pulmonary hypertension, clinical manifestations of ILD and ATA positivity. The findings of high CRP in SSc patients with ILD has been described before (101) and it may be explained by the presence of active alveolitis. With advanced disease and extensive fibrosis there is unlikely to be a rise in the CRP levels as there is only an attenuated inflammatory response.

In SSc, hypoalbuminaemia may be a marker of malnutrition or as a negative acute phase reactant. In this study, patients with upper GI pathology had lower albumin levels than those without and this was most likely nutrition related. Other associations with hypoalbuminemia at diagnosis included, the development of arthralgia/arthritis, joint contractures, proximal myopathy and pulmonary hypertension.
Hypoalbuminemia in these states most likely represents the role of albumin as a negative acute phase reactant. A negative correlation between albumin and CRP at diagnosis further supports this thought process. Hypoalbuminaemia in the context of pulmonary hypertension has been associated with poor outcomes (102) and in our cohort the development of pulmonary hypertension was strongly associated with mortality.

### 4.2 Limitations

Some limitations arise from the retrospective nature of this study. We were reliant on the clinical records for both clinical manifestations and investigations. In some cases the information pertinent to the study was insufficient. In a majority, we were not able to characterise the anaemia due to the absence of full haematinic profiles. Certain non-serological investigations were also done only if clinically warranted. The use of haematological parameters at last visit was limited due to the impact of immunomodulatory drugs on these parameters and incomplete documentation in this

regard. Our small sample in conjunction with a lost to follow up rate of 18% added to our limitations. Lastly, this is a single centre study and results are not generalisable to the overall population.

# CHAPTER 5: CONCLUSION

In this study, haematological abnormalities in SSc appear to be more common than previously described. Some novel as well as some neglected parameters of the full blood count and differential white cell count or derivatives thereof have been shown to be associated with organ manifestations and mortality in SSc. The main findings are that of an elevated NLR associated with cardiac disease and digital lesions, moreover, a NLR of greater than 3 was a positive predictive factor of mortality. Its utility as a surrogate marker for ongoing inflammation was also highlighted in this study. Similarly, a high RDW (>17%) at diagnosis was associated with cardiopulmonary disease. A high RDW at last visit was also strongly associated with death. Thrombocytosis and hypoalbuminaemia were associated predominantly with inflammatory manifestations of SSc. Similarly, elevated levels of the acute phase reactant, CRP, with manifestations of ILD.

Further prospective studies are needed with a larger cohort to validate the findings of this study. The use of NLR as a surrogate marker of inflammation, as well as an elevated RDW as a screening tool for cardiopulmonary disease might have clinical utility in the resource limited settings of sub-Saharan Africa.

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# REFERENCES

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# APPENDIX A - DATA SHEET

## DEMOGRAPHICS

| | | |
|---|---|---|
| **DATE OF RECORD EVALUATION** | DD/MM/YYYY | |
| **DATE OF DIAGNOSIS OF SSc** | DD/MM/YYYY | |
| **AGE AT DIAGNOSIS** | YEARS | |
| **DATE OF ONSET OF RAYNAUD'S PHENOMENON (RP)** | DD/MM/YYYY | |
| **DATE OF ONSET OF NON-RP FEATURES** | DD/MM/YYYY | |
| **DISEASE DURATION** | YEARS | |
| **DATE OF LAST VISIT ( RHEUMATOLOGICAL )** | DD/MM/YYYY | |

| | | |
|---|---|---|
| **GENDER** | MALE | FEMALE |

| | | | | | |
|---|---|---|---|---|---|
| **ETHNICITY** | BLACK | WHITE | INDIAN | COLOURED | |

| | | |
|---|---|---|
| **SMOKER** | EVER | NEVER |

| | | |
|---|---|---|
| **MINING EXPOSURE** | YES | NO |
| **TOXIN EXPOSURE** | YES | NO |

## HISTORY

| | | | |
|---|---|---|---|
| **RAYNAUDS PHENOMENON** | 0 | 1 | 2 |
| **UPPER GIT SYMPTOMS** | 0 | 1 | 2 |
| **LOWER GIT SYMPTOMS** | 0 | 1 | 2 |
| **ARTHRALGIA** | 0 | 1 | 2 |
| **DYSPNOEA** | 0 | 1 | 2 |
| **COUGH** | 0 | 1 | 2 |
| **PALPITATIONS** | 0 | 1 | 2 |

## EXAMINATION

| | | |
|---|---|---|
| **ACR 1980** | YES | NO |
| **ACR/EULAR 2013** | YES | NO |

| | | | |
|---|---|---|---|
| **SUBSET** | LIMITED | DIFFUSE | OVERLAP |

| | | |
|---|---|---|
| **MRSS** | **ONSET** | **HIGHEST** |
| | | |

| | | | |
|---|---|---|---|
| **DIGITAL PITS** | 0 | 1 | 2 |
| **DIGITAL ULCERS** | 0 | 1 | 2 |
| **DIGITAL GANGRENE** | 0 | 1 | 2 |
| **NAILFOLD CHANGES** | 0 | 1 | 2 |
| **SCLERODACTYLY** | 0 | 1 | 2 |
| **TELANGIECTASIA** | 0 | 1 | 2 |
| **CALCINOSIS** | 0 | 1 | 2 |
| **ARTHRITIS** | 0 | 1 | 2 |
| **JOINT CONTRACTURES** | 0 | 1 | 2 |
| **TENDON FRICITION RUBS** | 0 | 1 | 2 |
| **PROXIMAL MYOPATHY** | 0 | 1 | 2 |
| **PULMONARY BASAL CRACKLES** | 0 | 1 | 2 |

| | | |
|---|---|---|
| **MEDICATIONS** | **EVER** | **NEVER** |
| **CORTICOSTEROIDS** | | |
| **CYCLOPHOSPHAMIDE** | | |
| **METHOTREXATE** | | |
| **AZATHIOPRINE** | | |
| **MYCOPHENOLATE MOFETIL** | | |
| **RITUXIMAB** | | |
| **ILOPROST** | | |

| | | |
|---|---|---|
| **ASPIRIN** | | |
| **CALCIUM CHANNEL BLOCKER** | | |
| **ACE-I / ARB** | | |
| **PROTON PUMP INHIBITOR** | | |
| **NON-STEROIDAL ANTI-INFLAMMATORY AGENTS** | | |
| **DIURETICS** | | |

## ORGAN INVOLVEMENT

SKIN

| | | |
|---|---|---|
| **TELANGIECTASIA** | YES | NO |
| **CALCINOSIS** | YES | NO |
| **X-RAY ACRO-OSTEOLYSIS** | YES | NO |
| **DIGITAL LESIONS** | YES | NO |

MUSCLE

| | | |
|---|---|---|
| **PROXIMAL MYOPATHY** | YES | NO |
| **ELEVATION OF CK** | YES | NO |
| **EMG (MYOPATHIC)** | YES | NO |
| **BIOPSY (MYOSITIS)** | YES | NO |

JOINT / TENDON

| | | |
|---|---|---|
| **ARTHRITIS** | YES | NO |
| **JOINT CONTRACTURES** | YES | NO |
| **TENDON FRICTION RUB** | YES | NO |

GASTROENTEROLOGY

| | | |
|---|---|---|
| **UPPER GIT SYMPTOMS** | YES | NO |
| **LOWER GIT SYMPTOMS** | YES | NO |
| **GASTROSCOPY (GAVE)** | YES | NO |
| **BARIUM SWALLOW (GERD)** | YES | NO |

CARDIAC

| | | |
|---|---|---|
| **ECG : ARRYTHMIA / CONDUCTION ABNORMALITY** | YES | NO |
| **ECHO** | YES | NO |
| **LVEF < 50%** | YES | NO |
| **PAP > 35mmHg** | YES | NO |
| **DIASTOLIC DYSFUNCTION** | YES | NO |
| **PERICARDIAL EFFUSION** | YES | NO |

KIDNEY

| | | |
|---|---|---|
| **SCLERODERMA RENAL CRISIS** | YES | NO |
| **SERUM CREATININE > 120 micromol/litre** | YES | NO |
| **DIPSTIX - PROTEINURIA ≥ 1+** | YES | NO |

LUNG

| | | |
|---|---|---|
| **RESTRICTIVE PULMONARY FUNCTION TEST** | YES | NO |
| **FVC < 80% PREDICTED** | YES | NO |
| **DLCO < 80% PREDICTED** | YES | NO |
| **HIGH RESOLUTION CT CHEST - ILD** | YES | NO |

LABORATORY

| | | |
|---|---|---|
| **ANA** | POSITIVE | NEGATIVE |
| **ATA** | POSITIVE | NEGATIVE |
| **ACA** | POSITIVE | NEGATIVE |
| **RNP** | POSITIVE | NEGATIVE |
| **RO** | POSITIVE | NEGATIVE |
| **LA** | POSITIVE | NEGATIVE |
| **RF** | POSITIVE | NEGATIVE |
| **COOMBS** | POSITIVE | NEGATIVE |

| | | |
|---|---|---|
| **ACLA IgM** | POSITIVE | NEGATIVE |
| **ACLA IgG** | POSITIVE | NEGATIVE |
| **LA** | POSITIVE | NEGATIVE |
| **B2GP IgM** | POSITIVE | NEGATIVE |
| **B2GP IgG** | POSITIVE | NEGATIVE |

| | **AT DIAGNOSIS** | **AT LAST VISIT** | | **AT DIAGNOSIS** | **AT LAST VISIT** |
|---|---|---|---|---|---|
| **WHITE CELL COUNT (WCC)** | | | **C3** | | |
| **NEUTROPHILS** | | | **C4** | | |
| **LYMPHOCYTES** | | | **CREATININE** | | |
| **EOSINOPHILS** | | | **ALBUMIN** | | |
| **HAEMOGLOBIN** | | | **VITAMIN B12** | | |
| **MEAN CELL VOLUME (MCV)** | | | **RED CELL FOLATE** | | |
| **RED CELL DISTRIBUTION WIDTH (RDW)** | | | **SERUM IRON** | | |
| **MEAN PLATELET VOLUME (MPV)** | | | **TRANSFERRIN** | | |
| **ERYTHROCYTE SEDIMENTATION RATE (ESR)** | | | **TRANSFERRIN SATURATION** | | |
| **C-REACTIVE PROTEIN (CRP)** | | | **FERRITIN** | | |

| | **AT DIAGNOSIS** | | | **AT LAST FOLLOW UP** | | |
|---|---|---|---|---|---|---|
| **ANAEMIA** | YES | NO | | YES | NO | |
| | MICROCYTIC | NORMOCYTIC | MACROCYTIC | MICROCYTIC | NORMOCYTIC | MACROCYTIC |
| **THROMBOCYTOSIS** | YES | NO | | YES | NO | |
| **LEUKOPENIA** | YES | NO | | YES | NO | |
| **RDW** | LOW | NORMAL | HIGH | LOW | NORMAL | HIGH |
| **MPV** | LOW | NORMAL | HIGH | LOW | NORMAL | HIGH |

| | | | | | | |
|---|---|---|---|---|---|---|
| **LOW C3** | YES | NO | [redacted] | YES | NO | [redacted] |
| **LOW C4** | YES | NO | | YES | NO | |
| **HIGH ESR** | YES | NO | | YES | NO | |
| **HIGH CRP** | YES | NO | | YES | NO | |

## KEY

0 - NOT PRESENT

1 - PRESENT AT TIME OF DIAGNOSIS

2 - DEVELOPED DURING COURSE OF DISEASE

## REFERENCE RANGES

| **BLOOD RESULTS** | | | |
|---|---|---|---|
| **ANAEMIA (g/dL)** | Hb MALE < 13 | Hb FEMALE <12 | |
| **MICROCYTIC (fL)** | MCV < 79 | | |
| **NORMOCYTIC (fL)** | MCV 79 - 98 | | |
| **MACROCYTIC (fL)** | MCV > 98 | | |
| **LEUKOPENIA (x$10^9$/L)** | WCC < 4 | | |
| **NEUTROPENIA (x$10^9$/L)** | NEUTROPHIL < 2 | | |
| **LYMPHOPENIA (x$10^9$/L)** | LYMPHOCYTES < 1.0 | | |
| **THROMBOCYTOSIS (x$10^9$/L)** | PLATELET > 400 | | |
| **THROMBOCYTOPENIA (x$10^9$/L)** | PLATELET < 150 | | |
| **RDW (%)** | LOW | NORMAL | HIGH |
| | < 12.4 | 12.4 -17.0 | > 17.0 |
| **CRP (mg/L)** | NORMAL | HIGH | |
| | < 8 | ≥8 | |
| **ESR (mm/hr)** | NORMAL | HIGH | |
| | | ≥ 28 | |
| **ALBUMIN (g/L)** | LOW | NORMAL | |

| | | | |
|---|---|---|---|
| | < 40 | ≥ 40 | |
| **COMPLEMENT** | LOW | NORMAL | |
| | C3 < 0.50 | C3 0.50 - 1.53 | |
| | C4 < 0.20 | C4 0.20 - 1.00 | |
| **VITAMIN B12 (pmol/L)** | LOW | NORMAL | |
| | < 156 | 156 - 672 | |
| **FOLATE (ng/mL)** | LOW | NORMAL | |
| | < 3 | 3 - 20 | |
| **SERUM IRON (umol/L)** | LOW | NORMAL | |
| | < 11.6 | 11.6 - 31.3 | |
| **FERRITIN (ug/L)** | LOW | NORMAL | |
| | < 20 | 20 - 250 | |

**DEFINITIONS FOR DATA SHEET**

**Arrythmia** - Any documented ECG abnormalities deviating from sinus rhythm.

**LV dysfunction** :

**Systolic dysfunction** - Echocardiogram finding of reduced ejection fraction < 50%.

**Diastolic dysfunction** - Echocardiogram findings suggestive of diastolic dysfunction.

**Cor pulmonale** - altered structure and/or impaired function of the right ventricle that results from pulmonary hypertension that is associated with diseases of the lung parenchyma, pulmonary vasculature, upper airway disease or chest wall abnormalities.

**Pericardial disease** - diseases related to the pericardium of the heart

**Interstitial lung disease** - a diffuse parenchymal lung diseases, often collectively referred to as interstitial lung diseases (ILDs). Heterogeneous group of diseases classified together because of similar clinical, radiographic or pathologic manifestations. Clinical diagnosis based of fine bibasal inspiratory crackles (velcro). Plain chest X-ray features of low lung volumes with bilateral reticular or reticulonodular infiltrates. Array of signs on HRCT chest such as ground glass opacification, honeycombing or reticulonodular opacities.

**Isolated pulmonary hypertension** - mean pulmonary arterial pressure (PAP) ≥35 mmHg at rest (transthoracic echo).

**Pleural disease** - pathological processes affecting the parietal or visceral pleura or involving the pleural cavity eg pleural effusion.

**Restrictive lung disease -** group of disorders that may be extra pulmonary, pleural or lung parenchymal that leads to restriction of chest expansion and inadequate ventilation. Pulmonary function with a normal FEV1/FVC, with low vital capacity (VC) and decreased DLCO.

**Raynaud's phenomenon -** description of skin discolouration of the digits of the hand due to spasm of blood vessels. Initially the fingers turn white due to lack of blood flow, then blue to do hypoxia and finally red as the vessels open and local flushing occurs.

**Digital ischaemia induced changes** - tissue damage secondary to hypoxia e.g. digital ulcers - not related to trauma.

**Telangiectasia** - dilation of the capillaries which causes them to appear as small red clusters and often spidery in appearance on the skin surface

**Modified Rodnan skin score -** technique used to quantify skin thickness in scleroderma. 17 different regions of the body is palpated (fingers, hands, forearms, upper arms, face, chest, abdomen, thighs, lower legs, and feet) 0 = normal; 1 = mild; 2 = moderate; and 3 = severe (D). The maximum total score is 51.

**Upper GI tract symptoms -** constellation of symptoms including dysphagia, odynophagia, nausea, vomiting or heart burn

**Lower GI tract symptoms** - constellation of symptoms including constipation, bloating, diarrhoea or faecal incontinence

# APPENDIX B - ETHICS APPROVAL

R14/49 Dr Vaishak Vinod

## HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

## CLEARANCE CERTIFICATE NO. M160678

| | |
|---|---|
| **NAME: (Principal Investigator)** | Dr Vaishak Vinod |
| **DEPARTMENT:** | Internal Medicine<br>Chris Hani Baragwanath Academic Hospital |
| **PROJECT TITLE:** | Haematological and Immunological Parameters in Patients with Systemic Sclerosis Attending a Tertiary Care Hospital |
| **DATE CONSIDERED:** | 24/06/2016 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Dr C Ickinger and Prof M Tickly |
| **APPROVED BY:** | Professor P Cleaton-Jones, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 17/08/2016 |

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

**DECLARATION OF INVESTIGATORS**

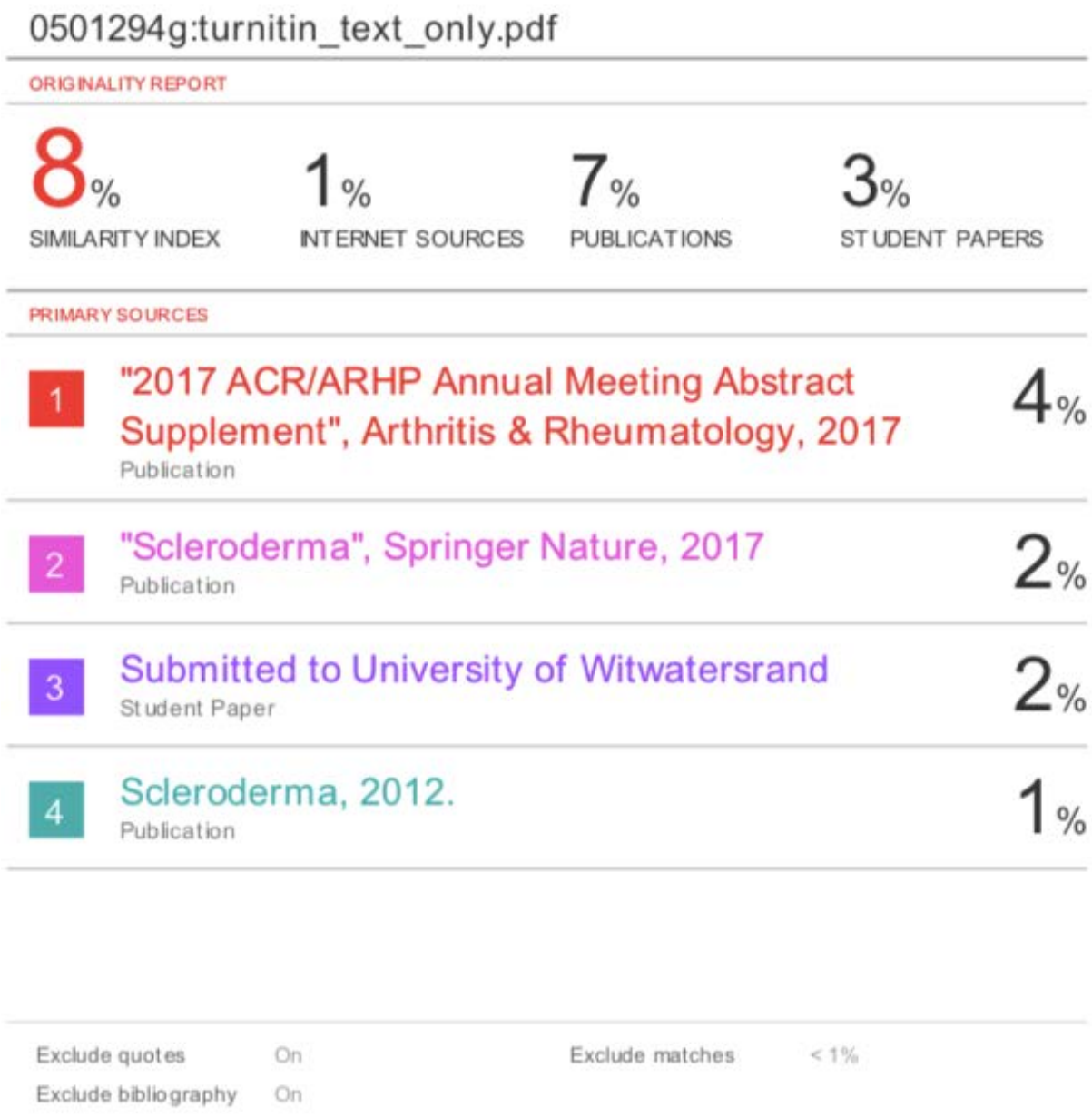
To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned 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 June and will therefore be due in the month of June each year.

Principal Investigator Signature

Date 20/8/2016

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

APPENDIX C - TURNITIN REPORT



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|---|---|---|---|
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PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | "2017 ACR/ARHP Annual Meeting Abstract Supplement", Arthritis & Rheumatology, 2017<br>Publication | 4% |
| 2 | "Scleroderma", Springer Nature, 2017<br>Publication | 2% |
| 3 | Submitted to University of Witwatersrand<br>Student Paper | 2% |
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This Turnitin report has been reviewed and approved by the supervisor of this study.

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