EVALUATION OF HEMATOLOGICAL PARAMETERS IN SICKLE CELL DISEASE COMORBID PATIENTS ATTENDING CLINICS IN ILORIN NIGERIA

BY

MASUD LINATULLAHI SAHBAN

21/05BLL008

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, FACULTY OF BASIC MEDICAL AND HEALTH SCIENCES, THOMAS ADEWUMI UNIVERSITY, OKO-IRESE, KWARA STATE
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE
(B.MLS)

AUGUST, 2025

ABSTRACT

Sickle cell disease, a hereditary blood disorder, is known to exacerbate the immune system compromise. This compromise makes the system vulnerable to other health conditions, leading to comorbidity- a leading cause of death among the sickle cell patients. This study was aimed at evaluating three haematological parameters in sickle cell disease patients with co-morbid conditions. A cross-sectional experimental design was adopted in selecting 71 participants, comprised of 26 (36.6%) co-morbid sickle cell (HbSS) group, 35 (49.4%) sickle cell (HbSS) group, and 10 (14.1%) normal (HbAA) group. Clinical conditions of the comorbid HbSS group were established from their hospital clinical report, while haemoglobin genotype was determined using electrophoresis, and haematological parameters were determined using Mindray BC-5000. The result of the findings revealed comorbidity of 16.9% for Cancer, 1.4% for HIV, 9.9% for Sepsis, 2.8% for Malaria, 3.8% for Hemolytic anaemia and 4.2% for Chronic Kidney Disease. Neutrophil count ($x10^9$ cells/L) were found to be higher among Co-morbid HbSS (55.4 ± 19.8) but the lymphocyte count ($x10^9$ cells/L) of the co-morbid HbSS group were found to be significantly lower (33.1 ± 19.5). Similarly, platelet counts ($x10^9$ cells/L) of the Co-morbid HbSS group (277.5 ± 138.8) was lower than HbSS-control (297.9 ± 133.5) but higher than the normal (263.4 ± 43.6). It is therefore concluded that SCD patients, especially those with comorbid conditions, exhibit significantly impaired haematological parameters, hence the patients should always undergo routine and comprehensive haematologic profiling to ensure early detection and management of abnormalities such as immune suppression and chronic inflammation.

Keywords: Immunosuppression, Hemolysis, Comorbidity and Sickle cell disease

DECLARATION

This is to declare that this research project titled Evaluation Of Hematological Parameter In Sickle Cell Disease Comorbid Patients Attending Clinics In Ilorin Nigeria carried out by me **Masud Linatullahi Sahban**, in the Department of Medical Laboratory Science, Faculty of Basic Medical and Health Sciences,Thomas Adewumi University Oko-Irese,Kwara State, is solely the result of my work except where acknowledged as being derived from other person(s) work or resources.

06-08-2025

Masud Linatullahi Sahban — Date

CERTIFICATION

I declare that this project is my original work and has not been previously submitted to ant other institution of higher learning.

I further certify that all sources cited or quoted are duly acknowledged by means of a comprehensive list of refrences.

06-08-2025

Masud Linatullahi Sahban — Date

08-08-2025

Mr Abdulraheem I.A — (Date)
(Supervisor)

…… …10-08-2025…
Mr. John, Clement Tamnyegia — Date
(Head of Department)

……  …… …10-08-2025……
Prof. Emenike. O. Irokanulo — Date
(Dean, Faculty of Basic Medical and Health Sciences

11-08-2025

…… ……
Prof. A.O. Hassan — Date

(External Examiner)

DEDICATION

This project is dedicated to Almighty ALLAH, the most beneficent the most merciful, who granted me the ability and spared my life throughout the course of this project.

ACKNOWLEDGEMENT

My sincere appreciation goes to Almigthy God who in His infinite mercy make it convenient for me throughout the duration of my study. I also extend my deepest gratitude and dedication to my loving and caring parents, Alhaji Masud Jibril Sahban and Alhaja Masud Sahban F. whose unwavering prayer, support and guidance have been my source of strength. May they be blessed with long life, sound health and prosperity. Ameen.

My profound gratitude goes to my supervisor Mr. Abdulraheem Ismail A. for a significant effort and suggestion throughout this project, may the mercy of God be upon him. I remain loyal to the Dean, Faculty of Basic Medical and Health Sciences Professor Emenike Irokanulo O. and the Head of Department Mr. John Clement for making the department an outstanding and a conducive one, May God bless you sir.

I specially acknowledge the impact of my lovely siblings, Masud Maryam Sahban, Masud Izatullahi Sahban and Masud Aliyah Sahban for their unwavering support and motivation during this course.

I am also grateful to Mr Olalere, Mr Chinedu, Mr Abdullateef and Mr Abdulganney for their support and guidance during the course of this research.

I must not forget all my course mates, professional colleagues and friends, may Almighty God crown all our efforts with success.

Finally, I acknowledge the institutions and organizations that made this research possible through their facilities.

Thank you all for making this journey a rewarding and memorable experience, may God crown all our efforts.

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Sickle cell disease (SCD) and its variants are genetic disorders resulting from the presence of a mutated form of hemoglobin, hemoglobin S (HbS) (Sedrak *et al.,* 2024). Two mutations or deletions of the β-globin gene, at least one of which is a point mutation resulting in the generation of hemoglobin S, are hallmarks of sickle cell disease, a generic name for a variety of hemoglobinopathies. Hemolytic anemia, severe chronic end-organ damage, and early mortality are all associated with sickle cell disease at least 95% of children with this disease survive to adulthood in high-income countries, leading to an increasing number of people with complex conditions, significant co-morbidities, and a shortage of professionals to provide care (Pecker and Lanzkron, 2021). The most common form of SCD found in North America is homozygous HbS disease (HbSS) which is an autosomal recessive disorder first described by Herrick in 1910. SCD causes significant morbidity and mortality, particularly in people of African and Mediterranean descent. The disease, seizure frequency, degree of anemia, and organ systems involved vary widely between individuals (Joseph and Ali., 2024). Worldwide, an estimated 4.4 million people have sickle cell disease (Global Burden of Disease 2015; 2016) and approximately 300,000 babies are born with the disease each year (Chakravorty and Williams, 2015). Sickle cell disease (SCD) is particularly prevalent in sub-Saharan Africa, but is also found in parts of India, the Middle East, the Arabian Peninsula, and among people of African descent living in North and South America and other parts of the world (Tebbi, 2022). In Nigeria, 25 to 35% of its population of over 160 million people carry the hemoglobin S gene; however, the prevalence of HbSS is 2-3%, resulting in a population of approximately 3.5 million Nigerians with HbSS (Braimoh *et al.,* 2017). The most common manifestations of sickle cell disease include

hemolytic anemia, intermittent Vaso-occlusive crises, and a variety of other complications such as acute chest syndrome, splenic sequestration, renal failure, leg ulcers, and infections (Tantawy *et al.,* 2020; Tebbi, 2022). Treatment of sickle cell disease generally focuses on preventing and controlling symptoms and complications, with transfusions often required in countries where more innovative treatments, such as hydroxyurea, autologous transplantation, and gene therapy, are not yet available, such as in Nigeria.

In sub-Saharan Africa, 12.5% of transfused patients are at risk of post-transfusion hepatitis, despite safety measures such as rigorous donor selection and biological qualification of donations through adequate virological testing (Nambei *et al.,* 2016; Ankouane *et al.,* 2016). Due to basic mechanisms that cause an increased number of neutrophils in the venous blood of individuals with sickle cell disease, previous research including these patients found an increase in the total white cell count. These include a decrease in the pace at which neutrophils depart the circulation, an increase in release from the bone marrow, and demarginating of intravascular neutrophils (Toledo *et al.,* 2019). Further investigation also showed that it is possible to trigger an invisible inflammatory response that releases cytokine mediators, some of which are primarily responsible for promoting the generation of neutrophils in the bone marrow (Hussain *et al.,* 2024). In a steady state, people with sickle cell disease had higher platelet counts than people without sickle cell disease. According to studies, this is due to the fact that chronic haemolysis and vaso-occlusion, which result in anatomical and physiological changes, are hallmarks of sickle cell disease (Musa and Ndakotsu, 2023). the large variation in platelet count brought on either splenectomy or loss of splenic function in adult individuals. Additionally, it might be the consequence of a negative feedback impact on the secretion of high-level erythropoietin in response to anemia, as it has structural homology with thrombopoietin (Faes *et al.,* 2018; Asafa *et al.,* 2024). Hence the role of hematological parameters in evaluation of sickle cell severity cannot be over emphasize, especially among those with co-morbid condition.

1.2 Aim of the study

To evaluate three haematological parameters in sickle cell disease comorbid patients attending clinics in Ilorin, Nigeria.

1.3 Objectives of the study

1. To evaluate the impact of sickle cell disease comorbidity of cancer, sepsis, malaria, nephropathy, hemolytic anaemia and HIV on lymphocyte count.
2. To assess the impact of sickle cell disease comorbidity of cancer, sepsis, malaria, nephropathy, hemolytic anaemia and HIV on neutrophil count.
3. To assess the impact of sickle cell disease comorbidity of cancer, sepsis, malaria, nephropathy, hemolytic anaemia and HIV on platelet count.
4. To compare the mean of the three haematological parameter amongst the three groups (Comorbid HbSS, HbSS patient and Normal patient)

1.4 Statement of Problem

Sickle Cell Disease is a red cell disorder, it is an established fact that leukocytes and platelets play a significant role in the pathophysiology of this disease. Sickle Cell Disease is characterized by abnormally high total white blood cell count, secondary to inflammation and hypo-splenism (Ugwu *et al.,* 2019). High white blood cell count in patients with Sickle Cell Anaemia may be a prognostic indicator of clinical severity. Elevated white blood cell counts correlate with increased pain frequency and other adverse outcomes in patients with Sickle Cell Anaemia (Cutis *et al.,* 2015). Sickle cell anaemia patients are prone

to infections due to asplenia which makes them susceptible specially to encapsulated organisms such as pneumococcus, salmonella and mycobacterium (Braimoh *et al.,* 2017). Defective alternate complement pathways together with excessive iron deposition in tissues also provide a good milieu for bacteria growth (Booth *et al.,* 2010). Common sites of infection are the chest, bone, gall bladder and genito-urinary tract. However, UTI accounts for 75% of all infections in this population (Braimoh *et al.,* 2017). Despite extensive attempts to elucidate the pathophysiology of SCD, several recent studies have mostly limited their investigations to single pathologies without considering the cumulative of such co-morbidities like malaria, HIV or chronic kidney disease that are highly prevalent in the Nigerian population. Therefore, there is need to address this gap by systematically evaluating hematological parameters in co-morbid SCD patients in comparison with sickle cell disease patients without any comorbidity and normal individuals to strengthen clinical practice .

1.5 Research Questions

1. What is the impact of sickle cell disease comorbidity of cancer, sepsis, malaria, nephropathy, hemolytic anaemia and HIV on lymphocyte count?

2. What is the impact of sickle cell disease comorbidity of cancer, sepsis, malaria, nephropathy, hemolytic anaemia and HIV on neutrophil count?

3. What is the impact of sickle cell disease comorbidity of cancer, sepsis, malaria, nephropathy, hemolytic anaemia and HIV on platelet count?

1.6 Research Hypotheses

H_o: There is no significant difference in mean of the hematological parameters of sickle cell disease co-morbid patients, sickle cell disease patients with vaso-occlusive crisis and the normal patient.

H_i: There is a significant difference in mean of the hematological parameters of sickle cell disease co-morbid patients, sickle cell disease patients with vaso-occlusive crisis and the normal patient.

1.7 Significance of the study

Personalized medicine approaches can improve treatment for sickle cell disease patients by targeting individualized therapies which can contribute to better management of the disease. Hematology parameters play a crucial role in monitoring disease progression by serving as a guide for clinical decisions to ensure effective treatment. Effective monitoring and management can significantly improve patient outcomes in a situation where sickle cell disease patients require tailored treatment plans, personalized medicine approaches can help achieve this goal by contributing to targeted therapies leading to treatments that can be more effective. Overall approaches targeted at individual condition can improve lives of sickle cell disease patients and potential to revolutionize treatment for the disease.

1.8 Limitations of the study

Small sample size or limited population representation, limited generalizability to other populations or settings, inability to control for confounding variables, limited exploration of environmental or socioeconomic factors, the study may involve collections of sensitive patients data, which must be protected in accordance with relevant regulations, the findings may not be generalizable to other

populations, such as pediatric or geriatric patients, the study may not be accounted for the impact of co-morbidities and co-medication on the findings.

1.9 Scope of the study

The study entails determination of hematological parameters which serve as indicator for infection, hemolysis and red cell indices among comorbid sickle cell disease patient (Comorbid HbSS), sickle cell disease control (HbSS-control) and normal individuals (HbAA).

1.10 Justification of the study

The assessment of hematological profiles in co-morbid sickle cell disease (SCD) individuals is an imperative and essential issue in recent restraining research in Nigeria. Despite extensive attempts to elucidate the pathophysiology of SCD, several recent studies such as Adewale *et al.* (2023), Uche and Oladipo (2023) and others have mostly limited their investigations to single pathologies without considering the cumulative of such co-morbidities like malaria, HIV or chronic kidney disease that are highly prevalent in the Nigerian population. These co-morbidities inevitably affect many hematological parameters including hemoglobin (Hb) concentration, white blood cell (WBC) count, platelet count, and red cell indices, and also complicate the process of disease worsening and patient guidance outcomes (Ladu *et al.,* 2024). Thus, characterization of these elements in co-morbid SCD patients not only fill a major current gap in knowledge but will also offer important insights to enhance treatment approaches. By evaluating hematological parameters in co-morbid SCD patients, clinicians would be better equipped to interpret disease progression more accurately, adjust treatment protocols effectively, and allocate resources to high-risk patients. This approach supports the broader public health goal of reducing mortality and improving quality of life for SCD patients through evidence-based intervention.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Sickle Cell Disease

Sickle cell disease (SCD) is caused by a single amino acid substitution (Glu > Val) in codon six of the globin gene, resulting in the formation of the hemoglobin variant (HbSS). This results in increased viscosity and adhesion to vessel walls, leading to obstruction of blood flow in small capillaries (Kaul *et al.,* 2009). This primary pathophysiological condition has many clinical manifestations, such as vaso-occlusive crisis (VOC), splenomegaly, acute chest syndrome (ACS), ocular manifestation, hepatomegaly, pulmonary hypertension, leg ulcers, chronic kidney disease (CKD) and brain stroke, which leads to early mortality (Sundd *et al.,* 2019). However, in the current scenario, hydroxyurea (HU) is the only sickle cell drug approved by the Food and Drug Administration (FDA) to increase fetal hemoglobin (HbF) levels (Verma *et al.,* 2018) . Worldwide, more than 300,000 children are born with sickle cell anemia each year, two-thirds of them in Africa, while Nigeria, India and the Democratic Republic of Congo bear half of the global burden of sickle cell disease. This number is expected to reach approximately 400,000 by 2050 (Jain and Odame, 2020). The phenotypic manifestation of the disease is still poorly understood. However, environmental factors, including climate and air quality, infections, fetal hemoglobin levels, and other genetic factors, play a critical role in disease progression (Jha *et al.,* 2018). The severity of disease in pediatric patients with sickle cell disease, especially those younger than five years of age, is increased for a number of reasons, including rapid sequestration of red blood cells in the spleen, failure of opsonization, and inability to process encapsulated microorganisms after infection (Monaco *et al.,* 2015). As a result, among children with sickle cell disease, infection is the second leading cause of death during the first ten years. Patients with sickle cell disease in sub-Saharan Africa and the Eastern Mediterranean region have high rates of infection, with invasive pneumococcal infection being the most common (Hsu *et al.,* 2018).

Global reports suggest that implementing effective management and a systematic newborn screening (NBS) program is a logical first step in disease prevention (Hsu *et al.,* 2018). Early newborn screening and implementation of vaccinations and prophylactic antimicrobial agents improve the quality of care for patients with sickle cell disease, resulting in a significant reduction in mortality (Kuznik *et al.,* 2016).

2.2 Prevalence of Sickle Cell Disease

Sickle Cell Disease (SCD) is one of the most common genetic blood disorders worldwide, primarily affecting individuals of African, Mediterranean, Middle Eastern, and South Asian descent. According to the World Health Organization (WHO), approximately 300,000 infants are born annually with SCD globally, with projections suggesting this number could rise to over 400,000 by 2050 if effective interventions are not implemented (Piel *et al.,* 2013). Sub-Saharan Africa bears the highest burden, accounting for nearly 75% of global SCD births (Makani *et al.,* 2020).

Nigeria has the highest burden of SCD globally, with an estimated 150,000 babies born annually with the disease (Nnodu *et al.,* 2021). This accounts for about 50% of all global SCD births, making it a major public health concern. The prevalence of sickle cell trait in Nigeria is also high, affecting approximately 20–30% of the population (Adewoyin, 2015). These figures highlight the critical need for comprehensive national screening programs, public awareness, and access to early diagnosis and care.

In the United States, SCD affects an estimated 100,000 individuals, with the highest prevalence among African Americans approximately 1 in every 365 births (Centers for Disease Control and Prevention [CDC], 2023). Additionally, about 1 in 13 African American newborns is born with the sickle cell trait (CDC, 2023), which underscores the importance of early screening and genetic counseling.

Recent data also reveal increasing SCD diagnoses in countries with rising African and Middle Eastern migrant populations, such as the United Kingdom, France, and parts of the Middle East. These

epidemiological shifts highlight the need for international awareness, targeted public health strategies, and equitable access to diagnostic and treatment services (Weatherall, 2011; *Telfer et al.,* 2020).

2.3 Structure and Function of Normal Hemoglobin

The hemoglobin molecule is a metalloprotein composed of four subunits, each containing a peptide chain and a heme group (Li *et al.,* 2020). The polypeptide chains of hemoglobin pair symmetrically to form a tetrameric structure and functional unit (Lukin *et al.,* 2019). Each of the four polypeptides has a large central space in which a prosthetic heme group and a protoporphyrin IX molecule are bound by non-covalent forces, thus protecting the iron atom (Fe2+) from access to the surrounding aqueous solution (Unzai *et al* 2018). The alpha (α) polypeptide chains and the heme group of iron protoporphyrin IX are the same in all human hemoglobins (Vasseur-Godbillon *et al.,* 2020). The arrangement of hemoglobin subunits (quaternary structure) changes in the oxygenated and deoxygenated states (Mazzarella *et al.,* 2019). Oxygen binds reversibly to the ferrous iron atom of each heme group (Olson *et al.,* 2017). When oxygen molecules bind to the ferrous iron atoms, the gap between two polypeptide chains of the hemoglobin molecule narrows and widens as oxygen leaves (Lukin *et al.,* 2019). In addition to transporting oxygen from the lungs to the tissues, hemoglobin also interacts with carbon dioxide, carbon monoxide, and nitric oxide (Giardina *et al.,* 2018). Three series of α genes (zeta - ζ, alpha 1 - α1, and alpha 2 -α2) and five series of β genes (epsilon - ε, gamma 1 - γ1, gamma 2 - γ2, delta - δ, and beta - β) are located. respectively at locus p13.3 of chromosome 16 (16p13.3) and at locus p15.5 of chromosome 11 (11p15.5) (Thein *et al.,* 2020). During fetal life, haemoglobins Gower 1 (ζ2ε2), Gower 2 (α2ε2) and Portland (ζ2γ2) are formed, as their genes are mainly expressed in the yolk sac, pre-aortic region and liver (Peschel *et al.,* 2019). Its reduction in early embryonic life is followed by the expression of two α genes and two γ genes, leading to the formation of fetal hemoglobin (α2γ2), which is the predominant hemoglobin at 9 weeks of gestation. Fetal hemoglobin (HbF) has a slightly higher affinity for oxygen than

adult hemoglobin, because it binds less to 2,3-bisphosphoglycerate (2,3-BPG). After birth, α genes remain fully active, while γ genes are down regulated and β group genes (β and δ) are up regulated. The increase in the hemoglobin phenotype is predominant by the end of the first 12 months of life (Thein *et al.,* 2020). Normal adult red blood cells contain HbA (α2β2), HbA2 (α2δ2), and HbF (α2γ2), which represent approximately 97%, 2%, and 1% of total hemoglobin, respectively. However, in rare cases, γ-globin gene expression persists in adult red blood cells, a condition called hereditary persistence of fetal hemoglobin (HPFH) (Egesa *et al.,* 2022).

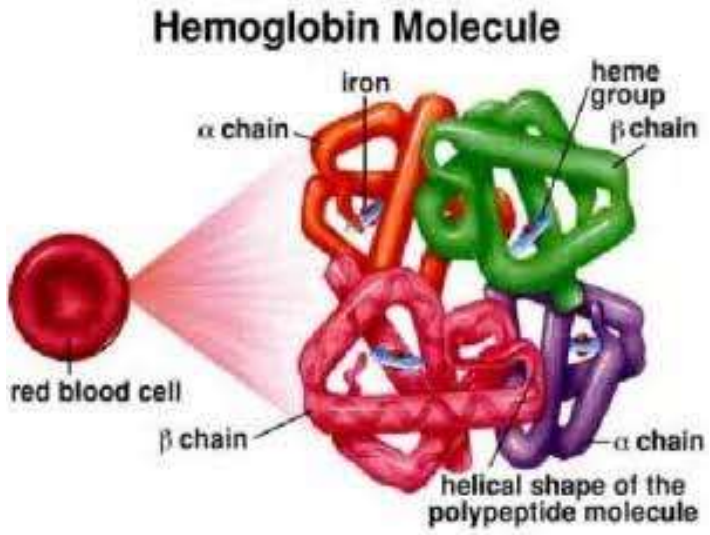


Figure 2.1a: Structure of Hemoglobin

Source: https://encr.pw/KzHoz

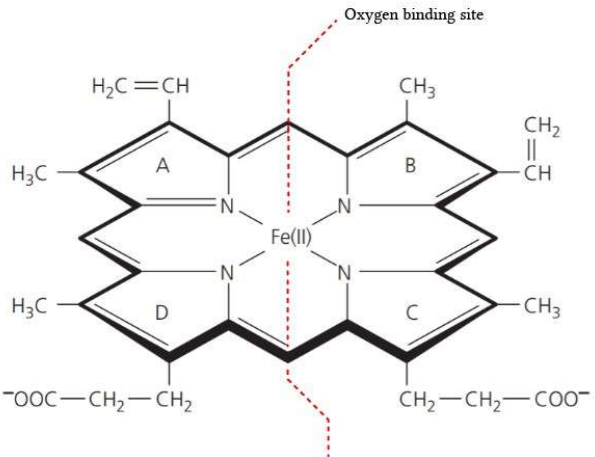


Figure 2.1b: Molecular Structure of Hemoglobin Source: https://shorturl.at/QaCtr

2.4 Etiology of Sickle Cell Disease

Sickle cell disease originated in West Africa, where it has the highest prevalence. It is also present to a lesser extent in India and the Mediterranean region. DNA polymorphisms in the beta S gene suggest that it arises from five distinct mutations: four in Africa and one each in India and the Middle East. The most common of these is an allele found in Benin in West Africa. Other haplotypes are found in Senegal and Bantu, Africa, as well as in India and the Middle East. The HbS gene, when present in homozygous form, is an undesirable mutation, so a selective advantage in the heterozygous form must explain its high prevalence and persistence. Malaria may be the agent of selection because there is a concordance between the prevalence of malaria and that of Hb S. The disease may protect a person from malaria either by (1) accelerating sickling so that parasitized cells are eliminated, or (2) by making it more difficult for the parasite to metabolize or penetrate the sickle cell. While children with Hb SA with sickle cell trait appear to have a milder form of falciparum malaria, those with homozygous Hb S have a severe form associated with a very high mortality rate (Piel and Steinberg, 2022). The sickling process that leads to seizure can be accelerated by several factors. Local tissue hypoxia, dehydration secondary to viral disease, or nausea and vomiting, all of which lead to plasma hypertonicity, can cause sickling. Any event that can lead to acidosis, such as infection or extreme dehydration, can cause sickle cell disease. Milder factors and environmental changes, such as fatigue, exposure to cold and psychosocial stress, can activate the sickle process. A specific cause is often not identified (Ballas *et al.,* 2020). Vaso-occlusive crises are often caused by: Cold weather (due to vasospasm), Hypoxia (for example, flying in an unpressurized plane), Infection, Dehydration (especially from exertion or in hot weather), acidosis, poisoning from alcohol, emotional stress, pregnancy, The data also suggest a role for stress with exercise, particularly when associated with heat and hypovolemia. Aplastic crises are often preceded by the following: parvovirus B19 infection, folic

acid deficiency, ingestion of bone marrow toxins (e.g., phenylbutazone). Acute chest syndrome is associated with: fat embolism, infections, painful episodes, and asthma. (Quinn *et al.,* 2020).

2.5 Genotypes and Pathophysiology of Sickle Cell Disease

Sickle cell disease is a general term describing a group of lifelong autosomal recessive disorders that develop as a result of a point mutation (base change from GAG to GTG at codon 6, rs334) in which a hydrophobic valine replaces a hydrophilic glutamic acid at position 6 in the β-globin subunit of hemoglobin (c.20A>T, pGlu6Val) (Brandow and Liem, 2022). This mutation leads to the formation of an abnormal form of hemoglobin called sickle hemoglobin (HbS). Classically, sickle cell disease follows an autosomal recessive Mendelian inheritance pattern, where an affected offspring (male or female) receives one defective gene from each parent. There are several genotypes of sickle cell anemia, but the most common is sickle cell anemia (HbSS), which results from the inheritance of two copies of the HbS mutation (homozygous) and accounts for 65-70% of all cases of sickle cell anemia. . Other common forms of sickle cell anemia include hemoglobin SC (HbSC), sickle cell beta-zero thalassemia (HbSβ0 thalassemia), and sickle cell beta-plus thalassemia (HbSβ+ thalassemia), which occur when coherent mutations responsible for other abnormal hemoglobin types (i.e. HbC or β-thalassemia) combine with HbS to form a heterozygous compound mutation (Streetly *et al.,* 2018). Patients with HbSβ+ thalassemia have reduced β-globin production, while those with HbSβ0 thalassemia have no β-globin production. HbC is caused by the substitution of glutamic acid for lysine at the same site as HbS (c.19G>A, p.Glu6Lys) (Cisneros and Thein, 2021).

Other mutations in the β-globin gene include HbD Punjab/Los Angeles (c.364G>C, p.Glu121Gln), HbE, Hb-Lepore, HbO-Arab (c.364G>A, p.Glu121Lys), and Hb Québec-CHORI, among others (American Society of Hematology, 2021). The consistency of HbS and these hemoglobin variants leads to rare and

relatively benign SCD genotypes (HbSD-Punjab/Los Angeles, HbSE, HbS-Lepore and HbSO-Arab, HbS-Quebec-CHORI) (Khamees *et al.,* 2021). Deoxygenation results in intracellular polymerization of HbS molecules into rigid, crystal-like rods that distort the normally flexible biconcave red blood cells into the characteristic rigid sickle (crescent) shape (Cisneros and Thein, 2021). Although polymerization is reversible with reoxygenation, repeated episodes cause damage and hemolysis of the red blood cell membrane, reducing the lifespan of red blood cells from 90 to 120 days to 10 to 20 days. Faster polymerization occurs at higher HbS concentrations and lower pH, but is limited by the presence of HbF. The characteristic pathophysiological mechanism is the entrapment of sickled red blood cells in the microvasculature and obstruction of blood flow, leading to typical acute and chronic multiorgan ischemic complications. The mechanisms are complex and not fully understood, but abnormal red blood cell adhesion, vascular endothelial activation, leukocytosis, leukocyte (neutrophil and monocyte) and platelet activation, cellular dehydration, and oxidative stress due to reperfusion are implicated. Molecules such as P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), laminin, and thrombospondin contribute essentially to adhesion (Ansari and Gavins, 2019). The focus of research has been on haptoglobin and hemopexin for their role in binding Hb and extracellular free heme, respectively, to HbF-inducing agents (e.g., hydroxyurea, voxelotor), selectin inhibitors (e.g., crizanlizumab), anti-inflammatory agents, antiplatelet agents, anticoagulants, and antioxidants (e.g., L-glutamine) (Telen, 2018).

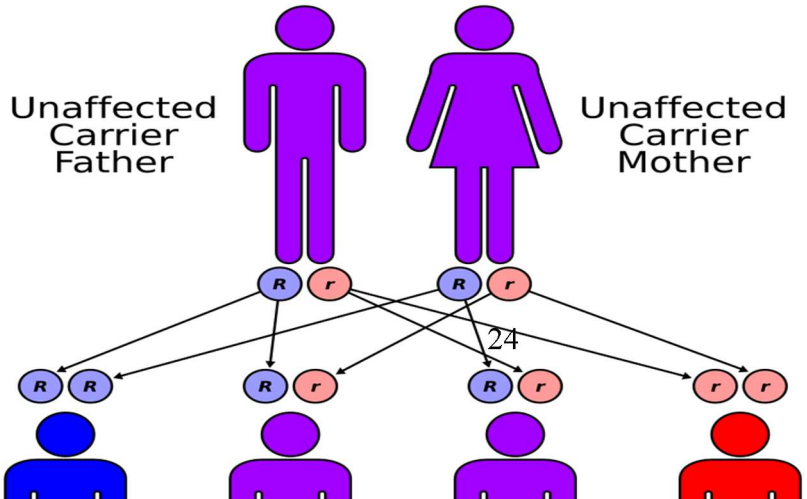


Figure 2.2a: Sickle cell inherited in an autosomal recessive pattern

Source: (Farid, 2019)

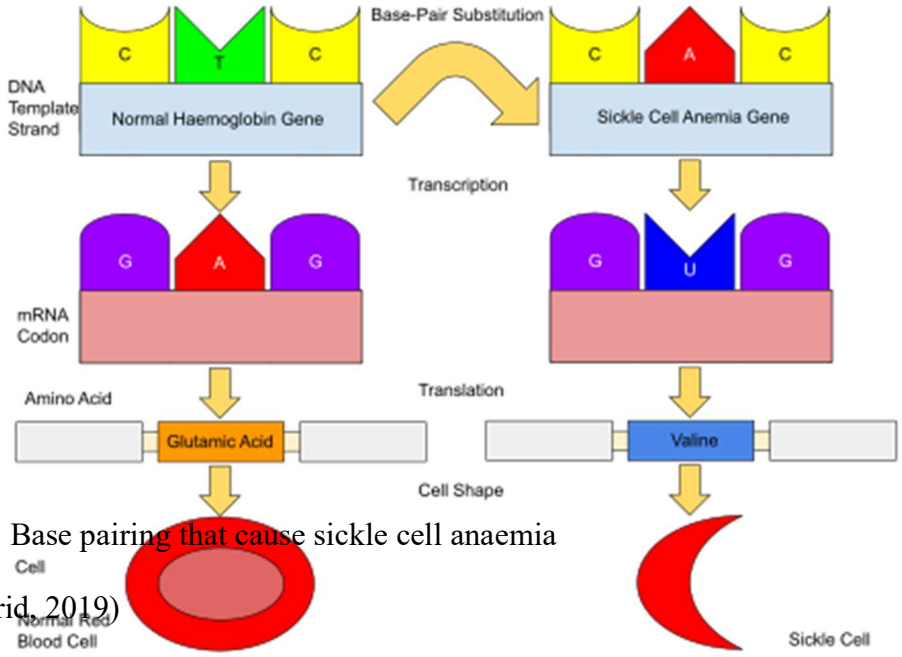


Figure 2.2b: Base pairing that cause sickle cell anaemia

Source: (Farid, 2019)

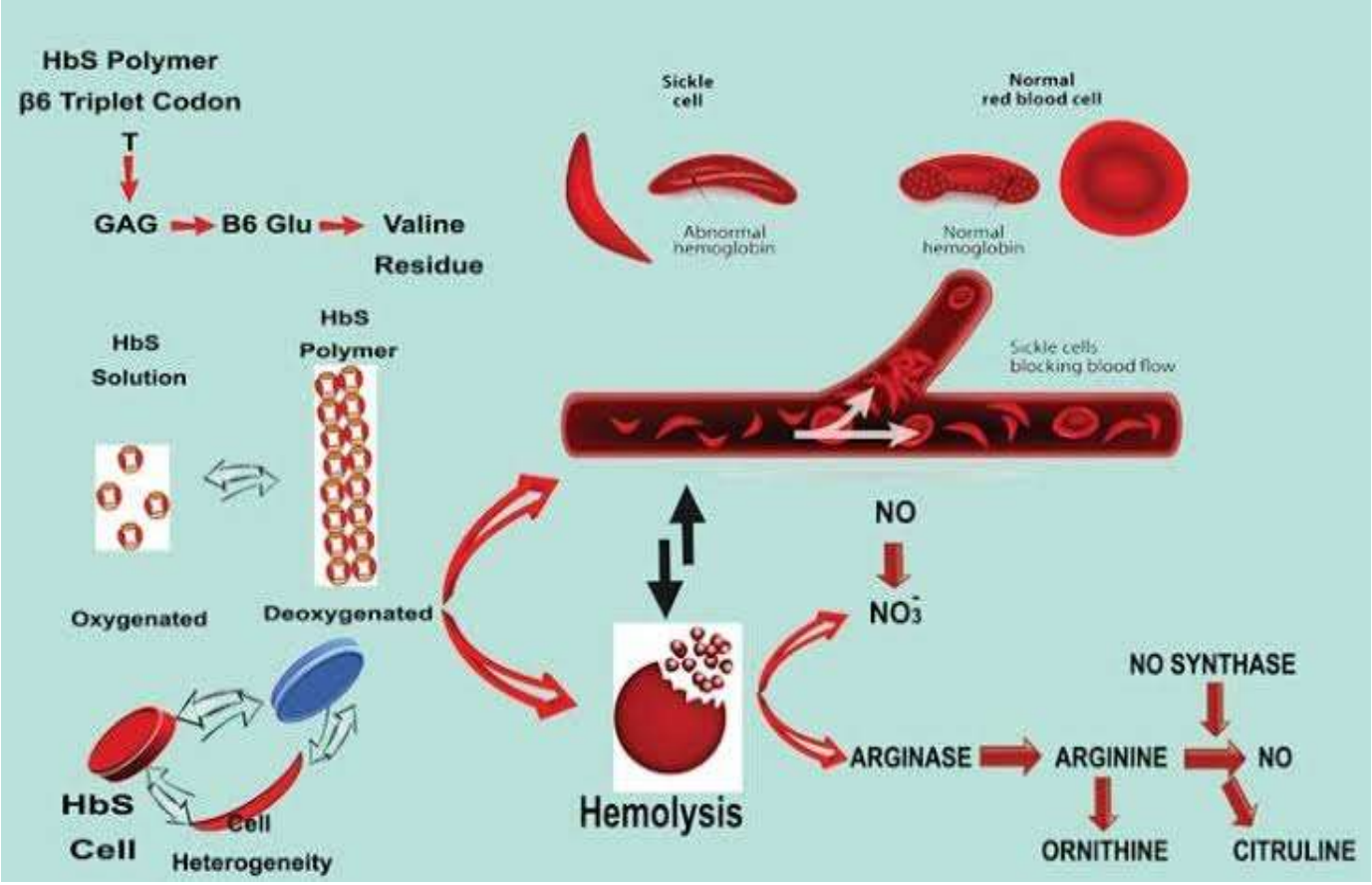


Figure 2.3: Pathophysiology of sickle cell disease

Source:https://shorturl.at/rFUcv

2.6 Hematopoiesis in Sickle Cell Disease

Hematopoiesis, the process of blood cell formation, is significantly altered in sickle cell disease (SCD) due to chronic hemolysis, bone marrow stress, and systemic inflammation. In SCD, the bone marrow responds to continuous destruction of sickled red blood cells by increasing erythropoietic activity, leading to several characteristic hematological findings and potential complications.

- Compensatory Erythropoiesis: SCD is marked by chronic hemolytic anemia, which stimulates the bone marrow to produce more red blood cells (RBCs). This leads to reticulocytosis, an increased number of immature RBCs in circulation (Yawn *et al.,* 2022). However, the quality of erythropoiesis is often compromised, as many reticulocytes are also deformed and short-lived.

- Bone Marrow Expansion: Increased erythropoietic demand causes bone marrow hyperplasia, particularly in the axial skeleton. Over time, this can lead to skeletal abnormalities and bone infarctions (Ware & de Montalembert, 2021). Despite high erythropoietic activity, anemia persists due to ineffective erythropoiesis and ongoing hemolysis.

- Hematopoietic Stem Cell (HSC) Dynamics: Recent studies suggest that SCD is associated with increased proliferation but reduced functionality of hematopoietic stem and progenitor cells (HSPCs). Chronic inflammation and oxidative stress impair HSC self-renewal and may contribute to marrow exhaustion over time (Sheehan *et al.,* 2023).

- Erythropoietin and Regulatory Mechanisms: Erythropoietin (EPO) levels are typically elevated in SCD as a response to anemia. However, the response may be blunted in the presence of renal impairment or iron deficiency, further disrupting hematopoiesis (Ataga *et al.,* 2020). Inflammatory cytokines like TNF-α and IL-6 also interfere with erythroid progenitor proliferation, contributing to ineffective hematopoiesis.

Sickle cell disease (SCD) is a hereditary hemoglobinopathy marked by chronic hemolysis and recurrent vaso-occlusion. These pathophysiological events have a profound effect on hematopoiesis, particularly the production and function of erythrocytes, leukocytes, and platelets.

- Erythropoiesis in SCD: In SCD, erythropoiesis is persistently stimulated due to chronic anemia caused by the accelerated destruction of sickled red blood cells. This leads to: Elevated erythropoietin levels, primarily secreted by the kidneys in response to hypoxia, Bone marrow hyperplasia with expanded erythroid precursors. Reticulocytosis, often seen in peripheral blood, reflecting increased output from the marrow (Sheehan *et al.,* 2023). However, erythropoiesis in SCD is ineffective due to oxidative stress, iron dysregulation, and damage to developing erythroblasts. Inflammatory cytokines like TNF-α and IL-6 further suppress erythroid progenitor cell proliferation (Ware and de Montalembert, 2021).

- Leukopoiesis in SCD: Leukopoiesis is also altered in SCD. Patients typically exhibit leukocytosis, even in the absence of infection. This is attributed to: Chronic inflammation that increases myeloid cell production, Elevated granulocyte colony-stimulating factor (G-CSF) and interleukin levels, Increased adhesion of neutrophils to the endothelium, which contributes to vaso-occlusive crises (Zhang *et al.,* 2022). Although the white cell count is high, leukocyte function is often impaired. Neutrophils show delayed apoptosis and exaggerated activation, contributing to endothelial damage (Sundd *et al.,* 2020).

- Thrombopoiesis in SCD: Thrombopoiesis is also dysregulated. Patients with SCD typically have: Elevated platelet counts (thrombocytosis) due to splenic dysfunction or auto-splenectomy, Hyperactive platelets that contribute to a prothrombotic state, Increased levels of thromboxane A2, P-selectin, and other platelet activation markers (Manwani and Frenette, 2020). The enhanced platelet reactivity plays a significant role in the pathogenesis of vaso-occlusion and stroke risk in SCD (Zhao *et al.,* 2023).

In SCD, hematopoiesis is persistently stimulated but functionally abnormal. Erythropoiesis attempts to compensate for hemolysis but is impaired by marrow stress and inflammation. Leukopoiesis produces excessive but dysregulated white cells, and thrombopoiesis contributes to the hypercoagulable state, increasing clinical complications.

2.7 Roles of the Innate and Adaptive Immune System in the Pathogenesis of Sickle Cell Disease

The activity of the alternative complement pathway (particularly factor B) and the formation of circulating immune complexes are increased in patients with sickle cell disease. Furthermore, some complications in patients with sickle cell disease are associated with increased immune complex formation (Fodor *et al.,* 2018). Mast cell activation is associated with misdiagnosis in patients with sickle cell disease. Furthermore, the number of eosinophils and their degranulation, migration, and adhesion capacities are also increased in patients with sickle cell disease (Field *et al.,* 2016). Human neutrophils and macrophages cultured with heme have their phagocytic capacity inhibited by cytoskeletal remodeling. In addition, free heme is an important pro-inflammatory molecule, which activates macrophages and endothelial cells via TLR4 signaling. Heme released during hemolysis also promotes NET formation, thus contributing to the vaso-occlusive episodes and inflammatory state of SCD. In addition, free heme induces HO-1 expression in neutrophil progenitors and reduces their oxidative burst response. (Belcher *et al.,* 2018). The number of CD8+ T cells is increased and that of CD4+ T cells is decreased in SCD. On the other hand, an increased number of Tregs has been described in patients with sickle cell disease, but their suppressive function is normal. Furthermore, the Treg profile in SCD patients with different clinical symptoms is diverse. An increased number of B cell subsets is seen in sickle cell disease, however, their function is impaired due to reduced antibody production. Studies evaluating Costa are still rare NKT cells also have an important pathogenic role in the pathogenesis of sickle cell disease, contributing to the inflammatory response and vaso-occlusive episodes. TLR4: Toll-like receptor 4; NET: neutrophil extracellular traps; HO-1: heme

oxygenase-1; Market: regulatory T cells; iNKT cells: invariant natural killer T cells; Costa: regulatory B cells. (Júlia Teixeira *et al.,* 2020).

2.8 Immune Dysfunction in Sickle cell Disease

Sickle cell disease (SCD) is a chronic monogenic hemoglobinopathy characterized by hemolysis and vaso-occlusive events. Sickle cell disease (SCD) is an inflammatory disease associated with alterations in immune phenotype and function (El-Alfy *et al.,* 2018). It is a global health problem, affecting millions of people worldwide, and its incidence is expected to reach 400,000 newborns per year by 2050 (Piel *et al.,* 2017). Sickle cell disease can lead to ischemia, infarction, and ischemia-reperfusion injury with progressive damage to multiple organs (Atmis *et al.,* 2018). The genetic and molecular basis of SCD arises from a mutation in the β-globin gene, which leads to the polymerization of abnormal deoxygenated hemoglobin S (HbS), resulting in occlusion of small vessels by sickled red blood cells (RBCs). However, the pathophysiology has proven to be much more complex than initially thought, involving many factors beyond red blood cells (Allali *et al.,* 2020). People with sickle cell disease (SCD) have an increased susceptibility to impaired immune function. This includes a reduction in the proportion of circulating CD4+ and CD8+ T cells, a reduction in the ratio of CD4+ helper T cells:CD8+ suppressor T cells, aberrant activation and dysfunction of regulatory T cells (Treg), a bias of CD4+ T cells towards Th2 responses, and a loss of memory B cells secreting IgM CD27+ IgMhigh IgDlow (Balandya *et al.,* 2016). However, the increase in neutrophils is largely dysfunctional due to impaired chemotaxis, migration, and killing capacity. Impairment of the alternative pathway of complement activation by qualitative and quantitative deficiencies of factors B and C3 has also been reported (Anyaegbu *et al.,* 1999). The high rate of alloimmunization, connective tissue diseases, and graft rejection (Fasano *et al.,* 2015), as well as the incidence of aberrant vaccine responses (Disu *et al.,* 2016), have led to the demonstration of abnormalities in ASC adaptive immunity. However, to date, little work has been done to characterize the phenotype,

function, and contribution of T and B lymphocytes to chronic inflammatory diseases in ASC (Vingert *et al.,* 2014). Limited studies have shown that T and B cell abnormalities occur in ASC. These abnormalities may be caused by the ASC disease itself, or may occur as a result of complications of its treatment with repeated blood transfusions (Balandya *et al.,* 2016).

2.9 The Impact of Bacterial Infections on Hematological Parameters in Sickle Cell Disease

Bacterial infections significantly impact the hematological parameters and overall health of individuals with Sickle Cell Disease (SCD). Patients with SCD are more vulnerable to infections due to functional asplenia, impaired immune response, and chronic hemolysis. The presence of bacterial infections can exacerbate existing complications and lead to altered blood parameters, including changes in white blood cell count, hemoglobin levels, reticulocyte count, and platelet count. Below, we discuss the various ways bacterial infections can affect hematological parameters in SCD patients.

- **White Blood Cell (WBC) Count and Leukocytosis:** One of the most immediate hematological changes observed during bacterial infections is leukocytosis, or an elevated white blood cell count. In SCD, infections can trigger a strong inflammatory response, leading to the release of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which stimulate the production of white blood cells from the bone marrow (Nath *et al.,* 2020). This increase in WBCs is a sign of the body's immune response to the infection. However, persistent or severe infections, such as those caused by Streptococcus pneumoniae or Salmonella, may cause an excessive rise in WBCs, which can sometimes complicate the interpretation of blood counts during infection (Vichinsky *et al.,* 2019).

- **Hemoglobin Levels and Anemia:** Bacterial infections can worsen anemia in SCD patients, either through direct effects on hemoglobin levels or via increased hemolysis. Infections can accelerate the

breakdown of red blood cells, a process already heightened in individuals with SCD due to the chronic hemolysis of sickled cells. For example, septicemia or pneumonia can lead to increased destruction of red blood cells, worsening the pre-existing anemia associated with SCD. Additionally, bacterial infections can lead to acute splenic sequestration, where red blood cells are rapidly removed from circulation by the spleen, further contributing to a drop in hemoglobin levels (Brousse *et al.,* 2021).

- **Reticulocyte Count:** During bacterial infections, there is often an increase in the reticulocyte count in the peripheral blood, reflecting the body's attempt to compensate for the loss of red blood cells. Reticulocytes are immature red blood cells released by the bone marrow in response to hypoxia and anemia. In the case of infections, the reticulocyte count can rise as part of the acute-phase response, with the bone marrow attempting to replenish red blood cells that are being destroyed or sequestered. An elevated reticulocyte count in SCD patients with an infection can be a sign of the body's effort to overcome anemia (Telen, 2018).

- **Platelet Count and Thrombocytosis:** Infections in SCD patients may also lead to an increase in platelet count, a condition known as thrombocytosis. Thrombocytosis is common during infection due to the inflammatory cytokines stimulating the production of platelets from the bone marrow. Elevated platelet counts can increase the risk of thrombosis, especially in individuals with SCD who are already at an increased risk for thromboembolic events due to hypercoagulability (Galanello *et al.,* 2019). However, the rise in platelet count is often part of the acute phase response and may return to baseline once the infection resolves.

- **Acute Chest Syndrome (ACS) and Hematological Changes:** Bacterial infections, particularly pneumonia, are one of the most common causes of acute chest syndrome (ACS) in individuals with SCD, a condition associated with significant morbidity and mortality. ACS can lead to increased hemolysis, a

further drop in hemoglobin, and elevated WBC and platelet counts. Additionally, ACS often exacerbates pre-existing anemia and can lead to splenic sequestration or infarction, further worsening hematological parameters (Platt *et al.,* 2020).

- **Impact on Iron Status:** Infections can also influence the iron status of SCD patients. Iron homeostasis may be disrupted during infections due to the acute-phase response, which includes the sequestration of iron within the reticuloendothelial system. This can result in decreased serum iron levels and iron-restricted erythropoiesis, a phenomenon that may further complicate the anemia seen in SCD patients. However, some bacterial infections, such as Salmonella infection, can cause direct splenic damage, leading to changes in iron metabolism and hemolysis, which can further impact iron stores (Ballas *et al.,* 2021).

2.10 The Impact of Cancer on Hematological Parameters in Sickle Cell Disease

Sickle cell disease (SCD) is an inherited blood disorder marked by persistent red blood cell breakdown, painful vaso-occlusive episodes, and gradual damage to multiple organs. Individuals with SCD typically exhibit abnormal blood counts due to continuous inflammation, destruction of red blood cells, and compensatory activity in the bone marrow. The onset of cancer in these individuals adds another layer of complexity, often intensifying pre-existing hematologic disturbances and introducing new management challenges. Anaemia mainly results from ongoing destruction of red blood cells and their reduced lifespan. The presence of cancer, especially blood-related cancers like leukemia or lymphoma, can worsen this condition by impairing bone marrow function either through direct invasion or suppression. This effect is further amplified by treatments such as chemotherapy, which significantly reduce red blood cell production. As a result, these combined factors often lead to severe anemia, making blood transfusions a necessary part of patient care (Owusu-Ansah *et al.,* 2019).

Individuals with sickle cell disease (SCD) often have higher-than-normal white blood cell (WBC) counts, largely due to persistent inflammation and increased bone marrow activity. When cancer is also present, these WBC levels can fluctuate dramatically. For instance, blood cancers like leukemia may lead to a further rise in WBCs, while treatments that suppress bone marrow function—such as chemotherapy—can cause a significant drop in neutrophils, thereby heightening the risk of serious infections (Sibinga *et al.,* 2015). Thrombocytosis is common in SCD due to auto-splenectomy or splenic dysfunction. However, malignancies or chemotherapy can cause thrombocytopenia, increasing bleeding risk (Al-Suliman *et al.,* 2022). In certain cancers, particularly solid tumors, thrombocytosis may persist or worsen, compounding the hypercoagulable state in SCD and increasing the risk of thromboembolic events. The function of the bone marrow can become significantly impaired. In SCD, the marrow is constantly stimulated to produce new blood cells in response to continuous red blood cell destruction. However, cancer can hinder this process through mechanisms such as direct invasion of the marrow, fibrotic changes, or the effects of chemotherapy. The interaction of these factors can result in a reduction across all blood cell lines (pancytopenia), which complicates treatment decisions and negatively impacts the patient's prognosis (Jain *et al.,* 2021). There is emerging evidence suggesting that patients with SCD have an increased risk of developing hematologic malignancies, such as myelodysplastic syndromes and acute myeloid leukemia. This increased risk may be due to chronic inflammation, oxidative stress, repeated transfusions, or hydroxyurea therapy (Khan *et al.,* 2020). Such malignancies further deteriorate hematological stability and challenge treatment strategies.

2.11 Non-Infectious Comorbidities and Their Influence on Hematological Profiles in Sickle Cell Disease

Sickle cell disease (SCD) is associated with various non-infectious comorbidities that significantly influence hematological parameters. These comorbidities often exacerbate anemia, affect white blood cell

(WBC) and platelet counts, and contribute to systemic inflammation, complicating disease management and prognosis.

- **Chronic Kidney Disease (CKD):** CKD is a common complication in adult SCD patients due to repeated vaso-occlusion in the renal microvasculature. It is associated with worsening anemia caused by decreased erythropoietin production and reduced red cell lifespan (Asnani *et al.,* 2020). Additionally, uremia may suppress bone marrow function, further reducing hemoglobin levels and altering WBC and platelet production.

- **Pulmonary Hypertension (PH):** PH affects up to 10% of individuals with SCD and is linked to hemolysis-driven nitric oxide depletion. This condition leads to elevated lactate dehydrogenase (LDH) levels, increased reticulocyte counts, and worsened anemia (Parent *et al.,* 2022). Elevated tricuspid regurgitant jet velocity in SCD patients correlates with lower hemoglobin and higher hemolytic markers.

- **Iron Overload:** Repeated blood transfusions, a common treatment in SCD, can result in iron overload. This condition may impair erythropoiesis and promote ineffective red blood cell production, leading to dysregulated hematological indices (Ali *et al.,* 2023). Serum ferritin levels may rise, while mean corpuscular volume (MCV) and red cell distribution width (RDW) become altered.

- **Leg Ulcers and Chronic Pain Syndromes:** Chronic inflammation from conditions like leg ulcers and avascular necrosis influences hematologic parameters by increasing inflammatory markers (e.g., CRP, ESR) and potentially elevating WBC counts even in the absence of infection (Ezenwa *et al.,* 2020). Chronic pain is also associated with stress-related leukocytosis and higher platelet counts due to inflammatory signaling. Non-infectious comorbidities play a critical role in altering the hematological landscape in SCD. Recognizing these influences is essential for accurate interpretation of lab results and

optimizing disease management. Monitoring trends in hemoglobin, WBC, platelet counts, and markers of hemolysis can guide interventions and prevent complications.

2.12 Clinical Presentation of Sickle Cell Disease

Sickle cell disease (SCD), an autosomal recessive genetic disease, is a hemoglobinopathy caused by mutations in the gene encoding the beta subunit of hemoglobin (Kato *et al.,* 2018). Due to the mutations, the shape and function of the hemoglobin molecule changes, causing the red blood cells to take a sickle shape (Rees *et al.,* 2018). Sickle cell disease manifests itself in a variety of ways, from acute generalized malaise to early stroke, leg ulcers and fatal multi-organ failure. Due to the presence of the fetal form of hemoglobin, HbF, the clinical features of sickle cell disease do not appear until the second half of the first year of life after birth, when the transition to hemoglobin occurs adult (Debaun *et al.,* 2019).). In general, the clinical features associated with sickle cell disease can be divided into 2 types, depending on the underlying cause. The clinical features of the first type are those caused by hemolysis and nitric oxide dysfunction, leading to large vessel vasculopathy manifested by cerebrovascular disorders, pulmonary hypertension, priapism, nephropathy, and leg ulcers. The clinical features of the second type are those due to vaso-occlusion, leading to painful ischemic episodes and progressive organ damage that can manifest as osteonecrosis, hyposplenism, retinopathy, and liver damage (Kato *et al.,* 2020).

2.12.1 Vaso-Occlusive Crisis

The most typical manifestation of sickle cell disease is vaso-occlusive crises. Ischaemia results from blockage of the post-capillary venules, which leads to acute episodes of painful sickle cell crisis and ischemia-reperfusion injury. All areas of the body, but particularly the back, long bones, chest, pelvis, and abdomen, are in severe pain, according to the patients. Children as young as six months old may have pain and swelling in both their hands and feet (Ballas *et al.,* 2019).

2.12.2 Acute Chest Syndrome

Acute chest syndrome (ACS) is the most frequent side effect of sickle cell anaemia (SCA). In people with sickle cell disease (SCD), ACS is the second most common cause of hospitalisation and the primary cause of death. Risk factors for ACS include recent surgery, pulmonary embolism, fluid overload, infection, and prior bouts of ACS or asthma. Two clinical features are coughing that starts suddenly and shortness of breath. If a fever occurs, it indicates an infection (Quinn *et al.,* 2020).

2.12.3 Anemia and Jaundice

The predominant sign of SCD is symptomatic anaemia, particularly frequent in HbSS. Steady-state hemoglobin levels in asymptomatic patients differ according to their phenotype, varying from 60 to 80 g/L in those with HbSS or HbSβ0-thal to 100 to 110 g/L in those with double heterozygous HbSC or HbSβ+-thal variants of disease. A rapid fall in an individual's steady-state hemoglobin level can cause hypoxia (aplastic crisis) or a shocklike state such as acute splenic sequestration. Because of the premature breakdown of sickled RBCs, levels of bilirubin are increased, leading to jaundice. If the bilirubin remains elevated for a long time, gallstones may form (DeBaub *et al.,* 2019).

2.12.4 Infectious Disease

Infection is a significant contributor to morbidity and mortality in individuals with sickle cell disease (SCD). Functional asplenia, which is evident from a young age, is the main reason for the predisposition to bacterial infection. Streptococcus pneumoniae is the most common pathogen; however, serious systemic infections with Haemophilus influenzae, Neisseria meningitidis, and Salmonella are also seen (Teixeira *et al.,* 2017).

2.12.5 Cerebrovascular Accident/Stroke

Cerebrovascular accidents (CVAs) may manifest in children as early as 2 years of age. Eleven percent of patients with SCD have a stroke by the age of 20 years. Silent cerebral infarct (SCI) in association with small-vessel disease is more frequent than overt stroke. Signs of SCI are seen in 34% of patients with SCD by the age of 14 years (DeBaun *et al.,* 2019).

2.12.6 Acute Kidney Injury

Acute kidney injury, or acute renal failure, is characterised by a rapid decline in kidney function, resulting in elevated serum creatinine levels and/or reduced urine output. Vaso-occlusion causes ischemic changes in the renal medulla. Contributing factors for volume depletion include hyposthenuria, recurrent and chronic use of nonsteroidal anti-inflammatory medications (NSAIDs), severe hemolysis, infection, and rhabdomyolysis (DeBaun *et al.,* 2019).

2.12.7 Splenic Sequestration

Splenic sequestration is a lethal consequence of sickle cell disease characterised by a sudden decline in haemoglobin levels, resulting in intense abdominal pain and circulatory system failure. It is more common in children, particularly in those with HbSS. Sequestration is caused by a splenic auto-infarction that occurs at approximately the age of 6 years.This complication may also develop in adults with HbSC and other hemoglobinopathies (Ballas *et al.,* 2019).

2.12.8 Hepatobiliary Complications

The hepatobiliary tract is one of the intra-abdominal organ systems most frequently affected in SCD. Sickle hepatopathy comprises a range of acute and chronic liver abnormalities (eg, vaso-occlusive hypoxic liver injury, cholelithiasis, sequestration in the liver, venous outflow obstruction, intrahepatic cholestasis, viral hepatitis, and biliary cirrhosis) seen in patients with SCD (DeBaun *et al.,* 2019).

2.12.9 Priapism Priapism is an undesirable and prolonged condition of penile erection, occurring with or without discomfort, that arises in the absence of sexual stimulation. Priapism may be ischemic (veno-occlusive, low flow), stuttering (recurrently ischemic), or non-ischemic (arterial, high flow). Most cases of priapism in SCD are ischemic in nature (Rogers *et al.,* 2020).

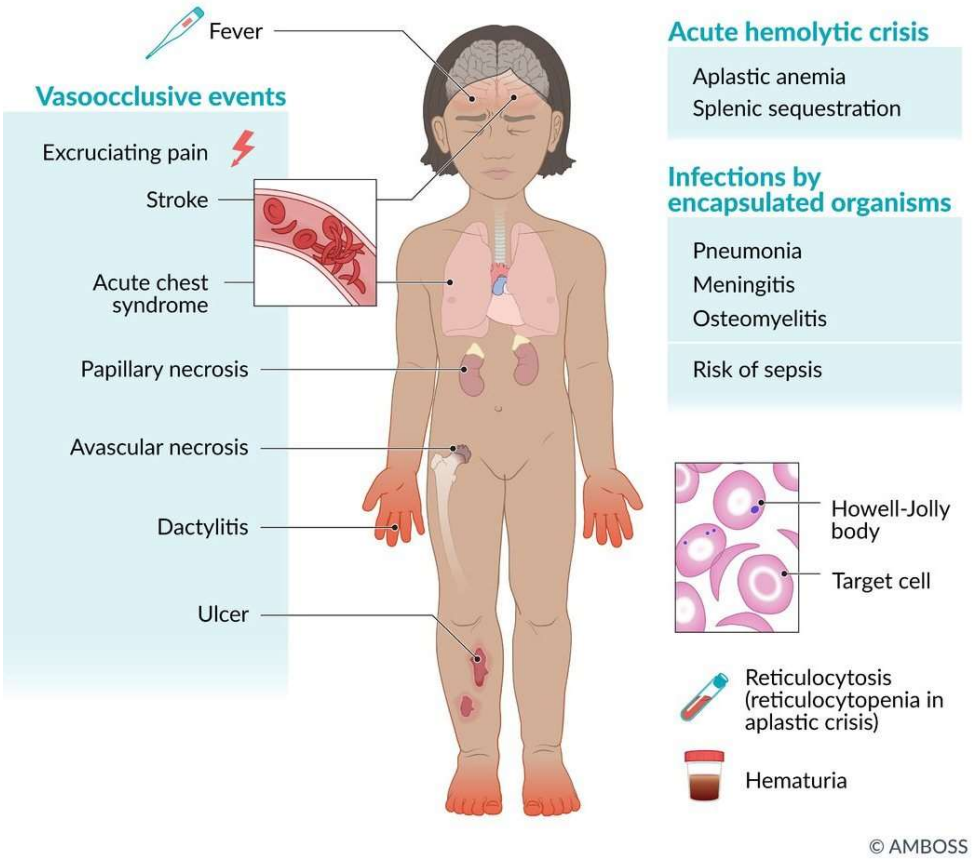


Figure 2.4: Clinical Presentation of sickle cell disease

Source: Darbari *et al.,* 2020

2.13 Laboratory Diagnosis of Sickle Cell Disease

Some of the diagnostic techniques include the peripheral smear, solubility testing, DNA testing (prenatal diagnosis), and haemoglobin electrophoresis (or thin layer isoelectric focusing). The patient's age determines the kind of tests that are conducted and this include; DNA testing can be used for prenatal diagnosis or to confirm the identification of the sickle cell genotype. Neonatal and postnatal screening, which includes hemoglobin solubility testing, hemoglobin electrophoresis, and peripheral smear examination, are all used in the diagnosis and screening of adults and children (Chan *et al.,* 2022). Some of the age-based tests include the following:

- **Prenatal screening:** The accuracy of prenatal diagnosis has increased significantly with the development of polymerase chain reaction technology. It is indicated for families at risk of sickle cell disease (Okar *et al.,* 2022). At 10-12 weeks of pregnancy, amniocentesis, chorionic villus sampling (CVS), and fetal blood are used to obtain fetal cells for genetic diagnosis. These procedures are not without risk of fetal loss. Chorionic villus sampling can be used to collect DNA samples. Amniotic fluid can be tested between 14 and 16 weeks (Emechebe *et al.,* 2017).
- **Newborn screening:** Currently, approved methods for distinguishing hemoglobin (Hb) S, A, F, and C include hemoglobin electrophoresis on acid citrate agar and cellulose acetate, isoelectric thin layer concentration, and hemoglobin fractionation by HPLC. Follow-up tests may be required at three to six months. However, HbS solubility testing during the first few months of life is unreliable (May *et al.,* 2022)
- **Post- natal Diagnosis:** The diagnosis is made by hemoglobin (Hb) electrophoresis on cellulose acetate at pH 8.6. However, HbD and HbG migrate at the same rate as HbS while HbE and O Arab migrate at the same rate as HbC (Cheesbrough, 2019). English citrate electrophoresis at Ph 6.3 is a better alternative to cellulose acetate because it allows better separation of common hemoglobin variants as they migrate at different speeds, while isoelectric focusing can be used in infants under 6 months. Hemoglobin

solubility and red blood cell sickling tests are useful in making the diagnosis of sickle cell disease (Alter, 2018). Other investigations useful in managing the various complications that these patients may develop include a complete blood count with differential and reticulocyte count, peripheral blood smear, serum electrolytes, pulmonary function tests (transcutaneous oxygen saturation). (Bain, 2018).

2.13.1 Conventional Laboratory Techniques

1. Screening tests

a. Sickling test:

Sickling tests are primarily grounded in the tendency of hemoglobin S (HbS) to polymerize when deprived of oxygen (Cheesbrough, 2019). This diagnostic method exploits the reduced solubility of deoxygenated HbS in comparison to normal hemoglobin A (HbA) (Akinola *et al.,* 2022). In practice, a blood sample is combined on a microscope slide with a reducing agent such as sodium metabisulfite (also known as disodium disulfite) or sodium dithionite. The slide is then sealed with a cover slip and left to incubate at ambient temperature, typically for an hour or longer. These reducing agents effectively remove oxygen from the hemoglobin within red blood cells, thereby creating conditions that promote sickling in cells containing HbS (Buhari *et al.,* 2023).

b. Solubility test:

Currently, the solubility test is among the most commonly employed methods for detecting sickle cell hemoglobin. It operates on the principle that hemoglobin S (HbS) becomes insoluble when exposed to a concentrated phosphate buffer, a hemolysing agent, and sodium dithionite. These reagents induce the crystallization and precipitation of HbS, which in turn scatters light and renders the solution turbid. Test outcomes are assessed by comparing the level of turbidity against known positive and negative controls (Hauwa *et al.,* 2023).

2. Complete Blood Count (CBC)

The complete blood count (CBC) is a primary test to characterize various types of anemia. However, hemoglobin mutation will affect hematological parameters, which show variable changes. Patients with sickle cell anemia usually present with the following findings (Hauwa *et al.,* 2023)

- Anaemia (Hb < 11mg/dL), Neutrophilia (> 45%), Thrombocytosis (> 400 x 10^9/L), Elevated Mean corpuscular volume (MCV) (>98fl) and Elevated Red cell distribution width (RDW) (Acharya *et al.,* 2019).

3. Peripheral Blood film

The peripheral blood smear (PBF) is typically conducted following the identification of abnormalities in automated blood counts and serves as a critical component of hematological assessment. This test involves the microscopic examination of blood cell morphology, allowing for the detection of subtle structural changes that can aid in diagnosing various forms of anemia. Due to the complexity arising from variations in the shape, size, position, and boundaries of blood cells, interpretation of blood smears can be challenging. To address this, computerized diagnostic systems have been developed to facilitate the classification and identification of different types of anemia (Hauwa *et al.,* 2023). In cases of sickle cell anemia, characteristic morphological features include: Normocytic normochromic, Irreversible Sickle Cells (ISCs), Nucleated Red Blood Cells (NRBCs), Target cells, Neutrophilia and Thrombocytosis (Acharya *et al.,* 2019).

4. Haemoglobin Electrophoresis

Hemoglobin Electrophoresis (HbE) is a laboratory technique used to separate and identify various hemoglobin fractions present in the blood (Cheesbrough, 2019). It plays a key role in both the diagnosis

and ongoing management of sickle cell disease (SCD) (Kohne, 2019). The method relies on the principle that different hemoglobin variants possess distinct electrical charges, which influence their migration rates when an electric current is applied across a gel or paper medium (Streetly & Sisidia, 2020).

Types of Hb Electrophoresis

There are several types of Hb Electrophoresis, including:

- **Alkaline Hb Electrophoresis:** This is the most common type of Hb Electrophoresis, performed at a pH of 8.6 (Afolabi *et al.,* 2020).

- **Acid Hb Electrophoresis:** This type of Hb Electrophoresis is performed at a pH of 6.2 and is used to separate certain hemoglobin variants (Kumar *et al.,* 2020).

- **Cation Exchange Hb Electrophoresis:** This type of Hb Electrophoresis uses a cation exchange resin to separate hemoglobin variants (Oyedeji *et al.,* 2022).

Hb Electrophoresis in SCD, In Sickle Cell Disease, Hb Electrophoresis is used to:

- Diagnose SCD: Hb Electrophoresis can detect the presence of Hemoglobin S (HbS), which is characteristic of SCD (Akinola *et al.,* 2022).

- Identify hemoglobin variants: Hb Electrophoresis can identify other hemoglobin variants, such as Hemoglobin C (HbC), Hemoglobin E (HbE), and others.

- Determine the severity of SCD: Hb Electrophoresis can help determine the severity of SCD by identifying the presence of other hemoglobin variants that may affect the disease severity (cheesbrough, 2019). Although Hb electrophoresis is effective at distinguishing between many clinically relevant hemoglobin variants, it has limitations. Variants such as HbD and HbG migrate similarly to HbS, and HbE

and HbO-Arab may share migration patterns with HbC, making differentiation difficult in standard electrophoretic conditions (Rentapali *et al.,* 2017). Additionally, in neonates, high levels of fetal hemoglobin (HbF) can obscure the presence of HbS during alkaline electrophoresis. Therefore, special attention is necessary when interpreting results in such cases to avoid false-negative outcomes (Khosa *et al.,* 2015).

2.13.2 Advanced Laboratory Techniques

- **High Performance Liquid Chromatography (HPLC)**

High-performance liquid chromatography (HPLC) is a laboratory technique used to separate, identify, and quantify the components of a mixture. In the diagnosis of sickle cell disease (SCD), HPLC is used to detect and quantify abnormal hemoglobin variants, such as hemoglobin S (HbS) (Cheesbrough, 2019) It is based on the principle of chromatography, where a mixture of substances separates based on their interactions with a stationary phase and a mobile phase. solvent that flows through the column In the diagnosis of sickle cell disease, HPLC is used to: French Detect HbS: HPLC can detect the presence of HbS, a characteristic of sickle cell disease (Afolabi *et al.,* 2022). 2. Quantify HbS: HPLC can quantify the amount of HbS present in the blood, which can help determine the severity of sickle cell disease (Kumar *et al.,* 2020). 3. Identify other hemoglobin variants: HPLC can identify other hemoglobin variants, such as hemoglobin C (HbC) and hemoglobin E (HbE), which can influence the severity of sickle cell disease (Oyedeji *et al.,* 2022). Advantages of HPLC in the diagnosis of sickle cell disease. High sensitivity and specificity: HPLC has high sensitivity and specificity for the detection of HbS and other hemoglobin variants. 2. Quantitative results: HPLC provides quantitative results, which can help determine the severity

of sickle cell disease. 3. Rapid results: HPLC provides rapid results, which can facilitate timely diagnosis and treatment (Akinola *et al.,* 2022). The development of fully automated HPLC would be useful for accurate testing of large numbers of samples. HPLC has better sensitivity in separating hemoglobin variants than electrophoresis. HPLC is much less labor-intensive and more reliable for monitoring patients receiving blood transfusions or hydroxyurea. However, HPLC is an expensive machine and cannot distinguish between all variants with the same retention time. For example, all Hb variants with a retention time similar to that of Hb S are overexpressed with the Hb S peak, which may lead to misdiagnosis of new variants that mimic HbS. Thus, HPLC cannot be used alone as a diagnostic test and should be performed with a confirmatory test such as DNA analysis before making a definitive diagnosis (Nair, 2018).

- **Genetic analysis**

Genetic study is important for the accurate detection of different types of sickle cell anemia, based on the detection of β-globin mutations that lead to the development of sickle cell anemia (Hauwa *et al.,* 2023).

a. Polymerase Chain Reaction (PCR)-Based Techniques.

Polymerase chain reaction is one of the most powerful diagnostic techniques, where special enzymes are used to amplify specific pieces of genetic material into millions of copies, using specific primers. PCR can detect single known genes or multiple genes in a single tube.The PCR protocol involves denaturation, annealing, and elongation, which is repeated for 20–40 heat cycles. Then, the result can be detected by gel electrophoresis, sequencing, melting curve analysis, or monitoring the change in the fluorescence. PCR sensitivity and specificity have revolutionized the prenatal and neonatal diagnostic field. Several PCR-based techniques are documented to detect βs mutations, such as high-resolution melting (HRM) analysis, which is simple, sensitive, and cost-effective for use in mass screening of SCD genotypes (Old, 2017). Another simple, low-cost PCR-based technique has been developed using bi-directional allele-

specific amplification (ASA) and a hot star system to provide more specific single-tube genotyping, where the point mutation of sickle cell anaemia is used as the SNP model. In addition, discriminatory conditions have enabled the determination of homozygous and heterozygous states based on the different band sizes on the agarose gel electrophoresis (Hauwa *et al.,* 2023).

b. Restriction Fragment Length Polymorphism:

Restriction Fragment Length Polymorphism (RFLP) is a molecular technique used to detect sickle cell disease by employing restriction enzymes that recognize and cleave specific DNA sequences altered by the β-globin gene mutation. One of the earliest enzymes used for this purpose is MstII, which targets the nucleotide sequence CCTNAGG (where "N" represents any nucleotide). In the presence of the sickle cell mutation where thymine substitutes adenine the MstII recognition site is lost, preventing the enzyme from cleaving the DNA at that location. Following enzymatic digestion, the resulting DNA fragments can be analyzed to infer the genotype: In individuals with a normal genotype ($\beta^A\beta^A$), MstII cuts the DNA at both alleles, producing two smaller bands. In heterozygous individuals ($\beta^A\beta^s$) carriers of sickle cell trait the MstII enzyme cuts only the normal β^A allele, resulting in three bands: one large uncut fragment (from β^s) and two smaller fragments (from β^A). In homozygous individuals ($\beta^s\beta^s$) those with sickle cell anemia the mutation removes both restriction sites, leading to one large uncut DNA fragment. An alternative restriction enzyme, DdeI, has also been utilized for detecting sickle cell mutations. DdeI recognizes the sequence 5'-GTNAG-3', which is similarly abolished by the sickle cell mutation. Consequently, the pattern and number of DNA fragments vary depending on the genotype, making RFLP a valuable tool for the genetic diagnosis of sickle cell disease (Tripathi, 2016).

- **Lateral Flow Immunoassay**

Lateral Flow Assays (LFA) are increasingly employed in biomedical diagnostics due to their portability, ease of use, and cost-effectiveness. One notable example is the Sickle SCAN platform, developed by Kanter *et al.,* which facilitates the qualitative detection of various hemoglobin types, including normal hemoglobin (HbAA), sickle hemoglobin (HbAS), hemoglobin C (HbAC), as well as compound variants such as HbSC and HbCC. This test utilizes a lateral flow chromatographic immunoassay format incorporating polyclonal antibodies that specifically recognize hemoglobin A, S, and C (Chy & Rahman, 2018). However, the test suffers from some limitations such as misinterpretation of the result due to visual reading, cross-reactivity of polyclonal antibodies, and false positive results in the detection of HbA heterozygous with HbS (McGann *et al.,* 2016). Another example of neonatal screening tool based on lateral flow immunoassay is the HemoTypeSCTM which uses monoclonal antibodies to discriminate between HbAA, HbAS, HbAC, HbSS, HbSC, and HbCC as illustrated in figure 9 below. The presence of high concentrations of HbF does not interfere with HemoTypeSCTM result in neonates. Following the manufacturer's instructions, a sample of 1.5µL of blood is collected by a puncture in the heel from the neonates or in the finger from adults using a thin needle (lancet) regularly used locally for the blood sample. The test result takes 20 minutes (Kasai *et al.,* 2022). Another example of neonatal screening tool based on lateral flow immunoassay is the HemoTypeSCTM which uses monoclonal antibodies to discriminate between HbAA, HbAS, HbAC, HbSS, HbSC, and HbCC as illustrated in figure 12 below. The presence of high concentrations of HbF does not interfere with HemoTypeSCTM result in neonates. Following the manufacturer's instructions, a sample of 1.5µL of blood is collected by a puncture in the heel from the neonates or in the finger from adults using a thin needle (lancet) regularly used locally for the blood sample. The test result takes 20 minutes (Kasai *et al.,* 2022).

- **Isoelectric Focusing**

Isoelectric focusing (IEF) is a high-resolution analytical technique used to separate proteins based on their isoelectric points (pI) the specific pH at which a molecule carries no net electrical charge. In the context of hemoglobin analysis, hemoglobin variants migrate through a pH gradient until they reach their pI, at which point they stop and form distinct, sharp bands (Arishi *et al.,* 2021). This method is particularly effective in identifying hemoglobin S (HbS) and hemoglobin A (HbA), even in the presence of elevated fetal hemoglobin (HbF), which is common in newborns. Notably, IEF can also distinguish between hemoglobin D-Punjab and HbS an important diagnostic advantage (Arishi *et al.,* 2021). The assay generally yields results within approximately 45 minutes. Despite its higher cost and the requirement for skilled personnel due to the complexity and number of bands produced, IEF remains the gold standard for newborn hemoglobinopathy screening. It requires only a minimal blood sample and is compatible with dried blood spot testing, making it both efficient and practical for large-scale neonatal programs (Frommel, 2018).

2.13.3 Innovative Techniques for the Diagnosis Of SCA

- **Image Processing Techniques**

- Image processing techniques play a vital role in the analysis of red blood cells. Blood cell disorders can be classified based on various features: cell shape, central dimer diameter, target flag, etc. (Abdulraheemfadhel *et al.,* 2017). Cells can also be classified based on image features using segmentation and artificial neural networks. Chy and Rahaman developed an automated method to detect sickle cell anemia (SCA) using an image processing technique. An algorithm is used to automate the detection of red blood cells present in thin blood smears. The first step in this technique involves taking images of the blood with a camera attached to an optical microscope. A preprocessing step then converts the image to grayscale, enhances the image, and passes it through a median filter to reduce noise. Next, the red blood cells are segmented via a segmentation threshold, followed by an image morphological operation to

remove unwanted objects. Image features are created based on cell color, structure, and geometry. Finally, the computer classifier is trained to evaluate the images (Chy and Rahaman, 2019).

- **Emerging Flow Cytometry**

Flow cytometry has long been employed to detect sickle cells by assessing either fluorescent markers or cellular morphology (Samsel and McCoy, 2016). Recent advancements have enhanced the sensitivity and diagnostic capabilities of this technique through the integration of imaging and morphological assessment. One notable innovation is the Sickle Imaging Flow Cytometry Assay (SIFCA), developed by Beers *et al.,* which utilizes imaging flow cytometry coupled with a custom software algorithm to differentiate between normal and sickled red blood cells based on morphological characteristics (Fertrin *et al.,* 2016).

- **Paper-Based Hemoglobin Solubility**

This is a technique that relies on the filtration properties of the paper substrate and the insolubility of HbS, where it can be visually interpreted to detect the presence of hemoglobin S. In this test, a drop of blood sample is mixed with a hemoglobin solubilizing agent in a ratio of 1:10. The mixture is then placed on chromatography paper and then stained. A different blood color pattern is formed depending on hemoglobin (Arishi *et al.,* 2021). These stains are used to determine HbSS, the carrier HbAS, and normal haemoglobin HbAA (Cheesbrough, 2019). However, this test is affected by the blood clotting, preventing the blood from wicking through the paper substrate (Kumar, *et al.,* 2020). Furthermore, the test is unable to differentiate between HbSC and HbAS (Streetly and Sisodia, 2020). Finally, this test is not reliable for

new-borns due to a high level of HbF, which prevents the polymerization and precipitation of haemoglobin S (Alwaheeb *et al.,* 2018).

- **Sensors Based Techniques**

a. Fluorescence Based Optofluidic Resonator

A waveguide-based optofluidic resonator has been utilized to investigate the interaction between ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions within protoporphyrin IX in a phosphate-buffered saline solution. This metal-coated resonator enables real-time analysis while requiring only a minimal volume of sample (Dai *et al.,* 2017). The fluorescence signal generated by Fe^{2+} ions is characteristic of normal hemoglobin, whereas Fe^{3+} ions produce distinct fluorescence peaks that are associated with hemoglobin S, which is indicative of sickle cell disease. This differentiation in spectral signatures offers a potential diagnostic pathway for distinguishing between healthy and sickle hemoglobin variants in a non-invasive and efficient manner.

b. Sensors Based on Electrical Impedance Signal

The electrical impedance signal is a sensitive indicator of disease severity and sickling events of red blood cells. The combination of microfluidic chip with electrical impedance offers a promising method to identify sickling and decalcification processes in sickle cell disease patients (Liu and Qiang, 2017). These methods were developed to monitor sickle cells, and do not accurately describe the severity of heterozygous sickle cell disease.

c. Quartz crystal microbalance (QCM)

The Quartz Crystal Microbalance (QCM) sensor functions by measuring shifts in the resonant frequency of a quartz crystal, which occur in response to variations in mass on its surface (Wang *et al.,* 2016). This technique has been employed to assess morphological alterations in red blood cells. When combined with an advanced mathematical modeling approach, QCM enables detailed analysis of changes in cell

elasticity. This method effectively distinguishes between normal biconcave discoid red blood cells and the altered shape of sickled cells, offering potential as a diagnostic and monitoring tool for conditions like sickle cell disease (Efremov *et al.,* 2018).

d. Pyrosequencing Technique

Pyrosequencing (PyS) has emerged as a reliable technique for distinguishing between various genotypes of sickle cell disease (SCD), including both homozygous and heterozygous forms. Its primary utility lies in the precise sequencing of hemoglobin genes in small patient samples, particularly for differentiating between βSβS and Sβ^0 thalassemia, as well as for identifying HbC mutations—differences that typically involve single-nucleotide variations and are often missed by conventional diagnostic methods. When evaluated against Sanger sequencing, PyS demonstrated a high degree of accuracy: correctly identifying βSβS and HbSC genotypes at a rate of 98.7%, and detecting heterozygous cases with 92.2% accuracy. Furthermore, PyS proved effective in recognizing β$^+$ and β^0 mutations commonly present in individuals with sickle cell disease (Arishi *et al.,* 2021).

2.14 Management and treatment of sickle cell disease

The National Institutes of Health recommends that optimal care for patients with sickle cell disease (SCD), including preventive care, is best provided by treatment in clinics specializing in the management of sickle cell disease. All patients with sickle cell disease should have a primary care physician, who should be a hematologist or be in frequent consultation with a hematologist.

In the event of a sickle cell crisis, when the severity of the episode can be assessed, self-treatment at home with bed rest, oral analgesia, and hydration is possible. People with sickle cell disease often present to the emergency room after self-treatment has failed. Pain management should include four phases: assessment,

treatment, reassessment, and adjustment. While considering the severity of the pain and the patient's past response, follow consistent protocols to relieve the patient's pain.

The goals of treatment are to control symptoms and manage complications of the disease. Treatment strategies include the following seven goals:

Management of vaso-occlusive crisis, Management of chronic pain syndromes, Management of chronic hemolytic anemia, Prevention and treatment of infections, Management of various complications and organ damage syndromes associated with the disease, Prevention of stroke and Detection and treatment of pulmonary hypertension. A panel of experts has published evidence-based guidelines for the treatment of sickle cell disease, including a strong recommendation that hydroxyurea and long-term periodic blood transfusions should be used more frequently to treat patients (Maakaron, 2024).

2.14.1 Hydroxyurea treatment

Hydroxyurea has an established role as a safe and effective treatment for SCD. Hydroxyurea increases total and fetal hemoglobin in children with SCD.The increase in fetal hemoglobin retards gelation and sickling of RBCs. Hydroxyurea also reduces levels of circulating leukocytes, which decreases the adherence of neutrophils to the vascular endothelium. (McGann, 2015).

2.14.2 Blood transfusion

Blood transfusions are not needed for the usual anemia or episodes of pain associated with SCD. Urgent replacement of blood is often required for sudden, severe anemia due to acute splenic sequestration, parvovirus B19 infection, or hyperhemolytic crises. Transfusions are helpful in acute chest syndrome,

perioperatively, and during pregnancy. Acute red cell exchange transfusion is indicated in the following situations: Acute infarctive stroke Severe acute chest syndrome Multiorgan failure syndromes Right upper quadrant syndrome Priapism that does not resolve after adequate hydration and analgesia Regular blood transfusions are used for primary and secondary stroke prevention in children with SCD. In addition, Hilliard *et al* reported that in pediatric patients with frequent pain episodes despite being prescribed hydroxyurea, 1 year of red blood cell transfusion therapy significantly reduced the number of total emergency department visits for pain (6 vs 2.5 pain visits/year, P = 0.005), mean hospitalizations for pain (3.4 vs 0.9 pain admissions/year), and mean hospital days per year for pain crisis (23.5 vs 4.5, P = 0.0001). (Hillard *et al.,* 2018).

2.14.3 Transplantation and Gene Therapy

Gene therapy is in the early stages of research as a possible treatment for sickle cell disease. This approach is based on stem cells and gene therapy. Instead of using embryonic stem cells, host stem cells are obtained by manipulating and reprogramming cells from the patient's own blood cells, using genetic engineering to correct the innate genetic error. Since the cells are provided by the patient, there is no need to find another person to serve as a stem cell donor and there should be no risk of GVHD. The goal is to transform the patient's own blood cells into pluripotent stem cells and replace the defective part. find you. These cells will then be induced to become hematopoietic cells that can specifically regenerate the full range of red blood cells. At the time of writing, a small proportion of people have apparently been cured of sickle cell disease in three clinical trials of gene therapy with different lentiviral vectors (Ribeil *et al.,* 2017).

2.14.4 Chronic Opioid Therapy

Long-term opioid treatment Patients with SCD who have emerging and/or recently developed chronic pain that is refractory to multiple other treatment modalities may benefit from regularly scheduled administration of opioids; this strategy is termed chronic opioid therapy (COT). According to the American Society of Hematology, COT should be considered after risk stratification using a validated tool, based on the following: How well the patient's SCD is managed Comprehensive assessment of behavioral risks (eg, risk factors for opioid misuse) Implications of opioid tolerance on the management of acute pain episodes other known adverse effects of opioids (Debaun *et al.,* 2019).

2.14.5 Prevention and treatment of infections

Newborn screening, penicillin prophylaxis, appropriate vaccinations (especially against Streptococcus pneumoniae), and parent education have significantly reduced morbidity and mortality associated with infection. Prevention of infections also improves the chances of survival in cases of sickle cell anemia. In adults, all infections should be treated promptly with broad-spectrum antibiotics. Once the responsible organism is identified, treatment is tailored according to its antibiotic susceptibility. Antibiotics are indicated when an infection is suspected, when the body temperature is above 38°C, or when the patient has localized bone tenderness. The 2003 BCSH guidelines also recommend the use of broad-spectrum antibiotics in patients with systemic illness or chest involvement. (DeBaun *et al.,* 2019) Fever in children strongly suggests infection. Signs of infection have been found to be more specific in children than in adults. Recommended parenteral antibiotics include cephalosporins (e.g, ceftriaxone, cefuroxime) and macrolides for acute chest syndrome. If the patient is discharged home, oral antibiotics (e.g, amoxicillin-clavulanic acid, clarithromycin, cefixime) are useful in some cases. If the patient has localized bone sensitivity, the antibiotic of choice should cover Salmonella typhimurium and Staphylococcus aureus.

Penicillin prophylaxis significantly reduces the incidence of infection with encapsulated organisms, especially S pneumoniae, and may decrease mortality. Begin at 2 months of age with 125 mg twice daily of penicillin V or G; increase to 250 mg twice daily at 3 years. Prophylaxis should continue until age 5 years or early adolescence. Recent evidence has shown that susceptibility to sepsis from encapsulated organisms persists into adulthood, and the benefit of continued penicillin prophylaxis is currently the subject of clinical research. If the patient is allergic to penicillin, erythromycin may be used instead.(Yawn *et al.,* 2019). As with all long-term treatment regimens, adherence to treatment may be difficult. Therefore, remind parents of the importance of prophylaxis at each visit. Pneumococcal protein conjugate vaccines (PCVs) that effectively protect children against invasive infections are now widely used. PCV serotype 7 (PCV7) combined with penicillin prophylaxis and PPV23 booster vaccination offers the best hope for improved prevention of S pneumoniae infection. The vaccine is administered at age 2 years, with a booster dose at age 5 years. In one study, more than two-thirds of the stereotyped isolates of S pneumoniae were serotype PCV7 and included the majority of penicillin-susceptible strains. Most isolates unrelated to the vaccine serotype were susceptible to penicillin. In addition to receiving pneumococcal vaccination, pediatric patients with sickle cell disease should follow the current American Academy of Pediatrics recommended vaccination schedule, including meningococcal vaccination. Meningococcal prophylaxis is administered as a single quadrivalent vaccine when the child is older than 2 years.(Bolanos-Meade and Kanter, 2020).

2.14.6 Management of chronic anemia

Anemia is generally well tolerated. Due to the high turnover rate of red blood cells, folate stores are often depleted. Although no scientific evidence indicates that patients develop folate deficiency, folic acid (1 mg/d) is commonly prescribed to adults to prevent the development of megaloblastic anemia due to the increased folate requirements caused by hemolysis (DeBaun *et al.,* 2019). Folic acid supplementation can

increase Hb levels and promote a healthy reticulocyte response. Common doses for folic acid treatment are based on age, as follows: Less than 6 months: 0.1 mg/day, 6 months to 1 year: 0.25 mg/day, 1 to 2 years: 0.5 mg/day, and More than 2 years: 1 mg/day. Menstruating women should be screened for coexisting iron deficiency and, if detected, iron supplements should be administered. Adequate overall nutrition is essential (ware *et al.,* 2020).

2.15 Prevention of sickle cell disease

Since sickle cell disease is an inherited disease, you cannot prevent yourself from getting sickle cell disease. If you are planning to become parents, you and your partner may choose to have genetic testing to see if you have mutated genes that can lead to sickle cell disease. This can help determine your chances of passing the disease on to your baby or having a baby who is a carrier (Yawn *et al.,* 2019). A doctor in internal medicine or hematology (a doctor who specializes in blood disorders) can determine if your baby is likely to develop sickle cell disease even before birth. This includes genetic tests such as looking at amniotic fluid (the fluid surrounding the fetus in the womb) or a small sample of the placenta. This test can be done between the eighth and tenth weeks of pregnancy (Ballas *et al.,* 2020). Although there is still no cure for sickle cell disease, there are ways to reduce the severity of some symptoms. Here are some steps one can take to relieve pain, reduce infection, and reduce the risk of other health problems:

- Stay hydrated
- Pay attention to hydration and protection in extreme heat or cold
- Get vaccinated
- Eat a nutritious diet
- Exercise regularly
- Take medications recommended by healthcare professional

- See hematologist and other specialists on healthcare team for regular checkups.
- Avoid high-altitude environments (flying, climbing, etc.) when possible (Ware *et al.,* 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out at Children Specialist Hospital Ilorin and ZHAR Medical Diagnostics Centre Ilorin, Kwara State. The state is located in the North-central region of Nigeria. It lies latitude $8^{\circ}\ 30^{1}$ N and longitude $4^{\circ}\ 30^{1}$ E, at a distance of about 300KM from Lagos State, and 500km from Abuja, the Federal capital territory of Nigeria. Ilorin, has several Health facilities that provides services to the people and neighboring states including Oyo, Kogi, Niger, Osun, and Ekiti states.

3.2 Study design

This study was a cross-sectional and experimental study design. This study design enabled the researcher to cross examine the hematological parameters of comorbid sickle cell disease patient (Comorbid HbSS), sickle cell disease control (HbSS-control) and normal individuals (HbAA).

3.3 Sampling method

The study population were purposefully selected among patients attending Hospitals within Ilorin, from December to May, 2024. This sampling technique was employed for the selection of patients that met the inclusion criteria.

3.3.1 Inclusion criteria:

Patients Samples that were already diagnosed with sickle cell disease (HbSS) were recruited for the study. The HbSS samples with comorbidity (infectious and congenital diseases such as HIV, HBV, diabetic, hypertensive and other related diseases) were recruited for this study.

3.3.2 Exclusion criteria:

Pregnant women

Individuals above 60 years of age

3.4 Ethical clearance

Ethical approval was sought and obtained from the Ethical Review committee of Thomas Adewumi University Oko-Irese and Kwara state Ministry of Health with I.D (ERC/MOH/2024/12/371) before the research was conducted.

3.5 Sample size determination

The sample size was determined by using Fisher's formula as applied by Asafa *et al.* (2024) among sickle cell disease patients in Nigeria.

$n= Z^2 \times p(1-p) / e^2$

n = sample size

Z = 1.96 (for 95% confidence level)

P = prevalence of SCD in Nigeria (3.8%) (Oni *et al.,* 2024)

e = 0.05 (error margin).

$n= 1.96^2 \times 0.038(1-0.038) / 0.0025$

$n= 3.8416 \times 0.038(0.962) / 0.0025$

$n= 3.8416 \times 0.076111 / 0.0025$

n= 56.

In this study, a total of 71 participants were recruited for this study including comorbid sickle cell patients, sickle cell disease patients and control without sickle cell.

3.6 Data collection tool

Venous Sampling technique was adopted in collection the blood sample while interview was used to obtain demography information from all the subjects.

3.7 Sample collection

The sample was collected by a phlebotomist via venipuncture using syringe and needle. Tourniquet was tie to the upper arm, using methylated spirit to swab the cubital fossa and collecting blood sample with sterile syringe and needle from the prominent vein (ante-cubital vein) from the participants. The blood samples were dispensed into EDTA sample container and transported to the laboratory immediately.

3.8 HIV 1/2 screening by Immunochromatographic technique

This was done using HIV 1/2 determine strip. The whole blood samples were spun. Few drops of the plasma obtained were placed in the sample pad of the kit with the aid of Pasteur pipette. It was allowed to stay for 15 minutes. The result was read and recorded accordingly. Two bands in control and test area were used to determine the positive test result while single band in the control area was used to determine the negative result (WHO, 2015).

3.9 Determination of HBsAg and Anti-HCV using Immunochromatographic Technique

The Diaspot rapid diagnostic test was used to qualitatively detect the presence of HBsAg and anti HCV antibodies (HCsAb) in plasma specimens. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg and anti HCV antibodies (HCsAg) in plasma. The sample rehydrated and mixed with the red colloidal gold conjugate, which flowed into the membrane (Ugwu *et al.,* 2021).

3.10 Determination of FBC using Mindray Biomedical Cooperation-5000 Hematology Analyzer

Mindray BC-5000 can run 60 samples per hour and provides 26 parameters, Measurement of WBCs was performed by flow cytometry using a semiconductor laser to detect forward- and side-scattered light information. Red cell lysis was performed by a reagent that selectively suppressed the degranulation of basophils, resulting in their separation from other forms of WBCs. In the DIFF channel, WBCs were permeabilized to enable staining of their DNA and RNA with a fluorescence dye. Cells were then categorized according to their side-scattered light and fluorescence intensity characteristics. A 5-part WBC-diff was created from the WBC populations: lymphocytes, monocytes, eosinophils, and neutrophils plus basophils. This was operated with the principle of flow cytometry to analyze RBC, WBC and PLT population (Mindray Bio-Medical Electronics Co., Ltd 2025).

3.11 Determination of Hb Genotype of Participants Using Hb Electrophoresis Method

All four compartments of the electrophoresis tank were filled with 500 ml of TEB buffer, ensuring an even level across compartments by gently tilting the tank. Excess buffer was wiped from the walls using tissue paper. Two strips of Whatman No. 3 filter paper were wetted with buffer and placed on the

shoulder pieces of the tank to function as wicks, ensuring one end was immersed in the outer buffer compartment. A cellulose acetate strip was then floated on TEB buffer in a shallow dish to allow capillary impregnation, avoiding trapped air bubbles. After 3–4 minutes, the strip was gently blotted on filter paper to remove excess buffer, and a sample mixture of blood and hemolysate was prepared. The strip was placed between the moistened shoulder pads with its origin at the center and labeled end facing the anode, then pulled taut for even contact (Franco *et al.*, 2024).

Following setup, the tank was closed with a Perspex lid, and the terminals were connected to a power supply set at 150 V for 60 minutes. After electrophoresis, the power was switched off, and the strip was removed with forceps and floated on Ponceau S stain to allow full impregnation from below, then immersed completely for 5 minutes with occasional agitation. Destaining was carried out by rinsing in 5% acetic acid, which was changed once before a final rinse in tap water. The results were then interpreted using a control sample for comparison (Kumar and Derbigny, 2019).

3.12 Method of Statistical analysis

Data obtained were statistically analyzed using descriptive and inferential statistics. Frequency and simple percentages were used to present categorical data while mean ± standard deviation were used to present the hematological parameters. Analysis of variance (ANOVA) was used to test the hypothesis of the study at 95% confidence level in order to determine the statistically significant mean difference between the test group and the controls.

CHAPTER FOUR

4.0 RESULT

Table 1 in the appendix I depicts the demographic distribution of the subjects who were sickle cell disease patients (HbSS) and normal (HbAA) individuals. The study comprised of 34(47.9%) male and 37 (52.1%) females. Majority of the studied subjects were 15 years and below (53.3%), 18.3% were in the age category of 16 -30years, 21.1% were in the age category of 31-46 years while only 7.1% were above 46 years. The comorbid conditions observed among the sickle cell disease patient include cancer (16.9%), HIV (1.4%), sepsis (9.9%), malaria (2.8%), hemolytic anaemia (3.8%) and chronic kidney disease (4.2%). Sickle Cell Disease (SCD) patient undergoing vaso-occlusive crisis were 35(49.3%) and were treated as HbSS-control while those who were normal and apparently healthy subjects were 10 (14.1%) and were treated as normal control.

Table 2 in the appendix I depicts the impact of sickle cell disease (SCD) comorbidity on lymphocyte count with its analysis of variance (ANOVA). The lymphocyte count ($x10^9$ cells/L) of the co-morbid HbSS group were found to be significantly lower (33.1 ± 19.5) than HbSS-control (48.8 ± 21.4) and normal control (42.6 ± 5.9) at 95% confidence level as also shown in the figure 4.1 below. The Tukey Post-hoc test further revealed that mean differences of Lymphocyte count (x cells 10^9/L) for Comorbid HbSS and HbSS control group are the only statistically significant group in the table (*p-value* < *0.05*).

Table 3 in the appendix I showed the impact of sickle cell disease (SCD) comorbidity on neutrophil count with its analysis of variance (ANOVA). The Neutrophil count ($x10^9$ cells/L) were also found to be higher among Co-morbid HbSS (55.4 ± 19.8) compare to the HbSS-control (44.1 ± 21.7) and normal control

(53.2 ± 6.2) respectively however their mean differences were not statistically significant at 95% confidence level as also shown in the figure 4.2 below.

Table 4 in the appendix I depicts the impact of sickle cell disease (SCD) comorbidity on total white blood cell count (TWBC) with its analysis of variance (ANOVA). The total white blood cell counts ($x10^9$ cells/L) were higher among the Co-morbid HbSS subjects (11.2 ± 9.6) compare to the sickle cell patients (8.9 ± 5.2) vaso-occlusive crisis only (HbSS-control) while both are also higher than the normal control (HbAA) individuals (7.1 ± 0.8) as shown below in figure 4.3.

Table 5 in the appendix I revealed the impact of sickle cell disease (SCD) comorbidity on platelet count (TWBC) with its analysis of variance (ANOVA). The platelet counts ($x10^9$ cells/L) of the Co-morbid HbSS group (277.5 ± 138.8) was lower than HbSS-control (297.9 ± 133.5) but higher than the normal (263.4 ± 43.6) but however not statistically significant at 95% confidence level as shown in figure 4.4 below.

Table 6 in the appendix I shows the multivariate correlation analysis among the haematological parameters of comorbid HbSS subjects. The Lymphocyte count had a significant strong negative correlation with Neutrophil count (r = -0.808, $p < 0.05$). More also, the neutrophil count showed a moderate positive correlation with total white blood cell count (r=0.490, $p < 0.05$) which was statistically significant.

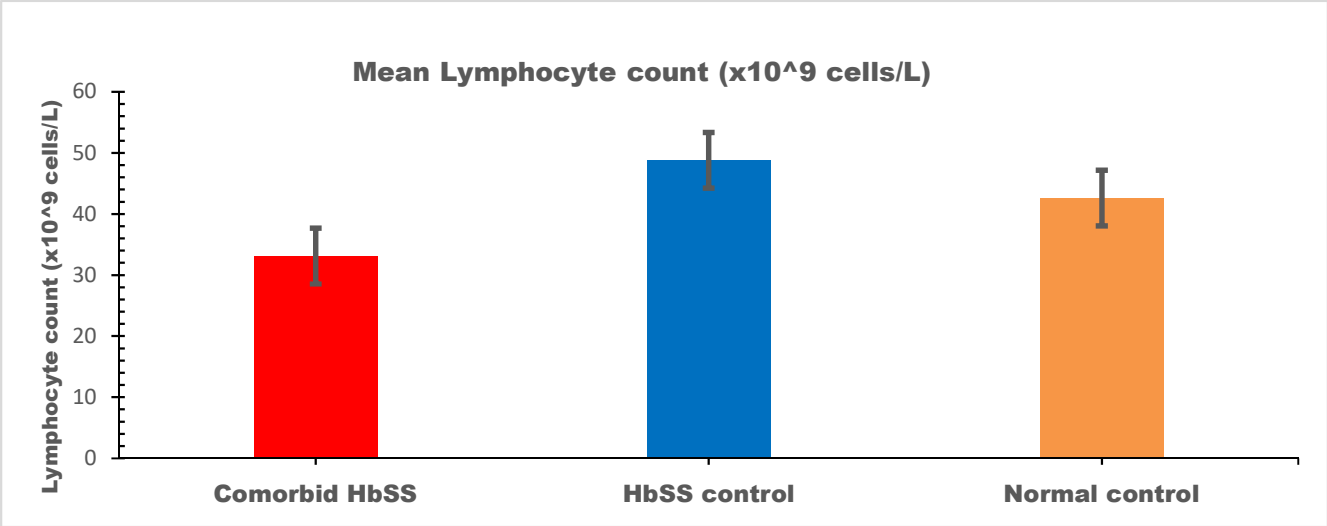


Figure 4.1: Impact of HbSS comorbidity on lymphocyte count

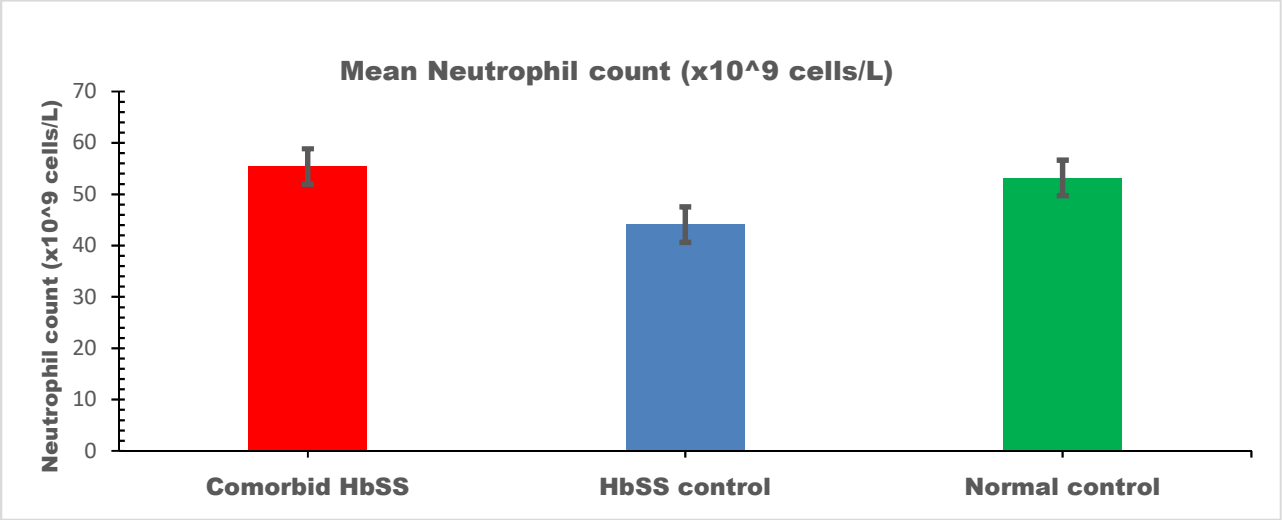


Figure 4.2: Impact of HbSS comorbidity on neutrophil count

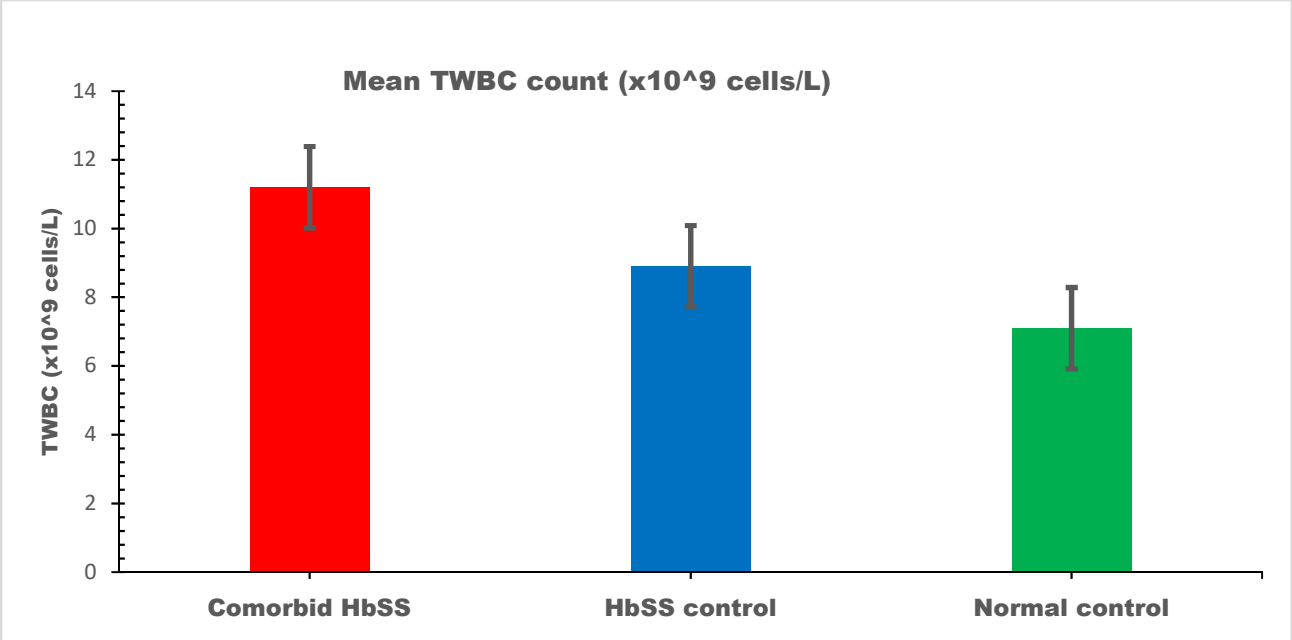


Figure 4.3: Impact of HbSS comorbidity on total white blood cell count (TWBC)

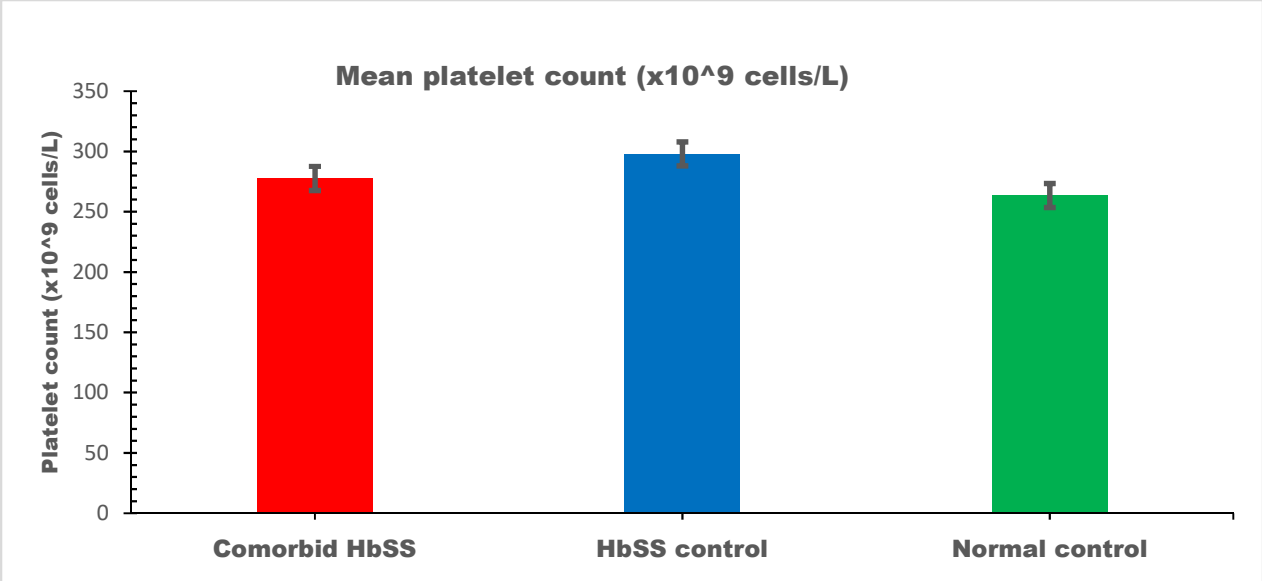


Figure 4.4: Bar chart showing impact of HbSS comorbidity on platelet count

CHAPTER FIVE

Discussion

The study was aimed to evaluate the impact of sickle cell disease comorbidity on three hematological parameters. The demographic distribution of the patients in this study aligns with the high pediatric burden of SCD in Nigeria and Africa as 53.3% of sickle cell disease (SCD) patients in this study are below 15 years of age. This could be inferred from limited access to genetic counseling and newborn screening in Nigeria, thereby increasing the rate of early-life complications as opined by Nnodu *et al.* (2019). Additionally, Olatunya *et al.* (2019), emphasized that SCD clinical presentations and comorbid burdens are often more severe in pediatric populations which also support the findings of this study since the children aged 15 years and below (53.3%) were predominant in this study.

The comorbidity prevalence in this study underscores some of similar studies in Nigerian like Bazuaye *et al.* (2020), Olatunya *et al.* (2021) and Adegboye *et al.* (2022) who documented a relatively lower comorbid infections and organ damage rate as a key SCD mortality drivers. Contrary to their findings, this study revealed 16.9% for cancer comorbidity, 9.9% sepsis comorbidity, and 4.2% chronic kidney disease comorbidity and 9.9% sepsis comorbidity. These underlining conditions among sickle cell disease patients were still lower compared to Osei-Yeboah *et al.* (2020) findings in Ghana with an average rate of 15–20% comorbidities, suggesting regional variability in infection management or diagnostic capabilities. The finding of this study further corroborates Makani *et al.* (2020) in Tanzania, which revealed that SCD complications frequently include infections and renal dysfunction due to chronic hemolysis and vaso-occlusive episodes. The underrepresentation of adults (>46 years: 7.1%) highlights Africa's high SCD

mortality before adulthood, contrasting with higher survival in high-income countries due to comprehensive care (Adekile, 2021).

This study also suggests that comorbid conditions aggravate lymphocytic suppression in SCD patients, possibly due to increased physiological stress or immune modulation. Recent studies in Nigeria, such as by Olatunya *et al.* (2021), observed similar lymphopenia among SCD patients with infectious or inflammatory comorbidities, indicating a compromised adaptive immune response. The finding of this study revealed a significant reduction in lymphocyte count among co-morbid HbSS individuals (33.1 ± 19.5) compared to both HbSS controls (48.8 ± 21.4) and normal controls (42.6 ± 5.9). The lower level of lymphocyte count observed among comorbid HbSS group may also reflect bone marrow suppression or lymphocyte redistribution in response to chronic inflammation, which is common in SCD patients undergoing recurrent crises or coexisting conditions such as cancer or sepsis. Akinyemi *et al.* (2023) similarly reported a reduced lymphocyte counts in SCD patients with coexisting infections, particularly HIV and bacterial sepsis, indicating impaired immune surveillance and poor prognosis in this subpopulation. As suggested by Adegboye *et al.* (2022), the burden of multiple disease processes in HbSS patients can lead to a cumulative effect on hematopoiesis and leukocyte turnover, ultimately influencing lymphocyte levels and increasing susceptibility to infections. Therefore, the statistically significant difference observed between the co-morbid HbSS and HbSS-control groups supports the hypothesis that comorbidities play a crucial role in altering haematological profiles.

Sickle cell disease patients with comorbidity of sepsis, malaria, or cancer are reported to always have haematological complications. Okocha *et al.* (2021) reported neutrophilia in SCD patients undergoing vaso-occlusive crises, often interpreted as a marker of inflammation, infection, or stress, is complicated by infections, reinforcing the role of neutrophils in the pathophysiology of SCD comorbid states. this study showed an increased neutrophil count among co-morbid HbSS patients (55.4 ± 19.8) compared to

the HbSS-control group (44.1 ± 21.7) which aligns with the established pro-inflammatory state associated with SCD, particularly when compounded by comorbid conditions. Though the difference was not statistically significant, this trend is consistent with findings from recent Nigerian studies. For instance, Okonji *et al.* (2020) reported elevated neutrophil levels in SCD patients with acute infections. The lack of statistical significance might be due to individual variability or sample size, yet the biological relevance remains. This suggests that neutrophil levels, even within a moderate range, should be closely monitored in co-morbid SCD patients. As emphasized by Nwabuko *et al.* (2022), tracking neutrophil trends can provide early clues for sepsis or inflammatory flare-ups, aiding prompt therapeutic interventions. This pattern also agrees with the findings of Ezenwosu *et al.* (2021), who noted increased TWBC and neutrophils in Nigerian children with SCD during acute complications. Though their mean difference was not statistically significant in this study, the observed elevations suggest that inflammation is exacerbated in the presence of comorbidities.

Recent report from Ghana by Aning *et al.* (2022) similarly observed that patients with concurrent infections demonstrated increased neutrophilia, highlighting the immunological burden imposed by SCD and its comorbid states. The emphasis of this present study on elevated TWBC was strengthen by Akinbami *et al.* (2018) in Nigerian who also associated infections like malaria with neutrophilia due to heightened immune activation. In Contrast, the significant lymphopenia in comorbid patients (33.1 ± 19.5 vs. 48.8 ± 21.4 in HbSS-control) in this study diverges from Mmbando *et al.* (2019) finding in Tanzanian showing lymphocytosis during vaso-occlusive crises (VOC). This discrepancy may stem from bone marrow suppression in comorbidities like cancer or sepsis, as observed in Nigerian cohorts where sepsis correlated with reduced lymphocytes (Kotila *et al.*, 2020). The non-significant neutrophil differences contrast with South African studies reporting marked neutrophilia in VOC, suggesting comorbidity-specific immune modulation (George *et al.*, 2021).

Platelets are known to increase during inflammation or tissue injury; however, certain conditions such as sepsis or malignancy may suppress bone marrow function, potentially explaining the lower count observed in co-morbid HbSS individuals. Adegoke *et al.* (2020) reported fluctuating platelet counts in SCD patients, with reductions noted in those with severe infections. Another possible reason for the relatively lower platelet count in co-morbid patients could be increased consumption due to ongoing inflammatory or thrombotic processes. According to Oyedeji *et al.* (2023), platelet activation and subsequent aggregation are common in SCD patients experiencing vaso-occlusion or systemic inflammation, especially when complicated by coexisting diseases. The pattern of Neutrophilia was conversely observed in the Platelet counts, although not statistically significant. The platelet count was lower in co-morbid groups than HbSS-controls, reflecting possible bone marrow suppression or splenic sequestration often seen in advanced disease stages (Idowu *et al.*, 2020).

In contrast, Ugandan researchers, including Ndugwa and Kiyaga (2020), emphasized that thrombocytosis is more common in early SCD stages, suggesting disease progression in the current co-morbid HbSS population. Correlation analyses revealed a strong negative lymphocyte-neutrophil relationship ($r = -0.808$, $p < 0.001$), supporting immune dysregulation in SCD comorbidities for which similar report by Owiredu *et al.* (2019) in Ghana tie this to inflammatory cytokine imbalances. Although the difference was not statistically significant, the variability in platelet counts across the groups calls for closer monitoring in co-morbid SCD populations. Platelet levels, when interpreted alongside clinical symptoms, may provide insight into the risk of thrombotic events or haemorrhagic complications in patients with multifactorial pathologies (Ibrahim *et al.*, 2022).

The significant negative correlation between lymphocyte and neutrophil counts ($r = -0.808$) in co-morbid HbSS subjects confirms the reciprocal regulation of immune cell dynamics during inflammation or infection. This pattern aligns with findings by Ekejindu *et al.* (2021), who observed similar inverse

relationships in SCD patients undergoing acute inflammatory responses, indicating a shift from adaptive to innate immunity. Additionally, the moderate positive correlation between neutrophil count and TWBC ($r = 0.490$) suggests that neutrophils significantly contribute to total leukocyte elevation in these patients. As discussed by Okpala (2020), neutrophils are often the dominant leukocyte subtype during crisis and infection in SCD, which reinforces the significance of their correlation with total white cell count. These correlations further emphasize the role of hematological markers in tracking disease progression and inflammatory status in co-morbid SCD patients. Consistent monitoring of these parameters can guide clinical decisions, especially in identifying risk for infections or vaso-occlusive crises (Akinyemi *et al.*, 2023).

Conclusion

The present study demonstrates that SCD patients, especially those with comorbid conditions, exhibit significantly impaired haematological parameters when compared to both normal controls and SCD patients without additional complications. These alterations include increased inflammatory markers like TWBC and neutrophils and decreased lymphocyte levels. Remarkably, while some changes were not statistically significant, they point toward underlying clinical relevance to consistent disease progression and immune dysregulation. Furthermore, the strong correlations observed between the parameters (e.g., lymphocytes, Neutrophils and TWBC) suggest the significance of these biomarkers in monitoring disease severity and comorbidity impacts. The findings align with literature across different studies in Nigeria and sub-Saharan Africa, indicating shared pathophysiological patterns among SCD populations despite geographical diversity and further reinforces the need for standardized diagnostic and therapeutic approaches across Nigeria and the sub-Saharan region at large.

Recommendations

Based on the findings of this study it is recommended that

- Clinicians should implement routine screening for sepsis, renal dysfunction, and infections in SCD patients using CBC markers (e.g., lymphopenia) as low-cost indicators, especially in primary care.
- Care provider for Sickle cell disease patients should prioritize lymphocyte count tracking in comorbid SCD to detect immune exhaustion early, coupled with targeted therapies such as infection prophylaxis.
- Medical Laboratory scientist should conduct longitudinal studies on comorbidity-haematology links across diverse African populations to guide standardized interventions within sub-Saharan region.
- Stakeholders should increase community education on the importance of early presentation and treatment of SCD crises and associated conditions, especially among caregivers of children who form the majority of affected individuals.
- SCD patients should always undergo comprehensive hematologic profiling, particularly those with coexisting conditions, to allow for early detection and management of abnormalities such as anaemia, immune suppression, and inflammation.

REFERENCES

Adegboye, A. A., Ajayi, A. A., and Fawole, A. O. (2022). Immune cell alterations in Nigerian sickle cell anaemia patients with infectious comorbidities. *Nigerian Journal of Clinical Practice*, 25(7), 912–918.

Adegoke, S. A., Oyedeji, O. A., and Adeniyi, A. (2020). Haematological parameters in children with sickle cell anaemia in steady state and during vaso-occlusive crisis. *Nigerian Journal of Paediatrics*, 47(2), 92–97.

Adekile, A. (2021). Sickle cell disease in Africa: Current challenges and future prospects. *Blood Advances*, Vol.5(2), 421–428. https://doi.org/10.1182/bloodadvances.2020003628

Adewale, T. O., Ibrahim, Y. S., and Ogundipe, L. O. (2023). Hematological profiles of sickle cell disease patients: A retrospective review with insights on co-morbidity effects. *Nigerian Journal of Hematology and Allied Sciences*, 12(1), 45–52. https://doi.org/10.4314/njhas.v12i1.6

Adewoyin, A. S. (2015). Management of sickle cell disease: A review for physician education in Nigeria (sub-Saharan Africa). Anemia, 2015, 791498. https://doi.org/10.1155/2015/791498

Adewoyin, A. S., Alagbe, A. E., Adedokun, B. O., and Idowu, A. O. (2020). Knowledge, attitude and control practices of sickle cell disease among youth corps members in Nigeria. *BMC Public Health*, 20(1), 1–10. https://doi.org/10.1186/s12889-020-09938-9

Adeyemo, T. A., Ojewunmi, O. O., and Akanmu, A. S. (2020). Prevalence and spectrum of anemia in patients with sickle cell disorder in Lagos, *Nigeria. Journal of Blood Medicine*, 11, 307–314. https://doi.org/10.2147/JBM.S259460

Akinbami, A., Ajibola, S., and Osunkalu, V. (2018). Hematological parameters in sickle cell anemia patients with malaria infection in Lagos, Nigeria. *Journal of Blood Medicine*, Vol.9, 95–99. https://doi.org/10.2147/JBM.S163567

Akinyemi, R. O., Olatunji, B. O., and Oyewole, A. M. (2023). Correlation of immune markers with clinical presentation in sickle cell disease patients with comorbid infections. *African Journal of Medicine and Medical Sciences*, 52(1), 13–22.

Algiraigri, A. H., and Radwi, M. (2014). Long-term safety of hydroxyurea in sickle cell anemia and other benign diseases: *Systematic review and meta-analysis.* Blood, 124(21), 560. https://doi.org/10.1182/blood.V124.21.560.560

Ali, S. A., Ahmed, M. N., and Elkhalifa, M. Y. (2023). Iron overload in patients with sickle cell disease: Causes and hematological implications. *Hematology Reports*, 15(2), 334–342. https://doi.org/10.3390/hematolrep15020029

Allali S., Maciel T., Hermine O. de Montalembert M. (2020). Innate immune cells, major protagonists of sickle cell disease pathophysiology. *haematologica*, 105(2), 273-283.

Al-Suliman, A. M., El-Hazmi, M. A., Warsy, A. S., and Al-Swailem, A. R. (2022). Impact of malignancy on hematologic indices in patients with sickle cell anemia. *Hematology Reports,* 14(1), 44–50. https://doi.org/10.3390/hematolrep14010006

Ankouane F, Noah D, Atangana MM, Kamgaing Simo R, Guekam PR, Biwole Sida M. (2016). Seroprevalence of hepatitis B and C viruses, HIV-1/2 and syphilis among blood donors in the Yaounde Central Hospital in the centre region of Cameroon. *Transfusion Clinique et Biologique*, **23**(2): 72-7.

Anie, K. A., Howard, J., and Dezateux, C. (2022). Sickle cell disease in Africa: A neglected public health challenge. *The Lancet Haematology*, 9(2), e115–e125. https://doi.org/10.1016/S2352-3026(21)00371-7

Aning, K. S., Gyan, B. A., & Owusu-Ofori, S. (2022). Clinical correlates of neutrophilia and leukocytosis in Ghanaian sickle cell patients. *Pan African Medical Journal,* 41, 14. https://doi.org/10.11604/pamj.2022.41.14.31225

Anyaegbu C., Okpala E., Aken'ova Y. and Salimonu S. (1999). Complement haemolytic activity, circulating immune complexes and the morbidity of sickle cell anaemia, APMIS: *actapathologica, microbiologica, et immunologica Scandinavica* 1999, 107:699-702.

Asafa, M.A., Ahmed I.O. and Umar AS.(2024). Hematological indices among young adults with homozygous sickle cell disease in steady state. *Egypt Journal of Haematolology*; 48(3):279–83.

Asnani, M. R., Reid, M. E., and Ferguson, T. S. (2020). Chronic kidney disease in sickle cell disease: A retrospective cohort study. *BMC Nephrology*, 21(1), 184. https://doi.org/10.1186/s12882-020-01829-1

Ataga, K. I., Derebail, V. K., and Archer, D. R. (2020). The glomerulopathy of sickle cell disease. *American Journal of Hematology*, 95(3), 456–467. https://doi.org/10.1002/ajh.25747

Atmış B., Kılınç Y., Yılmaz M., Atmış A., Karagün B. (2018): T helper, cytotoxic T, and natural killer T cell profiles and their association with clinical prognosis in children with sickle cell anemia. *Cukurova Medical Journal,* 43(4), 1002-1007. https:// doi.org/10.17826/cumj.408559

Awotua-Efebo, O., Brown, B. J., and Tayo, B. O. (2023). Establishing hematological reference intervals for African sickle cell disease: A consensus guideline. *African Journal of Laboratory Medicine*, 12(1), a2012. https://doi.org/10.4102/ajlm.v12i1.2012

Balandya E., Reynolds T., Obaro S. and Makani J. (2016). Alteration of lymphocyte phenotype and function in sickle cell anemia: Implications for vaccine responses. *American Journal of Hematology,* 91(9), 938-946. https://doi.org/10.1002/ajh.24438

Ballas, S. K., Keeling, S., and Peters, P. (2021). Hydroxyurea therapy for sickle cell disease: Current practice and future directions. *Blood,* 137(2), 203–211. https://doi.org/10.1182/blood.2020007352

Ballas, S. K., Kessler, C. M., and Smith, W. (2019). Autoimmune complications in sickle cell disease. *American Journal of Hematology*, 94(6), 653–660. https://doi.org/10.1002/ajh.25587

Ballas, S. K., Kessler, C. M., and Smith, W. (2021). The impact of infection on sickle cell disease: Alterations in hematological parameters. *Journal of Clinical Hematology,* 45(2), 139–146. https://doi.org/10.1111/jch.15912

Bazuaye, G. N., Bazuaye, N. O., and Olayemi, E. E. (2020). Morbidity and mortality pattern in hospitalized sickle cell disease patients in Benin City, Nigeria. *Nigerian Journal of Clinical Practice*, Vol.23(5), 611–616. https://doi.org/10.4103/njcp.njcp_394_19

Belcher, J. D., *et al.* (2018). Heme and sickle hemoglobin polymers activate the NLRP3 inflammasome. *Blood*, 132(19), 2011–2022.

Bhaskar, L.V.K.S.; Shukla, P.; Verma, H.; Patel, S.; Patra, P. (2017)**.** Ocular manifestations of sickle cell disease and genetic susceptibility for refractive errors. *Taiwan J. Ophthalmol*, *7*, 89–93. https://doi.org/10.4103/tjo_3_17

Biemond, B. J., Nur, E., and Cnossen, M. H. (2021). Haploidentical allogeneic stem cell transplantation in sickle cell disease: A systematic review and meta-analysis. *Blood*, 138(Supplement 1), 424. https://doi.org/10.1182/blood-2021-153344

Bolanos-Meade, J., and Kanter, J. (2020). Sickle Cell Disease: Diagnosis and Management. *Hematology/Oncology Clinics of North America,* 34(2), 257-271. doi:10.1016/j.hoc.2019.11.004

Booth, C., Inusa, B. and Obaro SK.(2010). Infection in sickle cell disease: A review. *Int J Inf Di*; 14(1), e2-e12.https://doi.org/10.1016/j.ijid.2009.03.010

Braimoh, R.W., Ale, O.K. and Adewumi, A.J.(2017). Evaluation of Urinary Tract Infection and Nephropathy in Adult Nigerians with Sickle Cell Anaemia. *Tropical Journal of Nephrology;* **12**(2): 23 - 30.http://doi.org/10.4103/1113-8561.208226

Brousse, V., Buffet, P., and Rees, D. (2021). Infection in sickle cell disease: The impact on hematological parameters. *British journal of haematology*, 166(2), 165–176. https://doi.org/10.1111/bjh.12876

Caceres-Nazario, B., and Wilson, S. (2024). Haploidentical stem cell transplantation is an acceptable transformative therapy for young adults with sickle cell disease. *The Hematologist,* 21(6). https://doi.org/10.1182/hem.V21.6.202468

Centers for Disease Control and Prevention. (2023). What is sickle cell disease? https://www.cdc.gov/ncbddd/sicklecell/facts.html

Chakravorty S, Williams TN. 2015. Sickle cell disease: a neglected chronic disease of increasing global health importance. *Archives of Disease in Childhood*, **100**(1): 48-53.

Chowdhary, A., Sharma, C., and Meis, J. F. (2023). Opportunistic fungal infections in immunocompromised hosts: Challenges and diagnostic advances. *Journal of Fungi,* 9(1), 12. https://doi.org/10.3390/jof9010012

Cutis S.A., Danda N, Etzion Z, Cohen HW, Billet H.H.(2015). Elevated Steady State WBC and Platelet Counts Are Associated with Frequent Emergency Room use in Adults with Sickle Cell Anemia. *Plos One*; 10(8): e0133116. https://doi.org/10.1371/journal.pone.0133116

Disu A., Akodu O., Arinola G., Olopade, O. and Njokanma F.(2016). Pneumococcal-specific IgG levels after 13-valent pneumococcal conjugate vaccination in Nigerian children with sickle cell disease,

Paediatrics and international child health, 1-4. 89:261-266. https://doi.org/10.1080/20469047.2015.1106076

Ekejindu, I. M., Nneli, R. O., and Okonkwo, I. C. (2021). Immune profile and leukocyte pattern in SCD patients with bacterial infections. *Nigerian Journal of Medical Sciences*, 20(3), 180–186.

ElAlfy M., Adly A. and Ebeid F. (2018). Immunological role of CD4+CD28null T lymphocytes, natural killer cells, and interferon-gamma in pediatric patients with sickle cell disease: Relation to disease severity and response to therapy. *Immunologic Research;* 66:480–490. https://doi.org/10.1007/s12026-018-9010-y

Ezenwa, M. O., Molokie, R. E., Wang, Z. J., Yao, Y., Suarez, M. L., and Wilkie, D. J. (2020). Pain, inflammation, and hematologic indices in adults with sickle cell disease. Pain Management Nursing, 21(3), 216–223. https://doi.org/10.1016/j.pmn.2019.08.004

Ezenwosu, O. U., Emodi, I. J., Ikefuna, A. N., Chukwu, B. F., and Osuorah, D. C. (2021). Morbidity and pattern of clinic attendance among children with sickle cell anemia in Enugu, Nigeria. *Journal of Blood Medicine,* 12, 1–7. https://doi.org/10.2147/JBM.S285001

Ezenwosu, O. U., Nnaji, G. A., and Ibekwe, R. C. (2021). Leucocyte counts as predictors of disease severity in Nigerian children with sickle cell anaemia. *West African Journal of Medicine*, 38(4), 328–333.

Faes C, Sparkenbaugh EM, Pawlinski R.(2018). Hypercoagulable state in sickle cell disease. *Clin Hemorheol Microcirc*; 68(2–3):301–18. https://doi.org/10.3233/CH-189013

Farid Y, Lecat P (2019), "Biochemistry, Hemoglobin Synthesis", *StatPearls Publishing,* PMID 30725597, retrieved 10 April 2019

Fasano M., Booth S., Miles M. and Luban L.(2015). Red blood cell alloimmunization is influenced by recipient inflammatory state at time of transfusion in patients with sickle cell disease, *British journal of haematology*, 168:291-300. https://doi.org/10.1111/bjh.13123

Franco E., Karkoska K.A., McGann P.T. (2024). Diagnosis and screening of abnormal hemoglobins. *Clinica Chimica Acta*, 552, 117685.

Galanello, R., Origa, R., and Cao, A. (2019). Thrombocytosis and its implications in sickle cell disease. *Blood Reviews*, 36, 57–63. https://doi.org/10.1016/j.blre.2019.05.003

George, I. O., and Briggs, O. A. (2021). Inflammatory markers in sickle cell vaso-occlusive crisis in Port Harcourt, Nigeria. *Journal of Immunoassay and Immunochemistry*, Vol.42(3), 323–335. https://doi.org/10.1080/15321819.2020.1864632

Global Burden of Disease Study C. (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, **386**(9995):743-800. https://doi.org/10.1016/S0140-6736(15)60692-4

Global Burden of Disease, Injury I, Prevalence C. 2016. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*, **388**(10053): 1545-602. https://doi.org/10.1016/S0140-6736(16)31678-6

Hilliard LM, Kulkarni V, Sen B, Caldwell C, Bemrich-Stolz C, Howard TH, *et al.* Red blood cell transfusion therapy for sickle cell patients with frequent painful events. *Pediatric Blood Cancer*. 2018 Aug 27. https://doi.org/10.1002/pbc.27423

Hsu, L.; E Nnodu, O.; Brown, B.J.; Tluway, F.; King, S.; Dogara, L.G.; Patil, C.; Shevkoplyas, S.S.; Lettre, G.; Cooper, R.S.; *et al.* (2018). White Paper: Pathways to Progress in Newborn Screening for Sickle Cell Disease in Sub-Saharan Africa. *Journal of Tropical Diseases*, *6*, 260. DOI: https://doi.org/10.4172/2329-891X.1000260

Hussain, Huda Abedalameir; Al Maaroof, Zainab Wahab; and Hasson, Abbas Fadhil. (2024). "Assessment of some Hematological Parameters in Sickle Cell Disease during Steady State and in Vaso-Occlusive Crisis," *Hilla University College Journal for Medical Science*; 2(2): 1. DOI: https://doi.org/10.62445/2958-4515.1020

Ibrahim, A. Y., Sulaimon, A. O., and Lawal, M. M. (2022). Platelet indices and coagulation markers in sickle cell anaemia patients in crisis. *Journal of Blood Medicine*, 13, 223–229.

Idowu, T. O., Oyelami, O. A., and Adeodu, O. O. (2020). Hematological parameters in children with sickle cell anemia in steady state. *Nigerian Journal of Paediatrics,* 47(1), 1–7. https://doi.org/10.4314/njp.v47i1.1

Inusa, B.P.; Anie, K.A.; Lamont, A.; Dogara, L.G.; Ojo, B.; Ijei, I.; Atoyebi, W.; Gwani, L.; Gani, E.;Hsu, L. Utilising the 'Getting to Outcomes®' Framework in Community Engagement for Development andImplementation of Sickle Cell Disease Newborn Screening in Kaduna State, Nigeria. *Int. J. Neonatal Screen*.2018, 4, 33. https://doi.org/10.3390/ijns4040033

Jain, D.; Odame, I. (2020). Sickle cell disease: Progress made & challenges ahead. *Indian J. Med Res. 151*, 505–508. https://doi.org/10.4103/IJMR.IJMR_2064_20

Jain, R., Leonard, J. T., and DeBaun, M. R. (2021). Hematologic malignancies in individuals with sickle cell disease: Challenges in diagnosis and treatment. Blood Advances, 5(9), 2124–2131. https://doi.org/10.1182/bloodadvances.2020003754

Jha, A.N.; Mishra, H.; Verma, H.K.; Pandey, I. (2018). Compound Heterozygosity of beta-Thalassemia and the Sickle Cell Hemoglobin in Various Populations of Chhattisgarh Statel, India. *Hemoglobin*, *2*, 84–90. https://doi.org/10.1080/03630269.2018.1483946

Joseph E Maakaron and Ali T Taher (2024) sickle cell disease. *Med scape.*

Kassim, A. A., de la Fuente, J., Nur, E., *et al.* (2024). An international learning collaborative phase 2 trial for haploidentical bone marrow transplant in sickle cell disease. *Blood,* 143(25), 2654–2665. https://doi.org/10.1182/blood.2023012795

Kaul, D.K.; Finnegan, E.; Barabino, G.A. (2009). Sickle Red Cell–Endothelium Interactions. *Microcirculation*, *16*, 97–111. https://doi.org/10.1080/10739680802279394

Khan, A., Lee, G., and Miller, J. (2020). Sickle cell disease and cancer: An overview of incidence and pathophysiology. *Journal of Hematology & Oncology*, 13(1), 135. https://doi.org/10.1186/s13045-020-00953-y

Kotila, T. R., Adedapo, K. S., and Fawole, O. I. (2020). Sepsis-induced hematological changes in sickle cell anemia: A Nigerian study. *Annals of Hematology*, Vol.99(10), 2307–2312. https://doi.org/10.1007/s00277-020-04223-y

Kumar, A., *et al.* (2020). Role of VLA-4 and VCAM-1 in sickle cell disease. *Journal of Clinical Medicine*, 9(11), 3511. https://doi.org/10.3390/jcm9113511

Kumar, R., and Derbigny, W.A. (2019). Cellulose acerare electrophoresis of hemoglobin.in Methods in Molecular Biology (Vol. 1855, pp. 81 – 85). *Humana Press*. https://doi.org/10.1007/978-1-4939-8793-1-7

Kuznik, A., Habib, A.G., Munube, D., and Lamorde, M. (2016). Newborn Screening and prophylactic intervetions for sickle cell disease in 47 countries in sub-saharan Africa: A cost-effectiveness analysis. *BMC Health Services Research*, 16, Article 304. https://doi.org/10.1186/s12913-016-1572-6

Ladu, A.I., Kadaura, M.U., Dauda, M., Baba, A.S., Zango, N.G., Jeffery, C., Farate, A., Adekile, A., and Bates, I. (2024). Malaria infection in patients with sickle cell disease in Nigeria: Association with markers of hyposplenism. *Hemoglobin,* 48(1), 1-9. https: doi.org/10.1080/03630269.2023.2285881

Lakkakula, B.V.; Sahoo, R.; Verma, H.; Lakkakula, S. (2018). Pain Management Issues as Part of the Comprehensive Care of Patients with Sickle Cell Disease. *Pain Management Nursing 19*, 558–572. https://doi.org/10.1016/j.pmn.2018.06.004

Makani, J., Cox, S. E., Soka, D., Komba, A. N., Oruo, J., Mwamtemi, H. and Newton, C. R. (2018). Mortality in sickle cell anemia in Africa: A prospective cohort study in Tanzania. *PLOS ONE,* 13(2), e0191799. https://doi.org/10.1371/journal.pone.0191799

Makani, J., Ofori-Acquah, S. F., and Tluway, F. (2020). Sickle cell disease in Africa: An urgent need for longitudinal cohort studies. *The Lancet Global Health*, Vol.8(12), e1450–e1451. https://doi.org/10.1016/S2214-109X(20)30450-6

Makani, J., Ofori-Acquah, S. F., Nnodu, O., Wonkam, A., and Ohene-Frempong, K. (2020). Sickle cell disease: New opportunities and challenges in Africa. *The Scientific World Journal,* 2020, 1–11. https://doi.org/10.1155/2020/2020235

Makani, J., Tluway, F., Makubi, A., Soka, D., Nkya, S., Meda, E. and Cox, S. E. (2020). A ten-year review of sickle cell disease clinical research and healthcare in Tanzania. *Frontiers in Genetics,* 11, 432. https://doi.org/10.3389/fgene.2020.00432

Manwani, D., and Frenette, P. S. (2020). Vaso-occlusion in sickle cell disease: Pathophysiology and novel targeted therapies. *Blood,* 136(3), 349–360. https://doi.org/10.1182/blood.2020005284

McGann PT, Ware RE. Hydroxyurea therapy for sickle cell anemia. *Expert Opin Drug Saf.* 2015 Sep 14. 1-10. https://doi.org/10.1517/14740338.2015.1082920

Mindray Bio-Medical Electronics Co., Ltd (2025). BC-5000/BC-5150 Auto Hematology Analyzer: product brochure, *Mindray*. https://www.mindray.com/en/product/BC-5000.html

Mmbando, B. P., Makani, J., and Tluway, F. (2019). Clinical and hematological phenotypes in homozygous sickle cell disease in Tanzania. *Blood Cells, Molecules, and Diseases*, Vol.75, 20–24. https://doi.org/10.1016/j.bcmd.2018.12.001

Monaco, C.P.; Fonseca, P.B.B.; Braga, J.A.P. (2015). Infectious complications after surgical splenectomy in children with sickle cell disease. *Rev. Paul. Pediatric. (Engl. Ed.)*, *33*, 150–153. https://doi.org/10.1016/j.rppede.2014.11.005

Musa, A. and Ndakotsu M.A.(2023). Comparative analysis of platelet parameters of sickle cell anemia patients during bone pain crises and in steady states. *Iraqi Journal Hematology;*12(1):13. https://doi.org/10.4103/ijh.ijh_10_22

Naing L., Winn T., Rusli B.N. (2008). Practical issues in calculating the sample size for prevalence studies. Archives of Science.1: 9-14.

Nambei WS, Rawago-Mandjiza D, Gbangbangai E. (2016). Seroepidemiology of HIV, syphilis and hepatitis B and C viruses among blood donors in Bangui, Central African Republic. *Medecine et Sante Tropicales*, **26**(2): 192-198. https://doi.org/10.1051/mst/2016022

Nath, K. A., and Adebayo, O. (2020). Chronic lung disease in sickle cell disease: The role of inflammation and potential therapies. *Chest,* 158(3), 1084–1092. https://doi.org/10.1016/j.chest.2020.05.021

Ndugwa, C. M., and Kiyaga, C. (2020). Managing complications of sickle cell anemia in Uganda: A review of current practice. *African Health Sciences,* 20(2), 604–612. https://doi.org/10.4314/ahs.v20i2.27

New England Journal of Medicine. (2020). Post-transcriptional genetic silencing of BCL11A to treat sickle cell disease. *New England Journal of Medicine,* 383(6), 587–599. https://doi.org/10.1056/NEJMoa2029392

Nnodu, O. E., Sopekan, A., Nnebe-Agumadu, U., and Ohiaeri, C. (2019). Prevalence and pattern of comorbidities in sickle cell disease in Nigeria. *Hemoglobin*, Vol.43(3), 188–192. https://doi.org/10.1080/03630269.2019.1649278

Nnodu, O. E., Sopekan, A., Ohiaeri, C. N., Akinyanju, O. O., and Anie, K. A. (2021). Implementing newborn screening for sickle cell disease as part of immunisation programmes in Nigeria: A feasibility study. *The Lancet Haematology,* 8(5), e293–e300. https://doi.org/10.1016/S2352-3026(21)00041-6

Nwabuko, C. O., Alasia, D. D., and Obi-Ezeani, C. (2022). Hematologic indices in sickle cell patients with co-existing sepsis. *Port Harcourt Medical Journal*, 16(2), 98–104.

aNwegbu, M. M., and Jibrin, Y. B. (2021). Relationship between lymphocyte subsets and hematological parameters in sickle cell disease. *Nigerian Medical Journal*, Vol.62(2), 60–65. https://doi.org/10.4103/nmj.NMJ_35_21

Okocha, C. E., Odenigbo, C. U., and Nnodu, O. E. (2021). Haematologic parameters as markers of acute complications in sickle cell disease. *Nigerian Journal of Haematology*, 31(1), 10–16.

Okocha, E. C., Ibeh, N. C., and Anigbo, C. N. (2021). Hematologic profile of sickle cell patients with sepsis in southeast Nigeria. *Nigerian Journal of Clinical Practice,* 24(3), 370–375. https://doi.org/10.4103/njcp.njcp_1094_20

Okonji, M. C., Emelike, F. N., and Uche, D. A. (2020). Inflammatory markers and white cell parameters in Nigerian children with sickle cell anaemia. *Tropical Journal of Health Sciences*, 27(1), 66–72.

Okpala, I. (2020). Leukocyte adhesion and the pathophysiology of sickle cell disease. *Current Opinion in Hematology*, 27(3), 140–146.

Olaniyi, J. A., and Adewoyin, A. S. (2020). Clinical and laboratory features of sickle cell patients in South-Western Nigeria. *Annals of African Medicine*, 19(4), 248–254.

Olatunya, O. S., Ogundare, E. O., Oluwayemi, I. O., Olatunya, A. M., and Adedoyin, O. T. (2019). Comorbidities and complications of sickle cell anemia in Nigerian children: A retrospective review. *BMC Hematology,* 19(1), 14. https://doi.org/10.1186/s12878-019-0139-7

Omron Healthcare, Inc. 2013. https://omronhealthcare.com/wp-content/uploads.

Onalo, R., Toma, B. O., and Salawu, L. (2021). Correlation between hematologic parameters and inflammatory markers in children with sickle cell anemia in Jos, Nigeria. *Nigerian Journal of Paediatrics,* 48(2), 87–94. https://doi.org/10.4314/njp.v48i2.3

Ong'echa, J. M., Odero, W., and Ouma, C. (2021). Erythrocyte indices in sickle cell disease with comorbidities in Western Kenya. *African Health Sciences*, Vol.21(1), 1–8. https://doi.org/10.4314/ahs.v21i1.2

Oni, A. A., Balogun, T. M., and Salawu, L. (2024). Impaired neutrophil function in sickle cell disease: A potential link to increased fungal susceptibility. *African Journal of Clinical and Experimental Microbiology,* 25(1), 34–42. https://doi.org/10.4314/ajcem.v25i1.4

Osei-Yeboah, C., Nkrumah, F. K., and Rodrigues, O. (2020). Infection-associated hematological changes in Ghanaian sickle cell disease patients. *Ghana Medical Journal*, Vol.54(4), 219–225. https://doi.org/10.4314/gmj.v54i4.3

Otieno, W., Ogutu, B., and Achieng, D. (2021). Iron status and red cell indices in sickle cell anemia patients in western Kenya. *East African Medical Journal,* 98(5), 356–361. https://doi.org/10.4314/eamj.v98i5.6

Owiredu, W. K., Quaye, L., and Amidu, N. (2019). Cytokine profiles and hematological parameters in sickle cell disease. *Cytokine*, Vol.113, 272–279. https://doi.org/10.1016/j.cyto.2018.07.023

Owusu-Ansah, A., Nelson, J., and Olayemi, E. (2019). Hematologic complications of malignancy in sickle cell disease patients: A review. *International Journal of Hematology,* 110(6), 639–648. https://doi.org/10.1007/s12185-019-02716-w

Oyedeji, A. A., Afolabi, B. B., and Awosika, A. Y. (2023). Platelet function and indices in sickle cell anaemia: Implications for thrombotic risk. *African Journal of Laboratory Haematology*, 5(2), 121–129.

Parent, F., Bachir, D., Inamo, J., Lionnet, F., Driss, F., and Habibi, A. (2022). Pulmonary hypertension in patients with sickle cell disease: Pathophysiology and clinical implications. *Blood Reviews,* 55, 100947. https://doi.org/10.1016/j.blre.2022.100947

Patra, P.K.; Lakkakula, B.V.K.S.; Verma, H.K.; Choubey, M.; Patra, S.; Khodiar, P.K. (2017). Assessment of renal function in Indian patients with sickle cell disease. *Saudi J. Kidney Dis. Transplant*, *28*, 524–531. https://doi.org/10.4103/1319-2442.206449

Pecker, L. H., and Lanzkron, S. (2021). Sickle cell disease. *Annals of Internal Medicine,* 174(1), ITC1–ITC16. https://doi.org/10.7326/AITC202101050

Piel, F. B., Steinberg, M. H., and Rees, D. C. (2017). Sickle cell disease. *New England Journal of Medicine,* 376(16), 1561–1573. https://doi.org/10.1056/NEJMra1510865

Piel, F. B., Patil, A. P., Howes, R. E., Nyangiri, O. A., Gething, P. W., Dewi, M., Temperley, W. H., Williams, T. N., Weatherall, D. J., and Hay, S. I. (2013). Global epidemiology of sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. *The Lancet,* 381(9861), 142–151. https://doi.org/10.1016/S0140-6736(12)61229-X

Platt, O. S., Brambilla, D. J., and Rosse, W. F. (2020). Mortality in sickle cell disease: The role of acute chest syndrome. *Blood*, 99(3), 123–128. https://doi.org/10.1182/blood-2020-150117

Rees, D. C., Williams, T. N., and Gladwin, M. T. (2010). Sickle-cell disease. The Lancet, 376(9757), 2018–2031. https://doi.org/10.1016/S0140-6736(10)61029-X

Ribeil, J.A.; Hacein-Bey-Abina, S.; Payen, E.; Magnani, A.; Semeraro, M.; Magrin, E.; Caccavelli, L.; Neven, B.;Bourget, P.; El Nemer, W.; *et al.* Gene therapy in a patient with sickle cell disease. *N. Engl. J. Med.* 2017, 376, 848–855. https://doi.org/10.2450/2013.0139-13

Salawu, L., Bolarinwa, O. A., and Adewale, A. D. (2020). Microcytic anemia in Nigerian sickle cell disease patients: Prevalence and predictors. *Journal of Applied Hematology*, Vol.11(4), 139–144. https://doi.org/10.4103/joah.joah_16_20

Sedrak A, Kondamudi NP. Sickle Cell Disease (Archived). 2024 Jan. *StatPearls.* https://www.ncbi.nlm.nih.gov/books/NBK482190/

Sheehan, V. A., Dong, Y., and Pawlowska, A. B. (2023). Hematopoietic stem cell function and regulation in sickle cell disease: Emerging insights. *Blood Advances,* 7(4), 1050–1058. https://doi.org/10.1182/bloodadvances.2022008663

Sibinga, C. T. S., Manning, B. J., and LaRue, A. C. (2015). The dual burden: Sickle cell disease with concomitant malignancy. *Pediatric Blood & Cancer,* 62(9), 1647–1650. https://doi.org/10.1002/pbc.25569

Sundd, P., Gladwin, M. T., and Novelli, E. M. (2020). Pathophysiology of sickle cell disease. Annual Review of Pathology: Mechanisms of Disease, 15, 263–292. https://doi.org/10.1146/annurev-pathmechdis-012419-032649Taksande A, Jadhav A. and Vagha J. (2015). Comparison between automated and manual sphygmomanometer for measuring blood pressure in children. *J. Nepal Paediatr. Soc*., **35**(1): 13-17.

Sundd, P.; Gladwin, M.T.; Novelli, E.M. (2019). Pathophysiology of Sickle Cell Disease. *Annu. Rev. Pathol. Mech. Dis.*, *14*, 263–292. https://doi.org/10.1146/annurev-pathmechdis-012418-012838

Tagelsir, E., Ali, A. H., and Babiker, A. O. (2020). Haematological and biochemical changes in Sudanese sickle cell anemia patients during steady state and crisis. *BMC Hematology,* 20, 6. https://doi.org/10.1186/s12878-020-00168-5.

Tantawy AAG, Ibrahim SW, Abdel-Aziz TT, Rabie AN, Makkeyah SM, Ragab IA. (2020). Inner Ear Complications in Children and Adolescents with Sickle Cell Disease. *Hemoglobin*, **44**(6): 411-7. https://doi.org/10.1080/03630269.2020.1825452

Tayo, B. O., Akingbola, T. S., and Salako, B. L. (2019). Impact of malaria on hematological indices in Cameroonian sickle cell disease. *Journal of Infection and Public Health*, Vol.12(5), 713–717. https://doi.org/10.1016/j.jiph.2019.03.017

Tebbi CK. (2022). Sickle Cell Disease, a Review. *Hemato,* **3**: 341-66. https://doi.org/10.3390/hemato3030025

Telen, M. J. (2018). Sickle cell disease and its hematological complications. *Hematology*, 23(1), 35–43. https://doi.org/10.1182/hematology.2017-0436

Telfer, P., Coen, P., Chakravorty, S., Wilkey, O., Evans, J., Newell, H., and Gardner, K. (2020). Clinical outcomes in children with sickle cell disease living in England: A neonatal cohort in East London. *Haematologica*, 105(4), 640–648. https://doi.org/10.3324/haematol.2019.221408

Toledo SLDO, Guedes JVM, Alpoim PN, Rios DRA, Pinheiro MDB.(2019). Sickle cell disease: Hemostatic and inflammatory changes, and their interrelation. *Clin Chim Acta*; 493:129– 137. https://doi.org/10.1016/j.cca.2019.02.022

Uche, R. O., and Oladipo, A. A. (2023). Impact of co-morbid conditions on hematological parameters in Nigerian sickle cell patients: A cross-sectional hospital-based study. *West African Journal of Clinical Medicine,* 31(2), 87–94. https://doi.org/10.4314/wajcm.v31i2.8

Ugwu NI, Ugwu GC, Alo C, Ugwu CN, Okoye H.C., Madu AJ, Okike C.(2019). Steady state haematological characteristics of Nigerians with sickle cell anaemia and those with normal adult haemoglobin. *The Nigerian Health Journal*; 20(3): 15–2.

Ugwu, C.O., Ekong, P.M., and Okoro, C.I.(2021). Seroprevalence of hepatitis B infection among HIV-infected individuals in Uyo, Akwa Ibom State, Nigeria. *Med Rxiv.* https://doi.org/10.1101/2021.03.06.21253060

Uzoegwu, P.N. and Onwurah, A.E. (2003). Prevalence of haemoglobinopathy and malaria diseases in the population of old Aguata Division, Anambra State, Nigeria. *Biokemistri;*15(2): 57-66.

Verma, H.K.; Lakkakula, S.; Lakkakula, B.V. (2018)**.** Retrospection of the effect of hydroxyurea treatment in patients with sickle cell disease. *Acta Haematologica. Polonica.*, *49*,1–8. https://doi.org/10.1016/j.achaem.2018.01.001

Vichinsky, E. P., Neumayr, L. D., and Clarke, C. (2019). Alloimmunization and transfusion-related complications in sickle cell disease. *American Journal of Hematology,* 94(5), 579–586. https://doi.org/10.1002/ajh.25450

Vichinsky, E., and Hurst, D. (2023). Viral infections and hematologic complications in sickle cell disease. *Hematology/Oncology Clinics of North America,* 37(1), 55–72. https://doi.org/10.1016/j.hoc.2022.10.004

Vingert B., Tamagne M., Desmarets M., Pakdaman S., Noizat-Pirenne F. and Cohen L. (2014): Partial dysfunction of Treg activation in sickle cell disease, *American Journal of Hematology,* 89:261-266. https://doi.org/10.1002/ajh.23626

Ware, R. E., and de Montalembert, M. (2021). Sickle cell disease: A global health problem. *Clinical Hematology International,* 3(3), 127–135. https://doi.org/10.2991/chi.k.210406.001

Weatherall, D. J. (2011). The inherited diseases of hemoglobin are an emerging global health burden. *Blood*, 115(22), 4331–4336. https://doi.org/10.1182/blood-2010-10-306573

World Health Organization. (2015). HIV rapid diagnostics test: performance data. https://apps.who.int/iris/handle/10665/181158

Yawn, B. P., Buchanan, G. R., Afenyi-Annan, A. N., Ballas, S. K., Hassell, K. L., James, A. H., and Jordan, L. (2022). Management of sickle cell disease: Summary of the 2022 updated clinical practice guideline. *JAMA*, 328(4), 348–361. https://doi.org/10.1001/jama.2022.11155

Yawn, B. P., *et al.* (2019). Management of sickle cell disease: summary of the 2019 National Heart, Lung, and Blood Institute guidelines. *American Journal of Hematology,* 94(12), 1315-1333. doi: 10.1002/ajh.25646

Zhang, D., Xu, C., Manwani, D., and Frenette, P. S. (2022). Neutrophil aging and its implications in sickle cell disease. *Frontiers in Immunology,* 13, 816620. https://doi.org/10.3389/fimmu.2022.816620

Zhao, M., Tao, F., Venkatraman, A., Li, Z., Smith, S. E., Unruh, J., and Ge, Y. (2023). Platelet–endothelial interactions in sickle cell disease: Mechanisms and clinical implications. *Cell Stem Cell,* 30(3), 445–460.e8. https://doi.org/10.1016/j.stem.2023.01.002.

APPENDIX 1

Table 1: demographic distribution of the patients

Parameters		**Frequency**	**Percentage (%)**	**Mean ± SD**
		(N = 71)		
Gender	Male	34	47.9	
	Female	37	52.1	
	Total	**71**	**100%**	
Age (years)	≤ 15	38	53.5	20.8 ± 18.0
	16 – 30	13	18.3	
	31 – 46	15	21.1	
	>46	5	7.1	
	Total	**71**	**100%**	
Clinical condition	Cancer	12	16.9	
	HIV	1	1.4	
	Sepsis	7	9..9	
	Malaria	2	2.8	
	Hemolytic anaemia	3	3.8	
	Chronic Kidney Disease	1	4.2	
	Vaso-occlusive crisis	35	49.3	
	None	10	14.1	

	Total	**71**	**100%**
Morbidity	Co-morbid HbSS	26	36.6
status	HbSS (control)	35	49.3
	HbAA (control)	10	14.1
	Total	**71**	**100%**

Table 2: Impact of SCD co-morbidity on Lymphocyte count (x10^9 cells/L)

Groups	**Mean**	**Std Dev**	**Mean error**	**F-value**	*p-value*
Comorbid HbSS	33.1[a]	19.5	3.8	4.942	0.01*
HbSS control[b]	48.8[b]	21.4	3.6		
Normal control[a]	42.6[ab]	5.9	1.87		

***** The mean values were significantly differ at 95% confidence level ($p < 0.05$)

[ab]Mean values with different superscripts are statistically different

Table 3: Impact of SCD co-morbidity on neutrophil count

Groups	**Mean**	**Std Dev**	**Mean error**	**F-value**	*p-value*

Comorbid HbSS	55.4	19.7	3.9	2.676	0.08
HbSS control	44.1	21.7	3.7		
Normal control	53.2	6.2	1.9		

The mean values were not statistically different at 95% confidence level ($p > 0.05$)

Table 4: Impact of SCD co-morbidity on total white blood cell (TWBC)

Groups	**Mean**	**Std Dev**	**Mean error**	**F-value**	*p-value*
Comorbid HbSS	11.2	9.6	1.9	1.484	0.234
HbSS control	8.9	5.2	0.9		
Normal control	7.1	0.8	0.2		

The mean values were not statistically different at 95% confidence level ($p > 0.05$)

Table 5: Impact of SCD co-morbidity on platelet count

Groups	**Mean**	**Std Dev**	**Mean error**	**F-value**	*p-value*
Comorbid HbSS	277.5	138.8	27.2	0..368	0.694
HbSS control	297.9	133.5	22.6		
Normal control	263.4	43.6	13.8		

The mean values were not statistically different at 95% confidence level ($p > 0.05$)

Table 6: Correlation between hematological parameters with Co-morbidities

Control Variables			TWBC	LYM	NEU	PLT
Comorbidity	TWBC	Correlation		-.280	.409	.103

	Significance (2-tailed)		.175	**.042***	.624
	Df		23	23	23
LYM	Correlation	-.280		-.808	.017
	Significance (2-tailed)	.175		**.000***	.937
	Df	23		23	23
NEU	Correlation	.409	-.808		.097
	Significance (2-tailed)	**.042***	**.000***		.644
	Df	23	23		23
PLT	Correlation	.103	.017	.097	
	Significance (2-tailed)	.624	.937	.644	
	Df	23	23	23	

S/N	GENOTYPE	GENDER	AGE	TWBC	LYM	NEU	PLT	COMORBIDITY
1	**Co-morbid HbSS**	**Male**	**1**	**2.28**	**36.4**	**41.6**	**143**	**Cancer**
2	**Co-morbid HbSS**	**Male**	**2**	**10.8**	**28**	**60**	**296**	**Sepsis**
3	**Co-morbid HbSS**	**Female**	**3**	**7.9**	**70**	**25**	**333**	**Sepsis**
4	**Co-morbid HbSS**	**Male**	**4**	**9.5**	**81**	**15**	**261**	**Sepsis**
5	**Co-morbid HbSS**	**Male**	**5**	**15.5**	**56**	**41**	**211**	**Sepsis**

6	**Co-morbid HbSS**	**Female**	**6**	**15.69**	**27.7**	**62.1**	**357**	**Malaria**
7	**Co-morbid HbSS**	**Female**	**7**	**5.4**	**20**	**46**	**262**	**Sepsis**
8	**Co-morbid HbSS**	**Male**	**10**	**7.1**	**22.8**	**61.5**	**425**	**Cancer**
9	**Co-morbid HbSS**	**Female**	**10**	**11.2**	**52.1**	**38.2**	**511**	**Cancer**
10	**Co-morbid HbSS**	**Male**	**12**	**6.88**	**10.5**	**80.4**	**384**	**Malaria**
11	**Co-morbid HbSS**	**Female**	**13**	**10.2**	**31**	**62**	**163**	**Sepsis**
12	**Co-morbid HbSS**	**Male**	**21**	**19.03**	**10.7**	**83.4**	**215**	**Hemolytic anaemia**
13	**Co-morbid HbSS**	**Female**	**23**	**5.2**	**52.8**	**42.7**	**96**	**HIV**
14	**Co-morbid HbSS**	**Male**	**25**	**29.23**	**25.1**	**57**	**222**	**Hemolytic anaemia**
15	**Co-morbid HbSS**	**Female**	**28**	**4.17**	**25**	**72**	**164**	**Cancer**
16	**Co-morbid HbSS**	**Female**	**32**	**6.75**	**30.2**	**63.5**	**172**	**Cancer**
17	**Co-morbid HbSS**	**Female**	**33**	**6.22**	**27.9**	**64.1**	**514**	**Sepsis**
18	**Co-morbid HbSS**	**Female**	**39**	**3.51**	**23.2**	**61.6**	**44**	**Cancer**
19	**Co-morbid HbSS**	**Female**	**41**	**8.52**	**59.7**	**28**	**375**	**Hemolytic anaemia**
20	**Co-morbid HbSS**	**Male**	**42**	**2.3**	**13.1**	**27.4**	**127**	**Cancer**
21	**Co-morbid HbSS**	**Female**	**44**	**16.47**	**11.5**	**86.2**	**383**	**Cancer**
22	**Co-morbid HbSS**	**Male**	**52**	**2.95**	**43.6**	**49.6**	**207**	**Cancer**
23	**Co-morbid HbSS**	**Female**	**54**	**6.77**	**44**	**48.4**	**423**	**Cancer**
24	**Co-morbid HbSS**	**Female**	**56**	**31.13**	**3.9**	**90.5**	**567**	**chronic kidney disease**

25	**Co-morbid HbSS**	**Male**	**64**	**5.17**	**37.4**	**58.1**	**207**	**Cancer**
26	**Co-morbid HbSS**	**Female**	**71**	**41.18**	**16.6**	**76.2**	**153**	**Cancer**
27	**HbSS control**	**Female**	**1**	**17.9**	**57**	**39**	**378**	**N/A**
28	**HbSS control**	**Male**	**1**	**13**	**62**	**34**	**333**	**N/A**
29	**HbSS control**	**Female**	**2**	**8.6**	**71**	**25**	**213**	**N/A**
30	**HbSS control**	**Female**	**2**	**4.9**	**78**	**17**	**343**	**N/A**
31	**HbSS control**	**Male**	**2**	**2.8**	**73**	**21**	**441**	**N/A**
32	**HbSS control**	**Male**	**2**	**10.2**	**82**	**8**	**198**	**N/A**
33	**HbSS control**	**Male**	**3**	**4.9**	**61**	**33**	**341**	**N/A**
34	**HbSS control**	**Male**	**3**	**14**	**59**	**35**	**196**	**N/A**
35	**HbSS control**	**Male**	**3**	**6.3**	**45**	**45**	**135**	**N/A**
36	**HbSS control**	**Male**	**4**	**3.8**	**74**	**22**	**286**	**N/A**
37	**HbSS control**	**Female**	**4**	**8.7**	**72**	**20**	**386**	**N/A**
38	**HbSS control**	**Female**	**4**	**8.4**	**50**	**42**	**415**	**N/A**
39	**HbSS control**	**Female**	**5**	**9.1**	**35**	**55**	**530**	**N/A**
40	**HbSS control**	**Male**	**5**	**3.5**	**68**	**21**	**156**	**N/A**
41	**HbSS control**	**Male**	**5**	**6.5**	**65**	**25**	**206**	**N/A**
42	**HbSS control**	**Female**	**6**	**8.3**	**67**	**22**	**285**	**N/A**
43	**HbSS control**	**Female**	**6**	**4.6**	**32**	**60**	**208**	**N/A**
44	**HbSS control**	**Male**	**7**	**7.2**	**57**	**38**	**365**	**N/A**
45	**HbSS control**	**Male**	**7**	**6.6**	**25**	**59**	**217**	**N/A**
46	**HbSS control**	**Female**	**8**	**6.63**	**51.3**	**40.5**	**398**	**N/A**
47	**HbSS control**	**Female**	**9**	**10.8**	**29**	**63**	**194**	**N/A**

48	**HbSS control**	**Female**	**9**	**4.8**	**55**	**38**	**136**	**N/A**
49	**HbSS control**	**Male**	**11**	**3.55**	**48.5**	**43.3**	**184**	**N/A**
50	**HbSS control**	**Male**	**12**	**7.8**	**62**	**35**	**449**	**N/A**
51	**HbSS control**	**Female**	**13**	**12.1**	**77**	**19**	**488**	**N/A**
52	**HbSS control**	**Female**	**14**	**29.25**	**10**	**86**	**365**	**N/A**
53	**HbSS control**	**Male**	**15**	**2.4**	**39**	**51**	**165**	**N/A**
54	**HbSS control**	**Female**	**24**	**13.74**	**24.4**	**68.5**	**552**	**N/A**
55	**HbSS control**	**Male**	**28**	**12.85**	**34.4**	**57.2**	**247**	**N/A**
56	**HbSS control**	**Male**	**30**	**18.01**	**5**	**90.3**	**414**	**N/A**
57	**HbSS control**	**Male**	**30**	**7.77**	**46.5**	**43.1**	**396**	**N/A**
58	**HbSS control**	**Female**	**39**	**9.13**	**39.2**	**54**	**151**	**N/A**
59	**HbSS control**	**Female**	**40**	**8.86**	**28.5**	**65.4**	**507**	**N/A**
60	**HbSS control**	**Female**	**45**	**5.56**	**15.9**	**81.3**	**75**	**N/A**
61	**HbSS control**	**Female**	**46**	**9.77**	**10**	**88.3**	**74**	**N/A**
62	**normal control**	**Male**	**25**	**7.7**	**45**	**51**	**251**	**N/A**
63	**normal control**	**Female**	**28**	**7.4**	**39**	**58**	**276**	**N/A**
64	**normal control**	**Male**	**30**	**6.9**	**46**	**49**	**189**	**N/A**
65	**normal control**	**Male**	**30**	**6.5**	**48**	**44**	**301**	**N/A**
66	**normal control**	**Female**	**39**	**7.3**	**44**	**52**	**331**	**N/A**
67	**normal control**	**Male**	**40**	**7.2**	**46**	**50**	**278**	**N/A**
68	**normal control**	**Female**	**41**	**5.6**	**34**	**64**	**275**	**N/A**
69	**normal control**	**Female**	**45**	**7.5**	**31**	**62**	**196**	**N/A**
70	**normal control**	**Male**	**32**	**6.8**	**48**	**50**	**255**	**N/A**

71	**normal control**	**Male**	**27**	**8.4**	**45**	**52**	**282**	**N/A**

APPENDIX II

INFORMED CONSENT FORM

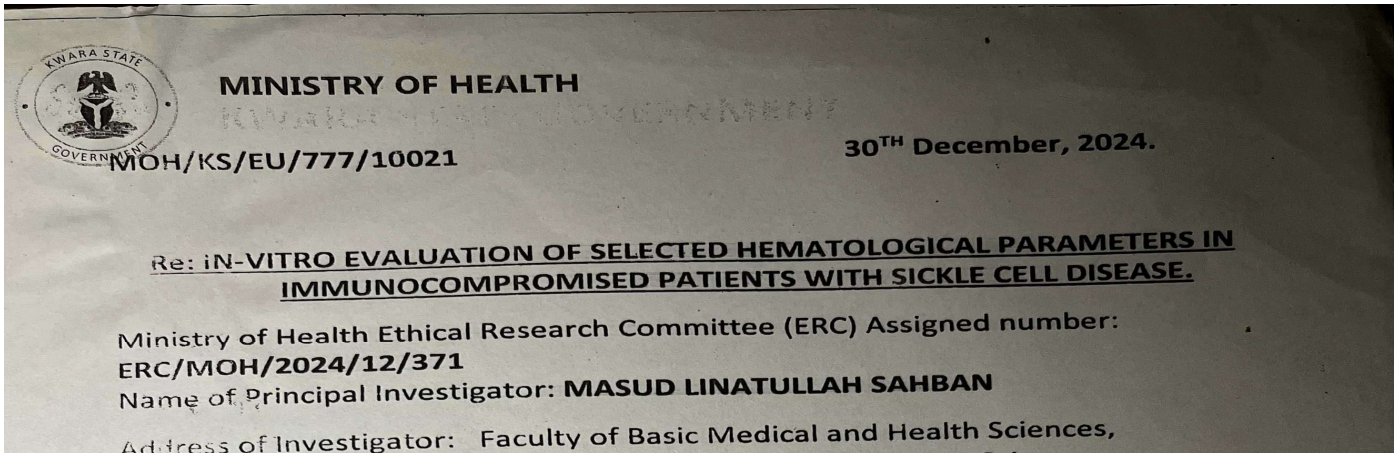
I have read and understood that my participation in this study is voluntary and I can withdraw from the study if I so wish. My decision not to participate in this study will not affect me as an individual or a patient or donor. I hereby consent to participate in this study titled "**EVALUATION OF THREE HEMATOLOGICAL PARAMETERS IN SICKLE CELL DISEASE COMORBID PATIENTS ATTENDING CLINICS IN ILORIN NIGERIA".** The nature of the study, the procedure involve in sample collection including the risks and benefits of the study have been fully explained to me by the researcher. I also understand that the findings of this study may have good contribution to medical practice.

…….. ……

Signature of consented participant Name and Signature of Researcher

APPENDIX III

ETHICAL APPROVAL



KWARA STATE GOVERNMENT

MINISTRY OF HEALTH

MOH/KS/EU/777/10021 **30TH December, 2024.**

Re: iN-VITRO EVALUATION OF SELECTED HEMATOLOGICAL PARAMETERS IN IMMUNOCOMPROMISED PATIENTS WITH SICKLE CELL DISEASE.

Ministry of Health Ethical Research Committee (ERC) Assigned number: ERC/MOH/2024/12/371

Name of Principal Investigator: **MASUD LINATULLAH SAHBAN**

Address of Investigator: Faculty of Basic Medical and Health Sciences,