Defining the Clinical Spectrum of Sickle Cell Disease in Tanzania: A Clinico-Epidemiological Study

Thesis

Defining the Clinical Spectrum of Sickle Cell Disease in Tanzania: A Clinico-Epidemiological Study

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ABSTRACT

INTRODUCTION: The burden of Sickle Cell Disease (SCD) in Africa is high; having over 75% of annual global births and mortality reaching 95% in childhood. Introduction of interventions, which could prevent 70% of deaths, have been limited because of lack of local evidence. This study described the clinical spectrum of SCD in Tanzania as the first step in providing evidence to guide targeted interventions.

METHODS: SCD patients attending Muhimbili Hospital in Dar-es-Salaam, Tanzania were recruited between 2004 and 2009. Prospective surveillance of clinical and laboratory information was done at outpatient clinic and during hospitalisation. Specific investigations included blood cultures on all hospitalised SCD patients, Transcranial Doppler (TCD) ultrasonography to measure cerebral blood flow velocity (CBFv) and HPLC to measure levels of foetal haemoglobin (HbF). The outcomes of interest were death, hospitalisation, malaria, bacteraemia and stroke.

RESULTS: 1,725 SCD patients [mean age 9.7 (SD 7.9) years; 10% below 2 years] were enrolled with information recorded from 14,000 visits during the study period. 12% of enrolled SCD patients were lost to follow up and 23% of 86 deaths occurred at the hospital. The mortality was 2 deaths/100 person years of observation (PYO); highest under 5 years and independently associated with low haemoglobin (Hazard ratio 0.7 95%CI 0.6-0.8; p<0.01). 504 (29%) of the SCD cohort were hospitalised with pain, fever and anaemia as the commonest cause of hospitalisation. SCD patients had less malaria than non-SCD patients at clinic (OR, 0.46; 95% CI, 0.25-0.94; P = .01) and during hospitalisation (OR, 0.53; 95% CI, 0.32-0.86; P = .008). In SCD patients, prevalence of malaria was higher during hospitalisation and associated with severe anaemia and death. 43 out of 890 hospitalisations had bacteraemia (4.8%) with *Staphylococcus aureus* (28%), non-typhii *Salmonella* (21%) and *Streptococcus pneumoniae* (7%) as the most common organisms. The mean CBFv in 372 patients (2 - 16 years) was 132 cm/sec with CBFv > 200cm/sec occurring in 40 (10%) patients. The incidence of stroke was 0.3 per 100PYO; associated with sickle haemoglobin and reticulocyte count but not with CBFv. The mean HbF level in 1,669 SCD patients was 6.3 (SD 4.7) % with no association with mortality and hospitalisation.

CONCLUSION: This study has highlighted the burden of disease to individuals and health system. The findings have important implications for policies to improve healthcare as well as identifying areas for further research.

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"Yote yamewezeshwa na Mwenyezi."

DEDICATION

To Abdu Simba

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List of Abbreviations

ACA	Anterior cerebral artery
ACS	Acute chest syndrome
ACT	Artemesinin - based combination therapy
ALP	Alkaline phosphatase
ALU	Artemether and lumefantrin
API	Analytical profile index
AS	Heterozygous state of sickle cell
AST	Aspartate transaminase enzyme
BA	Basilar artery
BP	Blood pressure
BT	Blood transfusion
CAR	Central African Republic
CBFv	Cerebral blood flow velocity
CFR	Case fatality rate
cM	Clinical malaria
CNS	Central nervous system
CT	Computerized tomography
CVA	Cerebrovascular accident
EA	East Africa
FACS	Fluorescence-activated cell sorting
FBC	Full blood count
FC	F cells
FCP	F-cell production locus
GDP	Gross domestic product
GGT	Gamma glutamyl transferase
GM	Geometric mean
GNI	Gross national income
Hb	Haemoglobin
Hb A_0	Haemoglobin A_0
Hb A_2	Haemoglobin A_2
HBB	B-globin gene region on chromosome 11
HBE	Haemoglobin electrophoresis
HbF	Foetal haemoglobin
HbS	Sickle haemoglobin
HbSS	Homozygous inheritance of sickle cell disease
HPFH	Hereditary persistence of foetal haemoglobin
HPLC	High performance liquid chromatography
HU	Hydroxyurea
ICA	Internal carotid artery
IEF	Isoelectric focusing
IMR	Infant mortality rate

IPD	Inpatient department
ISC	Irreversibly sickled cell
ITN	Insecticide treated nets
LDH	Lactate dehydrogenase
LFT	Liver function test
LIC	Low income country
LTBT	Long-term blood transfusion therapy
MCA	Middle cerebral artery
MCA-ACA	Bifurcation of the middle cerebral and anterior cerebral artery
MCV	Mean corpuscular volume
MNH	Muhimbili national hospital
MPS	Malaria parasite
MRI	Magnetic resonance imaging
NBS	Newborn screening
NO	Nitric oxide
OPD	Outpatient department
PCA	Posterior cerebral artery
PCR	Polymerase chain reaction
PYO	Person years of observation
QC	Quality control
QTL	Quantitative trait locus
RBC	Red blood cell
RDW	Red cell distribution width
RFLP	Restriction fragment length polymorphism
RDT	Rapid diagnostic test
SCA	Homozygous state (SS) of sickle cell disease
SCD	Sickle cell disease
SCT	Stem cell transplant
SMA	Severe malarial anaemia
SP	Sulphadoxine-pyrimethamine
SPN	*Streptococcus pneumoniae*
SSA	Sub Saharan Africa
STOP	Stroke prevention trial
TCD	Trans-cranial doppler ultrasonography
TIA	Transient ischemic attack
TTI	Transfusion transmitted infections
VOC	Vaso-occlusion
WBC	White blood cell

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Chapter One

Introduction

INTRODUCTION

Sickle Cell Disease (SCD) is the single most important genetic cause of childhood mortality world-wide. In May 2006 at the 59th World Health Assembly (WHA), resolution WHA 59.20 of the World Health Organisation (WHO) was to develop, implement and reinforce integrated national programs for the prevention and management of Sickle Cell Disease (SCD)[1].

Approximately 300,000 children are born every year with SCD in the world, with over 75% in Africa[2]. In Tanzania, a low income country (LIC) in Africa, SCD is of public health significance as the prevalence of the carrier state ranges between 15 – 20%,[3] which is amongst the highest in Africa. The estimated birth incidence of children with homozygous SS SCD in Tanzania is between 6 to 7 per 1000 children[4]. The majority of these children will not know that they have SCD and it is estimated that over 5% of infant mortality may be attributable to SCD[5]. Although there is a wide variation in the severity of disease in affected children, these children will require life-long access to hospital care for acute and chronic complications, with the average cost for managing one patient with SCD ranging between $4,425 in Jamaica to $7,000 in Canada per year[6].

Major benefits in the health and survival of children with SCD in high income countries have been attained through the implementation of a few, simple evidence-based interventions. The most striking achievements have been as a result of early diagnosis of SCD by newborn screening (NBS) and the subsequent enrolment of these patients into comprehensive care

programmes[7]. These programmes provide interventions which include prophylaxis against infections, for example with penicillin, early detection and treatment of acute clinical events such as anaemia, septicaemia, stroke and acute chest syndrome due to infections/infarctions. These interventions have not been introduced in Tanzania despite the fact that they have been shown to be highly effective in developed countries. Although there is every reason to believe that such an approach would have a similar impact in Africa, there are simply no data on even the most basic problems, such as the common clinical complications and the current mortality, to support this assertion. In addition, the limitation of resources in LIC mean that it is essential that proposed interventions are cost-effective and based on a clear definition of the problems.

The main objective of this study was to describe the clinical spectrum of SCD in Tanzania by defining the major causes of morbidity and mortality. This involved the establishment of a systematic framework for a prospective, descriptive study in a setting with an existing clinical sickle cell service. This thesis will focus on malaria, bacterial infections and neurological events, mainly cerebrovascular accidents or stroke, which are thought to cause high morbidity and mortality in the population of SCD subjects currently forming the clinical load at MNH. This is probably typical of many hospitals in East Africa (EA). In addition, these three clinical events have interventional strategies which are effective, could be implemented in resource-limited settings and would significantly improve the management of African patients. This work will also describe the clinical epidemiology of foetal haemoglobin (HbF) in the SCD cohort, as patients with high concentrations of HbF appear to be protected against severe consequences of SCD. Therefore, study of HbF has the potential both to increase our understanding of the mechanisms behind SCD and to provide effective therapeutic options in Africa.

This thesis starts with an outline of the scientific and clinical background to SCD, reviewing pertinent existing literature on malaria, bacterial infections, stroke and foetal haemoglobin. This is done with an emphasis on defining what needs to be done to improve outcome in an African setting as the rationale for this thesis. In the second section, I summarise the methodology and resources that were used to establish the framework for carrying out the work. The third section initially outlines an overall description of the SCD cohort, followed by individual chapters on findings in malaria, infections, neurological events and HbF. Finally, the concluding section summarizes the results as the baseline on which to propose locally relevant approaches to target resources for appropriate management, intervention and research. This information has the potential of having a significant impact on clinical course, quality of life and survival of SCD individuals in Tanzania.

Chapter Two

Literature review

SICKLE CELL DISEASE

In this section, the biology of haemoglobin, in particular sickle haemoglobin and the genetics of SCD will be reviewed. This will be followed by an overview of the mechanisms of disease in SCD and the clinical consequences of SCD. The third section will review the diagnosis, treatment, prevention and control of SCD, focusing on the interventional strategies that are pertinent for this study. Finally, there will be a summary of the situation of SCD in Africa.

Basic concepts

The function of haemoglobin (Hb) is to carry oxygen and carbon-dioxide between the lungs and tissues. It is a molecule that is carried within a red blood cell (RBC), a non-nucleated, bi-concave cell. RBCs are produced by the bone marrow, with erythropoietic activity occurring in almost every marrow cavity in childhood but later activity is limited to the ends of long bones, axial skeleton, ribs and skull. RBCs have a life span of 120 days. An average Man will have $5x10^{12}$ RBCs per litre of blood and the level of haemoglobin ranges between 11.5 – 15.5g/dl in women and 13-18 g/dL in men. Anaemia is defined as a decrease in haemoglobin below the lower limit of reference values for age and sex.

The structure of haemoglobin molecule

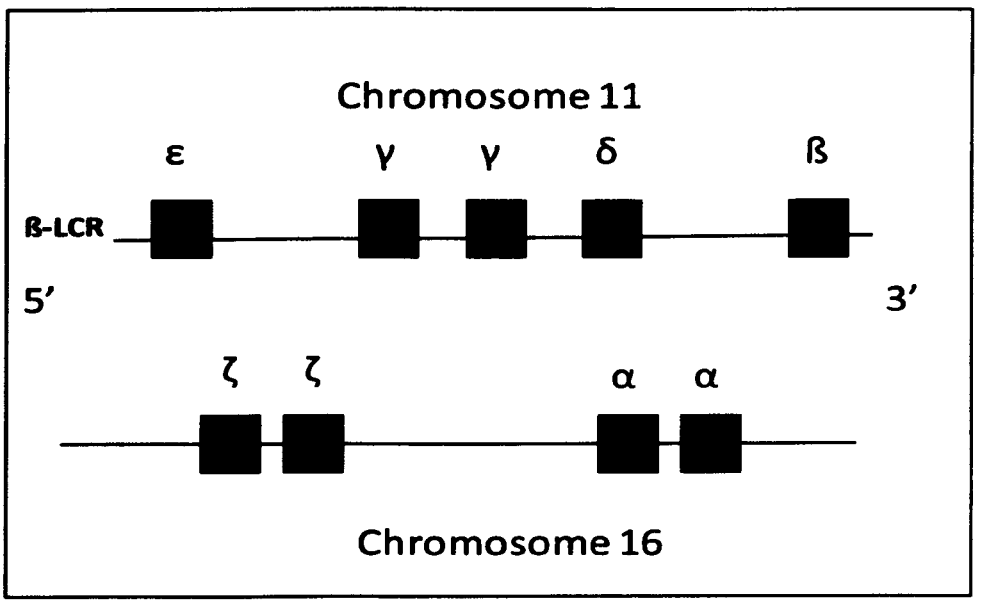
The haemoglobin molecule is a protein which consists of four polypeptide globin chains, with each globin chain containing one molecule of haeme. There are 2 alpha-globin chains and two non-alpha globin chains. In adults the non-alpha globin chains are beta (β), gamma (γ)

and delta (δ). Therefore, in adults there are three types of haemoglobins - haemoglobin A ($\alpha_2 \beta_2$), Haemoglobin A_2 ($\alpha_2 \delta_2$) and Haemoglobin F ($\alpha_2 \gamma_2$).

Genetic control of haemoglobin production

There are 2 alpha-like globins [zeta (δ) and alpha (α)] and the genes that control the synthesis of these globins are located on chromosome 16. Similarly for the non-alpha globins (ε, γ, δ, β), the genes are located on the short arm of chromosome 11. The β-globin gene (*HBB*) exists in a region of chromosome 11 called the "beta globin locus control region (LCR)." The sequence in which they are physically ordered on their respective chromosomes reflects the timing of their expression during development.

Figure 1 Schematic drawing of gene clusters that regulate haemoglobin molecules in humans

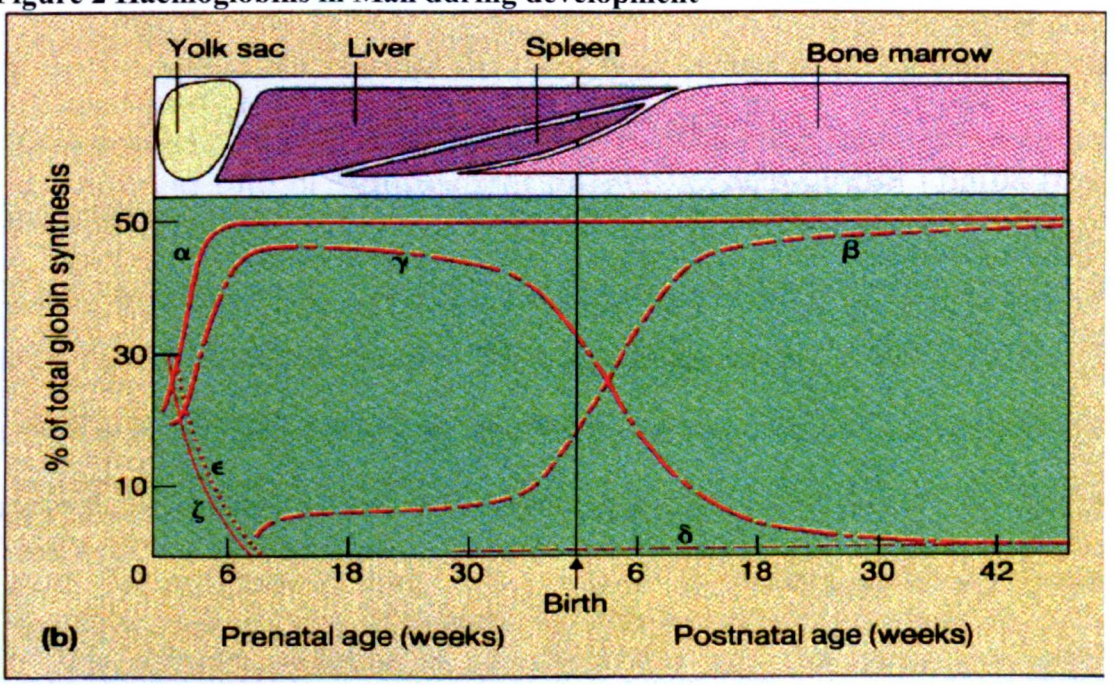


β-LCR stands for β locus control region

Distribution of haemoglobins during human development

Since the environment during embryonic, foetal and adult life with respect to availability and source of oxygen differs, so the properties of the haeme molecule in binding and releasing oxygen have to differ at each of these stages. This means that the distribution of globin chains making up the different haemoglobins in individuals gradually changes during development (Figure 2). During the embryonic period, (up to 6 weeks of gestation), embryonic haemoglobin is produced consisting of Gower 1 ($\zeta_2\ \varepsilon_2$), Gower 2 ($\alpha_2\ \varepsilon_2$) and Portland Hb ($\zeta_2\ \gamma_2$). As the embryo develops into a foetus, there is a reduction in the production of zeta and epsilon globin chains and an increase in the production of alpha chains resulting in a high concentration of HbF ($\alpha_2\ \gamma_2$). This haemoglobin has a high-affinity for oxygen and is therefore able to bind oxygen more efficiently from maternal haemoglobin, at any given oxygen concentration. After birth, oxygen is more readily available and therefore there is a gradual reduction in the synthesis of foetal haemoglobin and an increase in adult haemoglobin (HbA – $\alpha_2\ \beta_2$).

Figure 2 Haemoglobins in Man during development



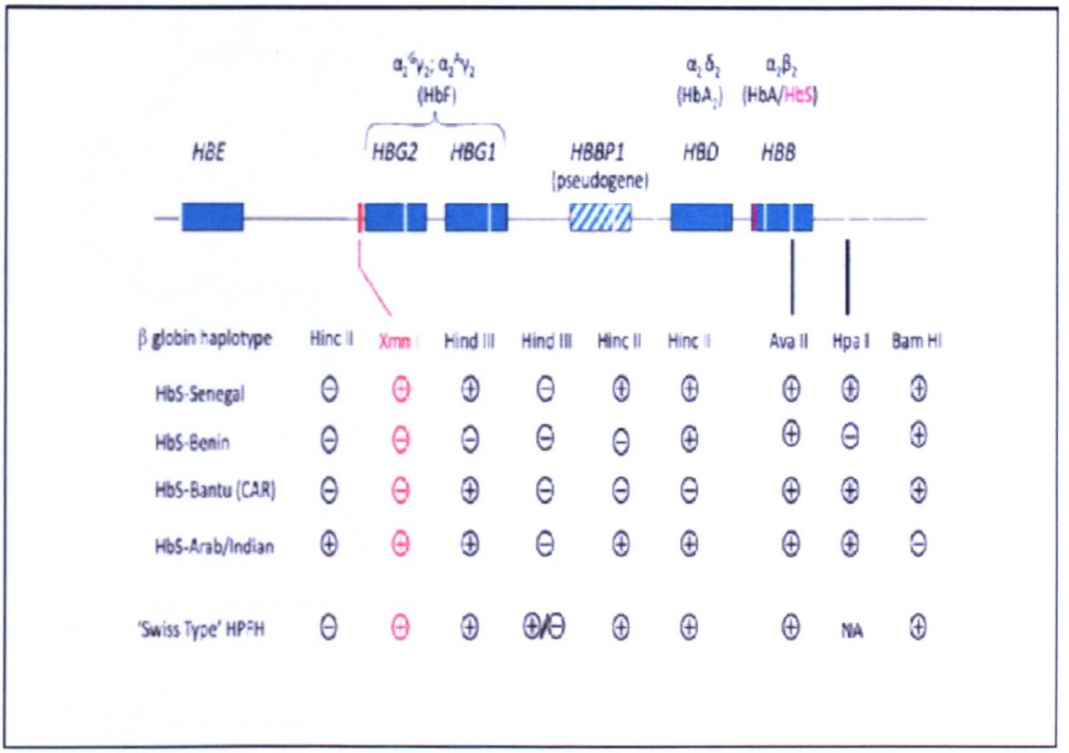
Schematic diagram showing the time course of development of globin genes in man from conception to adulthood. Sites of synthesis are shown as well as the proportion of different globins at different time points[8].

Sickle Gene

Random mutations can occur in the coding regions of *HBB,* resulting in abnormal structural variations in the haemoglobins leading to haemoglobinopathies. Sickle haemoglobin (HbS) occurs when one amino acid (valine) substitutes another amino acid (glutamic acid) at position six of the β-globin polypeptide chain. This substitution is caused by a single base mutation in codon 6 within the β-globin gene cluster, where the sequence GAG occurs instead of GTG. The sickle gene is autosomal recessive and follows Mendelian principles of inheritance. HbS is one of 3 haemoglobinopathies which have reached high population frequencies, the others being haemoglobin C and haemoglobin E, which result from single amino acid substitution (β 6 Glu →Lys) and (β 26 Glu →Lys) respectively.

Random mutations can also occur in the non-coding region of β-globin gene cluster, which are neither selected for or against. When a gene mutation occurs in the coding region of the β-globin gene, the surrounding non-coding region is not affected. The genetic background of the surrounding region is called the "haplotype" of that particular mutation. Since the chance is extremely small that another random mutation will occur in the non-coding region, the haplotype of a particular gene mutation event is fixed. The haplotypes can be identified by endonuclease enzymes that isolate the specific polymorphisms by restriction fragment length polymorphism (RFLP) typing. There are 4 β^S –haplotypes; β^S – Senegal, β^S – Benin, βS – CAR (Central African Republic, also known as Bantu) and β^S – Arab/Indian. Figure 3 shows the HBB cluster with the different haplotypes.

Figure 3 β-Haemoglobin gene (HBB) cluster of chromosome 11

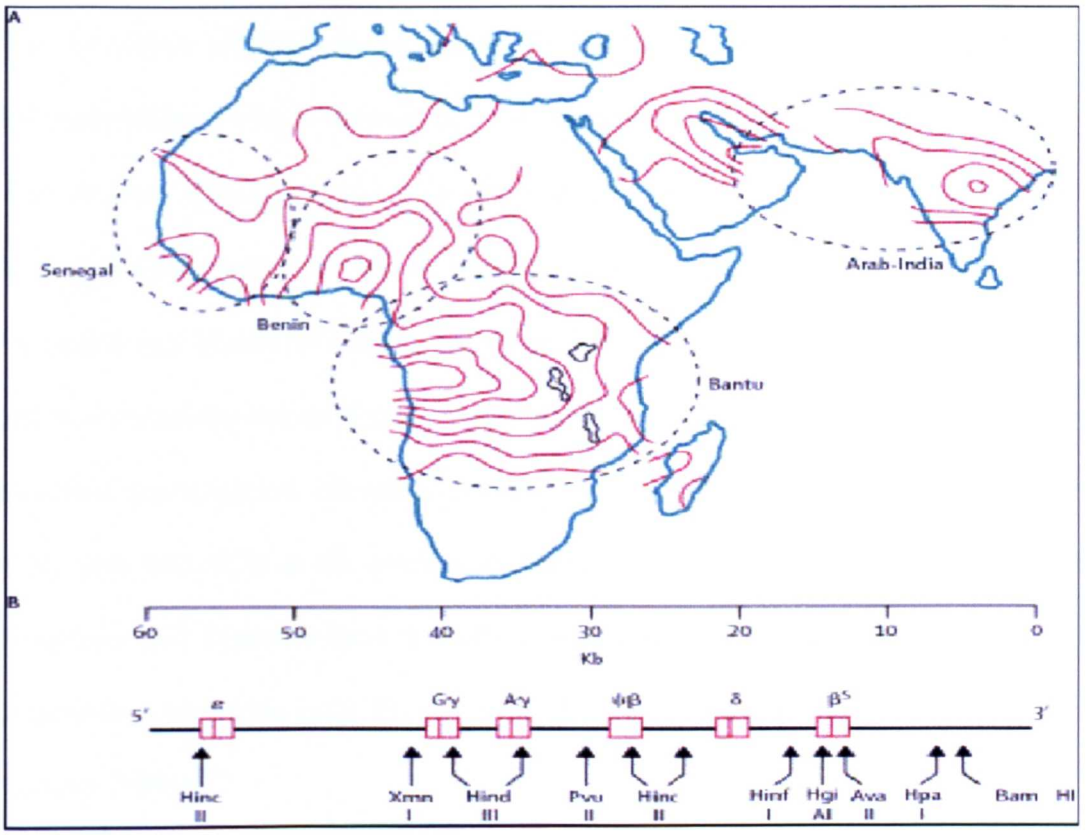


Genomic organisation of the HBB cluster with different polymorphic restriction enzyme sites[9].

Geographic distribution of the sickle gene

The sickle gene is present in three distinct areas in Africa. The Senegal and Benin haplotypes occur predominantly in West Africa and the Central African Republic (CAR), also known as the Bantu haplotype, is predominantly found in East and Central Africa. The Arab/Indian haplotype is found predominantly in the Arabian Peninsula and the Indian sub-continent. Epidemiological studies suggest that the number of individuals with β^S gene are higher near the coast and fall concentrically with progression inland[10].

Figure 4 Geographic distribution of the sickle gene



Schematic representation of geographical distribution of Sickle gene. The boxes are DNA polymorphic sites that determine the HBB-gene cluster haplotype, identified by RFLP. Bantu haplotype is now known as CAR[11].

Sickle cell disorders of clinical significance

SCD refers to a group of disorders that are characterised by the presence of sickle haemoglobin. The SCD syndromes which have significant clinical consequences include the homozygous sickle state (HbSS), known as sickle cell anaemia (SCA), and the compound heterozygous states, HbSC and HbSβthalassaemia. Within this thesis, SCD refers to the homozygous state HbSS.

Epidemiology of SCD

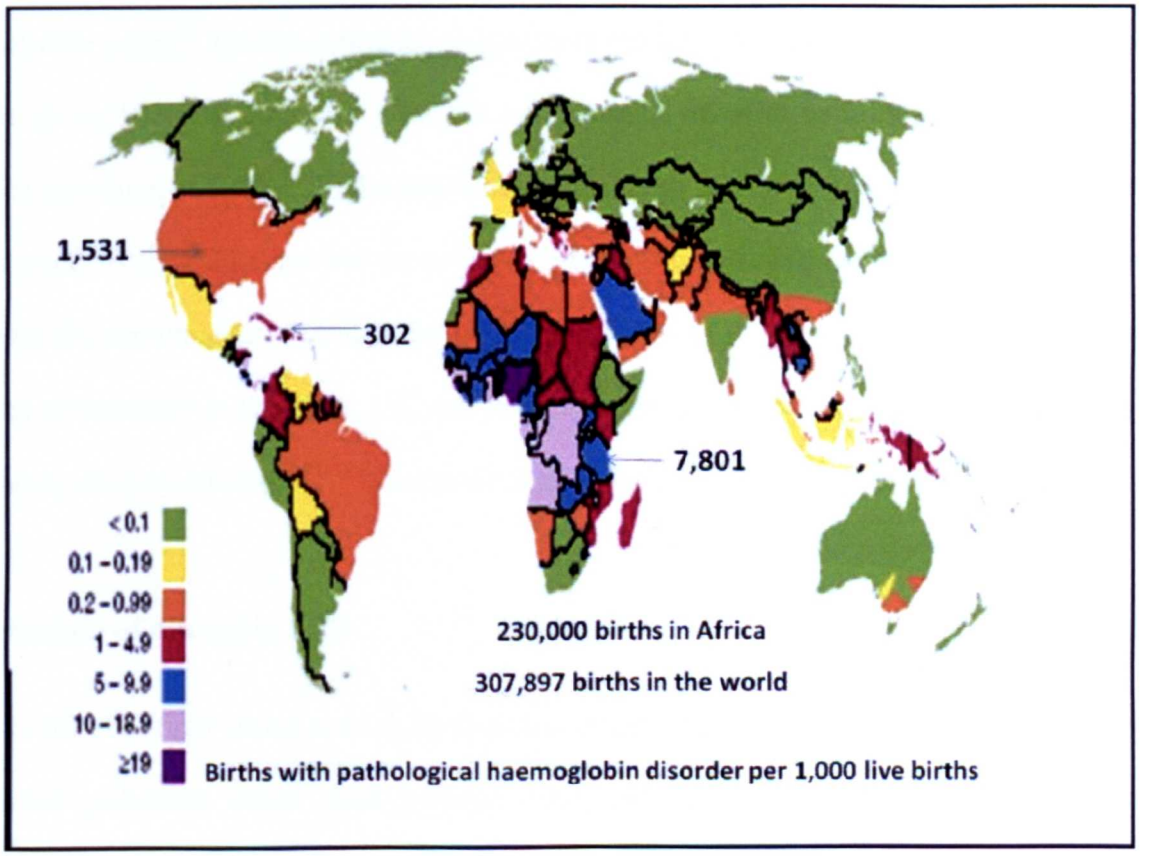
The prevalence of SCD can be objectively determined by calculating the birth prevalence of affected children, which requires accurate diagnosis and registration at birth. Since this is not done in most African countries, an alternative method is to use the prevalence of the carrier or heterozygous states (HbAS) to calculate the expected birth rate of SCA based on the gene frequency and Hardy-Weinberg equation. This has been done in several recent reviews[4,12] and is summarised below. Table 1 and Figure 5 show the global burden of children born with inherited haemoglobin disorders every year[13]. Approximately 300,000 children are born every year with SCD in the world, and countries such as the United States of America, United Kingdom and Jamaica have a well-documented SCD population. However, this SCD population constitutes only 1% of the global population of SCD, as over 75% are in Sub-Saharan Africa[2,14].

Table 1 Frequency of heterozygous state, birth prevalence and estimated annual births in selected areas of the world

Area	HbAS	Birth prevalence of SCD	Annual births
Americas			
USA (AA)	9%	0.4 (1:375)	1,531
Jamaica		5.6	302
Bahamas		7.7	46
Europe			
UK		0.3	196
France		0.4	220
Africa	15-25%		
Nigeria		18	84,636
Tanzania		5.6	7,801
Uganda		6.4	7,821
Kenya		1.2	1,296

AA - African-American; Source [4].

Figure 5 Global map showing the birth prevalence of inherited haemoglobin disorders



Births of individuals with pathological haemoglobin disorders (haemoglobinopathy). The predominant haemoglobinopathy in Africa is due to sickle cell gene whereas in Asia it is thalassaemia. Figures are shown in Tanzania, USA and Jamaica for comparison. Source[15].

The high birth prevalence of SCD has been successful in highlighting the burden of SCD, such that in 2006, the World Health Organisation recognised SCD as a public health priority[1]. However, the birth prevalence does not provide information about the burden to the health system and the impact that it has on individuals.

Individuals with SCD have high mortality, with reports suggesting that most children with SCD in Africa die in early childhood. A study done in Nigeria found that the prevalence of children with HbSS fell from 2.1% at birth, 0.4% at one to four years and to 0.05% over the age of nine years[16]. Recent estimates suggest that mortality has decreased and is more likely to be up to 50% by 20 years with the contribution of SCD to under-five mortality in Africa estimated at 6.4 %[17]. In the 1960s, the mortality in SCD in USA and UK was estimated to be over 50% by 20 years but recent reports have shown improved survival in childhood, with 85.6% surviving to 18 years in the USA[18] and 99.0% to 16 years in the UK[19]. The common causes of mortality in the USA, UK and Jamaica were infections, acute splenic sequestration and acute chest syndrome[20, 21, 22, 23], with the highest incidence between 1 to 3 years of age.

Mechanism of disease in SCD

Due to the abnormal amino acid in the β-globin chain, sickle haemoglobin (HbS) forms long, insoluble polymers when deoxygenated, and the RBC containing HbS become less deformable and form a 'sickle' shape. It was previously thought that the clinical

consequences were simply due to this abnormal, rigid sickle red blood cell occluding small blood vessels. However, there is increasing evidence that the pathogenesis of the various clinical events, both acute and chronic, result from a series of complex mechanisms which are not limited to the RBC.

The complexity of the pathophysiological mechanisms at play were recently reviewed by Stuart and Nagel[24]. Although the mechanisms have been broadly divided on the basis of whether they not they are directly related to RBC (Figure 6), this is an arbitrary division as the processes are closely linked and are probably even more complex than this simple classification. However, for purposes of this review, the discussion will outline factors related to RBC (erythrocyte related) and factors outside the RBC (extra erythrocyte factors).

Erythrocyte related factors

The rate of polymerisation of HbS into the pathogenic β^S-globin polymers is determined by the degree of oxygenation, intracellular pH and the concentration of HbS and HbF within the RBC. Well-oxygenated HbS remains in solution in RBC in the arterial vessels but in the venous circulation, with a reduction in the oxygen content, HbS polymerises and becomes insoluble. This process reaches a point when the RBC loses its capacity to return to its normal shape even with exposure to oxygen and remains an 'irreversibly' sickled cell (ISC)[25]. With regards to the intracellular concentration of Hb, studies have suggested that although the total concentration of Hb in cells influences the HbS polymerisation, it is the relative concentration of HbF which is of greater significance as the ability of HbS to polymerise is significantly reduced by the presence of HbF[26,27] and reduction in the concentration of HbS[28]. Kinetic studies of HbS gelation have also been able to demonstrate the role of oxygen concentration[29] and acidity in HbS polymerisation[30].

Under normal physiological conditions, the population of RBC in individuals is heterogeneous; however this is even more marked in SCD[31]. Pathological consequences are related to the wide variation in the density of SS cells[32], the most extreme one being the ISC. The density of RBC is determined by the volume of the RBC which is carefully controlled. However, there are disturbances in this homeostatic process in SCD which have been linked to two ion-transport mechanisms in the RBC membrane; the potassium-chloride cotransporter and the calcium-sensitive gardos channel which result in cellular dehydration[33]. In addition to the abnormalities to the transporter mechanisms in the RBC membrane, there is also dysfunction in the lipid bilayer, with abnormal exposure of phosphotidylserine components in the phospholipid layer. This mechanism seems to be independent of HbS polymerisation and itself causes membrane rigidity, increased RBC-endothelial interaction and pro-coagulation activity[34,35].

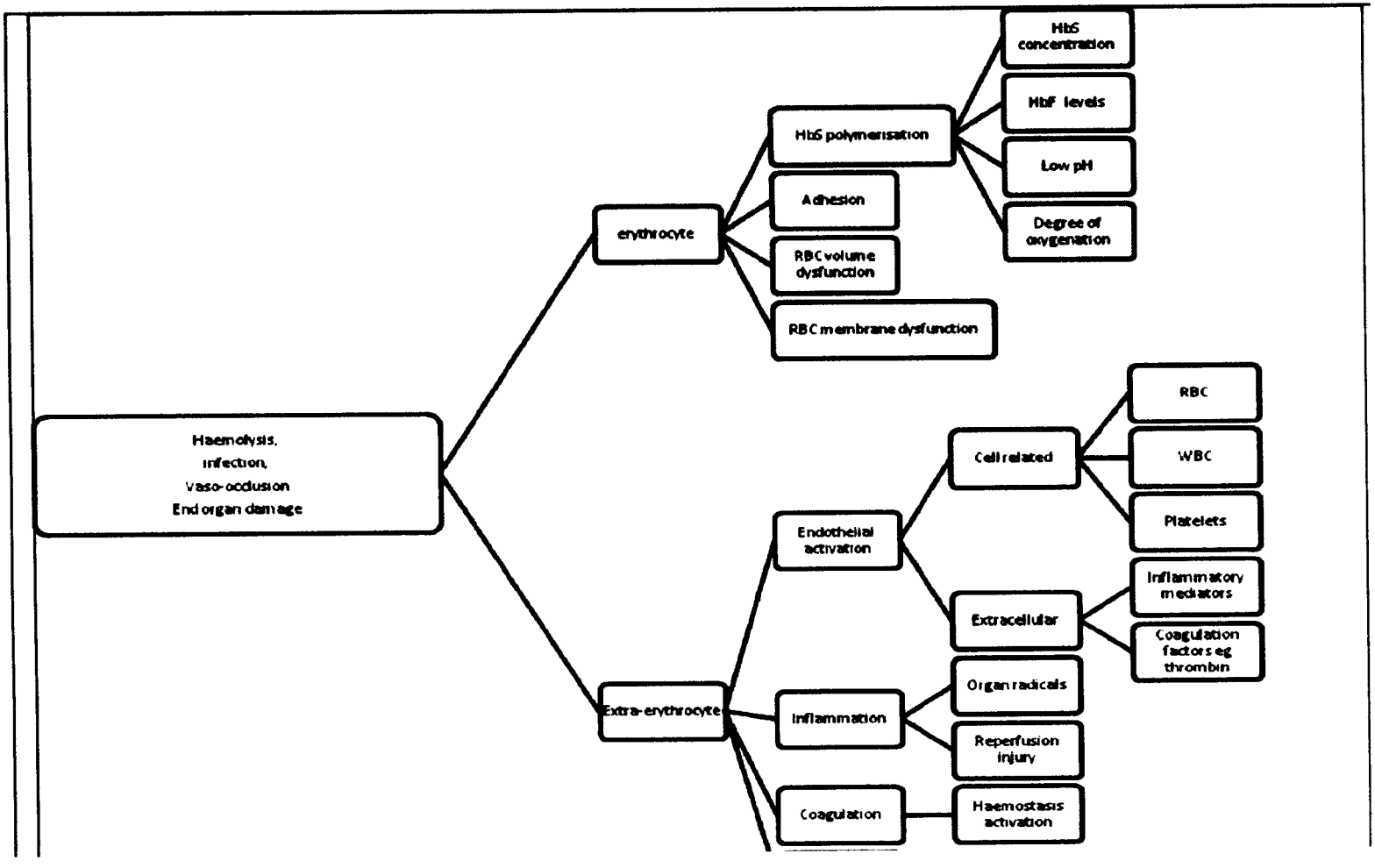
In SCD, vaso-occlusion in both the macro and microvasculature is also thought to result from increased adhesiveness between different components within the RBC, plasma and endothelial wall. The sickle RBC has increased adhesiveness to endothelium[36,37] as well as to other cellular components. In addition, within the plasma, there are factors such as thrombospondin (from platelets) and von Willebrand factor (from endothelial cells) which are also involved[38,39,40,41].

Extra-erythrocyte factors

The endothelium has been found to be activated from both cell-related (sickle RBC, white blood cell and platelets) and extra-cellular (inflammatory mediators, coagulation factors) [38,39,40,41,42,43]. There is increased inflammatory activity with what appears to be reperfusion injury

and an increase in free oxygen radicals[44]. There is also evidence of pro-coagulant activity as well as activation of haemostasis which may contribute to some of the acute and chronic clinical events[45,46,47]. Finally there is compelling evidence of the role of Nitric Oxide (NO) in SCD[48]. The increase in haemolysis in SCD results in an excess of haemoglobin which exceeds the protein-carrying capacity of plasma factors such as haptoglobin. The result is that there is abnormal 'cell-free' haemoglobin which circulates in plasma, binding to and destroying NO, so causing a reduction in the concentration of NO[49]. This results in vasoconstriction, increased adhesiveness of erythrocytes, leukocytes and endothelial cells and platelet aggregation. The clinical consequences of increased vaso-occlusion are particularly seen in the lungs and brain.

Figure 6 Mechanisms of disease in SCD

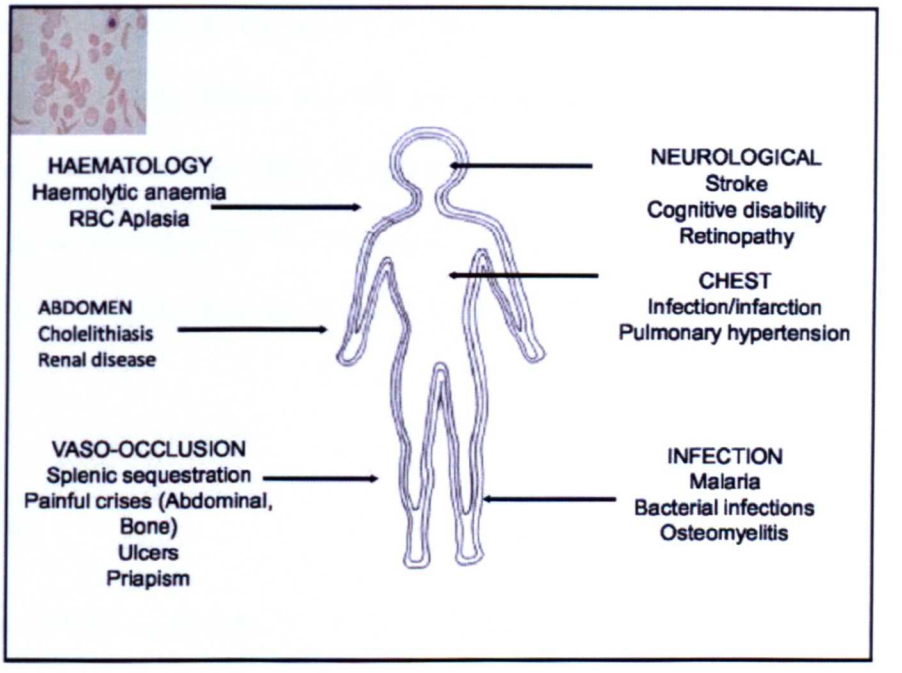


Mechanisms of disease and clinical events in Sickle Cell Disease. This is divided into factors related and not related to erythrocytes

Clinical events in SCD

Although SCD stems from an abnormality of the RBC, it is essentially a multi-system disorder, affecting almost every organ-system of the body, as shown in Figure 7.

Figure 7 Selected clinical consequences of SCD



The clinical consequences can be arbitrarily divided into 4 groups; haemolysis, vaso-occlusion, infection and organ dysfunction.

Haemolysis and haematological complications

At birth, individuals with SCD do not have anaemia, but with the synthesis of adult Hb, they develop chronic haemolytic anaemia that is present throughout life. This may be interspersed with

acute episodes of reduction in haemoglobin – 'anaemic crises'. The chronic haemolysis in SCD results in gall bladder disease due to high levels of bilirubin. Although the main cause of anaemia in SCD is chronic haemolysis, there are other types of anaemia. Hyperhaemolysis crises is defined by a sudden fall in steady state haemoglobin accompanied by increased reticulocytosis and exaggerated hyperbilirubinaemia. Acute splenic sequestration, when there is rapid onset of trapping of red blood cells in the spleen, is characterised clinically by a sudden increase in splenic size, at least 2cm below the left coastal margin, accompanied by a reduction in haemoglobin or haematocrit by 20% of baseline level has been described in SCD and is a significant cause of mortality[50,51,52,53]. Anaemia may be secondary to infections, notably RBC aplasia in the bone marrow that has been associated with infection with parvovirus serotype B19[54,55,56].

Vaso-occlusion

Vaso-occlusion (VOC) is thought to be the underlying cause of painful crises, acute splenic sequestration and priapism (painful and prolonged penile erection). Painful crises, considered the hallmark of SCD, are defined as severe pain lasting for 2 or more hours that is attributable to SCD. The sites that are normally affected include the arms, legs, back, abdomen, chest and head. Painful crises do not include other causes/types of pain in SCD such as dactylitis, acute chest syndrome, right upper quadrant syndrome, osteomyelitis and appendicitis. It is the most common cause of hospitalisation and frequent pain (defined as 2 or more painful events a year for three years) is associated with poor quality of life and increased risk of death[57].

Infection

In the absence of prophylaxis, infections are thought to be the leading cause precipitating clinical events and associated with increased mortality[22,23,58]. The role of bacterial infections and malaria in SCD will be reviewed in detail in the respective sections as they are the focus of the thesis.

End-organ dysfunction

With increase in survival, major organs in individuals with SCD are eventually damaged. The brain and chest are particularly affected, with stroke, defined as an acute neurological syndrome due to vascular occlusion or haemorrhage in which symptoms and signs last for more than 24 hours, being a well-described event. Acute chest syndrome (ACS) is an acute respiratory illness characterised by new pulmonary infiltrates on chest x-ray[59,60]. Both these events have been reported to occur with high prevalence in SCD and are also risk factors for death[23,60,61]. The mechanism of disease in stroke in SCD will be reviewed in subsequent section as it is a main focus of this thesis.

Heterogeneity of clinical events in SCD

The clinical consequences of SCD are extremely heterogeneous in several ways. There is inter-individual variability with some individuals who are completely asymptomatic while others have extreme, debilitating illness. There is also variability within an individual, with changes in

the type and frequency of clinical events with age. Finally, there is variability in clinical events depending on the geographical location. This is due to the differences in environmental factors such as nutrition, socioeconomic status, and climate that will influence the natural history of disease. The general pattern of clinical disease is characterised by quiescent periods interspersed with acute events, which are referred to as crises.

Table 2 The prevalence of selected clinical consequences of SCD

Clinical event	Prevalence	References
Haemolysis		
Anaemia	Chronic	[62,63, 64,65,66,67,68]
Cholelithiasis	Prevalence is 40% by adolescence	[69]
Aplastic anaemia	Associated with parvovirus B19 infection	[55,70,71]
Hyperhaemolysis	Not common in Africa	[72,73,74,75]
Vaso-occlusion		
Pain	More than 60% patients. Most common cause of admission	[23,57,76,77,78,79]
Acute splenic	Frequently occurs before the age of 3yrs	[23,50,51]
Leg ulcers	Prevalence is 10-25% adults	[80,81]
Priapism	Prevalence is 10-40% males Occurs frequently 5 - 14years age group	[82]
Organ dysfunction		
Neurological events		
Stroke	Prevalence is 10% in children Risk factor for mortality	[83]
Cognitive /silent	Prevalence is 20% Risk factor for overt stroke Leads to impairment of executive function	[84,85,86,87]
Retinopathy	Prevalence is >30% in HbSC	[88,89]
Chest		
Acute chest syndrome (ACS)	Prevalence is 40%. Occurs frequently in children, Has severe consequences in adults	[59,60,61]
Pulmonary hypertension	30% Risk factor for mortality	[90,91,92,93,94]
Avascular necrosis of femoral head	Prevalence is 10-50% in adults	[95,96,97]
Renal disease	Prevalence of chronic renal failure is 5% 20%	[98]
Infections		
Malaria		[99,100]
Bacterial infections	10% children under 5 years	[101]

Modified from [102,103].

Diagnosis of SCD

The laboratory diagnosis of SCD is based on the demonstration of HbS and the absence of HbA, with variation in the percentage of the other 2 haemoglobins - HbF, HbA_2 - in RBCs. The tests involve the initial detection of HbS by screening tests which include sodium metabisulphite sickling test and sickle solubility tests. This is followed by tests to confirm the sickle phenotype (SS/AS/SC/$S\beta^-$thalassaemia).

There are three tests that are widely used - haemoglobin electrophoresis (HBE), isoelectric focusing (IEF) and high performance liquid chromatography (HPLC). DNA-based tests can be done to precisely describe the genotype. However, for clinical purposes, the diagnosis usually involves screening (sickling or solubility test) followed by confirmation of phenotype using one or two of these tests (HBE, IEF or HPLC).

Screening tests

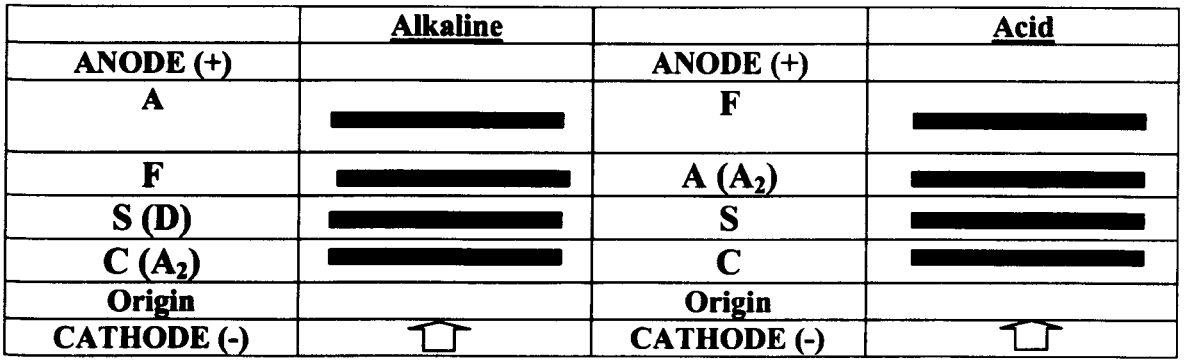
In most African hospitals, screening is done, using the 'sickling test', which involves making a thin blood film which is then put under hypoxic conditions by the addition of sodium metabisulphite. This will result in RBCs containing HbS forming sickle cells which are seen under a light microscope. A "positive" sickling test identifies the presence of sickled RBCs, which occur in both homo (SS) -and heterozygous (AS) states. So to confirm SS-SCD or other SCD, other tests need to be done. The sickle solubility test is another method used for screening which is based on the principle that Sickle Hb becomes insoluble when it is deoxygenated.

Confirmatory tests

These tests are based on the principle that the different haemoglobins have different overall ionic charge which makes them move with different velocities in an electric field. The principles and procedures for HBE and HPLC are in appendix III.

HBE can be done under alkaline or acidic conditions, where HbA, HA_2, HbF and HbS migrate towards the anode under an electric field with different speeds. The results are shown schematically in Figure 8, showing that during alkaline Hb electrophoresis the resolution between HbS and HbF can be poor, particularly in individuals with high HbF levels e.g. neonates. Under acidic conditions, HbF moves much faster and is therefore distinct from both HbA and HbS (Figure 8).

Figure 8 Electrophoresis of common haemoglobin variants under alkaline and acid conditions

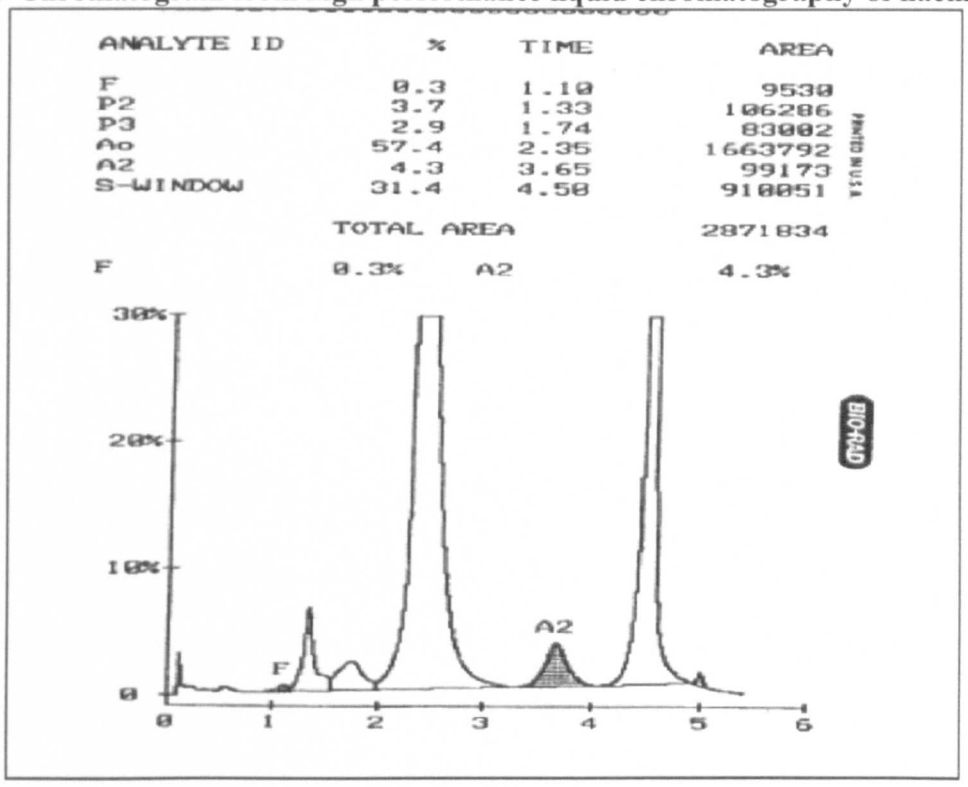


Isoelectric focusing uses the same principles but is slightly more expensive than HBE.

However, it is able to identify more Hb variants that would not be detected by HBE. It also has the advantage that it does not require reagents which are commercially prepared.

HPLC uses cation exchange chromatography to identify the various haemoglobins in an individual. It has the advantage in that it can also accurately quantify the Hb levels. In resource rich countries, screening has largely been replaced by HPLC and confirmation is then done by IEF or HBE. This is mainly because HBE and IEF are labour intensive, time consuming and would not identify abnormal bands or quantify Hb. Furthermore, the quantification of Hb fractions by HPLC is used to monitor patients who are on Hydroxyurea therapy or exchange blood transfusion.

Figure 9 Chromatogram from high performance liquid chromatography of haemoglobin



The peaks correspond to the quantity of each haemoglobin component as a fraction of total haemoglobin. Source [104]

The distribution of the different haemoglobins in individuals with different sickle phenotypes is shown in Table 3.

Table 3 Distribution of haemoglobin fractions in AA, AS and SS individuals

			Percentage of total haemoglobin		
		Foetus	Adult (AA)	Adult (AS)	Adult (SS)
HbA	$\alpha_2 \beta_2$	5-10	97	55-70	0
HbA_2	$\alpha_2 \delta_2$		2-3	2-3	2-3
HbF	$\alpha_2 \gamma_2$	60-80	0.5-1	0.5-1	2-10
HbS	$\alpha_2 \beta_{S2}$		0	30-45	90

Source[105]

Although detection of the abnormal protein (HbS, HbC, and HbE) is usually adequate for clinical diagnosis, definitive diagnosis of the genotype requires examination of DNA. This includes methods such as RFLP, PCR and sequencing, which would not only allow the correct description of the genotype pattern but would also allow further differentiation of the haplotypes. With recent advances in genomic medicine, genotypic diagnosis has become readily available and is routinely used in some reference centres for screening and diagnosis of SCD.

Interventions in SCD prevention, treatment and control

As a chronic disease, the natural history of SCD is such that it is characterised by quiescent periods interspersed by acute events, known as crises, leading to patients seeking health care and frequent hospitalisation. The 'crises' range from well characterised syndromes such as acute chest syndrome (ACS), acute splenic sequestration (ASS), to less well defined symptoms

that include pain, fever, anaemia, worsening of jaundice and leg ulcers. There are specific conditions or circumstances when SCD patients require extra care. Peri-operatively, when SCD individuals undergo general anaesthesia and surgery, there is an increased risk of developing acute sickling complications and sudden death. Other circumstances include pregnancy, dehydration, and extreme cold weather. With the increased life span of individuals with SCD, there has been an increasing awareness of the importance of improving the quality of life and well as preventing damage to organs such as the spleen, heart, lungs, brain, eyes, and kidney.

SCD is associated with increased mortality. The causes of mortality in the USA, UK and Jamaica included infections, ACS, ASS, aplastic crises[20,21,22,23]. Other events such as stroke, pulmonary hypertension, and haemolysis have also been reported to be associated with increased risk of death.

The management of patients with SCD involves interventions that improve survival, prevent and treat acute events and reduce end-organ damage. Over the past 3 decades there has been an improvement in the understanding of the different pathogenic mechanisms responsible for sickle cell events and organ dysfunction. Through a series of clinical trials, effective interventional strategies have been established. For purposes of this thesis, I will provide a brief overview of the interventions available, but will focus on interventions that are pertinent to the situation in East Africa.

Newborn screening (NBS)

The highest incidence of death occurs in the first 3 years of life[20,22,23,106]. Therefore, identification of children at birth by newborn screening (NBS) leads to improved survival[18, 107,108,109]. Patients who are identified at birth can be given proper counselling and advice about the course of illness. They can then be enrolled in comprehensive care programmes which provide prompt and effective interventions as well as providing prophylaxis against complications which has a positive impact in reducing mortality and improving quality of life. Countries with large SCD populations and adequate resources have started NBS programmes. These were initially targeting high-risk populations, but most States in the USA as well as other areas in Europe are now recommending universal screening[110].

Comprehensive care including dedicated day care facilities

The identification of SCD at birth has to be accompanied by enrolment into programmes that provide comprehensive care. These programmes provide appropriate advice, counselling and support to parents and affected individuals. This includes advice such as drinking adequate quantities of fluid to avoid dehydration and wearing proper clothing in cold weather. Specific health education that will enable them to recognise acute events and seek medical care is also essential. Teaching mothers to recognise enlargement of the spleen and anaemia was effective in diagnosing and treating anaemia due to ASS[51,53]. Patients are also seen on a regular basis and provided with folic acid supplements. Prompt treatment of crises (fever and pain), particularly at outpatient or in day-care facilities, has been found to be effective and reduces the burden of hospitalization to the individual and the health system[111,112,113, 114]. This has been found to

have a significant impact on not only quality of life but also mortality[7,114,115,116].

This approach that has been found to be effective with comprehensive, multi-disciplinary teams, with long term care being provided by professionals who have specialised in haematology and blood transfusion. In settings where there is a low prevalence of SCD or limited number of health care professionals, SCD patients can receive care from general health care workers. In such a setting, guidelines for management can be provided to general health care workers with a system of referral to specialised centres.

Prevention and treatment of infections

Bacterial infection in SCD is mainly due to *Streptococcus pneumoniae,* resulting in pneumonia, sepsis and meningitis. The highest incidence of invasive disease is in children less than 6 years of age[20,101]. In a landmark study in the USA, Gaston and colleagues demonstrated an 84% reduction in incidence of pneumococcal infection with the use of oral penicillin[117] and there has been a significant change in survival with acute treatment of infections[118].

Blood Transfusion (BT)

Individuals with SCD have chronic anaemia which is tolerated. However, rapidly worsening anaemia can occur and this presents as an emergency. It can be caused by ASS, aplastic crises, hyperhaemolysis or associated with other events such as bacterial infections and malaria. Under these circumstances, anaemia is life-threatening and requires prompt treatment with blood transfusion. The products that are used (whole blood or packed RBCs) and the method

of transfusion (simple or exchange) are determined by the clinical situation, availability of resources and the capacity to provide the blood product and establish venous access[119]. Acute BT is also effective in other conditions, such as acute stroke[120], ACS[121] and peri-operatively[122]. BT works by increasing the level of Hb, thus improving oxygen delivery. It also reduces the proportion of sickle RBCs in the circulation. Exchange or red cell transfusion has also been shown to be effective in reducing the level of HbS to less than 30%[123,124,125,126]. This is thought to reduce the deleterious effects of HbS and improve outcome.

Long term blood transfusion therapy (LTBT) has been found to be effective in the prevention of brain injury due to cerebrovascular disease[120]. Blood transfusion is associated with risks which have to be weighed against the benefits when considering implementing this is an intervention. These will be reviewed in the section on stroke.

Prevention and treatment of malaria

It is recommended that individuals with SCD who live in a malaria endemic area should receive prophylaxis against malaria[127]. Furthermore, any clinical suspicion or diagnosis of malaria in SCD should be treated promptly.

Hydroxyurea

Hydroxyurea (HU) (also known as hydroxycarbamide) has been reported to be effective in improving survival and reducing morbidity in some SCD patients (Table 4). The clinical outcomes include reduction in frequency of painful episodes, and hospital admissions[128].

Hydroxyurea therapy is also monitored by a number of laboratory parameters which include increase HbF levels, mean corpuscular volume (MCV) and reduction in WBC count. Hydroxyurea has been found to be effective in the prevention of brain injury due to cerebrovascular disease[129].

Table 4 Summary of study outcomes for hydroxyurea use in adults and children

Outcome	Impact in adults	Impact in adolescents
Clinical outcomes		
Pain crises	↓↓↓	↓↓
Hospitalisations	↓↓↓	↓↓↓
Blood transfusion therapy	↓↓↓	↔ (insufficient data)
Acute chest syndrome	↓↓↓	↔ (insufficient data)
Laboratory markers		
Foetal haemoglobin	↑↑↑	↑↑↑
Haemoglobin	↑↑↑	↔ (not significantly
Mean corpuscular haemoglobin	↑↑↑	↑↑↑
White blood cell count	↓↓↓	↓↓↓
Prevention of end organ damage		
Brain	↔	↔
Spleen	↔	↔
Kidney	↔	↔
Mortality	↓	↔=

↓↓↓=high-grade evidence for decrease; ↓=low grade evidence for a decrease; ↑↑↑=high-grade evidence for increase; ↑=low grade evidence for an increase; ↔=not evaluated/not significantly different/insufficient data. Source [130]

Nitric oxide

Lung dysfunction results from a combination of repeated pulmonary infections and infarctions as well as increased vasoconstriction leading to pulmonary hypertension[59, 60]. The latter has recently been shown to be due to reduced bioavailability of nitric oxide[49], which has resulted

in therapies such as L-arginine, citrulline and inhaled nitric oxide which is aimed at increasing NO levels through different pathways[131,132,133,134,135].

Stem cell transplant

The only potential cure that is available for SCD is stem cell transplantation (SCT), which replaces the host's bone marrow with stem cells containing normal β-globin genotype. Since the first successful transplant reported in 1984[136], there has been significant reduction in risks due to SCT and increasing success, with the best results, of up to 85% event free survival, occurring with HLA-matched sibling donors and transplantation early in the course of the disease before end-organ damage occurs[137,138,139,140]. One limitation of SCT is the availability of sibling donors[141], and therefore there have been attempts to improve survival for unrelated stem-cell donors[142,143]. However, the second limitation is more difficult to address because the clinical course of SCD is extremely heterogeneous. Despite the knowledge of various genetic and environmental factors known to alter disease severity, it is still difficult to accurately identify children with risk of severe disease before extensive damage has occurred. Until such time that a low-risk, definitive cure is available, the cornerstone of management of SCD is the prevention of early mortality, prevention of end organ damage and improvement of the quality of life.

Gene therapy

Since SCD is caused by a defective gene, definitive treatment would involve replacement of this gene with a normal gene. This has been done successfully in the sickle transgenic

mouse[144] but progress in humans has been limited by identification of appropriate vectors and efficacy for gene transfer and low level expression of globin genes.

Role of programmes for control and management of SCD

From a public health perspective, the policy for approaching the control of SCD in national health programmes needs to integrate two main areas: the provision of appropriate medical care to affected individuals (management) and the prevention of disease. Although, there is ongoing debate whether care of SCD should be integrated into existing health care services or whether there should be separate disease-specific programmes for SCD, the WHO recommends[3] that for countries where the birth rate of affected infants is above 0.5 per 1,000 births, they should develop separate programmes for these conditions.

Management

The management of SCD involves early diagnosis of affected people, the provision of the most appropriate basic, cost effective treatment and genetic counselling and psychosocial support. The long term goal is to ensure appropriate management at different levels of health care with development of referral centres for specialised diagnosis and treatment. This approach ensures a cost effective way of effectively dealing with a highly prevalent condition in areas where the resources are limited. However, it is important that these centres are not limited to urban areas or centred on academic or research oriented health facilities. In order to avoid this, there must be active strategies to ensure that appropriate management is built into services at all levels of health care with adequate support from these specialised centres.

Prevention

Management of SCD patients needs to be accompanied by strategies aimed at two levels of prevention: tertiary prevention which involves early diagnosis of SCD and prevention of complications and more ambitiously, secondary prevention which tries to reduce the number of children that are born with SCD. (Note that primary prevention aims to ensure that individuals are born free of SCD). Preventative services involves community education, population screening and genetic counselling that would encourage people to undergo screening before conception, during the antenatal or postnatal period. There are several issues that need to be addressed with regards to prevention of SCD. The aim of screening is to detect SCD in the foetus, discuss the consequences of a diagnosis of SCD, options for treatment and prognosis. Since SCD is a recessive disorder, during pre-conception screening, the chances of getting an affected child is shown in table below.

Table 5 Likelihood of children being born with SS SCD from parents with different sickle phenotypes

Parent	Parent	Possible phenotype of offspring	Chance of affected children (SS)
AA	AS	AA,AS	Nil
AA	SS	AS	Nil
AS	AS	AA, AS, SS	1:4
SS	AS	AS, SS	1:2
SS	SS	SS	1:1

This illustrates the difficulty in advising a couple not to have children as the risk of getting an affected child is relatively low (1 in 4), and does not increase with each pregnancy. The highest

risk would be for two individuals who are SS who wish to have children. This is different from thalassaemia, where children with the most severe form, thalassaemia major, will inevitably have severe disease. Therefore, one could argue that this therefore justifies the use of pre-natal diagnosis as this would identify pregnancies with SCD children and then parents would be given the appropriate information regarding the consequences and prognosis of SCD. However, although SCA is the most severe form of the disease (compared to SC/Sβ-thalassaemia etc), there is still wide variability in disease severity. Therefore, even with the correct identification and diagnosis of SS with screening, it would be difficult to predict those who would develop severe disease and have a poor outcome.

SICKLE CELL DISEASE IN AFRICA

In this section, the importance of SCD in Africa will be reviewed from a public health perspective, highlighting the burden of disease. This will be followed by an outline of the rationale in investing in SCD, from a public health as well as from a scientific perspective. Finally, there will be an overview of SCD in Tanzania.

Importance of SCD – public health perspective

In May 2006 at the 59th World Health Assembly (WHA), resolution WHA 59.20 of the World health Organisation (WHO) was to develop, implement and reinforce integrated national programs for the prevention and management of Sickle Cell Disease (SCD). The WHO identified SCD a problem of public health significance as it is has a high prevalence, causes significant burden to health systems by having a high morbidity and mortality and relatively simple, cost-effective interventions can prevent some of the complications associated with this condition[3].

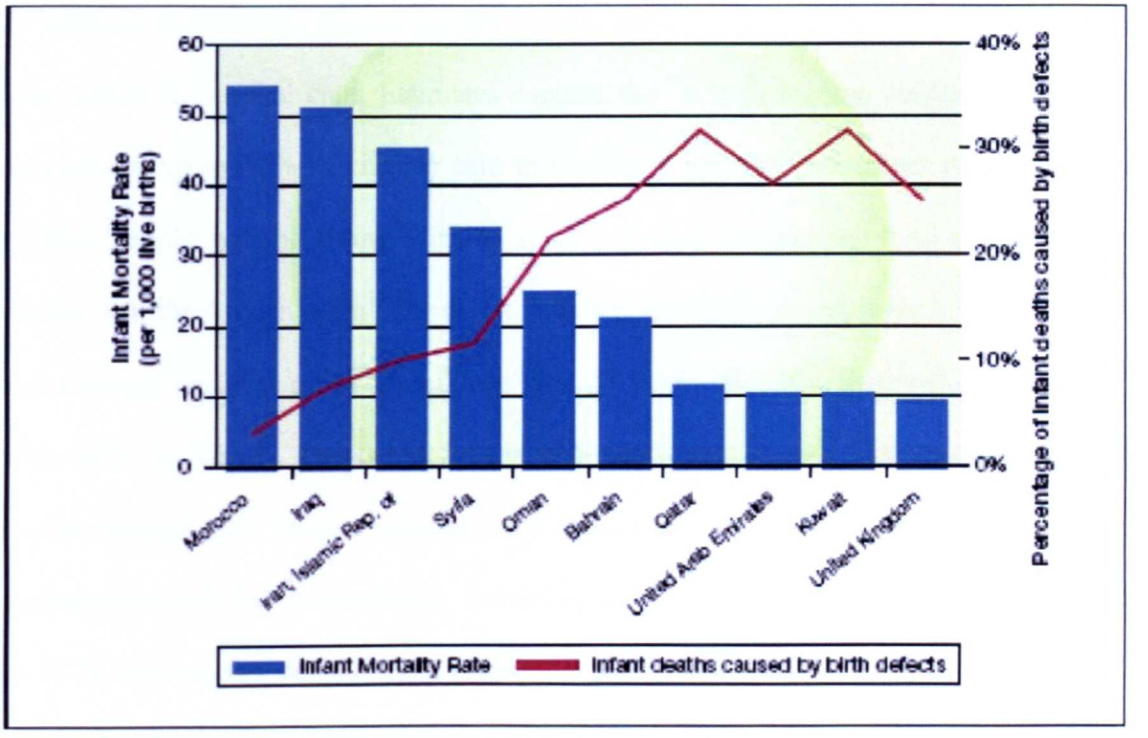
Health burden of SCD - prevalence

In order to determine the epidemiology and health burden of SCD in Africa, the WHO requires an accurate estimate of the magnitude of the disease and recognised consequences, specifically mortality and socio-economic burden of the disease to individuals, families and the health system.

It was previously thought, that although the birth prevalence of SCD is high in Africa, SCD

was not a significant health burden because the majority of children would not survive the high childhood mortality. However, there is evidence of increasing population prevalence of SCD[81,145,146,147,148,149,150]. Although this may be due to improvement in detection of SCD, this is more likely due to reduction in mortality due to infectious diseases. Although there is still much to be done, there is significant success in the control of infectious diseases such as malaria, lower respiratory tract infections, malnutrition and diarrhoeal diseases. Furthermore, African countries are becoming increasingly heterogeneous with improvement in education, health care and socio-economic status particularly in urban areas. This results in a demographic health transition where the impact of birth defects will increase (Figure 10). Genetic disorders, which include pathological haemoglobin disorders, account for up to 30% of birth defects[5]. The proportionate contribution of SCD to infant mortality is increasing, with estimates suggesting that the contribution by SCD to under-five mortality in Africa is 6.4%. However, without taking into account the inevitable health transition and within the context of infectious diseases, genetic diseases have a considerable burden on the health care system.

Figure 10 Effect of demographic health transition in selected countries



Modified from [15]

Health burden of SCD: morbidity and mortality

With an increase in detection of SCD children, the previously unrecognised burden of SCD on the health system will become more apparent. SCD has been estimated to account for up to 14% of general infant mortality rate (4-8% of under-five mortality)[5]. In West Africa, the under 5-year mortality rate that can be directly attributed to SCD has been estimated at 20 per 1,000[151]. In Africa, assuming a mortality of 50 to 80 percent under 5 years of age implies that there are at least 21,600 to 34,500 deaths per year due to SCD. This is most likely an underestimate as overall mortality may reach 173,000 per year. This accounts for between 1-2% of burden of mortality in the under 5-year age group[6].

In addition to mortality, because of the chronic nature of the disease, SCD patients require life-long access to hospital care. Estimates suggest that in high income countries, genetic diseases account for up to 40% of chronic care in paediatric practise[12]. A recent report suggests that 6 million people will be living with SCD in Africa if average survival of affected children reaches half the African norm[17] (by this we assume, that if the life expectancy in Africa is 50 years, and average survival of SCD children was 25 years, then there would be 6 million people with SCD in Africa). These individuals will need lifelong care with complications that will require hospital care. This includes anaemia without any other complications or anaemia requiring management with blood transfusion. Painful episodes, which are the most common complication in SCD also causes considerable burden to the individual and to the health system if they require hospitalisation. Other clinical events, such as orthopaedic complications due to avascular necrosis of the femoral head, neurological complications due to stroke and seizures, leg ulcers and retinopathy also contribute to the disability due to SCD[6].

The financial burden of SCD has been estimated to illustrate the need to develop and plan health services for SCD individuals. A report from the WHO estimates that the cost of health care for SCD in Jamaica is approximately $300 per patient per year[3]. This is based on estimates in resource-rich countries or with established SCD research programmes. Table 6 shows an estimate of the cost of providing care to 1000 SCD patients in Jamaica. Recent studies have shown that it is possible to provide comprehensive care, with significant improvement in morbidity and mortality in low income settings[152].

Table 6 Estimates of cost of care of 1000 SCD patients in Jamaica

	cost USD/ patient/year*	fraction of patients	number	Total (USD)
Outpatient care	250	1	1,000	250,000
Inpatient care	2,500.00	0.85	850	2,125,000
Transfusion	25,000.00	0.1	85	2,125,000
Exceptional problems	10,000.00	0.1	85	850,000.00
				5,350,000

*Exceptional problems include surgery and pregnancy.[3]

Control programmes for SCD in Africa

Most African countries do not have adequate resources to set up national programmes for control of SCD. This is 'justified' by the argument that low-income countries do not have the resources to deal with genetic diseases in the face of pandemics due to infectious disease such as Human Immunodeficiency Virus/ Acquired Immune Deficiency Syndrome (HIV/AIDS), tuberculosis and malaria.

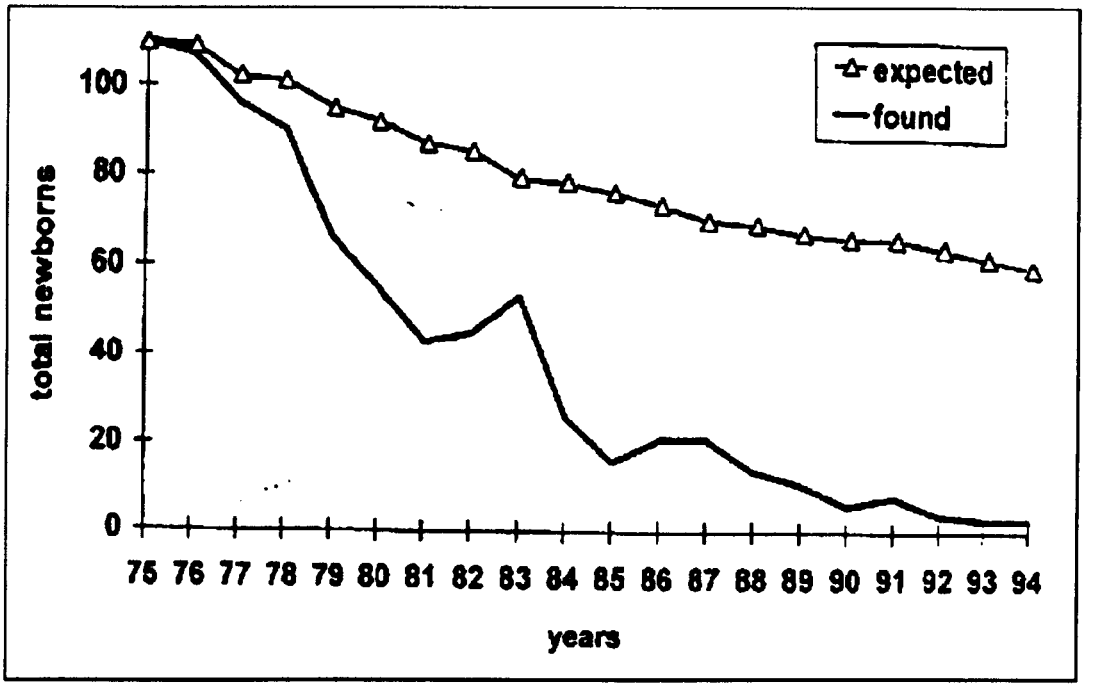
It is recommended that counties with a high prevalence of SCD, start planning effective control measures. In this context, control of SCD encompasses two elements: providing best possible care for affected individuals and preventing the birth of affected individuals. With regard to providing best possible care, the following are options, depending on available resources, that have been recommended by Weatherall et al in 2006[6]:

- *Option one:* best possible patient care with the use of prophylactic penicillin following diagnosis, together with retrospective genetic counselling.

- *Option two:* best possible patient care, together with a newborn screening program and the use of penicillin for all homozygous babies, together with retrospective screening and counselling.
- *Option three:* best possible patient care, together with newborn screening and the use of prophylactic penicillin from birth for homozygotes, together with population screening and prospective genetic counselling.
- *Option four:* as for option three, plus the availability of prenatal diagnosis, bone marrow transplantation, or both.

Some countries have been able to demonstrate a significant reduction in the birth of affected individuals by effective public education and screening programmes. Figure 11 below shows the experience in Sardinia with control of β-thalassaemia, where the number of affected individuals was reduced from 1:250 to 1: 4,000 live births[3].

Figure 11 Impact of effective control programme on birth rate of β-Thalassaemia in Sardinia[3]



Although there are differences between SCD and Thalassaemia, it would not be overly ambitious to aim for such successful results for SCD. SCD has the advantage that the screening and diagnosis are relatively cheap and easier to do (technologically) and the interventions e.g. oral penicillin, pneumococcal vaccination) could be integrated into existing programmes e.g. expanded programme of immunization (EPI), essential drug programme (EDP). However, it is difficult to justify termination of pregnancy in the event that prenatal diagnosis confirms that a child has SCD. This is because homozygous SCD is extremely heterogeneous and it is not possible to determine the course of the disease in an individual.

SICKLE CELL DISEASE IN TANZANIA

Burden of disease

The prevalence of SCD in Tanzania is not known. Estimates of the birth prevalence have been provided by calculating the birth prevalence of SCD from the prevalence of the heterozygous carriers. The most recent report estimates that up to 8,000 children with SCD are born in Tanzania every year[153]. This figure makes Tanzania the country with the fourth highest SCD birth rate in the world, after Nigeria, Democratic Republic of Congo and Angola.

The population prevalence of SCD in Tanzania is not known. It is assumed that in the absence of treatment, up to 95% of SCD born in Africa die in childhood[6]. However, it has been reported in Tanzania that some SCD individuals survive into adulthood[146]. Observations in hospital records (personal observation) suggest that there is an increase in the number of SCD patients who are hospitalised and who attend the clinic. It is difficult to know whether this reflects an increase in number as a result of improvement in diagnosis and identification or whether there is an increase in survival.

Healthcare for SCD in Tanzania

The healthcare in Tanzania is mainly provided by the government, through its health facilities organised in different levels of healthcare. The government has recognised that since individuals with SCD will require healthcare throughout their life, the cost would be a considerable burden to the individuals. Therefore, the government has a policy that SCD patients should receive free access to health care in government health facilities. At the level of

health care facilities, SCD patients receive symptomatic treatment for acute events such as pain, anaemia and infections, and this is normally provided by general health services, internal medicine or paediatrics. There are limitations in technical expertise to provide a correct diagnosis of SCD and its complications such as malaria, bacterial infections, and stroke. In addition, there are few health personnel who are adequately trained to provide care for SCD patients. There are no health facilities which provide comprehensive care programmes for SCD patients, despite the fact that this has been shown to have major impact on reducing morbidity and mortality as well as being more cost-effective. In order to improve the healthcare for SCD patients in Tanzania, it is critical to get local evidence. This will be used to tailor interventions that are known to be effective in other populations so that the interventions that are implemented are appropriate to the individuals, health system and environment.

Within the community, there is little awareness about the prevalence and social and psychological impact of SCD in Tanzania. Personal observation at the clinic and with individuals with SCD suggests that there is stigma associated with the condition with mothers being abandoned because of delivering a child affected with SCD. Within the health facilities, personal observation has highlighted that SCD patients who are hospitalised with pain receive minimal and inadequate analgesia and are often labelled as being narcotic-dependent or addicted. Individuals with SCD may have difficult in receiving a good education due to school absenteeism as a result of illness or some have reported opting not to attend school due to the stigma of chronic leg ulcers or jaundice. Furthermore, some SCD patients cannot get employment because of their illness. In order to address the lack of public awareness in

society, a non-governmental organisation known as the sickle cell foundation of Tanzania was founded by an individual with an affected child who approached me expressing her desire to establish an organisation that would raise awareness of SCD in Tanzania. This was registered in 2008. The foundation will work with other organisations and individuals in Tanzania to encourage patient advocacy and provide support to individuals and families affected by SCD.

As Tanzania goes through a health transition, it is expected that there will be reduction in mortality due to infectious diseases and malnutrition. Therefore, the proportionate contribution from non-communicable diseases, including genetic diseases, will significantly rise. The Ministry of Health and Social Welfare has recognized the increasing importance of SCD and has published a strategy for developing a national policy for the management and prevention of SCD in Tanzania.

SICKLE CELL DISEASE AND MALARIA

Introduction

There has been extensive research to understand the complex relationship between sickle haemoglobin and malaria, based on the evidence that sickle haemoglobin confers protection against malaria in individuals with AS. This section will review the literature that is available to support the protection as well as explore the mechanisms proposed for protection. The second section will look at malaria and SCD, exploring the ways in which the two conditions influence each other, with particular focus on evidence of protection and susceptibility to malaria in SCD patients. This will be followed by an outline of the approach to management to malaria in SCD. The final section will review the research questions that need to be explored to start providing evidence to guide the approach to malaria in SCD.

Sickle haemoglobin and protection against malaria

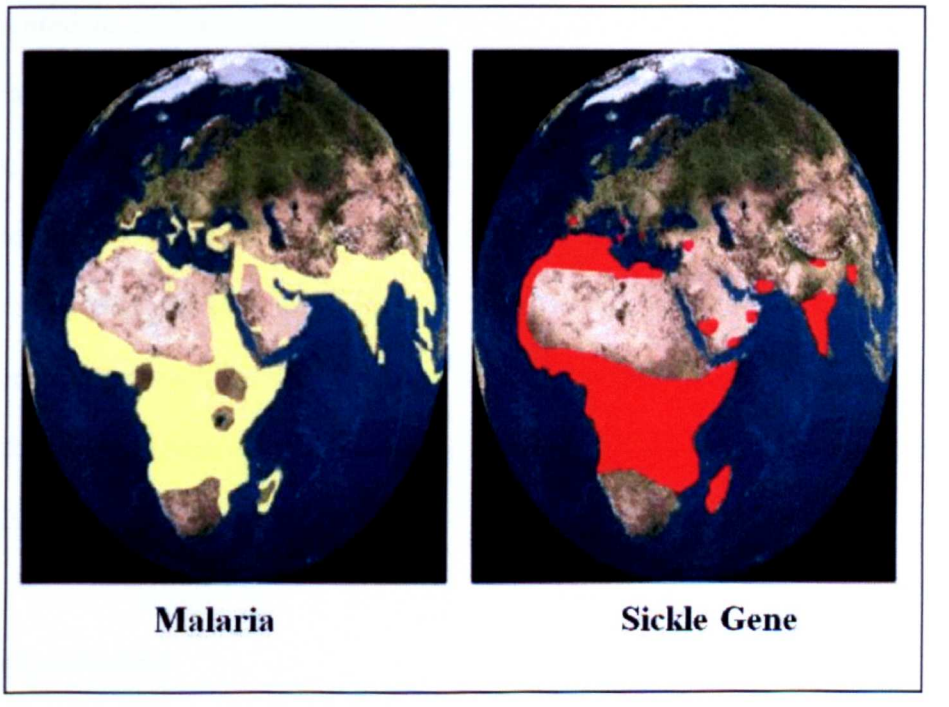
The sickle gene is regarded as the classical example of balanced polymorphism, a theory that was proposed by Haldane over 50 years ago with regards to thalassaemia[154]. Although individuals with homozygous state for sickle cell (SS) have an increased risk of premature death, this is balanced by the fact that individuals with the heterozygous state (AS) are protected from dying from severe malaria[155]. The consequence of this is that the sickle gene has achieved high frequencies in malaria endemic areas[156, 157].

Geographical distribution

The striking overlap of the geographical distribution of the sickle cell gene and malaria is

compelling evidence of balanced polymorphism. Figure 12 shows that the overlay between the sickle gene and malaria is almost complete, with the exception of South Asia and Oceania. In this area, the predominant haemoglobinopathies are haemoglobin E (HbE) and α-thalassaemia whereas in sub-Saharan Africa it is HbS. This is an example of negative epistasis between haemoglobinopathies, where the presence of one gene modifies the expression of another, resulting in the predominance of one haemoglobinopathy in a population[11]. Recent studies in Kenya suggested a reduced effect of protection by α-thalassaemia or sickle trait when they occur together, which may account for the predominance of the sickle gene in East Africa[158].

Figure 12 Global map showing geographical overlap of malaria and the sickle gene



Source: http://www.wellesley.edu/Chemistry/chem227/bindprotns/malaria.htm

Epidemiology

Although the geographical overlap is striking, the direct evidence for a protective effect was initially surprisingly limited since the first descriptions by Allison[159]. There was evidence of reduced parasite densities in AS individuals in some but not all populations. However, over the last fifteen years a number of case control and longitudinal studies have shown that individuals with AS have unequivocal protection against malarial disease, with the most striking effect on reducing the risk of severe life threatening malaria[158,160,161].

Mechanism of protection

In parallel with these epidemiological and clinical studies a number of studies in the laboratory have attempted to define the mechanisms underlying the protective effect of the HbS. Although, a detailed review of the proposed mechanism of protection will not be included in this thesis, proposed mechanisms include reduced parasite invasion[162], reduced parasite growth[163] as well as increased parasite death and removal[164,165,166]. Other putative mechanisms have been proposed, ranging from effects on potassium haemostasis, through effects of haemoglobin F, to interactions with the immune system. Although the precise mechanisms are not clear, the *sine qua non* is that malaria infection causes less severe disease in individuals with Hb AS.

Evidence of protection

The evidence that HbS is protective against malaria is compelling, and one might expect that the protection is 'dose-dependent', with higher concentrations of the HbS conferring more

protection. This hypothesis has been explored in another haemoglobinopathy, haemoglobin C (HbC). Although, HbC involves a mutation at the same position as HbS, i.e. position 6 on the β globin gene, the substitution for glutamic acid is with lysine (HbS it is valine). The clinical phenotype in homozygotes (HbCC) when compared to HbSS has a less severe course of disease, with less anaemia and reduced prevalence of the other end-organ consequences seen in SCA[23,167,168]. HbC has been demonstrated to be protective against malaria[169,170], with evidence that the protection is higher in individuals with homozygous states (CC) than heterozygous carriers (AC)[170]. The mechanism of protection proposed suggest that it acts by causing reduced adherence between parasitized RBC and endothelium, reduced rosetting and agglutination, with higher occurrence of features of RBC senescence. In all the experiments the effect was greatest in HbCC with some effects in HbAC and the least effect in HbAA[171,172].

From the evidence in HbC, it would be possible to hypothesize that individuals with SCD, who have higher concentrations of HbS, would be even more protected against malaria than heterozygotes (AS). However, it is widely believed that malaria is associated with severe morbidity and mortality in SCD. Therefore, the recommendation is that all patients with SCD who live in malaria-endemic countries should be on anti-malarial prophylaxis[100,127]. There have been significant advances in understanding of the two diseases as well as a reduction in prevalence of malaria by interventions with insecticide treated bed nets, intermittent presumptive therapy as well as the effect of environmental and climate change[173,174,175.] Therefore as part of these developments, there is a need to re-evaluate the interaction between SCD and malaria.

In order to do this one could look at the current evidence to answer three questions. The first is whether there is high malarial-related morbidity and mortality in patients with SCD. The second is whether there is an increased prevalence of SCD amongst patients with severe malaria. This alternative approach is to look at children admitted to hospital with severe malaria, where one might expect to see a significant proportion of individuals with SCD, if severe malaria is a special problem in this group. The third question is whether there has been an increase in mortality in SCD due to malaria with the spread of chloroquine (CQ) resistance.

Evidence of role of malaria in SCD

The evidence to determine the role of malaria as a cause of morbidity and mortality in SCD is conflicting; with some studies suggesting that individuals with SS-SCD have a higher susceptibility compared to individuals with AA or AS states, while other studies suggest protection against malaria.

Increased susceptibility to malaria

With regards to malaria as a cause of morbidity and mortality, work done in the Garki district in Nigeria in 1979, where they followed a cohort of children, found that the prevalence of SCD was 2.1% at birth which was maintained in the first year of life. However, this prevalence decreased to 0.4% between 1-4 years and 0.05 above 9 years, suggesting a high mortality amongst the SCD patients. In determining the cause of mortality, although there was less

parasitaemia in SS children compared to AS the assumption was that when SCD children did get malaria, it was fatal and concluded that malaria was responsible for the high mortality[16,176].

In recent years, two studies in Nigeria also found evidence of increased susceptibility to malaria in SCD individuals. One study looked at 104 consecutive cases of patients with SCD presenting with severe anaemia (packed cell volume < or = 15%). They found that the most common problem associated with SCD patients in anaemic crisis was malaria, which occurred in 68 (66%) of cases[177]. Similarly, in 102 adolescents and adults with SCD [mean age 20.5 years (SD 13.1)] who presented to an emergency unit in Nigeria, sixty-three per cent had infection of which 24.5% was malaria[76]. Other reports suggested that malaria precipitates crises and admission[178,179]. It is important to note that this is evidence from studies that were small, with the number of SCD patients not exceeding 105.

Increased protection from malaria

The evidence from other studies and case reports suggests a reduced prevalence and density of malaria in SS patients. The most recent studies in Kenya found malaria prevalence per genotypic group was 44.1% AA, 36.2% AS and 20% SS, giving a relative risk of malarial infection of 0.33 in SS (n=20) compared to AA (n=626) and AS (n=120)[180]. Another study in Nigeria found no significant difference in malaria parasitaemia (p=0.1) or frequency of malaria attacks (p=0.06) between SS (n=36) and controls (n=37)[181]. In another prospective study in Nigeria, which aimed to explore the relationship between size of spleen and malaria, tests were done to determine the prevalence of malaria and density of parasitaemia in 100 SCD patients

and 100 controls; 30% and 34% of the SCA patients and controls respectively had malaria parasitaemia. In SCA subjects, the mean parasite density was significantly lower (p value<0.05) compared to AA controls [1071.10 ± 895.5 per microlitre (range 33 - 4000) cf 1759±1382.87 per microlitre (range 180 - 5150)][182]. Again in 1992 in Nigeria, malaria parasitaemia was documented in fewer children with SCA (9%) (n=162) compared to controls (29%) (p<0.0001)[183]. Once again, the evidence is from small studies where the maximum number of SCD patients studied was 162[183].

Evidence of role of SCD in malaria

It is difficult to find convincing evidence on the prevalence of SCD in patients with severe malaria mainly because in most studies, the sickle status of patients with severe malaria is not known. In addition, if it is known, most of the SCD patients will be on anti-malarial prophylaxis or will receive prompt treatment (at home or empirically on arrival) because of the belief that malaria is the cause of hospitalisation. The few studies that have been able to look at children admitted with a diagnosis of malaria, found that severe cases of malaria had the same frequency of individuals with AA, AS and AS[184]. More recent, epidemiological studies suggest that SCD individuals are protected against malaria, not only in relation to malaria parasitisation with reduced parasite incidence, but also protection against severe malaria anaemia[158,161].

Lack of evidence of increase in malaria in SCD with ineffective anti-malarial chemoprophylaxis

It has been generally been accepted that individuals with SCD are susceptible to malaria and should be on malaria prophylaxis to prevent mortality[127]. Furthermore, there was evidence that more malaria attacks and deaths were observed in SCD individuals who were not on malaria prophylaxis[185,186]. For many years, weekly chloroquine provided a cheap, effective, convenient approach to chemoprophylaxis. However, over the past 2 decades, there has been development of malaria parasite resistance to chloroquine. During this period, when a drug that was no longer effective was being used to treat a potentially fatal condition, there has not been any reported evidence of an increase in morbidity or mortality in SCD due to malaria. The lack of evidence may be interpreted in two ways; that malaria does not have a role in SCD or it does have a role but studies are needed to illustrate this. A recent Cochrane review of the effect of malaria chemoprophylaxis in SCA found that only two studies, one conducted in 1962 and one in 2003, met the inclusion criteria of a randomised and quasi-randomised controlled trials comparing anti-malarial chemoprophylaxis given for a minimum of three months, compared with placebo or no intervention. The first study done in 1965 in Uganda on 126 children reported a reduction in the frequency of malaria episodes and dactylitis as well as an increase in mean haemoglobin values[187]. The second study done in 2003 in Nigeria on 97 children reported a reduction in sickle cell crises (RR 0.17, 95% CI 0.04 to 0.83; 97 children), hospital admissions (RR 0.27, 95% CI 0.12 to 0.63; 97 participants), and blood transfusions (RR 0.16, 95%CI 0.05 to 0.56; 97 participants)[188]. From these two studies, with a total of 223 children with SCD, the reviews concluded that it is beneficial to give routine malaria

prophylaxis in SCD in malaria-endemic areas[127].

Current management of malaria in SCD in Tanzania

Treatment

In Tanzania, there have been reports of increasing resistance of *Plasmodium falciparum* to chloroquine treatment[189,190]. There was a policy change in 2001, which recommended the use of sulphadoxine–pyrimethamine (SP) for uncomplicated malaria as first line therapy, quinine as second line drug when SP is contra-indicated or has failed and quinine as the recommended drug for severe malaria. In 2006, following an increase in parasite resistance to SP of up to 25.5%, it was recommended that first line therapy be changed to artemether and lumefantrine (ALu), an artemesinin-based combination therapy (ACT)[191].

For effective management of malaria, treatment needs to be accompanied by prompt and correct diagnosis. However, there is often over diagnosis of malaria[192,193,194] and this is more so in children with SCD[192], where malaria is perceived as a common cause of hospitalisation and mortality.

Prophylaxis

In Tanzania, until alternative agents are available or in place chloroquine remains the drug recommended for use for prophylaxis against malaria in SCD patients. The dosage is 300mg a week for adults and 50 mg/kg for children. However, since 2001, when there was the change in policy with regards to use of chloroquine for treatment, there has been reduced availability in

the market. Although, the drug is available in regional and tertiary hospitals, it has become increasingly difficult for patients to receive an adequate and regular supply, with the result that patients often do not take any or adequate prophylaxis.

There has been discussion with regards to what agents could be used as an alternative to chloroquine for prophylaxis that will be effective and affordable. In some countries, the agents that have been used include proguanil, pyrimethamine and mefloquine[183,195]. However, the dosage regime for these agents may not be convenient. In addition, the duration for prophylaxis is not known. Therefore the usual practise is to continue until at least the age of 15 years and often prophylaxis is used throughout life[196]. Unfortunately, there are few data on the safety profile of these alternative agents, when used over a long period of time. Furthermore, the impact of insecticide treated nets (an effective intervention in malaria prevention) has not been assessed specifically in the SCD patient group. These factors highlight a real urgency to determine what agents and interventions should be used for malaria prevention in SCD, and since prophylaxis may be required for a long period of time, there are issues of patient adherence and compliance as well as the efficacy and safety profile of the drugs that are used that need to be carefully explored[181,195]. Agents such as co-trimoxazole and methotrexate could be used in SCD as they have both anti-malarial and anti-inflammatory activity. An ideal way to resolve this would be to conduct a randomised controlled clinical trial, but due to the presumed relationship between SCD and malaria, it is difficult to justify a placebo-controlled trial[127].

Conclusion

Although the relationship between malaria and the HbS has been "known" for several decades, there are still basic questions that remain unanswered. With regards to mechanism of disease, it is not clear how HbS confers protection against malaria, particularly in SCD patients where one would expect more protection. However, from a clinical perspective, there is a need to collect evidence that will first elucidate whether malaria is a cause of morbidity and mortality in SCD. Against this background, this study set out to determine the role of malaria in SCD patients attending MNH. The purpose was to define the burden of malaria at outpatient clinic and during hospitalisation. This study also aimed to determine whether malaria was associated with adverse events such as severe anaemia and mortality. The evidence would allow the development of policies to guide the appropriate strategies for prevention and treatment of malaria in these individuals.

SICKLE CELL DISEASE AND BACTERIAL INFECTIONS

Introduction

Individuals with SCD are reported to be susceptible to infections. Besides malaria which is one of the specific foci of this thesis, SCD patients have increased risk of infection with encapsulated organisms such as *Streptococcus pneumoniae* [23,197,198]. Gaston *et al* showed that the use of oral penicillin in the USA had a significant impact on reduction in mortality[117], and it is now policy in many high-income countries to give penicillin prophylaxis and anti--pneumococcal vaccination to SCD patients[199].

The situation in Africa may be different. Aside from the fact that the data regarding the clinical spectrum of SCD are limited, there is controversy regarding the role and significance of pneumococcal disease in causing morbidity and mortality in SCD in this setting. Here I will briefly look at the factors responsible for susceptibility to infections in SCD and then review the epidemiology of bacterial infections in Africa, with particular focus on SCD. I will then consider the various interventions that have been used to prevent and treat infections in SCD.

Increased susceptibility to infections in SCD

The various factors that are associated with increased infections in SCD may be directly related to the immune system or not. Some infections may be the result of a complication or treatment of SCD itself. SCD patients are at high-risk of transfusion- transmissible infections particularly with human immunodeficiency virus and viral hepatitis since they receive frequent, often unplanned emergency BT[200,201,202,203,204,205]. This is particularly important in Africa, given the

high prevalence of HIV infection and the operational problems in providing adequate blood-transfusion services. In addition, long-term transfusion may result in iron overload, which in itself is associated with infections due to *Yersinia Enterocolitica*[206]. SCD causes end-organ damage to the lung, liver, kidney and skin, making these sites susceptible to infection by unusual organisms. In addition, skeletal complications, poor perfusion and blood supply to bone tissue are also thought to contribute to increased susceptibility to infections of the bone, osteomyelitis, which is often due to salmonella infections[207,208,209,210,211]. Other factors include high bone marrow turnover due to chronic haemolysis which results in increased susceptibility to viral infection. Parvovirus B19 infections is one of the viral infections that predispose to poor outcome with erythrocytic aplasia that may lead to life-threatening anaemia[54,212,213]. However, the epidemiology of this virus in Africa is poorly defined[214,215,216].

Impaired immunity in SCD

Individuals with SCD may have impairment of the immune system, which affects both cellular immunity and humoral immunity. The most well described immune defect is caused by reduced function of the spleen. Patients with SCD have repeated splenic infarction due to vaso-occlusion which causes loss of the splenic vasculature leading to hyposplenism[217]. Reports have suggested that 14% patients with SS-SCD are functionally asplenic at 6 months of age, with this number gradually increasing; 28% at 1 year, 58% at 2 years, 78% at 3 years and by 5 years, 94% are affected[218]. One of the roles of the spleen is filtration of unopsonised bacteria and remnants of red blood cells from intravascular space[219,220] as well as opsonised bacteria[221]. Furthermore, the spleen is involved in the synthesis of soluble mediators of immunity.

Therefore patients with SCD, with a functional asplenia, have been reported to have impaired antibody responses as well as lacking specific antibodies, particularly against *Salmonella* species and *Streptococcus pneumoniae*[222,223]. This is thought to be due to deficiency of a complement factor involved in the activation of the immune system. The classic pathway is activated by antigen-antibody interaction which causes fixation of complement components C1, C2 and C4 which then activate C3, whereas in the alternate pathway the antigen directly activates C3. Activation of C3, which is an opsonin, results in fixing of antigens on the microorganism[224,225,226] making them susceptible to enhanced phagocytosis by neutrophils and monocytes/macrophage. Johnston *et al* illustrated that patients with SCD have an abnormality in the activation of this pathway with failure of full activation and fixing of C3 to encapsulated bacteria[227]. This results in failure of SCD patients to phagocytose invading organisms, particularly *Streptococcus pneumoniae*.

The distinction between factors directly related to the immune system or not is somewhat arbitrary as there is a lot of overlap between the various factors. Although there have been reports of different patterns of infections in patients with SCD, reviewed above and summarised in table below (Table 7), for this study the focus is on invasive bacterial infections as detected by blood culture.

Table 7 Clinical syndromes and common causative organisms reported in SCD

Syndrome	Organisms	Reference
Septicaemia	*S. pneumoniae, H influenza, Salmonella* Spp, *E*	228,229, 230
Pneumonia	*S pneumoniae, M Pneumoniae, Chlamydiae*	
Meningitis	*S. pneumoniae*	
Osteomyelitis	*Salmonella* spp, *E Coli*, Gram negative organisms,	207, 208, 209, 210, 211
Aplastic anaemia	Parvovirus	54,212, 213
AIDS and Hepatitis	HIV Viral hepatitis B,C	200, 201, 202, 203, 204, 205
Abdominal pain	*Helicobacter pylori, Yersinia enterocolitica*	206

Epidemiology of bacteraemia in SCD

Streptococcus pneumoniae (SPN) is the most common cause of bacteraemia, sepsis, meningitis and pneumonia in children[231]. The rates of invasive infection with SPN have been summarized by Overturf[232] and have been presented in Table 8.

Table 8 Prevalence of invasive pneumococcal infections in healthy and high-risk children, expressed as the number per 100,000 population

Population	Age	Subgroup	Prevalence/100,000	Reference
US	Under 5 years	US African American	5.7 . 227.8 20.6 . 542.2	233,234
	Over 5 years	US total African American	5.7	233 234
SCD	Under 5 years		5,500 . 6,500	101 235,236
	Over 5 years		600 . 1,100	101,235
	Over 2 years		3,100 . 3,600	237,238

Modified from [232]

The prevalence is much higher in SCD individuals compared to the general population. This was first reported by Robinson in 1966, where she looked at 305 SCD patients and found 18 episodes

of meningitis, 87% of which were caused by SPN[239]. Since then there have been other reports that have confirmed that SCD patients have increased susceptibility to SPN and other encapsulated bacteria[208, 240, 241, 242]. Studies done in the 1970s and 1980s, reported an annual incidence of SPN septicaemia in SCD of 7-10 per 100 person-years[101, 118, 243, 244]. The rates of SPN infection in SCD compared to normal children exceeds 20 – 100 fold, with greatest risk in children under the age of 5 years[23, 101, 235, 245] and the highest risk under the age of 2 years[101, 245]. These bacterial infections, particularly those caused by encapsulated organisms, are a common cause of mortality among SCD cases[20, 23, 208]. The case fatality rate due to SPN infection has been reported to be between 18 – 35 %[101, 241, 243, 246].

Management and prevention of bacterial infection s in SCD

Treatment

The management of bacterial infections in children with SCD involves prompt diagnosis and aggressive treatment, which results in significant reduction in morbidity and mortality[7]. As a consequence, in countries where there are comprehensive programmes for SCD, the parents and carers of SCD children, usually detected by newborn screening, receive counselling and support so that they can assess fever and recognise respiratory distress, splenomegaly and jaundice[247]. It is recommended that they should receive prompt treatment with broad-spectrum antibiotics such ceftriaxone or cefotaxime[248]. However, there are various factors (Table 9) that are associated with increased risk of mortality. For patients with these features it is recommended that they are admitted for inpatient care and intravenous antibiotics, after the appropriate blood and urine cultures and haematological investigations

are taken.

Table 9 Indications for inpatient care with intravenous antibiotics

Age of child (<6 months)
Fever of 40°C
History of prior infections especially SPN bacteraemia
Recurrent abdominal pain with/without severe or increasing jaundice
Persistent pain in an extremity accompanied by acute signs of classic inflammation, increased
Chest pain accompanied by persistently decreased oxygen saturation and/or presence of
Increase in jaundice
Signs of toxicity or meningitis
Evidence of respiratory symptoms such as respiratory distress or dyspnoea
Central nervous system symptoms or signs e.g. severe headache, deteriorating level of
Anaemia and/or enlarging spleen, rapid worsening of anaemia or haematocrit less than 25%
Persistent haematuria or proteinuria
Priapism

Source [248, 249, 250]

There have been a few studies done to understand the significance of fever in children with SCD, as fever is not always associated with infection. A retrospective study published in 2001, found that fever was more commonly associated with painful crises (27.3%) and acute chest syndrome (21.8%) than with bacteraemia (6.1%)[198]. Both pain and ACS are associated with increased mortality[57,59,61]. This report highlights the need to take a holistic approach to management of fever in patients with SCD and not assume that all febrile episodes are infective in nature.

Since infections are a common cause of hospitalisation, there has been interest in improving management by determining whether care can be given without hospitalisation. Although this

would reduce the high costs that would be associated with inpatient care as well as improve the quality of life of patients, there is concern that outpatient care may be associated with the risk of severe morbidity or death. An initial study reported in 1990, suggested that selected patients with SCD could be treated as outpatients[251]. Another study by Wilimas *et al.* compared outpatient therapy and routine inpatient management of fever by conducting a prospective, randomised trial in SCD children (6 months to 12 years) who were seen in casualty department in a hospital in the USA with a temperature of 38.5°C and above[111]. Patients who were considered high risk were admitted; and this group included children with temperature > 40°C, severe pain, signs of dehydration, severe anaemia with Hb <5g/dL, WBC count <5 or >30 $x10^9$/UL and pulmonary infiltrates on chest X-ray. There were 233 febrile episodes in 197 patients. Of these, 98 episodes in 86 patients were randomised. Of the 86 patients, 44 patients (51%) could safely receive outpatient treatment which was estimated to save $1,195 US dollars per febrile episode. However, the rate of hospitalisation in this group after 2 weeks was higher compared to those who received inpatient treatment (22% vs. 2%). The data from this study on safety and efficacy would be more compelling as it was a prospective study.

In addition to outpatient treatment of infections, efforts have been made to reduce the number of days patients stay in hospitals. Bakshi *et al* reported that parenteral antibiotics, ceftriaxone, reduced the number of days of hospitalisation from 214 (6.3 +/- 1.6 days/patient) to 111 days (2.8 +/- 0.7 days/patient)[252].

Prevention

A prospective randomised trial was carried out in Jamaica, between 1978 and 1983 which showed effective prevention with penicillin. In this study the intervention was benzathine penicillin given as an intramuscular injection, once a month with no penicillin given to the control arm and 14-valent polysaccharide pneumococcal vaccine given to both arms. No SPN infection was reported in the patients receiving penicillin prophylaxis[253].

A landmark study done by Gaston et al was conducted between 1983 and 1985. This was a multi-centre, double-blind, placebo-controlled trial on children aged 3 to 36 months. 105 children received penicillin V (250mg bd/day) while 110 children received placebo. All children under 2 years of age also received pneumococcal vaccination. The study was terminated early because the incidence of infection in the penicillin groups was reduced by 84% (incidence rate of SPN bacteraemia per 100 patient years 1.5 vs. 9.5; p= 0.0025)[117].

The current recommendation is that all patients with SCD should be on oral penicillin prophylaxis up to the age of 5 years. The recommended dose is 125mg twice a day, for children from 2 months to 2 years. From 2 to 5 years, the dose in increased to 250 mg. There have been reports of occurrence of SPN infection in SCD patients on oral penicillin[237, 254, 255]. This may be due to lack of adherence or penicillin-resistance strains. Therefore, the concern of emergence of resistance weighed against the risk of SPN infection caused a debate to arise about continuing prophylaxis after the age of 5 years. The factors that need to be considered when debating continuing prophylaxis after 5 years using oral penicillin in SCD include

patient compliance, cost of the drug, adverse effects and the potential for emergence of resistance. Various studies found that the compliance after 5 years was difficult with reports ranging from 50 to 70 %[237, 256]. However, Falleta *et al.* conducted a study to determine the rate of SPN bacteraemia on discontinuing oral penicillin. A randomised controlled trial was conducted with 201 SCD patients continuing with oral penicillin (250mg twice a day) and the other groups received placebo. These were patients who had received penicillin for at least 2 years before their 5th birthday and had received pneumococcal vaccination between 2 and 3 years. The study found that the rate of invasive disease between these two groups was not statistically significant (0.33/100 person years vs. 0.67/100 person years; relative risk 0.5 95%CI 0.1-3.6)[257]. As a result, it is recommended that prophylaxis should stop at 5 years of age, after the second dose of pneumococcal vaccination. If there is occurrence of invasive disease, patients should receive prompt treatment with oral antibiotics and seek medical attention if the condition worsens and requires inpatient care with intravenous antibiotics. In one study, 570 patients with SCD were reviewed. 33 had sepsis due to SPN, with 3 dying with the first episode of sepsis. SPN infections recurred in 8 out of 30 with SCA who had had a previous episodes of sepsis [(odds ratio 5.2 95% CI 1.9-13; p=0.002)][258]. It is recommended that these patients continue with oral penicillin.

Changing trend of bacterial infections

Since the advent of prevention of SPN infections, using oral penicillin, pneumococcal vaccination as well as prompt treatment of infections there has been a significant decrease in the incidence of SPN infection in SCD patients[259]. However, although the mortality due

to SPN infections has fallen from 18-35% in the 1970s and 1980s, to 12.5% in 2001[259], it is still a major cause of morbidity and mortality in SCD patients[22]. Furthermore, there has been a reported increase in the frequency of other infections such as *Salmonella* spp and *Haemophilus Influenza* in the USA[106, 118].

Bacteraemia in SCD patients in Africa

Epidemiology

In Africa, anaemia, pain and infections or febrile episodes are the commonest problems reported in children with SCD; with bacteraemia considered to be an important cause of in-patient morbidity and mortality. In Benin, a definite or presumed bacterial infection was the cause of the febrile episode in 76.7% of cases[112]. Another study in Nigeria, found that 72% patients with SCD were admitted with fever or with a history of febrile illness, 63% had infection of which malaria was reported in 24.5%, bacterial in 17% and viral in 6%[76]. There was also an association between anaemia and bacterial infections, reported in 18 (17.3%) out of 104 SCA patients admitted with anaemia[177].

The role of infections in causing mortality in SCD has not been well described in Africa. A retrospective review of 69 children with SCD hospitalized between 1964 and 1985 in Kinshasa, Democratic Republic of Congo, reported that 18 children (26%) died, including 10 with pneumococcal septicaemia (5 cases) and 10 with salmonella septicaemia (5 cases)[260]. In 62 Zambian children with SCD, the common causes of death were infections (29.5%), vasoocclusive crises (22.7%) and splenic sequestration crises

(20.45%)[261] and similarly another study in Central African Republic found that after anaemia, which was responsible for 47.8% of deaths, infections accounted for 30.4% in 23 children with SCA[262]. Recently, a study conducted in Angola, reported that bacterial infections was the most common cause of death accounting for 40.1% of deaths in 64 individuals with SCD[263].

However, data regarding the pattern of bacterial infections, particularly the role of pneumococcal disease, in patients with SCD in Africa are limited and conflicting. There have been studies reporting pneumococcal isolates[179, 228, 240, 264, 265, 266]. However, other reports from Africa suggest that in SCD individuals who are hospitalised there is a predominance of organisms such as *Klebsiella* spp., *Staphylococcus Aureus* and non-typhoidal *Salmonellae*[210, 260, 267, 268]. This is contrary to reports from USA and Europe, where SPN is the commonest organism in blood stream infections. It is possible that important differences really do exist, in the epidemiology of invasive bacterial disease in patients with SCD, between Africa and the North. For studies that have shown a predominance of *Staphylococcus Aureus*, *Salmonellae spp* and *Klebsiella spp*, it has been argued that there may be high level of carriage of these organisms in the environment[269]. However, there is compelling evidence from large, systematic studies showing that in the general population SPN is the commonest cause of childhood bacteraemia in Africa[269, 270, 271, 272]. Therefore, considering that SCD patients are highly susceptible to SPN infections, there does not appear to be a plausible reason to explain why this pathogen should not be important in patients with SCD in Africa.

A study in Uganda, failed to find a high prevalence of SPN infection in SCD patients. The study was conducted on a cohort of 155 SCD with age ranging between 3 months to 15 years. Febrile episodes were documented in 1,265 occurrences and 47 (3.7%) blood cultures were positive. The pattern of bacteria was as follows: *S. Aureus* 28 (60%); *H. influenza* 9 (19%) and SPN 3 (6%). From this study, the authors questioned whether prophylaxis was justified in Africa[229] considering the lack of evidence. However, a review by Obaro questioned whether lack of evidence implies evidence of absence[273]. This is mainly because the evidence that SPN is one of the leading causes of bacteraemia is compelling[231], [270] and there is no reason to doubt that SCD patients would have higher rates of infection. Therefore, the possibility is high that the absence of evidence is due to limitations in the design of the studies that have been done to get this evidence. The failure to report SPN in African SCD studies may be due to three factors. First, studies on SCD patients in Africa are often conducted in hospitals on patients known to have the diagnosis of SCD. Since most African countries do not have newborn screening programmes; the diagnosis of SCD is made when they are older. However, most SCD patients will likely succumb to bacterial infections between 1-3 years of age, before the diagnosis is known. Therefore, it is highly likely that the SCD cohorts studied in hospital will not have a high incidence of SPN disease. Second, blood cultures may be taken after patients had already received prior antibiotic treatment either within the hospital or within the community due to widespread availability of over-the-counter antibiotics. Third, is that there may be limitations in microbiology facilities at hospitals in SSA since SPN is a particularly fastidious organism, and there is often difficulty in isolation of this bacterium from blood cultures.

In order to address these limitations, a study design needs to be community based or include all hospitalised patients. Recently, a research centre in Kilifi, Kenya has established a robust framework to capture bacteraemia events in all children who are hospitalised[270, 274]. Although the prevalence of SCD is low in Kenya (birth rate <5 per 1,000) compared to the other countries in east Africa[4, 275] this framework and the low likelihood that the SCD patients who are hospitalised have received antibiotics before hospitalisation allowed this question to be answered. A retrospective analysis to determine the phenotype in 1,749 (4.5%) of 38, 866 hospitalised patients in a ten year period was done. The study was able to determine the bacteraemia rates in 108 SCD patients who had been hospitalised during a 10 year period. *S. pneumoniae* was isolated in 44 (41%); non-Typhii *Salmonella* in 19 (18%) and *H. Influenza* 13 (12%) of the SCD patients[230]. This is compelling evidence that SPN has an important role in causing bacteraemia in SCD patients during hospitalisation who should receive prompt treatment with effective antibiotics.

Management

There are few countries in Africa with published national guidelines for management of fever and infections in SCD. However, due to the high prevalence of infections in these areas, most SCD patients who are admitted are given empirical antibiotics and antimalarial treatment. However, the assumed association between SCD and malaria is better known than that with bacterial infections. Therefore, in Muhimbili National Hospital, as is the case in most hospitals in Africa, SCD patients will receive antimalarial therapy, usually intravenous

quinine, which is first line treatment for severe malaria. However, they will not receive antibiotics unless the patient is severely ill or there is an obvious focus of infection or signs of septicaemia (personal observation). There are now increasing efforts to encourage health care providers to treat SCD patients who are febrile or have evidence of infections with antibiotics[273, 276, 277, 278]. In a study conducted in Benin, West Africa to assess the safety and feasibility of outpatient management of infections, 60 children with fever received intramuscular ceftriaxone. 76.6% of these febrile episodes were presumed to be due to infection. No complications, hospitalisation or death was associated with outpatient treatment suggesting safety in this approach. In addition, the cost of outpatient management was estimated to be lower compared to inpatient care ($30 vs. $140 per patient) which is particularly important as most African counties have limited resources[112].

With regards to prophylaxis, resistance to penicillin is relatively low and therefore there is justification in using daily oral penicillin as prophylaxis in SCD children who are less than 5 years. Pneumococcal vaccines have also been recommended[279]. However, the role of these vaccines needs to be assessed as the 7-valent conjugate vaccine does not cover the serotypes which have been reported to be common in Africa[278].

Conclusion

In summary, it is likely that bacterial infection, particularly with pneumococcal disease is an important cause of morbidity and mortality in SCD patients in Africa. There is a need to provide evidence that is sufficiently compelling to indicate that preventative measures, such as

prophylaxis with antibiotics and appropriate vaccines should be implemented in patients with SCD in Africa. In addition, there is a need to define the most effective antibiotic regimens to manage febrile episodes in SCD. Therefore, against this background, it remains a high priority to clearly define the importance of bacterial infections in SCD, identify the common causative organisms and outline clinical and laboratory features that would identify patients at risk of bacterial infections and complications and targeting interventions appropriately.

SICKLE CELL DISEASE AND STROKE

Introduction

The increase in survival of SCD patients has allowed long-term natural history and clinical observational studies. This has been complemented by an increase in the understanding of the various mechanisms involved in disease pathology. There are recognised clinical consequences of SCD which are the characteristic acute complications (crises). However, there are also long term chronic effects of the disease. This is related to the fact that although SCD is due to a defect in the haemoglobin molecule, the complex erythrocytic and extra-erythrocytic mechanisms result in a multi-system disease, where almost every organ in the body can be affected. The organs which have been most extensively studied are the brain (including the eye), lungs, kidneys, heart and lung.

Several studies, initially through clinical observation, have described the various neurological manifestations of SCD. The spectrum of disease includes central nervous system (affecting both the brain and spinal cord), peripheral nervous system, isolated neurological deficits as well as less well-defined syndromes. The presentation ranges from seizures, coma, mono- or hemiplegia, brainstem lesions, visual and hearing disturbances, spinal cord syndromes and peripheral neuropathy[280, 281, 282, 283, 284].

The best described consequences of SCD for the neurological system are those involving the brain[285]. Although patients with SCD may have infections directly affecting the brain e.g. meningococcal meningitis and cerebral malaria, the main burden of disease has been due to

non-infectious complications. Cerebrovascular disease (CVD) refers to disorders which result from pathology of the blood vessels in the brain. This is the most well-described and common complication in SCD[283, 286]. There is evidence of both small vessel and large vessel involvement, with patients presenting with stroke, transient ischaemic attack, seizures, coma, focal palsies and a range of other rare manifestations. Cerebrovascular accidents (CVA), commonly referred to as stroke, are sudden neurological deficits caused by injury to part of the brain due to lack of blood (infarction). They may also result from rupture of a blood vessel and subsequent haemorrhage into the brain. Transient ischaemic attacks are reversible, resolving within 24-48 hours and often result from lack of blood or ischaemia whereas CVAs are usually irreversible and may be caused by either ischaemia or haemorrhage. With an improvement in neurological imaging techniques, subtle cerebral damage due to infarction has become increasingly recognized[84, 85, 287]. These lesions were previously referred to as "silent infarcts since patients do not have a clinically apparent episode of neurological deficit. However, these "silent" infarcts have been associated with neurocognitive defects in children with SCD[84, 85, 288].

Epidemiology of stroke in SCD

In developed countries, the risk of developing CVA is 250 times higher among children with SCD than in their non-sickle counterparts[289]. In the U.S.A., approximately 25% of patients with SCD suffer a stroke by the age of 45 years[83], the peak incidence being between the ages of 2 and 5 years of age[283]. There is now evidence of "silent cerebral infarcts occurring in 15 to 20% of children by the age of 6 years[85, 86,290, 291, 292, 293]. This causes significant impairment of higher cognitive and executive function with reduced intellectual and academic performance and is

thought to be highly predictive of overt stroke[84, 85, 86, 291, 294].

Although, the overall incidence of stroke is estimated at 1.29 per 100,000 per year for children, the incidence of stroke in children with SCD was estimated at 285 per 100,000 per year [289]. These have been stratified by age with the rates shown in Table 10.

Table 10 Age-related incidence of first stroke in SCD in the USA (modified from [83])

Age group	Incidence of stroke
<2 years	0.13 per 100 patient years
2-5 years	1.02 per 100 patient years
6-9 years	0.79 per 100 patient years
10-19 years	0.41 per 100 patient years
20 49 years	0.52 0.74 per 100 patient years
>50 years	1.28 per 100 patient years

Stroke in SCD may result from haemorrhage, thromboembolism or infarction. Overall, stroke due to infarction is more common[295] and has two peaks; between 2-5 years and above 30 years. On the other hand haemorrhagic stroke, which is less common, occurs in young adults[83].

Figure 13 Hazard rates of infarctive and haemorrhagic stroke in SCD patients by age

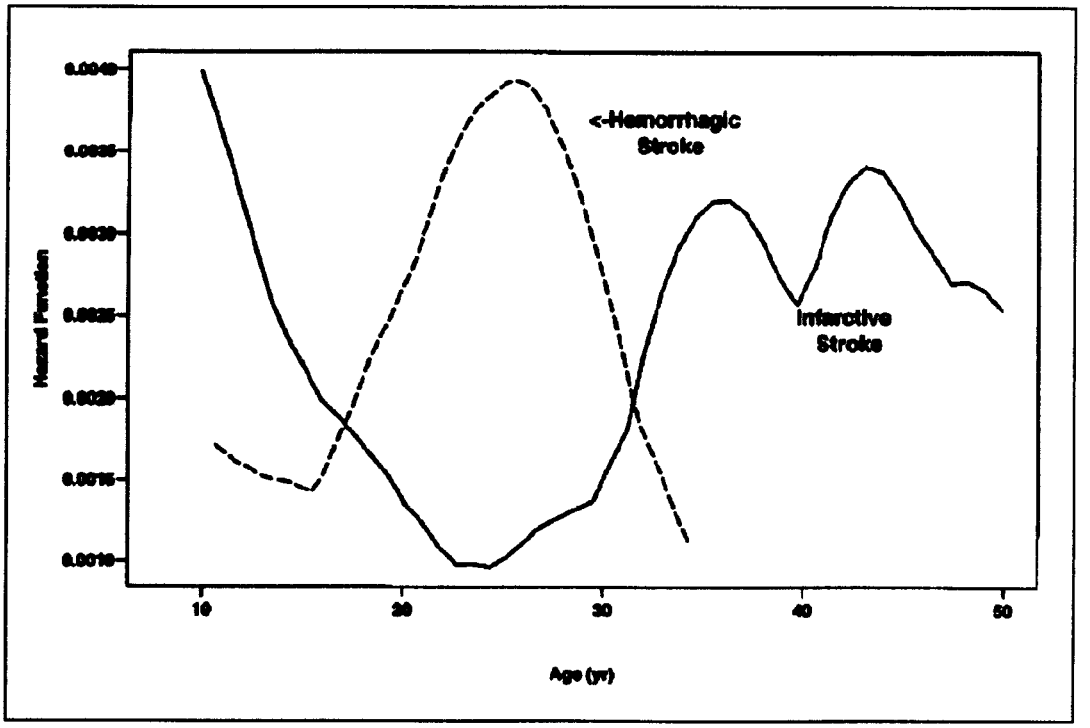


Figure showing hazard rates of stroke in SCD. The solid line shows the rates of infarctive stroke at different ages while the dashed line shows haemorrhagic stroke.[83]

In Africa, the evidence seems to suggest that the incidence of stroke amongst patients with SCD may be higher than in the U.S.A., with reported rates of 1.3 per 100 per year[296, 297, 298] compared to 0.61/100[83]. The age-group most affected, 2–5 years is similar in both regions. In Nigeria, a study looked at neurological events in SCD patients over a 12 month period. These occurred at a prevalence of 1.3% with CVA accounting for 57.5%, meningitis 22.5% and recurrent seizures 10% of these events[297]. In Kenya, a larger and longer study was conducted to assess neurological complications in stroke. This was a retrospective study done at Kenyatta National Hospital (KNH) which is the main hospital in Nairobi, the capital of Kenya. In this 5 year retrospective analysis, 18 SCD patients aged between 7 months and 21, years had

neurological complications. 12 (76%) of these were CVA, 6 (33.3%) were seizures and other complications were visual and hearing disturbances as well as confusion and hallucinations 298. The relatively high incidence of SCD-associated stroke in Africa has been suggested to be due to the presence of higher rates of some factors in Africa which are associated with increased risk. These include low haemoglobin, leucocytosis and CAR β-globin haplotype.

Risk factors

A number of factors have been found to be associated with an increase risk of stroke in SCD. These include age, which as mentioned above, the 2-5 year age group is particularly susceptible. Although most vascular events occur between the ages of 5 and 10 years, there is another peak above 50 years. It is thought that stroke may have different pathophysiological mechanisms which are dominant at different ages; vascular stenosis due to SCD in the younger age group, haemorrhage during adolescents and adulthood and infarction in the older age group[83].

Other risk factors for stroke in SCD children include low red blood cell indices particularly Hb, HbF, RBC count as well as high nucleated red cell, white cell and platelet count[83], [299], [300]. High homocysteine levels which result from low folate levels are also associated with increased risk of stroke; folate is required for homocysteine metabolism[301].

Some clinical syndromes that have been correlated with prediction of stroke include previous TIA, a recent history of acute chest crisis, high acute chest syndrome rate, recurrent painful

episodes (more than 3 a year)[23,302,303]. Hypoxia, particularly nocturnal hypoxaemia, has been associated with CVA[304].

Table 11 Factors associated with increased risk of cerebral infarction

Clinical factors
Age 2-8 years
Family history – sibling with a stroke
Repeated episode of convulsions
Acute anaemic episode (Hb 2g/dl below normal level)
Bacterial meningitis
Acute chest syndrome
Splenic dysfunction/infarctions near age 1 year
Priapism
Decreasing academic performance
Impaired neurocognitive function
Laboratory factors
Haemoglobin (steady-state_ concentration <7.5g/dl.
low red blood cell count
high nucleated cell counts
High reticulocyte count
Platelet count
Pitted/pocked RBC
Foetal Hb <13% by 2 years
CAR Beta globin haplotype
No alpha gene deletion

Modified from [305]

Mechanism of disease

Although SCD is often thought to cause small vessel disease, the most common pathology in the brain is due to large vessel involvement. A complex interplay of different pathophysiological processes causes a progressive proliferation of endothelial wall, collection and organisation of mural thrombi, resulting in narrowing or stenosis of the blood vessel.

These events have been summarised previously in the review of pathophysiology of disease in SCD. This can occur at any site but commonly affects basal cranial arteries, in the circle of Willis; predominantly in the distal internal carotid artery (ICA) and the proximal middle and anterior cerebral artery (MCA and ACA respectively)[306]. As a consequence, CVA frequently involves the watershed distribution between the ACA and MCA within the frontal lobes[307].

Although, one of the consequences of progressive stenosis is eventual occlusion and ischaemia, the body may respond by the development of small vessel collaterals, which bypass the stenotic portion of the large blood vessel[308]. This phenomenon which results in an angiographic pattern referred to as the Moyamoya syndrome has been reported in SCD patients[295], [309], [310] and found to be useful in predicting the recurrence of CVA in SCD patients[311]. The pathological consequences in small intracerebral vessels are cortical and sub-cortical branch occlusions, which normally affect the frontal lobe and manifest as subtle higher executive function abnormalities[312,313]. The effect of other mechanisms of CVAs in patients with SCD is not known. However it is suggested that hypoxia[304], which has been associated with increased risk of stroke promotes polymerisation of HbS and adhesion of RBC to endothelium and platelet and white blood cell adhesion[39, 313-315].

Diagnosis

Diagnostic techniques such as cerebral angiography, magnetic resonance imaging and Transcranial Doppler (TCD) ultrasonography are used to detect cerebrovascular disease[316]. However, for purposes of this thesis, the focus will be on TCD.

Transcranial doppler ultrasonography (TCD)

Transcranial Doppler (TCD) ultrasonography is a technique that was devised by Aaslid as a non-invasive technique for measuring cerebral blood flow velocity (CBFv) in the basal cranial arteries[317]. It uses ultrasound to measure the velocity of red blood cells and by application of Dopplers' principles can estimate the velocity of blood flowing in large cerebral vessels through an intact cranium. TCD measures the time-averaged maximum mean velocity of the blood flowing in large cerebral vessels (TAMMV). Although an increase in velocity may be due to stenosis or increased cerebral blood flow, studies have determined the abnormalities in blood velocity that are predictive for stenosis. Studies done in the USA found that a low haematocrit can increase the velocity up to 150 cm per second in the middle cerebral artery (MCA), but values greater than this are more likely to be due to stenosis[318].

TCD ultrasonography has been found to be highly sensitive and specific in identifying cerebrovascular abnormalities by detection of arterial stenosis and occlusion in both symptomatic and asymptomatic SCD patients[319, 320, 321]. In a study conducted in the USA, 9.7% of children were found to have CBFv abnormalities, with more children between 2 to 8 years of age (10.7%)[294]. The cut–off point that was found to be predictive of risk of stroke was a flow rate of 200cm per second in the MCA, which was associated with a 40 percent risk of stroke within three years[319]. Therefore, this is the cut-off point that is taken to define SCD patients who are at high-risk for stroke and therefore require more aggressive interventional strategies. Although TCD ultrasonography has not been used in SCD in Africa, it has the advantage of being relatively cheap, safe, well tolerated, and therefore an ideal investigation in hospitals in

resource limited countries where cerebral angiography and MRI are not feasible.

Magnetic resonance imaging

Imaging techniques such as high resolution computerized tomography (CT) and magnetic resonance imaging (MRI) are used for the diagnosis of CVA. The latter is particularly good in the detection of clinically silent infarcts which are associated with increased risk of stroke[86, 322]. However, neither technique is practical or economically feasible in many of the poorly resourced areas in sub-Saharan Africa (SSA). In addition, these investigations are mainly diagnostic of neurological events whereas TCD is used for identifying high-risk patients and therefore allowing primary intervention. In such circumstances, a combination of TCD examination and careful neuropsychometric evaluation has been suggested to be helpful in screening and identifying those children with early CNS pathology or silent infarcts[84, 294, 323] which can then be confirmed by more specialised techniques such as MRI or CT scanning.

Treatment and prevention

There has been progress in the primary and secondary prevention of stroke as there is improvement in the ability to identify SCD patients who are at particularly high risk of stroke and therefore require aggressive interventional strategies.

Several randomised controlled trials have shown that initiating aggressive management strategies such as long-term transfusion therapy in these patients can significantly reduce the incidence of stroke. In a randomised controlled trial conducted in 1998, 130 children with SCD

who had abnormal CBFv (above 200cm/sec) documented on two occasions with no history of previous stroke were randomised to receive standard care of treatment or blood transfusion[120]. Standard care of treatment included penicillin prophylaxis, pneumococcal vaccination, folic acid supplementation, surgery for chronic hypersplenism, treatment of acute infections including blood transfusion when needed for transient episodes but excluding use of Hydroxyurea. The aim of blood transfusion was to reduce the HbS levels to less than 30% within a period of 21 days without exceeding a haemoglobin concentration of 12g/dL or a haematocrit of 36%. There was a 92% reduction in risk of stroke in the patients receiving blood-transfusion, which led to early termination of the trial[120]. It is now recommended that children with SCD between 2-16 years of age, should have annual TCD ultrasonography to measure their CBFv, and for those with a CBFv of more than 200cm/sec, which is classified as abnormal, the recommendation is that they should be started on long term blood transfusion (LTBT) programmes[324]. However, there have been difficulties in ensuring compliance and adherence to LTBT[325, 326]. The regime involves blood transfusion every 3-6 weeks and it is not entirely clear what should be the duration of the regime since there is evidence to suggest that discontinuation of therapy results in a high risk of recurrence[322]. Despite this high risk of recurrence, patients and their caregivers have difficulties complying. This is because of having to go to hospital every 3-4 weeks as well as venous access[326]. In addition, there are concerns with LTBT such as iron overload and alloimmunisation, as well as a risk of transfusion transmissible infections (TTIs)[327]. These limitations are particularly pertinent in SSA where there is the added problem of availability of adequate blood supply, with use of replacement donors and there is often no screening for RBC antibodies before transfusion.

Alternatives for LTBT for prevention of stroke have been investigated. Hydroxyurea which has been found to be beneficial for other adverse clinical outcomes in SCA[128] may also be a useful alternative in preventing stroke in high risk patients[328, 329, 330]

Conclusion

A more comprehensive picture of the burden of stroke in SCD in Africa needs to be established. Due to the variability of phenotype and the different pathophysiological mechanisms for the same event, it is important to establish the associated risk factors in Africa. This will involve looking at known risk factors as well as looking at factors that can be established in a cost-effective manner in a resource limited setting. In this context, given that TCD ultrasonography is relatively cheap, safe and well-tolerated, it should be feasible to use this technique to determine the spectrum of cerebral blood-flow velocities in African children with SCD and, subsequently, to define a high-risk group for targeted investigations such as MRI and CT scanning of the brain with potential interventions with LTBT or Hydroxyurea.

SICKLE CELL DISEASE AND FOETAL HAEMOGLOBIN

Introduction

As reviewed previously, there are three haemoglobins in normal individuals HbA, HbA_2 and foetal haemoglobin (HbF). This chapter will focus on reviewing the physiology and measurements of foetal haemoglobin (HbF) followed by a review of its role in SCD and malaria.

Physiology

The main function of red blood cells (RBC), transporting oxygen and carbon dioxide between lungs and other tissues, is carried out by haemoglobin. Hb has properties that allow it to bind, transport and release oxygen and carbon dioxide. Since the environment during embryonic, foetal and adult life with regards to availability and source of oxygen differs the properties of the haeme molecule in binding and releasing oxygen have to differ at each of these stages. Therefore, physiologically there are 6 types of haemoglobin, embryonic (Gower 1 and 2, Portland); foetal Hb (HbF) and adult haemoglobin (HbA and HbA2). The distribution of these haemoglobins in individuals gradually changes during development.

During the intra-uterine period, the foetus receives oxygen from the maternal circulation in the placenta. The main Hb present in foetal RBCs is HbF which has a higher affinity for oxygen then Hb A, which is the main Hb in maternal RBCs. Since the oxygen saturation of the maternal blood in the placenta is low, difference in affinity allows effective gas exchange across the placenta. At birth, HbF constitutes 60 to 80% of total Hb and during the first 2 years of life, there is gradual transition to adult haemoglobin, consisting of Hb A, with small amounts of Hb A_2 and Hb F (0.5 to 1%).The composition of Hb in RBC remains fairly

constant throughout adult life. The variation in the haemoglobin from embryonic to intrauterine HbF to adult HbA means that during development there are two switches in the β – like gene expression from ε to γ and then to β– globin genes. This process of switching from one gene to another during development has been the focus of a lot of research in order to understand the factors controlling γ-globin gene expression, in order to increase HbF production in SCD.

Factors influencing HbF levels

There is a wide variation of Hb F in the population, with 85 – 90 % individuals having HbF less than 0.6 – 0.7% and F-cells (red blood cells containing any HbF) are less than 4.5%. The other 10-15% of the population has values above these levels. The factors that influence the HbF levels include age, sex (females have higher levels), pregnancy and a – globin gene number. Genetic factors that influence HbF levels may or may not be linked to the β – globin gene region, with the former associated with the variation in HbF levels with the different sickle haplotypes. On the other hand, the F-cell production locus (FCP) on the X-chromosome account for the difference between 11% men and 21% females who have higher HbF level. The three factors that determine HbF levels are number of F cells (determined by percentage of F-reticulocyte), amount of HbF per F-cell and the preferential survival of F-cells over non-F cells[331].

F-Cells (FC), non-F cells and F-reticulocytes: HbF is restricted to a sub-set of specific red blood cells (RBCs) called F-Cells (FCs) and the rest of the RBCs which do not have detectable levels of HbF are known as non-F cells. Although, the percentage of FCs within an individual is stable, there is a wide variation between individuals. The percentage of FCs in normal

individuals ranges between 0.5 to 7%, while in SCD, the range is broader and levels are higher (up to 60%)[331].

Hb F per F Cell: To add complexity to the situation, HbF is not evenly distributed in FCs. Studies have shown that concentration of HbF per FC is not constant, and ranges between 4-6 picograms. The average amount of HbF per FC varies amongst individuals with SCD[332], but the maximum percentage of HbF in each FC does not exceed 25% of total Hb.

Survival of F-cells: There is selective destruction of Non-F cells compared to FCs. This preferential survival of F-cells can be calculated by the ratio of FCs to F reticulocyte, which is known as the enrichment ratio. Although the presence of HbF in FCs, protects it from sickling and therefore ensures its survival over non FCs, it appears that there may be other factors that are responsible for the preferential survival of FCs[331, 333].

Hereditary persistence of foetal haemoglobin (HPFH)

HPFH refers to a group of conditions characterised by synthesis of HbF into adult life in the absence of evidence of haematological disease. The distribution of HbF may be pancellular or heterocellular. In hereditary HPFH, the distribution of HbF is pancellular/even and levels are higher in African (25-35%) than Mediterranean (12-25%) populations[334]. In heterocellular HPFH, the distribution of HbF is uneven between the cells and levels are often less than 10%, having been described in Swiss and Northern European populations.

Table 12 Laboratory characteristics of haemoglobin disorders

	Foetus	AA	AS	SS	S – β^0 Thal	S – β^+	S-HPFH	HPFH
Hb electrophoresis		AA	AS	SF	SF	SAF	SF	
HbA ($a_{2\beta2}$) (%)	5-10	97	55-70	0	0	5-30	0	0
HbA_2 ($a_{2\delta2}$) (%)		2-3	2-3	<3.5	> 3.5	>3.5	<2.5	
HbS ($a_2\beta^S{}_2$) (%)		0	30-45	90	>80	>60	>60	
HbF (%) ($a_2\gamma_2$) (%)	60-80	0.5-2	0.5- 2	2-10	<20	<20	35-40	100
F-cells (range) (%)	20-25	0.5 - 7	0.5 – 7	50-60	50-60	50-60	100	100
F cells (mean) (%)				55.9	61.6	51.3		
HbF/F-cells (%)		15	15	25 - 40				
Haemoglobin g/dL		11-14	11-14	6-8	7-9	9-12	11-14	
Mean corpuscular Volume		N	N	N or ↑	↓	↓	N or ↓	

Modified from [105, 331, 332, 335]

Laboratory measurement of foetal haemoglobin

Quantification of HbF

The quantification of HbF can be done using a number of methods. Betkes method uses an acid-elution technique which allows the estimation of HbF per cell[336]. This is normally reported as a percentage of total haemoglobin. However, this method is limited because the laboratory procedures are complex and require diligent, labour-intensive skills.

The second method is done by initially performing haemoglobin electrophoresis and identifying the HbF bands which are then measured by densitometry. A third method uses radial immunoprecipitation to quantify HbF levels. However, the HbF levels using radial immunoprecipitation have been reported as being higher and this could be misleading[337].

The quantification of HbF by high performance liquid chromatography (HPLC) uses ion-exchange chromatography to identify the different haemoglobins in blood, and determine the levels of Hb in each individual[338]. This method is increasingly becoming more widely used as it is automated and not user-dependent and is therefore the gold-standard for Hb quantification. With the increase of availability of HPLC, it will be possible to accurately describe the range of HbF in normal and SCD individuals in resource limited settings.

Detection of F-cells

The Kleihauer - Betke method has been used to stain and count the number of FCs present in individuals and is reported as a percentage of RBC[28]. This method is slide based and requires microscopy and is therefore labour intensive, time consuming and prone to methodological errors. The use of fluorescent-labelled anti-F antibody allows the detection of FC on fixed smears. However this method cannot measure the HbF concentration per cell. Flow cytometry using anti-HbF antibody, is a simpler method of counting the number of FCs, and flow cytometry, both simple and fluorescence activated cell separator (FACS) systems have been used to accurately and rapidly detect and quantify FCs[332, 339, 340].

FOETAL HAEMOGLOBIN AND SICKLE CELL DISEASE

Physiology of HbF in SCD

In individuals without SCD, the levels of HbF are between 0.5 and 2%. Most patients with SCD have elevated levels of HbF compared to normal individuals, with marked variation within individuals, and has been found to be up to 20-fold in some populations 9. The factors responsible for this variation include age, sex and a-globin gene number. The β-globin haplotype have also been associated with different HbF levels, with the Arab - Indian haplotypes having higher levels of HbF followed by Senegal, Benin and CAR haplotypes. This probably explains the findings that the levels of HbF go up to 10% in Africa[150, 341, 342, 343, 344, 345;] and 30% in the Arab region[346]. However, there are genetic

factors that are not linked to the β-globin gene, one of which is an area located on the X chromosome known as the F-cell production locus (FCP). Although the FCP locus is thought to be the most important determinant identified, more than 50% of the variation cannot be explained by known factors. These other factors are thought to act by varying the levels of FCs, HbF concentration per cell and preferential survival of FCs.

Amelioration of severe disease by HbF in SCD

There is a wide variation in the clinical severity of disease in individuals with SCD and this has been attributed to various genetic and environmental factors. High levels of HbF is one of the more important factors that has been associated with modifying clinical and laboratory features, reducing the severity of disease and mortality [57,61]. Furthermore, epidemiological studies have reported that certain populations have milder forms of the disease, attributable to high HbF levels[346, 347, 348, 349].

There has been considerable debate regarding the levels of HbF required to ameliorate disease. In 1980, Powars *et al* specifically looked at 14 clinical and haematological parameters in a cohort of 272 patients with SCA in the USA and were unable to establish the effect of HbF on their phenotype[350]. This led to studies that suggested that for HbF to be associated with mild disease, the levels have to be above a 10% to prevent major organ failure e.g. stroke and above 20% to prevent recurrent clinical events e.g. painful crises[351, 352]. In Africa, there are few studies, mainly in West Africa to evaluate HbF and its association with disease severity. The results from these studies have been conflicting

with some reporting association with severe disease and others not showing any significant association[150,341,342,343,344,345].

Mechanism

As previously mentioned, the rate of polymerisation of HbS depends on the total concentration of Hb, as well as the relative composition of HbS and non-HbS Hb within a RBC. The ability of HbS to polymerise is significantly reduced by the presence of HbF[10, 26, 27]. In addition, the high level of HbF "automatically" reduces the amount of Hb S in the RBC[28]. The result is fewer irreversibly sickle cells, reduced rates of haemolysis and preferential survival of FCs. However, even with high HbF levels, there is evidence that suggests that other factors such as FC production, concentration of HbF per FC and FC survival, may also influence the ameliorating effect of HbF[331].

Therapy

Since high levels of HbF are associated with protection from severe consequences of SCD, interventions that would increase the levels of HbF have been used in the treatment of SCD. These interventions include pharmacological agents that are known to augment HbF levels and other interventions involving the manipulation of genetic switching to ensure that HbF levels are produced at high levels, similar to the intrauterine, foetal period.

Pharmacological augmentation of HbF

There are two groups of drugs which have been found to be associated with an increase in HbF levels: cytotoxic drugs which interfere with DNA synthesis such as Hydroxyurea, 5-azacytidine & its analogues and short chain fatty acids. The second group are agents that stimulate the production of erythropoiesis such as erythropoietin.

Hydroxyurea

Hydroxyurea (HI) has been studied extensively and found to increase the number of F-reticulocyte, F-cells and HbF levels[353 354]. A series of clinical trials have found that the use of HI is associated with improvement in various clinical and laboratory events in SCD (Table 13).

Table 13 Studies showing effects of hydroxyurea

Outcome	Year	Number	Type or aim of study	Reference
Reduce haemolysis	1990	10	Effect of HU	[355]
Rate of haemolysis	1990	5	Treatment	[353]
Painful episodes Hospitalisations	1995	Cases n=152 Controls n=147	Double-blinded, randomised controlled trial	[128]
Increase in Hb, MCV, HbF.	1996	15	Pilot trial	[356]
Reduced number and duration of hospitalisation	1996	25	Randomised, cross-over	
Reduced number and duration of hospitalisation	2001	93	Prospective registry	[357]
Mortality	2008	233 Treatment 152 Control 147	Observational	[358]
Increased Hb, MCV, HbF	2004	122		[359]
Secondary stroke	2004	35		[360]
Reduced cerebrovascular flow velocity	2007	59		[361]
Increase HbF, F retics, F cells, the amount of HbF		29	Phase II therapeutic assay	[362]

A landmark double-blind, randomised control trial was published in 1995 which was stopped after a mean follow-up of 21 months. Amongst 148 men and 151 women studied at 21 clinics, the 152 patients assigned to hydroxyurea treatment had lower annual rates of crises than the 147 patients given placebo (median, 2.5 vs. 4.5 crises per year, $P < 0.001$). The median times to the first crisis (3.0 vs. 1.5 months, $P = 0.01$) and the second crisis (8.8 vs. 4.6 months, $P < 0.001$) were longer with hydroxyurea treatment. Fewer patients assigned to hydroxyurea had chest syndrome (25 vs. 51, $P < 0.001$), and fewer underwent transfusions (48 vs. 73, $P = 0.001$)[128]. The effect of HU on mortality was shown by Steinberg *et al* in 2003, where they reported a 40% reduction in mortality ($P = .04$). However, patients with reticulocyte counts less than 250 000/mm3 and haemoglobin levels lower than 9 g/dL had increased mortality ($P = .002$)[363].

However, there are limitations with HU therapy. In about one third of patients, HU does not seem to have any effect on HbF levels, and the reason for this is not entirely clear[364]. It is therefore important to identify very early on the patients who are likely to respond in order to avoid patients being on a cytotoxic drug that will have no benefit. Attempts have been made to identify factors that may be predictive of a good response; and these include plasma hydroxyurea level, initial white blood count and initial HbF concentration. Others include high baseline counts of reticulocytes, two or more episodes of study-defined myelotoxicity (note that myelosuppression is target of therapy and this has to be balanced with myelotoxicity), and absence of a CAR haplotype[354,364].

HU is also associated with toxicity including gastrointestinal disturbance (nausea), hair loss, skin rash and the most significant being myelosuppression. The latter is reversible on

stopping HU intake, and therefore patients on HU require monitoring to be done on a monthly basis. A long term side affect of HU is that as a cytotoxic drug that interferes with DNA synthesis, it has the potential of causing DNA mutation. Although there is a suggestion that prolonged use of HU may be associated with an increase risk of carcinogenesis, in particular with acute leukaemia, there has not been such an increase risk seen when HU was used in other conditions[365-367].

Other agents that increase HbF levels

Azacytidine and its analogues

Decreased HbF production is associated with DNA methylation (by DNA methyltransferase [DNMT]) at the gamma-globin (HbF) gene promoter. Therefore cytosine analogs such as 5-azacytidine and 5-aza-2'-deoxycytidine (decitabine) hypomethylate DNA by inhibiting DNMT, resulting in an increase in HbF levels[368]. Clinical trials with Azacytidine showed a consistent response in increasing HbF levels[369]. Other azacytidine analogues such as decitabine are being tried for use in patients who do not respond to HU[370, 371]. The further development and use of azacytidine was stopped due to concerns of toxicity and increased risk of cancers. However, there is currently interest in use of decitabine as it has been found to be useful in preventing certain cancers[372, 373].

Short chain Fatty Acids

Butyrate and other short chain fatty acids have been found to also increase HbF levels[374-377]. However, the limitation of this group of pharmacologic agents is that in order to achieve adequate HbF levels, a continuous intravenous infusion is required. Moreover, this increased is not sustained with continuous therapy, requiring an intermittent dosing schedule[377, 378].

Orally administered butyrates have been tried but are again limited by the formulation; requiring an intake of over 30 tablets a day[375].

Erythropoietin

Studies in animal models, showed that HbF production could be induced by the use of erythropoietin[379, 380]. Although some studies suggested that use of erythropoietin in SCD patients may not be of measurable benefit[353], Rodgers *et al* showed that if it is used with HU, the effects of HU are augmented, more so than if HU is used alone[381].

Switching

Although the mechanism of switching of globin gene expression resulting in production of HbF in foetal life to adult haemoglobin in the first year of life is beyond the scope of this thesis, it is worth mentioning that there is extensive research into understanding the mechanisms responsible for controlling the switching. This is because by understanding the mechanisms responsible for production of foetal haemoglobin in adult life, this will provide alternative routes for therapeutic manipulation.

FOETAL HAEMOGLOBIN AND MALARIA

Protection against malaria in SCD

There is now substantial evidence that individuals with SCD have resistance against malarial infection *per se*. Such protection has been shown in a number of epidemiological studies [159-161] and is supported by the results from *in-vitro* studies that indicate a range of potential mechanisms, including reduced invasion of HbAS erythrocytes by malarial parasites, reduced parasite multiplication, and accelerated parasite clearance by the spleen[164, 382, 383]. The protective role of HbS in malaria has been studied extensively and is covered in detail in chapter 5 in this thesis. In this section, I will concentrate on the role of foetal haemoglobin (HbF) in malaria, particularly in SCD.

The protection that is described in SCD has been attributed to HbS. However, SCD individuals possess HbF, which has properties that may contribute to malaria resistance. The epidemiological evidence of protection of HbF against malaria can be seen in the first year of life. During this period (birth to 9 months) children have been reported to have few episodes of malaria, and they have not developed immunity[384]. The protection during this period is partially thought to be due to maternal antibodies, but HbF may also contribute to immunity during this period as it is the only Hb present in circulation. (Note Hb in neonatal period is almost 100% HbF) and has been shown to be protective against malaria. The proposed mechanism of protection by HbF is backed by evidence from clinical, *in-vitro* and studies in animal models is as follows: there is preferential invasion of young RBCs by the malaria parasite over HbA containing RBCs[385]. Therefore, parasites are "directed" to invade HbF containing cells (foetal, F-cells). However, HbF has been shown to be resistant to parasite digestion with malaria parasites showing slow rates of growth in HbF containing cells[386, 387].

Therefore parasitised, HbF-containing RBCs will have a retarded growth, shortened life span and will be rapidly cleared from the body[388]. This model seems plausible, as in the first 6-9 months the main Hb is HbF, and it is only after this period that the protective effect of HbS starts having a role[384].

Conclusion

Foetal haemoglobin has an important role in ameliorating disease severity in patients with SCD, with agents that increase HbF levels being effective options in treatment of SCD. In addition, the role of HbF in protection against malaria has not been entirely elucidated. In this thesis, I will examine the spectrum of HbF levels in patients with SCD and explore the relationship with disease morbidity and mortality, with particular focus on malaria, bacterial infections and stroke.

CONCLUSION

On reviewing the literature on SCD, I conclude that although the single gene mutation in SCD has allowed extensive research to understand the role of sickle Hb in clinical disease, it is now apparent that there is a complex interplay between the genotype and the extremely heterogeneous phenotype. From a public health perspective, there is a high prevalence of SCD in Africa and it causes considerable burden to individuals, communities and the health system. Therefore, Tanzania needs to develop a policy that will guide interventions that can be used in the short-term, and start planning a more detailed management plan for the long term. In the medium to long-term, with economic development in low income countries such as Tanzania, there will be a demographic transition, making chronic, non-communicable diseases such as SCD have increasing importance. Research in high income countries has shown evidence of reduction in disease morbidity and mortality with the application of relatively simple interventional measures. There is every reason to believe that the application of some of these interventions would have similar results in Africa.

Model for control of genetic disorders

Within the past 10 years, there have been significant advances in the field of genomics, marked by the publication of the human genome sequence. Since then, various technologies which were previously confined to academic or research oriented centres, have become available in health care. Although the growth of the field of medical genetics (which deals with the application of genetics to medicine) has rapidly expanded in resource-rich countries, it is inevitable that developing counties will need to engage with medical genetics[5 389]. It is therefore important that within developing countries there is a strategy that is formulated to

form the basis of the introduction of medical genetic services for care and control in these countries[5]. SCD could be considered the most important public health genetic disorder because it is common and severe, causing considerable burden to the health system. The problems that hinder establishment and implementation of a control programme of genetic disorders include lack of awareness (of prevalence and significance) and lack of organisation which can be addressed by strategic advocacy to key policy makers, patient/parent support groups and community education[2 3]. Once this is done, there is compelling evidence that SCD could be reduced because it can be diagnosed by simple, cheap tests and the care and treatment can be incorporated into the primary health care system, with only one "central reference laboratory/treatment centre[151]. For these reasons, SCD would be an "ideal disease that could be used as a good model for developing a frame-work for delivering cost-effective genetic services to the community in developing countries[390].

Understanding disease mechanisms, population and evolutionary genetics

Since the discovery of SCD as a molecular disease in 1949 and the subsequent identification of the single gene defect in 1956, there has been extensive research in trying to understand the relationship between genotype and phenotype, resulting in significant progress in our understanding of disease mechanisms and factors modifying disease severity. SCD is also a disease that could be used to understand population and evolutionary genetics by studying recent selection pressures, like malaria, on the development of the human genome. Therefore, it is thought that by using the tools and techniques that are now available and with the appropriate investment, it is possible that major leaps in scientific knowledge, would most likely happen with a simple monogenic disorder like SCD.

RATIONALE FOR THE STUDY

This thesis will describe the clinical and laboratory characteristics of SCD patients in Tanzania. The focus will be on malaria, bacterial infections, neurological events and foetal haemoglobin as these are considered to have a significant role in causing disease morbidity and mortality. In addition, these areas have various interventional options that can be applied to the magnitude of illness and death in individuals with SCD. Unfortunately these interventions have not been used in Africa probably due to the limited resources available in most of the countries in sub-Saharan Africa. The aim is to provide an evidence-base that is locally appropriate which will guide the development of policies for the management and control of SCD in Tanzania.

Chapter Three

Materials and Methods

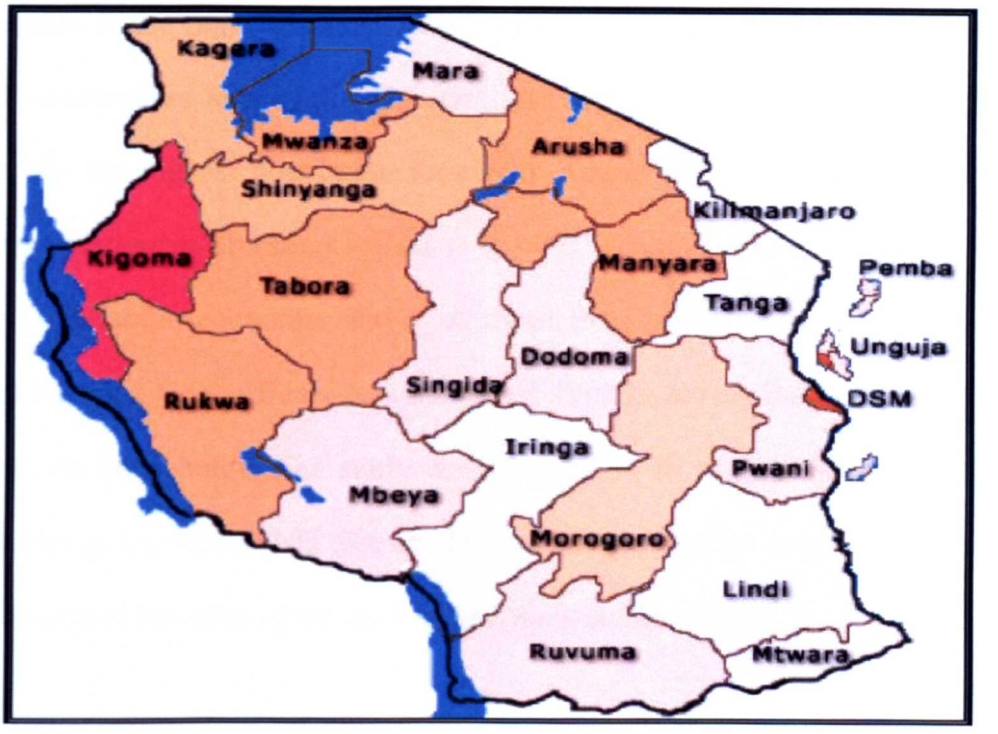
STUDY AREA

Tanzania

Tanzania is situated on the eastern coast of Africa between longitude 29° and 41° East, Latitude 1° and 12° South covering 940,000 square kilometres. It has a population of 40 million with a population growth rate of 2.1 %. 36% of the population lives in an urban area. Tanzania is a low income country which has one of the lowest gross national income (GNI) per capita in the world at 730$, with 36% of the population living below the national poverty line. Note the GNI is converted to international dollars using purchasing power parity rates. An international dollar has the same purchasing power over GNI as a U.S. dollar has in the United States[391].

The national expenditure on health is 4% of gross domestic product (GDP). This covers the total health expenditure and is the sum of public and private health expenditures figured as a percentage of a country's GDP. It covers the provision of health services (preventive and curative), family planning activities, nutrition activities, and emergency aid designated for health but does not include provision of water and sanitation. The physician to population ratio is 0.04 per 1,000 people. The annual birth rate is 1,400,000 per year with a life expectancy at birth of 52 years. The infant and under five mortality rates are high at 104 and 125.6 per 1,000 live births respectively[391]. Tanzania mainland is divided into 21 administrative regions which are further divided into 106 districts (Figure 14).

Figure 14 Map of Tanzania showing administrative regions



Source http://www.tanzania.go.tz/census/census/map2.htm. The shading denotes population density.

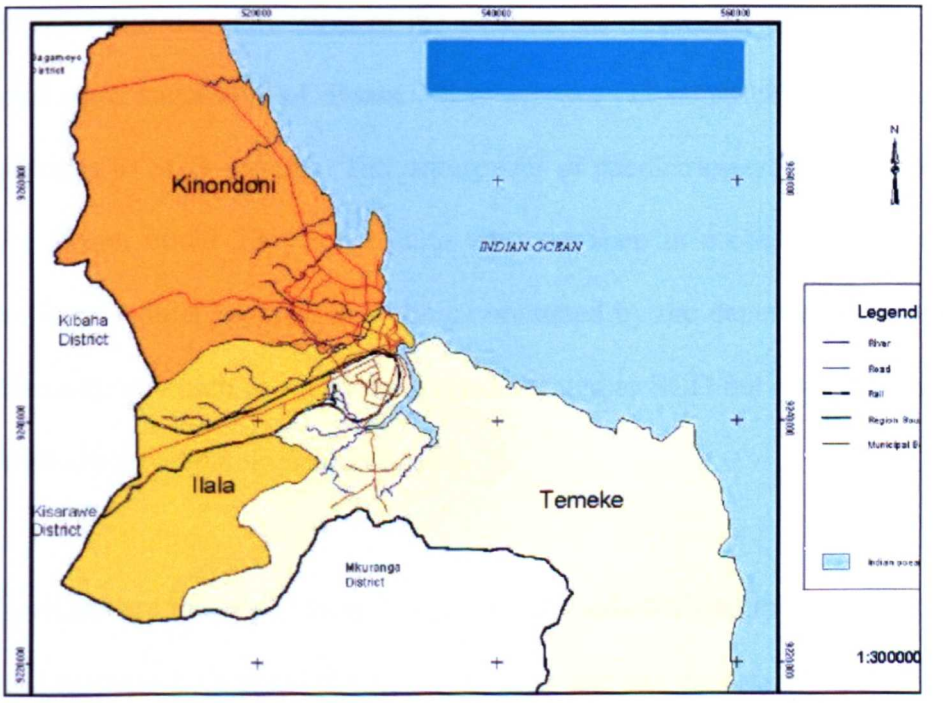
Organisation of health services

The health services in Tanzania are organised into three levels with health centres and district hospitals at primary level, regional hospitals at secondary and tertiary level hospital services being provided by national, referral and specialised hospitals. Tanzania is divided into 4 geographical zones, each with one referral hospital. MNH is the national hospital and also serves as the referral hospital for the eastern zone. The other hospitals are Kilimanjaro Christian Medical Centre (KCMC) based in Kilimanjaro region and serving the northern zone, Bugando referral hospital based in Mwanza region and serving the Western Zone and Mbeya Referral Hospital, based in Mbeya region and serving the southern zone.

Dar-es-Salaam

Dar-es-Salaam is the main administrative region of Tanzania, with a population of 2.5 million. It occupies an area of 1,392 square kilometres and at 1,800 persons per square kilometre has the highest population density in Tanzania. Dar-es-Salaam region has 3 districts (population in brackets) - Ilala (640,000), Kinondoni (1,100,000) and Temeke (780,000). The socio-economic status of these three districts is heterogeneous with Kinondoni having a more affluent population and Temeke having the highest concentration of low income households. The study was carried out in Muhimbili National Hospital (MNH), which is located in Ilala district. This district has the lowest population density in Dar-es-Salaam and has areas of the most intense malaria transmission[391].

Figure 15 Map showing the three districts in Dar-es-Salaam



Muhimbili

Muhimbili consists of the Muhimbili University of Health and Allied Sciences (MUHAS) and Muhimbili National Hospital (MNH). MUHAS is the only public higher learning institution in health sciences in Tanzania and is responsible for training all cadres of health care workers (including doctors, nurses, laboratory technologists and pharmacists). It is closely affiliated to MNH. MNH serves approximately 1,000 outpatients every day (Monday to Friday) and 1,000 admissions a week for inpatient care.

Clinical Services for SCD in Muhimbili

The clinical services for patients with SCD require a multidisciplinary approach and are therefore shared between three departments; haematology and blood transfusion, internal medicine and paediatrics and child health. There is no system in place for screening and diagnosis of SCD and therefore most of the patients are diagnosed when they present with symptoms and signs suggestive of illness. There are two outpatient clinics a week providing outpatient services to SCD patients. The department of paediatrics and child health provides services to children under 13 years of age who are seen in a clinic that is specifically dedicated to SCD. Adults are seen in a clinic conducted by the department of haematology and blood transfusion which is not exclusively dedicated to SCD but also provides a service to patients with other haematological disorders.

Since SCD patients are thought to have a high risk of morbidity and mortality due to malaria, the policy in Tanzania is provide chemoprophylaxis against malaria and prompt diagnosis and treatment of malaria. The recommendation for chemoprophylaxis is chloroquine despite the high resistance. This was because of a failure to identify and propose another agent that

would be affordable, effective and had a known safety profile. All patients attending the paediatric clinic were prescribed chloroquine at a dose of 5mg/kg once a week. During the period of the study, first line therapy for malaria changed from monotherapy with chloroquine to sulphadoxine pyrimethamine (SP), with the current recommendation being artemisinin-based combination therapy (ACT), artemether lumefantrine (ALu)[191] For patients enrolled in the study management continued as recommended by the policy and followed hospital guidelines. SCD patients do not receive chemoprophylaxis with oral penicillin V or vaccination with pneumococcal vaccination to prevent infection against *Streptococcus pneumoniae*.

For patients who require inpatient care, children below the age of 8 years are admitted under the care of the department of paediatrics and child health whereas older children and adults are admitted in the department of internal medicine. The haematology department provides additional care in collaboration with these two departments. The management of SCD patients who were hospitalised followed hospital guidelines. Patients who were hospitalised with pain received hydration with oral fluids (water) and intravenous fluid (1 to 2 litres of normal saline alternating with dextrose-saline over 24 hours depending on the weight and degree of dehydration). The analgesia included paracetamol, diclofenac and pethidine following recommended dosage regimes. Pethidine was often limited to the first 24 hours. SCD patients who were hospitalised with fever were investigated for malaria by a blood slide for malaria parasite. Following this, they received presumptive treatment for malaria following national recommendations mentioned above; uncomplicated malaria was treated with chloroquine, SP or ALu due to changing treatment therapy during the course of the study. Severe malaria was treated with quinine. Patients who were suspected to have bacterial

infections from symptoms and signs (such as a focus of infections e.g. abscess; signs of septicaemia) received antibiotics. The hospital recommendation which was followed for the SCD patients includes a combination of benzylpenicillin and chloramphenicol in children. In adults the same combination was used although in some cases, ceftriaxone was used as first line, presumptive treatment of septicaemia including meningitis. The indication for blood transfusion (whole blood 20mls/kg or packed red blood cell 10mls/kg) included a haemoglobin level of <5g/dL or clinical features of severe anaemia including symptoms of anaemia and the presence of pulmonary oedema and/or right heart failure. Severe pancytopaenia that was not transient (>1 month) and occurred in the presence of evidence of active erythropoiesis (high reticulocyte count above 2%) was discussed with the surgeons to make a decision about splenectomy. Osteomyelitis and avascular necrosis of the femoral head were managed after consultation with the orthopaedic surgeons.

DESCRIPTION OF STUDY

Study design

This thesis is based on an observational study which consisted of systematic longitudinal surveillance of clinical and laboratory events in a cohort of SCD patients recruited at Muhimbili National Hospital. Cross sectional analysis was done to describe pattern of clinic attendance, recruitment and hospitalisation.

In order to explore the pattern, cause and outcome of hospitalisation during the study period, a series of approaches were used. The first was a case-control approach, where cases were patients within the SCD cohort who were recruited into the study and hospitalised at MNH

during the course of the study. Controls for this analysis, were SCD patients who were recruited into the study and never hospitalised at MNH during the course of the study. The second approach was a cross-sectional study, looking at risk factors and outcome for SCD patients hospitalised at MNH. In this analysis, the outcome was survival and therefore comparison was between patients who survived and did not survive during hospitalisation at MNH.

In order to address the role of malaria in precipitating hospitalisation, we used a case-control approach with cases defined as SCD and controls were non-SCD individuals. Details of the analytical approach have been outlined in chapter five, SCD and malaria.

In order to determine the role of bacterial infections in SCD during hospitalisation, this was a prospective cohort study. All SCD patients who were hospitalised at MNH were identified and blood cultures were done to identify the rates and risk factors of bacteraemia. Some patients were identified during hospitalisation and therefore their first contact and recruitment into the study was during hospitalization. In this case, patients were advised to return to clinic after discharge from hospital. For the majority of patients, they were identified and recruited at outpatient clinic. Therefore, events that occurred during hospitalisation could be compared to outpatient visits.

To establish the association of increased CBFv and CVA in SCD patients, the study had two components; the first phase was a cross sectional, observational study to determine the spectrum of CBFv using TCD in SCD patients in MNH. The second phase was a cohort study exploring the relationship between clinically evident CVA, confirmed by CT scan of brain,

and CBFv. Other risk factors for stroke, including hypoxia, frequency of painful crises, hospitalisations etc were also to be assessed. Further details of analytical approach have been outlined in chapter seven, SCD and stroke.

The study design to determine HbF levels in SCD was a cross-sectional survey on all SCD patients who had been recruited into the study. The HbF data were then used to explore association with clinical events collected as part of the longitudinal surveillance of the cohort study.

Study population

SCD population

Estimates suggest that there are approximately 5.6 to 9.9 per 1000 children born every year in Tanzania with SCD[4]. With an annual birth rate of up to 1,500,000 this means that approximately 7,800 to 14,600 children are born with SCD every year. Due to the magnitude of the disease and a reported high rate of mortality in childhood, the Ministry of Health and Social Welfare in Tanzania has a policy that all individuals with SCD should have access to free health care. However, there are no national guidelines for specific comprehensive care or regular follow up at outpatient clinic. Therefore, individuals who are known or suspected to have SCD are diagnosed only when symptomatic and are often seen when they have an acute clinical event or require inpatient hospital care. Outpatient care is offered as part of general medical or paediatric care. MNH is the only hospital which provides specialised health care services for SCD.

Study population

Study population: The study population was patients with SCD who came to MNH for health services and had an entry visit as defined below. Recruitment was done twice a week from patients attending the pre-existing OPD clinics. In addition, patients who were admitted during the study period were also advised to come to the clinic when they were discharged. A patient was considered to be in the SCD cohort if they fulfilled the following criteria:

- Entry visit at outpatient clinic.
- Laboratory diagnosis of SCD. This was defined as SS on haemoglobin electrophoresis and on HPLC their haemoglobin levels were as follows: HbS>70%; HbA_0 = 0%. The assumption was made that the contribution of patients with S-β°thalassaemia and those with S and hereditary persistence of foetal haemoglobin into this cohort of SCD patients would be minimal. This was based on the supposition that the clinical phenotype of S-HPFH is mild and it is unlikely that these individuals would come to the clinic. Individuals with S-β°thalassaemia would also present with SF on haemoglobin electrophoresis and HbS of above 70%, similar to SS individuals. Although, microcytic hypochromic indices may be used to distinguish SS from S-β°thalassaemia, the high prevalence of iron deficiency anaemia in our setting would make it difficult to use this parameter. The definitive diagnosis of S-HPFH and S-β°thalassaemia requires family studies and genotyping for Beta globin mutations which was not done as part of this thesis. Co-inheritance of α-thalassemia, which would also result in low MCV, also requires genotyping for confirmation.
- Patients who had conflicting results or had a history of blood transfusion had a repeat

investigation to confirm the sickle phenotype. If a repeat investigation was not possible, either because the patient died or was lost to follow-up, the patient was not considered to be in the SCD cohort.

The following patients were not considered to be part of the main SCD study population:

- Laboratory diagnosis of AS or AA. These patients were considered to be controls. Further details are included below in section of control population.
- Failure to confirm the diagnosis of SCD. These are patients who had equivocal laboratory results either due to blood transfusion and a repeat laboratory test was not possible either due to death or loss to follow-up.
- No entry visit at outpatient clinic. This includes patients who were identified in the wards during hospitalisation or who were found to have SCD but did not return to clinic for an entry visit.

Hospitalised SCD population: Clinical surveillance was done every day to recruit all patients known or suspected to have SCD who were admitted to the hospital. They were given a unique admission number for each hospitalisation. Since recruitment and the issuing of unique SCD cohort number was only done at the clinic, there were some SCD patients who were identified at hospitalisation but had not been recruited into the study. In this case their hospitalisation date preceded the date of recruitment into the study. Therefore, they were not included in analysis of the rates and risk factors for hospitalisation of the cohort. However, these patients were included in the risk factors for death during hospitalisation.

TCD population: In order to establish the cerebral blood flow velocity using Transcranial

Doppler ultrasonography, SCD children who were aged between 2-16 years were selected during scheduled clinic visits. Between November 2004 and June 2005, all consecutive SCD patients who attended the clinic were given an appointment to return to the hospital for TCD ultrasonography.

Control population

There were two groups of individuals who were recruited into the study as the "control" population. The first population consisted of siblings and relatives of SCD patients attending clinic who were requested to enrol into the study as family members would have similarity with regards to environment as well as some genetic factors. In addition, individuals who came to hospital requesting investigation to exclude SCD were also recruited. Individuals who were found to have a diagnosis of AS or AA were used as the control group for individuals with SCD.

The second population was patients who were recruited from those hospitalisations to the paediatric wards at MNH between 2005 and 2007. These were patients who were not known to have SCD and were screened for malaria using malaria rapid diagnostic tests (RDT) and microscopy to provide data of the background prevalence of malaria parasitaemia during the study period. It is acknowledged that ideally this control population should have included adults. I decided to focus on the paediatric population as from an epidemiological point the prevalence, morbidity and mortality of malaria is highest in children. This was acknowledged as a limitation of the study.

PROCEDURES

Clinical procedures

Procedures at the outpatient clinic

Three types of visits to the outpatient department resulted in the collection of clinical surveillance data.

1) Recruitment: this was the clinic attendance during the study period (25th March 2004 to March 2009) when patients were recruited and enrolled into the study. This is also referred to as the baseline or entry visit.
2) Follow-up visit: three monthly visits, which were scheduled for all SCD patients who were well.
3) Acute visit: unscheduled visits, when the patient attends due to a problem or the attending clinician gave the patient an appointment for review earlier than the scheduled visit.

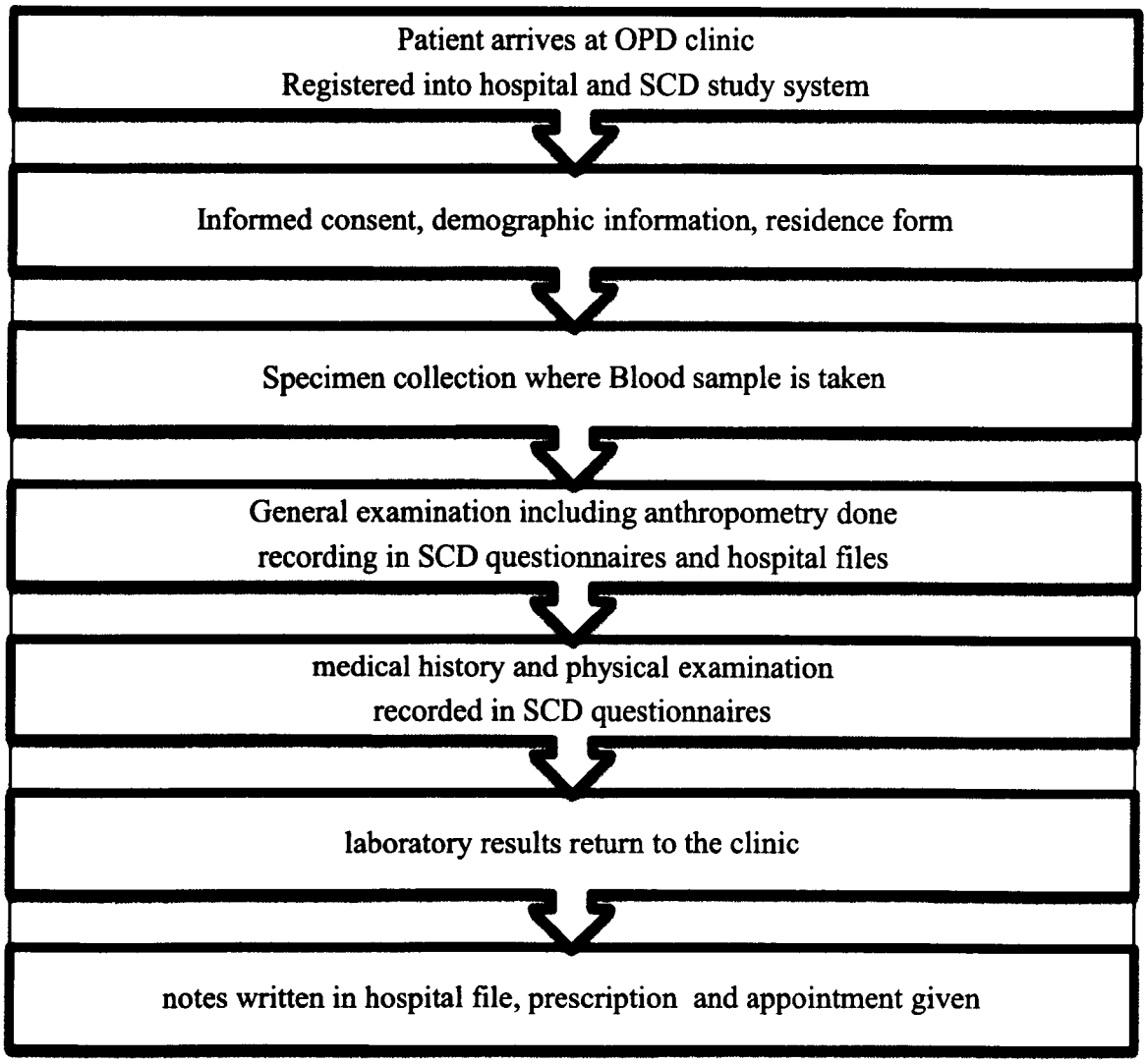
Baseline visit (entry)

At the first clinic attendance during the study window, patients were enrolled into the study. This visit was referred to as the entry visit. All patients who fulfilled the inclusion criteria were recruited into the study after informed consent was obtained from the patients or relatives. All the information was recorded onto standardized proformas that were specifically designed for the study. Clinical information collected included demographic details, past and current symptoms. Physical examination was performed which included general anthropometric measurement and thorough systematic examination. Detailed neurological evaluation was done if clinically indicated as well as assessment of cranial and peripheral nervous system for focal deficits.

Patients were given a SCD number which was their unique identifier that would be used for all subsequent SCD visits. In addition, patients were given SCD booklets where their haemoglobin levels and any other pertinent details were recorded. These books were also used to record details of visit to any health care facility outside Muhimbili.

For patients who were identified as having SCD during hospitalisation, data was collected during this event but recruitment into the study was only done when the patient came to the clinic after discharge from the hospital. Therefore some patients will have hospitalisation events which precede recruitment into the study. This information was collected as it was thought that important data regarding causes of hospitalisation or mortality may not be captured. Therefore, the data were used to determine the rates and risk factors for mortality during hospitalisation. It was not used in the analysis of the rates of hospitalisation for the cohort.

Figure 16 Flow chart for procedures at baseline visit



Scheduled (Follow up) visit

Patients with SCD were scheduled to come to the clinic every three months. During this visit information was collected on a follow-up proforma. This included clinical history with symptoms as well as any acute events between scheduled clinic visits. In addition, physical examination was performed. Investigations that were done included full blood picture and examination for malaria parasites. Additional investigations were done as clinically indicated. During the course of the study, there was an increase in the number of SCD patients. Therefore, the outpatient visits were scheduled between 3 to 9 months.

Unscheduled (Acute) visit

Unscheduled visits were precipitated by an event that required patients to seek health care. During this visit patients were handled as during scheduled visits with particular attention paid to precipitating event. In the event that the patients required in patient care, they were admitted and subsequently handled as an admission. Doctors were also able to ask patients to return to clinic before the 3 month period to follow up a particular problem. This was also regarded as an acute outpatient visit.

Procedures for hospitalisation

Clinical surveillance of the adult and paediatric admitting wards to identify individuals admitted with known or suspected SCD was maintained throughout the period of study. On arrival to the ward, SCD patients were admitted following normal hospital procedures. A general examination was performed that included a record of temperature, weight, blood pressure, peripheral oxygen saturation pulse and respiratory rate. Detailed clinical history and physical examination were undertaken to determine the events leading to hospitalisation.

Blood samples were collected for the following investigations; FBC, reticulocyte count, malaria parasitaemia and blood cultures, serum creatinine, lactate dehydrogenase [LDH], bilirubin total and direct and alkaline phosphatase [ALP]. Additional examinations such as chest X-rays were performed as clinically indicated. All this information was recorded in the "admission questionnaire.

Procedures for neurological events

For patients admitted with suspected neurological events, a more detailed history and examination was taken focusing on the neurological system and the information was recorded in a specific questionnaire for CNS events. Additional investigations that were done included a computerised tomogram of the head for patients suspected to have a cerebral event. Similar procedures were followed at outpatient if a SCD patient was suspected to have a neurological event as an outpatient.

Procedures for TCD ultrasonography

From November 2004 to June 2005, SCD patients who were between 2-16 years of age were recruited during scheduled clinic visits and given appointments to return to the hospital for TCD ultrasonography. On the day of the TCD examination, patients were registered on arrival. Information was given about the procedures and consent was given to allow TCD examination. Personal and demographic details were recorded and a general examination was done with recording of temperature, weight, blood pressure, peripheral oxygen saturation pulse and respiratory rate. Following this, a detailed history and examination was taken focusing on the neurological system and the information was recorded in a specific questionnaire for CNS events. TCD examination was then done on the patient to measure the

CBFv. Patients with abnormal results were informed of the high readings and this information was documented in their SCD books so that they were identified as high risk for CVA. All the patients were then advised to return to the OPD clinic on the day of their scheduled appointment.

Procedures for control populations

Siblings and relatives of SCD patients were invited to attend the OPD clinic where after informed consent, history and examination was taken. This included a detailed family history. Physical examination was done followed by blood sample collection. All the information was recorded in a questionnaire for control patients. Other individuals who were recruited as control populations were patients who attended the hospital requiring screening for SCD. The individuals received their results after 2 weeks. For those who were found to have SCD, they were given an appointment to attend OPD clinic. For those whose results were AS were advised that they had sickle trait but would not require hospital care. However, they were informed of a risk of having children with SCD. These patients were not followed up.

The control population for malaria parasitaemia was recruited from hospitalisations to general paediatric wards. All hospitalisations were seen daily and screening information that included age, sex, presenting complaint was collected. Malaria testing was done by rapid diagnostic testing and microscopy. The results of the malaria test were immediately made available to guide clinical management. Note that any discordant results were read by another laboratory technologist. In the event that the two reports were discordant, a third independent technologists read the slides.

The ideal control population should have been adults and children hospitalised during the study period. This was not possible during this study and is acknowledged as a limitation. For purposes of this sub-study, recruitment was done for all consecutive hospital admissions into the paediatric ward from July 2006 to July 2008.

Procedures for tracing patients who were lost to follow up

In a cohort study, it is imperative that one tries to have complete data on all patients who have been recruited. Therefore, tracing patients who were lost to follow up was an integral part of ensuring completeness of data.

Definition of loss to follow up

Lost to follow up was defined as any SCD patient after recruitment into the study, who did not come to clinic for a follow up visit for a period of at least 6 months. This definition did not include patients who were identified to have SCD during hospitalization but had not yet been seen at the OPD clinic and therefore did not have a unique SCD number.

Tracing patients who were lost to follow up

The procedures for tracing patients who were lost to follow up consisted of 3 stages: 1) identifying from the database patients who were lost to follow up; 2) telephone tracing for those who had telephone numbers 3) physically tracing the patients by going to their place of residence.

Identifying patients who were lost to follow up from database: All patients who were recruited into the study were identified by analyzing the entry database. Two timelines were

identified: date of recruitment and date of last visit to the clinic. For some patients who had only been recruited and never returned to the clinic, this date would be the same. Patients who did not have SCD were excluded, as were those who had been recruited within the last 6 months. From this list, patients who were known to have died either in hospital during hospitalization or when death was known or was reported were identified and excluded. In addition there were a few patients who were known not to attend the clinic for a specific reason e.g. migration. Therefore a list was generated with 3 groups of patients: those with telephone details, those with no telephone but with physical details and those who had no details at all. This information was verified by checking in the proformas in the case notes to confirm the following details: attendance at clinic implying that they are not really lost to follow up but there was an error in the database. Telephone and residence details were also checked to identify if additional information had been provided on other visits. All information was recorded in a new form that was specifically designed with detailed residence information (appendix).

Telephone tracing: A list of lost to follow up for telephone tracing was generated. A new variable was inserted, telephone tracing (available/not available). This information was verified for the second time to ensure that all the information was available. Patients were then telephoned and information was recorded as to whether they were available. For those who were available, a specially formulated proforma known as LTF was completed and entered into LTF database. Traced patients were given an appointment to come to the hospital where a follow up proforma was completed. For those who were not traced by telephone, they were then moved to the list of patients who were going to be physically traced.

Physical tracing: a list of patients who had to be physically traced was generated which included those without telephone numbers, or those with telephone numbers but who had not been found by telephone. The residence information was clustered in the following manner: District>Division>ward>street>ten cell unit leader>school>health post>bus stop so as to make it logistically easy for patients to be traced in the same geographical location. This follows the administrative hierarchy of local government organisation within districts in Tanzania. Before going to the community, information was verified for the third time to confirm that all information is most current.

Procedures in the field: The physical tracing of patients was limited to patients living in Dar-es-Salaam. This has the administrative divisions outlined with local government officers starting at street level with an executive officer who is responsible for "streets which may have several ten cell units. The ten cell unit leaders are "political leaders but often are responsible and know individuals in the households within their units. Therefore, the tracing followed the administrative system, with households being visited with the ten cell unit leader. On finding the patient a previously designed questionnaire for non attenders was completed. This included a verbal autopsy for patients who were reported to have died.

Laboratory procedures

Procedures for collection of samples

Procedures for collection of samples at outpatient clinic

At the baseline visit a total of 6 mls of blood was drawn in all individuals, irrespective of age 2 mls of blood was collected in a tube with EDTA anticoagulant. 2 vacutainers were filled with 1ml of blood each. The vacutainers were either empty sterile containing no additive or

had an additive that separated the serum. One thick blood slide was made for malaria microscopy and a thin blood film was made for reticulocyte staining. All the samples were labelled with SCD number and date when sample was collected. For follow up visits, whole blood was collected and a malaria film was made, with additional specimens being collected as clinically indicated.

Procedures for collection of samples during hospitalisation

During hospitalisation, following informed consent and aseptic procedures, 8 mls of blood was collected: 2 mls into an EDTA container, 2 mls into an empty sterile or serum separator container and 4 mls taken for blood culture. Before 2006, the blood culture samples were collected in bottles that were prepared with appropriate media in the hospital laboratory but after 2006, samples for blood culture were collected in commercially prepared BacTec® bottles. In addition, a urine sample was collected in an empty sterile bottle. All the samples were appropriately labelled.

Procedures for collection of samples for the control population

For siblings and other individuals who came to the outpatient department, the procedures for sample collection were similar to those for patients at baseline visit. For paediatric hospitalisations, following informed consent and explanation of procedures, a capillary blood sample was taken from a finger prick. The blood drop was used for malaria RDTs and an additional sample was used to make a thick film for malaria microscopy.

Procedures for processing of samples

All samples from outpatient clinics were taken during normal working hours and were

transported to the laboratory. The specific investigations were done as outlined. Samples from patients admitted out of working hours were processed within 24 hours.

Processing and separation of samples

Processing of EDTA sample: Two films were prepared from whole blood. A thick film was stained for malaria parasites and a thin film was stained for reticulocytes. A full blood count was then performed using the remaining sample. This included peripheral blood cell count, both total and differential white cell count as well as red blood cell indices. For samples from baseline visits or control individuals, haemoglobin electrophoresis was done using the alkaline cellulose acetate method. The sample was then centrifuged and aliquoted into plasma, which was stored for immunological studies; buffy coat for DNA extraction and subsequent sickle genotyping.

Processing of serum separated samples: Biochemistry analysis was done on the serum and the sample was then stored for processing for HIV and viral hepatitis at a later stage. This is not part of the thesis. The summary of processing of samples is outlined in appendix.

Archiving of samples; There were some investigations that could not be performed immediately. In this case, the appropriate samples were stored for analysis at a later stage. The following components were stored in 10x10 boxes, and stored at -20 degrees centigrade. Buffy coat, packed red blood cells, serum and plasma were also stored. A template for archived samples was written and filed.

Procedures for laboratory investigations

Malaria

Microscopy for malaria parasitaemia: Thick films for malaria diagnosis were made from whole blood at the time of collection from the patients or from EDTA bottle in the laboratory. Thick films were stained using Giemsa and examined by microscopy for the presence of malaria parasites using standard methods. The number of parasites (trophozoites) was counted against 200 white blood cells. A slide was reported negative for malaria parasites after at least 100 high-powered microscopic fields were examined. Note that counting malaria parasites against 200 white blood cells may have resulted in underestimating malaria density as the white cell count in SS patients is higher due to inflammation and haematology analysers counting nucleated RBC as neutrophils. Therefore ideally, the white cell count should have been corrected and "normal" reference values for SS and non-SS individuals calculated and used. For purposes of this thesis, the parasite density was not used. The quality assurance process involved the following: all positive slides were read by a second person. If there was a discrepancy between the two readings, then a third person read the slide. For the negative slides, one out of every ten slides was reread by a second person.

Rapid diagnostic testing for malaria: Rapid diagnostic testing (RDT) to detect malaria Histidine rich protein (HrP-2) antigen was conducted using the Parahit test (Parahit, Span Diagnostics, India or Paracheck, Orchid Biomedical Systems, India). This was reported as positive or negative. These tests come with a commercially-prepared quality assurance method for positive and negative results.

Haematology

Full blood count: Full blood counts were performed on an automated haematology analyser (Pentra 60, Horiba ABX, Kyoto, Japan) was used. Quality assurance was done using commercially prepared reagents that were run on a daily basis with the readings documented and acted on, following the laboratory protocol. On a regular basis, samples were run on 3 different haematology analysers in the university to ensure that the results were valid. The comparison was made with haematology analysers which were part of an external QA programme.

Reticulocyte count: Reticulocyte counts were performed using the new methylene blue method as outlined in appendix III. Using a high-powered lens, the number of reticulocytes was counted against 1,000 mature red blood cells and the results were reported as a percentage of total red blood cells. One in ten slides was read by a second person and the results were compared.

Haemoglobin electrophoresis: Haemoglobin electrophoresis was done for all patients to determine the sickle phenotype using the cellulose acetate method (Helena, Sunderland, Tyne & Wear, UK). (See appendix III). The cellulose acetate membranes were stored and filed for references. The results were reported as SS, AS or AA. For some patients, the results were reported as SF. These results were considered to be SS. Quality assurance was done using commercially prepared controls that were run on for each electrophoresis gel.

The quantitative analysis of haemoglobin sub-types by HPLC: From June 2005, haemoglobin was analysed quantitatively using the Beta thalassaemia short programme on a Variant

Classic HPLC machine (BioRad, Hercules, CA, USA). This machine uses ion-exchange high performance liquid chromatography (HPLC) to identify the different haemoglobins in whole blood. The haemoglobins of interest were haemoglobin A_0, A_2, F and S and the results were used to confirm the diagnosis of sickle haemoglobin as well as to determine the levels of HbF in each individual. The chromatograms and results were photocopied and filed. The data for the corrected area percentage of the different haemoglobins were entered using FoxPro (TM) and subsequently into MySQL database. Patients were considered SS if HbS>70%; HbA=0%; HbA_2<3.5%$_2$. For a definitive differentiation of SS from S/β°-thalassaemia and S/HPFH, DNA and/or family studies are required. HPLC (Hb Fractions and value with units in percentage), however, are extremely useful in distinguishing the 3 genotypic groups as outlined below.

- SS: HbA = 0; HbA_2<3.5; HbS >70; HbF 0 - 25
- S/ β°-thalassaemia: HbA_0 = 0; HbA_2 >3.5; HbS >70; HbF <20
- S/ HPFH: HbA_0 = 0; HbA_2 <2.5; HbS >70; HbF >30

The following patterns were labelled as non-SCD and were distinguished further as follows:

- AS: HbA >50; HbA_2 2-3; HbS 30-40%; HbF 0 -2
- AA: HbA_0>90; HbA_2 2 - 3; HbS 0%; HbF 0 -2

For purposes of this thesis, the analysis was assumed to predominantly include individuals with SS genotype based on Table 12. It is acknowledged that this cohort may include individuals with S/β°-thalassaemia and S/HPFH. Quality assurance was done using commercially prepared reagents that were run for each batch of reagents with the readings documented and acted on, following the laboratory protocol.

DNA diagnosis for haemoglobinopathies: The diagnosis of SCD and other haemoglobinopathies particularly S/β°-thalassaemia and S/HPFH is made by DNA studies. DNA was extracted and stored for all individuals recruited into the study, but DNA studies were not part of the thesis.

Chemistry

Blood: The following parameters were assayed using a chemistry analyser (Roche Cobas Mira, New York, USA or Abbott Architect, New York, USA) - bilirubin (total and direct); lactate dehydrogenase, (LDH) alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and creatinine. Quality assurance was done using commercially prepared reagents that were run on a daily basis with the readings documented following laboratory protocol.

Microbiology

Blood cultures

From the start of the study until July 2005, blood cultures were processed using standard hospital laboratory procedures. Culture media was prepared in the hospital microbiological laboratory and identification followed conventional routine techniques. The organisms that were identified were archived in -70°C freezers. The hospital laboratory is part of a project known as Network for Surveillance of Pneumococcal Disease in the East African Region (netSPEAR). netSPEAR (http://www.aboutus.org/NetSpear.org) supported laboratories to improve the quality of microbiology techniques, particularly in hospital laboratories where resources were limited and the yield of positive microbiology culture was low. This was done by providing reagents, reviewing standard operating procedures and providing a quality assurance system. The organisms that were identified were archived in -70°C freezers and

sent to Nairobi, Kenya for re-identification. The re-identification was done in a laboratory that has accreditation at international standards for good laboratory practise. The results from the re-identification revealed that the differences in the results between the two laboratories were more than 70%. This was discussed in detail with the laboratory and strategies were proposed to address the potential causes of the differences in the results. Some of the strategies included using commercially-prepared horse blood instead of human blood to make blood agar media plates, use of automated blood culture machines and regular positive quality assurance method to ensure that the media supported the growth of fastidious organisms such as SPN.

For purposes of this thesis, it was felt that in order to ensure scientific rigour in achieving this objective, it was necessary for blood cultures to be done in an environment where I would be able to achieve the standards required to answer the question. Therefore, from July 2005, blood cultures were processed using the BACTEC 9050 system (BACTEC, Becton Dickinson, Franklin Lakes, NJ, USA). Positive samples were examined under Gram staining and sub-cultured to standard media plates (chocolate agar, blood agar and CLED). Isolates were identified using routine microbiological techniques which included microscopy and Gram-staining, API biochemical testing, catalase and specific identification for streptococcus and staphylococcus. Isolates were stored in freezing media which was specifically prepared and stored at minus $70^{O}C$ for identification in the laboratory in Kenya. When this was done, the results that were obtained were accurate. All batches of blood culture bottles and agar media plates were tested by inoculation with known organisms suspended in transport media the blood culture bottle was then inserted into the BACEC machine to check that positive growth would be identified. For agar media plates, organisms were inoculated, grown and

identified to ensure that the media supported microbial growth and resulted in correct diagnosis.

Cultures of other samples

Samples from other sites such as wounds, urine etc were collected as clinically indicated. They were processed using routine microbiological procedures. These results will not be presented in this thesis.

Procedures for TCD ultrasonography

TCD ultrasonography

I conducted TCD examination following intensive training for 3 months in Kilifi, Kenya. TCD examination was conducted to measure CBFv using a Nicolet EME companion II (Nicolet, Warwick, UK). This instrument uses pulsed ultrasound at a frequency of 2MHz generated by a transducer that is held against the temporal portion of the scalp to assess velocities of middle cerebral artery (MCA), anterior (ACA) and posterior cerebral arteries (PCA) on the left and right hand side. The basilar artery (BA) was assessed using the suboccipital approach. Additional signals were obtained from the internal carotid artery (ICA) in the neck. The highest time-averaged maximal mean velocities (Vmean), depth at which the artery was insonated, pulsatility index (PI) and resistance index (RI) were recorded.

Quality assurance of TCD: The blood vessels were identified by depth, waveform pattern, sample volume, direction of blood flow and angle of insonation. The quality of the TCD measurements was done by ensuring that the CBFv that was recorded came from the

waveform with the best audiovisual signal and envelope. Before recording, the vessel was "tracked" up and down by 2mm increments to ensure that the best waveform was identified. Snapshots of all the recordings were taken and saved. The results were saved on the TCD machine. The data were backed up on an external hard drive which was stored in a different physical location from the TCD machine. The results were independently reviewed by a consultant neurologist, Fenella Kirkham, Institute of child health, to ensure that the readings were accurate.

Reference ranges for the parameters were derived from studies done in Kilifi, Kenya[392]. These were calculated from CBFv from individuals who were well and not known to have SCD. The range was defined as -2 standard deviation (SD) as the lower limit and +2SD as the upper limit around the median value of CBFv readings.

Computerised tomography

For patients who presented with features suggestive of a neurological event due to a cerebrovascular accident, computerised tomography of the head was done. The scans were reported by a radiologist and the results were filed. The CT scans were also digitised. These results will not be presented in this thesis.

Radiography of chest, bones and other sites

X-rays were taken at Muhimbili National Hospital when clinically indicated. The results were documented in the patients file notes. These results will not be presented in this thesis.

Other radiological and imaging investigations

Other radiological investigations including ultrasonography and echocardiography were conducted as clinically indicated. The results of these investigations were documented and filed in the patients records.

Personnel

Clinical personnel

The clinical-epidemiological information from the hospital visits was collected by the author with the assistance of two medical officers. Additional assistance was increased during the course of the study to take into the account the higher number of study participants than anticipated. Supervision and monitoring was done through daily meetings, reviewing of questionnaires as well as reviewing hospitalised patients. For the TCD, I was responsible for performing all the procedures of neurological and TCD examinations.

Laboratory personnel

I was responsible for setting up and performing the following laboratory tests: haemoglobin electrophoresis, reticulocyte staining, and malaria microscopy. These tests were subsequently done by a laboratory technician with a Diploma in Medical Laboratory Sciences, under my supervision. Microbiology procedures were similarly set up following a period of training at the KEMRI-Wellcome Trust programme in Kilifi, Kenya. This site has established laboratory systems and has received accreditation for international good laboratory practise standards. The microbiology procedures were consequently supported jointly with experienced microbiologists in both Muhimbili and Kilifi.

I was responsible for interpretation of the results and troubleshooting problems that arose with equipment or procedures. Investigations using automated machines such as Haematology and chemistry analysers were done following standard operating procedures for the machines. The tests were performed by hospital laboratory personnel. The author was responsible for ensuring that quality assurance procedures were put in place as well as the interpretation of normal and abnormal results. Once the procedures were in place and functioning adequately, they were performed by 2 laboratory technologists under the supervision of the author.

Data personnel

I was responsible for development of the proformas, coding of the variables, and design of the database (both the FoxPro and MySQL). The data were entered by data entry clerks who I supervised.

DATA MANAGEMENT

Data collection

Clinical data

All clinical information was collected in pre-formatted questionnaires or proformas that were designed specifically for the study. There were specific proformas for different visits or events. During outpatient visits a baseline/entry or follow up visits proforma was completed and during hospitalisation an admission proforma was completed. A CNS proforma was completed in the event of a suspected neurological event. For the control population, a control proforma was completed. Information for paediatric hospitalisations was recorded in

a log book. For patients who were lost to follow up, a proforma was completed and in the event of death a verbal autopsy section was completed. Residence information was collected into residence proformas. Proformas were reviewed annually to amend variables and improve the quality of data collected. All proformas are attached in appendix.

Laboratory data

Laboratory data were collected from different sources. For haematology data, the results were printed from haematology analysers. For the results that were generated from hospital laboratory, the printed results were photocopied and the copy was stored in the study patient file. For other laboratory results, this was recorded into pre-printed laboratory results forms and filed with the patients' case notes.

Other data

TCD data were automatically generated during TCD examination. These data were entered into FoxPro and subsequently into the MySQL database.

Data entry

From 2004 to 2007, clinical and laboratory data were entered in FoxPro (version 6 (Microsoft Corp., Seattle, WA, USA). In 2007, due to the increase in data that were being collected, a new database system was designed that used a web-based system known as MySQL (Sun Microsystems Inc, Santa Clara, California, USA). The data that had already been entered were transferred into the new database system.

Data cleaning

Data verification and cleaning was done following double entry and inconsistencies were corrected. Missing data, that was independent of time of collection, was collected at subsequent visits.

Data security

The questionnaires were stored in filing cabinets and locked, with access only to personnel involved in the study. Access to the data was limited to a few key personnel who were authorised by the author. Data were backed up on a weekly basis onto CDs which were stored in a different physical location.

STATISTICAL METHODS

Introduction

An analysis plan was formulated during proposal development. This was refined after 18 months of data collection to outline an approach to the data. An initial description of the cohort was made. In order to address the four specific objectives, regarding malaria, infections, stroke and HbF, a series of different analytical approaches were used to take into account the complexity of the study design and magnitude of the data. Details of the analyses conducted will be described in the methods section of each chapter. The analysis was done using STATAv10 (StataCorp, College Station, TX).

Definition of variables

Outcome variables

SS Sickle cell disease: This was defined as any patient who had sickle cell phenotype SS as detected by Hb Electrophoresis and HPLC. Patients who were SS were defined as sickle cell disease, those with phenotype AS as sickle cell trait and AA as normal. This was generated into binary outcome variables where 1=SS was sickle and 0= Non-sickle and included AA or AS.

Hospitalisation: This was defined as hospitalisation during the study period and included any patient who was admitted for inpatient hospital care. A binary variable was generated from hospitalisation or no hospitalisation during the study period where 0=no hospitalisation and 1=hospitalisation. At entry into the study patients were asked if they had ever been admitted and this was defined as history of hospitalisation where 0=no history and 1=history of hospitalisation.

Malaria: Malaria was defined as positive malaria rapid test or the presence of malaria trophozoites on microscopy.

Stroke: A neurological deficit caused by injury to part of the brain due to either lack of blood (infarction) or rupture of a blood vessel and subsequent haemorrhage into the brain. This was characterized by a combination of symptoms (weakness of limb or part of body, loss of consciousness, seizures) and signs (loss of muscle bulk; either decrease or increase in muscle tone; loss of muscle power) lasting for more than twenty four hours.

Bacterial infection: Bacterial infection was defined as binary variable with presence or absence of bacteria on blood culture during hospitalisation (bacteraemia). Further categorisation of bacteraemia was done according to the species isolated.

Exposure variables

Demographic variables This included the gender, age and place of birth. Ethnic origin was defined by coding the tribe of mother. The residence at time of recruitment was coded according to the frequency of occurrence of a place.

Clinical variables This included the presence or absence (coded 0 absent 1 present) of any of the following features during surveillance. Symptoms included any history where the individual or parent reported the presence of fever, pain, anaemia, jaundice, cough, difficulty in breathing and CNS event (weakness (hemiplegia), seizures, transient ischaemic attacks, cranial nerve deficit and gross cognitive impairment) Physical signs were categorised as follows: fever was axillary temperature > 37.5°C; hypertension was blood pressure > 150/100 mmHg and hypoxia as peripheral oxygen saturation <95%.

Laboratory variables: Haematological variables included red blood cell indices [haemoglobin (Hb), red blood cell count (RBC), and mean corpuscular volume (MCV)], white cell indices (total white blood cell count (WBC)) and platelet count. In addition, reticulocyte count and HbF levels were also defined. Clinical chemistry variables included bilirubin (total and indirect), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminase (AST) and creatinine.

The laboratory variables were analysed as continuous variables. They were then categorised using cut-offs from published international reference values. I decided to use the cut – off values from published reference values as this would allow me to compare the results from this study with the normal population. The level of haemoglobin below 5g/dL was defined as severe anaemia. The details of cut-off points that were used to define abnormal CBFv and high HbF as generated from this study are presented in their respective chapters.

Patient level variables

Variables that remained constant for each individual throughout the study were sex and sickle status. Sex was recorded as female=0, male=1, with female used as the reference category. Sickle status has been defined previously.

Time dependent variables

Variables that were known to change their value over time were age of individuals, clinical and laboratory variables and month in study.

Age: The age was calculated at date of visit from the date of birth. The age was calculated in years rather than months. Age was then examined as a continuous variable with increments of 1 year. Three collapsed age groups variables were defined with categories as follows: <2, 2-4, 5-9, 10-14, 15-19, 20-29, 30-39 and >40. The second age group was: <2, 2-4, 5-9, 10-19, >20. The third age group was <5, 5-10 and >10 years. Different age groups were defined to allow comparison with published data.

Steady state: although the clinical and laboratory variables changed with each visit, it was

assumed that when well there would be minimal variability within an individual. The values during this "steady-state" were determined from visits at outpatient clinic where patient was well and there was no hospitalisation event within the preceding or subsequent 3 months. These data were not used in the thesis.

Date and month of visit: month of visit was categorized into a variable that included month and year of study, beginning with March 2004 and ending in March 2009 for recruitment.

Descriptive analyses

Description of study populations for analyses

The initial analyses involved an overall description of the study outlining the total number of SCD patients and control individual recruited into the study. This was followed by a description of the SCD patients at two different time points – at recruitment into the study and during hospitalisation.

Description of SCD cohort during recruitment: This was a description of the clinical and laboratory features of SCD patients at the time of recruitment into the study. This group of patients was compared with patients who did not have SCD.

Description of SCD cohort during hospitalisation: A number of cohort members were hospitalised during the study period. A description of the rates and risk factors for hospitalisation was examined by comparing the admitted and non-admitted patients. A separate analysis was done on all SCD individuals hospitalised during the study period to determine risk factors that determine adverse outcomes during hospitalisation.

Description of SCD cohort during follow-up visits (longitudinal surveillance) : There was initial analysis of the pattern and factors that determine follow up visits. This included examination of adherence/compliance to follow up. Major clinical events that were known to be a cause of morbidity and mortality in SCD were then examined. This included mortality and hospitalisation. The three events that were the specific aims of the study – malaria, infection and stroke are examined in their individual chapters. Longitudinal analysis of specific clinical events such as pain, anaemia, jaundice and any other event that had a high occurrence will not be included in this thesis.

Disease estimates

Prevalence: The unit of observation was at the visit level i.e. the clinical and laboratory results from one individual during a visit. The visit was either recruitment, follow-up, hospitalisation or tracing during lost to follow up. The prevalence estimates for any outcome represent the outcome prevalence (number of positive outcome/total number of outcomes in the relevant population).

Incidence rates: Incidence rates were calculated for the following disease outcomes; mortality, hospitalisation, malaria and stroke. An incident case was defined as a case in any individual at that particular visit. Therefore incidence rate estimates included recurrent cases. The exception was for stroke, where clinical examination had to determine whether the neurological deficit was due to a new event. Incidence rates were expressed as number of events per patient years of observation.

Calculation of incidence using STATA

All the data from outpatient clinic visits and hospitalisation were combined to make one dataset. The data therefore comprised of records of individuals with prospectively collected observations at different time points. In order to do longitudinal analysis, the data need to look at events at different time points. This analysis is referred to as survival analysis and involves the event of interest and time. The event is referred to as a 'failure' and the time until 'failure' is 'survival time'. In order to do longitudinal analysis, the data has to be set so that the analysis focuses on times until the event of interest occurs. In order to do this, the following command was used: *stset datetod, id (demographic_id) failure(death) exit(time end_date) scale(365.25)* where *datetod* is the date that the individual was seen, *id* is the unique identifier of the individual, which in my data base was coded as 'demographic id'. *Failure* is the event under analysis. The event was the outcome for which the incidence was being calculated. This was death, hospitalisation, malaria and stroke. *Exit* is the time at which the individual exited the study and *scale* gives the time scale in years.

Following this, the incidence was calculated with the outcome expressed in person years of observation. This was done by using the STATA command *stsum* to calculate the overall incidence of the event and *stsum, by (agegp),* provided the incidence rate stratified by age at which the event occurred. The result was expressed per 100 PYO.

Univariable analyses

Univariable analyses of exposure variables were conducted to assess the strength and direction of association with the outcome of interest. The statistical significance of univariable associations was determined using χ^2 or Fisher's exact contingency table analysis.

The level of statistical significance was set at 0.05 with values between 0.05 and 0.09 considered to be marginally statistically significant. Crude odds ratios were determined with ordinary logistic regression and reported with 95% confidence intervals (95% CI) around the odds ratios (OR).

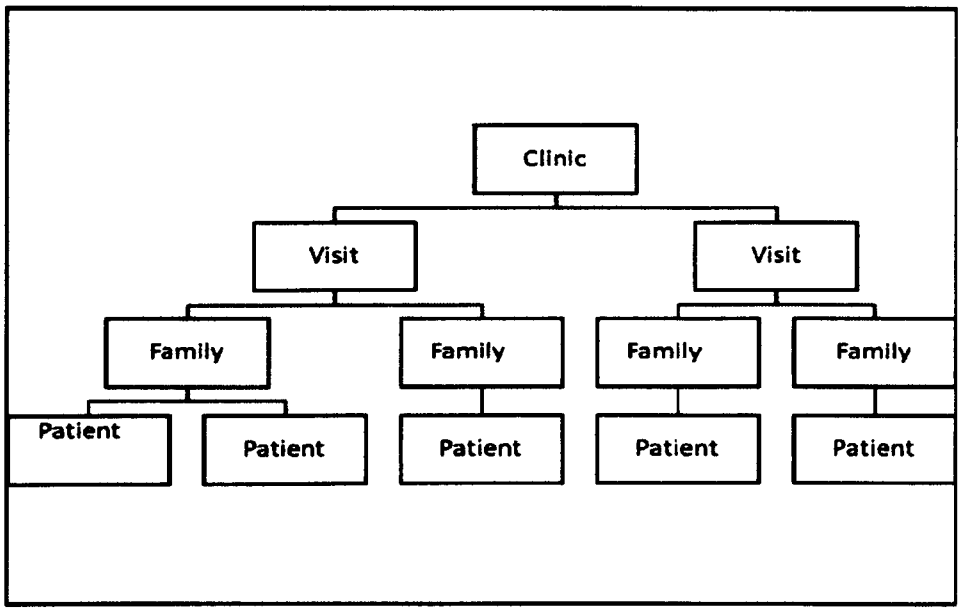
Multivariable analyses

Multivariable analyses was done using logistic regression which assumes that exposure variables are independent of each other. This allows one to determine the effect of exposure factor on the risk of disease outcome after controlling for the effect of another exposure factor. Exposure factors that had a level of significance of ≤ 0.05 during univariable analysis were included in a multivariable model. A stepwise procedure was used to arrive at a final multivariable model. The level of significance and confidence interval was reported as above.

Accounting for clustering of data

Multivariable analyses assumes that exposure variables are independent of each other. However, the design of the study is such that there was clustering of data at different levels leading to a multi-level hierarchical dataset which is shown below (Figure 17)

Figure 17 Hierarchical structure of data



Clinic is defined as visit to hospital clinic. Family: members of one family who share one or both parents.

Individual: data were clustered within an individual, as a patient could have data from different visits during the study period.

Family; There were observations that were clustered within families as there were siblings and twins who may have either SCD or were controls. Family was defined as individuals who shared one or both parents.

Visits: It was likely that there would be unmeasured factors that were specific for a particular visit. For example, haematology variables from Thursday and Friday clinic would be different as they use different haematology analysers on each day. Results from the different analysers were compared to ensure that they were comparable to ensure that the use of different analysers did not affect or account for the differences. Therefore there was clustering of individuals within visits.

Cross classification: although there was clustering within visits, an individual could have measurements from different visits, therefore visits could be said to cluster within an individual. For example, an individual could have 2 hospitalisations and four follow up visits. This type of data would require modelling to take into account such a complex data structure. For purposes of this thesis, the analysis used the building of models with 2-level mixed effects logistic regression, with visit included as a fixed effect and individual as a random effect. This would account for clustering at visit and individual level. The clustering within families was not taken into account.

ANALYSIS PLAN

Baseline description

This included a description of pattern of clinic attendance and recruitment. This was followed by a detailed description of the clinical and laboratory features of patients at the time of recruitment into the study. The group used as a control population was individuals with sickle phenotype AA and AS.

Hospitalisation

This included a description of the pattern, cause and outcome of hospitalisations during the study period. On determining risk factors of hospitalisation, the association of the clinical and laboratory features of the SCD patients at baseline visit was explored with the risk of hospitalisation during the course of the study. For this analysis, a comparison of characteristics at baseline visit between the individuals in the SCD cohort who were

hospitalised at MNH during the course of the study and those individuals who never hospitalised at MNH during the course of the study. The aim was to identify factors that will identify patients at the first visit with a likelihood of hospitalization

A second analysis looked at risk factors for mortality during hospitalisation by analysing the association between clinical and laboratory factors at hospitalisation with mortality. The aim was to identify factors at hospitalisation that could identify those with a higher risk of mortality during hospitalisation. In this analysis, all SCD individuals hospitalised during the study period were included. Therefore, this included SCD individuals who were identified in the ward, but may not have returned to clinic and may not have been recruited into the SCD cohort.

Longitudinal data (follow up)

This is a description of pattern of follow-up visits and rates of patients who are lost to follow up. There is a description of key events which include death, hospitalisation, malaria, stroke and bacteraemia during the course of the study. The aim was to identify the rates of these events during the study period and identify the risk factors associated with these events.

Malaria

The analysis for this section was to answer the question; is malaria a common cause of morbidity and mortality in SCD? To do this a description of the prevalence of malaria in SCD compared to non-SCD population was made. Further analyses were done to assess malaria in SCD patients and control during hospitalisation. Further details of analytical approach are covered in the individual chapter.

Infections

The analyses in this section were to describe the major bacterial pathogens in blood associated with all SCD patients on hospitalisation. The objective was to determine the magnitude of bacteraemia in SCD patients and to describe the major pathogens identified. Further details of the analytical approach are covered in the relevant chapter.

Stroke and other CNS events

This includes a descriptive analysis of prevalence of stroke and other neurological events at entry into the study. This was followed by a description of the spectrum of CBFv in SCD. For longitudinal analysis, the incidence rate of stroke and risk factors associated with increased risk was determined. This is covered in detail in the relevant chapter.

Foetal haemoglobin

Initially there was a description of the spectrum of levels of HbF in SCD. Analysis was done to look for demographic, clinical and laboratory factors that could be used to identify individuals with high HbF levels. This was followed by analysis to determine whether HbF levels were associated with specific clinical events including death, hospitalisation, malaria and stroke. Details of further analyses are included in relevant chapter.

Study design and statistical analysis

Study design

During planning the study, I contacted individuals and centres considered to be experts in the field of SCD research and healthcare. Therefore, I visited and discussed the most appropriate

study design for the questions that I wanted to answer. The centres and people I discussed included David Weatherall, Swee Lay Thein, David Rees, and Fenella Kirkham in the UK; Graham Sergeant, Terrence Forrester in Jamaica and in the USA Martin Steinberg, Mohandas Narla, Griffin Rogers, Thomas Wellems, Alan Schechter, Kwaku Ohene-Frempong. The statistical methods for planning the study were done with the statistician of the KEMRI Wellcome research programme, Kilifi, Kenya. This included estimating sample size, advice on the size of control populations as well as statistical methods for analysis.

Data analysis

Developing skills in clinical epidemiology and statistical analysis was part of the PhD training programme. I attended two courses at the London School of Hygiene & Tropical Medicine, UK; the intensive course in epidemiology and medical statistics and the advanced course in epidemiological analysis. During the course, I acquired skills in analysing data using STATA. As part of the course, the course organisers provided study clinics, where they provide advice regarding strategic approaches to data analysis. Since I had already been collecting data for over 12 months when I attended the course, I had several discussions and advice during the data clinics. Following these discussions, I reviewed and modified my analysis plan.

The analysis of the data was done under supervision of Greg Fegan, the statistician of the programme. The analysis of the malaria chapter was done in collaboration with one of the PhD supervisors, Thomas Williams. For analysis of survival data, the approach followed the methods used by Platt et al. This was supervised by Greg Fegan with advice from Ian Hambleton (Jamaica) and Emelda Okiro (Kenya). Both have expertise in longitudinal

analysis of cohort data.

Analysis of longitudinal data requires time-series analysis and advanced statistical techniques in handling data with multiple levels of hierarchy and clustering. This was noted, discussed and agreed that for purposes of this thesis, the analysis would be limited to approach described. In order to further utilise the data that has been collected, I would pursue further analytical skills during the post-doctoral period.

Chapter Four

General Results

INTRODUCTION

The prospective, cohort study at Muhimbili started on 25th March 2004. In this chapter, the first section will be a description of pattern of recruitment, comparison between SCD patients and controls and characteristics of main study population at recruitment. Section two will include the rates and risk factors of hospitalisation during the study period. Section three will describe the rates and risk factors for mortality in SCD patients. Within each section, there will be a brief introduction of the objectives of the section, methods and results. The discussion of the three sections will be covered at the end of the chapter.

DESCRIPTION OF STUDY POPULATION

Introduction

In Tanzania there are no screening programmes to identify SCD individuals at birth or in the community. Therefore, it is likely that SCD individuals who are symptomatic visit several health facilities and receive symptomatic treatment for clinical events such as fever, pain, anaemia and stroke before a diagnosis is suspected and the appropriate laboratory investigations are done. Recognising that most SCD individuals will visit a health facility which may not have expertise and resources for diagnosis of SCD, we wanted to identify symptoms and signs that could be used in areas with limited resources to identify those individuals likely to have SCD. These individuals would then be prioritised to be sent for further investigations. Therefore, in this study, the aim was to compare clinical and laboratory

characteristics of SCD and non-SCD individuals at OPD clinic.

Methods

SCD patients

This is the main SCD population with the following inclusion criteria:

- An entry visit at the outpatient clinic at MNH between March 2004 and March 2009.
- Confirmed laboratory diagnosis of SCD.

SCD individuals who were identified during hospitalisation but did not return to the clinic for an entry visit were not included in this analysis.

SCD patients who did not live in Dar-es-Salaam were included in the main study cohort. This is because most of these patients would travel to Muhimbili once or twice a year for follow-up clinics. Furthermore, those patients who lived outside Dar-es-salaam agreed to be contacted by telephone.

Non-SCD individuals

The ideal control population for this study would be age-matched individuals, recruited in the same period and from the same community as the SCD population. This would have required considerable resources (including a demographic surveillance system) that were not available during the course of my study. At the beginning of the study it was agreed following consultation with my supervisors and statisticians, that the control population should have a minimum of a 100 control patients in each age band < 2 years, 2 – 4 years, 5 – 9 years, 10 – 19 years and over 20 years. A decision was made to recruit siblings and relatives of SCD patients attending the MNH. The assumption was that family members would have similarity

with regards to environment as well as some genetic factors. Therefore, the differences would be due to the diagnosis of SCD. Therefore, family members of SCD patients were invited to enter the study. After 3 months of recruitment, the number of individuals who were identified was small. Therefore, after discussion with my supervisors, a decision was made to include individuals who came to hospital requesting investigations for SCD. These were referred either because they were symptomatic or because the attending clinician wanted to exclude a diagnosis of SCD.

This group consisted of individuals who had the following criteria:

- A visit at the outpatient clinic between March 2004 and March 2009.
- Confirmed laboratory diagnosis of AS or AA.

Statistical methods

A general outline of descriptive statistics, univariable and multivariable analyses is provided in the methods chapter.

In this analysis all individuals who fulfilled the inclusion criteria for SCD and non-SCD as defined above were included in one dataset. Individuals where the laboratory diagnosis of SCD status was equivocal or was not available were excluded. The outcome was SCD status. Descriptive analyses were run to determine the mean, median and range of individuals recruited per month during the study period. The non-SCD individuals were then separated into AS and AA individuals to determine the composition of the non-SCD population.

To explore the clinical and laboratory characteristics of the SCD and non-SCD populations

the two groups were compared. The initial comparison was done by stratifying the two populations into the 5 age bands. Following this, clinical and laboratory characteristics were compared between the two groups.

Continuous variables were compared using two sided t-tests while categorical variables were compared using the χ^2 test and results presented as odds ratios (ORs) with 95% confidence intervals. Multivariable logistic regression was used to identify independent associations with malaria, using all variables that were significantly associated ($p<0.05$) on univariable analysis.

Results

Patients were recruited serially from the paediatric SCD clinic and adult general haematology clinic. The study is ongoing, but the analysis is limited to patients recruited from March 2004 to March 2009, giving a total period of follow up of 60 months. 2,487 individuals were recruited into the study with the sickle phenotype as follows: SS 1,725 (69.4%); AS 455 (18.3%) and AA 307 (12.3%) (Figure 18). The pattern of recruitment is shown in the figure below (Figure 19). The mean number of individuals recruited per month was 41.4 (SD 35.9) with a range of 2 to 209 individuals seen per month. For individuals with SCD, a mean 28.7 (SD 36.5) number of patients were recruited every month with a range of 2 to 206 patients. For non-SCD individuals, the mean number of individuals seen every month was 13.1 (11.9) patients with a range of 1 to 71 individuals every week.

Figure 18 Summary of individuals recruited into the study

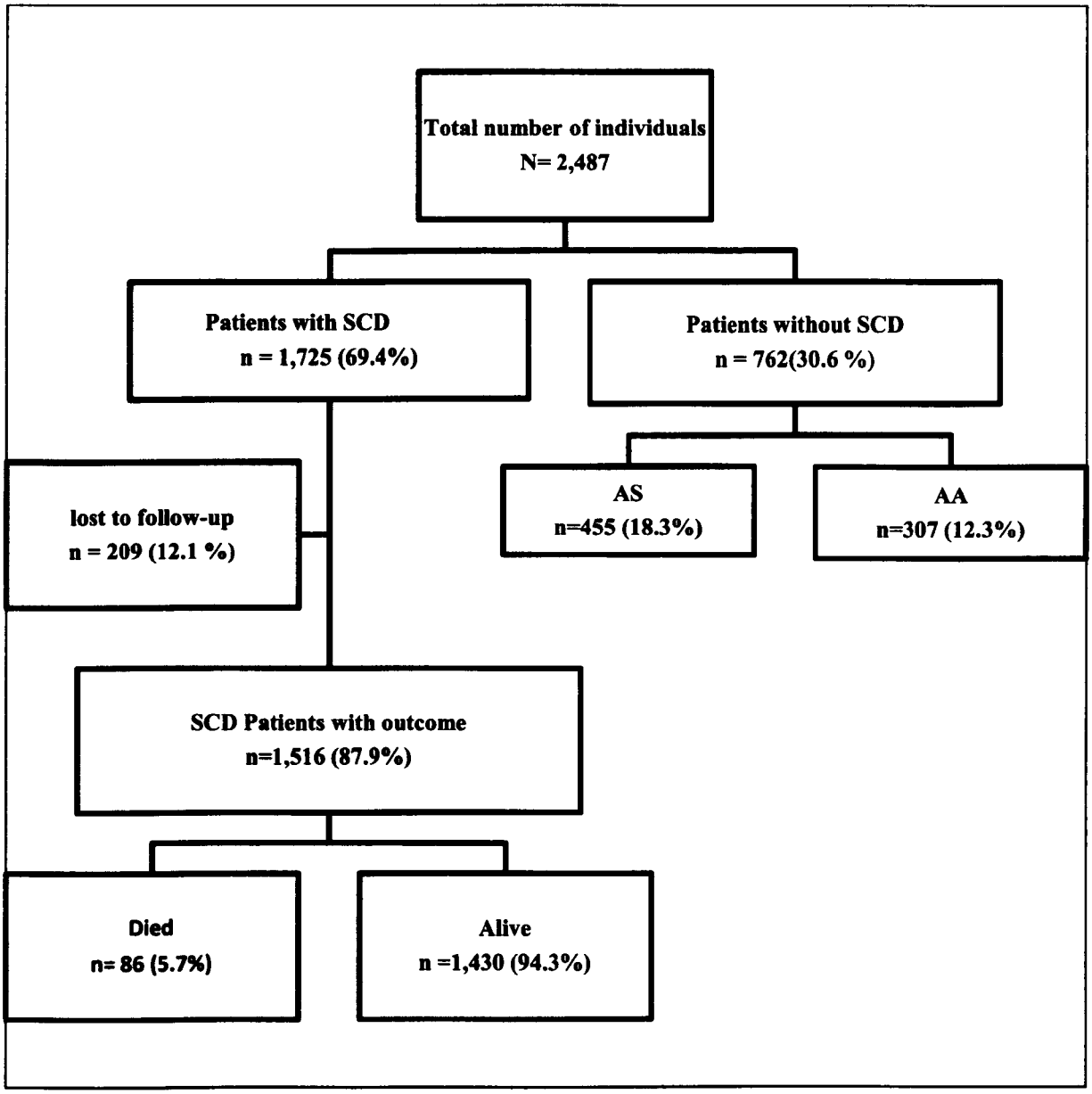
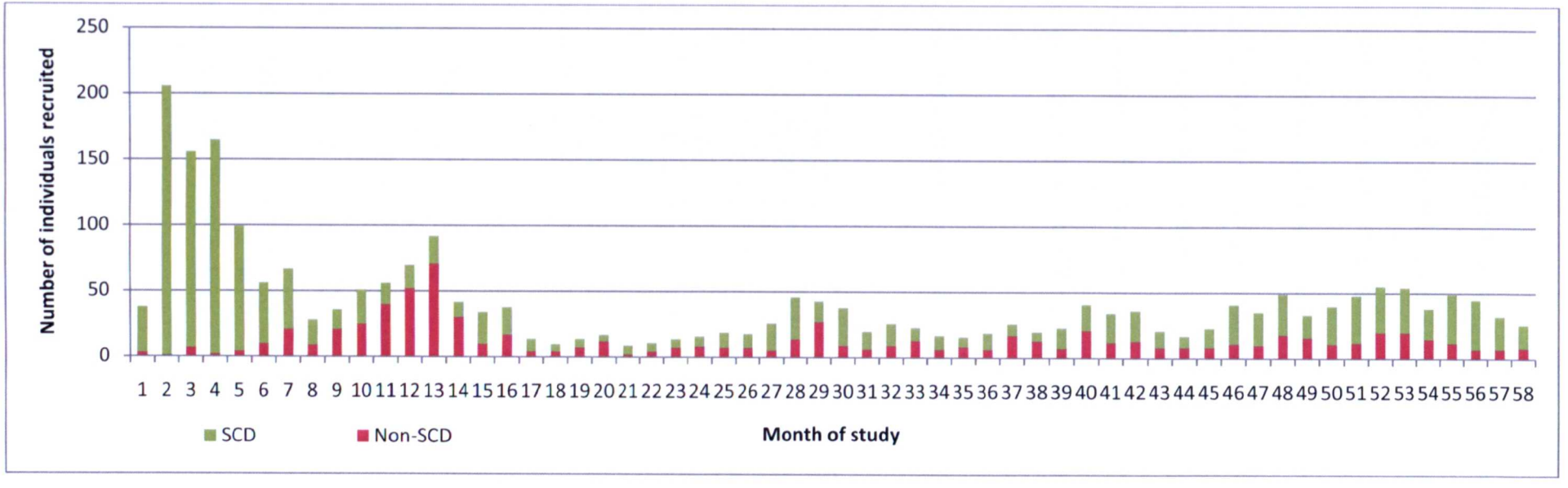


Figure 19 Pattern of recruitment of SCD and non-SCD individuals over study period



The x-axis shows the number of months with 1 being March 2004.

Recruitment: Mean (SD): 41.4 (35.9)	Median: 33 patients	range: 9 206	Period: 60 months
Recruitment of SS: Mean (SD): 28.7 (36.5)	Median: 20 patients	range: 5 206	Period: 60 months
Recruitment of Non - SS: Mean (SD): 13.1 (11.9)	Median: 10 patients	range: 1 71	period: 60 months

Mean denotes the mean number of individuals seen per month, with the standard deviation in brackets.

Characteristics of SCD and non-SCD individuals at recruitment

The composition of SCD and non-SCD patients stratified by age group is shown in Table 14. SCD patients were younger than controls (9.7 vs. 14.4 years; p<0.01). There was a significant difference in the two groups, SCD and non-SCD, in children under 2 years and adults over 20 years. For the age group under 2 years, SCD patients were older compared to Non-SCD patients (0.81 vs. 0.59 years; p<0.01). For the individuals over 20 years, the SCD group was younger (26.8 years vs. 34.9 years; p<0.01). The difference between the groups from 2 years to 19 years was not statistically significant.

Table 14 Number, mean and median age of different age groups in SCD and non-SCD individuals.

	Non-SCD			SCD				
		Mean (SD)	P^{50}		Mean (SD)	P^{50}	QR	P
<2	104	0.59 (0.49)	1	166	0.81 (0.39)	1	3.05 (1.75-5.29)	<0.01
2-4	114	2.96 (0.86)	3	368	3.06 (0.81)	3	1.16 (0.89-1.49)	0.26
5-9	139	6.98 (1.39)	7	478	6.7 (1.44)	7	0.89 (0.79-1.02)	0.11
10-19	193	13.3 (2.76)	13	518	13.59 (2.8)	13	1.02 (0.96-1.09)	0.49
20+	195	34.9 (56.4)	30	195	26.8 (6.0)	25	0.92 (0.89-0.95)	<0.01
Total	745	14.4 (31.6)	10	1725	9.7 (7.9)	8	0.96 (0.95-0.97)	<0.01

P_{50} is the median. QR is the interquartile range.

The clinical characteristics of SCD patients compared to individuals who are non-SCD are shown in Table 15. On univariable analysis, more SCD patients than controls reported to have been born in Dar-es-Salaam (71.3% vs. 62.8%; p<0.01). On measurement of vital signs, SCD patients had lower diastolic and systolic BP; lower saturation and higher pulse rate. On examination, they were more likely to have jaundice and a palpable spleen.

Table 15 Clinical features of SCD and non-SCD patients at outpatient clinic during recruitment into the study

	All	SCD		Non-SCD				
	n=2407	n=1,711		n= 762		OR	P	95 %CI
	Total	Total	% / mean(SD)	Total	% or mean(SD)			
Demographic								
Male (n %)	2487	1725	856(49.6)	762	363(47.6)	1.08	0.36	0.91-1.28
Age(years) GM (SD)[1]	2407	1725	9.7(7.9)	745	14.4(31.6)	0.96	<0.01	0.95-0.97
Individuals less than 18years	2407	1725	1472(85.3)	745	530(71.1)	0.65	<0.01	0.55-0.77
Born in Dar-es-salaam	2378	1661	1,185(71.3)		450(62.8)	1.48	<0.01	1.23-1.78
Clinical history								
Hospitalisation in lifetime	1400	1396	1,239(90.5)	31	25(80.6)	2.29	0.07	0.92-5.68
Hospitalisation in previous 12 months		1187	678(57.1)	30	13(43.3)	1.74	0.14	0.84-3.62
Physical examination								
Pulse rate (beats per minute)	2185	1502	95.3 (18.5)	683	89.7(20.7)	1.02	<0.01	1.01-1.02
Temperature (^{0}c)	2275	1575	36.5(0.52)	700	36.5(0.51)	0.98	0.81	0.82-1.16
Febrile	2275	1575	14 (0.9)	700	11(1.6)	0.56	0.16	0.25-1.24
Systolic hypertension	1991	1.321	10(0.8)	670	27(4.0)	0.18	<0.01	0.09-038
Systolic blood pressure () (mmHg)	1991	1321	105.6(15.8)	670	112.9(19.5)	0.98	<0.01	0.97-0.98
Diastolic blood pressure (DBP) (mmHg)	1824	1187	66.9(11.1)	637	73.8(13.9)	0.96	<0.01	0.95-096
Diastolic hypertension	1824	1187	6(0.5)	637	31(4.9)	0.09	<0.01	0.04-0.24
Peripheral oxygen saturation (percent)	1671	1008	97.3(3.1)	663	98.6(2.8)	0.83	0.07	0.79-087
Hypoxia	1671	1008	27(2.7)	663	9(1.4)	2	<0.01	0.93-4.28
Jaundice	2292	1587	857(54)	705	99(14.0)	7.19	0.11	5.69-9.08
Pallor	1651	1622	635(39.2)	29	7(24.1)	2.02	<0.01	0.86-4.76
Palpable spleen	1812	1608	232(14.4)	204	7(3.4)	4.75	<0.01	2.20-10.21
Palpable liver	1552	1522	79(5.2)	30	1(3.3)	1.59	0.65	0.21-11.81

GM (SD) this is the geometric mean with standard deviation in parenthesis.

Table 16 Laboratory features of SCD and non-SCD patients at outpatient clinic during recruitment into the study

	All	SCD		Non-SCD				
	n= 2407	Total n=	% or Mean(SD)	Total n=	%or Mean (SD)	OR	P	95%CI
WBC count ($x10^9/l$) GM (95%C.I)	2271	1579	15.7(6.8)	692	8.1(4.2)	1.47	<0.01	1.42-1.52
Haemoglobin (g/dl)	2256	1579	7.5(1.4)	677	11.1(2.6)	0.40	<0.01	037-0.43
Severe anaemia (Hb<5g/dl)	1615	1373	51.4(54.1)	242	16.6(32.7)	1.02	<0.01	1.02-1.03
Red blood cell count	2244	1568	2.9(0.8)	676	4.7(0.9)	0.15	<0.01	0.12-0.17
Mean corpuscular volume (fL)	2221	1568	79.0(9.5)	653	74.2(10.1)	1.05	<0.01	1.04-1.06
Red cell distribution width (%)	2233	1562	23.2(4.4)	671	17.3(3.5)	1.59	<0.01	1.53-1.66
Platelet count ($x10^9/l$)	2241	1567	427.2(192.6)	674	354.6(155.6)	1.00	<0.01	1.002-1.003
Reticulocyte count (% of RBC)	1562	958	12.6(7.1)	594	6.1(9.4)	1.13	,0.01	1.11-1.15
Bilirubin (μmol/L) GM (95%C.I)	2009	1530	70.1(58.1)	479	20.3(37.4)	1.06	<0.01	1.05-1.07
Bilirubin-indirect (μmol/L)	1654	1391	21.7(35.2)	263	8.04(13.3)	1.19	<0.01	1.16-1.23
Lactate dehydrogenase (IU/L)	1232	640	974.1(465.9)	592	571.6(387.9)	1.00	<0.01	1.002-1.003
Alkaline phosphatase (IU/L)	2219	1579	264.9(126.60	640	288.8(194.8)	0.99	<0.01	0.998-0.999
Aspartate transaminase (IU/L)	2222	1577	49.9(27.6)	645	35.8(25.7)		<0.01	1.03-1.04
Creatinine (μmol/L)	2217	1575	41.2(19.8)	642	53.5(42.3)	0.98	<0.01	0.97-0.98

GM (SD) this is the geometric mean with standard deviation in parenthesis.

Table 16 shows the laboratory characteristics of SCD patients compared to individuals who are non-SCD. With regards to haematological factors, SCD individuals had lower Hb; but higher levels of WBC, Reticulocyte count, RDW and MCV. With regards to biochemical tests for markers of haemolysis and liver function, SCD patients had higher levels of bilirubin, AST, ALP, LDH and creatinine.

Multivariable analysis

On multivariable analysis, factors that were independently associated with likelihood of having SCD were low diastolic blood pressure [(OR 0.97 (95% CI 0.94 – 0.99); p=0.01] and jaundice [2.77 (1.66 – 4.64); p=<0.01] on examination. Laboratory factors that were independently associated with SCD status were high WBC count [1.27 (1.19 – 1.30); <0.01] and RDW [1.17 (1.08 – 1.27); <0.01] and low haemoglobin [0.75 (0.63 – 0.89); <0.01]. For the laboratory markers of haemolysis and liver function, individuals with SCD had lower ALP [0.99 (0.99 – 0.99); 0.01] and high total bilirubin [1.01 (1.00 – 1.01); 0.02].

Table 17 Multivariable analysis of clinical and laboratory features associated with SCD status n=991

Factor	OR	P
Age (years)	0.99 (0.96 – 1.02)	0.41
Pulse rate (beats per minute)	0.99 (0.98 – 1.01)	0.52
Systolic blood pressure (mmHg)	1.01 (0.99 – 1.03)	0.19
Diastolic blood pressure (mmHg)	0.97 (0.94 – 0.99)	0.01
Peripheral oxygen saturation (percent)	0.94 (0.87 – 1.02)	0.12
Jaundice	2.77 (1.66 – 4.64)	<0.01
White blood cell count ($X10^9$/L)	1.27 (1.19 – 1.36)	<0.01
Haemoglobin (g/dl)	0.75 (0.63 – 0.89)	<0.01
Red blood cell count (x 10^{12}/L)	0.79 (0.56 – 1.12)	0.19
Mean corpuscular volume (fL)	1.02 (0.99 – 1.05)	0.23
Red cell distribution width (%)	1.17 (1.08 – 1.27)	<0.01
Platelet count ($x10^9$/l)	1.00 (0.99 – 1.00)	0.84
Aspartate transaminase (IU/L)	0.99 (0.99 – 1.01)	0.53
Alkaline phosphatase (IU/L)	0.99 (0.99 – 6.99)	0.01
Bilirubin – total (μmol/L)	1.01 (1.00 – 1.01)	0.02

For the second model, we limited analysis to laboratory factors to determine whether there were any parameters that could be used to identify individuals who were more likely to have SCD. The results are shown in table below (Table 18).

Table 18 Multivariable analysis of laboratory features associated with SCD status (n=1,805)

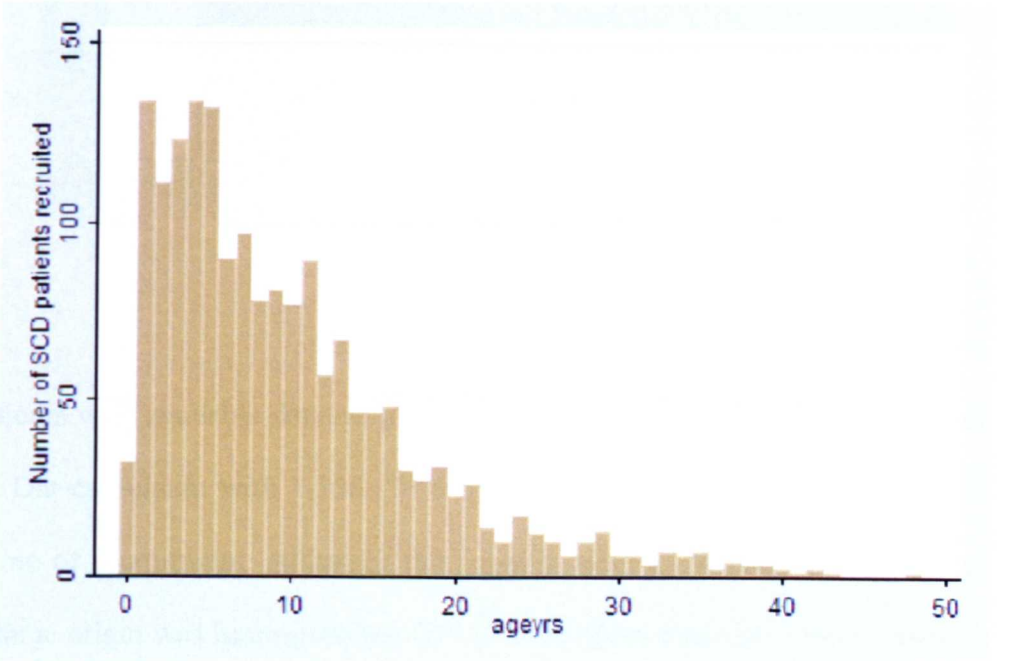
Factor	OR	P
White blood cell count (x109/l)	1.25 (1.19 – 1.32)	<0.01
Haemoglobin (g/dl)	0.79 (0.69 – 0.91)	<0.01
Red blood cell count (x 10^{12}/L)	0.64 (0.49 – 0.82)	< 0.01
Mean corpuscular volume (fL)	1.01 (0.99 – 1.04)	0.33
Red cell distribution width (%)	1.18 (1.10 – 1.00)	<0.01
Platelet count (x10^{9}/l)	1.00 (0.99 – 1.00)	0.55
Aspartate transaminase (IU/L)	0.99 (0.99 – 1.00)	0.86
Alkaline phosphatase (IU/L)	0.99 (0.996 – 0.998)	<0.01
Bilirubin – total (μmol/L)	1.02 (1.02 – 1.03)	<0.01

STUDY POPULATION

Baseline characteristics of SCD patients

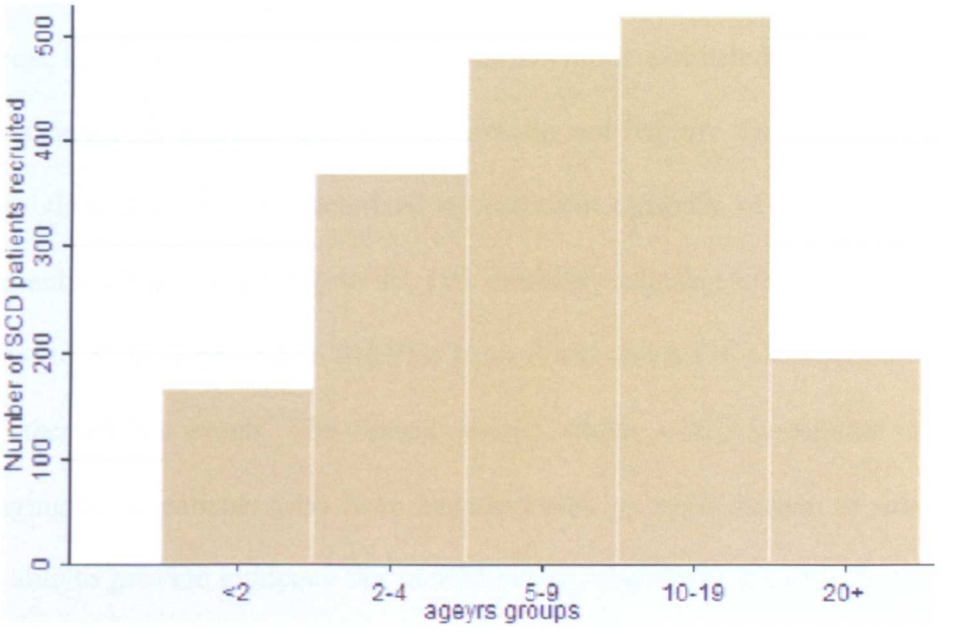
During the study period, 1,725 patients with SCD were recruited into the study and 209 (12.1%) were lost to follow up as 31st March 2009. In the SCD cohort, there were 856 (49.6%) male. Figure 20 shows the age distribution at time of recruitment into the study.

Figure 20 Age distribution of SCD patients at time of enrolment



When age was grouped, the distribution was as shown in Figure 21, with 166 (9.6%) below 2 years and the majority of patients (79.3%) between 2 years and 20 years, which would be considered children and adolescents.

Figure 21 Age distribution, grouped into 5 age groups, of SCD patients at time of recruitment



For 1,661 patients with available data on place of birth, 1,185 (71.3%) of SCD patients had been born in Dar-es-Salaam with 1,158 (72.5%) patients reported to be living in Dar-es-Salaam, at time of recruitment. Although the patients were born and resident in Dar-es-salaam, the ethnic origin was heterogeneous; 418 (24.7%) were from the 2 main tribes on the coast; Zaramo and Ndengereko. 379 (22.4%) were from North Tanzania, around the region of Lake Victoria. 121 (7.0%) were from Zanzibar or had Indian or Arab origin. The rest were from tribes from different parts of Tanzania.

HOSPITALISATION IN SCD

Introduction

The burden of disease as a result of being affected by SCD can be estimated determining the birth and population prevalence of disease. These estimates do not capture the disability that SCD patients face due to chronic illness, characterized by recurrent episodes of acute clinical illness. Weatherall *et al* recently estimated that up to 20, 194 disability adjusted life year (DALY) losses occur every year due to SCD, with 13,856 (68.9%) from death and 6,338 (31.4%) due to chronic anaemia, pain and other clinical events[6] The clinical severity within a SCD population is extremely heterogeneous, ranging from patients who have asymptomatic or mild disease to severe forms. However, if I was able to provide evidence that would aid in identifying individuals who were at high-risk of hospitalisation, this would help in targeting interventions towards these individuals.

Acute clinical events such as pain, fever and anaemia may require visits to a health facility or hospitalisation. In this thesis, I wanted to focus on those individuals who were hospitalized. The aim was to determine the outcome of hospitalisation and to determine whether there were any clinical or laboratory features at the time of at hospitalisation that could be used to identify those at risk of having an adverse outcome.

Therefore, in this section, I set out to do the following; 1) determine the pattern and incidence of hospitalisation in SCD; 2) determine factors associated with likelihood of hospitalisation in SCD; 3) identify factors associated with mortality during hospitalisation.

Methods

Patients

In order to determine the rates and risk factors of hospitalisation in SCD, the study population consisted of SCD patients who were part of the main study population i.e. individuals who had an "entry visit" at outpatient clinic as described previously and were part of prospective surveillance. For this analysis, SCD patients who had been hospitalised but did not have a clinic visit were not included.

In order to determine the factors associated with death during hospitalisation, the study population consisted of all SCD individuals who were hospitalised during the study period. In this analysis, individuals who were identified as having SCD in the wards but did not have an entry visit were included.

Procedures

At the first clinic attendance during the study window, patients were enrolled into the study. This visit was referred to as the entry visit. All patients who fulfilled the inclusion criteria were recruited into the study after informed consent was obtained from the patients or relatives. Clinical information collected included demographic details, past and current symptoms. Physical examination was performed which included general anthropometric measurement and thorough systematic examination. Detailed neurological evaluation was done if clinically indicated as well as assessment of cranial and peripheral nervous system for focal deficits. Patients were given a SCD number which was their unique identifier that would be used for all subsequent visits to MNH. For patients who were identified as having SCD during hospitalisation, data was collected during this event but recruitment into the study was only done when the patient came to the clinic after

discharge from the hospital. Therefore some patients will have hospitalisation events which precede recruitment into the study. This information was collected as it was thought that important data regarding causes of hospitalisation or mortality may not be captured.

Statistical methods

Rates and risk factors for hospitalisation

Data were analyzed using STATAv10 (StataCorp, College Station, TX). The SCD cohort was defined to include patients who had an enrolment visit. The overall and age-specific incidence of hospitalisation was estimated for the cohort. This was calculated from the ratio of number of hospitalisations divided by the number of person years of observation (PYO), expressed as hospitalisation rate according to age. The analysis did not include SCD patients who were identified at hospitalisation, but had not returned to outpatient clinic for enrolment into the cohort. Data were summarized as means, medians or proportions.

In order to determine the factors at enrolment associated with hospitalisation, I grouped the individuals in the cohort into two groups; those who had never been hospitalised at MNH and those who had been hospitalised at MNH (once or more) during the study period. Individuals with multiple hospitalisations during the course of the study were classified in the latter group. Logistic regression analysis was used to analyze factors at enrolment associated with hospitalisation, with results presented as odds ratios (ORs) with 95% confidence intervals (95%CIs). Multivariable logistic regression was used to identify independent associations with hospitalisation, using variables that had significant association ($p<0.05$) on univariable analysis.

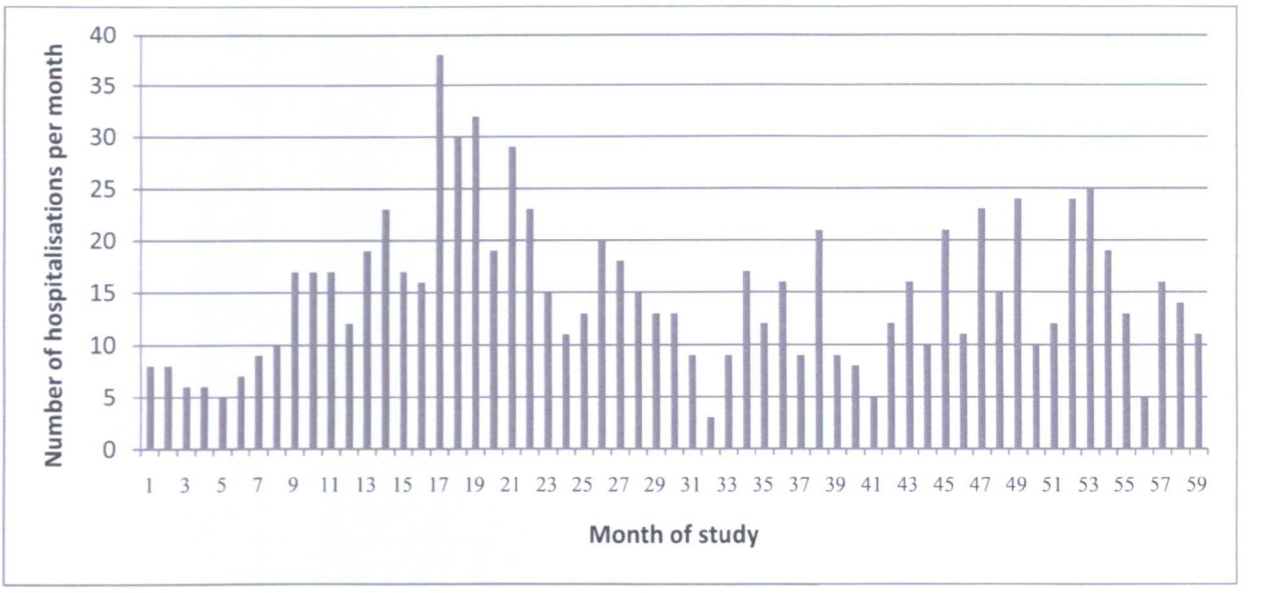
Risk factors for mortality during hospitalisation

This analysis included all SCD individuals who were hospitalised at MNH during the course of the study. This included some SCD patients who were not included in the main cohort population. Therefore this was a cross-sectional study of all hospitalised SCD patients with comparison made between those who survived during hospitalisation and those who died. Logistic regression analysis was used to analyze factors at hospitalisation associated with death, with results presented as OR with 95% confidence intervals (95%CIs). Since some individuals may have multiple hospitalisations, I had to take this into account during the analyses. There are two approaches; accounting for clustering using the following command in Stata; *logistic death_inp var cl(demographic_id)*. The other approach would be using a random effects model (*xi: xtlogit death_inp var re i(demographic_id) or nolog).* I analysed the data using both approaches and following statistical advice, the results presented are from the first model. Multivariable logistic regression (accounting for clustering as done during univariable analysis) was used to identify independent associations with death, using variables that had significant association ($p<0.05$) on univariable analysis.

Results

During the course of the study, of 1,725 SCD patients who had a baseline visit, 504 (29.2%) were hospitalised at MNH during the five year period. The mean number of hospitalisations per month was 15 (SD 7.2) with a minimum of 3 and maximum of 38 hospitalisations per month. The hospitalisation pattern during the course of the study is shown in Figure 22.

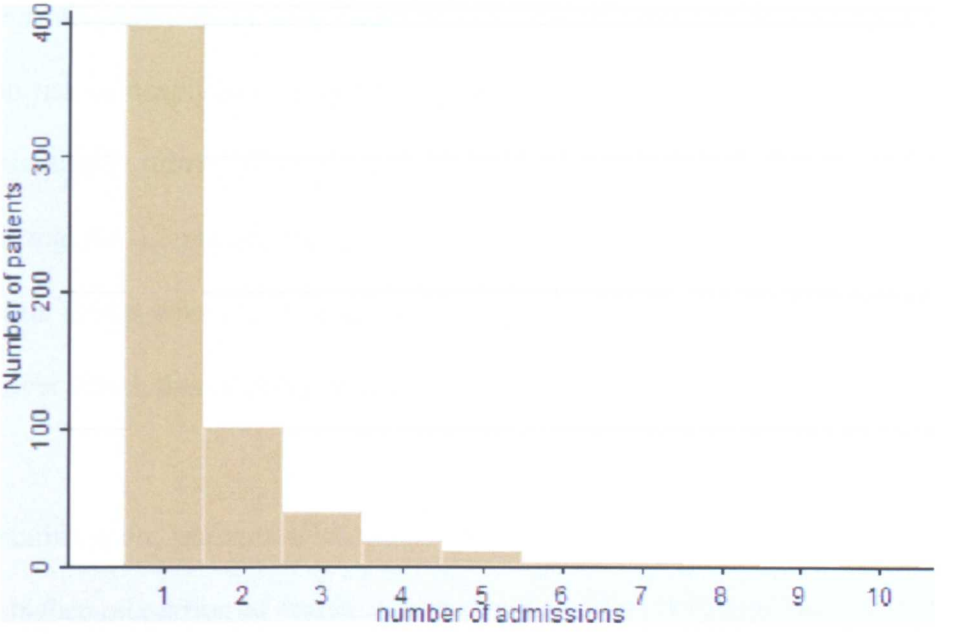
Figure 22 Number of hospitalisations per month during course of the study



The x-axis shows the number of months with 1 being March 2004.

For the 572 patients who were admitted, the range of number of hospitalisations (Figure 23) was 1 10 times, with a mean of 1.6 (1.1) hospitalisations. 398 (69.6%) of patients were admitted only once during the course of the study; 149 (28.7%) admitted between 2-5 times and 6 (1.2%) patients were admitted between 6-10 times.

Figure 23 Frequency of hospitalisation for 572 patients hospitalised during the study



Rate of hospitalisation during the study

During the course of the study, the incidence rate of hospitalisation was 0.15 per 100 PYO. This rate was calculated from 1,496 of 1,725 enrolled SCD patients with 620 hospitalisation events. This gave 4,167 PYO.

Table 19 Rate of hospitalisation for SCD patients during study

Age (Group)	Patients[1]	Observation(yrs)[2]	Events[3]	Incidence[4] (/100 PYO)
<2	100	49.3	24	0.49 (0.33-0.73)
2-4	361	453.4	109	0.24 (0.19-0.29)
5-9	584	1182.9	186	0.16 (0.14-0.18)
10-19	698	1871.9	195	0.10 (0.09-0.12)
20+	256	610.5	106	0.17 (0.14-0.21)
Total	**1,496**	**4167.9**	**620**	**0.15 (0.14-0.16)**

Note: includes 1,496 out of 1,725 SCD patients with complete data. [1] Number of patients in that age group [2] Person-years of observation [3] events or hospitalisation [4] Incidence of hospitalisation per 100 PYO

Factors associated with hospitalisation during the study

The clinical and laboratory features at baseline were analysed to explore whether there was an association with risk of hospitalisation during the course of the study. At the baseline visit, patients who were subsequently admitted were more likely to report having a febrile episode or being hospitalised during the 12-month period preceding the baseline visit. There was also a higher proportion in this group who reported to have noticed worsening of jaundice. The duration or period of time over which this occurred was not documented.

On physical examination, patients who were subsequently admitted had lower diastolic blood pressure and a higher proportion of jaundice, pallor and bossing of frontal bone of skull was noted. Furthermore, this group of patients had a higher proportion of individuals with palpable liver and spleen.

Table 21 shows laboratory features at baseline, comparing patients who were hospitalised at least once during the course of the study and those who were never hospitalised. The group of patients who were likely to be admitted had lower Hb, wider RDW and higher LDH.

Table 20 Clinical features at baseline visit associated with hospitalisation during the study

	Total	No	Hospitalisation	OR	P
Total	**1725**	**1221 (70.8)**	**504 (29.2)**		
Male	856	602 (49.3)	254 (50.4)	1.04 (0.85-1.29)	0.68
Age (years)	1725	9.8 (7.9)	9.5 (8.1)	0.99 (0.99-1.01)	0.49
Born in Dar	1185	823 (70.3)	362 (73.9)	1.19 (0.94-1.52)	0.14
Clinical events reported in past 12 months					
Hospitalisation	678	405 (50.8)	273 (70.0)	2.26 (1.75-2.92)	<0.01
Blood transfusion	548	374 (30.9)	174 (34.8)	1.19 (0.95-1.48)	0.12
Pain	743	523 (43.3)	220 (44.2)	1.04 (0.84-1.28)	0.73
Fever	480	320 (27.4)	160 (32.7)	1.29 (1.02-1.62)	0.03
Chest symptoms	141/1	90 (7.5)	51 (10.2)	1.41 (0.98-2.03)	0.06
Seizures	1720	8 (0.7)	1 (0.2)	0.3 (0.04-2.41)	0.26
Symptoms at baseline visit					
Fever	1650	69 (5.9)	40 (8.3)	1.44 (0.96-2.15)	0.08
Pain	1651	120 (10.3)	52 (10.8)	1.05 (0.75-1.49)	0.77
Anaemia	1628	22 (1.9)	14 (2.9)	1.55 (0.78-3.05)	0.21
Jaundice	1630	9 (0.8)	11 (2.3)	2.98 (1.23-7.24)	0.02
Cough	1648	81 (6.9)	43 (8.9)	1.31 (0.89-1.93)	0.17
CNS event	1645	6 (0.5)	3 (0.6)	1.21 (0.30-4.85)	0.79
Physical examination					
Temperature (°C)	1575	36.5 (0.5)	36.5 (0.5)	1.04 (0.84-1.28)	0.70
Febrile (≥37.5^{0}C)	1575	19 (1.7)	8 (1.8)	1.03 (0.45-2.37)	0.94
SBP (mmHg)	1321	105.8 (15.8)	105.7 (15.8)	0.99 (0.99-1.01)	0.94
DBP (mmHg)	1187	67.4 (11.2)	65.7 (10.7)	0.95 (0.97-0.99)	0.02
SpO_2 (%)	1008	97.2 (3.3)	97.6 (2.7)	1.04 (0.99-1.09)	0.09
SaO_2<95%	1008	23 (3.2)	4 (1.4)	0.42 (0.14-1.23)	0.11
Jaundice	1587	581 (52.1)	276 (58.5)	1.29 (1.04-1.61)	0.02
Pallor	1622	422 (36.8)	213 (44.8)	1.39 (1.12-1.74)	<0.01
Frontal bossing	1576	255 (23.0)	145 (30.9)	1.49 (1.18-1.90)	<0.01
Palpable spleen	1608	146 (12.8)	86 (14.4)	1.54 (1.15-2.06)	<0.01
Palpable liver	1522	42 (3.9)	37 (8.3)	2.23 (1.42-3.53	<0.01
Death	1516	45 (4.2)	41 (9.1)	2.27 (1.47-3.53)	<0.01

Table 21 Laboratory features at baseline visit associated with hospitalisation during the study

	Total	No hospitalisation	Hospitalisation	OR	P
White blood cell count ($X10^9$/L)	1579	15.6 (6.8)	16.1 (6.9)	1.01 (0.99-1.03)	0.22
Haemoglobin (g/dl)	1579	7.6 (1.4)	7.4 (1.4)	0.88 (0.82-0.96)	<0.01
Severe anaemia (Hb <5g/dL)	1579	32 (2.9)	30 (6.5)	2.34 (1.40-3.89)	<0.01
Red blood cell count (x 10^{12}/L)	1568	3.0 (0.9)	2.9 (0.8)	0.87 (0.76-1.00)	0.06
Mean corpuscular volume (fL)	1568	79.1 (9.6)	78.8 (9.5)	0.99 (0.99-1.01)	0.57
Red cell distribution width (%)	1562	23.0 (4.4)	23.6 (4.4)	1.03 (1.00-1.05)	0.02
Platelet count (x10^9/l)	1567	430.4 (182.6)	419.6 (214.5)	0.99 (0.99-1.00)	0.31
Reticulocyte (% of RBC)	968	12.4 (6.9)	12.9 (7.6)	1.01 (0.99-1.03)	0.29
Haemoglobin F (%)	1491	6.4 (4.6)	6.2 (4.7)	0.99 (0.97-1.02)	0.58
Bilirubin – total (μmol/L)	1530	69.6 (57.3)	71.3 (60.0)	1.00 (0.99-1.00)	0.59
Bilirubin – indirect (μmol/L)	1373	51.8 (54.5)	50.5 (53.3)	0.99 (0.99-1.00)	0.68
Bilirubin – direct (μmol/L)	1391	20.9 (35.9)	23.6 (33.2)	1.00 (0.99-1.01)	0.19
Aspartate transaminase (IU/L)	1577	49.1 (27.2)	51.8 (28.5)	1.00 (0.99-1.01)	0.09
Lactate dehydrogenase (IU/L)	640	947.8 (418.7)	1047.9 (573.6)	1.00 (1.00-1.00)	0.02
Alkaline phosphatase (IU/L)	1579	264.7 (125.6)	265.4 (129.3)	1.00 (0.99-1.00)	0.92
Creatinine (μmol/L)	1575	40.8 (18.2)	42.1 (23.4)	1.00 (0.99-1.01)	0.26

Multivariable analysis

On multivariable analysis, two models were built. For the first model all variables that were significant at 0.05 during univariable analysis were included. 703 events, with complete data were included in this analysis. The factors that were independently associated with hospitalisation during the course of the study were hospitalisation and RDW (Table 22).

For the second model, we did not include LDH which was available for only 640 (37.1%) hospitalisation events. 238 events were included in this analysis. In this case, hospitalisation, febrile episode in past 12 months and RDW were independent risk factors for hospitalisation during the course of the study (Table 23).

Table 22 Multivariable analysis: Factors at baseline visit associated with hospitalisation during the study (n=703)

Factor	OR	P
Hospitalisation in past 12 months	2.64 (1.85-3.76)	<0.01
Febrile episode in past 12 months	0.88 (0.60-1.31)	0.56
Reported worsening jaundice	2.29 (0.57-9.19)	0.24
Diastolic blood pressure (mmHg)	0.99 (0.98-1.02)	0.99
Jaundice on examination	1.13 (0.80-1.61)	0.48
Pallor	1.31 (0.92-1.88)	0.13
Bossing	1.22 (0.82-1.80)	0.32
Palpable spleen	0.78 (0.49-1.26)	0.31
Palpable liver	1.95 (0.93-4.12)	0.08
Haemoglobin (g/dl)	0.97 (0.85-1.10)	0.62
Red cell distribution width (%)	1.05 (1.01-1.10)	0.02

Table 23 Multivariable analysis: Factors at baseline visit associated with hospitalisation during the study (n=238

	OR	P
Hospitalisation in past 12 months	2.92 (1.41-6.07)	<0.01
Febrile episode in past 12 months	0.37 (0.15-0.94)	0.04
Reported worsening jaundice	1.62 (0.21-12.75)	0.65
Diastolic blood pressure (mmHg)	0.99 (0.97-1.02)	0.73
Jaundice on examination	1.65 (0.90-3.03)	0.11
Pallor	0.94 (0.49-1.77)	0.85
Bossing	2.29 (0.96-5.50)	0.06
Palpable spleen	1.02 (0.40-2.58)	0.97
Palpable liver	4.65 (0.60-36.05)	0.14
Haemoglobin (g/dL)	0.92 (0.72-1.18)	0.5
Red cell distribution width (%)	1.13 (1.04-1.24)	<0.01
Lactate dehydrogenase (IU/L)	1.00 (0.99-1.00)	0.29

The factors that were independently associated with likelihood of hospitalisation during the course of the study were history of hospitalisation in previous 12 months and RDW.

Description of events during hospitalisation

There were 885 hospitalization events identified from 572 SCD patients (note that this includes SCD patients who did not have a baseline visit), and the analysis included all these events. For 3 of these events, the outcome (death or discharge) was not known. Death occurred in 20 out of 882 (2.3%) hospitalizations.

From 882 hospitalisation events, 401 (46.5%) events were from male patients; with no difference between the two groups (gender was not available for 3 events). The mean age at hospitalisation

was 10.9 (SD 8.72) years, with 78.9% below the age of 18 years. 605 of 849 hospitalisation s (73.3%) were from people who were born in Dar.

The commonest clinical event in the 12 months preceding hospitalisation was pain, occurring in 226 of 865 events (26.1%) and fever (24.3%) . 99 of 865 (11.5%) reported to have received blood transfusion .

The most common symptoms at hospitalisation were pain, occurring in 667 of 885 events (76.1%) fever in 520/871 (59.6%) and anaemia in 191/868 (21.9%). 110/804 (12.4%) events reported worsening of jaundice.

On physical examination, 246/868 (28.3%) were febrile, with body temperature above 37.7°C with 9 out of 755 patients (1.2%) having peripheral oxygen desaturation. 77% were jaundiced with palpable spleens and liver in 23.5% and 12.9% respectively.

Factors associated with mortality during hospitalisation

For 882 hospitalisation events, 862 (97.4%) were discharged and 20 (2.3%) died. The age group with the highest proportion of deaths was 10 – 19 years (3.9%), followed by over 20 years (2.7%) . the proportion of deaths in the other 2 age groups was 2 – 4 years (2.4%) and 5-9 years (0.8%). No deaths were recorded in the 62 children admitted who were below 2 years of age at hospitalisation (OR 1.38(0.94-2.02); p=0.09).

Univariable analysis

For this analysis, the association between clinical and laboratory features at hospitalisation and

whether the outcome was survival or death was explored. There were no statistical differences between those who survived and those who died with respect to history of clinical events in the 12 months neither preceding each hospitalisation nor presenting symptoms. Although, jaundice was reported to occur in more patients who died (23.5%) compared to those who survived (12.3%), this was not statistically significant (OR=2.21; p=0.18). On physical examination at hospitalisation, the group of patients who died had higher temperatures, lower systolic blood pressure and palpable liver at hospitalisation.

Comparing patients who survived to those who died, there were differences between the laboratory values of these groups at hospitalisation. The laboratory factors that were associated with increased risk of death were haemoglobin level, reticulocyte and platelet count. Individuals who died had a lower haemoglobin (5.1g/dL) compared to those who survived (6.5g/dL; OR-0.88; p<0.01). Those with severe anaemia, haemoglobin <5g/dL, had a higher risk of death compared to those without severe anaemia (Hb>5g/dL) but this was not statistically significant (OR 2.46; p=0.07). For the other laboratory parameters, bilirubin, AST and creatinine were significantly higher in the group of patients who died.

Table 24 Clinical features at hospitalisation associated with mortality during hospitalisation

	Individuals	Events	Survival	No survival	OR	P
Total	569	882	862 (97.4)	20 (2.3)		
Male	569	882	401 (46.5)	12 (60.0)	1.72 (0.69-4.26)	0.09
Age (years)		882	10.9 (5.2)	14.3 (10.4)	1.04 (0.99-1.08)	0.24
Birth - Dar	543	605/849 (73.3)	592 (71.2)	13 (72.2)	1.05 (0.37-2.96)	0.93
Past events						
Blood transfusion	562	99/865 (11.5)	99 (11.5)	2 (10.0)	0.86 (0.19-3.74)	0.84
Pain	565	226/865 (26.1)	221 (26.2)	5 (25.0)	0.94 (0.34-2.63)	0.91
Fever	553	205/845 (24.3)	201 (24.4)	4 (20.0)	0.78 (0.26-2.34)	0.65
Current symptoms						
Fever	565	520/871 (59.7)	507 (59.6)	13 (65.0)	1.26 (0.49-3.19)	0.63
Pain	567	667/877 (76.1)	652 (76.1)	15 (75.0)	0.94 (0.34-2.59)	0.91
Anaemia	565	191/868 (22.0)	186 (21.9)	5 (25.0)	1.19 (0.42-3.34)	0.75
Jaundice	526	100/804 (12.4)	96 (12.32)	4 (23.5)	2.21 (0.70-6.99)	0.18
CNS event	553	29/839 (3.5)	28 (3.4)	1 (5.6)	1.67 (0.21-12.99)	0.63
Examination						
Temperature (°C)	559	868 (37.2)	37.2 (0.9)	37.9 (1.4)	2.00 (1.22-3.28)	<0.01
Febrile (Temperature >37.5^{0}C)	559	246/868 (28.3)	233 (27.5)	13 (65.0)	4.9 (1.94-12.36)	<0.01
Pulse rate (beats per minute)	552	853 (101.6)	101.5 (18.6)	106.8 (23.4)	1.02 (0.99-1.04)	0.30
Systolic blood pressure (mmHg)	539	826 (108.0)	108.1 (15.0)	103.4 (10.4)	0.98 (0.95-1.00)	0.05
Diastolic blood pressure (mmHg)	513	764 (67.8)	67.8 (11.2)	64.5 (10.9)	0.97 (0.92-1.03)	0.29
Peripheral oxygen saturation SpO_2 (%)	508	755 (97.9)	97.9 (2.5)	97.5 (2.4)	0.95 (0.82-1.09)	0.44
Hypoxia ($SpO_2 \leq 95\%$)	501	9/755 (1.2)	9 (1.2)	0 (-)		
Jaundice	560	664/862 (77.0)	646 (76.6)	18 (94.7)	5.49 (0.72-41.6)	0.09
Pallor	552	529/850 (62.2)	515 (61.9)	14 (73.7)	1.72 (0.61-4.82)	0.30
Bossing	535	158/829 (19.1)	152 (18.8)	6 (30.0)	1.85 (0.69-4.94)	0.22
Palpable spleen	550	200/853 (23.5)	193 (23.2)	7 (35.0)	1.79 (0.69-4.57)	0.23
Palpable liver	527	105/815 (12.9)	99 (12.4)	6 (31.6)	3.25 (1.21-8.69)	0.02

Table 25 Laboratory features at hospitalisation associated with mortality during hospitalisation

	Individuals	Events	Survival	No survival	OR	P
White blood cell count ($X10^9$/L)	529	784 (22.4)	22.3 (12.8)	28.5 (15.2)	1.03 (1.00-1.05)	0.02
Haemoglobin (g/dl)	529	784 (6.5(1.8))	6.5 (1.8)	5.1 (2.0)	0.65 (0.49-0.88)	<0.01
Red blood cell count (x 10^{12}/L)	516	761 (2.66(1.2))	2.7 (1.2)	2.3 (0.6)	0.59 (0.33-1.05)	0.07
Mean corpuscular volume (fL)	521	762 (81.1(10.3))	81.0 (9.9)	83.7 (11.7)	1.03 (0.98-1.08)	0.32
Red cell distribution width (%)	528	785 (21.9(4.1))	21.9 (4.1)	21.4 (3.5)	0.96 (0.86-1.08)	0.55
Platelet count (x10^9/l)	520	764 (379.3(211.1))	381.4 (212.0)	295 (150.6)	0.99 (0.995-0.999)	0.05
Reticulocyte count (% of RBC)	436	614 (13.6(6.7))	13.5 (6.6)	19.9 (9.0)	1.12 (1.04-1.19)	<0.01
Haemoglobin F (%)	517	816 (6.4(4.9))	6.4 (4.9)	6.1 (6.0)	0.99 (0.86-1.13)	0.84
Bilirubin – total (µmol/L)	444	636 (64.7(75.4)	62.2 (69.4)	166.2 (181.5)	1.01 (1.00-1.01)	<0.01
Bilirubin – direct (µmol/L)	393	535 (25.6(52.8)	24.5 (51.4)	70.7 (85.1)	1.01 (1.001-1.009)	<0.01
Bilirubin – indirect (µmol/L)	379	513 (46.0(59.9)	44.4 (56.2)	115.8 (135.6)	1.01 (1.00-1.01)	<0.01
Aspartate transaminase (IU/L)	468	663 (66.9(52.3))	66.2 (51.9)	96.6 (64.5)	1.01 (1.00-1.01)	<0.01
Lactate dehydrogenase (IU/L)	424	577 (1245.7(705.4)	1238.2 (698.4)	1631 (968.4)	1.00 (0.99-1.00)	0.07
Alkaline phosphatase (IU/L)	474	674 (243.2(138.0))	243.3 (138.7)	240.2 (114.4)	0.99 (0.99-1.00)	0.93
Creatinine (µmol/L)	476	682 (48.0(34.8)	47.0 (33.0)	89.7 (69.5)	1.01 (1.01-1.02)	<0.01

'Individuals' refers to the number of individuals who were included in the analysis. 'Events' refers to the number of hospitalisations that have been included in the analysis. Individuals can have more than one hospitalisation. The analysis took into account clustering of hospitalisations in individuals who had multiple episodes of hospitalisation.

Multivariable analysis

On multivariable analysis, two models were built to identify the factors that were independently associated with mortality. For the first model, 405 events were included (388 individuals) with complete data. From this model, total bilirubin was independently associated with mortality.

Table 26 Multivariable analysis: Risk factors for mortality during hospitalisation n= 405 (388 individuals)

Factor	OR	P
Temperature (°C)	1.61 (0.47-5.51)	0.45
Systolic blood pressure (mmHg)	0.99 (0.92-1.07)	0.89
Palpable liver	1.64 (0.19-13.64)	0.65
White blood cell count (x109/l)	1.02 (0.97-1.16)	0.46
Haemoglobin (g/dl)	0.67 (0.39-1.16)	0.15
Reticulocyte count (%)	1.02 (0.90-1.16)	0.70
Platelet count ($x10^9$/l)	0.99 (0.99-1.00)	0.22
Bilirubin – total (µmol/L)	1.01 (1.00-1.01)	0.04
Aspartate transaminase (IU/L)	1.01 (1.00-1.01)	0.39
Creatinine (µmol/L)	1.00 (0.99-1.02)	0.58

For the second model, the following variables were excluded because they had more than 25 % of variables missing; reticulocyte had 616 (69.7%) with available data and total bilirubin had 637 (71.9%) with available data. From this model, haemoglobin was independently associated with mortality during hospitalisation. Temperature, platelet and creatinine showed an association but this was not statistically significant.

Table 27 Multivariable analysis: Risk factors for mortality during hospitalisation n= 532 (388 individuals)

Factor	OR	P
Temperature (°C)	2.13 (0.94-4.81)	0.07
Systolic blood pressure (mmHg)	0.97 (0.92-1.02)	0.24
Palpable liver	1.47 (0.37-5.77)	0.58
White blood cell count (x109/l)	1.00 (0.97-1.04)	0.81
Haemoglobin (g/dl)	0.66 (0.44-0.99)	0.05
Platelet count ($x10^9$/l)	0.99 (0.99-1.00)	0.11
Aspartate transaminase (IU/L)	1.00 (0.99-1.01)	0.24
Creatinine (µmol/L)	1.00 (0.99-1.02)	0.09

Reticulocyte and total bilirubin excluded from this model.

MORTALITY IN SCD

Introduction

Estimates suggest that 50-80% of SCD children born in Africa patients will die before adulthood[6]. The World Health Organization estimate that up to 70% of these deaths are preventable with simple, cost-effective interventions such as early identification of SCA patients by newborn screening (NBS) and the subsequent provision of comprehensive care. Identification of risk factors has led to improved survival through targeted interventions. In the West, reported risk factors for death include infections, low haemoglobin and foetal Hb (HbF), high white blood cell count and hemolysis[22, 61, 73]. Therefore comprehensive care includes prompt treatment of acute events and prophylaxis against infections, mainly with oral penicillin and vaccination against *Streptococcus pneumoniae* and hydroxyurea. Countries that have introduced these interventions have achieved significant reduction in mortality; with up to 94% surviving to 18 years in the United States of America (USA)[18] and 99% to 20 years in the UK[19]. In most African countries, the lack of an evidence-base has led to inertia in terms of implementation of these interventions, such as penicillin prophylaxis[273]. One of the first steps in addressing this lack of knowledge is to provide an estimate of mortality rates to highlight the burden of disease due to SCA. The ideal approach is to establish a cohort of SCA patients, diagnosed at birth, and follow them up to determine rate and cause of death. However, most countries in Africa do not have NBS programs, therefore such evidence will rely on hospital-based studies. Information from such cohorts is biased, as it will consist of healthy survivors or those with severe disease, who seek health care. This situation is similar to that in Jamaica and USA in the early 1970s, when NBS for SCA was not established and evidence relied on prospective studies in hospital-based cohorts, where most of the patients (92% and 65% respectively) were not identified at birth. Despite the limitations of hospital-based studies, these

studies provided important evidence on morbidity and mortality due to SCA. The aim of this analysis was to determine the rate and risk factors of mortality in SCD patients.

Methods

Individuals were enrolled at outpatient clinics when they were stable without evidence of acute illness. Those patients with acute illness were seen in casualty or hospitalized. All the SCA patients attending the clinic followed the existing clinical practice of referrals and diagnosis on the basis of clinical suspicion or family history. There is no NBS programme in Tanzania. The methods have been described previously[393]. A detailed history and examination was recorded onto standardized proformas. Blood samples were collected for a full blood count, haemoglobin electrophoresis, HPLC and biochemical analysis. Nucleated RBC could not be differentiated from neutrophils by the haematology analyzer.

SCA patients who did not attend clinic for more than 12 months were defined as defaulters and were actively contacted by telephone or, for those residents in Dar-es-Salaam, home visits. Patients who were not found after three attempts of tracing were considered to be lost to follow-up.

Statistical analysis

Data were analyzed using STATAv10 (StataCorp, College Station, TX, USA). The study period started at enrolment clinic visit and the end was date of death or date last known to be alive. For patients who died outside the hospital where date of death could not be ascertained, I used the date of last hospital attendance. The Cnaan and Ryan approach to data analysis was used[394], which takes into account patients entering and leaving the study cohort with the observation beginning after disease onset, which in this case was at birth. According to this method, a patient contributes

to the population at risk for a given death only if that patient is enrolled in the study at the age at which death occurs. Data were set using the stset command in STATA; *stset date_end, fail(death) origin(dob) enter(date_recruit) exit (date_end) id(demographic_id) scale(365.25).* This command takes into account three time points; origin which is the date of birth; enter which is the date that the patient was enrolled into the study (in this case this is the entry visit at outpatient clinic) and exit which is the date of censoring. This was date of death or date last known to be alive.

The overall and age-specific incident rate of mortality was estimated. This was calculated from the ratio of number of deaths divided by the number of person years of observation (PYO), expressed as mortality rates. In STATA, the command *strate, per(100)* generated the overall mortality rate and the command *strate age_exit_5y_grp, per(100)* gave the age-specific incident rate, with age in 3 years; < 5 years, 5 – 9 years and above 10 years. The results are presented as incidence per 100 PYO.

To determine life expectancy, I modelled age at death rather than the length of time from enrolment to death, similar to method used by Platt *et al*[61]. This was done using the *stci* command in STATA to determine the median survival after the data were set as described above and the survival curve was drawn using the *sts graph* command in STATA The data were summarized as means, medians or proportions. Cox regression was used to analyze factors at enrolment associated with death, with results presented as hazard ratios (HRs) with 95% confidence intervals (95%CIs). STATA command *stcox `var'* where var is the clinical or laboratory characteristic that is being analysed. Multivariable Cox regression was used to identify independent associations with death, using variables that had significant association ($p<0.05$) on univariable analysis. For the SCA patients who died in hospital, the clinical and laboratory findings were reviewed and likely cause of death

was ascertained.

The cause of death could only be ascertained in the SCD patients who died in hospital. For these patients, the hospital notes, proformas and laboratory results were reviewed. I classified possible cause of death into infection if the patient presented with fever; presence of malaria or bacterial infection and marked increase in white cell count. Cause of death was anaemia if the patient presented with anaemia and haemoglobin was <5 g/dL or there was a drop of haemoglobin level of more than 2 g/dL from the nearest clinic visit when patient was not unwell.

Results

Mortality rate

From March 2004 to March 2009, 1,725 SCD patients were recruited into the cohort. Two hundred and nine were lost to follow up. Therefore, longitudinal information was available for 1,516 (80%) patients with 4,295.9 person years of observation (PYO) [median: 2.9 (range: 0.02 – 5.14) years]. Death occurred in 86 (5.7%) patients, with only 20 (23.3%) occurring in MNH. The overall incidence of death was 2.0 (95%CI 1.6, 2.5) per 100 PYO, with the highest rate below 5 years (Table 28).

Table 28 Mortality rates stratified by age at exit from study

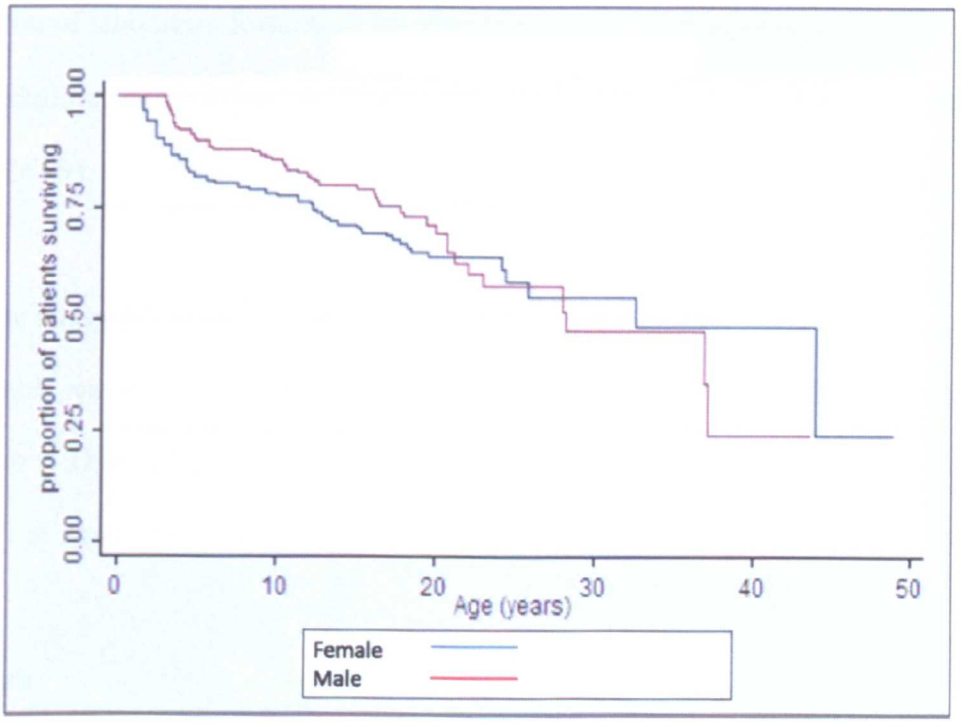
	Age at exit or death*			
Age (group)	**Patients[1]**	**Observation[2]**	**Deaths[3]**	**Incidence[4]**
<5years	252	285.4	23	8.1 (5.4 12.1)
5-9 years	1017	3,212.5	48	1.5 (1.1 1.9)
>10 years	247	797.9	15	1.9 (1.1 3.1)
Total	**1,516**	**4,295.9**	**86**	**2.0 (1.6 2.5)**

*Date begin is date of birth and date of entry is date of enrolment into the study at outpatient clinic. [1]patients; [1]Number of individuals in each year group; [2]person-years of observation; [3]Deaths; [4]incidence of death per 100 PYO

Survival of patients enrolled into the study

The estimated median survival of patients enrolled into the study was 32.7 (95%CI 25.9, 43.9) years, with no difference between genders (p=0.8). The survival of patients enrolled in the study was above 85%, during the period of observation.

Figure 24 Survival estimates of SCD patients enrolled into study.



The survival curve takes into account that patients were identified and recruited into the study after onset of disease. Using the Cnaan and Ryan approach[393], a patient contributes to the population at risk for a given death, only if that patient is enrolled in the study at the age at which death occurs (p=0.8)

Risk factors for mortality

The characteristics of 1,516 patients at the visit when they were recruited into the cohort were compared for those who were alive at last contact and those who died to explore whether any of these factors could be used to identify high-risk individuals. Older age at exit from the study, frontal bossing, and pallor on examination were significantly associated with increased risk of mortality (Table 29).

On examination of laboratory features at baseline visit, lower haemoglobin, higher direct bilirubin and higher alkaline phosphatase were associated with increased risk of death on univariable analysis (Table 29).

Multivariable analysis

On multivariable analysis, low haemoglobin (HR 0.693, 95%CI 0.580, 0.829; $p<0.0005$), and high direct bilirubin (HR 1.004, 95%CI 1.001, 1.007; $p=0.018$) were associated with death independently of age at exit from the study and the other variables, including indirect bilirubin.

Cause of death

Of the 20 deaths that occurred in hospital, 5 (20%) were likely due to anaemia, 3 (15%) due to infections and 12 (60%) were difficult to ascertain. No post-mortem studies were done.

Table 29 Clinical and laboratory features at baseline visit associated with survival in SCD patients

	Survived **n=1,430;**	**Died** **n=86 (5.7%)**	**HR (95% CI)**	p
	n (%) or	n (%) or		
Male (n %)	696 (48.7)	44 (51.2)	1.02 (0.66, 1.57)	0.92
Age at entry (years)	7.9 (0.25-47.7)	9.3 (0.8-42.3)	1.003 (0.975, 1.032)	0.83
Age at exit (years) Median	11.2 (0.7-49.0)	12.0 (1.5-44.0)	0.965 (0.933, 0.997)	0.03
Clinical symptoms and signs				
Pain	145 (10.6)	8 (9.4)	0.97 (0.46 2.02)	0.93
Symptoms of anaemia	25 (1.9)	5 (5.9)	2.44 (0.98 6.12)	0.06
Jaundice	692 (52.6)	50 (60.9)	1.23 (0.77 1.95)	0.38
Pallor on examination	525 (38.9)	44 (53.7)	2.04 (1.31 3.16)	<0.01
Bossing	306 (23.5)	32 (39.0)	1.59 (1.01 2.51)	0.05
Palpable spleen	189 (14.1)	16 (20.0)	1.49 (0.84 2.66)	0.17
Palpable liver	60 (4.8)	8 (10.3)	1.69 (0.79 3.59)	0.17
Hb F	6.3 (4.7)	6.7(5.0)	1.044 (0.991, 1.099)	0.11
White blood cell count	15.9 (7.0)	15.7 (7.3)	1.014 (0.98 1.049)	0.45
Haemoglobin (g/dl)	7.5 (1.3)	6.9 (1.6)	0.678 (0.578, 0.794)	<0.01
RBC	3.0 (0.8)	2.8 (0.8)	0.729 (0.519, 1.024)	0.07
RDW	23.1 (4.3)	24.4 (5.1)	1.039 (0.993, 1.088)	0.09
MCHC	32.9 (2.9)	33.1 (2.5)	0.902 (0.804, 1.011	0.08
Total bilirubin	69.7 (56.7)	87.5 (81.4)	1.002 (0.999, 1.005)	0.14
Direct bilirubin	20.9 (34.2)	33.1 (57.6)	1.003 (1.000, 1.006)	0.05
Aspartate transaminase	50.1 (28.4)	49.3 (24.1)	0.997 (0.987, 1.006)	0.43
Alkaline phosphatase (IU/L)	266 (124)	277 (153)	1.002 (1.000, 1.004)	0.05
Creatinine	40.8 (19.9)	43.3 (22.8)	1.003 (0.992, 1.013)	0.62

DISCUSSION

This study presents data from one of the largest cohorts of SCD patients from a single centre in the world. Although the prevalence of SCD is high in African countries, the largest cohorts have been established outside of Africa where the prevalence is much lower (estimated annual birth prevalence of SCD: Tanzania 7,800; USA 1,531 and Jamaica 302)[4]. The reasons for this are threefold; first, for many low income countries (LIC), it is reported that there are few individuals with SCD who survive and require health care as the assumption is that there is high mortality in childhood. Since there is a high incidence of infections such as malaria, lower respiratory infections, diarrhoeal diseases, the focus and priority has been on addressing these conditions. Second, as a genetic disorder it is assumed that management (diagnosis and treatment) would require resources that are beyond the means of health ministries in LIC. Third, prospective surveillance of patients for clinical and research purposes requires considerable resources in terms of personnel, time and funding. However, the situation is changing, with SCD having been recently recognised as a public health priority[1 12] and reports that show how cost-effective interventions can be introduced in LIC[277]. Finally, identification of 1,725 SCD patients from one centre and without active surveillance in the community or health facilities illustrates the magnitude of disease in this setting.

The SCD population

Previous reports from Africa suggest that most children with SCD die in early childhood with prevalence of SS reported to fall from 2.1% at birth, 0.4% at one to four years and to 0.05% over the age of nine years[16]. In our study, 70% of the SCD population was above 5 years,

suggesting that although there is no active intervention, SCD patients are surviving. More recent estimates suggesting that Hb disorders contribute the equivalent of 6.4% of under 5 mortality in Africa[17]. Although almost 90% of our study population were paediatric (under 18years), only 11.3% were < 2 years. This is of particular significance for SCD, because this is the period of highest incidence of mortality[22]. In order to address this information gap of spectrum of disease in first 2 years of life, natural history and survival of SCD, accurate information on the birth and population prevalence as well as the age-specific mortality rates is required.

In this study, we wanted to determine clinical and demographic features that could be used to identify patients likely to have SCD as well as describe the clinical and laboratory features of SCD patients in this setting. Clinical features classically described in SCD were seen in our SCD populations, but were not found to be useful in identifying SCD patients. The finding that hypoxaemia, measured by SpO_2, was associated with SCD is interesting as it has been reported to be a marker of disease severity [315] and has been associated with pulmonary[60] and neurological events[304 314]. This study also found that enlargement of the spleen was independently associated with SCD status. The detection of a spleen can be easily taught and could be used to identify patients who should be screened for SCD. In addition, teaching SCD patients parents and carers to recognize splenic enlargement is of relevance with regards to early detection and treatment of acute splenic sequestration. This is one of the common causes of death in SCD[20 53 58] and recognition of splenic enlargement has been reported to improve survival[106].

The haematological and biochemical parameters in both SCD and the control population in

Tanzania have been described, which would be locally-appropriate reference values of SCD as well as non-SCD individuals in this setting. Comparison with SCD populations from other geographical areas (Table 30) suggests that there are differences[52, 63, 68, 114, 395-398]. In addition, our study has reported laboratory values that were independently associated with SCD status (low levels Hb and ALP and high WBC, HbF and Bilirubin). This is of particular importance in African countries, as there are limited resources to set up confirmatory tests for SCD (Hb electrophoresis or HPLC) in all laboratories. Therefore, these values could be used to screen and identify children that should be referred for confirmatory SCD testing.

In the selection of SS individuals to include in the cohort there are two limitations of note. First, SS individuals who received blood transfusion within 3 months of enrolment visit were excluded from the SCD cohort, on the basis of having HbA_0 from transfused blood. Second, confirmation of diagnosis of S-β^0thalassemia and S-HPFH requires genotyping. Although hypochromic microcytic red cell indices may be used to identify individuals with co-inheritance of thalassemia, the high prevalence of iron deficiency anaemia in our population limits the use of these parameters in this setting. In order to identify individuals with S-β^0thalassemia who will have severe disease, genotyping should be done. Although this description is limited in that it may have included individuals with S-β^0thalassemia, published reports suggest that the predominant sickle genotype in most East African countries is SS, with a low prevalence of S-β^0thalassemia. For those with S-HPFH, the assumption is that they will have mild disease and will most likely not present to hospital or require hospital follow-up. The plan is to establish genotyping facilities in Tanzania that would allow us to determine the prevalence of S-β^0thalassemia and S-HPFH as well as to understand the genetic determinants of clinical disease.

Table 30 Laboratory values in SCD from different studies

		SCD populations					
	Std ranges	**This study**	**Nigeria ***	**Congo**	**UK France**	**Jamaica**	**Saudi Arabia**
Haematology							
White blood cell count ($x10^9$/l)	4-11	15.7 (6.8)	10.6 (4.0)	17.35 (9.8)			8.7 (3.1)
Hb (g/dl) GM (95%C.I.)	10 - 18	7.6 (1.4)	7.3 (1.1)	6.6 (1.3)	7.8 / 8.0 (1.5)	7.7 (0.9)	11.2 (2.2)
Red blood cell count	4.1 – 6.0	2.9 (0.9)	3.6 (0.8)	2.55 (0.7)		2.78 (0.4)	4.1 (0.8)
MCV		78.5 (10.9)	85.3 (9.4)	83.82 (11.8)	79/ 83.9 (9.0)	83.8 (6.7)	
RDW (%)		23.2 (4.4)					
Platelet count		423.5 (196.0)		319.13 (165.4)	394	452 (109)	
Reticulocyte		12.6 (7.9)	9 (0.16)			10.5 (4.0)	
HbF (percent)	<2% or	6.3 (4.6)	7.2 (3.7)	8.7 (5.73)	10.2/ 9.8	5.8 (5.3)	10.3 (3.7)
HbA_2		4.5 (1.5)		3.44 (2.2)		3.11 (0.46)	
Chemistry							
Bilirubin total (μmol/L)	5.1-17	70.4 (59.9)		36.69 (25.7)	55		
Bilirubin – indirect (μmol/L)	3.4-12	21.7 (35.2)		20.80 (23.4)			
Lactate dehydrogenase (IU/L)	200-450	1033.7 (450.4)		674.03 (527.7)	580		
Alkaline phosphatase (IU/L)		276.4 (175.9)			176		
Aspartate transaminase (IU/L)	0-35	49.9 (27.2)			55		
Creatinine (μmol/L)	<133	40.6 (18.5)			52		

Some results are missing as they were not reported in the publications.

This study has been able to demonstrate that with relatively limited resources it is feasible to set up a cohort study, integrated into health care provision in LIC in Africa. However, we acknowledge limitations of hospital based cohorts; selection bias, since the SCD population have described may be those with mild disease who have survived or those with severe disease who come to the hospital. In addition, MNH is the national hospital, linked to the main medical school. Although the resources are limited, the situation is likely to be worse in small, rural health facilities. It is therefore important to interpret the results in this context as the SCD population in these areas is likely to be worse. As part of future studies, I hope to collaborate with other sites to describe the spectrum of SCD in other areas of Tanzania.

The second limitation is that the proportion of the SCD population under 2 years (166/1,725; 9.6%) and above 20 years (195/1,725; 11.3%) was low. Therefore, the information from my study should be interpreted with caution for these age groups. This limitation highlights the need to identify SCD children at birth with prospective follow up of this birth cohort, which is of importance for research as well as development of clinical programmes[276 277]. It also highlights the importance of population-based approach to epidemiological studies. With regards to the small number of patients above 20 years of age, this may be due to mortality or it may be due to patients having mild disease when older and therefore not requiring regular visits to health facilities. Further studies are required to determine the burden of disease in the older age group. The third limitation is related to the controls. The control population should ideally be matched for age, geographical residence and time of recruitment. They should also be followed up in order to determine key events such as death and health facility visits which could be part of a demographic surveillance system. The controls in this study were recruited from siblings and individuals referred to the hospital which could result in bias.

There was no information on mortality. This was not done due to limitations of resources and it is proposed that future studies should be planned bearing in mind these limitations.

Notwithstanding these limitations, I have provided a description of SCD in Africa and have developed the framework for further studies into understanding disease mechanisms and role of different disease modifying factors SCD. In addition, this framework will allow the development of an evidence-base, through clinical trials, to determine the interventions that would be locally-appropriate.

Hospitalisation

The burden of SCD is frequently quantified by estimating the birth prevalence of SCD individuals and the mortality rates. However, SCD is a lifelong illness and causes significant burden on individuals, communities and health systems with episodes of acute clinical events which sometimes require care at hospitals. During the course of the study, 29% of the 1,725 SCD patients recruited into the cohort were hospitalised. This rate is higher than the estimate quoted that 10% of SCD require inpatient care[2, 3, 6]. The reason for the higher rate of hospitalisation in this study may be due to more severe disease but is more likely due to lack of comprehensive dedicated services for SCD. In settings with specialised care for SCD, hospital care is organised in such a way that patients may receive treatment such as blood transfusion, antibiotic treatment, or undergo investigations on an outpatient basis in the form of day wards. In many African countries, services are not organised in that way. Therefore, SCD patients are either seen in the emergency department (ED) or are hospitalised. A dedicated SCD service, particular with day ward facility has been found to be an efficient way of providing care which results in reduced need, as well as reduced length of stay during

hospitalisation[399]. In the UK, the frequency of hospitalisation in a SCD cohort in South London, UK in 1981, reported that 63/211 (29.8%) SCD patients were hospitalised[400] which is similar to the frequency in this study. However, day wards were reported to reduce hospital admissions by 43% and resulted in a 49% reduction in length of stay in hospitals[401]. In Africa, ambulatory care (which includes care in the community) has also been found to be feasible and effective[402].

The main causes of hospitalisation in this study were pain (76.1%), fever (59.6%) and anaemia (21.9%). Painful episodes, are the commonest cause of hospitalisation in SCD[23 403]. They account for the following proportion of hospitalisation; 26.7% in Congo Brazzaville[404], 36% in Yemen[405], 63.2% in Kuwait[406], 65% in Senegal[149] and 50% in USA[407]. However, some studies have reported infections as being more common than pain as a cause of hospitalisation; a study in Congo Brazzaville reported cause of admission as infections (36.6%), pain (26.7%) and anaemia (20.3%)[404] and in another study in Nigeria, infections accounted for 69% of admissions[408]. Therefore, pain and febrile episodes appear to be the two most common causes of hospitalisation in SCD.

At baseline visit, the 3 factors that were independently associated with increased risk of hospitalisation were a history of hospitalisation or febrile episode within 12 month of baseline visit and high RDW. The former two factors are useful indicators, as they are relatively reliable from reports of parents or guardians. Therefore they can be established easily from taking a good clinical history. This information would then be used to alert health care providers of SCD patients who are at increased risk of hospitalisation. Therefore, the high-risk individuals would be monitored more closely. The relationship between RDW and

hospitalisation is less clear. A high RDW suggests that there is mixed population of red blood cells, both small and large cells. Small cells or microcytic RBCs may be due to iron deficiency whereas large cells, macrocytes, may be due to deficiency of vitamin B12 or folate. Macrocytes may also be due to increased erythropoiesis resulting in large RBCs circulating in peripheral blood. The increased erythropoiesis may be in response to low Hb levels. On univariable analysis, low Hb was significantly associated with risk of hospitalisation but not on multivariable analysis. This relationship, between anaemia and risk of hospitalisation requires further exploration in more detailed studies.

During hospitalisation, 20 of 882 hospitalisation events resulted in death; giving a case fatality ratio (CFR) of 2.3%. This rate is lower than that reported in Zambia, where a study that tried to determine cause of death in 62 SCD deaths for 3 years, between January 1987 and December 1989, found a CFR of 6.6%. The lower CFR in this study may be due to a 'healthy-survivor effect'; where the SCD patients that are seen at MNH are those with less severe disease and therefore will have lower mortality.

The information from this study is an initial step in identifying the causes and risk factors for hospitalisation. I acknowledge that some SCD patients do not come to MNH for healthcare. Factors such as physical distance, referral system, and socioeconomic factors may influence who is hospitalised at MNH. It is therefore important to develop a system that will capture events that occur at home and that are managed in health facilities outside MNH.

Mortality

This is the first study that presents the rates and risk factors for mortality in SCD in Africa,

where over 75% of SCD patients in the world live. The median survival amongst patients with SCD was 33 years, which is 19 years less than life expectancy at birth (52 years) in Tanzania[391]. This life expectancy is markedly lower than the 40 to 60 years of SCD patients in the United States of America[61] and Jamaica[409]. The mortality rate in this cohort is 2.0 per 100 PYO (95%CI 1.6–2.5) is not dissimilar to the 3 per 100 PYO reported from the USA in the 1970s before use of penicillin prophylaxis[410, 411], but is markedly higher than the current incidence of 0.13 to 1.39 per 100 PYO reported from Europe and the USA[18, 19, 61]. The second important finding is that most deaths occur in children rather than adolescents or adults. This is similar to reports from Jamaica and USA where, before prophylactic penicillin, the highest incidence of death was reported between 1-3 years[19, 20, 22, 23, 106]. This highlights the importance of identifying children at birth by newborn screening as SCD children are probably dying before the diagnosis is made. Pilot studies have shown that newborn screening is feasible in Africa[276] and its establishment with comprehensive care will most likely significantly improve survival.

In this study, low haemoglobin was independently associated with mortality. It has been previously reported that there is an association between low haemoglobin and mortality[19, 20 22, 23, 61, 71, 106, 177, 408]. However, more evidence is needed to identify underlying cause of anaemia so that interventions that ameliorate the effects can be considered and targeted. So far, interventions to reduce mortality have been aimed to address the recognised causes of death, namely infections, acute splenic sequestration and acute chest syndrome. Blood transfusion therapy is necessary in SCD, particularly during acute episodes and perioperatively[125, 125], but chronic blood transfusion is not a viable treatment option in Africa due to problems of inadequate blood supply, risk of transmission of infections and

alloimmunization[205, 412]. A potentially effective therapy is hydroxyurea as it is relatively cheap, easy to administer and has been shown to reduce blood transfusion requirements and to modestly increase haemoglobin and improve survival[128, 358]. However, despite these advantages, it is not widely used in SCD patients in Africa and there have been no trials to determine its role in this setting.

There is increasing evidence of the role of hemolysis in pathogenesis of clinical events in SCD, such as pulmonary hypertension and stroke[73-75], via a mechanism involving interference with endothelial nitric oxide by free haemoglobin. However, hemolysis and the hyperhaemolysis phenotype are thought not to be common in Africa[78]. In this study the relationship between haemolysis and mortality was carefully explored. Analysing, the risk factors at baseline associated with overall mortality, markers of hemolysis, such as lactate dehydrogenase[73] and total and indirect bilirubin did not show significant association. However, during hospitalisation, total bilirubin and indirect bilirubin, were significantly associated with increased risk of mortality on univariable analysis. This may suggest that during OPD visits, SCD patients are stable with low levels of haemolysis, hence the absence of an association with mortality. However, during the acute phase, there is an increase in haemolysis which is associated with mortality. It is therefore essential to conduct further studies to accurately elucidate the role of haemolysis in SCD as interventions that reduce haemolysis, such as hydroxyurea, may be effective in improving survival.

In this study, direct bilirubin was an independent predictor of death when analysing the relationship between levels at baseline with overall mortality as well as during levels at admission during hospitalisation. The association between direct hyperbilirubinemia and

mortality may be related to liver disease, as there was also a strong trend in univariable analysis for an effect of alkaline phosphatase, although aspartate transaminase was not a predictor. It is possible that the direct hyperbilirubinemia is related to the upregulation of haem-oxygenase in response to the hypoxia associated with SCD. This may have protective effects via the antioxidant effects of bilirubin and the vasodilatory effects of carbon monoxide[413]. Adaptations may have downstream costs, however, and there is a suggestion that upregulation of haeme-oxygenase may have detrimental effects in patients who are acutely ill[414]. Haeme-oxygenase also up-regulates Vascular Endothelial Growth Factor, potentially leading to further angiogenesis[415, 416]. Dysfunctional angiogenesis, haeme--oxygenase and Nitric oxide are factors that are implicated in the pathogenesis of clinical events and disease in SCD.

One of the aims of the study was to determine the cause of mortality in SCD. However, in this study it was difficult to make valid conclusions from the data that I was able to collect as only 20 (23%) out of 86 deaths occurred in hospital. On reviewing the notes of these 20 cases, I was able to confidently ascertain the likely cause of death in 8 cases (9.3%); in which anaemia and infection may have been the cause in 5 (20%) and 3 (15%) cases respectively. In order to ascertain cause of death, it is important to conduct verbal autopsy on deaths that occur outside health facilities. For those that occur within health facilities, such as Muhimbili, efforts should be made to conduct post-mortem studies. This information is critical to ascertain cause of death which would improve interventions to prevent future mortality.

This cohort study highlights four key areas to improve survival in SCD and identifying knowledge gaps to guide further research. First, over 85% of patients enrolled into the study

survived, providing evidence to challenge the dogma that SCD patients do not survive beyond childhood in Africa. Modell *et al* estimated that 6 million Africans will be living with SCD if average survival of affected children reaches half the African norm[12]. The burden of disease to individuals, communities and health systems has not been quantified yet, but these patients will suffer from anaemia, painful crises, infections, stroke and other complications[6]. Undoubtedly, SSA countries need to develop programmes to provide appropriate care for these individuals as the burden will be considerably high. Second, only 25% of deaths occurred in the MNH. This is probably an underestimate as we did do not have data on deaths in undiagnosed SCD children < 5 years. Although it is possible that these deaths occurred in other health care facilities, the burden of disease survey reported that 50% of deaths in Tanzania occur outside a health facility[417]. The finding has important implications for planning services as it highlights the need to develop a strong network of community-based health care and not focus resources in specialist hospitals. The responses on questioning relatives about events surrounding death suggested that most deaths occurred at home but further studies following recognised verbal autopsy procedures are required. Third, although 1,903 patients were identified at clinic and in wards, 10% did not return to the clinic and for those who did return to clinic and were recruited into the cohort, 12% were lost to follow-up. Therefore, SCD patients may be seen at a health care facility only once. This is in contrast to US, Jamaica and Europe where steady state values (average annual routine measurements) [22, 61, 418] are used, as the health system in these countries ensures that patients can be seen regularly, or at least easily traced if they are found to be high risk. Therefore, in this study, factors at baseline clinic visit were explored to identify high-risk patients. Attendance may be improved by health education to SCD patients and their caregivers on the importance of regular follow-up as well as improving access to health care.

This study provides the first description of mortality rate of SCD in Africa. As expected, there is a high mortality in SCD, with children significantly affected. Level of haemoglobin and direct bilirubin at baseline visit were independently associated with mortality. Although I have provided data that will guide initial policies and guidelines of management, the study has highlighted the areas where there are gaps in knowledge. I propose that priority should be given to the establishment of a SCD birth cohort to provide accurate survival data and identify causes of mortality particularly in early childhood. Furthermore, since anaemia was associated with mortality, detailed studies to identify causes of anaemia and randomised clinical trials to determine appropriate interventions to ameliorate the effects of anaemia are recommended.

Chapter Five

Sickle Cell Disease and Malaria

INTRODUCTION

Malaria is widely considered to be one of the major causes of illness and death in patients living with SCD in SSA[14, 100]. However, the evidence to support this has been conflicting. An association between malaria and both admission to hospital and anaemic crises [76, 177-179, 202, 419] and increased mortality[19, 100] have been reported in a number of studies. However, these studies included a relatively small number of SCD patients and were generally conducted in areas with a high prevalence of malaria in the general population.

On the other hand, the evidence of increased protection from malaria in SCD individuals is more compelling; a lower prevalence of malaria infection[180, 181, 183] and a lower parasite density[182] have been reported. The evidence that the HbSS genotype may confer resistance against malarial infection *per se* would make more sense, given that subjects heterozygous for the β^s-gene (Hb AS) show unequivocal protection from malaria[158-161]. This raises the question of whether, if HbS protects the heterozygotes against malaria, those with SCD, who have even higher levels of HbS, might be even better protected.

Recently there have been three important developments in the field of malaria. First, effective interventions against malaria are available, in the form of insecticide-treated nets[173], and artemisinin-based combination therapies[420]. Second, there are reported changes in the epidemiology of malaria in some parts of East and West Africa, with reductions in both

transmission rates and the incidence of disease[174, 175, 421]. It is possible that this reduction is due to effective interventions and control measures as well as climate change. Third, due to increasing resistance by *Plasmodium falciparum* parasites most countries have had to stop using chloroquine. In Tanzania, during the period of the study, first line therapy for malaria changed from monotherapy with chloroquine to sulphadoxine pyrimethamine (SF), with the current recommendation being artemisinin-based combination therapy (ACT) – artemether lumefantrine (ALu)[191] . However, it has been difficult to determine which drug to use for prophylaxis. SF has anti-folate properties and is not recommended for prophylaxis in patients with SCD who are considered to be folate deficient. The folate deficiency is thought to be a result of chronic haemolysis and compensatory increase in erythropoiesis. Most malaria-endemic countries have therefore been unable to decide which drug to use and have continued to recommend chloroquine for prophylaxis in SCD.

The changes in malaria epidemiology and management as well as the increasing evidence of protection against malaria by HbS, emphasized the need to determine the role of malaria as a cause of morbidity and mortality in SCD. However, the general perception that malaria is a major risk in patients with SCD means that the prescription of anti-malaria chemoprophylaxis is now standard recommended practise in endemic areas[127]. As a consequence, ethical considerations make it difficult to study this question directly. The cohort of SCD in Tanzania provided a unique opportunity to study the importance of malaria as a cause of morbidity in SCD. Tanzania stopped using chloroquine for treatment of malaria in 2001, due to high resistance. Although the national malaria control programme continues to recommend that children with SCD should receive prophylaxis with chloroquine, the importation of chloroquine is banned and in practise most patients are not on effective prophylaxis. Dar-es-

Salaam has "urban malaria , a pattern that is characterised by low transmission intensity and variable parasite prevalence, ranging from 0.8% to 10% [422-424]. The primary aim of this study was to determine the role of *Plasmodium falciparum* infection as a cause of ill health in individuals with SCD in Tanzania.

METHODS

Patients

All SCD patients were seen at the outpatient department (OPD) clinic. Routine appointments were scheduled at 3-6 month intervals and the majority of patients attending these appointments were well. During these routine visits, the results of a standardised clinical history and examination were recorded and blood samples were collected for full blood count, biochemical analysis and malaria investigation. Patients admitted for inpatient care were identified by active daily ward surveillance. Routine clinical and laboratory data, including a full blood count, biochemical analysis and malaria diagnostic tests, were collected at admission onto standard proformas. Additional investigations were performed as clinically indicated. Data were collected from two populations of non-SCD individuals: (1) subjects invited for SCD screening at the OPD clinic, including relatives and friends of SCD patients, and (2) non-SCD patients who were admitted to the paediatric wards of MNH between July 2006 and July 2008.

Laboratory methods

Regardless of their clinical status, all individuals who were enrolled into the study were investigated for malaria at all visits. Malaria was confirmed using rapid diagnostic tests

(RDTs) (Parahit, Span Diagnostics, India or Paracheck, Orchid Biomedical Systems, India) and or Giemsa-stained thick blood films following standard methods. *Plasmodium Falciparum* densities were assessed by counting the number of asexual-stage parasites per 200 WBCs. The parasite density was calculated and expressed in parasites/μL by reference to the mean WBC count in this study population of 14.8 x 10^{12} /μL. These results have not been presented in this thesis.

Statistical methods

A general outline of descriptive statistics, disease estimates, univariable and multivariable analyses is provided in the methods chapter. Malaria events were analyzed according to three definitions: (1) malaria parasitaemia (MPS) - positive blood film and/or a positive RDT irrespective of clinical status; (2) clinical malaria (cM) - positive test in the presence of fever (an axillary temperature of >37.5^{0}C) and; (3) severe malarial anaemia (SMA) - Hb <5g/dl in the presence of malaria parasitaemia. For the purposes of this analysis, the diagnosis of SMA did not require a threshold parasite density of >10,000 parasites/μL, the definition used by WHO[425]. This assumption was made for two reasons; first, an Hb <5g/dL is a life-threatening condition as it associated with high risk of mortality. Second, since patients with SCD often receive treatment for malaria when febrile. Therefore, malaria parasitaemia may be low, but it may have already triggered events leading to severe anaemia.

The factors that were examined to determine an association with malaria included demographic (age, sex, place of birth, residence), clinical variables (fever, pain, anaemia, chest or neurological event) and laboratory parameters (haematology, clinical chemistry and microbiology). Time dependent variables were age, date of visit, clinical and laboratory

parameters at each visit. For purposes of the analyses, the parameters of each individual at recruitment into the study (baseline visit) for SCD patients and first visit for control individuals were used.

Continuous variables were compared using two-sided Student's *t*-tests while categorical variables were compared using the χ^2 test and results presented as odds ratios (ORs) with 95% confidence intervals. Multivariable logistic regression was used to identify independent associations with malaria, using all variables that were significantly associated ($p<0.05$) on univariate analysis. Here the focus will be on the analytical groups and outcomes of interest. The analytical approach that was used was to answer the 4 specific questions.

What is the prevalence of malaria in SCD during OPD visits? The data of all visits to OPD clinic by SCD and control population were examined. The groups being compared were SCD and non-SCD patients at OPD clinic.

What is the prevalence of malaria during admission? For this, the analysis was limited to data of patients who were admitted, with the two groups being SCD and non-SCD populations. The prevalence of malaria in the two inpatient population groups was compared with the SCD population. Comparison was then done looking at hospitalised SCD patients with malaria and without malaria. A description of specific clinical syndromes of malaria that SCD present with and then looked at factors associated with malaria was made.

What is the difference in malaria prevalence and associated factors during OPD and IPD visits of SCD patients? Data from OPD and IPD were combined. The groups that were

being compared were SCD at OPD and SCD who were hospitalised.

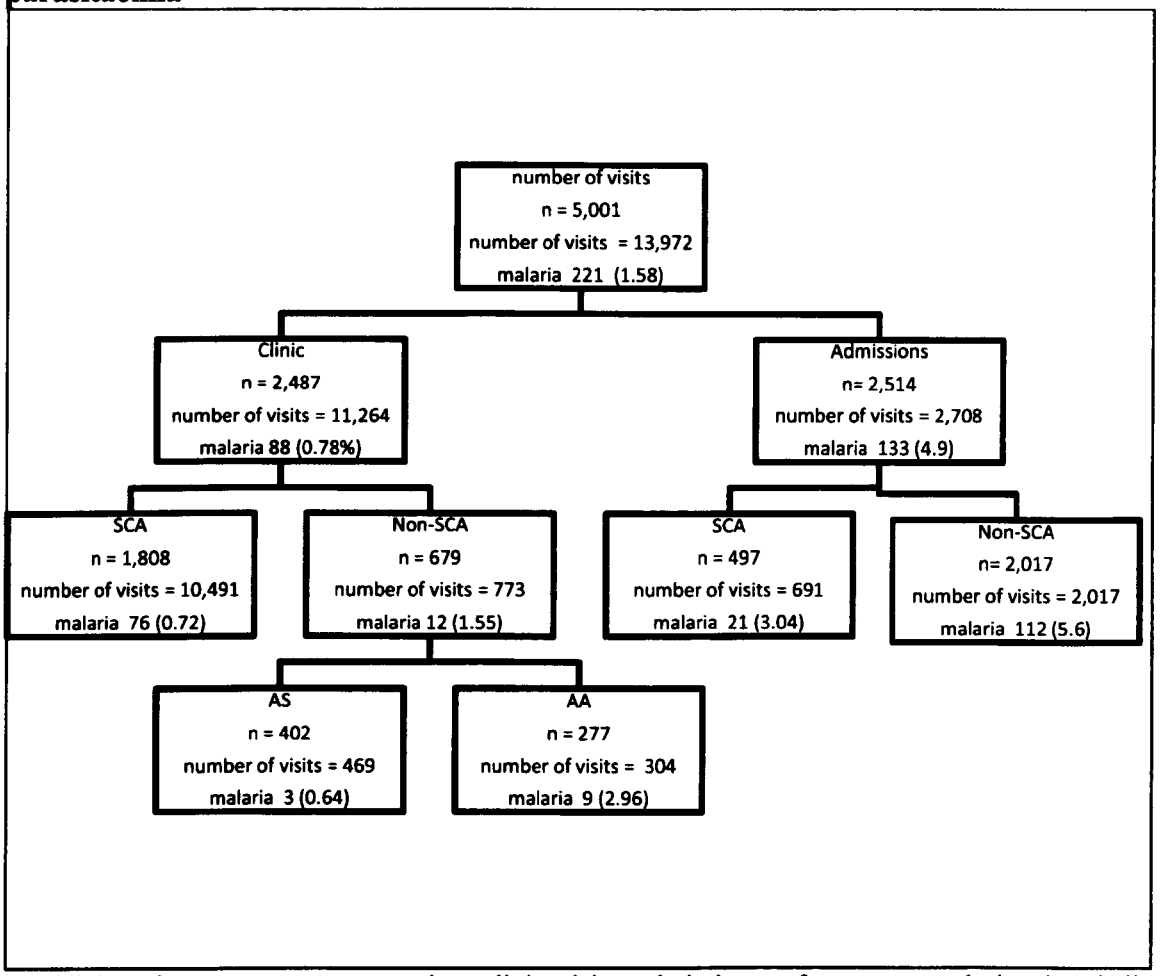
What is the incidence of malaria in SCD? The incidence rates for malaria events were calculated for the whole study period, combining events at OPD clinic and during hospitalisation. The data was set for survival analysis using the following command: *stset datetod, id (demographic_id) failure(malariaanaemia) exit(time end_date) scale(365.25).* The incidence was calculated by the ratio of malaria events (MPS, cM and SMA as defined above) divided by the number of person-years of exposure. This was done for the 3 malaria events using the following STATA commands *strate, per(100).* In order to calculate the incidence of the malaria event stratified into 2 age groups, above and below 5 years, the following command was used: *strate agegp, per(100)*. The result was expressed as the number of events per 100 person years of follow up, with 95% confidence intervals and a p - value of 0.05 was considered to be of statistical significance. Note that in the example given, the event is severe malaria anaemia, coded as malariaanaemia. This analysis was repeated for clinical malaria and malaria parasitaemia as defined above.

RESULTS

The analysis period was from March 2004 to March 2009. The OPD clinic data derived from 10,491 visits amongst 1,808 SCD patients (median age 11 years; range 4 months to 47 years) and 773 visits from amongst 679 non-SCD individuals (12.8 years; 3 months–64 years) while the inpatient data derived from 691 admission events in 497 SCD patients (10.8 years; 5 months–43 years). Please note that this chapter included all patients at OPD clinic visits and hospitalisation. There are some SCA patients who were seen at clinic or in wards who did not

return for enrolment/baseline visit. Therefore the numbers differ from the baseline description. Control data were collected from 2,017 non-SCD patients aged 6 months to 10 years who were admitted between 2006 and 2008. The prevalence of malaria in the various subject groups is summarized in figure below (Figure 25). Note that the prevalence of malaria in the control population during hospitalisation is limited to children.

Figure 25 Summary of individuals and visits in the study with prevalence of malaria parasitaemia

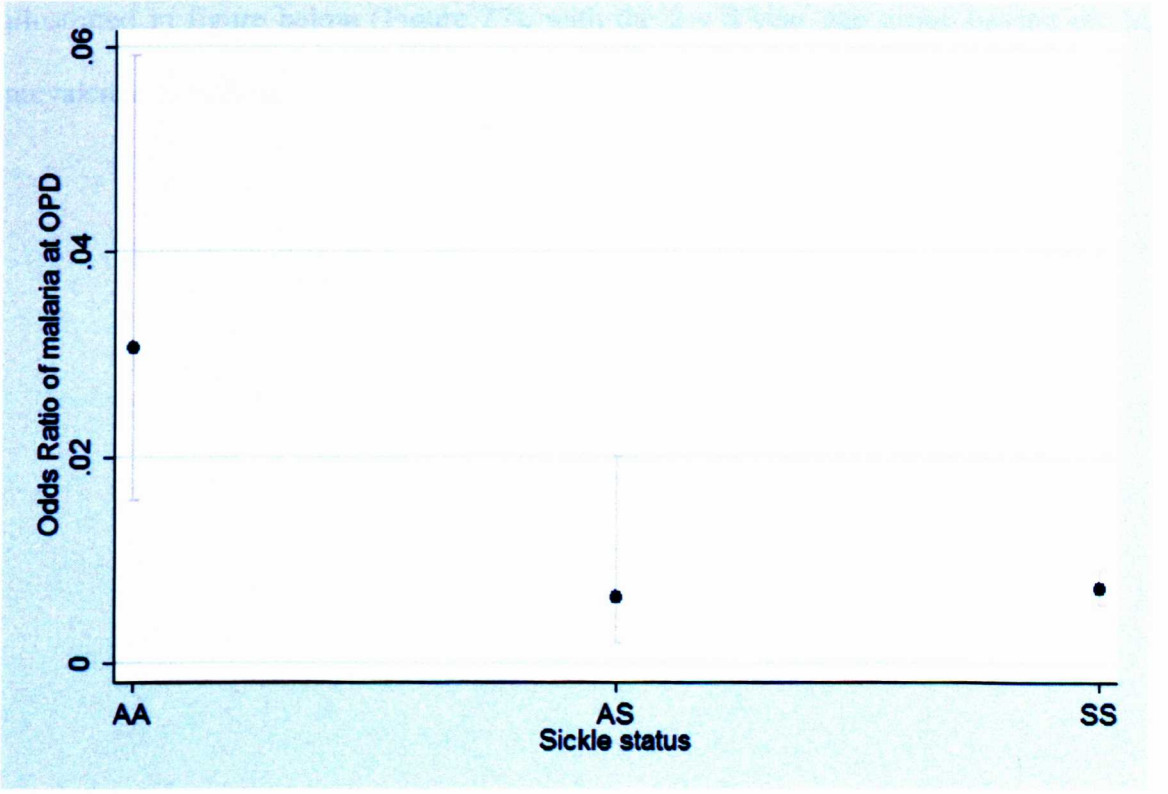


Clinic refers to events at outpatient clinic visits; admissions refer to events during hospitalization. Number of visits refers to all visits; n is number of individuals. The number after malaria is the number of episodes of malaria parasitaemia, figures in parentheses is the prevalence per visit (not per individual).

Malaria in the outpatient clinic

The overall prevalence of malaria parasitaemia at the OPD clinic was 88/11,264 (0.78%) (Figure 25), with the proportion in SCD patients being 76/10,491 (0.72%) and non-SCD patients being 12/773 (1.55%) [OR (95%CI) 0.46 (0.25 – 0.85); p=0.01]. Further subdividing the groups into the three sickle phenotypes (SS 1,808; AS = 402; AA = 304), the prevalence was significantly lower both in subjects with AS 0.64% and SS 0.72% than those with HbAA (2.96%). This is illustrated by showing likelihood (odds ratio) of malaria parasitaemia (Figure 26) in the three phenotypes.

Figure 26 Odds ratio of malaria parasitaemia in AA, AS and SS individuals at OPD clinic

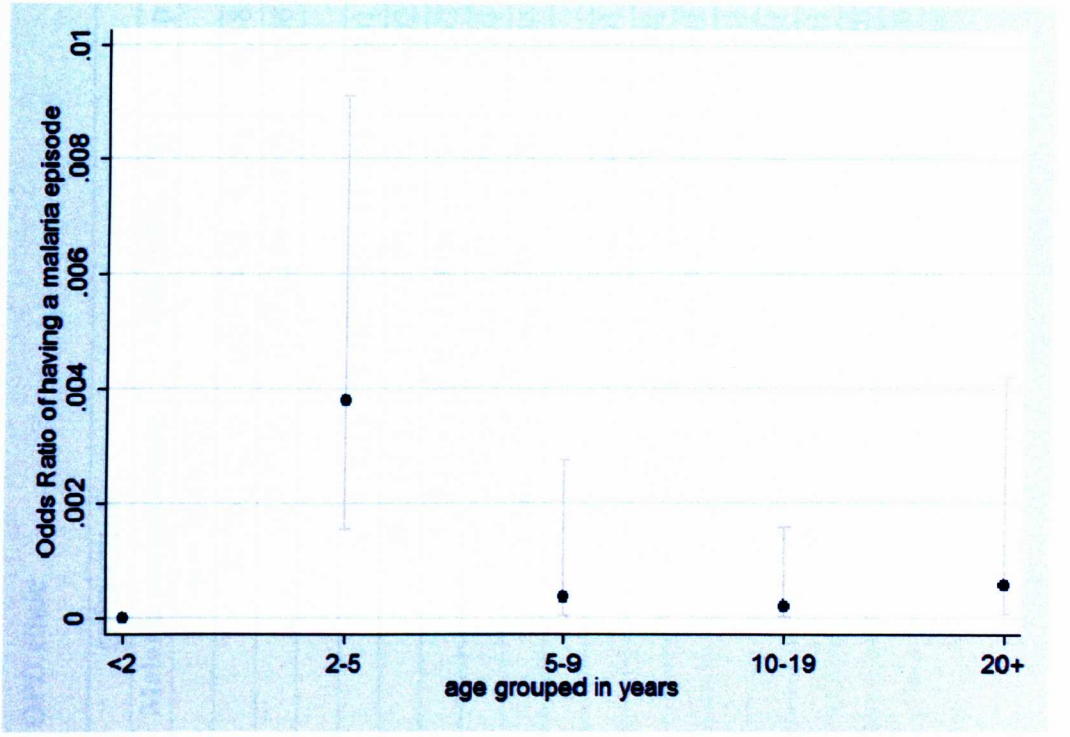


HbAS 0.64 %; (OR 0.21 (95% CI 0.06 – 0.79); p= 0.02) and HbSS 0.72 % (0.24 (0.12 – 0.48); p<0.001) than those with HbAA (2.96%).

Due to the variation in malaria prevalence with age, comparison was made between SCD and non-SCD at different age groups. The proportion with malaria in SCD compared to non-SCD was as follows: < 5 years; SCD 20 (1.17) vs. non-SCD 3 (1.33) [OR (95%CI) 0.88 (0.26 – 2.99); p=0.84]. 5 – 9 years; SCD 20 (0.77) vs. non-SCD 4 (2.44) [0.32 (0.11 – 0.93); p=0.04]. 10 – 19 years; SCD 27 (0.60) vs. non-SCD 3 (1.61) [0.37 (0.11 – 1.23); p=0.10]. Over 20 years; SCD 9 (0.53) vs. non-SCD 2 (1.07) [0.49 (0.11 – 0.29) p=0.37].

Limiting analysis of malaria in SCD, the prevalence of malaria parasitaemia, out of 10,488 OPD visits where data of age was available, was as follows: <2 years 2 (0.53%); 2 – 5 yrs 18 (1.36%); 5 – 9 yrs 20 (0.77%); 10-19 yrs 27 (0.60%); over 20 years 9 (0.53%). This is illustrated in figure below (Figure 27), with the 2 – 5 year age group having the highest prevalence of malaria.

Figure 27 Odds ratio of malaria parasitaemia in SCD at OPD clinic stratified by age



Factors associated with malaria at OPD clinic

The factors associated with malaria parasitaemia in SCD patients at OPD clinic are summarised in table below (Table 31). During OPD clinic visits, factors that were significantly associated with parasitaemia on univariable analysis included fever, higher oxygen saturation, spleen and liver enlargement, high WBC and MCV and low Hb.

Table 31 Factors associated with malaria parasitaemia in SCD individuals at OPD clinic

	OPD Clinic			
Clinical features	**No Malaria parasitaemia**	**Malaria parasitaemia**	**OR (95% CI)**	**p**
	n= 10,415 (99.3)	**n = 76 (0.72)**		
Male (n %)	4,916 /10,415 (47.2)	32/76 (42.1)	0.81 (0.52 – 1.28)	0.38
Age (years) GM (SD)	12.5 (8.0)	10.7 (7.9)	0.97 (0.94 - 1.00)	0.06
Physical signs				
Febrile (>37.5^{0}C)	184/10,152 (1.8)	6/74 (8.1)	4.78 (2.05 – 11.15)	<0.001
Peripheral oxygen saturation (SpO_2), (%)	97.2 (3.15)	98.1 (2.8)	1.13 (1.02 – 1.25)	0.02
Hypoxia ($SpO_2 \leq 95\%$)	229/9,952 (2.3)	1/71 (1.4)	0.61 (0.08 – 4.39)	0.62
Spleen palpable on examination	953/9,761 (9.8)	13/67 (19.4)	2.23 (1.21 – 4.09)	0.01
Liver palpable on examination	173/7,206 (2.4)	3/38 (7.9)	3.48 (1.06 – 11.44)	0.04
Laboratory features				
White blood cell count (x109/l) (x10^9/l)	14.7 (6.3)	21.8 (11.7)	1.07 (1.05 – 1.09)	<0.001
High WBC count (x109/l) (> 17.7 x10^9/l)	2,250/9,656 (23.3)	38/69 (55.1)	4.03 (2.50 – 6.49)	<0.001
Haemoglobin (g/dl)	7.5 (1.3)	6.7 (1.4)	0.64 (0.54 – 0.75)	<0.001
Severe anaemia (Hb<5)	305/9.658 (3.16)	8/70 (11.4)	3.96 (1.88 – 8.34)	<0.001
Mean Corpuscular volume (fL)	80.4 (9.3)	85.8 (12.9)	1.06 (1.04 – 1.09)	<0.001
Macrocytosis (MCV > 93fL)	763/9,602 (7.95)	19/70 (27.14)	4.32 (2.54 – 7.35)	<0.001
Reticulocyte count (%)	13.7 (8.8)	15.2 (7.6)	1.01 (0.99 – 1.01)	0.20
Aspartate transaminase (IU/L)	45.9 (23.4)	48.4 (23.4)	1.0 (0.98 – 1.02)	0.66
High AST (AST > 97.2 IU/L)	83/3,586 (2.31)	1/18 (5.56)	2.48 (0.33 – 18.87)	0.38

On multivariable analysis, high WBC was the only feature that remained significantly associated (OR 3.3; 95% CI 1.5-7.18; p<0.01), while fever (3.3; 0.97-11.29; 0.057) showed an association but did not reach the conventional cut-off for statistical significance.

Table 32 Multivariable analysis of factors associated with malaria parasitaemia at OPD clinic in SCD

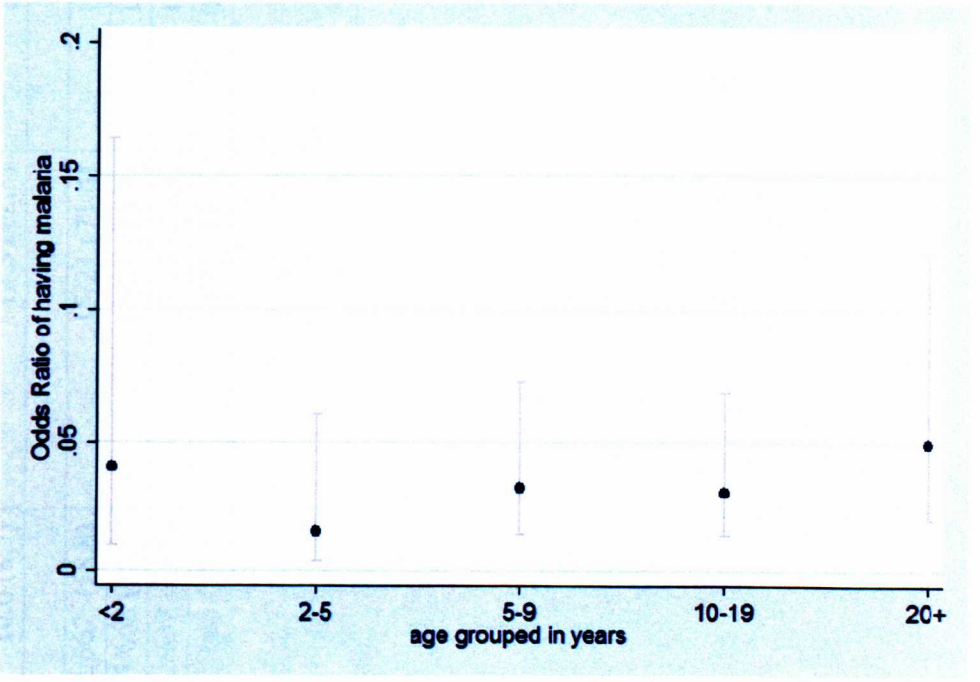
OPD clinic visits		
Variable	**OR (CI)**	**P**
Age	1.00 (0.951 - 1.06)	0.916
Febrile (>37.5^{0}C)	3.30 (0.97 11.29)	0.057
Saturation (SpO2), (%)	1.07 (0.94 1.21)	0.300
Hepatomegaly	2.95 (.85 - 10.18)	0.087
Splenomegaly	1.09 (0.40 - 2.95)	0.867
High WBC (> 17.7 x10^9/l)	3.29 (1.50 7.18)	0.003
Haemoglobin (g/dl) *	0.79 (0.59 - 1.047)	0.101

Malaria during hospitalisation

During the course of the study there were 691 hospital admissions amongst 487 patients with SCD. This is limited to SCD patients who were hospitalised and had malaria data. Malaria parasitaemia was detected in 21 (3.4 %) admissions, only 1 patient testing positive during more than one admission. The overall prevalence of malaria parasitaemia during hospitalisation was 133/2,708 (4.9%) (Figure 25), with the proportion in SCD being 21/691 (3.04%) and non-SCD 112/2,017 (5.6%) [OR (95%CI) 0.53 (0.320.86); p=0.08]. Due to the variation in malaria prevalence with age, comparison was made between SCD and non-SCD below 10 years of age. The proportion with malaria in SCD was 10/380 (2.63) compared to 112/2,017 (5.6) in the non-SCD group [OR (95%CI) 0.46 (0.21 - 0.89); p=0.02].

Limiting analysis to SCD only, the figure below shows the odds ratio of having malaria parasitaemia stratified by age group (Figure 28).

Figure 28 Odds ratio of malaria parasitaemia in SCD patients during hospitalization, stratified by age



Clinical features associated with malaria during hospitalisation

The factors associated with malaria parasitaemia during hospitalisation on univariate analysis are summarised in table below (Table 33). At admission, SCD patients with parasitaemia were older and had lower Hb concentrations and higher MCVs, reticulocyte counts and AST than those without parasitaemia. The risk of death during hospitalization was higher in those with parasitaemia (OR 4.9; 95%CI 1.04 – 23.20); 0.04).

Table 33 Factors associated with malaria parasitaemia in SCD individuals during hospitalisation

Clinical features	No Malaria parasitaemia	Malaria parasitaemia	OR (95% CI)	p
	N=670 (96.9)	N=21 (3.04)		
Male (n %)	308/670 (45.9)	13/21 (41.0)	1.9 (0.78 – 4.67)	0.16
Age (years) GM (SD)	10.6 (8.3)	14.3 (12.3)	1.04 (0.99 – 1.09)	0.05
Symptoms on admission				
Fever	392/667 (58.8)	15/21 (71.4)	1.75 (0.67 – 4.58)	0.25
Pain	505/668 (75.6)	18/21 (85.7)	1.94 (0.56 – 6.66)	0.29
jaundice	102/638 (15.9)	5/19 (26.3)	1.88 (0.66 – 5.32)	0.24
Anaemia	141/666 (21.2)	3/21 (14.3)	0.62 (0.18 – 2.14)	0.45
Physical signs				
Febrile (>37.5^{0}C)	197/661 (29.8)	7/21 (33.3)	1.18 (0.47 – 2.96)	0.73
Peripheral oxygen saturation (SpO_2), (%)	98.1 (2.4)	98.9 (1.9)	1.24 (0.94 – 1.62)	0.12
Hypoxia (SpO_2 ≤95%)	7/681 (1.03)	0	-	-
Spleen palpable on examination	146/648 (22.5)	6/20 (30)	1.47 (0.56 – 3.9	0.44
Liver palpable on examination	80/604 (13.25)	2/19 (10.5)	0.77 (0.17 – 3.39)	0.73
Laboratory features				
White blood cell count (x109/l) (x10^9/l)	23.3 (13.6)	28.8 (19.9)	1.02 (0.99 – 1.05)	0.09
High White blood cell count (> 17.7 x10^9/l)	390/623 (62.6)	11/20 (55.0)	0.73 (0.29 – 1.79)	0.49
Haemoglobin (g/dl)	6.59 (1.8)	5.56 (2.1)	0.74 (0.58 – 0.93)	0.01
Severe anaemia (Hb<5)	114/623 (18.3)	11/21 (52.4)	4.91 (2.04 – 11.84)	0.01
Mean Corpuscular volume (fL)	81.2 (10.1)	87.9 (10.2)	1.07 (1.02 – 1.11)	0.003
Macrocytosis (MCV > 93fL)	58/606 (9.57)	6/21 (28.6)	3.78 (1.41 – 10.12)	0.008
Reticulocyte count (%)	13.5 (6.7)	16.4 (5.4)	1.06 (0.99 – 1.12)	0.05
Aspartate transaminase (IU/L)	65.7 (50.4)	117.57 (83.4)	-	<0.001
High AST (AST > 97.2 IU/L)	85/546 (15.6)	8/19 (42.1)	3.94 (1.54 – 10.09)	0.004
Death during admission	14/668 (2.1)	2/21 (9.5)	4.9 (1.04 – 23.2)	0.04

The factors that were independently associated with parasitaemia were severe anaemia (OR 3.46; 1.279.45; 0.02) and high AST (3.41; 1.23-9.45; 0.02) (Table 34).

Table 34 Multivariable analysis of factors associated with malaria parasitaemia during hospitalisation in SCD

IPD		
Variable	**OR (CI)**	**P**
Age (years)	1.04 (0.99 1.09)	0.088
Severe anaemia (Hb<5g/dl)	3.46 (1.27 - 9.45)	0.016
Macrocytosis (MCV > 93fL)	2.22 (0.73 6.72)	0.158
Reticulocyte count (%)	1.04 (0.97 - 1.10)	0.264
High AST (AST > 97.2 IU/L)	3.41 (1.23 - 9.45)	0.018
Death	0.49 (0.04 5.74)	0.573

Malaria in SCD at OPD clinic versus hospitalisation

The prevalence of parasitaemia was lower in SCD compared to non-SCD individuals during hospitalization (3.0 vs. 5.6; OR 0.46; 0.25-0.94; p=0.01). Table 35 shows the events in SCD patients both at the outpatient clinic and during hospitalization. The prevalence of parasitaemia, malaria and SMA were all higher during hospitalization than at the outpatient clinic. Amongst SCD patients visiting the OPD clinic, subjects were clinically ill (with either fever or severe anaemia associated with parasitaemia) on 14/10,466 (0.13%) of visits. 6 (0.06% of the total visits) had fever alone, 8 (0.08%) had severe anaemia alone and 4 (0.04%) had both fever and severe anaemia. During hospitalization, 11 out of 691 (1.59%) had either clinical malaria or SMA, 7/682 (1.03%) had clinical malaria alone, 11/644 (1.71%) had SMA and 4/691 (0.58%) had both clinical malaria and SMA. The difference between malaria events during OPD clinic and hospitalisation is summarised in table below (Table 35).

Table 35 Malaria in SCD at the OPD clinic and during hospitalisation

	Total	Clinic	Hospitalization	OR (95% CI)	p
Malaria	97/11,182 (0.87)	76/10,491 (0.72)	21/691 (3.04)	4.29 (2.63–7.01)	<0.001
Clinical malaria only[1]	13/10,908 (0.12)	6/10,266 (0.06)	7/682 (1.03)	17.66 (5.92–52.71)	<0.001
Severe malaria anaemia only[2]	19/10,372 (0.18)	8/9,728 (0.08)	11/644 (1.71)	21.11 (8.46–52.67)	<0.001
Clinical malaria and severe malaria anaemia	8/11,157 (0.07)	4/10,466 (0.004)	4/691 (0.58)	15.23 (3.80-61.00)	<0.001
Clinical malaria or severe malaria anaemia	25/11,157 (0.22)	14/10,466 (0.13)	11/691 (1.59)	12.08 (5.46–26.70)	<0.001

OR describe odds ratios for events hospitalized patients versus the outpatient clinic. OPD clinic was used as baseline. [1] Malaria and temperature above 37.5^{0}C; number with percentages in parentheses. [2] Malaria and hemoglobin <5g/dl; number, parentheses percentage

Incidence of malaria in SCD

During the 5 years of study, 3,827 person years of observation (PYO) were recorded. The incidence rate (95% CI) of parasitaemia was 2.53 (2.06–3.09), malaria 0.37 (0.20–0.61) and SMA 0.50 (0.30–0.78) episodes per 100 PYO. Please note that the number of SCD patients included in this analysis is different from that presented in the general results chapter. This is because the numbers are determined by SCD patients who had malaria data and included patients who did not have an enrolment visit.

Table 36 Age - specific incidence of malaria, clinical malaria and severe malarial anaemia in 1,862 SCD patients

	Total	<5 years	>5 years	OR(CI)	p
MPS	1,862 (100.00)	380 (20.4)	1,482 (79.6)	0.57 (0.29 1.08)	0.08
CM[1]	85 (4.6)	11 (2.9)	74 (5.0)	2.96 (1.02 8.57)	0.05
SMA[2]	14 (0.8)	6 (1.6)	8 (0.5)	2.29 (0.89 5.88)	0.08
	19 (1.0)	7 (1.84)	12 (0.81)		

MPS – Malaria; [1]Malaria and temperature above 37.5°C; [2]Malaria and haemoglobin <5g/dl; number, parentheses percentage

Table 36 shows malaria events stratified into two age bands (above and below 5 years), excluding events clustered in individuals. Compared to individuals over 5 years, children under 5 years had a lower prevalence of malaria (OR 0.57; 95%CI 0.29–1.08; p=0.08) but a higher prevalence of clinical malaria (2.96; 1.02–8.57; 0.05) and SMA (2.29; 0.89–5.88; p=0.08), with clinical malaria reaching statistical significance.

DISCUSSION

This is the first detailed report to have focused on the importance of malaria as a cause of morbidity and mortality in patients living with SCD. This cohort of patients living with SCD in Dar-es-Salaam provided an opportunity to study this question in more detail; although chloroquine is recommended for prophylaxis, resistance rates are high and may not provide adequate protection. Ethical concerns about conducting an observational study of SCD subjects, generally considered to be at risk of malaria, in a group who were essentially not on effective chemoprophylaxis, was countered by the fact that the malaria prevalence in Dar-es-Salaam is generally low (0.8%-14%) [423, 424, 426] and interventions such as insecticide treated nets and prompt, effective treatment with ACTs is available. The overall prevalence of malaria parasitaemia at the OPD clinic was 0.78%, which is considerably lower than 3.9% reported in a recent health facility survey conducted in Dar-es-Salaam[423].

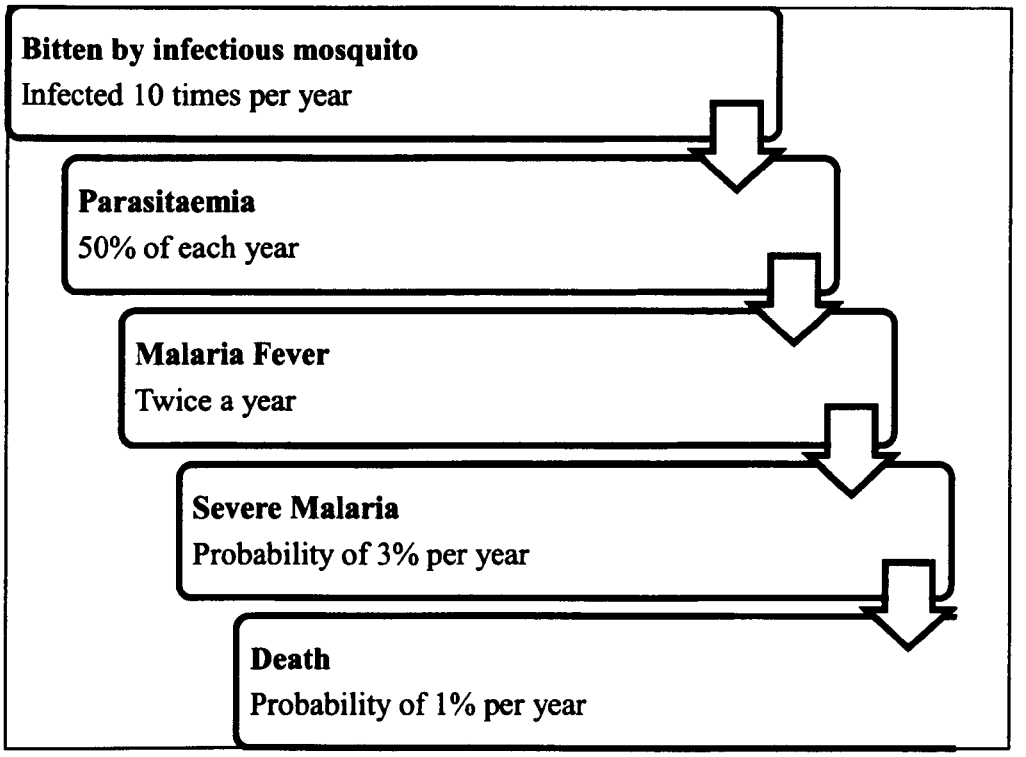
The most striking finding in this study was the lower prevalence of malaria parasitaemia in SCD than in non-SCD patients, both at the outpatient clinic and during hospitalization. A degree of malaria resistance in patients with SCD seems plausible, given that protection is unequivocal in heterozygotes (HbAS)[158, 161]. The prevalence of malaria parasitaemia and parasite densities were significantly higher in AA than in AS subjects (OR 0.21; p=0.02) while the prevalence and density of infections was similar in SS and AS subjects. These findings suggest protection from malaria parasitaemia and raise the question of the view that malaria is a major cause of morbidity and mortality in SCD. This observation raises the question of whether, if HbS protects the heterozygote individuals against malaria, those with SCD, who have even higher levels of HbS, might be even better protected. A dose-dependent effect is certainly seen in HbC, another haemoglobinopathy, commonly found in West Africa,

where both heterozygous (HbAC) and homozygous (HbCC) individuals are protected against malaria[170] , with protection considerably greater in HbCC[171].

The incidence of malaria parasitaemia in this study was 2.53 events per 100PYO. The prevalence of parasitaemia in children under 5 years was lower than in those over 5 yrs, a finding that may be due to protection of younger children by insecticide treated nets (ITN), provided free in Tanzania to children of this age-group[426].

The spectrum of malarial disease includes asymptomatic infection, febrile episodes and severe illness (anaemia and cerebral complications). The likelihood of a child progressing from one stage to the next in a malaria endemic area is as follows – parasitaemia (50% of each year); clinical febrile (twice per year) and severe disease (anaemia or cerebral malaria – 3% per year)[427] . This is illustrated in Figure 29. There was a similar trend in this study, with high prevalence of malaria parasitaemia (4.6%) and fewer episodes of malaria fever (0.8 %). However, in this study more patients had SMA (1.0%) most likely due to pre-existing haemoglobinopathy. As expected, younger children had a significantly higher prevalence of malaria ($p=0.05$) since they have not yet developed immunity.

Figure 29 Progression of malarial disease in a malaria-endemic region



Source[427]

Malaria events occurred more frequently in SCD patients during hospitalization than at the outpatient clinic. This suggests that, although SCD patients are at lower risk of malaria than non-SCD individuals, protection is not complete and malaria is an important factor that needs to be considered during hospitalization. It is acknowledged that this finding may simply reflect the fact that patients with malaria are more likely to have severe illness and to be hospitalized. Parasitaemia was significantly associated with mortality during hospitalization (9.5 vs. 2.2%; OR 4.9; p=0.04) and was independently associated with severe anaemia and high AST. Therefore, the consequences of malaria in SCD appear to be severe during acute illness, as previously suggested by Molineaux and colleagues[16] . These findings highlight the importance of prompt and effective management of malaria in patients with SCD during hospitalization, even though such patients appear to benefit from a degree of protection from the disease.

There are a number of limitations. First, the overall prevalence of malaria parasitaemia at the OPD clinic was only 0.78%, which is lower than the 3.9% reported in a recent survey of health facilities conducted in Dar-es-Salaam[424]. Therefore, it is necessary to validate these results in areas with higher and more intense malaria transmission. The second limitation is that the control (non-SCD) population for hospitalized individuals was collected for only 2 years during the 5 year period of study and it did not include adults. The other limitation is that since this study was conducted in a referral hospital there is a possibility that some events may have been missed as a result of treatment at casualty, other health-care facilities or at home. Furthermore, although there was no change of protocols of referral and management in the hospital, the SCD patients were both part of a cohort study and were encouraged to present early in the event of an intercurrent illness, a fact that may have introduced a bias towards higher rates of presentation in those with SCD.

In summary, through this study, conducted in an area of low but perennial malarial transmission, it was found that malaria was less common in SCD than in non-SCD subjects both at the outpatient clinic and during hospitalization. This raises the question of whether, and under what circumstances, malaria prophylaxis is required in patients with SCD. Further studies are required to establish the role of malaria as a cause of ill-health in such patients under conditions of higher malarial transmission. Nevertheless, it was also found that malaria was significantly associated with severe anaemia and death in hospitalized patients with SCD and therefore it is recommended that malaria should be promptly and effectively diagnosed and treated in such patients. Evidence is needed, ideally in the form of randomized clinical trials, regarding the most appropriate approach to the prevention and treatment of malaria in patients living with SCD in malaria-endemic environments.

Chapter Six

Sickle Cell Disease and Bacterial Infections

INTRODUCTION

In the absence of intervention, bacterial infection is the leading cause of mortality in individuals with SCD in developed countries, and the age group that is most affected is between 1 to 3 years[20, 22, 208, 428] . Interventions with daily oral penicillin and vaccination against pneumococcal infections have successfully reduced mortality in developed countries[18, 117, 429] .

In Africa, these interventions have not been implemented as the evidence to demonstrate a similar role of bacterial infections has been lacking. This has made it difficult for hospitals and governments in developing countries to implement these interventions. Furthermore, published reports have actually questioned the role of prophylaxis against *Streptococcus pneumoniae* (SPN), in Africa[229] . However, there has been increasing evidence of the role of bacterial infections, particularly due to SPN in causing high childhood mortality[270, 272] . Since SCD patients are highly susceptible to SPN infections due to impaired immunity, this makes it even more likely that SPN infections will have a more significant role in SCD mortality. Therefore, there has been an increase in the appeal to implement these interventions[273, 278].

With this background, this study set out to determine the rates and pattern of bacterial infections in SCD who were hospitalised at MNH. During the course of this study, Williams *et al*, started another study in Kilifi, Kenya that set out to address the same objective[230] .

METHODS

Patients

The study population was all SCD patients who were hospitalised at MNH. Every day, active surveillance was done in the medical and paediatric ward to identify any SCD patients who had been hospitalised. For SCD patients who had not yet been recruited into the study at outpatient clinic, confirmation of SCD phenotype was done by HPLC.

Clinical and laboratory data were collected onto standard proformas. The clinical data collected included age, gender, symptoms that caused them to come to the hospitals. Physical examination included pulse and respiratory rate, peripheral oxygen saturation and temperature. Systemic examination was done noting physical signs including pallor, jaundice and the presence of a palpable liver and spleen. The laboratory data included a full blood count, biochemical analysis and malaria diagnostic tests, were collected at admission. Additional investigations were performed as clinically indicated.

Clinical and laboratory methods

All SCD individuals who were hospitalised had a sample taken for blood culture, regardless of their clinical status. The skin at the site for venous sampling was cleaned with 70% ethanol and allowed to dry. Blood was drawn and the needle was changed. The top of the blood culture bottle was also cleaned and allowed to dry.

Blood cultures were initially done in the microbiology laboratory within the hospital. The results were received and documented and the isolates were frozen at -70°C. These isolates were then taken to the microbiology laboratory at the KEMRI Wellcome programme in Kilifi, Kenya, to

confirm the status. The results revealed a significant difference from Muhimbili. It was therefore felt that the data collected during this period were not reliable. Furthermore, there were procedures within the routine hospital laboratory that were suboptimal and were likely to reduce the possibility of growing organisms and particularly fastidious organisms such as SPN, *Neisseria Meningitis* and *Haemophilus Influenza B.* First the use of human blood to make blood agar was unlikely to successfully grow bacteria as human blood had microcidal properties. Horse blood was initially sourced from Dar-salaam, but this was not reliable and there were difficulties in collecting blood. Two sheep were bought and were kept at a farm that belonged to the hospital. But this system also did not work because of the difficulties in bleeding the sheep. Therefore, commercially prepared blood was ordered from overseas and used. The limitation in setting this up in the first place was the high cost in doing this as horse blood had a short shelf life and needed to be brought every 3-4 weeks. Considering that the number of SCD patients who were being hospitalised was small, it was very expensive to set this system up. The second limitation was that the blood culture media were prepared locally and there were no external quality control systems in place. This was considered not to fulfil good laboratory practise (GLP). It was therefore decided that an automated blood culture system would be used. Second, media were commercially bought and prepared by the study laboratory team. Third an external QC system was set up. This was initially with the NetSPEAR programme in Nairobi, Kenya. This is the network for pneumococcal surveillance programme that was established to support the development of capacity in the surveillance of pneumococcal infection in laboratories in hospitals in east African countries.

For these reasons, in 2006, the BACTEC system (BACTEC, Becton Dickinson, Franklin Lakes, NJ, USA) was established in the SCD research laboratory. When the BACTEC system was established, blood culture bottles were weighed before and after inoculation to make sure that

adequate amount of blood were collected. The blood culture bottle was then inserted into the BACTEC system. Positive blood cultures were sub-cultured on standard mediums with the use of routine microbiological techniques. Positive QC systems were done on the blood culture bottles as well as the media for sub-culture.

Statistical methods

A general outline of descriptive statistics, disease estimates, univariable and multivariable analyses is provided in methods chapter. The organisms that were identified were reported with frequency and proportions. The clinical and laboratory features that were associated with bacteraemia were explored using logistic regression with results presented as odds ration and 95% confidence intervals. Clustering of admissions within individuals was accounted for during analysis by random effects modelling. Multivariable logistic regression was used to identify independent associations with bacteraemia for variables that were significantly associated at p-0.05 on univariable analysis.

RESULTS

The results were limited to a description of the rates and pattern of infections for three calendar years, 2006, 2007 and 2008. During this period, a total of 890 blood cultures were done. Of these, 143 (16.1%) were positive. 118 (13.3%) were gram positive.

There were a total of 143 organisms isolated. The following organisms were considered contaminant and were excluded from analysis: Coagulase negative *Staphylococcus Aureus* (88), *Bacillus* species (11) and *Micrococcus* species (1). Therefore, 43 pathogenic organisms were isolated from 890 blood cultures, giving a bacteraemia prevalence of 4.8%.

The pattern of bacteraemia from SCD individuals hospitalised at MNH is shown in table below (Table 37). Of 43 organisms cultured, the commonest organisms was *Staphylococcus Aureus* 12 (27.7 %), *Salmonella* species 11 (25.6%) and *Streptococcus* species 6 (14%). The commonest organisms were *Staphylococcus Aureus (1.3%)* and Non-Typhii *Salmonella* (1.0%). *Streptococcus pneumonia* had a rate of 0.3% which was similar to *E. Coli, Klebsiella* Species, *Pseudomonas* Species, *Salmonella Typhii* and *Proteus* Species.

Table 37 Bacterial isolates from 890 blood cultures of hospitalised SCD patients

Organism identified	Isolates	Percent	Bacteraemia
Staphylococcus Aureus	12	27.9	1.3
Non-Typhii *Salmonella*	9	20.9	1.0
Streptococcus pneumoniae	3	7.0	0.3
Streptococcus species	3	7.0	0.3
Escherichia coli	3	7.0	0.3
Klebsiella species	3	7.0	0.3
Pseudomonas Species	3	7.0	0.3
Salmonella Typhii	2	4.7	0.2
Proteus Species	2	4.7	0.2
Acinetobacter species	1	2.33	0.1
Aeromonas salmonicida	1	2.3	0.1
Morganella morganii	1	2.3	0.1
Total number of isolates	43 (4.8%)		
Negative bacteraemia	847 (95.2%)		
Total number of cultures done	890		

Factors associated with bacteraemia

Univariable analysis

The clinical and laboratory features of those with bacteraemia were compared to those who did not have bacteraemia. The differences in means or proportions were compared taking each hospitalisation as a separate event.

However, on univariable analysis, the clustering within individuals was taken into account by applying a random effects model. Although patients with bacteraemia were more likely to have symptoms of anaemia and worsening of jaundice, this did not reach statistical significance. A palpable liver was the only factor on physical examination that was significantly associated with bacteraemia. Of the laboratory findings, patients with bacteraemia were more likely to have lower haemoglobin, higher level of direct bilirubin and creatinine. Bacteraemia was not associated with an increased risk of death [OR 1.19 (1.51 . 9.38); p=0.87].

Table 38 Clinical and laboratory features in SCD patients' associated with bacteraemia during hospitalisation

	No Bacteraemia	Bacteraemia	OR (95% CI)	p
	847 (952)	43 (4.8)		
Clinical features at hospitalization				
Fever	137/600 (22.8)	9/25 (36.0)	1.90 (0.78-4.63)	0.16
Pain	187/478 (39.1)	11/23 (47.8)	1.43 (0.61-3.36)	0.42
Anaemia	50/600 (8.3)	5/25 (20.0)	2.75 (0.98-7.73)	0.06
Jaundice	39/595 (6.6)	4/25 (16.0)	2.72 (0.90-8.17)	0.08
Examination				
Jaundice	430/630 (68.3)	20/78 (71.4)	1.16 (0.49-2.73)	0.73
Pallor	284/589 (48.2)	12/25 (48.0)	0.99 (0.46-2.13)	0.98
Temperature	36.7 (0.7)	36.9 (1.0)	1.57 (0.94-2.62)	0.08
Febrile	64 (10.2)	4 (16.0)	1.68 (0.58-4.85)	0.33
SpO_2 (%)	97.7 (2.8)	98.4 (2.2)	1.12 (0.93-1.35)	0.22
Hypoxia ($SpO_2 \leq 95\%$)	11 (1.9)	0 (-)	-	-
Palpable spleen	129/588 (21.9)	4/26 (15.4)	0.65 (0.22-1.94)	0.44
Palpable liver	43/551 (7.8)	3/25 (12.0)	1.61 (0.45-5.72)	0.05
Laboratory features				
White blood cell count (x109/l)	18.5 (11.6)	23.0 (15.9)	1.02 (0.99-1.05)	0.06
Haemoglobin (g/dl)	7.1 (1.6)	6.2 (1.8)	0.72 (0.56-0.91)	<0.01
Mean corpuscular volume (fL)	80.6 (9.9)	82.1 (10.8)	1.01 (0.97-1.06)	0.49
Red cell distribution Width (%)	22.7 (4.3)	22.6 (4.1)	0.99 (0.90-1.09)	0.89
Platelet count ($x10^9/l$)	399.2 (206.1)	371.1 (167.7)	0.99 (0.99-1.00)	0.47
Reticulocyte count (%)	13.9 (6.6)	13.9 (7.9)	1.00 (0.93-1.07)	0.98
Bilirubin – total (μmol/L)	67.6 (71.5)	49.6 (34.1)	0.99 (0.98-1.00)	0.23
Bilirubin – direct (μmol/L)	20.8 (26.6)	70.3 (164.3)	1.01 (1.00-1.01)	<0.01
Bilirubin – indirect (μmol/L)	53.2 (41.3)	30.4 (42.8)	0.99 (0.98-1.00)	0.18
Aspartate transaminase (IU/L)	56.1 (41.3)	56.2 (29.5)	1.00 (0.99-1.01)	0.98
Alkaline phosphatase (IU/L)	265.7 (144.3)	225.9 (119.6)	0.99 (0.99-1.00)	0.28
Creatinine (μmol/L)	42.4 (26.9)	58.4 (51.8)	1.01 (1.00-1.02)	0.05
Lactate dehydrogenase (IU/L)	1068.2 (610.9)	1141.4 (471.1)	1.00 (0.99-1.00)	0.55
Haemoglobin F (%)	6.8 (5.2)	7.9 (6.4)	1.04 (0.96-1.12)	0.33
Death	19/606 (3.1)	1/26 (3.7)	1.19 (1.51-9.38)	0.87

Multivariable analysis

During multivariable analysis, two models were built. In the first model, direct bilirubin was not included as data were not available for many patients. In this model, none of the factors that were significantly associated during univariable analysis were independently associated with increased likelihood of bacteraemia during hospitalisation.

Table 39 Multivariable analysis of factors associated with bacteraemia in hospitalised SCD patients n = 308; admissions = 530 (excluding bilirubin)

	OR	p
History of anaemia	1.99(0.55-7.13)	0.29
History of jaundice	2.25(0.55-9.25)	0.26
Temperature (°C)	0.92(0.49-1.70)	0.78
Haemoglobin (/dL)	0.77(0.57-1.04)	0.09
White blood cell count ($x10^9$/l)	1.00(0.97-1.04)	0.94

In the second model, bilirubin was included in the model resulting in the analysis of 245 events in 208 individuals. Direct bilirubin was independently associated with increased likelihood of bacteraemia during hospitalisation.

Table 40 Multivariable analysis of factors associated with bacteraemia in hospitalised SCD patients n=208; admissions = 245 (including bilirubin)

	OR	P
History of anaemia	3.15(0.75-13.27)	0.12
History of jaundice	1.60(0.29-8.73)	0.59
Temperature (°C)	0.95(0.45-2.00)	0.89
Haemoglobin (g/dL)	0.82(0.57-1.17)	0.27
White blood cell count ($x10^9$/l)	0.99(0.95-1.04)	0.81
Bilirubin direct – (μmol/L)	1.01(1.00-1.02)	0.05

DISCUSSION

This is the largest study of episodes of bacteraemia in SCD patients in Africa. The prevalence and pattern of bacterial infections in SCD hospitalised at MNH during a three year period is reported. During this period, data were analysed from 890 hospitalisation events from 667 SCD patients. 143 (16.1%) of the 890 blood cultures grew organisms. 100 of these organisms (69.9%) grew coagulase negative staphylococci (88), bacillus (11) and micrococcus (1). These were considered contaminants and were classified as being negative for infection. 43 out of 890 hospitalisation events in SCD patients had a positive infection, giving a prevalence of bacteraemia of 4.8%.

A study conducted in Kenya reported a prevalence of bacteraemia of 1,094 from 16,750 (6.6%) patients admitted to Kilifi district hospital between 1998 and 2002 [270]. The age range was from 1 day to 13 years. In patients who were above 1 year of age, the prevalence was 5.4%. A retrospective study at the same site analysed 38,441 blood cultures from admissions between 1998 and 2008 of children less than 14 years. 2,157 (6%) cultures grew important bacterial pathogens[230] . Despite the small number of patients with SS SCD (108 (6%) of 1,749), the robust framework in Kilifi was able to clearly demonstrate the increased risk of bacteraemia in SCD between 0 to 13 years. This was reported as 26.3 (95%CI 14.5 - 47.6). Within SCD patients, the highest incidence reported at 5.34 (95%CI 4.38 - 6.44) was in the 12-23 month age group. Furthermore, the study was also able to unequivocally illustrate the role of *S. pneumoniae* infection in patients with SCD. The strongest association was with *S. pneumoniae,* non-Typhii *Salmonella* and *H. Influenza* type b, with reported incidence of 2.18, 1.58 and 0.64 per 100 person years of observation respectively.

The prevalence of bacteraemia in SCD patients in this study was 4.8% compared to 6.6% in Kenya[230], 6.1 % in Jamaica[198] and 5.2 in the USA[235] . Although these rates could be considered comparable, three possible factors can account for the marginally lower rate in Dar-es-Salaam. First, the age structure of the SCD population in Dar-es-Salaam was older, with a median age of 10.5 (8.5) years. Although studies in Kenya, Jamaica and USA did not report a median age, the study population from other studies was limited to children. Furthermore, although only 30% of the study population were less than 5 years, 43% of the positive isolates were in children in this age group. Therefore, these results were probably an underestimate of the true bacteraemia rates as there was no information on the younger population where the incidence of bacteraemia is likely to be highest. The second factor may be that this patient group may represent the individuals with mild phenotype who have less severe disease (as the ones with severe disease have already died), and therefore less susceptible to bacterial infections. The third possible factor that may have contributed to the low prevalence of bacteraemia in Dar is that this study was conducted in a referral hospital. Therefore, there are differences in access to care with patient's most likely receiving care before being hospitalised at MNH. Prior treatment with antibiotics would therefore reduce the prevalence of bacteraemia.

The pattern of bacteraemia from hospitalised SCD individuals found that the commonest organisms isolated were .*S Aureus*, non-Typhii *Salmonella* species and *S. Pneumoniae*. *Staphylococcus Aureus*, non-Typhii *Salmonella* and *Mycobacterium tuberculosis* were the top three causes of bacteraemia in patients who were older than 15 years hospitalised at MNH[430]. *Salmonella* species has been reported as one of the top three causes of bacteraemia in both hospitalised[230 270] as well patients attending outpatient clinic in Kenya[431].

Table 41 Bacteraemia in SCD patients in Africa from selected studies

Site	Period	Age	Sample size	Indication	Prevalence	Isolates	Author
Tanzania	2006 – 2008	5 months - 43 yrs	890	Hospitalisation	4.8%	SAU 27.9% NTS 20.9%	This study
Kenya	1998 – 2008	<14years	108	Hospitalisation	6.6%	SPN 41% NTS 18%	Williams[230]
Uganda	Oct 01 – Jan 2002	<15 years	167	Temperature ≥38°C	28.4%	SAU 60% HIB 19%	Kizito[229]
Benin	1995 – 1996	6m to 138 m	60	Fever	13.3%	SAL 37.5% SAU 37.5%	Rahimy[112]
Nigeria	1989	4mo-13yrs	60	Crisis	32%	KLE 31% SAB 31%	Akinyanju[432]
Nigeria	1987	6m – 16 yrs	162	Temperature ≥38°C	33%	SAL 26% KLE 26%	Okuonghae[228]
Nigeria	Not specified	11 – 36yrs	269	Temperature ≥ 38°C	36%	KLE 22% SAU 14%	Akuse[265]
Nigeria	June to Nov 1980	<15 yrs	57	Crisis	35%	SPN 40% SAL 35%	Maharajan[179]
Nigeria			269	Temperature	36.1%	KLE	Akenova[266]

SAU Staphylococcus Aureus; NS Non-Typhii Salmonella; SPN Streptococcus pneumoniae; HIB Haemophilus influenza; KLE *Klebsiella;* SEP *Streptococcus epidermidis;* SAL Salmonella; ENT enterobacter; SAB Staphylococcus albus; ECOL Escherichia Coli: SPYo Staphylococcus pyogenes

The age of the SCD patients greatly influences the type of bacteraemia. In a study in the USA[235], *S. Pneumoniae* accounted for 66% infections under 6 years whereas Gram negative rods accounted for over 50% infections above 6 years. The commonest Gram negative rod was salmonella species. This has been reported in other SCD populations where *S. pneumoniae* is more common in the younger age group[209]. Norris *et al* reported that the mean age was 3.5 years in a group of SCD patients where *S. pneumoniae* accounted for 42% of total pathogens isolated whereas the mean age was 8.1 years in the group where the Gram negative rods, mostly Salmonella species, were the commonest organisms accounting for 28% of total isolates[433]. These findings are in keeping with the evidence of the epidemiology of pneumococcal infection. It has a higher burden before 5 years. It is therefore not surprising that in Africa, where the SCD population that is seen in hospitals is usually older, the prevalence of bacteraemia due to *S pneumoniae* is low. This may be because children with SCD and *S. Pneumoniae* infections die before presenting to hospital.

The other factor that could explain the low prevalence of *S Pneumoniae* bacteraemia in this population was prior treatment with antibiotics. During the course of the study a methodological issues was identified as a limitation to the data that were being collected. Since MNH was a tertiary health facility and in an urban setting, SCD patients were likely to seek health care at a health facility near their home or take medication that they had bought over the counter. This would either be an anti-malarial or antibiotic. The antimalarial that were available were chloroquine, Sulphadoxine - pyremethamine (SP) or Artemether Lumefantrine (ALu). SP and ALu both have anti-bacterial activity. Furthermore, when patients were hospitalised, they received anti-malarial medication within 24 hours. In order to address this limitation, blood cultures were taken before patients received antibiotics in the

hospital. However, it was difficult to influence the use of antibiotics before SCD patients were hospitalised. It was felt that since patients had easier access to peripheral clinics, discouraging them from receiving treatment that was prescribed would be unethical.

The association been salmonella osteomyelitis with SCD was reported almost 50 years ago[207] and mechanisms involve deficiency of opsonising factors[222] . Although over 70% of haematogenous osteomyelitis is due to salmonella[434] and over 77% of salmonella bacteraemia is associated with osteomyelitis[235], salmonella bacteraemia is not frequently considered as a cause of infection in SCD patients[435] . However, there is evidence that salmonella infection is common in hospitalised SCD patients suspected to be infected, with[210, 267, 268] or without involvement of bone [198, 433, 436, 437].

In summary, this study has reported high prevalence of bacterial infection in SCD patients who are hospitalised at MNH. The commonest organisms were *S. Aureus*, non-Typhii *Salmonella* and *S. pneumoniae*. Guidelines for prompt management of infection in patients who are hospitalised are recommended. This study has limited information on bacteraemia in SCD children who are less than 2 years old and highlights the need of a birth cohort to identify natural history of infection. There were only 3 episodes of bacterial infection due to *S. pneumoniae* and the number of children under 5 years was small, making it difficult to make conclusive recommendations from this study. However, during the course of this thesis, published reports have provided this evidence. It is now recommended that all SCD children below 5 years should receive prophylaxis against infection, particularly due to *S. pneumoniae*. The questions that need to be addressed are the appropriate agent that should be used and the mechanism of delivering this agent to ensure compliance and access.

Chapter Seven

Sickle Cell Disease and Stroke

INTRODUCTION

Stroke and transient ischaemic attacks (TIA) are the most common neurological complications of SCD, occurring in 11% of patients with SCD by the age of 20 years [245 438]. Approximately 25% patients with SCD will have had a stroke by the age of 45 years[83]. Most vascular events occur between the ages of 5 and 10 years and are thought to be cerebral infarction resulting from cerebral blood vessel occlusion. High-risk patients can be predicted by raised cerebral blood flow velocity (CBFv) in the middle cerebral artery (MCA) as measured by Transcranial Doppler Ultrasonography (TCD)[319] . Interventional strategies for primary and secondary prevention of stroke include blood transfusion and hydroxyurea [120 129 361]. All this is information from studies conducted in the USA and Europe with limited information from Africa.

Prior to the work reported in this thesis, I conducted a pilot study in Kilifi where I looked at the rates and risk factors of high CBFv in SCA population in Kenya. In a cross-sectional study of 105 Kenyan children, the mean CBFv was 120±34.9 cm/sec. However, we found that only 3 had conditional CBFv (170-199 cm/sec), but none had abnormal CBFv>200 cm/sec. After adjustment for age and haematocrit, CBFv≥150 cm/sec was predicted by peripheral oxygen saturation (SpO2) ≤95% and history of fever. Four years later, 10 children were lost to follow-up, none had had neurological events and 11/95 (12%) had died, predicted by history of fever but not low Sp O2[392]

In Tanzania, there has been no description of the magnitude of neurological events in patients with SCD, but there have been a few case reports[439] and anecdotal evidence that suggest that with an increase in the prevalence of SCD surviving past 5 years of age, CVA may be a common cause of morbidity and mortality, as found elsewhere in the world. Therefore, the third aim of my PhD was to investigate the relationship between cerebral blood flow velocities (CBFv) and cerebrovascular accidents (CVA) in SCD patients in East Africa. Initially, I measured the CBFv in a proportion of SCD patients in order to assess the range of CBFv in this population in Tanzania and to determine the factors at enrolment that were associated with abnormal CBFv. I then determined the prevalence of neurological events in the cohort of SCD patients. During the period of follow-up, I evaluated the incidence of stroke and tried to establish an association with CBFv in order to determine a cut-off point for CBFv that would be used to identify high-risk patients who would benefit from intervention.

METHODS

Procedures

Description of CBFv by TCD measurement

Between November 2004 and June 2005, TCD examination was undertaken in a cross-section of patients between the age of 2 to 16 years of age who were attending the outpatient clinic and had consented to participate in the study. Patients were recruited consecutively at the OPD clinic and were given an appointment to return for a TCD examination. All patients had TCD examination done which was recorded. For those patients who had an inadequate examination, a repeat TCD was performed.

Prevalence of neurological events

All patients recruited into the study had screening questions to determine whether there were any neurological deficits that were present or had occurred in the past. For those patients who had a CNS event during the course of the study, a thorough neurological assessment with detailed clinical history and neurological examination. Neurological events included stroke, seizures, transient ischaemic attacks, lower limb weakness, cranial nerve deficit and cognitive impairment.

Incidence of neurological events

The incidence of neurological events was determined at follow up clinic in the outpatient department (OPD) as well as during admission. At the clinic, all patents coming for follow-up visit would be specifically asked for a history of any neurological deficits that occurred at home, required care at any health facility. During admission, any evidence of neurological deficit was assessed and documented.

Imaging

High resolution computerised tomography (CT) was performed on patients who were either admitted or had attended outpatient clinic and suspected to have a stroke. The CT scan was performed in the radiology department at Muhimbili National Hospital. The images were scanned and saved in JPEG format. The results of this will not be included in this thesis.

Statistical methods

Description of range of CBFv in SCD population

CBFv was measured following the STOP protocol[440] using the Companion II (Nicolet, Warwick, UK) machine. The highest *CBFv* [time-averaged maximal mean velocity in the distal internal carotid artery (ICA) and MCA on either side] was determined. We classified the higher (right or left) CBFv as low (<50 cm/sec), normal (50-169 cm/sec), conditional (170 - 199) or high (>200 cm/sec) based on Adams' (1990) criteria. The lower limit of normal was based on CBFv data from TCD measurements in Kenya[441] .

I documented the factors at enrolment visit that have been previously reported to be associated with increased risk of abnormal or high CBFv including clinical features such as history of specific clinical events e.g. painful episodes, admissions as well as laboratory features previously suggested to be predictors of high CBFv, such as haemoglobin[392 442], haemoglobin oxygen saturation, MCV, WBC, HbF levels and markers of haemolysis such as lactate dehydrogenase levels[398, 443, 444]. Effect modifier variables included age, sex, past history of CNS event e.g. seizures, stroke.

Continuous variables were analysed using two-sided t-tests. Categorical variables were analysed by χ-squared test. Logistic regression was used to explore associations between high *CBFv* (>200 cm/sec) and clinical and laboratory variables, presenting the results as odds ratio (OR) with 95% confidence interval. A p-value <0.05 was considered statistically significant. Multivariable analysis included all variables with univariable significance of <0.05. Using backward elimination, the final model included all variables significant at the 0.05 level. Binary logistic regression was also used to assess factors associated with abnormal CBFv.

Determining the prevalence of CNS events

The prevalence of neurological event was determined at enrolment where gross neurological impairments such as hemiplegia, dense cognitive impairment or neurological deficit was recorded. During the course of the study, any neurological event was identified and documented. The results presented include the prevalence of neurological events identified throughout the study period.

The prevalence of neurological events was described. This included stroke, transient ischaemic attack, loss of consciousness, seizures and any other focal or general neurological deficit. Explanatory variables included clinical and laboratory features with specific focus on the following variables as confounders/effect modifiers: age, sex, Hb, MCV, WBC, oxygen saturation, LDH, AST, unconjugated bilirubin, reticulocyte percentage.

Determining the incidence of CNS events

This analysis was done to determine the incidence of neurological events during the study period. Therefore the outcome was any neurological event captured during the study period and if possible confirmed by CT scan. The explanatory variables were clinical and laboratory features including TCD reading, which were explored to look for any association. Confounders/effect modifiers: age, sex, Hb, MCV, WBC count, oxygen saturation. Some individuals will have more than one event and for some patients, the exact time of event may not be known.

Inclusion criteria;

1) Enrolled into the main SCD study population. This is defined previously but requires an entry visit at outpatient clinic.
2) Laboratory confirmation of SCD

Exclusion criteria;

1) SCD patients who were identified at hospitalisation, but had not returned to outpatient clinic for enrolment into the cohort.
2) No laboratory confirmation of SCD

Data were analyzed using STATAv10 (StataCorp, College Station, TX). The overall incidence of stroke was estimated for the cohort. This was calculated from the ratio of number of strokes that occurred during the course of the study divided by the number of person years of observation (PYO), expressed as stroke rate per 100 PYO.

In order to determine the factors at enrolment associated with stroke, I grouped the individuals in the cohort into two groups; those who had a stroke documented at MNH and those who had never had a stroke during the study period. Data were summarized as medians or proportions. Logistic regression analysis was used to analyze factors at enrolment associated with stroke, with results presented as odds ratios (ORs) with 95% confidence intervals (95%CIs). Multivariable logistic regression was used to identify independent associations with stroke, using variables that had significant association ($p<0.05$) on univariable analysis.

In order to determine the association between CBFv and TCD, the analysis was limited to the sub-group who had a TCD examination done. Note that the TCD sub study was conducted from November 2004 to June 2005. During this period, I conducted detailed neurological examination on all patients who came for a TCD examination. Individuals who had a previous stroke were not included in the analysis.

The overall and age-specific incidence of stroke was estimated for this sub-group. This was calculated from the ratio of number of strokes divided by the number of person years of observation (PYO), expressed as stroke rate per 100 PYO stratified by age. In order to explore the association between CBFv and stroke, I explored this relationship using different categories of CBFv. Data were summarised as means and proportions. Logistic regression was done to explore this relationship with results presented as OR (95%CI).

The categories for CBFv were as follows:

1) Definition used in the trials in the USA (STOP trial). STOP definition[120] uses the following cut-off points: >200cms/sec abnormal, 170-199cms/sec conditional, 50 - 170cms/sec normal.
2) I used the interquartile range of the CBFv in this study to group CBFv (in cms/sec) into low (0 - 103), medium (104 - 156) and high (157 and above)
3) I used the 150cms/sec cut off point used from Kilifi data[392] to group CBFv into low (below 150cms/sec) and high (above 150cms/sec).
4) I used the 150cms/sec cut off point used from Kilifi data[392] to group CBFv into low and high. In this instance, I excluded all individuals with CBFv less than 70 cms/sec.

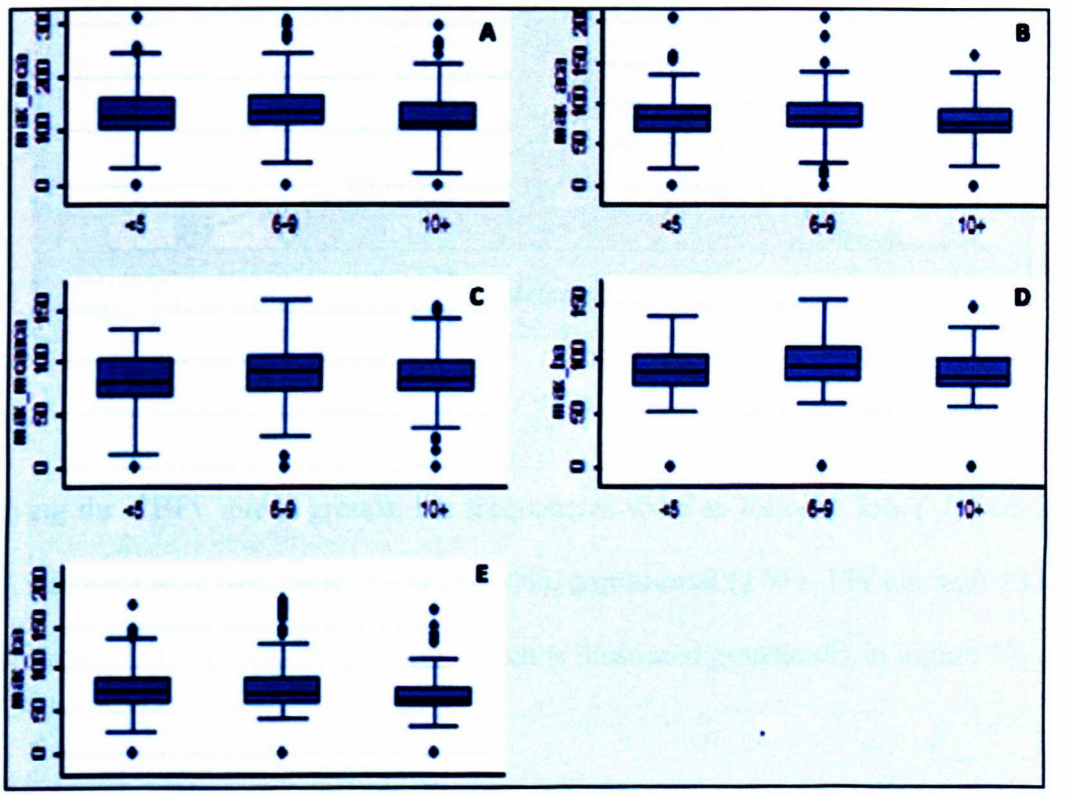
The rationale for this is that these individuals either have low velocity due to stenosis and Moya Moya syndrome or the examination was not valid

RESULTS

Description of cerebrovascular flow velocity (CBFv)

A total of 6,416 cerebral blood flow velocities (CBF_v) were measured in 401 SCA patients. 6,096 of these measurements were successful and we selected 1,934 CBFv representing the maximum velocity measured in each vessel, irrespective of side. The velocities for the five vessels are shown (Figure 30) shows the maximum CBFv of the five blood vessels which were measured, stratified into 3 age groups as well as summary of the velocities for each vessel.

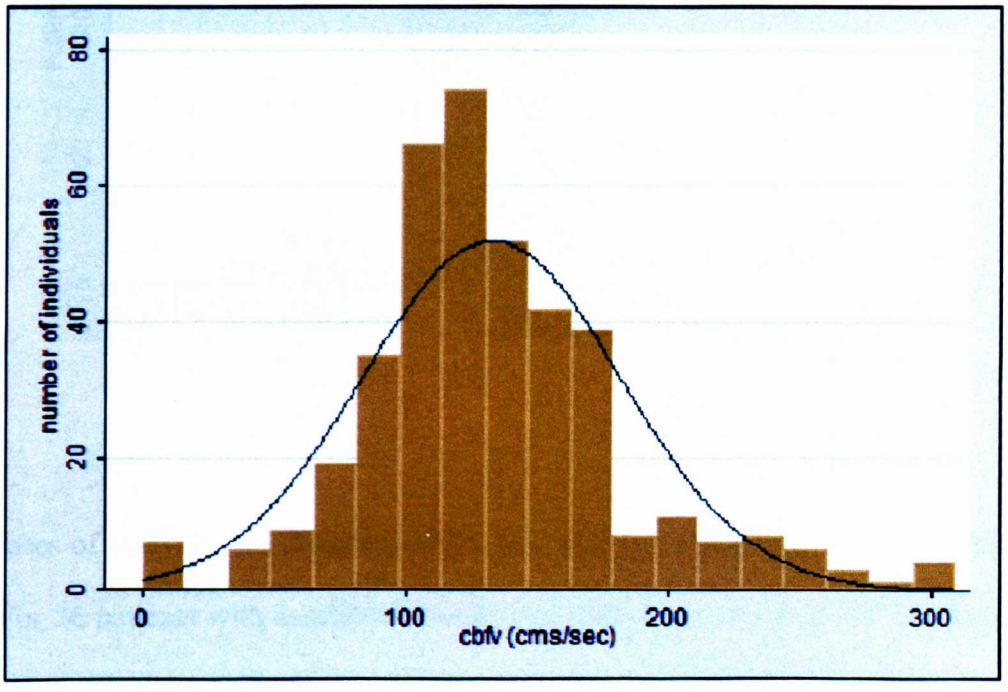
Figure 30 Age stratified maximum CBFv of specified blood vessels or sites



A. Middle cerebral artery (MCA), B. Anterior cerebral artery (ACA). C. Bifurcation of MCA and ACA. D. Basilar artery E. Internal carotid artery. The y-axis is cerebral blood flow velocity. The x-axis is age in years in 3 groups.

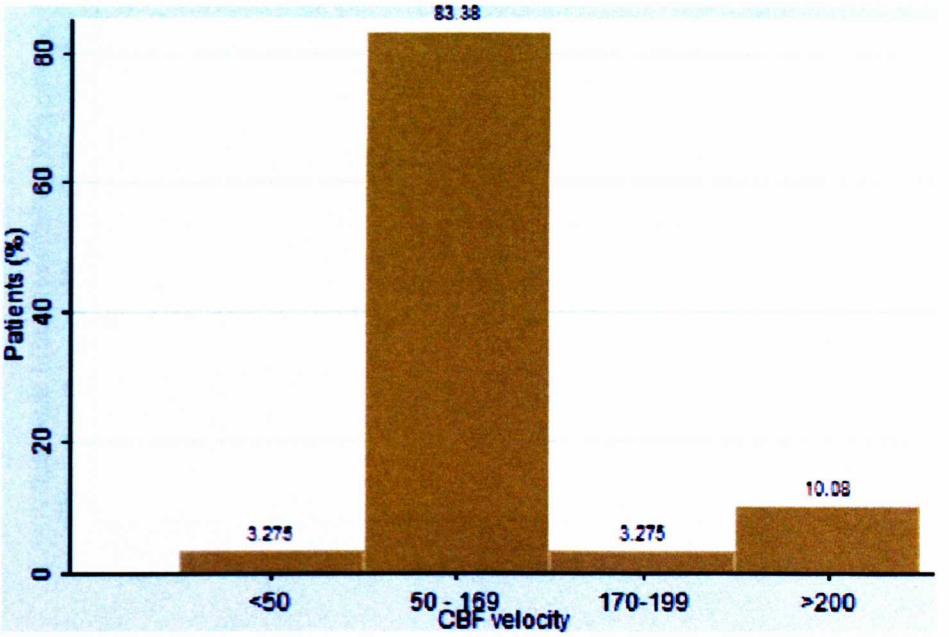
Following this, the highest velocity at the MCA or bifurcation of MCA and ACA was selected for each person. The spectrum of maximum CBFv is shown in Figure 31 with a mean velocity of 132.2 (49.4) and a range between 0 and 309.

Figure 31 Histogram of maximum CBFv in 397 SCD patients



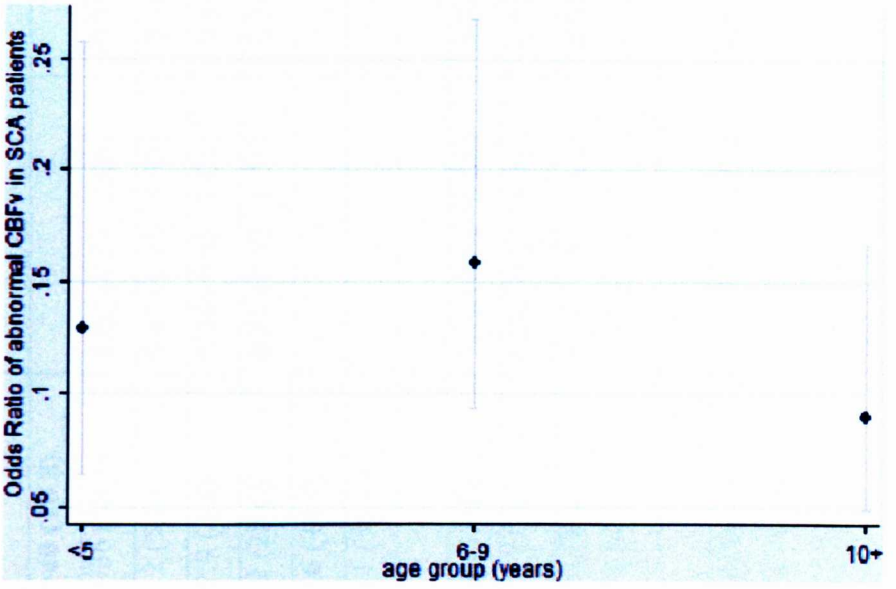
Classifying the CBFv into 4 groups, the frequencies were as follows: low (<50 cm/sec) 13 (3.3%), normal (50 – 169 cm/sec) 331 (83.4%), conditional (170 – 199 cm/sec) 13 (3.3%) and abnormal (>200 cm/sec) 40 (10.1%), which is illustrated graphically in Figure 32.

Figure 32 Distribution of CBFv in SCD patients



For purposes of analyses, we classified CBFv into normal or abnormal using 200cms/sec as a cut off. For 36 patients with available information about age, 16 out of 117children (13.7%) were between 6-9 years with abnormal CBFv compared to 9 out of 79 (11.4%) under 5 years and 11 of 133 (8.3%) above 10 years. The difference in odds ratio is shown in Figure 33. This is not statistically significant (p=0.38)

Figure 33 Odds ratio of having abnormal CBV in 3 age groups



Associations with abnormal CBFv

Univariable analysis

To explore the association of clinical and laboratory features with abnormal CBFv, univariable analysis was done and the results are shown in Table 42. Individuals with abnormal CBFv complained more often of symptoms of anaemia and were reported to have received more frequent blood transfusions within 12 months of the baseline visit. On examination, these patients also had higher levels of peripheral oxygen saturation and for laboratory factors, direct bilirubin showed a significant association with abnormal CBFv.

Table 42 Clinical characteristics in SCD patients with normal and abnormal CBFv

	Total	Normal	Abnormal	OR (95% CI)	P
	372	**n=332 (89.3)**	**n=40 (10.8)**		
Male (n %)	330	170/294 (57.8)	21/36 (58.3)	1.02 (0.51 2.06)	0.95
Death	330	20 (6.8)	2 (5.6)	0.81 (0.18 3.59)	0.78
Age (years) Mean (SD)	329	8.6 (4.1)	7.9 (3.7)	0.96 (0.88 1.05)	0.39
<5	79 (24.0)	70 (23.9)	9 (25.0)	0.82 (0.53 1.27)	0.38
6-9	117 (35.6)	101 (34.5)	16 (44.4)		
10-	133 (40.4)	122 (41.6)	11 (30.6)		
Symptoms on day of TCD					
Fever	315	17 (6.1)	4 (11.1)	1.93 (0.61 6.08)	0.26
Pain	315	20 (7.2)	2 (5.6)	0.76 (0.17 3.4)	0.72
Anaemia	308	3 (1.1)	3 (8.8)	8.74 (1.69 45.19)	0.01
Jaundice	308	3 (1.1)	1 (2.9)	2.73 (0.28 27.08)	0.39
History in preceding 12 months					
Blood transfusion	329	122 (41.6)	7 (19.4)	0.34 (0.14 0.79)	0.01
Pain	329	172 (58.7)	18 (50.0)	0.70 (0.35 1.41)	0.32
Fever	329	145 (49.5)	14 (38.9)	0.65 (0.32 1.32)	0.23
Physical signs					
Peripheral oxygen saturation (SpO_2) (%)	71	97.1 (3.5)	99.3 (0.9)	1.89 (1.00 3.57)	0.05

Table 43 Laboratory characteristics in SCD patients with normal and abnormal CBFv

	Total	Normal	Abnormal	OR (95% CI)	P value
White blood cell count ($x10^9/l$)	310	15.5 (4.8)	16.4 (5.2)	1.04 (0.97 – 1.11)	0.28
High white blood cell count (x109/l)	310	132 (48)	21 (60)	1.63 (0.79 – 3.33)	0.18
Haemoglobin (g/dl)	310	7.6 (1.2)	7.6 (1.1)	1.03 (0.77 – 1.39)	0.83
Severe anaemia (Hb<5g/dl)	310	8 (2.9)	0 (-)		0.31*
Mean corpuscular volume (fL)	308	77.6 (8.2)	76.1 (8.1)	0.98 (0.94 – 1.02)	0.32
Red cell distribution width (%)	306	23.9 (4.6)	23.9 (4.4)	0.99 (0.92 – 1.08)	0.91
Reticulocyte count (%)	87	6.7 (4.7)	7.7 (1.9)	1.04 (0.91 – 1.19)	0.53
Creatinine (µmol/L)	323	40.8 (15.8)	47.9 (38.0)	1.01 (0.99 – 1.02)	0.07
Aspartate transaminase (IU/L)	322	56.4 (24.6)	51.9 (20.4)	0.99 (0.98 – 1.01)	0.30
Alkaline phosphatase (IU/L)	320	264.8 (103.7)	275.9 (109.2)	1.00 (0.99 – 1.00)	0.55
Total bilirubin (µmol/L)	319	82.2 (55.4)	83.9 (67.7)	1.00 (0.99 – 1.01)	0.86
Direct bilirubin (µmol/L)	309	21.7 (15.9)	29.9 (38.4)	1.01 (1.00 – 1.02)	0.04
Indirect bilirubin (µmol/L)	309	62.3 (54.6)	54.3 (66.9)	0.99 (0.99 – 1.00)	0.43
Lactate dehydrogenase (IU/L)	62	1,229 (409)	956 (339)	0.99 (0.99 – 1.00)	0.07
Haemoglobin F (%)	322	5.3 (3.8)	5.3 (3.5)	1.00 (0.92 – 1.10)	0.93
High haemoglobin F (> 8.5%)	322	47 (16.4)	7(20.0)	1.27 (0.53 – 3.09)	0.59
Haemoglobin A2 (%)	322	4.8 (1.9)	5.1 (2.1)	1.06 (0.92 – 1.22)	0.42

All patients with severe anaemia had normal TCD.

Multivariable analysis

Two separate models were built for multivariable analysis of factors associated with abnormal CBFv. In the first model, we did not include peripheral oxygen saturation and the analysis included 291 individuals. The results are shown in Table 44. In this model, all the three factors, symptoms of anaemia, history of blood transfusion and direct bilirubin were independently associated with abnormal CBFv.

Table 44 Multivariable analysis of factors associated with abnormal CBFv (n=291); excluding saturation

	OR (95% CI)	p
Symptoms of anaemia	8.55 (1.58 – 46.4)	0.01
History of blood transfusion	0.36 (0.15 – 0.88)	0.03
Direct bilirubin (μmol/L)	1.02 (1.00 – 1.03)	0.03

For the second model, this was restricted to those individuals who had peripheral oxygen saturation at time of TCD and included 64 individuals. During analysis, the variable reporting symptoms of anaemia was dropped from the model because of collinearity. The results are shown in Table 45 and show the association of history of blood transfusion, direct Bilirubin and peripheral oxygen saturation. Higher peripheral oxygen saturation was independently associated with abnormal CBFv (p=0.04).

Table 45 Multivariable analysis of factors associated with abnormal CBFv including saturation (n=64)

	OR (95% CI)	p
History of blood transfusion	0.51 (0.09 – 2.72)	0.43
Direct bilirubin (μmol/L)	1.03 (0.98 – 1.07)	0.26
Peripheral oxygen saturation (%)	2.04 (1.04 – 4.00)	0.04

Prevalence of neurological deficits

Data were available from 435 CNS forms from 1,573 patients. Please note that neurological events forms were only completed during TCD examination or when there was clinical suspicion of neurological event. There were 77 recorded neurological deficits with 47 patients reporting history of seizures (10.3%) and 16 episodes of stroke (3.8%). This is shown in Table 46.

Table 46 Neurological deficits in 435 SCD patients

Neurological event	Total	Number	Percent
Stroke	426	16	3.8
Seizures	434	47	10.8
TIA	423	9	2.1
Weakness	365	14	3.8
Cranial nerve deficit	428	9	2.1
Cognitive impairment	425	10	2.4
Total	435	77	17.7

The numbers vary depending on data available.

The table below (Table 47) shows the prevalence of neurological deficits in 1,573 SCD patients, with stroke occurring in 16 (1.02) patients.

Table 47 Prevalence of neurological deficits in 1,573 SCD patients

	Total	N	Percent
Stroke	1,573	16	1.02
Transient ischemic attack	1,573	9	0.57
Seizures	1,573	47	2.99
Weakness	1,573	14	0.89
Cranial nerve deficits	1,573	9	0.57
Cognitive impairment	1,573	10	0.64
	1573	77	4.9

Incidence of stroke

During the course of the study there were 16 episodes of stroke from 1,573 SCD patients (1.02%) with available neurological data. 2 of the patients had repeated episodes. The incidence of stroke was 0.3 (0.18 . 0.52) per 100 PYO for patients who were not lost to follow-up (n=1,516).

Factors at baseline associated with stroke

Univariable analysis

The risk factors for stroke during the course of the study were explored by looking at the association of clinical and demographic features at baseline with stroke. A reported history of painful (p=0.03) or febrile episode (p=0.04) was more common amongst the patients who had a stroke.

With regards to laboratory values, patients with stroke had higher MCV (83.6 vs. 77.2; p=0.01) and reticulocyte count (12.7 vs. 6.9; p=0.01) but a lower level of HbS (83.5 vs. 87.5; p=0.02) compared to the patients who did not have a stroke.

Table 48 Factors at enrolment visit associated with stroke during the study

	No Stroke	Stroke	OR	p
	412 (96.3)	16 (3.7)		
Male	222 (55.8)	6 (46.2)	0.68 (0.22 - 2.06)	0.49
Age in years Mean (SD)	8.5 (3.8)	7.8 (4.6)	0.95 (0.72 - 1.24)	0.69
<5	76 (22.7)	1(25)	1.1 (0.31 - 4.01)	0.88
5-10	120 (35.8)	1 (25)		
>10	139 (41.5)	2 (50)		
History of hospitalisation	125 (44.8)	7 (70)	2.87 (0.78 - 11.3)	0.13
History of blood transfusion		3 (23.1)	0.47 (0.13 - 1.73)	0.26
Pain	220 (55.4)	3 (23.1)	0.24 (0.07 - 0.89)	0.03
Fever	191 (48.1)	2 (15.4)	0.19 (0.43 - 0.89)	0.04
Laboratory features				
White blood cell count ($x10^9$/l)	15.9 (5.1)	15.8 (4.4)	0.99 (0.89 - 1.11)	0.92
Haemoglobin (g/dl)	7.5 (1.2)	7.9 (1.1)	1.29 (0.79 - 2.11)	0.30
Red blood cell count ($x10^{12}$)	2.9 (0.8)	2.8 (0.5)	0.70 (0.28 - 1.75)	0.45
Mean corpuscular volume (fL)	77.2 (8.3)	83.6 (6.1)	1.11 (1.03 - 1.19)	0.01
Red cell distribution width (%)	244.1 (4.9)	22.5 (2.9)	0.93 (0.81 - 1.06)	0.27
Platelet count ($x10^9$/l)	431.5 (178.0)	527.7 (269.2)	1.00 (0.99 - 1.01)	0.06
Reticulocyte count (% of RBC)	6.9 (5.2)	12.7 (5.9)	1.17 (1.05 - 1.30)	0.01
Creatinine (μmol/L)	40.9 (18.6)	42.8 (35.0)	1.00 (0.98 - 1.03)	0.73
Aspartate transaminase (IU/L)	56.2 (28.4)	52.5 (13.3)	0.99 (0.97 - 1.02)	0.65
Alkaline phosphatase (IU/L)	264.8 (102.9)	285.3 (153.0)	1.00 (0.99 - 1.01)	0.50
Bilirubin (μmol/L) - total	80.0 (55.2)	58.3 (37.4)	0.99 (0.89 - 1.05)	0.18
Haemoglobin F (%)	5.3 (3.8)	6.6 (55.6)	1.07 (0.95 - 1.22)	0.26
Haemoglobin S (%)	87.5 (5.1)	83.5 (10.7)	0.92 (0.86 - 0.99)	0.02

Multivariable analysis

Two separate models for multivariable analysis of factors associated with stroke were built. In the first model, we did not include reticulocyte count because there were not enough data points for reticulocyte at the baseline visit. The analysis included 363 and the results are shown in table below Table 49. Sickle haemoglobin levels were independently associated with risk of stroke.

Table 49 Multivariable analysis of factors associated with stroke (n= 363)

	OR (95% CI)	p
Pain	0.42 (0.89 - 1.96)	0.27
Fever	0.35 (0.06 - 1.99)	0.24
Mean corpuscular volume (fL)	1.09 (1.01 - 1.18)	0.04
Haemoglobin S (%)	0.89 (0.82 - 0.97)	0.01

For the second model, reticulocyte count was included and the model included 101 individuals. The results are shown in Table 50. Reticulocyte and sickle haemoglobin were independently associated with risk of stroke.

Table 50 Multivariable analysis of factors associated with stroke including reticulocyte count (n= 101)

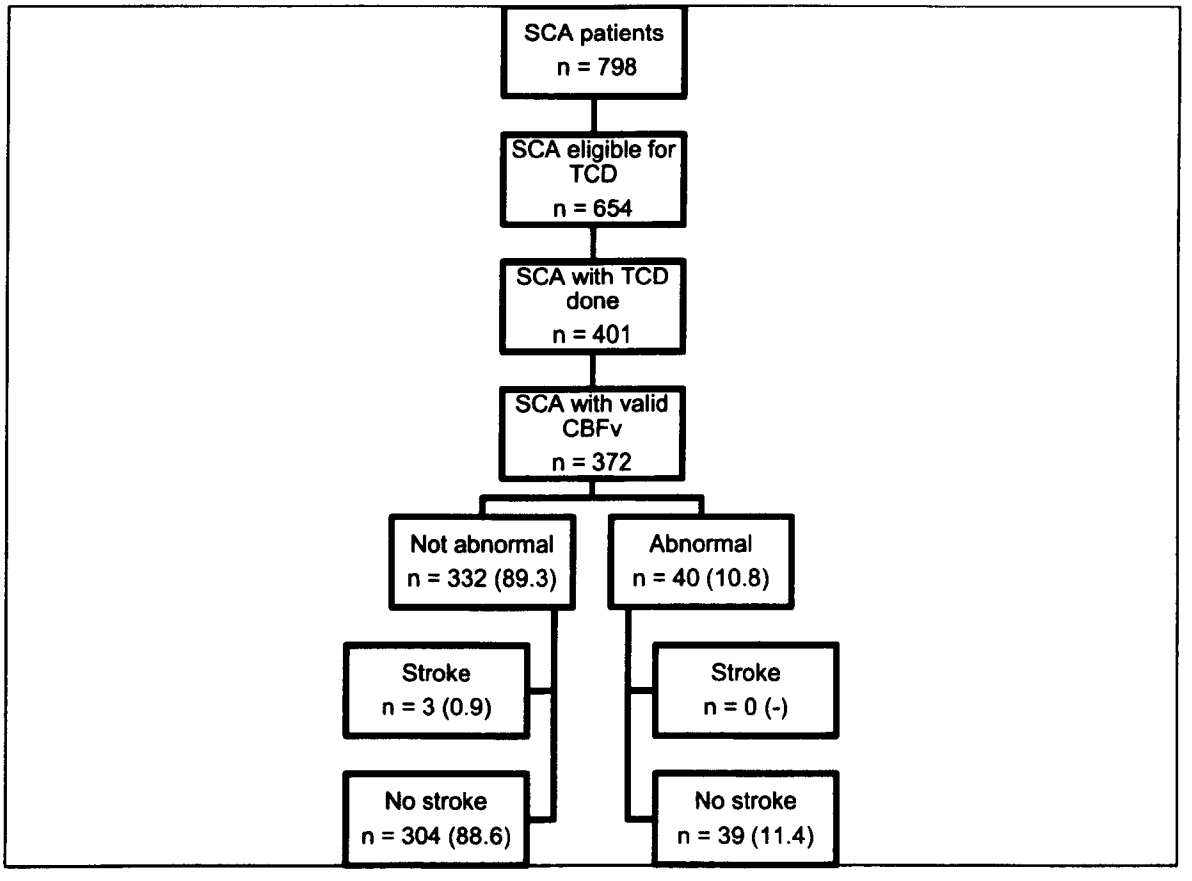
	OR (95% CI)	p
Pain	0.71 (0.05 - 9.23)	0.79
Fever	0.32 (0.03 - 4.08)	0.38
Mean corpuscular volume (fL)	1.02 (0.92 - 1.13)	0.73
Reticulocyte count (%)	1.19 (1.02 - 1.39)	0.03
Haemoglobin S (%)	0.87 (0.77 - 0.99)	0.04

Incidence of stroke and relationship with CBF

Incidence of stroke

The association between CBFv and stroke was explored by focusing on the individuals who had CBFv that was considered valid. The figure below (Figure 34) shows the flow of patients in the study who had CBFv measurement and known stroke during the course of the study.

Figure 34 Summary of patients to explore relationship of CBFv and stroke



There were 372 SCD patients who had valid CBFv of which 348 had documented presence or absence of stroke. Amongst the 348 SCD patients, 3 individuals (0.86%) had a stroke and the CBFv was 135, 155 and 188 cms/sec. The patients had 240.1 patient- years of observation

(PYO). The incidence of stroke was 1.25 per 100 PYO, with the rate decreasing from 2.0 (0.29 – 14.5) in under 5 year age group, 1.13 (0.16 – 8.01) between 5 -9 years and 0.98 (0.14 – 6.93) above 10 years (Table 51). This was not statistically significant (p=0.85).

Table 51 Incidence of stroke in patients with CBFv, stratified by age at TCD

Age (group)	# patients[1]	Observation (yrs)[2]	# stroke	Incidence[4]
<5years	81	48.9	1	2.0 (0.29 – 14.5)
5-9 years	121	88.6	1	1.13 (0.16 – 8.01)
>10 years	146	102.5	1	0.98 (0.14 – 6.93)
Total	**348**	**240.1**	3	1.25 (0.40 – 3.87)

The mean CBFv was higher amongst the individuals who had a stroke compared to those who did not have a stroke (133.5 vs. 159.0). The odds ratio was 1.01 (95% CI 0.99 – 1.03) but this did not reach statistical significance (p=0.37). The relationship between CBFv was explored using different cut-offs for CBFv (Table 52) and there was a trend of association between CBFv and stroke with a lower CBFv cut-off of 150cms/sec. However, there was a very wide confidence interval since there were very few episodes of stroke.

Table 52 Association between CBFv and stroke during course of the study: The relationship using different cut-off points to predict risk.

	No stroke	Stroke	OR	p
CBFv (cms/sec)	133.5 (49.7)	159 (26.8)	1.09 (0.99 1.03)	0.37
STOP definition[1]				
50-169	292 (85.1)	2 (0.7)	1.16 (0.24 5.69)	0.85
170 199	12 (3.5)	1 (7.7)		
>200	39 (11.4)	0 (-)		
Inter quartile range[2]				
Low (0 103)	88 (24.0)	0 (-)	1.95 (0.35 10.79)	0.45
Medium (104 156)	184 (50.3)	2 (1.1)		
High (157+)	94 (25.7)	1 (1.1)		
Using 150[3] (include CBFv<70)				
0-150	247 (67.5)	1 (0.4)	4.15 (0.37 46.2)	0.25
>150	119 (32.5)	2 (1.7)		
Using 150 (exclude CBFv < 70)				
50 149	236 (66.5)	1 (0.4)	3.97 (0.36 44.2)	0.26
150+	119 (33.5)	2 (1.65)		

STOP definition[120]: uses the following cut-off points: >200cms/sec abnormal, 170-199cms/sec conditional, <170cms/sec normal. Interquartile range of the CBFv in the Dar cohort, 150cms/sec is cut off point used from Kilifi data[391].

DISCUSSION

This is the first description of neurological events and *CBFv* in SCD in Tanzania. Most of the information that is available on the neurological events and CBFv in SCD is from studies that were conducted in the USA and Europe. Although there have been studies in Nigeria and Kenya, these studies involved small number of patients. There are key differences between the spectrum of CBFv in Tanzania, Kenya and USA. The mean CBFv in the Tanzanian population was (132 cms/sec) higher than what was found in Kilifi (120cm/sec) and that reported in the US (129cm/sec). One possible explanation for the higher CBFv in the Tanzanian population may be that SCD children in this

population have lower levels of haemoglobin. However, anaemia can only explain the difference in CBFv up to 150cms/sec, as studies done in the US and UK suggests that velocities beyond 150cm/sec are most likely due to stenosis.

The second important finding in this study was that the prevalence of abnormal CBFv was 10%. This is similar to the finding from studies in the USA where prevalence rates were between 3-17%[319, 444,-446] . It was expected that that the spectrum of CBFv would be similar in the two areas in Africa because the populations are similar with regards to sickle haplotype and well as genetic and environmental exposures. On the contrary, SCD patients in Kilifi had lower mean CBFv and there were no abnormal CBFv detected. This unexpected result may be because the SCD patients in Kilifi are different from that in Dar as it is rural with the highest rates of people living below the poverty line, low educational and nutritional status and poor access to health care[447] . Furthermore, the site where the study was done is a district hospital. In comparison, Dar-es-Salaam is a large city with a more heterogeneous population and MNH is the national referral hospital. It is therefore possible that the patients seen at Muhimbili have more severe disease.

Another factor that may explain the difference is that children in Kilifi die before there is a chance for them to develop stenosis which would be detected by TCD. Reports from Kilifi suggest that the mortality in SCD in Kilifi is particularly high with only 5% of SS births reported to be alive by five years of age (personal communication). However, in Kilifi, CBFv was not associated with mortality [392] as was the case in this study.

The other explanation for this finding is that the natural history of neurological complications

in SCD may be different in Kilifi. According to proposed pathophysiological mechanisms, progressively worsening stenosis may result in complete occlusion of the vessel leading to infarcts/stroke. In this case, patients will have gradually increasing CBFv which can be identified by TCD measurements. However, it is possible that, rather than leading to occlusion, the progressive stenosis, may precipitate the development of collaterals. In this situation, there is a decrease in CBFv as less blood flows in the affected vessel. This phenomenon, Moyamoya syndrome, has been described in SCD children[309 311 448]. In Kilifi, low *CBFv*, compatible with[449] but not diagnostic of Moyamoya, was documented in 7 SCD patients, one of whom had had seizures; none of these patients was known to have died or have neurological complications 4 years later. It is not clear whether the development of cerebrovascular disease in Dar-es-Salaam would involve stenosis leading to occlusion or stenosis leading to Moya Moya syndrome.

To determine the factors that could be used to identify SCD patients who may have abnormal CBFV, we looked at the association between clinical and laboratory features at time of TCD measurement. For some of the laboratory investigations, e.g. chemistry the investigations were not available at time of TCD. In this instance we used the measurement done at the closest time point to TCD measurement. On univariable analysis, patients with high CBFv had symptoms of anaemia, history of blood transfusion within the past 12 months and had higher peripheral oxygen saturation. High levels of direct bilirubin were associated with abnormal CBFv. On multivariable analysis, peripheral oxygen saturation was independently associated with high CBFv; however the model included only 64 SCD patients because at this visit, patients did not have peripheral oxygen saturation measured. However, evidence from Kilifi and USA suggests that low oxygen saturation predicts neurological events [304, 392, 442],

whereas in this case patients with abnormal CBFv had higher oxygen saturation. However it is known that hypoxia promotes polymerisation of HbS and adhesion of RBC to endothelium 314 and platelet and WBC adhesion[39]. The hypoxia may be caused by upper airway obstruction, since enlarged adenoids are associated with CVA[304]. It is difficult to make any conclusions from the study as there were incomplete data for peripheral saturation. However, this requires further investigation as the evidence suggests that peripheral oxygen saturation has a significant role in cerebrovascular dynamics.

The second objective of our study was to describe the prevalence of neurological events in SCD patients. Neurological events that have been described in SCD patients include seizures, coma, and stroke, visual and hearing disturbances. In a study reported from Nigeria, neurological events were more common in individuals with SCD (76%) compared to controls (32.1%). Stroke, febrile convulsions and headaches were common in children whereas paraplegia, epileptic seizures and localizes sensory neuropathy were more common in adolescents and adults[450] . During the study period we identified 77 neurological events out of 1,573 SCD patients (4.9%). Of the 77 events, the commonest events were convulsions (2.99%) and stroke (1.02%). Seizures were more common than stroke as they accounted for 47 out of 434 individuals (10.8%) examined for neurological events, when compared to reports in Kenya where they accounted for 6 out of 18 events (33.3%) and were less frequent than stroke[298] . Seizures have been previously reported in SCD in Nigeria where ten out of 96 patients (1.1%) satisfied the criteria for clinical diagnosis of epilepsy, with a prevalence of epileptic seizures of 10.4%[451]. The question of whether seizures are more common in SCD children than in the normal population cannot be addressed by the study but the results highlight the need for further investigations.

During the course of the study, the incidence of stroke was 0.3 (0.2-0.5) per 100 PYO while or the subset with CBFv the incidence was 1.25 (0.4 – 3.9) per 100 PYO. The reason for this difference is not clear, but may be due to the different ages since CBFv was measured only in children below 16 years. It is possible that there is an increase in incidence of stroke when the patients get older. This would be in keeping with previous descriptions of stroke risk, which suggests that there are two peaks, during childhood between 2-5 years, where stroke is due to cerebral infarction. The second peak occurs during adulthood, above 30 years, and is commonly due to cerebral haemorrhage[83]. Both of these rates are higher than the rate of 0.61/100 PYO reported from the USA[83]. Previous studies reported ranges of prevalence of stroke between 1% in Saudi Arabia and 18% in Brazil illustrating marked variation of rates between populations. In this study stroke occurred in 1.02% of patients. In Kenya, strokes were reported to be more common than seizures, accounting for 67% of neurological events[298] and in 4 out of 6 patients with SCD admitted within a 18 month period with a neurological event had a stroke[284] . Furthermore, stroke causes significant morbidity to individuals and causes a high burden of disease to the individual, family and the community. Therefore, there have been concerted efforts to understand the natural history of stroke in SCD and improve the outcome either by preventing the occurrence or prevention of recurrence. Good diagnostic methods in the form of TCD and neuroimaging to identify high-risk individuals[319 452]. Interventions, such as chronic blood transfusion and Hydroxyurea, have also been shown to be effective [120, 328, 330, 361, 453].

The risk factors for stroke were determined by assessing clinical and laboratory features at the baseline or TCD visit. This visit was chosen as the health care system in Tanzania, particularly for SCD patients, is not comprehensive. Therefore, since SCD patients may be

seen at a specialist clinic only once, it is vital that the patients are assessed at this stage for risk of stroke. In this study, the factors that were associated with stroke were history of pain and fever as well as high MCV, reticulocyte count and HbS level. HbS level and reticulocyte count were independently associated with stroke. The finding that HbS concentration is associated with risk of stroke is of interest. This is a recognised disease mechanism as therapeutic intervention aimed at a reduction of HbS by exchange transfusion to below 30% has been found to improve outcome during acute events such as stroke, acute chestsyndrome[120, 122, 125, 454]

The association between reticulocyte count and stroke may be due to an increase in erythropoiesis. It is interesting that reticulocyte numbers were associated with stroke but haemoglobin levels were not as one would expect both to be related as an increase in reticulocyte normally occurs with a decrease in haemoglobin. The exception to this is when the bone marrow fails to respond. However, this analysis was done on a small subset of patients. Therefore, further studies are required to determine the natural history of the relationship between stroke and red blood cell indices. This is of particular interest as previous studies have reported an association with low haemoglobin level, low HbF level, low red blood cell count and high nucleated cell counts[83, 455]. In this study, there was no association with any of these factors. This may reflect a true difference in the pathophysiology of stroke in the two populations. Alternatively, it may be that we have not been able to see the association because this was a clinic-based cohort rather than a birth cohort and therefore the SCD patients that have been studied may be survivors with mild forms of the disease.

The final objective was to explore the relationship between CBFv and incidence of stroke. Patients who had a stroke during the study had higher levels of CBFv (159cms/sec) compared to those who did not have a stroke (133.5cms/sec). However, this was not statistically significant (p 0.37). The level of 200cm/sec used in the STOP trial to define high-risk individuals[120], was not associated with risk of stroke, as none of the 3 patients with stroke had a CBFv above 200cms/sec and only 1 had conditional velocity (188cms/sec). The OR of having a stroke when CBFv was above 200cms/sec was 1.16 (95% CI 0.24 – 5.69); p=0.85. When a CBFv of 150cm/sec was used, the OR was higher at 4.15, suggesting a trend towards a positive association. However, the confidence interval was very wide, 0.37 – 46.2 and this association did not reach statistical significance (p = 0.3). These results suggest that the cut-off value that was used in the STOP trial may be too high for the SCD population in Africa. This has certainly been the case in other populations outside of the USA. A study done in Brazil found that a CBFv of 123.4cm/sec in the MCA was able to accurately identify stenosis in these vessels with a sensitivity of 100% and specificity of 73%[456]. It is therefore vital that the threshold for CBFv that is used in identifying patients with high risk of stroke should be guided by the STOP trial but local-evidence should be determined for each population.

In summary, this study has reported the spectrum of CBFv in a cohort of children with SCD in Tanzania. High peripheral oxygen saturation was independently associated with risk of having an abnormal CBFv. During the course of the study, stroke occurred in 1.02% of patients, and these were independently associated with HbS levels and reticulocyte count. It is difficult to accurately determine the cause-effect relationship of the various factors, including CBFv, and their change over time with stroke. In order to determine this relationship accurately, detailed examination during steady-state and during acute clinical

events are required to ascertain whether there are any changes at different time points. In this way, accurate descriptions of the natural history of cerebrovascular disease in SCD in children will be possible. It is possible that the course and mechanisms of cerebral vasculopathy and neurological events in Africa are different from North America and Europe. More detailed neuroimaging studies with magnetic resonance angiography are required to elucidate this further.

Chapter Eight

Sickle Cell Disease and Foetal Haemoglobin

INTRODUCTION

Foetal haemoglobin (HbF) is a major ameliorating factor of clinical illness in individuals with SCD. High HbF levels are associated with milder forms of the disease with patients having less pain[57, 457,] lower morbidity and improved survival[61] . Clinical epidemiological studies in the Eastern region of Saudi Arabia, where the Asian βS - globin haplotype predominate, SCD patients have haemoglobin F levels of 10-40%, and rarely have severe disease[458], [346.] . α - thalassaemia is also common in these regions and therefore the relative importance and correlation between HbF, α - thalassaemia, and other factors, is not clear[459]

The association of HbF and SCD severity has led to several interventional studies undertaken using agents to increase HbF levels in SCD individuals[354]. Hydroxyurea (HU) is one of these agents and has been effective in reducing painful episodes, rate of blood transfusion requirement and rate and duration of hospitalisation[354]. HU is also effective in reducing mortality[363 459]. However, not all individuals with SCD have a similar response to HU. About 60-80% of SCD patients, received HU to a maximum tolerated dose, and were not able to increase the HbF levels. The factors that determine the response of individuals to HU are not well understood[364] .

In Tanzania, although there is a high prevalence of SCD, HbF levels are not measured for

clinical purposes to assist with therapy or for research purposes. This is because the laboratory measurement of HbF levels has been difficult, requiring meticulous laboratory skills which have not previously been available. However, with the increasing use and availability of high performance liquid chromatography (HPLC), HbF levels can be measured easily, with reliable results that can be validated and compared with other centres around the world. In 2005, Hb fraction quantification by HPLC was established in our laboratories.

We hypothesized that the spectrum of HbF levels in Tanzania was likely to be different from that elsewhere because of the different ethnic origins of people in Dar-es-Salaam. There is an admixture of people with African, Arab and Indian ethnicity and these 3 groups have different βS -gene haplotypes. People of Bantu descent will most likely have the Central African Republic (CAR) haplotype which is associated with low levels of HbF and severe disease. On the other hand people of Arab or Indian descent are most likely to have the Asian haplotype which is associated with high HbF levels and milder forms of SCD. Because HbF has such a key role in determining clinical severity in SCD, I included a study to determine role of HbF in SCD as part of this thesis. The aim was to describe the spectrum of HbF in SCD patients and look at the association of HbF with disease morbidity and mortality. With regards to morbidity, the clinical factors that I examined included malaria, CBFv, stroke and hospitalization.

METHODS

Procedures

From June 2005, HPLC for quantification of Hb fractions was included as one of the investigations to be done on all SCD patients who came to the clinic. Since all patients had a sample of whole blood for FBC, an aliquot was taken for HPLC. Measurement of HbF, HbS, HbA0 and HbA2 was done. For patients who were being recruited into the study, HPLC also served as a test to confirm the sickle phenotype. Patients who were already in the cohort also had HPLC measurements. All patients had a single HbF measurement at OPD clinic which was then taken as representative of his or her HbF level at that age. HbF measurements were not available for some patients within the cohort who were either lost to follow up or deceased before HbF levels were determined.

HPLC testing was repeated 3 months later in patients who had received a previous blood transfusion. HPLC was also repeated in some patients in whom the HPLC results did not match previously undertaken haemoglobin electrophoresis. For both these groups of patients, a thorough assessment with detailed clinical history and examination was undertaken to determine whether they had received blood transfusion or had symptoms and signs suggestive of SCD. Only patients who had confirmed SCD were included in this analysis.

Statistical methods

HbF measurements were undertaken consecutively on all SCD patients from June 2005. However, there were some patients who were already lost to follow up or had died when HbF measurements were established. Therefore, for purposes of the thesis, the analysis was

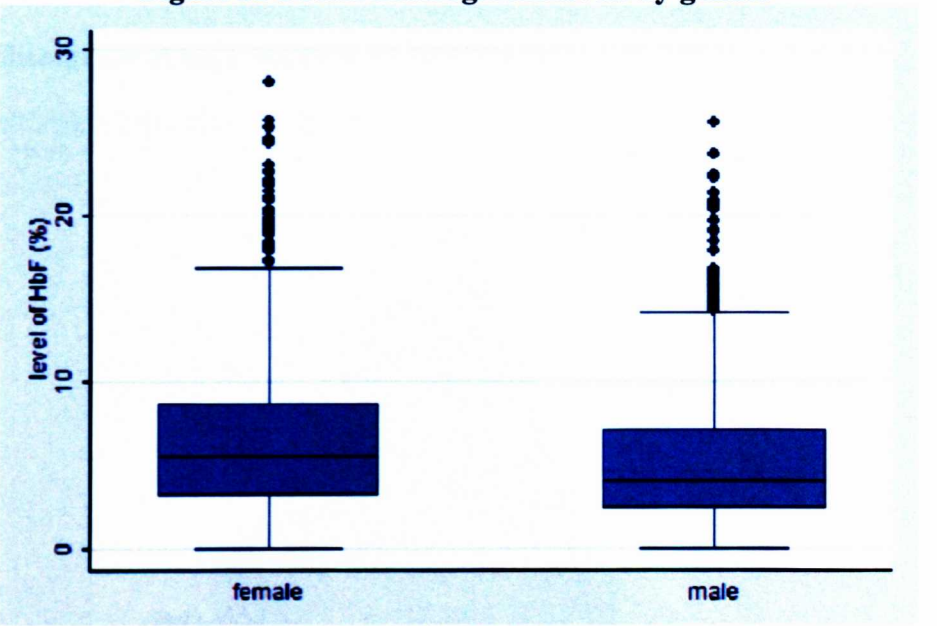
limited to SCD patients who had HbF with data on the primary outcome measure, death, at time of analysis.

The explanatory variables included were laboratory factors such as Hb, MCV, WBC, and markers of haemolysis and liver function such as bilirubin, AST and LDH levels. Effect modifier variables included age and sex. For purposes of this thesis, clinical factors at baseline were not included. Continuous variables were analysed using two-sided t-tests. Categorical variables were analysed by χ-squared test. Logistic regression was used to explore associations between HbF and clinical and laboratory variables, presenting the results as odds ratio (OR) with 95% confidence interval. A p-value <0.05 was considered statistically significant. Multivariable analysis included all variables with univariable significance of <0.05. Using backward elimination, the final model included all variables significant at the 0.05 level.

RESULTS

The analysis included 1,668 SCD (HbSS) patients with HbF measurements and known survival status. The median HbF level was 5.3 with an interquartile range of 2.9 – 8.6%. The mean HbF level was 6.3% (SD 4.7) with men having a lower mean of 5.8% (4.4) compared to females, who had a mean of 6.9% (4.8) (OR (95%CI): 0.68 (0.55 – 0.85); $p<0.01$).

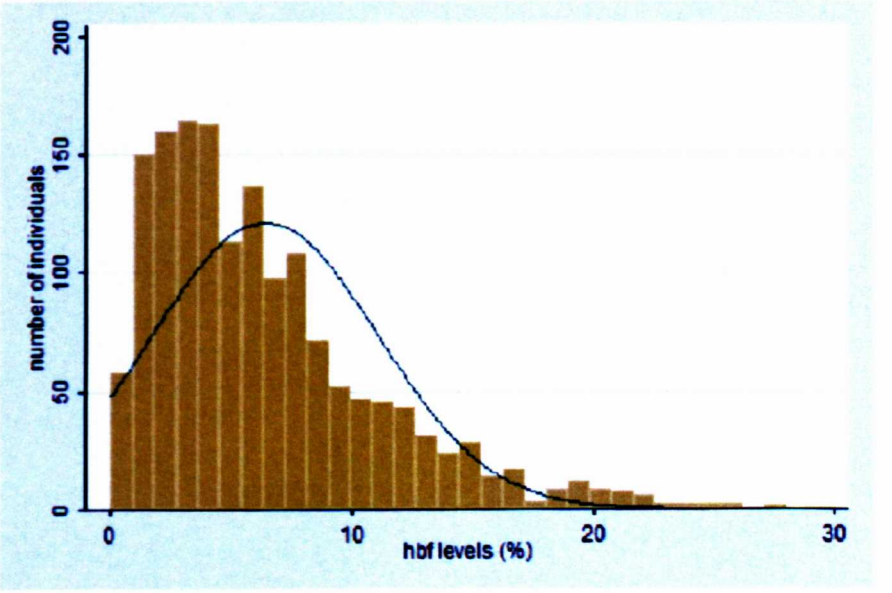
Figure 35 Foetal haemoglobin levels by gender



Levels of HbF in female and male. N=1,668. OR 0.68 (0.55 - 0.86); p<0.01

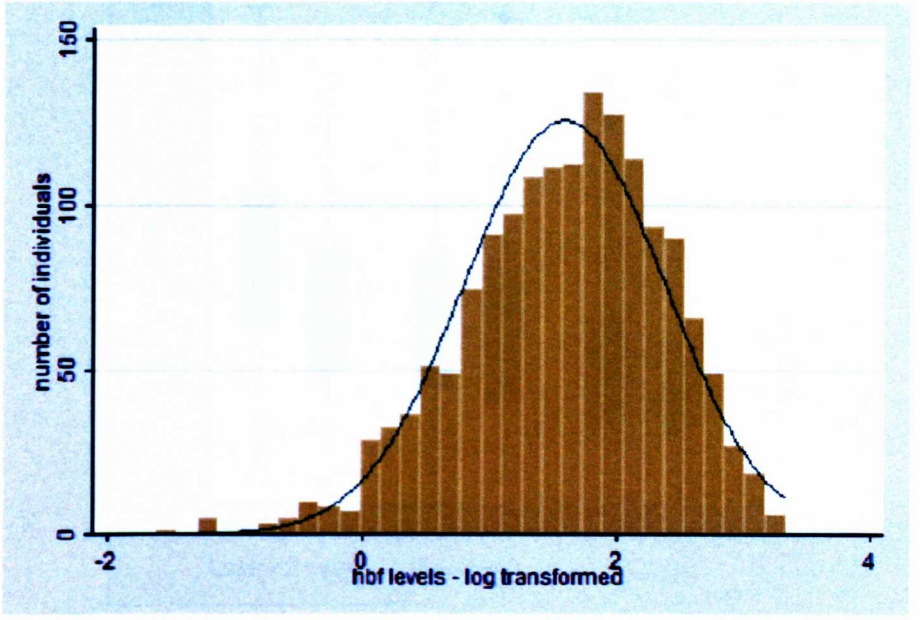
The spectrum of HbF levels is shown below, with a minimum of 0 and maximum of 25.7%. It does not show a normal distribution.

Figure 36 Histogram of HbF levels



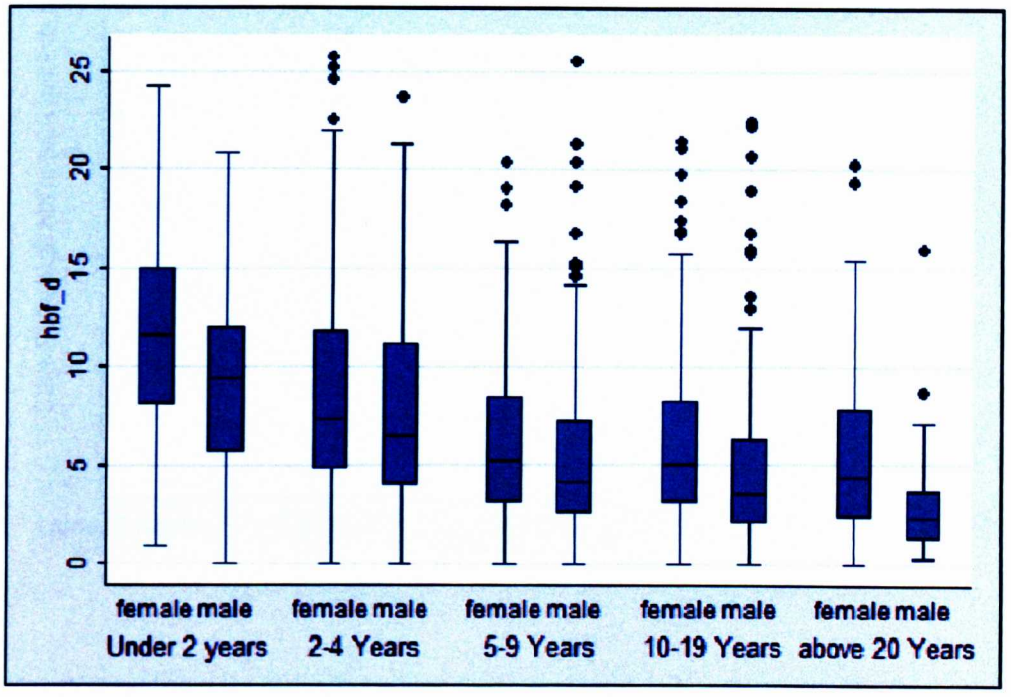
Due to the distribution, the data were log transformed and the histogram shown below.

Figure 37 Histogram of log transformed HbF levels



The levels of HbF are known to vary within the first 5 years of life, following which they remain relatively stable although there is a slow fall in levels with age. This pattern was similar in this study, which showed higher HbF variation levels in patients in this age group;

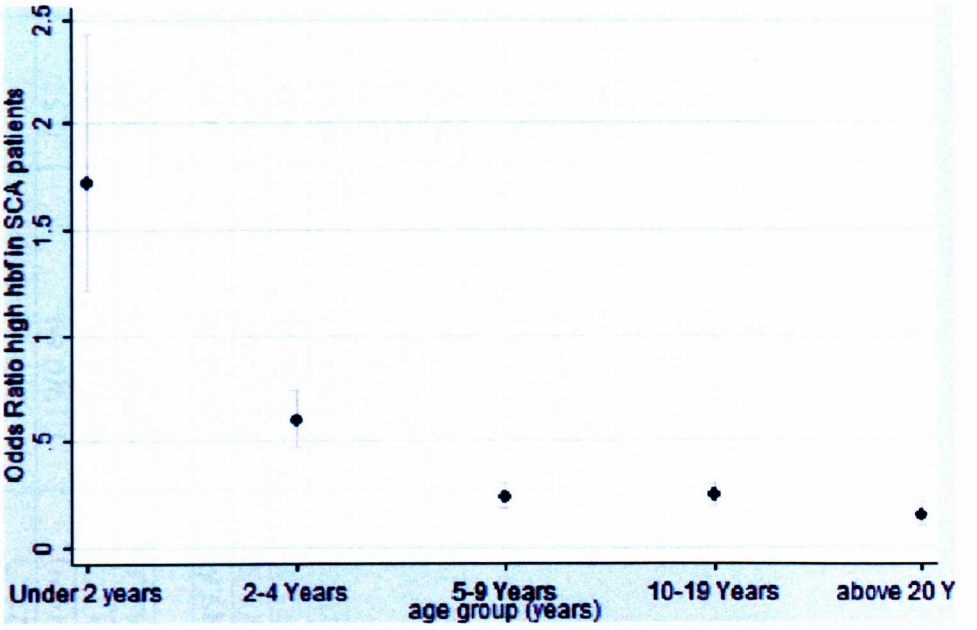
Figure 38 Distribution of HbF stratified by age and sex in SCD patients



For the purpose of analysis, we used HbF level of 8.5% which was the 75th percentile of the study population, as the cut-off to differentiate patients as having high or normal HbF. There were 428 SCD patients out of 1,688 (25.7%) with high HbF. With regards to variation of HbF levels with age, there were 86/136 individuals under 2 years (63.3%) and 123/330 (37.3%) of those between 2- 5 years having high HbF. After this, the HbF levels were lower, with less variation.

To illustrate the age-stratification of HbF levels, the odds ratio of high HbF in the 5 age groups was plotted, showing that in the younger age group, the likelihood of having high HbF is high but there is wide variation as evidenced by the wide confidence interval.

Figure 39 Odds ratio of high HbF in 5 age groups



Factors associated with high HbF

Univariable analysis

To explore the association of laboratory features with HbF, univariable analysis was done and the results are shown in Table (Table 53). Since the HbF level is variable until the age of 5 years, the analysis excluded children below the age of 5 years. Individuals with high HbF had significantly higher haemoglobin (7.6 vs. 7.4), and Mean Cell Volume (80.5 vs. 78.5) compared to those with normal HbF and there was a trend for an association with RBC (3.1 vs. 2.9). The platelet count (383.9 vs. 439.2 $x10^9/l$) and RDW (22.2 vs. 23.5 %) were significantly lower in the group with high HbF compared to in group with normal HbF. Total bilirubin was significantly lower and there was a strong trend for lower creatinine. Alkaline phosphatase level was higher in those with high haemoglobin F (Table 53).

Table 53 Laboratory factors associated with high HbF in SCD patients

	Total	Normal HbF	High HbF	OR	P
Total	1668	1240(74.3)	428(25.7)		
Death	1508	47/1124(4.2)	19/384(4.9)	1.19(0.69-	0.53
Age (years)		11.3(8.1)	7.5(7.2)	0.93(0.91-	<0.01
Male	1668	642/1240(51.8)	181/428(42.3)	0.68(0.55-	<0.01
Haematology					
White blood cell count (x109/l)	1594	16.3(7.5)	16.9(9.6)	1.01(0.99-	0.25
Haemoglobin (g/dl)	1593	7.4(1.3)	7.6(1.5)	1.13(1.04-	<0.01
Red Blood cell count (x1012)	1587	2.9(2.9)	3.0(2.9)	1.06(0.94-	0.36
Mean Cell Volume (fL)	1577	78.9(9.5)	81.4(9.8)	1.03(1.02-	<0.01
Red Cell Distribution Width (%)	1592	22.2(3.9)	21.5(3.7)	0.95(0.92-	<0.01
Platelets (x109/l)	1574	464.8(191.1)	377.1(185.6)	0.99(0.997-	<0.01
Reticulocyte count (%)	1523	13.8(6.1)	14.7(6.6)	1.02(1.00-	<0.02
Clinical Chemistry					
Bilirubin (μmol/L) – Total	58	64.9(60.5)	57.8(56.7)	0.99(0.99-	0.13
Bilirubin (μmol/L) – Direct	585	20.6(42.5)	16.6(13.7)	0.99(0.98-	0.26
Bilirubin (μmol/L) Indirect.	576	49.9(61.8)	45.4(59.9)	0.99(0.99-	0.40
Alkaline Phosphatase (IU/L)	793	265.7(128.2)	291.0(135.8)	1.00(1.000-	0.01
Aspartate Transaminase (IU/L)	782	48.9(33.1)	48.7(25.4)	0.99(0.99-	0.96
Lactate Dehydrogenase (IU/L)	552	979.9(482.2)	1015.5(522.7)	1.00(0.99-	0.44
Creatinine (μmol/L)	792	41.5(28.7)	36.7(13.5)	0.99(0.98-	0.02

Multivariable analysis

On multivariable analysis, the results are shown in table below (Table 54). In this model, sex, haemoglobin, MCV, RDW and platelets were independently associated with high HbF.

Table 54 Multivariable analysis of factors associated with high HbF (n= 1,434);

	OR	P
Sex	0.57 (0.41 .0.82)	<0.01
Haemoglobin (g/dl)	0.97 (0.95 .1.01)	0.20
Mean cell volume (fL)	1.35 (1.16 .1.56)	<0.01
Red Cell distribution width (%)	1.06 (1.04 .1.08)	<0.01
Platelet count ($x10^9$/l)	0.93 (0.88 .0.98)	<0.01
Reticulocyte count (%)	0.99 (0.996 .0.998)	<0.01

DISCUSSION

This is the first study to report the spectrum of HbF in SCD patients in East Africa. The mean HbF was 6.3 (SD 4.7) with a range of 0 to 25.7%, which is lower than that reported in Congo Brazzaville (8.8 (5.8)[397]. Various reports from Nigeria have reported mean HbF levels ranging between 5.9 and 9.5[150, 460 ,461] . This may suggest either a wide variation in mean HbF in SCD patients within the same geographical location or differences in methodological approaches in measuring HbF levels. In the studies in Nigeria, the alkaline denaturation method was used.

In this study the mean HbF levels were significantly higher in females compared to males, similar to reports in Jamaica where adult males had a mean HbF level of 4 compared to the mean level in females of mean 5.5[462] . The HbF levels from SCD patients in the USA were higher, with mean values of 7% in those aged <24 years and 6% in older patients in the

Co-operative study of sickle cell disease[463] . As expected, the levels in Tanzania were much lower than the mean levels in countries in the Arab peninsular, where the mean HbF is 27.8 [346, 464]. Although it is likely that both the CAR and Arab-Indian haplotype exist in the population in Dar-es-Salaam, the predominant haplotype is expected to be the CAR haplotype, as reports from Kenya suggest that this is the common haplotype in this region[465, 466]. Reports suggest that within the CAR haplotype, there are two chromosomal types that determine different levels of HbF and G-gamma expression[467], which in turn influence HbF levels. Therefore, there may be differences in the sub-haplotypes that could be affecting the HbF levels. In this study, the β_S-haplotypes were not characterised but this will be an area of further research.

The laboratory factors that were independently associated with high HbF levels were sex, Hb, MCV, RDW and platelets. High levels of Hb have been associated with HbF[468]. The reason for this association is due to the fact that high levels intraeyrthocytic HbF improves RBC survival[469]. Individuals with higher HbF levels have higher MCV values which could be explained by a pleiotropic effect of the genetic factors underlying the increase in HbF[470] One of the suggested mechanisms for the increase in HbF relates to an accelerated differentiation of erythropoiesis leading to the release of relatively 'younger' erythroid precursor cells, hence the lower RBC and larger MCV values. These 'younger' erythroid precursor cells are still synthesising relatively more HbF. The association of higher F cells (erythrocytes that contain HbF) with lower RBC and higher MCV values, has also been shown in healthy non-anaemic white Europeans[471]. Independent genetic association has also been demonstrated with the *HBS1L-MYB* locus, one of the three major HbF-associated quantitative loci (QTLs), with these haematological variables, including platelet counts.

In summary, this study has the first report of the spectrum on HbF in a cohort of SCD patients in Tanzania. There is a significant gender difference, with females having higher levels than male. Factors that were independently associated with high HbF levels were female gender, haemoglobin, MCV, RDW and platelet count. The association with clinical events such as death, hospitalisation and high CBFv was difficult to elucidate as the differences did not reach statistical significance. HbF levels are highly genetically controlled and recently three major loci (QTLs) have been shown to account for 20-50% of its variation in diverse populations including healthy individuals of European descent as well as Sardinians, African-Americans, Brazilians, African-British, Chinese and Thai, healthy and with thalassaemia or SCD. Genotyping of the sequence variants in the three HbF QTLs is underway to investigate if these three loci are also associated with modulation of HbF production in Tanzanian sickle populations. If so, we will explore the incorporation of these variants into a prediction algorithm as part of the care pathway for sickle cell disease. Information on the HbF-associated QTLs should also be of value in better understanding of therapeutic reaction of HbF using agents such as hydroxyurea.

Chapter Nine

Discussion

INTRODUCTION

Although SCD results from a single gene mutation, it is a complex disease with marked variation in clinical presentation of disease. There are environmental and genetic factors that influence disease patterns within individuals at different stages, as well as within populations in different geographical locations. Therefore, the approach with regards to interventions and management differs within individuals at different time points and the interventions that are applicable in one setting may not be appropriate in another setting. Much of the knowledge that is available regarding SCD is based on evidence from SCD populations in Europe, United States of America and Jamaica. There is limited information on the causes of illness and death in SCD in Africa, where 75% of global population of SCD individuals live.

The work presented in this thesis increases the knowledge of the clinical spectrum of SCD in Africa. A prospective study was conducted between 2004 and 2009 in Muhimbili National Hospital in Dar-es-Salaam, Tanzania. This is the largest cohort established in Africa for research from a single centre. Clinical events, at outpatient clinic and during hospitalisation were described in order to understand the course of disease in this setting. The rates and risk factors for hospitalisation and death were described. The decision to focus on malaria, bacterial infections and stroke was due to the previously described role of these conditions in SCD within Africa or in the case of stroke, within SCD populations outside Africa. There was evidence, in some cases conflicting, that these conditions cause significant morbidity and

mortality. However, this study has provided a description of factors that can be used to identify SCD patients who have high risk or are protected from these outcomes. The details of the study findings have been reported in chapter 4, 5, 6, 7 and 8.

This chapter will summarize the key findings and discuss the validity of the study findings with regards to the SCD population at Muhimbili (internal validity) as well as the generalisability or validity of the findings in relation to other SCD populations (external validity). This will be followed by a brief outline of the implications of the findings to current policy for management of SCD as well as future research.

FINDINGS AND EVIDENCE TO SUPPORT FINDINGS

General results – baseline, hospitalisation and mortality

This study has illustrated that it is possible to conduct a prospective, cohort study on a genetic disease in a country with limited resources. During the study period, 1,725 patients were enrolled, with a survival rate of 80% and loss to follow-up rate of 12%. The mean age at recruitment was 9.7 (7.9) years. Since SCD is an inherited disease that is present at birth, the high median age at recruitment in this cohort suggests that children remain undiagnosed for a long period of time. The natural history of the disease is such that there are many clinical events in the first 3 years of life and that the highest incidence of death is between 1-3 years. This finding highlights the need for newborn screening to identify children at birth. Once identified, these children can be managed optimally with prophylaxis against infections as well as prompt treatment of acute clinical events such as anaemia, pain and infections. Identification of a birth cohort, would also allow accurate description of clinical events in

early childhood in this setting.

504 (29.2%) of the cohort were hospitalised during the course of the study. Pain was the most common cause of admission, followed by fever and anaemia. A history of hospitalisation within the preceeding 12 months period was a risk factor for hospitalisation during the course of the study.

The median survival amongst patients with SCD in the study was 33 years, which is 19 years less than the life expectancy in Tanzania, which in 2008 was52 years[472] . The mortality rate in the study was high, with an incidence of 2 per 100 PYO. The age group that was most affected was under 5 years, although, the confidence interval was wide because of the small number of children within this age group. The risk factors for mortality were low level of haemoglobin and high total bilirubin. 23% of the deaths occurred in Muhimbili.

There were several issues that highlighted the importance of having a good health system that includes good service delivery at different levels of health care starting from within their homes. The first is that there were some SCD patients who were identified, during hospitalisation, or were screened and found to have SS phenotype, but never returned for a baseline visit. Second, from the 1,725 SCD individuals recruited during the study period, 209 (12.1%) individuals were lost to follow up. Although this rate is acceptable to make conclusions about the information collected from the cohort, it is important to address the factors that contribute to patients not attending the clinic. Third, only 23% of deaths occurred at MNH. There may be different factors that contribute to these findings. However, these issues suggest that SCD patients may only be seen once at a specialist clinic in a tertiary

centre. Furthermore, acute clinical events and death occur at home or outside these specialised centres. Therefore it is recommended that if any SCD patient is identified, risk-stratification is done immediately at this stage. Health education and counselling of patients and their guardians about the importance of regular attendance to clinics may improve attendance. It is also important to explore whether there are any issues within the health system that can be addressed to improve access to care. First, health services provided for SCD patients in peripheral clinics and in the community can be improved. National centres should work with the public health system to ensure that there are good systems in place for delivering care to SCD patients at different levels of care. Second, outreach services can be improved by the use of telephone and community based health workers so that doctors in MNH or other specialist centres can provide advice to patients or health care workers. Third, day care facilities would also reduce the time that patients spend at hospitals for clinic or inpatient care. This would also improve adherence as spending long periods of time at hospital may contribute to patients not coming to MNH.

Malaria

Falciparum malaria is usually considered a significant cause of morbidity and mortality in SCD. In this study, the prevalence of parasitaemia was lower in SCD than in non-SCD patients both at clinic (0.7% versus 1.6%; OR 0.53, 95% confidence interval 0.32–0.86; p=0.008) and during hospitalization (3.0% versus 5.6%; 0.46; 0.25 - 0.94; 0.01). However, SCD patients had higher rates of malaria during hospitalization than at clinic, the ORs being 4.29 (2.63 - 7.01; p<0.001) for malarial parasitaemia, 17.66 (5.92 - 52.71; p<0.001) for clinical malaria, and 21.11 (8.46 - 52.67; p<0.001) for severe anaemia associated with malarial parasitaemia respectively. The implications of these findings have significant consequences

to the management of malaria in SCD. First; since malaria was rare amongst SCD patients at outpatient clinic, this suggests that prophylaxis may not be necessary. The caveat to this is that this result may only be pertinent to areas with low malaria transmission like Dar-es-Salaam. Further studies in areas with high prevalence would need to be done to validate this finding. The second implication, is that malaria parasitaemia during hospitalization was associated with both severe anaemia and death. It is therefore vital to ensure that during acute illness prompt diagnosis of malaria is made and effective treatment is given for malaria as well as anaemia.

Infections

From this study, the pattern of bacterial infection in SCD patients admitted with acute clinical events was ascertained. There was a low yield of pathogens from blood cultures during hospitalisation of SCD patients. There were concerns that this was due to methodological limitations in laboratory techniques within microbiology. After these issues were addressed, the prevalence of bacteraemia was 4.8%, and the commonest organisms isolated were *Staphylococcus Aureus*, non-Typhii *Salmonella* and *Streptococcus pneumoniae*. It is therefore recommended that SCD patients who are hospitalised should receive prompt diagnosis and treatment of bacterial infection.

Reports from Kenya, Uganda and other areas in the worlds suggest that bacterial infection (most likely due to *streptococcus pneumonia*) is the most likely cause of death in SCD individuals who are less than 5 years. Evidence is required to advise on the most appropriate vaccination and chemoprophylaxis for SCD individuals in Africa.

Stroke

The spectrum of CBFv in SCD patients in Tanzania was described in 372 SCD patients, with the mean CBFv [132 (SD 49.4) cms/sec] higher in Tanzania than that previously reported in Kenya and the US. However, the prevalence of abnormal velocities (above 200cms/sec) was 10% which was comparable to that reported by the US studies. Peripheral oxygen saturation was independently associated with likelihood of having an abnormal CBFv. Further studies are required to elucidate the role of hypoxia in cerebrovascular events.

During the course of the study, stroke occurred in 1% of patients. The incidence of stroke was 3.0 per 100 PYO and the factors that were independently associated with increased risk of stroke were level of HbS and reticulocyte count. For the subset with CBFv measurements; the rate was lower at 1.3 per 100 PYO. This is most likely due to the age difference as the latter group were younger. Although patients with stroke had higher CBFv compared to those who did not have a stroke, this was not statistically significant. Furthermore, the level of CBFv of 200cm/sec was not useful in predicting stroke. It is recommended that further evidence is required to determine the relationship and establish whether a lower threshold of CBFv would be more appropriate for predicting stroke. The fact that this was a self-selecting, hospital based cohort may bias these findings as this patients group may have less severe disease, including cerebrovascular disease. It is therefore essential that this study is conducted in a cohort of SCD patients identified at birth while also expanding the SCD population by either recruiting from several sites or including all SCD patients that can be recruited from the community so that the selection and study is not limited to a hospital cohort.

Foetal haemoglobin

The spectrum of HbF levels was described in 1,668 SCD patients. The mean HbF level was 6.3% (4.7) with females having significantly higher levels (6.9%) compared to males (5.8%). There was variation of HbF with a gradual reduction in the first 5 years of life. The factors that were independently associated with high levels of HbF were gender, haemoglobin, MCV, RDW and platelets.

Analysis of relationship between HbF with significant clinical events, in their respective chapters, did not show a significant association between HbF levels with mortality, hospitalization, high CBFv and stroke. This is a surprising result as HbF has been shown to be associated with mild forms of SCD and agents such as hydroxyurea which increase HbF have been found to improve survival and quality of life in SCD. However, such results have been reported from other studies and the recommendation is that more detailed description of the phenotype of HbF is required to understand the role of HbF in the clinical heterogeneity of SCD.

VALIDITY

The validity of a study refers to how accurately the study measured its findings. Internal validity refers to the adequacy with which the findings of the study can be used to draw inferences about the study population. On the other hand, external validity refers to the findings and how they relate to other populations. The lack of validity of a study results in systematic error.

Internal validity

The effect of selection bias

Selection bias can arise from errors in the study design that causes a difference in the way individuals are recruited into the study.

This was a hospital based study, with limited recruitment in the community. The SCD patients seen at Muhimbili were those who had been referred for investigation or had already been diagnosed as having SCD. Therefore, most of these patients had symptoms suggestive of SCD. This means that the patients that are at MNH may consist of those patients who have not died in early childhood and therefore have relatively mild disease, i.e. the "healthy survivors . However, this population may also present patients with disease severe enough to warrant seeking health care.

This study has described the rates and patterns of bacterial infections during hospitalisation in SCD patients. Although the SCD population is older, the evidence from this study reports that SPN is one of the most common cause of infection. Further research is required to determine the rates and patterns of bacterial infections in the younger age group and before antibiotic use.

The effect of information bias

Information bias refers to errors that are caused by collection of inaccurate information. This may be due to recall; reporting or observer bias. Recall bias may have arisen when interviewing children and adults about past events. It is likely that the caretakers of young children will have a more accurate recollection of events. Older children, adolescents and

adults may not have an accurate recollection of events that occurred in childhood. In order to address or reduce this error, it is necessary to include age in the multivariable analysis of association between outcome and exposure. Furthermore, designing a study where individuals are recruited at birth would allow the careful and accurate documentation of rates and risk factors of events.

Reporting bias may have resulted from individuals with SCD seeking health care or health care workers referring individuals to Muhimbili because of the knowledge that there was an ongoing study. During this study, attempts were made to reduce this error by not influencing the criteria for referral or hospitalisation. However, when exploring the role of malaria in SCD, it is acknowledged that SCD patients may be more likely to report and be investigated for malaria compared to non-sickle individuals.

The other issue that may have influenced the information that was collected was related to access to care. As a referral hospital there are some events which would be managed at home or in peripheral clinics. This is because there is a referral system which encourages patients to initially seek care at a health facility near their homes. Furthermore, patients may come to the hospital and receive care at casualty without being admitted. In these instances, clinical events would not be captured during the study. The other factor that may have caused information bias is physical and economic barriers to access to care. Individuals who live far away from the hospital and those who cannot afford to travel to come to hospital are less likely to come to the hospital.

Because of the potential for information bias, it is vital to reduce the factors that may

contribute to this bias. Health education should be provided to SCD individuals and their caretakers about the importance of attending clinic or contacting the health care providers about clinical events that occurred and were managed at home. The second intervention that can be put in place is to improve the care in the community by forming a network of care with health facilities in the periphery. In this way, clinical events will be identified and the appropriate intervention recommended. There is an urban demographic health surveillance system that is being established in Dar-es-Salaam and in addition, there are community based health workers that are providing care at home or in primary health care facilities for individuals with chronic illnesses such as HIV/AIDS, Tuberculosis and Diabetes Mellitus. As such, rather than set up a separate network of community-based and primary health care for SCD, it is recommended that care for SCD uses these existing networks.

The effect of losses to follow up

In a cohort study, individuals are followed up over a period of time to determine the rate and risk factors for clinical events. Patients may be lost to follow up because they drop out or stop coming to the hospital because they have died in the community and this information has not been collected. Alternatively, patients may move away from the study area or have physical or economic reasons that prevent them from coming to the hospital. In this study there were 209 patients who were lost to follow up. It is difficult to conclude whether the patients who were lost to follow-up are those with mild disease who stopped coming to the clinic because they did not feel there was any advantage or benefit from coming to the hospital. It may also reflect patients with severe disease who have not returned to clinic because they have died or have a severe event like stroke that makes it difficult for them to come to hospital. However, from the patients who were successfully traced, the suggestion was that there was no excess

of deaths or severe events in those who were lost to follow-up.

Confounding

Confounding occurs when an estimate between outcome and exposure is mixed up with a third factor. The third factor is associated with exposure and independently associated with the outcome.

Within this study, attempts were made to minimise confounding. In the design of the study and analysis of risk factors for key events of interest, confounding was controlled by restriction of the analysis only to individuals with SCD. Therefore, comparison was made between individuals who all had SCD. The other way that confounding was minimised was by multiple regressions analysis. Variables that were significant during univariable analysis were included in a multivariable regression model.

External validity - Generalisability of study findings

The external validity of the study refers to how the results from this study can be applied to other populations. The findings from this study reflect the spectrum of SCD in a hospital based-cohort in an area with low but variable malaria transmission. As it is in an urban setting, the socio-economic status, level of education and access to health care is relatively better than in a rural community. However, the rate of urbanisation in many African countries is high and there is considerable success in public health interventions such that there is an increasing number of SCD individuals who will be living in a setting similar to that where the study was conducted.

However, is acknowledged that these results may not be applicable to rural communities. It is therefore important for evidence to be gathered in these settings. In this case, the results from this study would be used as a reference point.

APPLICATION OF FINDINGS AND RECOMMENDATIONS FOR FURTHER WORK

Implications for policy

The results from this study have demonstrated the burden of disease and highlighted the rates and risk factors of hospitalization and mortality. Furthermore, it has provided initial evidence with regards to the role of malaria, bacterial, infections stroke and HbF in SCD. The aim is to start developing guidelines for the diagnosis and management of SCD patients at MNH. Within MNH, guidelines for prophylaxis and treatment of malaria in SCD in Dar-es-Salam have been proposed. Effective treatment for malaria during acute illness is of utmost importance to minimise morbidity and mortality due to malaria and its complications such as severe anaemia.

With regards to infection, it is recommended that SCD individuals who are below 5 years of age receive prophylaxis against infection with oral penicillin and pneumococcal vaccination. It is recommended that the introduction of prophylaxis should be accompanied by collecting further evidence to determine the most appropriate intervention.

During the course of the study, a programme for control and management of SCD was developed and submitted to the Ministry of Health. The aim is to establish a comprehensive programme for detection, management and prevention of SCD in Tanzania. In addition it will

aim to promote education and awareness in health professionals and the community regarding the magnitude of sickle cell anaemia in Tanzania and develop active research programmes that will provide an evidence-base for the detection, management and prevention of sickle cell anaemia in Tanzania.

It has been proposed that Muhimbili is established as the national centre for the comprehensive care and management of SCD. It will also act as the national referral diagnostic centre that will provide laboratory and other diagnostic facilities for the detection of SCD and its complications. Muhimbili will also support the establishment of regional centres that will provide comprehensive care for the prevention and management of SCD in other areas of Tanzania. The programme will explore appropriate strategies to introduce a minimum package of simple, evidence-based essential interventions amongst SCD patients at different levels of health care. This will include anti-malarials, antibiotics and guidelines for prompt diagnosis and treatment of acute clinical events. All this will be supported by active research programmes that will provide evidence for locally relevant policies and interventions.

Implications for research

It is recommended that molecular studies should be established in Tanzania to identify individuals with genotypes such as S/β^0Thalassemia and S/HPFH. This would improve the characterisation of the clinical spectrum of SS cohort. It is also recommended that further work should be done to define the clinical events in SCD in early childhood. This should be done by identifying SCD at birth by newborn screening. With the SCD birth cohort, we would understand the natural history of SCD in Tanzania in the first 5 years of life. This

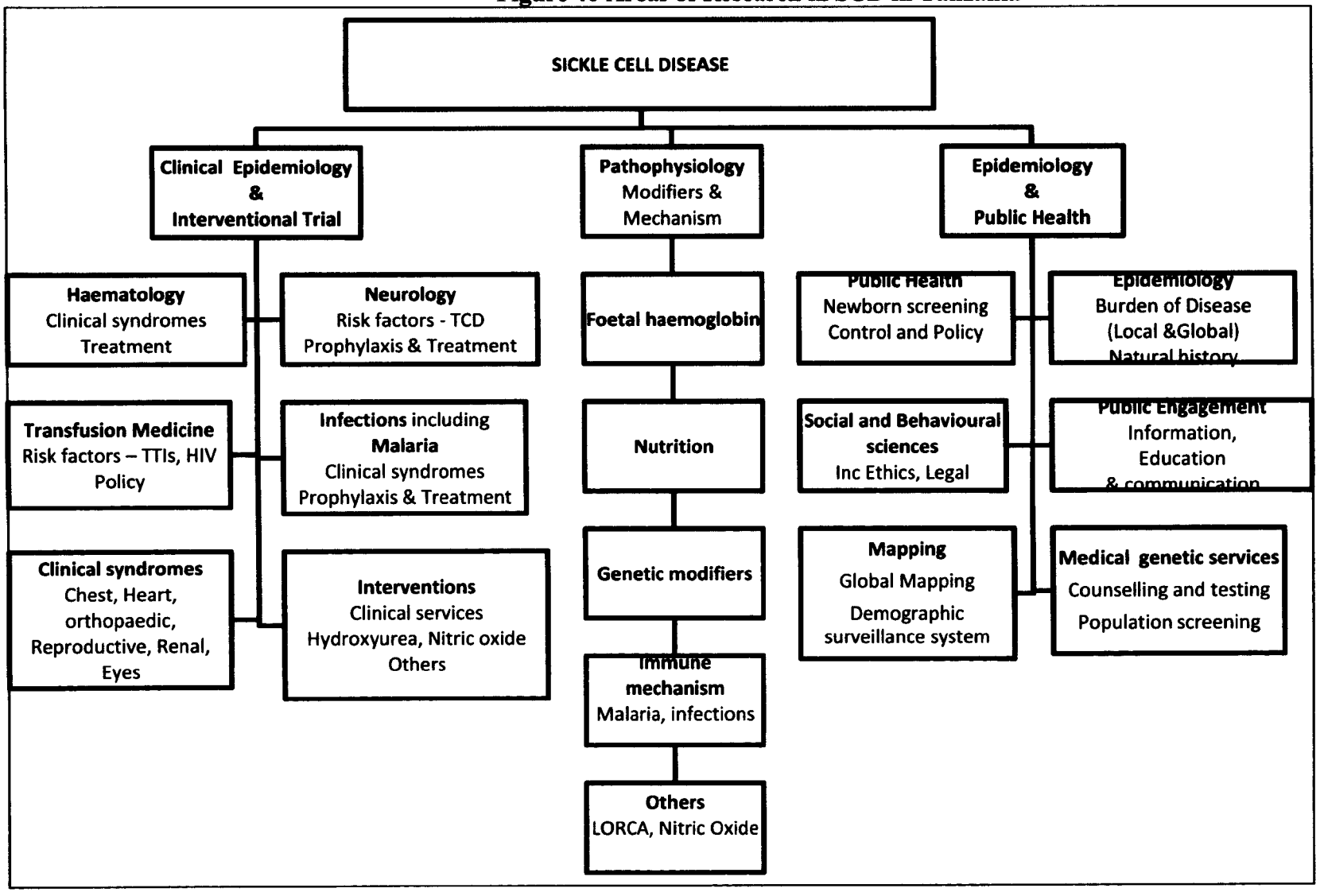
would allow us to determine the most appropriate interventions. Second, the logistical issues of NBS would be identified and used to guide and advise the ministry of health on how to introduce NBS within Tanzania. In addition, the birth cohort would allow the study of mechanisms of disease as well as understanding the genetic and environmental factors that determine disease severity.

With regards to stroke, it is recommended that all SCD individuals between 2-16 years should have measurement of CBFv by TCD. This should be done at least once a year, and those with high risk of stroke, determined by locally appropriate CBFv as well as other associated factors, should also have careful neurocognitive assessment and neuroimaging to further define neurologic events. Furthermore, the natural history of neurological events can be explored within the SCD birth cohort.

The role of foetal haemoglobin needs to be examined further by improving the phenotypic description of HbF, by more descriptive epidemiologic studies. This needs to be accompanied by careful genotyping to understand the genetic factors that determine Hb F levels.

This thesis forms part of a programme of work that is aimed at integrating research, clinical care and education in SCD. There are various research questions that cut across different disciplines, ranging from laboratory science aimed at understanding disease mechanisms to social sciences which seeks to understand the social and behavioural factors that influence disease and interventions. This is in partnership with clinical sciences focusing on morbidity and mortality at individual level and public health sciences which approaches the same issues at population level.

Figure 40 Areas of Research in SCD in Tanzania



CONCLUSION

This thesis has defined the spectrum of SCD in individuals at MNH. The study described the characteristics of SCD patients at recruitment and determined the rates and risk factors for hospitalisation and mortality. The role of malaria as a cause of morbidity and mortality was determined and the usefulness of CBFv and other factors in identifying risk factors for stroke was evaluated. Although the data were limited, information about the rates and patterns of bacterial infections was described. Pilot data was gathered on the spectrum of HbF which has important implications for interventions with agents such as Hydroxyurea.

This study has provided important evidence for developing policy for management of SCD at MNH which will also help to guide principles of care at other levels of health care; it has also highlighted important areas where there are gaps in knowledge which require further research.

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Appendices

APPENDIX 1 INFORMED CONSENT FORMS

MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES

P.O Box 65001 Dar es Salaam. Tanzania

INFORMED CONSENT FORM

Site name	Site number	Serial number (site specific)	Participant number				
MUCHS							

STUDY TITLE

The Sickle cell disease (SCD) Study

THE RESEARCHERS

Our research team consists of researchers from Muhimbili University College of health sciences and Muhimbili National Hospital. This includes doctors, nurses, laboratory technologists etc. We also work with other people from other universities and hospitals from all over the world including Kenya, and UK.

PURPOSE OF STUDY

Sickle cell disease (SCD) is an inherited condition that affects many people in Tanzania. As you know some people become very ill with frequent complications and risk of death while others remain relatively healthy despite the fact that both have SCD. We need to find out why this is so. If we can discover how some people do not have severe disease we may be able to use information to make a medicine that we can give to everyone or to develop ways to prevent them from developing severe complications of SCD.

The aim of the study is to describe the spectrum of clinical disease in patients with SCD attending and/or admitted to Muhimbili National Hospital (MNH). This will be the first step in trying to understand the causes of illness in SCD in Muhimbili and will focus on some areas which are relevant in determining treatment guidelines: malaria, infections, nutrition and stroke.

WHO WILL BE IN THE STUDY

All patients with proven or suspected SCD who attend the outpatient clinic or who are admitted to the paediatric or medical wards at MNH will be told about the study and given the option to participate in the study. We will also be asking relatives or people without SCD to participate in the study.

WHAT PARTICIPATION INVOLVES

We will ask you to attend the Haemotology clinic every 3 months where a doctor will ask you questions regarding the symptoms of SCD that you have had that may or may not have brought you to hospital to seek medical attention. You will also be asked questions about medical problems in the past, demographic, family history etc. This is because it is thought that SCD is thought to present with various problems in different people and levels of severity is determined by various factors. You will then be examined to determine and document any physical signs and investigations will be taken. A blood sample will also be collected – which is a normal practice for SCD patients attending the haematology clinics.

For patients who do not have SCD, the same procedures will be taken and you will be informed of the results of the tests. If you do not have SCD you will not be required to attend the clinic.

WHAT WILL HAPPEN TO THE SAMPLE

We will take a sample of blood from you or your child to do the tests that should be done on all patients attending the hospital, whether or not you are participating in the study. These tests will be done in the laboratory at Muhimbili. In addition, there will be some special tests that will be done which were not previously done because of lack of resources.

There will be some tests that will also be done as part of the research to improve our understanding of SCD. Some of these tests will be done immediately in Muhimbili while others will be done in a laboratory outside Tanzania.

We will also study the genetic material that is in the blood sample, and we would like to explain to you what that genetic material is. The genetic material is what makes everyone different from birth - in our height, in our looks, and in many other ways, including why people have different complications of SCD. We are studying the genetic material from some children who are very ill with SCD, and other children who are not ill with SCD, to try to discover what art of the genetic material that is responsible for this difference.

There will be some tests that take many years to develop. Therefore we are asking your permission to store the sample, including genetic material, so that we can perform other tests it in the future. We are also asking for permission to perform tests on samples which had been stored from your previous visits to hospital since your enrolment to the SCD study.

BENEFITS OF THE STUDY

There will be no direct benefit, financial or otherwise that you will get from this study. Your participation in the study will mean that questions about SCD, which is known to cause death, will be answered. This information will therefore be of use in understanding this condition and thus develop medicines and prevent complications by developing better ways of looking after patients with SCD in Tanzania and other places in the world.

POTENTIAL RISKS OF THE STUDY

There will be slight pain and discomfort at the site of blood collection. For the blood collection we will use equipment that is free of germs and that is made to reduce pain during pricking. There will be no new or special drug or treatment that you will receive.

If your child has any evidence that suggests increase risk of developing a complication, we will discuss the situation with you and we will offer increased frequency of follow up to try to prevent this causing any problem in the future. As new ways are developed to prevent these problems they will be discussed with you and offered to your child.

FREEDOM TO PARTICIPATE IN THE STUDY

We would like to stress that your participation in this study is strictly voluntary- it is your decision. Should you decide not to participate, it will not affect the treatment or management that you will receive from the hospital. In addition, at any time point during the study, decide that you do not wish to participate any further, you are free to end you participation, effective immediately. Any such decision will be respected and will not influence the quality of health care we will give you or your child.

If you miss some visits for any reason, but wish to come back into the study or attend the clinic, we will be ready to accept you back

CONFIDENTIALITY

All the information that you give will be confidential. You will be given a code number and we will be very careful with the information that we have collected. We will make absolutely sure that when we tell people about our findings on the genetic material, no-one will be able to discover that this genetic material came from you or your child."
Only the principal researcher or somebody authorized by him or her will be able to link the sample back to each participant

Please feel free to ask any questions about the information you have just been given or anything else to do with SCD and the care of your child.

There is more information in the form of leaflets, published papers that is available for you to learn more about SCD. Please feel free to take this home with you and you can contact us or ask us during your next visit for more information.

For the study, we will ask you to sign this paper to confirm that you have received this information and that you consent to participate in this study.

In case there is any further information that you require with regard to the study please ask to speak to Dr J Makani, Department of Haematology and Blood Transfusion, Muhimbili University College of Health Sciences. Tel: 0787 7680688 or any of the other investigators. If you ever have questions about your rights as a participant, you may call the chairman of the college research and Publications Committee, P. O. Box 65001. Tel: 2150302.

MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES

P.O Box 65001 Dar es Salaam. Tanzania

INFORMED CONSENT FORM

Site name	Site number	Serial number (site specific)	Participant number				
MUCHS							

Informed consent for participants:
I have read or I have been read the attached information regarding the SCD study in English / Swahili (please circle one), a language I speak fluently. I have also had an opportunity to discuss the study with the investigators and I am satisfied that I understand what the study involves.

I agree to or allow my child / children (listed below) to take part in this study:
(1)_____
(2)_____
(3)_____
(4)_____
(5)_____
(6)_____

Patients/ Parent / Guardian's
Signature or thumb print _____ **Date**_____

Parent / Guardian's Name: _____
(Please print name)

Witness
Signature (if caretaker cannot read)_____**Date**_____

Witness' Name: _____
(Please print name)

I certify that the above was explained verbally to the parent/guardian, and that s/he understands the nature and the purpose of the study and consents to the participation in the study of the above patients.

I have given them the opportunity to ask questions which have been answered satisfactorily.

Research Officer
Signature _____ **Date**_____

Research Officer Name: _____
(Please print name)

MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES/MUHIMBILI NATIONAL HOSPITAL

MAELEZO KUHUSU UTAFITI WA KUCHUNGUZA MADHARA YANAYOTOKEA KWA WATU WANOATHIRIWA NA UGONJWA WA "SICKLE CELL"

HOSPITALI YA TAIFA YA MUHIMBILI

Kama ujuavyo wewe / mtoto wako huhudhuria kliniki ya wagonjwa wenye maradhi ya upungufu wa damu mwilini ya "sickle cell" katika hospitali ya Taifa ya muhimbili.

Tatizo hili linatokea kwa watu wengi hapa nchini. Hali hii inamaanisha kwamba wakati mwingine inasababisha matatizo kama homa, maumivu mwilini na kupungukiwa damu. Baadhi ya matatizo kama haya yanaweza kuzuiliwa, na hii ndiyo maana mwanao analuja mara kwa mara kwa uchunguzi katika hospitali na wakati mwingine huhitaji matibabu.

Chuo cha Muhimbili (MUCHS) Dar-es-salaam kinashirikiana kikazi na hospitali ya taifa ya muhimbili. Virus hivi vina mamlaka ya kufanya uchunguzi wa magonjwa mbali mbali hapa Tanzania. Lengo la uchunguzi wote taifani, hospitalini na katika jamii, ni kusaidia kuelewa matatizo ya kiafya ya watu taifani, na kusaidia kuboresha huduma zilizoko, kukinga na kutibu maradhi. Kwa sababu tatizo hili la "sickle cell" linawaletea watu wengi, MUCHS inajishughulisha na tatizo hili ili kujua zaidi kuhusu hiyo hali na kutafuta njia mpya za kuboresha afya za watoto wa watu wazima wenye hali hii.

MAELEZO KUHUSU UCHUNGUZI

Ugonjwa wa "Sickle cell" ni ugonjwa unaojulikana kusababisha matatizo mbali mbali na hatari ya kifo. Madhumuni ya uchunguzi huu ni kujaribu kuangilia ni matatizo gani yanayowakabili wagonjwa wa "sickle cell" wanaohudhuria kliniki au wanaolazwa muhimbili.

Tutaangalia matatizo makubwa matatu: malaria, homa na sababu mbali mbali zinazoleta homa na kiarusi. Virus hivi vitatu tunaona ni muhimu tupate majibu ili tuweze kujua jinsi gani tutaweza kutibu au kuzuia matatizo haya.

UTARATIBU UTAKAOFUATWA

Tunawaomba wale wote wenye ugonjwa wa "sickle cell" wanaohudhuria kliniki na wanaolazwa hapa muhimbili washiriki kwenye utafiti huu.

Kipimo cha damu

Kipimo cha damu kitachukuliwa kwenye mkono badala ya kwenye kidole. Vipimo vitakavyofanywa ni vipimo ambavyo unahitaji kufanyiwa ukiwa na matatizo ya "sickle cell" hata kama hauchukiki kwenye utafiti. Kwa kawaida vipimo hivi vimekuwa kwifanyiki hapa muhimbili kwa sababu hospitali ilikuwa haina uwezo wa kufanya kwa sababu gharama yake ni kubwa. Majibu ya damu yatapatikana baada ya muda mfupi na baada ya hapo utaonwa na daktari kama ilivyo kawaida.

Kutoa maelezo

Utaulizwa maswali kuhusu matatizo yaliyokupata ambayo yalikufanya utafute huduma hospitalini. Pia utaulizwa maswali kuhusu matatizo ya afya yaliyokupata hapo zamani. Aidha utaomba utoe maelezo kuhusu mahali unapoishi, familia yako nk. Hii ni kwa sababu ugonjwa wa "'sickle cell'" unatokea na matatizo mbali mbali kwa watu tofauti. Baada ya hapo, utapimwa na daktari.

FAIDA YA UCHUNGUZI

Lengo kubwa la uchunguzi ni kutaka kujua matatizo yanaowapata watu wenye upungufu wa damu wa "sickle cell". Hakuna kishawishi chochote kitakachotolewa, na tunamatumaini kwamba watu watahimili lengo na kuruhusu watoto wao kwenye uchunguzi. Lengo kubwa ni kuleta maendeleo na kuboresha tiba kwa wenye "sickle cell" wa Dar-es-salaam na nchi nzima ya Tanzania. Hivyo basi utafiti utaleta manufaa kwa jamii nzima.

ATHARI YA UTAFITI

Madaktari na wachunguzi wanaoshiriki katika uchunguzi huu wamepata mafunzo maalum.

Utaratibu huu hauna madhara na hausababishi maumivu wala hero. Kipimo cha damu kitachukuliwa na wataalamu wa kutoa damu ili kupunguza maumivu kwa watoto.

Hakutakuwa na dawa wala matibabu ambayo ni mapya ambayo yatatolewa.

Ikiwa mwanao atapatikana kuwa ana madhara yanayoweza kuleta athari kubwa, tutajadiliana nawe na tutakuona kliniki mara kwa mara ili tuzuiye hili tatizo sikuza usoni. Njia mpya za kuzuia matatizo haya zinapogunduliwa tutazungumza nawe na kumtibu mwanao.

SIRI

Habari zote zitakazopatikana kutokana na uchunguzi hazitatolewa kwa mtu yeyote ambaye hahusiki na tiba za mgonjwa. Majina ya wahusika yataondolewa na hakuna mtu mwingine atakayeweza kuyapata, ama kuyatumia kwa shughuli nyingine.

KUJIHUSISHA NI HIYARI

Si lazima kwa yeyote atakayekuja hospitalini Muhimbili kuhusika kwenye uchunguzi. Kukataa kujihusisha katika uchunguzi hakutakuletea madhara yeyote. Mawaidha yafaayo, maelezo kuhusu vipimo na matibabu yatatolewa kwa wagonjwa wote ambao wanahusika ama hawahusiki katika uchunguzi. Vivyo hivyo, unaweza kujiondoa kwenye uchunguzi wakati wowote bila adhabu wala madhara.

Tafadhali kuwa huru kuuliza maswali kuhusu maelezo tuliyokupa, au kitu chochote kuhusu malezi ya mwanao. Tutakuomba uweke sahihi kwenye cheti cha makubaliano kwamba umeelewa haya maelezo na umekubali kushiriki kwenye utafiti huu.

Kama una swali lolote au unahitaji maelezo zaidi kuhusu utafiti huu, tafadhali omba kuongea na Dr J Mahani, idara ya damu, hospitali ya muhimbili. Simu: 0744 381 551 au wahusika wengine.

MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES

CHETI CHA MAKUBALIANO KUCHUNGUZA MADHARA YANAYOTOKEA KWA WATU WANOATHIRIWA NA UGONJWA WA "SICKLE CELL"

The Sickle Cell Disease Study.

Wahusika: Dr Julie Makani (PI), Dr F Kalokola, P Magesa, E Nkya

Cheti cha makubaliano kwa washiriki:

Nimesoma/nimesomewa maelezo kuhusu utafiti utakaoshirikisha watu wenye ugonjwa wa sickle cell kwa lugha nnayoelewa. (Kiswahili/kiingereza). Pia, nimepata nafasi ya kuongea na kuuliza maswali madaktari wanaofanya uchunguzi huu. Nimeridhika kwamba nnaelewa utafiti huu unahusu nini.

Ninakubali mimi/mtoto wangu (waliotajwa hapo chini) kushiriki kwenye utafiti huu:

(1)_____
(2)_____
(3)_____
(4)_____
(5)_____
(6)_____

Sahihi ya Mhusika/Mzazi/mlezi: _____ **Tarehe** _____

Jina la Mzazi au Mlezi: _____

(Tafadhali andika kwa herufi kubwa)

Ninashuhudia kwamba mzazi/mlezi/mgonjwa amepata maelezo yote juu ya utafiti huu na kwamba anaelewa nini na madhumuni ya kazi hii. Hivyo, amekubali kushiriki/watoto waliotajwa hapo juu kushiriki. Amepewa muda wa kutosha kufikiri na nafasi ya kuuliza maswali ambayo yamejibiwa kwa kuridhisha.

Sahihi ya Shahidi: _____ **Tarehe** _____

Jina la Shahidi: _____

(Tafadhali andika kwa herufi kubwa)

MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES

CHETI CHA MAKUBALIANO KUCHUNGUZA MADHARA YANAYOTOKEA KWA WATU WANOATHIRIWA NA UGONJWA WA "SICKLE CELL"

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Ninakubali mimi/mtoto wangu (waliotajwa hapo chini) kushiriki kwenye utafiti huu:

(1)_____
(2)_____
(3)_____
(4)_____
(5)_____
(6)_____

Sahihi ya Mhusika/Mzazi/mlezi: _____ **Tarehe** _____

Jina la Mzazi au Mlezi: _____

(Tafadhali andika kwa herufi kubwa)

Ninashuhudia kwamba mzazi/mlezi/mgonjwa amepata maelezo yote juu ya utafiti huu na kwamba anaelewa nini na madhumuni ya kazi hii. Hivyo, amekubali kushiriki/watoto waliotajwa hapo juu kushiriki. Amepewa muda wa kutosha kufikiri na nafasi ya kuuliza maswali ambayo yamejibiwa kwa kuridhisha.

Sahihi ya Shahidi: _____ **Tarehe** _____

Jina la Shahidi: _____

(Tafadhali andika kwa herufi kubwa)

APPENDIX II PROFORMAS

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL DISEASE STUDY – CONTROL

SCREENING

- Informed consent (N/Y) ..... |__|
- Today's Date (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| DATETOD

DEMOGRAPHIC HISTORY

- Name ..... |_____| NAME
- Sex (M/F) ..... |__| SEX
- Date of birth ..... |__|__|-|__|__|-|__|__| DOB
- Place of birth (District) ..... |_____| POB
- Tribe of mother ..... |_____| TRIBEMUM
- Tribe of father ..... |_____| TRIBEDAD

RESIDENCE INFORMATION

District ..... |_____|DISTR
Ward ..... |_____|WARD
Contact telephone number (N/Y) ..... |__|-|__|__|__|__|__|__|__|__|__|__|TEL

FAMILY and SOCIAL HISTORY

- Number of siblings (ALIVE) ..... |__|__|-|__|__|-|_____| SIBLIVE
- Number of siblings (DEAD) ..... |__|__|-|__|__|-|_____| SIBDEAD
- Siblings known to be affected by SCD (LIVE) ..... |__|__|-|_____| SIBSSCDLIVE
- Siblings known to be affected by SCD (DEAD) ..... |__|__|-|_____| SIBSSCDDEAD

GENERAL EXAMINATION

- Temperature ..... |__|__|.|__|__|^{0}C TEMP
- Pulse rate ..... |__|__|__| bpm PULSE
- Respiratory rate (per minute) ..... |__|__| breaths per min RESP
- Systolic blood pressure ..... |__|__|__|mmHg SBP
- Diastolic blood pressure ..... |__|__|__|mmHg DBP
- Oxygen saturation ..... |__|__|__|% SAT
- Length /Height ..... |__|__|__|.|__|cm HEIGHT
- Weight (kgs) ..... |__|__|__|.|__|kg WEIGHT
- Head circumference ..... |__|__|.|__|cm HC
- Mid-upper arm circumference ..... |__|__|.|__| cm MUA

PHYSICAL EXAMINATION

- Jaundice (**Nil / Tinge/Moderate/Deep**) ..... |__| JAUN
- Tonsillar enlargement (**Normal/Large**) ..... |__|TONSILS
- Other (Details) ..... |__|-|_____| OTHER EXAM

LABORATORY INVESTIGATIONS

SAMPLES TAKEN

- EDTA (N/Y) ..... |__| EDTA
- Serum separator (N/Y) ..... |__| SS
- Other (N/Y) ..... |_____| other

OVERALL ASSESSMENT

Issue/problem	PROB	Action	ACT
1.			

- Follow up appointment (months) ..... | | |APPOINTMENT

- Initials of investigator ..... |__|__|__|

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL DISEASE STUDY – ENTRY

SCREENING

- Informed consent (N/Y) ..... |__|
- Today's Date (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| DATETOD

DEMOGRAPHIC HISTORY

- Hospital ID number (HID) ..... |__|__|__|__|__|__| HID
- Name ..... |_____| NAME
- Sex (M/F) ..... |__| SEX
- Date of birth ..... |__|__|-|__|__|-|__|__| DOB
- Place of birth (District) ..... |_____| POB
- Name of mother ..... |_____| MUMNAME
- Tribe of mother ..... |_____| TRIBEMUM
- Name of Father ..... |_____| DADNAME
- Tribe of father ..... |_____| TRIBEDAD
- Respondent (Self/Parent/Guardian/Other) ..... |__| - |_____| RESPON

RESIDENCE INFORMATION

District ..... |_____|DISTR
Division ..... |_____|DIVISION
Ward ..... |_____|WARD
Ten Cell leader ..... |_____|TEN CELL LEAD
Nearest School ..... |_____|**SCHOOL**
Nearest Health post ..... |_____|**HEALTHPOST**
Nearest Bus Stage ..... |_____|**BUSSTOP**
Contact telephone number (N/Y) ..... |__| - |__|__|__|__|__|__|__|__|__|__|TEL

GENERAL EXAMINATION

- Temperature ..... |__|__|. |__|__|^{0}C TEMP
- Pulse rate ..... |__|__|__| bpm PULSE
- Respiratory rate (per minute) ..... |__|__| breaths per min RESP
- Systolic blood pressure ..... |__|__|__|mmHg SBP
- Diastolic blood pressure ..... |__|__|__|mmHg DBP
- Oxygen saturation ..... |__|__|__|% SAT
- Length /Height ..... |__|__|__|.|__|cm HEIGHT
- Weight (kgs) ..... |__|__|__|.|__|kg WEIGHT
- Head circumference ..... |__|__|.|__|cm HC
- Mid-upper arm circumference ..... |__|__|.|__| cm MUA

LABORATORY INVESTIGATIONS

SAMPLES TAKEN (Please tick)

2mls EDTA		2mls serum separator	
Blood slide		Urine	
Other			

- Initials of investigator ..... |__|__|__|

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL STUDY – ENTRY

PERSONAL HISTORY

- Age at diagnosis (years -months) ..... |__|__| - |__|__| AGED
- How were you referred (Self/Illness/Other) ..... |__| REF

FAMILY and SOCIAL HISTORY

- Number of siblings (Full/Half) ..... |__|__|-|__|__|-|_____| SIBN
- Siblings affected by SCD (Untested/Tested - Full/Half) ..... |__|-|__|__|-|__|__|-|_____| SIBSCDN
- Education (N/Y – Grade: Kindergaden/Primary/Secondary/Tertiary/Not applicable) ..... |__| - |__|__| EDU
- Academic performance (Good/Average/Poor/Not applicable) ..... |__|-|__|__|ACADEM
- Employment (Not Applicable/Description) ..... |__|__|-|_____| EMPLOY
- Marital status (/Not Applicable/Single/Married/ Widow/Common law)....|__|__|--|_____| MARIT

MEDICAL HISTORY

Previous Hospital Admissions

- Have you/your child ever been admitted to hospital (N/Y) ..... |__| ADM
- If yes, please give history of number of admissions in lifetime and details of last admission

Lifetime(#/<5/ 5-10/ 10+) NUM ADMIT LIFE	Number in last 12 mths NUM ADMIT 12	Date (mm-yy) and Place DATE PLACE	Cause of last admission PROBLEM

- **Have you/your child had any of the Sickle cell crises below that required OPD attendance or admission ? If yes, what was the total number in last 12 months and what was the date of last crisis ?**

	N/Y	Date of last episode	Number in past 12 months
Blood transfusion	|__| BLOOD	|__|__|-|__|__| BLOOD DATE	|__|__| BLOOD NUM
Dactylitis	|__| DAC	|__|__|-|__|__| DAC DATE	|__|__| DAC NUM
Painful crises	|__| PAIN	|__|__|-|__|__| PAIN DATE	|__|__| PAIN NUM
fever for >3 days	|__| FEVER	|__|__|-|__|__| FEVER DATE	|__|__| FEVER NUM
Acute Chest illness	|__| CHEST	|__|__|-|__|__| CHEST DATE	|__|__| CHEST NUM
Convulsion	|__| FITS	|__|__|-|__|__| FITS DATE	|__|__| FITS NUM
Neurological deficit	|__| NEURDEF	|__|__|-|__|__| NEURDEF DATE	|__|__| NEURODEF NUM
Leg ulcers	|__| L/ULCER	|__|__|-|__|__| L/ULCER DATE	|__|__| L/ULCER NUM
Priapism	|__| PRIAP	|__|__|-|__|__| PRIAP DATE	|__|__| PRIAP NUM
Other	OTHER	|__|__|-|__|__| OTHER DATE	|__|__| OTHER NUM

- **Medical history of other illness (N/Y)** ..... |__| MHOTHER
- Details (Age at onset, Duration, Outcome) .....
  ..... MH OTHER DETAILS
- **Other related information of interest to attending physician** ..... OTHER

CURRENT SYMPTOM HISTORY

- Are you well today? (Y/N – If No, answer below) ..... |__| CURRENT STATUS
- Febrile illness > 3 days (N/Y –details) ..... |__| -|_____| CURR FEV
- Painful episode >3days (N/Y –details) ..... |__| -|_____| CURR PAIN
- Anaemia (Y/N –details) ..... |__| -|_____| CURR ANAEMIA
- Jaundice is worsening (N/Y-details) ..... |__| -|_____| CURR JAUNDICE
- Acute Chest illness (Y/N –details) ..... |__| -|_____| CURR CHEST
- CNS event (N/Y –details) ..... |__| -|_____| CURR CNS
- Other symptom (N/Y –details) ..... |__| -|_____| CURR OTHER

SCD Study No|__|__|__|__|

CURRENT MEDICATION

- Folic acid (N/Y – dose per day) ..... |__|-|_____| FA
- Chloroquine (N/Y – dose per week) ..... |__|-|_____| CQ
- Iron (N/Y - dose) ..... |__|-|_____| IRON
- Other (N/Y - dose) ..... |__|-|_____| OTHR MED

IMMUNISATION HISTORY (if less than 5 years)

- Has your child completed childhood immunisation? (N/Y-card seen-details) .... |__|-|__|-|_____| VACC
- Has your child received any other immunisation ..... |__|-|__|__|/|__|__|/|__|__|| OTHER VACC

REPRODUCTIVE HISTORY for female teenagers/adults

- Menarche (N/Y – age) ..... |__|-|__|__|MEN AGE
- Menstruation (Regular/Irregular - details) ..... |__| -|_____|MENSES REG
- Pregnant (N/Y – EDD) ..... |__|-|__|__|/|__|__|/|__|__||PREG EDD
- Previous pregnancy (Y/N/NA - details) ..... |__|-|_____|PREV PREG

PHYSICAL EXAMINATION

- Jaundice (Nil / Tinge/Moderate/Deep) ..... |__| JAUN
- Pallor (N/Y) ..... |__| PALL
- Bossing (N/Y) ..... |__| BOSS
- Prognathia (N/Y) ..... |__| PROGN
- Gingival hypertrophy (N/Y) ..... |__|GING HYPER
- Tonsillar enlargement (Normal/Large) ..... |__|TONSILS
- Lymph nodes (N/Y - Details) ..... |__|-|_____| NODES
- Skin (Normal/Abnormal -details) ..... |__|-|_____| SKIN
- Other (Details) ..... |__|-|_____| OTHER EXAM

CHEST (CARDIOVASCULAR & RESPIRATORY SYSTEM)

- Heart sounds (Regular / Irregular) ..... |__|-|_____| RHYTM
- Murmur (N/Y – details) ..... |__|-|_____| MURM
- Chest auscultation (Normal / Abnormal) ..... |__|-|_____|AUSC - DET

ABDOMEN

- Spleen palpable (Size) ..... |__|__|cm SPLEEN
- Liver palpable [Size - tenderness (T/N)] ..... |__|__|cm-|__| HEP

MUSKULOSKELETAL SYSTEM

- Upper limbs (Normal/Abnormal -details) ..... |__|-|_____| UL
- Lower limbs (Normal/Abnormal -details) ..... |__|-|_____| LL
- Joints (Normal/Abnormal -details) ..... |__|-|_____| JOINTS
- Vertebrae (Normal/Abnormal -details) ..... |__|-|_____| VERT

NEUROLOGICAL SYSTEM (if yes to any below, fill in CNS event form)

- Comprehension (Normal/Abnormal -details) ..... |__|-|_____|COMPREH
- Cranial nerves (Normal/Abnormal -details) ..... |__|-|_____|CRNL NS
- Peripheral nerves (Normal/Abnormal -details) ..... |__|-|_____|PERI NS
- Reflexes (Normal/Abnormal -details) ..... |__|-|_____|REFLEX
- Gait (Normal/Abnormal -details) ..... |__|-|_____|GAIT

SCD Study No|__|__|__|

RESULTS (fill in shaded results)			
Date of test	Test	Result	Comment
HAEMATOLOGY			
FBC	White blood cells	10^9/L	
	Neutrophils	10^9/L	
	Lymphocytes	10^9/L	
	Haemoglobin	g/dL	
	Red blood cell count		
	MCV	Fl	
	MCH	pg	
	MCHC	g/dl	
	RDW		
	Platelets	10^9/L	
	ESR	Mm/hr	
	Reticulocyte	%	
	HB electrophoresis		
Malaria	Rapid test (pos/neg)		
	Thick film	Mps/200wbc	
BIOCHEMISTRY	Createnine	µmol/L	
	Sodium	mmol/L	
	Potassium	mmol/L	
	Chloride	mmol/L	
	AST	U/L	
	ALP	U/L	
	Bilirubin – T	µmol/L	
	Bilirubin - D	µmol/L	
	LDH		
URINE	Blood / protein		
	Glucose / nitrite		
HB QUANTIFICATION	Hb F		
	Hb A2		
	Hb S		
MICROBIOLOGY	Hepatitis B		
	Hepatistis C		
	Culture (site/result)		

OVERALL ASSESSMENT			
Issue/problem	PROB	Action	ACT
1.			
2.			
3.			

- Follow up appointment (months).....|__|__|APPOINTMENT
- Date of next scheduled visit (DD-MM-YY).....|__|__|-|__|__|-|__|__| DATE NEXT VISIT

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL DISEASE STUDY – FOLLOW UP

SCREENING

- Today's Date (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| **DATE TOD**
- Reason for visit (**R**outine, **F**ollow up, **A**cute event , **O**ther)..... |__| **REASON VIS**

DEMOGRAPHIC HISTORY

- Hospital ID number (HID)..... |__|__|__|__|__|__|HID
- Name..... |__| NAME
- Sex (M/F)..... |__| SEX
- Date of birth..... |__|__|-|__|__|-|__|__| DOB
- Name of mother ..... |__| MUMNAME
- Date of entry (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| **DATE ENT**
- Date of last OPD visit (DD-MM-YY)..... |__|__|-|__|__|-|__|__| **DATELV**

RESIDENCE INFORMATION

- **Change in personal details, If Y, complete a new form (N/Y) ..... |__|CHANGE IN DETAILS**
  - Residency/Caretaker/Other information. ..... |__| **RESIDFORM**
- Contact telephone number if available (N/Y)..... |__|-|__|__|__|__|__|__|__|__|__|__|PHONE

GENERAL EXAMINATION

- Temperature ..... |__|__|. |__|__|^{0}C TEMP
- Pulse rate..... |__|__|__| bpm PULSE
- Respiratory rate (per minute)..... |__|__| breaths per min RESP
- Systolic blood pressure ..... |__|__|__|mmHg SBP
- Diastolic blood pressure..... |__|__|__|mmHg DBP
- Oxygen saturation ..... |__|__|__|% SAT
- Length /Height ..... |__|__|__|.|__|cm HEIGHT
- Weight (kgs)..... |__|__|__|.|__|kg WEIGHT
- Head circumference ..... |__|__|.|__|cm HC
- Mid-upper arm circumference ..... |__|__|.|__| cm MUAC

LABORATORY INVESTIGATIONS

SAMPLES TAKEN (Please tick)

2mls EDTA		2mls serum separator	
Blood slide		Urine	
Other			

EXIT

- Excluded from study (N/Y) ..... |__| **EXCLUDED**
- Refused consent/Unable to obtain sample/other..... **REASON**

- Initials of investigator ..... |__|__|__|

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL STUDY – FOLLOW UP

CURRENT SYMPTOM HISTORY

- Are you well today? (Y/N – If No, answer below) ..... |__| CURRENT STATUS
- Febrile illness > 3 days (N/Y –details) ..... |__| -|_____|CURR FEV
- Pain (N/Y –details) ..... |__| -|_____|CURR PAIN
- Anaemia (Y/N –details) ..... |__| -|_____|CURR ANAEMIA
- Jaundice (N/Y-details) ..... |__| -|_____|CURR JAUNDICE
- Acute Chest illness (Y/N –details) ..... |__| -|_____|CURR CHEST
- CNS event (N/Y –details) ..... |__| -|_____|CURR CNS
- Other symptom (N/Y –details) ..... |__| -|_____|CURR OTHER

CURRENT MEDICATION

- Folic acid (N/Y – dose per day) ..... |__|-|_____| FA
- Chloroquine (N/Y – dose per week) ..... |__|-|_____| CQ
- Iron (N/Y - dose) ..... |__|-|_____| IRON
- Other ..... |__|-|_____| OTHR MED

MEDICAL HISTORY

Since the last visit, have you/your child had any of the Sickle cell episodes below that required OPD attendance or admission ? If yes, what was the total number since last visit and the date of last episode ?

	N/Y	Date of last episode	Number in past 12 months
Blood transfusion	|__| BLOOD	|__|__|-|__|__| BLOOD DATE	|__|__| BLOOD NUM
Dactylitis	|__| DAC	|__|__|-|__|__| DAC DATE	|__|__| DAC NUM
Painful crises	|__| PAIN	|__|__|-|__|__| PAIN DATE	|__|__| PAIN NUM
fever for >3 days	|__| FEVER	|__|__|-|__|__| FEVER DATE	|__|__| FEVER NUM
Acute Chest illness	|__| CHEST	|__|__|-|__|__| CHEST DATE	|__|__| CHEST NUM
Convulsion	|__| FITS	|__|__|-|__|__| FITS DATE	|__|__| FITS NUM
Neurological deficit	|__| NEURDEF	|__|__|-|__|__| NEURDEF DATE	|__|__| NEURODEF NUM
Leg ulcers	|__| L/ULCER	|__|__|-|__|__| L/ULCER DATE	|__|__| L/ULCER NUM
Priapism	|__| PRIAP	|__|__|-|__|__| PRIAP DATE	|__|__| PRIAP NUM
Other	OTHER	|__|__|-|__|__| OTHER DATE	|__|__| OTHER NUM

- **Medical history of other illness (N/Y)** ..... |__| MHOTHER
- Details (Age at onset, Duration, Outcome) .....
  ..... |_____|MH OTHER DETAILS
- **Other related information of interest to attending physician** .....OTHER

IMMUNISATION HISTORY (if less than 5 years)

- Has your child completed childhood immunisation? (N/Y-card seen-details) .... |__|-|__|-|_____| VACC
- Has your child received any other immunisation..... |__|-|__|__|/|__|__|/|__|__|| OTHER VACC

REPRODUCTIVE HISTORY for female teenagers/adults

- Menarche (N/Y – age) ..... |__|-|__|__|MEN AGE
- Menstruation (Regular/Irregular - details) ..... |__| -|_____|MENSES REG
- Pregnant (N/Y – EDD)..... |__|-|__|__|/|__|__|/|__|__||PREG EDD
- Previous pregnancy (Y/N/NA - details)..... |__|-|_____|PREV PREG

SCD Study No|__|__|__|__|

PHYSICAL EXAMINATION

- Jaundice (**Nil / Tinge/Moderate/Deep**) ..... |__| **JAUN**
- Pallor (N/Y) ..... |__| **PALL**
- Bossing (N/Y) ..... |__| **BOSS**
- Prognathia (N/Y) ..... |__| **PROGN**
- Gingival hypertrophy (N/Y) ..... |__|**GING HYPER**
- Tonsillar enlargement (**Normal/Large**) ..... |__|**TONSILS**
- Lymph nodes (N/Y - Details) ..... |__|-|_____| **NODES**
- Skin (Normal/Abnormal -details) ..... |__|-|_____| **SKIN**
- Other (Details) ..... |__|-|_____| **OTHER EXAM**

CHEST (CARDIOVASCULAR & RESPIRATORY SYSTEM)

- Heart sounds (**Regular / Irregular**) ..... |__|- |_____|**RHYTM**
- Murmur (N/Y – details) ..... |__|-|_____| **MURM**
- Chest auscultation (Normal / Abnormal)..... |__|- |_____|**AUSC - DET**

ABDOMEN

- Spleen palpable (Size)..... |__|__|cm **SPLEEN**
- Liver palpable [Size - tenderness (T/N)]..... |__|__|cm-|__| **HEP**

MUSKULOSKELETAL SYSTEM

- Upper limbs (Normal/Abnormal -details)..... |__|-|_____| **UL**
- Lower limbs (Normal/Abnormal -details) ..... |__|-|_____| **LL**
- Joints (Normal/Abnormal -details) ..... |__|-|_____| **JOINTS**
- Vertebrae (Normal/Abnormal -details)..... |__|-|_____| **VERT**

NEUROLOGICAL SYSTEM (if yes to any below, fill in CNS event form)

- Comprehension (Normal/Abnormal -details) ..... |__|-|_____|**COMPREH**
- Cranial nerves (Normal/Abnormal -details) ..... |__|-|_____|**CRNL NS**
- Peripheral nerves (Normal/Abnormal -details)..... |__|-|_____|**PERI NS**
- Reflexes (Normal/Abnormal -details)..... |__|-|_____|**REFLEX**
- Gait (Normal/Abnormal -details)..... |__|-|_____|**GAIT**

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL DISEASE STUDY – IN PATIENT

SCREENING

- Informed consent (N/Y) ..... |__|
- Today's Date (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| **DATE TOD**

DEMOGRAPHIC HISTORY

- Hospital ID number (HID) ..... |__|__|__|__|__|__|__| **HID**
- Name ..... |_____| **NAME**
- Sex (M/F) ..... |__| **SEX**
- Date of birth ..... |__|__|-|__|__|-|__|__| **DOB**
- Place of birth (District) ..... |_____| **POB**
- Name of mother ..... |_____| **MUMNAME**
- Date of entry (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| **DATE ENT**
- Date of last visit (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| **DATELV**
- Respondent (Self/Parent/Guardian/Other) ..... |__| -|_____| **RESPON**

RESIDENCE INFORMATION

District ..... |_____|**DISTR**

Contact telephone number if available (N/Y) ..... |__|-|__|__|__|__|__|__|__|__|__|__|**PHONE**

GENERAL EXAMINATION

- Temperature ..... |__|__|. |__|__|^{0}C **TEMP**
- Pulse rate ..... |__|__|__| bpm **PULSE**
- Respiratory rate (per minute) ..... |__|__| breaths per min **RESP**
- Systolic blood pressure ..... |__|__|__|mmHg **SBP**
- Diastolic blood pressure ..... |__|__|__|mmHg **DBP**
- Weight (kgs) ..... |__|__|__|.|__|kg **WEIGHT**

LABORATORY INVESTIGATIONS

SAMPLES TAKEN (Please tick)

2mls EDTA		2mls serum separator	
Blood slide		Urine	
Blood Culture		Other	

CURRENT SYMPTOM HISTORY

- Febrile illness > 3 days (N/Y –details) ..... |__| -|_____|**CURR FEV**
- Pain (N/Y –details) ..... |__| -|_____|**CURR PAIN**
- Anaemia (N/Y –details) ..... |__| -|_____|**CURR ANAEMIA**
- Jaundice (N/Y-details) ..... |__| -|_____|**CURR JAUNDICE**
- CNS event (N/Y –details) ..... |__| -|_____|**CURR ANAEMIA**
- Dactylitis (N/Y –details) ..... |__| -|_____|**CURR DACTY**
- Acute Chest illness (N/Y –details) ..... |__| -|_____|**CURR CHEST**
- Fits (N/Y –details) ..... |__| -|_____|**CURR FITS**
- Additional history .....

..... **CURR OTHER**

CURRENT MEDICATION

- Folic acid (N/Y – dose per day) ..... |__|-|_____| **FA**
- Chloroquine (N/Y – dose per week) ..... |__|-|_____| **CQ**
- Iron (N/Y - dose) ..... |__|-|_____| **IRON**
- Other ..... |_____| **OTHR MED**
- Other ..... |_____| **OTHR MED**

SCD Study No|__|__|__|__|

MEDICAL HISTORY

- **Since the last admission, have you/your child had any of the Sickle cell crises below that required OPD attendance or admission ?** ..... |__| SCD CRISIS
- **If yes, what was the total number since last visit (OPD/IPD) and what was the date of last crisis ?**

	N/Y	Date of last crises (MM-YY)	Comments
Blood transfusion	|__| BLOOD	|__|__|-|__|__| BLOOD DATE	
Dactylitis	|__| DAC	|__|__|-|__|__| DAC DATE	
Painful crises	|__| PAIN	|__|__|-|__|__| PAIN DATE	
fever for >3 days	|__| FEVER	|__|__|-|__|__| FEVER DATE	
Acute Chest illness	|__| CHEST	|__|__|-|__|__| CHEST DATE	
Convulsion	|__| FITS	|__|__|-|__|__| FITS DATE	
Neurological deficit	|__| NEURDEF	|__|__|-|__|__| FITS DATE	
Leg ulcers	|__| L/ULCER	|__|__|-|__|__| L/ULCER DATE	
Priapism	|__| PRIAP	|__|__|-|__|__| PRIAP DATE	
	|__| OTHER	|__|__|-|__|__| OTHER DATE	

- **Medical history of other illness (N/Y)** ..... |__| MHOTHER
- Details (Age at onset, Duration, Outcome) ..... MH OTHER DETAILS
- **Other related information of interest to attending physician** ..... OTHER

PHYSICAL EXAMINATION

- Jaundice (**Nil / Tinge/Moderate/Deep**) ..... |__| JAUN
- Pallor (N/Y) ..... |__| PALL
- Bossing (N/Y) ..... |__| BOSS
- Prognathia (N/Y) ..... |__| PROGN
- Gingival hypertrophy (N/Y) ..... |__|GING HYPER
- Tonsillar enlargement (**Normal/Large**) ..... |__|TONSILS
- Lymph nodes (N/Y - Details) ..... |__|-|_____| NODES
- Skin (Normal/Abnormal -details) ..... |__|-|_____| SKIN
- Other (Details) ..... |__|-|_____| OTHER EXAM

CHEST (CARDIOVASCULAR & RESPIRATORY SYSTEM)

- Heart sounds (**Regular / Irregular**) ..... |__| RHYTM
- Murmur (N/Y – details) ..... |__|-|_____| MURM
- Auscultation (Normal / Abnormal) ..... |__|-|_____|AUSC - DET

ABDOMEN

- Spleen palpable (Size) ..... |__|__|cm SPLEEN
- Liver palpable [Size - tenderness (T/N)] ..... |__|__|cm-|__| HEP

MUSKULOSKELETAL SYSTEM

- Upper limbs (Normal/Abnormal -details) ..... |__|-|_____| UL
- Lower limbs (Normal/Abnormal -details) ..... |__|-|_____| LL
- Joints (Normal/Abnormal -details) ..... |__|-|_____| JOINTS
- Vertebrae (Normal/Abnormal -details) ..... |__|-|_____| VERT

NEUROLOGICAL SYSTEM (if yes to any below, fill in CNS event form)

- Comprehension (Normal/Abnormal -details) ..... |__|-|_____|COMPREH
- Cranial nerves (Normal/Abnormal -details) ..... |__|-|_____|CRNL NS
- Peripheral nerves (Normal/Abnormal -details) ..... |__|-|_____|PERI NS
- Reflexes (Normal/Abnormal -details) ..... |__|-|_____|REFLEX
- Gait (Normal/Abnormal -details) ..... |__|-|_____|GAIT

SCD Study No|__|__|__|

WORKING DIAGNOSIS

Issue/problem	PROB	Action	ACT
1.			
2.			
3.			
4.			
5.			

PROGRESS IN WARD

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OVERALL ASSESSMENT

Issue/problem	PROB	Action	ACT
1.			
2.			
3.			
4.			
5.			

- Date of Discharge/Death ..... |__|__|-|__|__|-|__|__| DISCHARGE DATE
- Diagnosis on Discharge ..... |_____|DISCHARGE DIAGNOSES
- Follow up appointment (months) ..... |__|__|APPOINTMENT
- Date of next scheduled visit (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| DATE NEXT VISIT
- Other related information of interest to attending physician .....OTHER

EXIT

- Excluded from study (N/Y) ..... |__| EXCLUDED
- Refused consent/Unable to obtain sample/other..... REASON

- Initials of investigator ..... |__|__|__|

SCD Study No|__|__|__|

WORKING DIAGNOSIS

Issue/problem	PROB	Action	ACT
1.			
2.			
3.			
4.			
5.			

PROGRESS IN WARD

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OVERALL ASSESSMENT

Issue/problem	PROB	Action	ACT
1.			
2.			
3.			
4.			
5.			

- Date of Discharge/Death..... |__|__|-|__|__|-|__|__| DISCHARGE DATE
- Diagnosis on Discharge ..... |_____| DISCHARGE DIAGNOSES
- Follow up appointment (months)..... |__|__| APPOINTMENT
- Date of next scheduled visit (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| DATE NEXT VISIT
- Other related information of interest to attending physician ..... OTHER

EXIT

- Excluded from study (N/Y) ..... |__| EXCLUDED
- Refused consent/Unable to obtain sample/other..... REASON

- Initials of investigator ..... |__|__|__|

SCD Study No|__|__|__|__|

PROFORMA FOR SICKLE CELL DISEASE STUDY – CNS EVENT

SCREENING

- Today's Date (DD-MM-YY) ..... |__|__|-|__|__|-|__|__| **DATE TOD**
- Name ..... |_____| **NAME**
- Sex (M/F) ..... |__| **SEX**
- Date of birth ..... |__|__|-|__|__|-|__|__| **DOB**
- Name of mother ..... |_____| **MUMNAME**

TYPE OF EVENT

- Convulsion (N/Y) ..... |__| **CONVUL**
- Stroke (N/Y) ..... |__| **STROKE**
- Transient ischaemic attack (N/Y) ..... |__| **TIA**
- Cranial nerve lesion (N/Y) ..... |__| **CRAN NERVE**
- Mental/personality change (N/Y) ..... |__| **PERSON CHANGE**
- Difficulty in walking (N/Y) ..... |__| **S CORD**
- Other (not headache) (N/Y) .....
- ..... |__|-|_____| **OTHER**

PRECIPITATING EVENT

- Known/Uknown ..... |__| **KNOWN**
- Fever (N/Y) ..... |__| **PPT FEVER**
- Painful crisis (N/Y) ..... |__| **PPT PAIN**
- Infection (N/Y) ..... |__| **PPT INF**
- General anaesthetic (N/Y) ..... |__| **PPT GA**
- Pregnancy (N/Y) ..... |__| **PPT PREG**
- Anaemia symptoms (N/Y) ..... |__| **PPT ANAE**
- Trauma (N/Y) ..... |__| **PPT TRAUMA**
- Other (N/Y) ..... |__| **CONVUL**

NEUROLOGIC HISTORY

Convulsions

- Has child ever had a convulsion? (N/Y-Type-) ..... |__|-|__|__| **CONVUL**
  - **G**: Generalized **P**: Partial **PG**: Partial becoming generalized
- If Partial, side involved (**R/L**) ..... |__| **SIDE**
- Duration (start to restoration) (hours/minutes) ..... |___|___|___| : |___|___| **EVENT DUR**
- Loss of consciousness (N/Y) ..... |___| **EVENT LOSS OF CON**
- Body tone (tonic/flaccid) ..... |__| **EVENT TONE**
- Was it associated with fever (N/Y) ..... |__| **FEB CON**
- Ever had a fit NOT associated with a fever (N/Y) ..... |__| **NON-FEB CON**
- Recurrent fits (N/Y) ..... |__| **REC FIT**
- Date of first fit ..... |__|__|-|__|__|-|__|__| **CON DATE 1**
- Date of last fit ..... |__|__|-|__|__|-|__|__| **CON DATE 2**
- Number of fits in past 12 months ..... |__|__|__| **CON NUM**
- Are you/your child on anticonvulsants (N/Y) ..... |__|-|_____| **ANTI CON**

SCD Study No|__|__|__|__|

Neurological deficit

- Have you/your child ever noticed weakness of part of body ?(N/Y - Type).....|__|-|__| **WEAK**
  - **G:** Generalised **M**: Monoparesis **H**: Hemiparesis **P**: Paraparesis F: Facial weakness
- If Partial, Limb involved.....|__| **LIMB**
- Was it associated with loss of consciousness (N/Y).....|__| **CONC**
- Date of weakness .....|__|__|-|__|__|-|__|__| **DAT WEAK**
- How long did weakness last?.....|__|__|__| **Days DUR WEAK**

Sleep hypopnoea

- Noisy breathing / snoring at night (N/Y-Duration) .....|__|-|__|__|months **OSA1**
- Wake up at night due to difficulty in breathing (N/Y-Duration).....|__|-|__|__|months **OSA2**

FAMILY HISTORY

- Family History of fits (N/Y).....|__| **FH FITS**
- Family History of febrile convulsions/epilepsy (N/Y-F/E).....|__| **FH FEB EPI**

CRANIAL NERVES

- Handedness (Right/Left/Undetermined).....|__|-|_____|**HD**
- Sense of smell (Normal/Abnormal).....|__|-|_____|**SM**
- Eye movements (Full/Impaired - squint/nystagmus).....|__|-|_____|**EM**
- Pupils (Normal/Abnormal - reaction to light).....|__|-|__|-|_____|**PUP**
- Facial Weakness (No/Yes).....|__|-|_____|**FW**
- Uvula movement (Normal/Abnormal- Left/Right deviation).....|__|-|_____|**UVM**
- Tonsillar enlargement (Normal/Large).....|__| **TONS**
- Tongue deviation (No/Yes-Left/Right deviation).....|__|-|_____|**TONG**
- Other cranial nerve lesion (N/Y).....|__|-|_____|**OCN**

PERIPHERAL NERVES

	Upper Limbs	Lower Limbs
Bulk (Normal/Wasted-site)	**ULB**	**LLB**
Tone (Normal/Increased/Decreased)	**ULT**	**LLT**
Power (Normal/Decreased)	**ULP**	**LLP**

Reflexes	Left		Right	
Biceps (0/+/++/+++)		**BL**		**BR**
Triceps (0/+/++/+++)		**TL**		**TR**
Knee (0/+/++/+++)		**KL**		**KR**
Ankle (0/+/++/+++)		**AL**		**AR**
Plantars (Up/Down/Equivocal)		**PL**		**PR**

- Cerebellar function (Normal/Abnormal) .....|__|-|_____|**CER**
- Gait (Abnormal/Normal) .....|__|-|_____|**GAIT**
- Foqs sign **(N/Y)**.....|__|-|_____|**FOQ**
- Sensory deficit (N/Y).....|__|-|_____|**SEN**

OVERALL ASSESSMENT

Issue/problem	PROB	Action	ACT
1.			**CNS 1**
2.			**CNS 2**
3.			**CNS 3**

- Initials of investigator .....|__|__|__|

Date of today|__|__|-|__|__|-|__|__| ..... *SCD Study No*|__|__|__|__|

RESIDENCE FORM FOR SICKLE CELL STUDY

To be filled in if residence information has changed

- **Hospital Identification number** .....|__|__|__|__|__|__|**HID**
- **Name of patient** ..... |_____|**NAME**
- **Sex (M/F)**.....|__|**SEX**
- **Date of birth**.....|__|__|-|__|__|-|__|__|**DOB**
- **Name of mother/Guardian**..... |_____|**MUMNAME**
- **District/Location** ..... |_____|**DIS/LOC**
- **Division** ..... |_____|**DIVISION**
- **Ward/Village** ..... |_____|**WARD/VILL**
- **Ten cell leader** .....|_____|**TENCELL**
- **Nearest:**
  - **School** .....|_____|**SCHOOL**
  - **Health post**..... |_____|**HEALTHPOST**
  - **Bus stage** ..... |_____|**BUSSTOP**
  - **Neighbour 1** .....|_____|**NEIGHBOUR1**
- **Telephone Number (N/Y-Details)**..... |__|-|__|__|__|__|__|__|__|__|__|__|**TEL**
- **Investigators initials**.....|__|__|

Date of today |__|__|-|__|__|-|__|__| ..... **SCD Study No**|__|__|__|__|

RESIDENCE FORM FOR SICKLE CELL STUDY

To be filled in if residence information has changed

- **Hospital Identification number** .....|__|__|__|__|__|__|**HID**
- **Name of patient** ..... |_____|**NAME**
- **Sex (M/F)**.....|__|**SEX**
- **Date of birth**.....|__|__|-|__|__|-|__|__|**DOB**
- **Name of mother/Guardian**..... |_____|**MUMNAME**
- **District/Location** ..... |_____|**DIS/LOC**
- **Division** ..... |_____|**DIVISION**
- **Ward/Village** ..... |_____|**WARD/VILL**
- **Ten cell leader** .....|_____|**TENCELL**
- **Nearest:**
  - **School** .....|_____|**SCHOOL**
  - **Health post**..... |_____|**HEALTHPOST**
  - **Bus stage** ..... |_____|**BUSSTOP**
  - **Neighbour 1** .....|_____|**NEIGHBOUR1**
- **Telephone Number (N/Y-Details)**..... |__|-|__|__|__|__|__|__|__|__|__|__|**TEL**
- **Investigators initials**.....|__|__|

residence form

APPENDIX III LABORATORY PROCEDURES

PROCESSING OF SCD SAMPLES

SAMPLES TO BE RECEIVED FROM SCD PATIENTS

Entry visit	Scheduled visit	Admission
EDTA (pink) 4 mls	EDTA (pink) 2 mls	EDTA (pink) 2 mls
EDTA (pink) 2mls	Blood Film 2 slides	Blood Film 2 slides
Serum separator 1ml vials		Serum separator 1ml
		Blood culture 4mls

PROCESSING OF EDTA SAMPLES

EDTA sample – 3mls in HAEMATOLOGY

1. **Prepare two blood films**
    a. **Malaria parasitaemia** – Giemsa stained. mps/200wbc
    b. **Reticulocyte counting** - stained by new methylene blue. See protocol for reporting
2. **EDTA sample:** Perform a **FULL BLOOD COUNT** using the haematology analyser and record the result onto the accompanying form and into the laboratory counter book.
3. EDTA sample: perform HB ELECTROPHORESIS by cellulose acetate method (see protocol). If electrophoresis is not done immediately, prepare the haemolysate.

Preparation of haemolysate

1. Place 100µl of whole blood into eppendorf tube
2. Wash the whole blood with large volume of normal saline
3. Mix well with vortex
4. Centrifuge at 3000rpm for 3 minutes
5. Pour out the normal saline
6. Repeat step 2-5 three times

Commercially prepared haemolysing reagent

1. Place 60µl of haemolysate reagent into eppendorf tube
2. Add 10µl of prbc
3. Mix well with vortex
4. Spin for 3000rpm for 3 minutes

Alternate method of haemolysis using distilled water

1. Place 60µl of distilled water into prelabelled eppendorf tube
2. Add 30µl of prbc. (1volume RBC: 2 volume distilled water)
3. Add chloroform (2 volume lysed RBC: 1 volume of chloroform)
4. Centrifuge at 3000rpm for 10 minutes to remove stroma

PROCESSING OF EDTA SAMPLES

1. Centrifuge @ 3000rpm for 10 minutes.
2. Using a P1000 Gilson Pipette, aliquot the plasma into a pre-labelled 2ml alpha apex vial.
    a. Store for immunological studies-malaria, parvovirus, pneumococcus and possible iron studies
3. Transfer the **buffy coat, for DNA storage**, into a separate 2ml-freezing vial.
    a. Store for store for sickle genotyping.
4. Transfer 2 drops of packed red cells onto pre-labelled filter paper.
    a. Store for Hb quantification and sickle genotyping.
5. Wash the RBC two times with Normal saline.
    a. Store for malaria PCR.

PROCESSING OF FILTER PAPER SAMPLES

1. Cut filter paper into triangles
2. Label with patients number and date
3. Place two drops of blood from packed cells/whole blood onto filter paper
4. Filter paper samples are left to air dry for 48 hours.
5. Each filter paper sample is wrapped in paper and will be stored at –4.
6. HbF and HbA2 quantification by HPLC will be done at KCL, UK until HPLC is set up at MUCHS.
7. This sample can also be used for sickle genotyping.

PROCESSING OF SERUM SEPARATED SAMPLES IN SPL LAB

1. Perform a **BIOCHEMISTRY SCREEN** using the clinical chemistry analyser
2. Print out the results and record them in laboratory record book.
3. **Store the Serum at**
4. This will be processed for **HIV, Hepatitis B and C** in microbiology laboratory.

PROCESSING OF BLOOD FROM CONTROL POPULATION

SAMPLES TO BE RECEIVED FROM CONTROLS

EDTA sample	**Haematology**
Serum separated sample	**Clinical chemistry**

PROCESSING OF EDTA SAMPLE

1. **Prepare two blood films**
    a. **Malaria parasitaemia** – Giemsa stained. mps/200wbc. (See protocol for reporting)
    b. **Reticulocyte counting** - stained by New methylene blue. (See protocol for reporting)
2. Transfer 2 drops of packed red cells onto pre-labelled filter paper.
    a. Store for Hb quantification and sickle genotyping.
3. Perform a **FULL BLOOD COUNT** using the haematology analyser and record the result onto the accompanying form and into the laboratory record sheet.
4. Centrifuge @ 3000rpm for 10 minutes.
5. Using a P1000 Gilson Pipette, aliquot the plasma into a pre-labelled 2ml alpha apex vial.
    a. Store for immunological studies-malaria, parvovirus, pneumococcus and iron studies
6. Transfer the **buffy coat, for DNA storage**, into a separate 2ml-freezing vial.
    a. Store for store for sickle genotyping.
7. Wash the RBC two times with Normal saline.
8. Perform **HB ELECTROPHORESIS** by cellulose acetate method (see protocol)
    a. If electrophoresis is not done immediately, prepare haemolysate
    b. Store prbc for malaria PCR.

PROCESSING OF SERUM SEPARATED SAMPLES

1. Perform a **BIOCHEMISTRY SCREEN** using the clinical chemistry analyser
2. Print out the results and record them in laboratory record book.
3. **Store the Serum at -4^0C.**
4. This will be processed for **HIV, Hepatitis B and C** in microbiology laboratory.

SUMMARY OF PROCESSING OF SAMPLES RECEIVED FROM SCD PATIENTS

Bottle			**Laboratory**	**Tests**	**Method/reporting**	**Immediate**	**Stored**
EDTA	2mls		Haematology	FBC	Coulter counter	**Immediate**	
			Haematology	Hb electrophoresis	Cellulose acetate	Delayed	Haemolysate
Film			Haematology	MPS		**Immediate**	Film
Film			*Haematology*	*RBC morphology*		*Delayed*	
Film			Haematology	Reticulocyte		Delayed	
Filter paper			SPL	HbF, HbA2 quantification	HPLC	Delayed	Stored
			SPL	Genotyping - DNA	Sickle genotype	Delayed	Stored
EDTA			*SPL*	*Malaria Ag test*		***Immediate***	
			SPL	Genotyping - DNA	Sickle genotype	Stored	Buffy coat
				Malaria PCR	PCR	Delayed	Packed cells
				Immunological studies- malaria, parvovirus, pneumococcus & iron studies		Stored	Plasma
Serum separator	2mls	1ml	SPL /CRL	U&Es, LFTs		Stored – weekly	
		1ml	Microbiology	HbsAg, Hep B and C, HIV		Delayed	Serum
Urine			SPL	Chemistry	Urine dipstick	**Immediate**	
Blood culture		4mls	Microbiology	Blood culture		Immediate	

SUMMARY OF PROCESSING OF SAMPLES RECEIVED FROM CONTROLS

Bottle		**Laboratory**	**Tests**	**Method/reporting**	**Immediate**	**Stored**
EDTA	2mls	Haematology	FBC	Coulter counter	Immediate	
		Haematology	Hb electrophoresis	Cellulose acetate	Delayed	Haemolysate
Film		Haematology	MPS		Immediate	Film
Film		Haematology	Reticulocyte		Delayed	Film
Filter paper		SPL	HbF, HbA2 quantification	HPLC	Delayed	Dried blood
		SPL	Genotyping - DNA	Sickle genotype	Delayed	
		SPL	Genotyping - DNA	Sickle genotype	Stored	Buffy coat
			Malaria PCR	PCR	Delayed	Packed cells
			Immunological studies Malaria, parvovirus, SPN & iron		Stored	Plasma
Serum separator	2mls	SPL /CRL	U&Es, LFTs		Stored – weekly	
		Microbiology	HbsAg, Hep B and C, HIV		Delayed	Serum

FORM FOR STORAGE OF SAMPLES

RACK NUMBER										
	A.	**B.**	**C.**	**D.**	**E.**	**F.**	**G.**	**H.**	**I.**	**J**
1.										
2.										
3.										
4.										
5.										
6.										
7.										
8										
9										
10.										
	A.	**B.**	**C.**	**D.**	**E.**	**F.**	**G.**	**H.**	**I.**	**J**

Place		**Temperature**	
Freezer		**Nature**	
Intended use			

BLOOD SLIDE EXAMINATION FOR MALARIA PARASITES

PRINCIPLE OF GIEMSA STAINING METHOD

Giemsa stain is a Romanowsky stain which has basic and acidic stain components. The basic component which is eosin stains the nucleus of the malaria parasite giving it a pinkish colour. The acid component which is methylene blue stains the cytoplasm giving it a bluish colour. The red blood cells stain greyish pinkish. Neutrophil nucleus stain pinkish while monocytes and lymphocytes nucleus stain blue. Parasites are examined qualitatively and quantitatively. During the staining period the red cell in the thick film lyses releasing the parasites. The thin film is alcohol fixed hence the parasite remain in the red cells

SPECIMEN:

Whole blood. Or Capillary blood All thick blood smears shall be prepared using whole blood directly from the syringe or from a finger prick.

EQUIPMENT

Microscope, Coplin jars, Slides – grease-free and labels. Slide racks, Measuring cylinders of 50 mls and 100 mls, Forceps, Para film and Scissors, Filter paper, Timer, 100 ml measuring cylinder, Filter papers, Slides storage boxes

REAGENTS

Absolute methanol (GPR), Giemsa stain – Stock solution, Oil immersion, Buffer pH 7.2, 70% v/v industrial methylated spirit, 10% Giemsa stain.

PROCEDURE

1. The malaria blood smears are left to completely air dry before staining.
2. Fix the thin smears and stain only one slide per patient using 10% giemsa stain for 10 to 15 minutes by dipping the slides placed back to back in staining jar containing the stain.
3. Remove the slide and gently wash off the staining by dipping the slides in a beaker containing water.
4. Place the slides in a slide rack to drain and dry.
5. Using 40x objective to check the staining pattern, cell and parasites morphology and their distribution is satisfactory. Add oil immersion on the film then move the 100x objective and focus
6. Using power 100 examines the thick film for the presence of parasites and count the number of parasites per white cells.
7. Examine at least 100 high-powered microscope fields for parasites before the slide is reported negative.
8. Count the number of parasites against the cells depending on the thick film per 200 WBC.

If the counted parasites are greater than 500 before 200 wbc are counted, stop the count after counting all the parasites in that field, then report i.e. 564 parasites per 41 wbc

REPORTING OF RESULTS.

The final report shall be the number of counted parasites per 200 WBC.

SOURCE OF ERRORS

1. Anticoagulated blood may be washed away during staining or when washing the slide.
2. Over- staining can also affect the stain take- up of parasites.
3. The quantity of blood should also be just enough not more or less.

RETICULOCYTE COUNT

INTRODUCTION

Reticulocytes are young blood cells, newly released from the bone marrow, which contains two or more ribosomal RNA (blue staining small material. When the cells are exposed to certain dyes like brilliant cresyl blue or new methylene blue, the ribosomes are precipitated and stained by the dye to appear as a reticular network while the cells are still alive when exposed. This is referred to as supravital staining. With New Methylene Blue (NMB), red cells stain a pale greenish-blue while the reticulum stain bluish-purple.

Apparatus

20µl pipettes, 75 x 10 mm test tubes, Water bath (adjusted to 37^0C), Thermometer, Glass slides, Microscope, Spreader.

Reagents

- New Methylene Blue, Oil immersion

SAMPLE

Fresh EDTA blood.

PROCEDURE

1. Deliver 20µl of patient's blood into a 75x10mm glass or plastic tube.
2. Add 20 l of NMB dye to the patient's blood and mix.
3. Incubate the mixture in a water bath at 37^0C for 10 minutes.
4. Re-suspend the red cells by gentle mixing.
5. Make a thin film on a glass slide and air dry.
6. Examine the film using x100 objective with oil immersion.

COUNTING RETICULOCYTES:

The amount of reticulum in a reticulocyte varies from a large clump in the most immature cells (group 1 reticulocyte) to a few granules in the most mature forms (group IV reticulocytes). The minimum requirement varies from a single dot, to two or three dots to a maximum network.

The number of cells counted to achieve an acceptable degree of reproducibility is 1000 cells. The reticulocyte count is stable with storage of EDTA – anticoagulated blood for up to 24 hours at room temperature and for several days at 4°C . A common mistake is to make the film too thin.

CALCULATIONS:

Reticulocytes percentage (%)---------- $\frac{\text{Number of reticulocytes counted x 100}}{\text{1000 number RBC 1000 cells.}}$

NORMAL RANGE .

- Adult and children ---------- 0.5 to 2.5 %
- Infant (full term, cord blood---------- 2 to 6 %

SOURCES OF ERROR .

- Wrong sample used.
- Poor staining technique.
- Poor spreading which leads poor distribution of reticulocytes.
- Poor counting technique.

HAEMOGLOBIN ELECTROPHORESIS

PRINCIPLE

The sample is applied to the medium and under the effect of the electric field, groups of particles with similar charge, size and shape characteristics migrate at similar rates. This results in the separation of the particles into bands.

REAGENTS

Supreme-Heme Buffer, Haemolysate reagent, Ponceau S, 5 % Acetic Acid, Absolute methanol, Clearing solution, Hemo control

APPARATUS

Graduated pipette, Timer, Eppendorf tubes, 5 µl microdispenser , Hellena sample well plate , Blotter pads, Cellulose acetate paper, Hellena sample applicator, Power pack, Electrophoresis tank, Wicks, Glass slides, Staining jars, Bufferizer, Microhood

SPECIMEN

Whole blood collected in EDTA tubes. May be stored up to one week at 2-6^{0}c or Haemolysate

PREPARATION OF SAMPLE

1. Place 100µl of whole blood into eppendorf tubes
2. Label the eppendorf tubes 1,2,3,5,6,7. Leaving number 4 for the control
3. Wash the whole blood with large volume of normal saline
4. Mix well with vortex
5. Centrifuge at 3000rpm for 3 minutes
6. Pour out the normal saline
7. Repeat step 2-5 three times
8. Place 60µl of haemolysate reagent to eppendorf tubes (150 µl haemolysate reagent)
9. Add 10µl of prbc (20 µl of prbc)
10. Mix well with vortex
11. Spin for 3000rpm for 3 minutes

NB: -Haemolysate may be stored at –20^{0}c for one year or more.

Alternate method of haemolysis using distilled water

1. Place 60µl of haemolysate reagent to eppendorf tube
2. Add 30µl of prbc. (1volume RBC: 2 volume distilled water)
3. Add chloroform (2 volume lysed RBC: 1 volume of chloroform)
4. Centrifuge at 3000rpm for 10 minutes
5. Using a P20 Gilson pipette take 5-7µl of clear red haemolysate which is between a layer of chloroform and stroma

Haemolysates

1. Dilute 40µl of 10g/dl haemolysate with 150µl haemolysing reagent

Control

1. Centrifuge sample of control at 3000rpm for 5 minutes.
2. Aliquot the serum using a P100 Gilson pipette
3. Wash the red blood cells 3x with large volumes of normal saline.
4. Add 1part of packed cells to 6 parts haemolysate reagent Or to 20 µl of prbc add 150 µl haemolysate reagent
5. Mix well and allow to stand for 5mins.

PREPARATION OF ELECTROPHORESIS CHAMBER

1. Place 100mls of TEB/Supreme heme buffer in each of the outer buffer compartments. The centre 2 compartments should be empty.
2. Wet 2 chamber wicks in the buffer
3. Place one along each divider ensuring that they make good contact with the buffer.
4. The buffer in the tank should be changed every week.

PREPARATION OF CAM PLATES

1. Cut the top left hand corner of CAM to indicate sample position 1.
2. Place in soaking rack
3. Pour buffer from lower reservoir into top reservoir ensuring that you have covered the hole in the top reservoir.
4. Place the soaking rack into the empty lower reservoir and quickly replace the top reservoir.
5. Leave it to slowly soak with buffer trickling into bottom reservoir.

LOADING THE SAMPLE

1. Place 5µl - 7µl of the prepared patient haemolysate in the wells of the sample plates using a Gilson's pipette.
2. Place 5µl - 7µl of commercially prepared control into sample well plate (position 4)
3. Cover the sample well plate with a glass slide or cover slip if the samples are no to be used within 2 minutes.
4. Prime the applicator by depressing the tips into the sample wells 3 or 4x.
5. Apply this loading to a blotting paper. **NB**-Priming the applicator makes the second loading much more uniform.
6. Remove the CAM from the buffer with the fingertips
7. Blot twice between two layers of clean blotting paper. Do not allow the CAM to dry
8. Place the CAM (**CAM side up**) on the aligning base with the cathodal end on the indicator line. (This will give a sample application point of 2.5cm from the cathodal end of the plate.)
9. Load the applicator by depressing the applicator tips into the sample wells 3-4x.
10. Promptly transfer the applicator to the aligning base. The applicator should be used within 15seconds of being loaded.
11. Press the button down and hold it for 5 seconds.

ELECTROPHORESIS

1. Place the CAM across the bridges with the **plastic side up**
2. Place two glass slides across the strip to maintain good contact. Make sure the slides do not touch the wicks
3. Switch on the current at 350V for 25 minutes.
4. Remove the plates from the electrophoresis chamber

STAINING

1. Stain with Ponceau S stain for 10 minutes.
2. Decolourise the CAM by washing in 2 successive washes of 5% acetic acid.
    - 1st 2minutes
    - 2nd 2 minutes
3. Dehydrate the plates in 3 successive washes of absolute methanol for 2mins
4. Place the plates in clearing solution for 10 minutes then drain off excess solution
5. Place the plates with **CAM side up** on blotter pad under the micro-hood for 10mins.
6. Place the labels on the glossy/plastic side of the now clear CAM
7. Interpret the position of the bands.

EXPECTED VALUES:

At birth, the majority of haemoglobin in the erythrocytes of the normal individual is foetal haemoglobin, HbF . At the end of the first year of life and through adulthood, the major haemoglobin present is HbA with up to 3.5 % HbA_2 and less than 2 % HbF.

ANODE (+)

A

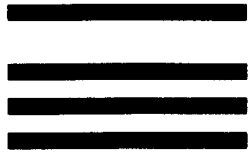
F

S (D, G, Lepore)

C (E, O, A2)

+++ Origin

CATHODE (-)



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR DIAGNOSIS OF HAEMOGLOBINOPATHIES

INTRODUCTION / PRINCIPLES

The Bio-rad variant beta thalassaemia short **program** (VARIANT™ β-thalassaemia Short Program) is intended for the separation and area percent determinations of haemoglobins A_2 and F and as an aid in the identification of abnormal haemoglobins in whole blood using ion-exchange high performance liquid chromatography (HPLC). The VARIANT™ β-thalassaemia Short Program is intended for use only with the Bio-Rad VARIANT Haemoglobin Testing System.

The β-thalassaemia Short Program utilises the principles of cation-exchange high performance liquid chromatography (HPLC). Specimens are sequentially injected into the analysis stream at 6.5-minute intervals for a throughput of 9 samples per hour. Two dual-piston pumps and a pre-programmed gradient control the elution buffer mixture passing through the analytical cartridge. The ionic strength of the elution buffer mixture is increased by raising the percent contribution of elution buffer 2. As the ionic strength of the mixture increases, more strongly retained haemoglobins elute from the analytical cartridge. A dual-wavelength filter photometer (415 and 690 nm) monitors the haemoglobin elution from the cartridge, detecting absorbance changes at 415nm. The 690 nm secondary filters corrects the baseline for effects caused by mixing buffers with different ionic strengths. Changes in absorbance are monitored and displayed as a chromatogram of absorbance versus time. Analysis data from the detector is processed by the built-in integrator and printed on the sample report.

To aid in the interpretation of results, windows (e.g., ranges) have been established for the most frequently occurring haemoglobins based on their characteristic retention times. The retention time is the elapsed time from the injection of the sample to the apex of a haemoglobin peak. Each of these common haemoglobin variants i.e. S, C, D^{Punj} has a characteristic retention time. Minor differences in the separation efficiency of individual analytical cartridges are corrected by the use of the Haemoglobin A_2/F (HbA2/F) Calibrator. At the end of each sample analysis, a copy of the chromatogram and report data is automatically printed. The report table includes the corrected area percent for haemoglobins A_2 and F for all subsequent samples in the run.

The Hb A2/F Calibrator which has assigned values (in units of area percent of total haemoglobin) for both haemoglobins A_2 and F is analysed at the beginning of each run. Analysis of the calibrator yields separate calibration factors for both haemoglobins A2 and F. Calibration factors are the calculated ratio of the assigned value (value imprinted on the labelling) to the observed value (value determined per run). These calibration factors are applied to the observed area percent for haemoglobins A_2 and F in all subsequent analyses in the run. The calibration corrects for the resulting differences in the allocation of area to the haemoglobin A_2 and F peaks.

EQUIPMENT

Bio-Rad II Variant HPLC analyser, PC, Printer, Sample racks, Automatic pipettes to deliver 5jJ, 250>l, 1 ml and 10ml, Micro-vials and micro-vial adapters

REAGENTS

Bio-Rad VARIANT Beta Thalassaemia Short Reorder Pack

Each pack contains:

	Storage temperature
Elution buffer 1 (A)	Room Temperature (15-30°C)
Elution buffer 2 (B)	Room Temperature (15-30°C)
Whole blood primer	2 - 8°C
Wash solution	Room Temperature (15-30°C)
Analytical cartridge - 2 supplied per kit CD	Room Temperature (15-30°C)
1,5ml plastic vials - 500 supplied per kit	Room Temperature (15-30°C)
Haemolysis reagent	Room Temperature (15-30°C)
HbA_2/F calibrator set/Diluent set	2 - 8°C
ROM Card	Room Temperature (15-30°C)

REQUIRED ITEMS

Latex medical gloves	
Disposable towelettes	
Micropipettes 5µL, 1mL, 10mL	
Transfer pipettes	
Deionized water	
Syringes 20mls	

ADDITIONAL ITEMS

Haemoglobin A_2 control	
Autodiluter, for sample preparation	
Variant Thermal printer paper (12 rolls)	

PROCEDURES

Preparation of reagents

General procedures

1. Wear latex gloves during reagent preparation procedures
2. Do not use reagents after their labelled expiration dates or mix different reagent lots
3. Do not use controls, calibrators or prime if the lyophilized pellet is brown or the vial is broken
4. Adherence to the protocol specified is necessary to ensure proper performance of this product
5. If aberrant results are obtained contact Bio-Rad technical services

Elution buffers, wash solution and analytical cartridges

To install or change elution buffers, wash solution and analytical cartridges; follow the procedures described in the VARIANT operation manual section 3.0

HbA_2/F Calibrator

1. Reconstitute the HbA_2/F Calibrator by adding 10mL HbA_2/F Calibrator diluent per vial
2. Swirl gently to dissolve and ensure complete mixing
3. Allow calibrator to stand for 10 minutes at 15 - 30°C. reconstituted calibrator is stable for 10 days when stored at 2-8°C.
4. When reconstituted, label vial with date of reconstitution, expiry date and sign vial.

Haemoglobin A_2 control

The following controls should be run at the beginning and end of each group of patient specimens:

1. EDTA blood from known normal subject (HbF 1-2%, HbA_2 1.8-3.2%)
2. Bio-Rad Lyphocheck HbA_2 Control 1
3. Bio-Rad Lyphocheck HbA_2 Control 2

Each vial is reconstituted with 1ml distilled water. Stand at 15-30°C for 10 minutes.
Reconstituted lyphochecks are stable for 21 days when stored at 2-8°C.
When reconstituted, label vial with date of reconstitution, expiry date and sign vial.

Haemoglobin primer set

Use the whole blood primer at the beginning of each run to condition the cartridge for analysis

1. Add 1 ml of deionized water to vial,
2. Swirl gently to dissolve and ensure complete mixing
3. Allow to stand for 10 minutes at 15-30°C.

Reconstituted primer is stable for 21 days when stored at 2-8°C.
When reconstituted, label vial with date of reconstitution, expiry date and sign vial. Note: each primer used up in one go.

SPECIMEN

EDTA anticoagulated blood sample - 5µL
Patients specimens are stable for 7 days when stored at 2-8°C

Sample preparation

1. Pipette 5µL whole blood from each patient sample into separate 1.5mL sample vials
2. Add 1.0mL of haemolysis reagent to each sample vial
3. Vortex sample at medium speed.
4. Place the sample vial into the VARIANT. Note: This will be in position 5 in variant sample tray
5. Patient haemolysates are stable for 24 hours when stored at 2-8°C

Method selection

1. Press POWER
    a. The system performs a 5-minute warm up program, after which the system enters IDLE set up. The RUN SETUP screen is then displayed.
2. Press the TEST MENU key followed by '1' to select the β thalassemia short program.
3. Press ENTER to confirm the selection

Preparation of analyser prior to run

Check buffer and wash solution reservoir levels and printer paper supply. Note: Do not mix components of different batches or kits.

1. When the machine is in stand-by mode:
    - Flush piston/seal wash port: open port half a turn, counter clockwise
    - Insert 10ml distilled water using a disposable syringe.
    - Close port.
    - Check for leaks; tighten any connections showing evidence of leakage.
    - Wipe up any spillage.
2. To put the machine into active/ready state click on the top left hand side box which says V2-BTHAL INACTIVE'. The communication box will appear and one left click on the return button will put the machine in a ready state and perform the auto warm-up operation. You will be asked:
    Q: Do you want to perform automatic warm-up operation?
    A: Yes
3. Each day the maintenance/cleaning log MUST be filled in by the operator
4. Place the laboratory number of the sample onto the micro-vial. Place the sample vials into the appropriate positions in the sample rack and place the rack on the analyser.

Run Set Up

1. Prepare patient samples as outlined above
2. Transfer 250µL of each of the following reconstituted reagents into sample vials and place in stated position on variant sample tray:

Well #	**Reagent**
STAT well	Haemoglobin primer
1	Deionised water
2	HbA_2/F Calibrator
3	Normal control
4	Abnormal control
5 to N*	Patient haemolysates
N+1	Normal control
N+2	Abnormal control

3. Initiate analysis:

The machine will be in the ready state (see preparation of analyser prior to run) so click onto the box on the left hand side of the screen which says RUN. Make sure you are in the work list screen and then clear the previous work list. Click onto the start button and click start again then the run will activate.

4. Post-analysis:

The calibration response factors for HbA_2 and F are automatically calculated. The calibration response factors are used in the calculation of area percentages for HbA_2 and F for all subsequent analyses in the run.

CALIBRATION REPORT	
F	**Response factor: 0.97**
A2	**Response factor: 0.93**

If the calibration response factor for either HbA_2 or HbF is less than 0.80 or greater than 1.20, the system prints an error message and automatically aborts the run. In such cases, contact Bio-Rad technical Service

5. At the completion of each cycle, the system automatically initiates a five-minute WASH cycle. At the completion of wash cycle, the system enters into IDLE status

INTERPRETATION OF CHROMATOGRAMS

1. Check water blanks show a flat, straight line.
2. Check baselines on samples analysed are flat.
3. Check that controls are within given ranges.
4. Check the total area of each sample analysed is within 1-4 x 10^6.
   Note: If any of the above are unsatisfactory, individual tests or the whole run will have to be repeated.
5. Record chromatography results against patient's demographics on worksheet. Typical examples of haemoglobin patterns are recorded as follows:
   NAHD
   A+S
   A+C
   A+ fraction co-eluting with A_2
   A+ A2 variant
   Record the percentages found of Hb A_{2l} Hb F and any variant fraction.
6. After reading and recording the values, sign the worksheet. The results must be checked by a senior member of staff. The senior must also sign the worksheet as having checked the data.

EXPECTED RESULTS / RANGE (S)

Normal haemoglobin pattern: No evidence of a haemoglobin variant. (Hb A detected as the major fraction present)

	Normal levels	**Variant maximum reportable range**
Normal Hb A_2	**2.2 - 3.3%**	**13%**
Normal Hb F	**Less than 1%**	**40%**

Patient state	**Hb A_2 level**	**HbF level**
Heterozygous β-thalassemia	4-9%	1-5%
Homozygous β-thalassemia	Normal or increased	80-100%
Heterozygous HPFH	<1.5%	10-20%
Homozygous HPFH	Absent	100%

INTERPRETATION

Detection of a haemoglobin variant requires second line testing in either heterozygous, compound heterozygous or homozygous conditions. Borderline Hb A_2 values (3.4 -3.7%) may require second line testing and results must be referred for comment. Elevation of HbA2 is diagnostic of β-thalassaemia trait, but may also be found in unstable p-globin chain variants. If differentiation is required, heat stability testing must be undertaken. Elevation of Hb F may be indicative of α-thalassaemia trait and further testing is indicated if the elevation of F is accompanied by microcytic, hypochromic indices.

REPORTING / VERIFICATION / PRINTING

Enter haemoglobin type, A_2 and F results. See reporting SOP -Interpretation and verification is undertaken by senior personnel.