BMJ Open

ORGAN DAMAGE IN SICKLE CELL DISEASE STUDY (ORDISS): DESIGN OF A LONGITUDINAL COHORT STUDY BASED IN GHANA

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-016727
Article Type:	Protocol
Date Submitted by the Author:	06-Mar-2017
Complete List of Authors:	Anie, Kofi; London North West Healthcare NHS Trust, Haematology and Sickle Cell Centre; Imperial College London, Medicine Paintsil, Vivian; Komfo Anokye Teaching Hospital, Directorate of Child Health Owusu-Dabo, Ellis; Kumasi Center for Collaborative Research in Tropical Medicine; Kwame Nkrumah University of Science and Technology Ansong, Daniel; Komfo Anokye Teaching Hospital, Directorate of Child Health; Kwame Nkrumah University of Science and Technology, Child Health Osei-Akoto, Alex; Komfo Anokye Teaching Hospital, Directorate of Child Health; Kwame Nkrumah University of Science and Technology, Child Health Ohene-Frempong, Kwaku; Sickle Cell Foundation of Ghana; Children's Hospital of Philadelphia Aikins Amissah, Kofi; Komfo Anokye Teaching Hospital, Directorate of Child Health Addofoh, Nicholas; Kumasi Center for Collaborative Research in Tropical Medicine Bonwin Ackah, Ezekiel; Kumasi Centre for Collaborative Research in Tropical Medicine Owusu-Ansah, Amma; University of Pittsburgh, Heart, Lung, Blood and Vascular Medicine Institute Ofori-Acquah, Solomon; University of Pittsburgh, Heart, Lung, Blood and Vascular Medicine Institute
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Genetics and genomics, Global health
Keywords:	Sickle Cell Disease, Organ Dysfunction, Genetic Markers, Biorepository, Complications, Cohort

ORGAN DAMAGE IN SICKLE CELL DISEASE STUDY (ORDISS): DESIGN OF A LONGITUDINAL COHORT STUDY BASED IN GHANA

Kofi A Anie[1,2], Vivian Paintsil[3], Ellis Owusu-Dabo[4,5], Daniel Ansong[3,6], Alex Osei-Akoto[3,6], Kwaku Ohene-Frempong[7], Kofi Aikins Amissah[3], Nicholas Addofoh[4], Ezekiel Bonwin Ackah[4], Amma Twumwaa Owusu-Ansah[8], Solomon Fiifi Ofori-Acquah[8]

[1]Haematology and Sickle Cell Centre, London North West Healthcare NHS Trust, Central Middlesex Hospital, London, UK

[2]Faculty of Medicine, Imperial College London, London, UK

[3]Directorate of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana

[4]Kumasi Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[5]School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[6]Department of Child Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[7]Sickle Cell Foundation of Ghana, Ghana

[8]Center for Translational and International Hematology, Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Correspondence to:

Dr Kofi A Anie
Email: kofi.anie@nhs.net
Tel: +44 (0) 20 8453 2050
Fax: +44 (0) 20 8453 2051

ABSTRACT

Introduction

Sickle cell disease is highly prevalent in Africa with a significant public health burden. Nonetheless, morbidity and mortality in sickle cell diease that result from the progression of organ damage is not well understood. The Organ Damage in Sickle Cell Disease Study (ORDISS) is designed as a longitudinal cohort study to provide critical insight into cellular and molecular pathogenesis of chronic organ damage for the development of future innovative treatment.

Methods and analysis

ORDISS aims to recruit children aged 0-15 years who attend the Kumasi Centre for Sickle Cell Disease at the Komfo Anokye Teaching Hospital in Kumasi, Ghana. Consent is obtained to collect blood and urine samples from the children during specified clinic visits and hospitalisations for acute events, to identify candidate and genetic markers of specific organ dysfunction and end-organ damage, over a three-year period. In addition, data concerning clinical history and complications associated with sickle cell disease are collected. Samples are stored in biorepositories and analysed at the Kumasi Center for Collaborative Research in Tropical Medicine, Ghana and Center for Translational and International Hematology, University of Pittsburgh, USA. Appropriate statistical analyses will be performed on the data acquired.

Ethics and dissemination

Research ethics approval was obtained at all participating sites. Results of the study will be submitted for publication in peer-reviewed journals, and the key findings presented at national and international conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- A longitudinal cohort study of children with sickle cell disease that intends to provide novel insights into cellular and molecular pathogenesis of chronic organ damage.
- The prospective design will allow risk factors for organ dysfunction associated with sickle cell disease complications to be determined in a naturalistic study of children in a specialist Centre.
- Attrition or loss to follow-up of children with sickle cell disease after the initial study visit at the specialist Centre may lead to a bias and reduction in the internal validity of the study.
- This is a study in a single setting.

INTRODUCTION

Sickle cell disease (SCD) comprises a group of inherited red blood cell conditions that result from the abnormal production of haemoglobin. Over 400,000 babies are born worldwide annually with SCD mostly in low and middle income countries, and about 75% or more of these births occur in sub-Saharan Africa, posing an increasing health burden[1]and contributing to early childhood mortality[2]. SCD affects approximately 2% of newborns in Ghana[3].

Clinical syndromes of SCD include anaemia, infection, and the consequences of blood vessel blockage (vaso-occlusion). The latter deprives tissues of oxygen and is indicated as the cause of acute painful episodes, the hallmark of SCD, and other complications such as stroke, acute chest syndrome, priapism, leg ulceration and chronic organ failure. Stem cell transplantation offers curative possibilities although this is not universal, and other treatment options are generally limited in Africa[4]. Improved knowledge has greatly advanced medical management over the past four decades. Nonetheless, progressive deterioration of organ function and end-organ damage is inevitable and appears to be irreversible[5,6]. The mechanisms that lead to these complications, studied mostly outside sub-Saharan Africa, are not fully understood. Further understanding through a longitudinal cohort study of patients with SCD may provide novel insights into cellular and molecular pathogenesis of chronic organ damage, and opportunities for the development of innovative treatment.

In Ghana, a pilot Newborn Screening (NBS) project for SCD was established in Kumasi (the second largest city) and Tikrom (a nearby rural community) from 1993 as an international collaborative study[3]. Newborns identified with SCD are registered in the Kumasi Centre for Sickle Cell Disease (K-CSCD) at the Komfo Anokye Teaching Hospital (KATH), and followed up until 15 years of age through the Child Health Directorate. This NBS project was

subsequently adopted by the Government of Ghana in 2010 to scale it up as a national public health programme.

STUDY OBJECTIVES

There are currently no data on the spectrum of organ dysfunction and end-organ damage in the SCD patient cohort attending K-CSCD. The Organ Damage in Sickle Cell Disease Study (ORDISS) was designed as a longitudinal cohort study of children with SCD attending K-CSCD to document acute events and the progressive deterioration in organ function with age, and to identify candidate and genetic markers of specific organ dysfunction and end-organ damage. Specific objectives are:

1. To determine the proportion of children with SCD who develop specific organ dysfunction.
2. To determine levels of biomarkers of organ dysfunction (heart, kidney, liver, lung, brain and skeletal muscle) from multiple candidate plasma and urine samples.
3. To determine haematological and haemolytic markers in the recruited children attending clinic for routine evaluations or acute illness management.
4. To compare clinical evidence of organ dysfunction with biochemical markers of organ dysfunction.

METHODS AND ANALYSES

ORDISS is an international collaborative study conducted at three institutions: Department of Child Health, KATH/Kwame Nkrumah University of Science and Technology (KNUST); Kumasi Center for Collaborative Research in Tropical Medicine (KCCR); and University of

Pittsburgh's Center for Translational and International Hematology. Research Ethics approvals were obtained from both the KNUST/KATH and University of Pittsburgh Institutional Review Boards.

Participants and Recruitment

i. Eligibility Criteria

Eligible participants are families of children with SCD comprising all genotypes confirmed with both isoelectric focusing and cellulose acetate electrophoresis who are registered at K-CSCD, aged 14 years and younger at recruitment, and receive outpatient or inpatient care at KATH. Patients who are not known to KATH, and older than 12 months of age must be registered at K-CSCD for at least 12 months prior to becoming eligible for enrolment.

ii. Exclusion Criteria

Exclusions are children with SCD and co-morbid chronic conditions including malignancies, seizure disorders, and history or clinical signs and symptoms of HIV infection. In addition, patients who cannot be followed-up for a minimum of 12 months during the study, and families who decline informed consent or assent are excluded.

iii. Recruitment and Enrolment Procedures

ORDISS was initially introduced to the families of children with SCD at the monthly Sickle Cell Disease Association meeting, a national support group for parents and patients with SCD, which is held at KATH premises.

Enrolment (Entry) Visit

Families are opportunistically approached during routine clinic visits, and the study introduced to them prior to phlebotomy. Signed or thumb-printed informed consent are

obtained from parents/caregivers and assent from children with SCD aged 7 years and over. Consenting families (participants) are enrolled into the study, and the child (subject) is assigned a unique study identification number that will be used as the subject identifier throughout the study. Participants' demographics, clinical information, and past medical history are recorded. These include the subject's age, gender, standing height, weight, head circumference, heart rate, respiratory rate, blood pressure, oxygen saturation (SpO2), and SCD complications relating to eyes, ears, head, nose, and mouth. Examinations of the throat, lymph nodes, chest with auscultation, heart with auscultation, abdomen, liver, spleen, genitalia, extremities, joints, and neurological examinations are recorded. In addition, data on parental ethnicity, religion, marital status, and educational level are collected. All information gathered is written in the subject's medical records, and entered into an electronic Case Report Form (e-CRF).

Using standard practice of phlebotomy[7], blood is collected from each subject into di-potassium ethylenediaminetetraacetic acid (K_2EDTA) tube and serum separator (SS) tube with gel, each 3-4 ml; 10-20 ml of urine is also collected from each subject at specific visits. The blood in the K_2EDTA tube is inverted 8-10 times to ensure adequate mixing of the blood with the EDTA anticoagulant; the blood in the SS tube is allowed to adequately clot. The samples from each subject are duly labelled with the specific study identification number. The K_2EDTA-anticoagulated blood samples are sent, in a cryobox at room temperature, to the KATH Laboratory where aliquots are taken and immediately used for hematologic analyses; these include full blood count (FBC) with white blood cell (WBC) differential, performed electronically, and reticulocyte count, performed manually. The remainder of the blood sample and the urine sample are then placed on wet ice in a cold box and transported to KCCR for further processing, storage and analyses.

Demographic, clinical information, and FBCs collected from the subjects are entered into a tablet adapted specifically for ORDISS with CommCare software, which allows creation and management of mobile applications through a website. The study database was developed at KCCR where the server is also held. Data are transmitted via a mobile phone network, subject to strength of connectivity, at the end of each clinic day from KATH to the KCCR server for cloud storage and management. This is subsequently extracted into Microsoft Excel spreadsheet format for statistical analyses.

Interim (Follow-up) Visit

On subsequent annual visits after recruitment (i.e. interim visits) over 3 years, clinical procedures and data collection will be replicated, with emphasis on each subject's current ailments, episodes of acute illness not presented at KATH, episodes of enuresis, current medications, and school performance. Furthermore, blood and urine samples will be collected and identical procedures applied. Additionally, the process of data transmission with tablets via internet to KCCR will be repeated for each interim visit.

Acute Illness Visit and Hospitalisation

During acute illness of subjects, blood and urine samples will be collected together with samples for acute illness blood tests requested by attending clinicians. These blood tests will also help to rule out illness due to other infections such as malaria, and allow comparison with steady-state laboratory values. Identical volumes of blood and urine will be collected from each subject during an acute illness; the samples will be processed using equivalent outlined procedures before these are transported on wet ice to KCCR.

Biorepository Sample Collection and Analyses

At KCCR, the blood samples collected at each ORDISS visit are centrifuged for segregation of the major blood components. Each of plasma (from K_2EDTA tube) and serum (from SS tube) is harvested and aliquoted into two (2) tubes for storage. Buffy coat is then collected from each sample into single tubes. Genomic DNA is manually extracted from aspirated buffy coat samples using the QIAamp DNA blood mini kit (QIAGEN, # 51106). DNA extracts are stored in double eluates/aliquots. Each sediment of red cells is stored in a single tube after washing three times with phosphate buffered saline. A 4.5 ml single aliquot of each urine sample is also stored. All samples at each stage (i.e. recruitment, interim and acute illness) of the study will be processed with a consistent approach at KCCR, and stored at -80^0C.

Duplicate biorepository (i.e. single aliquots) of DNA extracts, plasma and serum samples are held at the Heart, Lung, Blood and Vascular Medicine Institute (VMI), University of Pittsburgh, Pennsylvania, USA. The samples are transferred carefully into intact stockings, and organised into bundles in the stockings; the mouth of each stocking is tied with a string and labelled with a sticker that bears the stocking number, sample type (i.e. serum, plasma or DNA), and stage (visit) collected. The stockings are then placed in a tank containing liquid nitrogen (at -196^0C), the tank stoppered tightly, labelled and shipped via air flight to VMI, observing all protocols. An electronic file in Microsoft Office Excel format showing the samples in the various stockings and stocking bundles being shipped will also be sent electronically to VMI. The shipment of duplicate biorepository to VMI will take place once every year.

A sub-aliquot of each deposit of red cells will be used for haemolysate preparation. The haemolysates will be analysed to ascertain the haemoglobin (Hb) phenotype and determine the percentage of foetal Hb (HbF) of subjects at KCCR. DNA extracts will also be analysed for genetic markers of organ injury at VMI. Plasma, serum and urine samples will be batch

assayed for chemical biomarkers of haemolysis, organ dysfunction and end organ damage both at KCCR and VMI. Assays will be performed using standardised validated enzyme-linked immunosorbent assays and colorimetric techniques. Laboratory investigations are presented in the Table 1, and concise definitions of organ damage with diagnostic criteria are shown in Table 2.

ETHICS AND DISSEMINATION

Ethical and safety considerations

ORDISS is currently in an active phase which commenced in May 2015. Informed consent (and assent where suitable) is obtained from all participants. Blood samples are routinely collected from children with SCD attending the K-CSCD at KATH and collection of urine samples is a non-invasive procedure. Data transmission from the K-CSCD at KATH to KCCR is secure. Biorepository samples are transported from KATH to KCCR and from KCCR to VMI, and stored appropriately according to international standards.

Dissemination

The results of ORDISS will be submitted for publication in peer-reviewed journals, and the key findings presented at national and international sickle cell disease and haematology conferences.

CONCLUSION

It is envisaged that ORDISS will achieve its objectives and will substantially add to the modest amount of existing data on organ damage and progression in SCD. ORDISS will also

provide new insights into organ dysfunction and end-organ damage for future therapeutic inventions.

ACKNOWLEDGMENTS

We are grateful to the participants of the study, and to all staff involved at Komfo Anokye Teaching Hospital, Kumasi Centre for Collaborative Research in Tropical Medicine and University of Pittsburgh.

FOOTNOTES

Contributors: SFO-A, KAA, EO-D, VP, DA, AO-A, KO-F made substantial contributions to the conception, and design of the study. KA, NA, EBA, ATO-A are involved in the acquisition or analyses of data. All the authors are accountable for all aspects of the work.

Funding: ORDISS is supported by the University of Pittsburgh, USA.

Competing Interests: None declared.

Ethics Approval: Research Development Unit of Komfo Anokye Teaching Hospital, Committee on Human Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology and Komfo Anokye Teaching Hospital. University of Pittsburgh Institutional Review Board.

Provenance and Peer Review: Not commissioned; externally peer reviewed.

REFERENCES

1. Weatherall DJ. The challenge of haemoglobinopathies in resource-poor countries. British Journal of Haematology 2011; 154: 736–44.

2. Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, et al. Sickle cell disease in Africa: a neglected cause of early childhood mortality. American Journal of Preventive Medicine 2011;41(6 Suppl 4):S398–S405.

3. Ohene-Frempong K, Oduro J, Tetteh H, Nkrumah F. Screening newborns for sickle cell disease in Ghana. Pediatrics 2008;121: S120-S121.

4. Ansong D, Osei-Akoto A, Ocloo D, Ohene-Frempong K. Sickle Cell Disease: Management options and challenges in developing countries. Mediterranean Journal of Hematology and Infectious Diseases. 2013; 5(1): e2013062.

5. Powars DR. Sickle cell anemia and major organ failure. Hemoglobin. 1990;14:573-98.

6. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. Blood Reviews, 2007; 21:37–47.

7. World Health Organization, 2010. WHO guidelines on drawing blood: best practices in phlebotomy.

Table 1.

Organ Damage in Sickle Cell Disease Study (ORDISS): Laboratory Investigations

Classification	Investigations Conducted
Fundamentals	FBC with WBC differentials, reticulocyte count, Hb phenotype, percentage foetal haemoglobin (HbF), urinalysis
Haemolysis	Plasma Hb, haem, total and fractionated bilirubin, haptoglobulin, haemopexin, haem oxygenase-1, soluble C91 and CD163, arginase
Heart and Skeletal Muscle	Total creatine phosphokinase (CPK), CPK-2, troponin T and I, CPK-3
Lung and Brain	CPK-1, brain-derived neurotrophic factor
Kidney and Liver	Creatinine, blood urea nitrogen, alanine transaminase, aspartate transaminase, total and fractionated protein
Vascular and Systemic Inflammation	Panel of cytokines
Oxidative Stress	Methaemoglobin, oxidized phospholipids, alpha-1 macroglobulin

Table 2.

Organ Damage in Sickle Cell Disease Study (ORDISS): Definitions and Diagnostic Criteria for Organ Dysfunction

Classification of Complications	Clinical Manifestations of Organ Damage	Definition	Diagnostic Criteria
Cardiac	Cardiomegaly	Enlargement of the heart and may involve the ventricles, the atria or both	Evidence of enlargement on CXR or ECG
	Hypertension	BP exceeding the 90th centile for age	BP as measured sitting or supine in the steady state in a warm environment on 3 separate occasions separated by 15min. the BP values greater than the 90th centile for age, sex and height.
	Cardiomyopathy	Heart disease affecting the musculature of the heart leading to impairment of function. Chronic high cardiac output leads to cardiac hypertrophy and development of hypertrophic cardiomyopathy while iron overload causes dilated cardiomyopathy.	ECHO is the most commonly used technique used to measure cardiac function. MRI measurement of volume may be used.
Pulmonary	Acute chest syndrome	Acute illness characterised by fever and/or	Radiographic evidence of consolidation. A

		respiratory symptoms accompanied by a new pulmonary infiltrate on CXR	new segmental (involving at least one segment) radiographic pulmonary infiltrate. Temperature >38.50C, >2% decrease in SPO2, tachypnea, intercostal retractions, nasal flaring, use of accessory muscle, chest pains, cough, wheezing.
Musculoskeletal	Dactylitis	Inflammation caused by ischaemia/infarction of bone/bone marrow of the hands/feet resulting in swelling, redness and pain. It is seen primarily in children from 6months to 3years and generally does not occur beyond 5years of age due to lack of haemopoietic marrow activity in the hands and feet.	Soft tissue swelling of hands/feet and limited range of motion of extremities or pain and tenderness of hands and feet.
	Avascular necrosis of joints	Condition resulting in dead bone tissue due to an interruption in blood supply most likely as a result of vaso-occlusion.	Radiographic evidence of necrosis and subsequent bone changes. Plain films may be normal early in disease whereas MRI demonstrate early changes and provide more detail on the degree of bony involvement.
Neurological	Seizures	Acute onset of uncontrolled electrical activity in the brain which may produce a physical convulsion with minor physical signs, and thought disturbances.	EEG consistent with seizure, sustained abnormal electrical discharges that have a relatively discrete beginning and end or based on clinical history and neuroimaging(CT or MRI)
	Stroke – aneurysm /	Circumscribed blood filled dilatation of a	Visualization by MRA or angiogram of brain/

	haemorrhage / infarctive	cerebral artery caused by weakening of arterial wall / intracranial haemorrhage / acute neurological syndrome resulting from impaired cerebral blood flow without evidence of haemorrhage.	demonstration of haemorrhage on CT scan or MRI on brain/ MRI or CT Scan showing an infarctive CNS event consistent with symptoms and signs.
Renal	Haematuria	Presence of red blood cells in the urine, due to acute papillary necrosis, UTI and less commonly glomerulonephritis, obstruction, analgesic toxicity, mycobacteria infection, tumours, arterio-venous malformation and vasculitis.	Greater than 3 red blood cells per high power field on urine microscopy.
Hepatobiliary	Cholecystitis	Inflammation of gallbladder lining, generally caused by impairment of bile flow, gallstones in the biliary tract, infections, spasms of gall bladder.	Upper quadrant pain-colicky and one or more of the following: Pericholecystic fluid and gallbladder wall thickening>4mm. non visualization of gall bladder by 60min after cholescintigraphy. Positive murphy sign.
	Cholelithiasis/Sludge	Presence or formation of gallstones in biliary tract usually in gallbladder or common bile duct.	Ultrasound evidence of stones or sludge
	Hepatic sequestration	Sequestration of red blood cells in hepatic sinusoids leading to liver enlargement and decreased haemoglobin concentration.	Decrease of >2g/dl in haemoglobin concentration from baseline with reticulocytosis without other explanation, and Liver enlargement of >3cm without other explanation.

Splenic	Acute splenic infarction	Acute ischemic necrosis of spleen as a result of venous or arterial compromise	Acute (L) upper quadrant pain which may be referred to the (L) shoulder, and Imaging evidence of necrotic or ischaemic splenic parenchyma or surgical evidence of acute splenic parenchymal necrosis.

Organ dysfunction definitions adapted from: Ballas et al. Definitions of the phenotypic manifestations of sickle cell disease. American Journal of Hematology 2010; 85:6–13.

Stronger Together

London North West Healthcare NHS Trust

The Editor
BMJ Open

Haematology and Sickle Cell Centre
Central Middlesex Hospital
Acton Lane
London
NW10 7NS

Tel: 020 8453 2050
Fax: 020 8453 2051
Email: kofi.anie@nhs.net

5 March 2017

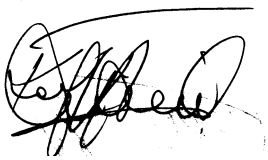
Dear Sir / Madam

Organ Damage in Sickle Cell Disease Study (ORDISS): Design of a Longitudinal Cohort Study Based in Ghana

Please find the above titled manuscript that we are submitting solely to BMJ Open. This manuscript has not been previously published, either in whole or in part.

I corresponding author confirm that I have full access to all aspects of the work and writing process, and take final responsibility for the manuscript.

Yours Sincerely



Dr Kofi A Anie
Consultant Psychologist and
Honorary Clinical Senior Lecturer (Imperial College London)

STROBE Statement—Checklist for ORDISS

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract: **Title Page (1)**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found: **Abstract Page (2)**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported: **Pages 3-4**
Objectives	3	State specific objectives, including any prespecified hypotheses: **Page 4**
Methods		
Study design	4	Present key elements of study design early in the paper: **Pages 4-5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection: **Page 5**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up: **Pages 5-7**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable: **Pages 6-9**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group: **Pages 6-9**
Bias	9	Describe any efforts to address potential sources of bias: **Pages 6-9**
Study size	10	Explain how the study size was arrived at: **Not applicable**
Quantitative variables	11*	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12*	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
		***Not applicable for this manuscript**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
		***Not applicable for this manuscript**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		***Not applicable for this manuscript**
Outcome data	15*	Report numbers of outcome events or summary measures over time
		***Not applicable for this manuscript**
Main results	16*	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and

		their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ***Not applicable for this manuscript**
Other analyses	17*	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ***Not applicable for this manuscript**
Discussion		
Key results	18*	Summarise key results with reference to study objectives ***Not applicable for this manuscript**
Limitations	19*	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ***Not applicable for this manuscript**
Interpretation	20*	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ***Not applicable for this manuscript**
Generalisability	21*	Discuss the generalisability (external validity) of the study results ***Not applicable for this manuscript**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Page 10**

BMJ Open

ORGAN DAMAGE IN SICKLE CELL DISEASE STUDY (ORDISS): PROTOCOL FOR A LONGITUDINAL COHORT STUDY BASED IN GHANA

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-016727.R1
Article Type:	Protocol
Date Submitted by the Author:	01-Jun-2017
Complete List of Authors:	Anie, Kofi; London North West Healthcare NHS Trust, Haematology and Sickle Cell Centre; Imperial College London, Medicine Paintsil, Vivian; Komfo Anokye Teaching Hospital, Directorate of Child Health Owusu-Dabo, Ellis; Kumasi Center for Collaborative Research in Tropical Medicine; Kwame Nkrumah University of Science and Technology Ansong, Daniel; Komfo Anokye Teaching Hospital, Directorate of Child Health; Kwame Nkrumah University of Science and Technology, Child Health Osei-Akoto, Alex; Komfo Anokye Teaching Hospital, Directorate of Child Health; Kwame Nkrumah University of Science and Technology, Child Health Ohene-Frempong, Kwaku; Sickle Cell Foundation of Ghana; Children's Hospital of Philadelphia Aikins Amissah, Kofi; Komfo Anokye Teaching Hospital, Directorate of Child Health Addofoh, Nicholas; Kumasi Center for Collaborative Research in Tropical Medicine Bonwin Ackah, Ezekiel; Kumasi Centre for Collaborative Research in Tropical Medicine Owusu-Ansah, Amma; University of Pittsburgh, Heart, Lung, Blood and Vascular Medicine Institute Ofori-Acquah, Solomon; University of Pittsburgh, Heart, Lung, Blood and Vascular Medicine Institute
Primary Subject Heading:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Genetics and genomics, Global health
Keywords:	Sickle Cell Disease, Organ Dysfunction, Genetic Markers, Biorepository, Complications

ORGAN DAMAGE IN SICKLE CELL DISEASE STUDY (ORDISS): PROTOCOL FOR A LONGITUDINAL COHORT STUDY BASED IN GHANA

Kofi A Anie[1,2], Vivian Paintsil[3], Ellis Owusu-Dabo[4,5], Daniel Ansong[3,6], Alex Osei-Akoto[3,6], Kwaku Ohene-Frempong[7], Kofi Aikins Amissah[3], Nicholas Addofoh[4], Ezekiel Bonwin Ackah[4], Amma Twumwa Owusu-Ansah[8], Solomon Fiifi Ofori-Acquah[8]

[1]Haematology and Sickle Cell Centre, London North West Healthcare NHS Trust, Central Middlesex Hospital, London, UK

[2]Faculty of Medicine, Imperial College London, London, UK

[3]Directorate of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana

[4]Kumasi Centre for Collaborative Research in Tropical Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[5]School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[6]Department of Child Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[7]Sickle Cell Foundation of Ghana, Ghana

[8]Center for Translational and International Hematology, Heart, Lung, and Blood Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Correspondence to:

Dr Kofi A Anie
Email: kofi.anie@nhs.net
Tel: +44 (0) 20 8453 2050
Fax: +44 (0) 20 8453 2051

ABSTRACT

Introduction

Sickle cell disease is highly prevalent in Africa with a significant public health burden. Nonetheless, morbidity and mortality in sickle cell disease that result from the progression of organ damage is not well understood. The Organ Damage in Sickle Cell Disease Study (ORDISS) is designed as a longitudinal cohort study to provide critical insight into cellular and molecular pathogenesis of chronic organ damage for the development of future innovative treatment.

Methods and analysis

ORDISS aims to recruit children aged 0-15 years who attend the Kumasi Centre for Sickle Cell Disease based at the Komfo Anokye Teaching Hospital in Kumasi, Ghana. Consent is obtained to collect blood and urine samples from the children during specified clinic visits and hospitalisations for acute events, to identify candidate and genetic markers of specific organ dysfunction and end-organ damage, over a three-year period. In addition, data concerning clinical history and complications associated with sickle cell disease are collected. Samples are stored in biorepositories and analysed at the Kumasi Center for Collaborative Research in Tropical Medicine, Ghana and the Center for Translational and International Hematology, University of Pittsburgh, USA. Appropriate statistical analyses will be performed on the data acquired.

Ethics and dissemination

Research ethics approval was obtained at all participating sites. Results of the study will be submitted for publication in peer-reviewed journals, and the key findings presented at national and international conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The establishment of a longitudinal cohort study of children with sickle cell disease that intends to obtain biologic samples and clinical data to allow for future studies aimed at elucidating cellular and molecular pathogenesis of chronic organ damage.
- The prospective design will allow risk factors for organ dysfunction associated with sickle cell disease complications to be determined in a naturalistic study of children in a specialist Centre.
- Attrition or loss to follow-up of children with sickle cell disease after the initial study visit at the specialist Centre may lead to a bias and reduction in the internal validity of the study.
- This is a study in a single setting, and risk factors for organ damage characteristic of the particular environment and setting may not be generalizable to populations elsewhere. Further ecological studies will be required examine risk factors for organ damage in multiple populations of children with sickle cell disease.

INTRODUCTION

Sickle cell disease (SCD) comprises a group of inherited red blood cell conditions that result from the abnormal production of haemoglobin. Over 400,000 babies are born worldwide annually with SCD mostly in low and middle income countries, and about 75% or more of these births occur in sub-Saharan Africa, posing an increasing health burden[1] and contributing to early childhood mortality[2]. SCD affects approximately 2% of newborns in Ghana[3].

Clinical syndromes of SCD include anaemia, infection, and the consequences of blood vessel blockage (vaso-occlusion). The latter deprives tissues of oxygen and is indicated as the cause of acute painful episodes, the hallmark of SCD, and other complications such as stroke, acute chest syndrome, priapism, leg ulceration and chronic organ failure. Stem cell transplantation offers curative possibilities although this is not universal, and other treatment options are generally limited in Africa[3]. Improved knowledge and successful primary public health prevention strategies have positively impacted childhood survival transforming SCD into a chronic disease. Nonetheless, progressive deterioration of organ function and end-organ damage is inevitable and appears to be irreversible[4-6]. The mechanisms that lead to these complications, studied mostly outside sub-Saharan Africa, are not fully understood. Further understanding through a longitudinal cohort study of patients with SCD may provide novel insights into cellular and molecular pathogenesis of chronic organ damage, and opportunities for the development of innovative treatment and precisely timed interventions to prevent onset of organ damage.

In Ghana, a pilot Newborn Screening (NBS) project for SCD was established in Kumasi (the second largest city) and Tikrom (a nearby rural community) from 1993 as an international collaborative study[7]. Newborns identified with SCD are registered in the Kumasi Centre for

Sickle Cell Disease (K-CSCD) at the Komfo Anokye Teaching Hospital (KATH), and followed up until 15 years of age through the Child Health Directorate. This NBS project was subsequently adopted by the Government of Ghana in 2010 to scale it up as a national public health programme.

All patients enrolled in the K-CSCD have their haemoglobin (Hb) genotype confirmed with isoelectric focusing (IEF) in the neonatal period, and alkaline Hb electrophoresis beyond the neonatal period. K-CSCD provides comprehensive care for patients with available facilities and services including blood transfusion, radiology, laboratory, pharmacy, orthopaedics, and ophthalmology. There is a team of two consultant paediatricians, two specialist paediatricians, three residents, three house officers, a nurse in charge, eight other nursing staff, and three auxiliary personnel who help with data recording and retrieval of medical records on clinic days. Clinics are held every day.

STUDY OBJECTIVES

There are currently no data on the spectrum of organ dysfunction and end-organ damage in the SCD patient cohort attending K-CSCD. The Organ Damage in Sickle Cell Disease Study (ORDISS) was designed as a longitudinal cohort study of children with SCD attending K-CSCD to document acute events and the progressive deterioration in organ function with age, and to identify candidate and genetic markers of specific organ dysfunction and end-organ damage. Specific objectives are:

1. To determine the proportion of children with SCD attending K-CSCD who develop specific organ dysfunction.
2. To determine levels of biomarkers of organ dysfunction (heart, kidney, liver, lung, brain and skeletal muscle) from multiple candidate plasma and urine samples.

3. To determine haematologic and haemolytic markers in the recruited children attending clinic for routine evaluations or acute illness management.
4. To compare clinical evidence of organ dysfunction with biochemical and genetic markers.

METHODS AND ANALYSES

ORDISS is an international collaborative study conducted at three institutions: Department of Child Health, KATH/Kwame Nkrumah University of Science and Technology (KNUST), Ghana; Kumasi Center for Collaborative Research in Tropical Medicine (KCCR), Ghana; and Center for Translational and International Hematology at the Heart, Lung, and Blood Vascular Medicine Institute (VMI), University of Pittsburgh, USA.

Participants and Recruitment

A consecutive purposive sampling method of all individuals willing to participate is being employed to recruit and follow up participants for the next three years in a longitudinal design.

i. Eligibility Criteria

Eligible participants are families of children with SCD comprising all genotypes, confirmed with both IEF and alkaline electrophoresis with cellulose acetate membrane, who are registered at K-CSCD, aged 0 to 15 years and younger at recruitment, and receive outpatient or inpatient care at KATH. Patients not known to KATH and older than 12 months of age must be registered at K-CSCD for at least 12 months prior to becoming eligible for enrolment.

ii. Exclusion Criteria

Exclusions are children with SCD and co-morbid chronic conditions including malignancies, seizure disorders, and history or clinical signs and symptoms of HIV infection. In addition, patients who cannot be followed-up for a minimum of 12 months during the study, and families who decline informed consent or assent are excluded.

iii. Recruitment and Enrolment Procedures

ORDISS was initially introduced to the families of children with SCD at the monthly Sickle Cell Disease Association meeting, a national support group for parents and patients with SCD, which is held at KATH premises.

Enrolment (Entry) Visit

Consecutive clinic attending families are opportunistically approached during routine clinic visits, and the study introduced to them prior to phlebotomy. Signed or thumb-printed informed consent is obtained from parents/caregivers and assent from children with SCD aged 7 years and over. Consenting families (participants) are enrolled into the study, and the child (subject) is assigned a unique study identification number that will be used as the subject identifier throughout the study. Participants' demographics, clinical information, and past medical history are recorded. These include the subject's age, gender, standing height, weight, head circumference, heart rate, respiratory rate, blood pressure, oxygen saturation (SpO2), and SCD complications relating to eyes, ears, head, nose, and mouth. Examinations of the throat, lymph nodes, chest with auscultation, heart with auscultation, abdomen, liver, spleen, genitalia, extremities, joints, and neurological examinations are performed by specialist paediatricians or residents (registrars), and recorded. In addition, data on parental ethnicity, religion, marital status, and educational level are collected. All information gathered is written in the subject's medical records, and entered into an electronic Case Report Form (e-CRF).

Using standard practice of phlebotomy[8], blood is collected from each subject into di-potassium ethylenediaminetetraacetic acid (K_2EDTA) tube and serum separator (SS) tube with gel, each 3-4 ml; 10-20 ml of midstream urine is also collected from each subject at specific visits. Blood and urine samples are collected from 8am to 12pm on the clinic day. Urine samples are collected from children aged 3 years and over using sterile urine containers, while infant urine collection bags are used for younger children. The latter urine samples are subsequently decanted into sterile urine containers. The blood in the K_2EDTA tube is inverted 8-10 times to ensure adequate mixing of the blood with the EDTA anticoagulant; the blood in the SS tube is allowed to adequately clot[8]. The samples from each subject are duly labelled with the specific study identification number. The K_2EDTA-anticoagulated blood samples are sent, in a cryobox at room temperature, to the KATH Laboratory where aliquots are taken and immediately used for hematologic analyses; these include full blood count (FBC) with white blood cell (WBC) differential, performed electronically, and reticulocyte count, performed manually[9]. The remainder of the blood sample and the urine sample are then placed on wet ice in a cold box and transported to KCCR for further processing, storage and analyses.

Demographic and clinical information, as well as FBC results, of the subjects are entered into a tablet adapted specifically for ORDISS with CommCare software, which allows creation and management of mobile applications through a website. The study database was developed at KCCR where the server is also held. Data are transmitted via a mobile phone network, subject to strength of connectivity, at the end of each clinic day from KATH to the KCCR server for cloud storage and management. This is subsequently extracted into Microsoft Excel spreadsheet format for statistical analyses.

Interim (Follow-up) Visit

On subsequent annual visits after recruitment (i.e. interim visits) over 3 years, clinical procedures and data collection will be replicated, with emphasis on each subject's current ailments, episodes of acute illness not treated at KATH, episodes of enuresis, and current medications. In addition, educational performance is assessed and documented from preceding school-term reports to determine whether this is maintained during the study. Furthermore, blood and urine samples will be collected and identical procedures applied. Additionally, the process of data transmission with tablets via internet to KCCR will be repeated for each interim visit.

Acute Illness Visit and Hospitalisation

During acute illness of subjects, blood and urine samples will be collected together with samples for acute illness blood tests requested by attending clinicians. These blood tests will also help to rule out illness due to other infections such as malaria, and allow comparison with steady-state laboratory values. Identical volumes of blood and urine will be collected from each subject during an acute illness; the samples will be processed using equivalent outlined procedures before these are transported on wet ice to KCCR.

Biorepository Sample Collection and Analyses

At KCCR, the blood samples collected at each ORDISS visit are centrifuged for segregation of the major blood components. Each of plasma (from K_2EDTA tube) and serum (from SS tube) is harvested and aliquoted into two (2) tubes for storage. Buffy coat is then collected from each sample into single tubes. Genomic DNA is manually extracted from aspirated buffy coat samples using the QIAamp DNA blood mini kit (QIAGEN, # 51106). DNA extracts are stored in double eluates/aliquots. Each sediment of red cells is stored in a single tube after washing three times with 1X phosphate buffered saline. A 4.5 ml single aliquot of each urine sample is also stored. All samples at each stage (i.e. recruitment, interim and acute

illness) of the study will be processed with a consistent approach at KCCR, and stored at -80^{0}C.

A duplicate biorepository (i.e. single aliquots) of DNA extracts, plasma and serum samples is maintained at the VMI. The samples are transferred carefully into intact stockings, and organised into bundles in the stockings; the mouth of each stocking is tied with a string and labelled with a sticker that bears the stocking number, sample type (i.e. serum, plasma or DNA), and stage (visit) collected. The stockings are then placed in a tank containing liquid nitrogen (at -196^{0}C), the tank stoppered tightly, labelled and shipped via air flight to VMI, observing all protocols. An electronic file in Microsoft Office Excel format showing the samples in the various stockings and stocking bundles being shipped are also sent electronically to VMI. The first shipment of duplicate biorepository to VMI has already been completed, and will subsequently take place once every year.

A sub-aliquot of each deposit of red cells will be used for haemolysate preparation. The haemolysates will be analysed to ascertain the haemoglobin (Hb) phenotype and determine the percentage of foetal Hb (HbF) of subjects at KCCR. DNA extracts will also be analysed for genetic markers of organ injury at VMI. Plasma, serum and urine samples will be batch assayed for chemical biomarkers of haemolysis, organ dysfunction and end organ damage both at KCCR and VMI. Assays will be performed using standardised validated enzyme-linked immunosorbent assays and colorimetric techniques. Laboratory investigations[10-27] are presented in the Table 1, and concise definitions of organ damage with diagnostic criteria[28] are shown in Table 2.

Statistical Analysis

Subjects will be allocated to the two extreme quartiles of biochemical and clinical evidence of organ dysfunction in a case-control design. The primary analysis will be ANOVA with

either the actual measurements of biomarkers or those normalized by appropriate transformations. Genotypes will be independent variable and the dependent outcomes will be biomarkers. Analyses will be run using the most recent version of the STATA software. In the event that there is evidence for a significant interaction between single nucleotide polymorphism (SNP) and a clinical event, analyses will also be run on organ damage and non-organ damage subjects independently. As with other phenotypes, it is likely that multiple genetic variants operate to affect the risk of specific clinical events more than any single SNP or plasma biomarker, independently. Thus, SNPs will be tested in 2-way-ANOVA and if a significant interaction term is observed, the effect size will be compared to the linear model. The results will be interpreted in light of known pathways and feedback loops for specific biomarkers. An exploratory analysis will be performed examining the relationship among biomarkers belonging to the same pathways. Specifically, we will interrogate data to see if correlations among these factors differ by the status of a clinical phenotype, which would suggest differences in the overall network of factors; this will be performed using Spearman's rank correlation and testing for heterogeneity among organ damage phenotypes using a t-test on the Fisher *r*-to-*z* transformations of the Spearman correlation coefficients performed. SNPs will be initially tested for association with the occurrence of acute organ damage as a dichotomous trait (e.g. +ACS/-ACS). Statistical tests for differences in single locus allele and genotype frequencies will be calculated using PLINK. All loci will also be tested for Hardy-Weinberg equilibrium to assess the possibility of genotyping error. Genetic association will be concluded if the frequencies of either genotypes or alleles differ significantly between the extreme quartile classes ($p<0.05$). Odds ratios will be calculated using logistic regression.

ETHICS AND DISSEMINATION

Ethical and safety considerations

Research ethics approvals for ORDISS were obtained from both the Committee on Human Research, Publications and Ethics of KNUST (Approval No. CHRPE/AP/325/14) and subsequently renewed approvals (No. CHRPE/AP/104/16 and No. CHRPE/140/17), and University of Pittsburgh Institutional Review Board (Approval No. PRO14010452). The study is currently in an active phase which commenced in May 2015, and just began year three. Informed consent (and assent where applicable) is obtained from all participants. Blood samples are routinely collected from children with SCD attending the K-CSCD at KATH and collection of urine samples is a non-invasive procedure. Data transmission from the K-CSCD at KATH to KCCR is secure. Biorepository samples are transported from KATH to KCCR and from KCCR to VMI, and stored appropriately according to international standards. Samples sent to VMI are de-identified, and there is an ethics (institution review board) approved material transfer agreement between the collaborating institutions.

Dissemination

The results of ORDISS will be submitted for publication in peer-reviewed journals, and the key findings presented at national and international sickle cell disease and haematology conferences.

CONCLUSION

It is envisaged that ORDISS will achieve its objectives and will substantially add to the modest amount of existing data on onset and progression of organ damage in children with SCD. ORDISS will also provide new insights into organ dysfunction and end-organ damage for appropriate and more precise timing of future therapeutic inventions.

ACKNOWLEDGMENTS

We are grateful to the participants of the study, and to all staff involved at Komfo Anokye Teaching Hospital, Kumasi Centre for Collaborative Research in Tropical Medicine and University of Pittsburgh.

FOOTNOTES

Contributors: SFO-A, KAA, EO-D, VP, DA, AO-A, KO-F made substantial contributions to the conception, and design of the study. KA, NA, EBA, ATO-A are involved in the acquisition or analyses of data. All the authors are accountable for all aspects of the work.

Funding: ORDISS is supported with a collaborative seed grant from the Center for Translational and International Hematology, Vascular Medicine Institute, University of Pittsburgh, USA.

Competing Interests: None declared.

Provenance and Peer Review: Not commissioned; externally peer reviewed.

REFERENCES

1. Weatherall DJ. The challenge of haemoglobinopathies in resource-poor countries. British Journal of Haematology 2011; 154(6): 736–44.

2. Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, et al. Sickle cell disease in Africa: a neglected cause of early childhood mortality. American Journal of Preventive Medicine 2011;41(6 Suppl 4):S398–S405.

3. Ansong D, Osei-Akoto A, Ocloo D, Ohene-Frempong K. Sickle Cell Disease: Management options and challenges in developing countries. Mediterranean Journal of Hematology and Infectious Diseases. 2013; 5(1): e2013062.

4. van Beers EJ, van Tuijn CF, Mac Gillavry MR, van der Giessen A, Schnog J-JB, Biemond BJ, Group CS: Sickle cell disease-related organ damage occurs irrespective of pain rate: implications for clinical practice. Haematologica 2008, 93(5):757-760.

5. Powars DR. Sickle cell anemia and major organ failure. Hemoglobin. 1990;14:573-98.

6. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. Blood Reviews, 2007; 21:37–47.

7. Ohene-Frempong K, Oduro J, Tetteh H, Nkrumah F. Screening newborns for sickle cell disease in Ghana. Pediatrics 2008;121: S120-S121.

8. World Health Organization, 2010. WHO guidelines on drawing blood: best practices in phlebotomy.

9. Bain BJ, Bates I, Laffan MA. Dacie and Lewis Practical Haematology 12th ed. London: Elsevier Health Sciences; 2016; 27-30.

10. Rees DC, Gibson JS: Biomarkers in sickle cell disease. British Journal of Haematology 2012, 156(4):433-445.

11. Driscoll MC: Sickle cell disease. Pediatrics in review 2007, 28(7):259.

12. Bender M, Seibel GD: Sickle cell disease. 2014.

13. Rother RP, Bell L, Hillmen P, Gladwin MT: The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA 2005, 293(13):1653-1662.

14. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman H-J, Law SA, Moestrup SK: Identification of the haemoglobin scavenger receptor. Nature 2001, 409(6817):198-201.

15. Philippidis P, Mason J, Evans B, Nadra I, Taylor K, Haskard D, Landis R: Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis. Circulation research 2004, 94(1):119-126.

16. Pathare A, Al Kindi S, Alnaqdy AA, Daar S, Knox□Macaulay H, Dennison D: Cytokine profile of sickle cell disease in Oman. American journal of hematology 2004, 77(4):323-328.

17. Wagener F, Feldman E, de Witte T, Abraham NG: Heme induces the expression of adhesion molecules ICAM-1, VCAM-1 and E selectin in vascular endothelial cells (44197). 1997.

18. Akohoue SA, Shankar S, Milne GL, Morrow J, Chen KY, Ajayi WU, Buchowski MS: Energy expenditure, inflammation, and oxidative stress in steady-state adolescents with sickle cell anemia. Pediatric research 2007, 61(2):233-238.

19. Milne GL, Musiek ES, Morrow JD: F2-isoprostanes as markers of oxidative stress in vivo: an overview. Biomarkers 2005, 10(sup1):10-23.

20. Klings ES, Christman BW, McClung J, Stucchi AF, McMahon L, Brauer M, Farber HW: Increased F2 isoprostanes in the acute chest syndrome of sickle cell disease as a marker of oxidative stress. American journal of respiratory and critical care medicine 2001, 164(7):1248-1252.

21. B Jeter C, J Hylin M, W Hergenroeder G, L Hill J, R Johnson D, A Barrera J, C Shields T, B Redell J, Zhao J, N Moore A: Biomarkers of Organ Injury. Recent Patents on Biomarkers 2014, 4(2):98-109.

22. Arslan G, Gemici AA, Yirgin IK, Gulsen E, Inci E: Liver trauma grading and biochemistry tests. Emergency radiology 2013, 20(5):379-384.

23. Schinstock CA, Semret MH, Wagner SJ, Borland TM, Bryant SC, Kashani KB, Larson TS, Lieske JC: Urinalysis is more specific and urinary neutrophil gelatinase-associated lipocalin is more sensitive for early detection of acute kidney injury. Nephrology Dialysis Transplantation 2013, 28(5):1175-1185.

24. Fremont RD, Koyama T, Calfee CS, Wu W, Dossett LA, Bossert FR, Mitchell D, Wickersham N, Bernard GR, Matthay MA: Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. The Journal of trauma 2010, 68(5):1121.

25. McLean AS, Huang SJ, Salter M: Bench-to-bedside review: the value of cardiac biomarkers in the intensive care patient. Critical Care 2008, 12(3):215.

26. Saenger AK: A tale of two biomarkers: the use of troponin and CK-MB in contemporary practice. Clinical laboratory science 2010, 23(3):134.

27. Guild CS, deShazo M, Geraci SA: Negative predictive value of cardiac troponin for predicting adverse cardiac events following blunt chest trauma. Southern medical journal 2014, 107(1):52-56.

28. Ballas SK, Lieff S, Benjamin LJ, Dampier CD, Heeney MM, Hoppe C, Johnson CS, Rogers ZR, Smith□Whitley K, Wang WC: Definitions of the phenotypic manifestations of sickle cell disease. American journal of hematology 2010, 85(1):6-13.

Table 1.

Organ Damage in Sickle Cell Disease Study (ORDISS): Laboratory Investigations

Classification	Parameters	Marker of Interest	References
Fundamentals	Basic/general organ function	FBC with WBC differentials, reticulocyte count and percentage, Hb phenotype, percentage HbF	[10-12]
Index of Injury	Haemolysis	Plasma Hb, haem, haptoglobulin, haemopexin, haem oxygenase-1, total and fractionated Hb, soluble C91, soluble CD163, arginase	[13-15]
	Vascular and Systemic Inflammation	IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, soluble VCAM-1, soluble ICAM-1, P-selectin, E-selectin, nitric oxide metabolites, TNF-α, IFN-	[13, 15-17]
	Oxidative Stress	Methaemoglobin, oxidized phospholipids, alpha-1 microglobulin, isoprostanes	[18-20]
Organs of Interest	Kidney and Liver	Creatinine, blood urea nitrogen, alanine transaminase, aspartate transaminase, total and fractionated	[21-23]

		protein	
	Lung and Brain	CPK-1, brain-derived neurotrophic factor	[21, 24]
	Heart and Skeletal Muscle	Total CPK, CPK-2, troponin T and I, CPK-3	[21, 25-27]

FBC=full blood count, WBC=white blood cells, Hb=haemoglobin, IL=interleukin, VCAM=vascular cell adhesion molecule, ICAM=intravascular cell adhesion molecule, TNF=tumour necrosis factor, IFN=interferon, CPK=creatine phosphokinase

Table 2.

Organ Damage in Sickle Cell Disease Study (ORDISS): Definitions and Diagnostic Criteria for Organ Dysfunction

Classification of Complications	Clinical Manifestations of Organ Damage	Definition	Diagnostic Criteria
Cardiac	Cardiomegaly	Enlargement of the heart and may involve the ventricles, the atria or both	Evidence of enlargement on CXR or ECG
	Hypertension	BP exceeding the 90th centile for age	BP as measured sitting or supine in the steady state in a warm environment on 3 separate occasions separated by 15min. the BP values greater than the 90th centile for age, sex and height.
	Cardiomyopathy	Heart disease affecting the musculature of the heart leading to impairment of function. Chronic high cardiac output leads to cardiac hypertrophy and development of hypertrophic cardiomyopathy while iron overload causes dilated cardiomyopathy.	ECHO is the most commonly used technique used to measure cardiac function. MRI measurement of volume may be used.
Pulmonary	Acute chest syndrome	Acute illness characterised by fever and/or respiratory symptoms accompanied by a new pulmonary infiltrate on CXR	Radiographic evidence of consolidation. A new segmental (involving at least one segment) radiographic pulmonary infiltrate.

			Temperature >38.50C, >2% decrease in SPO2, tachypnea, intercostal retractions, nasal flaring, use of accessory muscle, chest pains, cough, wheezing.
Musculoskeletal	Dactylitis	Inflammation caused by ischaemia/infarction of bone/bone marrow of the hands/feet resulting in swelling, redness and pain. It is seen primarily in children from 6months to 3years and generally does not occur beyond 5years of age due to lack of haemopoietic marrow activity in the hands and feet.	Soft tissue swelling of hands/feet and limited range of motion of extremities or pain and tenderness of hands and feet.
	Avascular necrosis of joints	Condition resulting in dead bone tissue due to an interruption in blood supply most likely as a result of vaso-occlusion.	Radiographic evidence of necrosis and subsequent bone changes. Plain films may be normal early in disease whereas MRI demonstrate early changes and provide more detail on the degree of bony involvement.
Neurological	Seizures	Acute onset of uncontrolled electrical activity in the brain which may produce a physical convulsion with minor physical signs, and thought disturbances.	EEG consistent with seizure, sustained abnormal electrical discharges that have a relatively discrete beginning and end or based on clinical history and neuroimaging(CT or MRI)
	Stroke – aneurysm / haemorrhage / infarctive	Circumscribed blood filled dilatation of a cerebral artery caused by weakening of arterial wall / intracranial haemorrhage / acute	Visualization by MRA or angiogram of brain/ demonstration of haemorrhage on CT scan or MRI on brain/ MRI or CT Scan showing an

		neurological syndrome resulting from impaired cerebral blood flow without evidence of haemorrhage.	infarctive CNS event consistent with symptoms and signs.
Renal	Haematuria	Presence of red blood cells in the urine, due to acute papillary necrosis, UTI and less commonly glomerulonephritis, obstruction, analgesic toxicity, mycobacteria infection, tumours, arterio-venous malformation and vasculitis.	Greater than 3 red blood cells per high power field on urine microscopy.
Hepatobiliary	Cholecystitis	Inflammation of gallbladder lining, generally caused by impairment of bile flow, gallstones in the biliary tract, infections, spasms of gall bladder.	Upper quadrant pain-colicky and one or more of the following: Pericholecystic fluid and gallbladder wall thickening>4mm. non visualization of gall bladder by 60min after cholescintigraphy. Positive murphy sign.
	Cholelithiasis/Sludge	Presence or formation of gallstones in biliary tract usually in gallbladder or common bile duct.	Ultrasound evidence of stones or sludge
	Hepatic sequestration	Sequestration of red blood cells in hepatic sinusoids leading to liver enlargement and decreased haemoglobin concentration.	Decrease of >2g/dl in haemoglobin concentration from baseline with reticulocytosis without other explanation, and Liver enlargement of >3cm without other explanation.
Splenic	Acute splenic infarction	Acute ischemic necrosis of spleen as a result of	Acute (L) upper quadrant pain which may be

		venous or arterial compromise	referred to the (L) shoulder, and Imaging evidence of necrotic or ischaemic splenic parenchyma or surgical evidence of acute splenic parenchymal necrosis.

Organ dysfunction definitions adapted from: Ballas *et al*., 2010[28]

STROBE Statement—Checklist for ORDISS

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract: **Title Page (1)**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found: **Abstract Page (2)**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported: **Pages 3-4**
Objectives	3	State specific objectives, including any prespecified hypotheses: **Page 4**
Methods		
Study design	4	Present key elements of study design early in the paper: **Pages 4-5**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection: **Page 5**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up: **Pages 5-7**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable: **Pages 6-9**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group: **Pages 6-9**
Bias	9	Describe any efforts to address potential sources of bias: **Pages 6-9**
Study size	10	Explain how the study size was arrived at: **Not applicable**
Quantitative variables	11*	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12*	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
		***Not applicable for this manuscript**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
		***Not applicable for this manuscript**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		***Not applicable for this manuscript**
Outcome data	15*	Report numbers of outcome events or summary measures over time
		***Not applicable for this manuscript**
Main results	16*	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and

		their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period ***Not applicable for this manuscript**
Other analyses	17*	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ***Not applicable for this manuscript**
Discussion		
Key results	18*	Summarise key results with reference to study objectives ***Not applicable for this manuscript**
Limitations	19*	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ***Not applicable for this manuscript**
Interpretation	20*	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ***Not applicable for this manuscript**
Generalisability	21*	Discuss the generalisability (external validity) of the study results ***Not applicable for this manuscript**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Page 10**