Sickle cell disease

Russell E Ware, Mariane de Montalembert, Léon Tshilolo, Miguel R Abboud

Sickle cell disease is a common and life-threatening haematological disorder that affects millions of people worldwide. Abnormal sickle-shaped erythrocytes disrupt blood flow in small vessels, and this vaso-occlusion leads to distal tissue ischaemia and inflammation, with symptoms defining the acute painful sickle-cell crisis. Repeated sickling and ongoing haemolytic anaemia, even when subclinical, lead to parenchymal injury and chronic organ damage, causing substantial morbidity and early mortality. Currently available treatments are limited to transfusions and hydroxycarbamide, although stem cell transplantation might be a potentially curative therapy. Several new therapeutic options are in development, including gene therapy and gene editing. Recent advances include systematic universal screening for stroke risk, improved management of iron overload using oral chelators and non-invasive MRI measurements, and point-of-care diagnostic devices. Controversies include the role of haemolysis in sickle cell disease pathophysiology, optimal management of pregnancy, and strategies to prevent cerebrovascular disease.

Lancet 2017; 390: 311–23

Published Online January 31, 2017 http://dx.doi.org/10.1016/S0140-6736(17)30193-9

Division of Hematology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA (Prof RE Ware MD PhD); **Department of Pediatrics, Hopital Necker, LABEX GR-Ex, Paris, France** (M de Montalembert MD PhD); **Centre Hospitalier Monkole, Kinshasa, Democratic Republic of Congo** (Prof L Tshilolo MD PhD); **and Department of Pediatrics, American University of Beirut, Beirut, Lebanon** (Prof MR Abboud MD)

Correspondence to: Dr Russell E Ware Division of Hematology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA **russell.ware@cchmc.org**

Introduction

Sickle cell disease is familiar to physicians but the term is actually a misnomer, since it refers not to a single disease but rather to a collection of inherited blood disorders that feature the propensity for erythrocytes to change into crescent or so-called sickle shapes. This characteristic morphology is akin to the common agricultural cutting tool bearing the same name. The condition is well-recognised in many African tribal cultures by distinct names such as *chwechweechwe*, *ahututuo*, or *nuidudui*, which are onomatopoeic and reflect the painful symptoms and cries from affected patients. Additional names such as *kibeka* or *malari ya mifupa ina pasuka* describe signs and symptoms, such as large spleen or broken bones. Perhaps the most sobering name is *ako kufa lobi*, which translates loosely to "he will die tomorrow". Although sickle cell is a common phrase among Anglophones, "drepanocytose" from Greek *drepane* (sickle) or "anemia falciforme" from Latin *falcicula* (sickle or false shape) are common in Europe and used throughout Francophone and Lusophone Africa.

Health and survival of children with sickle cell disease have improved considerably with the advent of newborn screening, penicillin prophylaxis, pneumococcal immunisation, and education about disease complications. Unfortunately, the average projected lifespan of affected adults has not improved beyond the fifth decade,[1] although wider use of hydroxycarbamide and newer therapeutic approaches offer hope for decreased mortality and improved health-related quality of life. Sadly, sickle cell disease in low-resource countries carries a bleak prognosis, especially in sub-Saharan Africa, where it is associated with high early childhood mortality.

This Seminar does not provide an encyclopaedic or exhaustive summary of the history, pathophysiology, and management of sickle cell disease; readers are referred to recent summaries,[2,3] and National Heart, Lung, and Blood Institute Evidence-Based Guidelines.[4] Instead, we offer a concise review of clinically relevant issues including current treatments, new advances, novel therapies, areas of controversy, and assessment of the global disease burden. We attempt to document exciting developments in the field reflecting activity and interest by academic institutions, pharmaceutical industries, biotechnology companies, and governments. We predict the near future will provide a long overdue paradigm shift with unprecedented opportunities for prolonged and improved life for people with sickle cell disease.

Diagnosis

Sickle cell disease is caused by the inheritance of abnormal beta-globin alleles carrying the sickle mutation on the *HBB* gene (Glu6Val, β^s). The most common and severe form is homozygous HbSS (sickle cell anaemia) with inheritance of β^s from both parents, which permits formation of the pathological sickle haemoglobin tetramer ($\alpha_2\beta^s{}_2$, HbS). Other forms of sickle cell disease include compound heterozygous conditions, such as haemoglobin C (HbC) with HbS (HbSC), HbS with β-thalassaemia (HbS/β^0-thalassaemia or HbS/β^+-thalassaemia), and HbS with other beta-globin variants such as HbSD or $HbSO_{Arab}$, all of which express sufficient HbS to cause intracellular sickling. The inheritance of both HbA and HbS (HbAS) is sickle cell trait; strictly not a form of sickle cell disease, sickle trait might be associated with adverse health outcomes

Search strategy and selection criteria

We did a careful search of the scientific literature in English up to July, 2016, using the terms "sickle cell", "hydroxyurea or hydroxycarbamide", "transfusions", "transcranial Doppler", and "alloimmunization"; we also reviewed the 2014 National Heart, Lung, and Blood Institute (NHLBI) evidence-based guidelines. We searched through ClinicalTrials.gov for all open trials identified as "sickle cell", "hydroxyurea", and "open or recruiting". We identified and reviewed all published phase 3 trials for children and adults with sickle cell anaemia, with a focus on trials involving cerebrovascular disease, transfusion therapy, and hydroxyurea.

	HbA (%)	HbS (%)	HbC (%)	HbF (%)	HbA_2 (%)	Clinical Course	Prevalence (%)*
Normal	95–98%	0	0	<1%	<3·5%	..	..
Trait conditions							
Sickle trait HbAS	55–65%	30–40%	0	<1%	<3·5%	Benign	1–8%
Haemoglobin C trait	55–65%	0	30–40%	<1%	<3·5%	Benign	1–3%
β-thalassaemia trait	90–95%	0	0	1–3%	>3·5%	Benign	1–2%
Disease conditions							
Sickle cell anaemia	0	80–95%	0	5–15%	<3·5%	Severe	50–60%
Sickle-C disease	0	50–55%	40–45%	<3%	<3·5%	Moderate	25–30%
$S/β^0$ thalassaemia	0	80–90%	0	5–15%	>3·5%	Severe	1–3%
$S/β^+$ thalassaemia†	10–25%	70–80%	0	<3%	>3·5%	Mild	5–10%
S/Other (Hb variant)	0	50–60%	0	Variable	<3·5%	Variable	1–2%

Trait conditions refer to beta globin heterozygous states, while disease conditions refer to compound heterozygous or homozygous states. Concomitant alpha-thalassaemia can coexist with all of these conditions and affects the ratio of HbA to HbS or HbC, as shown by the range of values for each haemoglobin listing. Sickle cell genotypes are shown with the typical haemoglobins present on electrophoresis, clinical course, and prevalence. The sickle/other disease conditions typically have 50–60% HbS with 20–45% of a variant haemoglobin such as HbD, HbE, or HbO_{Arab}. Thalassaemic trait and disease also feature microcytosis with low mean corpuscular volume. *Prevalence refers to persons living in the USA, Caribbean, UK, and Europe; the haemoglobin trait percentages refer to the general population and the disease percentages refer to sickle cell patient cohorts. †$S/β^+$ thalassaemia in the USA, Caribbean, UK, and northern Europe typically has 10–25% HbA, but more mild and severe forms of $β^+$ thalassaemia have been identified, particularly in southern Europe. Accurate prevalence figures from high burden regions such as sub-Saharan Africa are not available. HbA=. HbS=sickle haemoglobin. HbC=haemoglobin C. HbD=haemoglobin D. HbE=haemoglobin E. HbF=fetal haemoglobin. HbO_{Arab}=haemoglobin OArab.

***Table 1*: Common forms of sickle cell disease and related haemoglobinopathies by genotype**

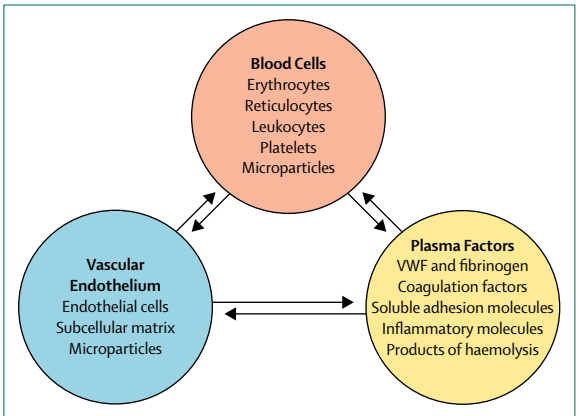

***Figure 1*: Complex interactions that lead to vaso-occlusion and tissue injury in sickle cell disease**
Older models of vaso-occlusion featured primary erythrocytes undergoing hypoxia-induced sickling with resulting blood hyperviscosity and stasis, which damaged the endothelium and led to transient ischaemia and subsequent intimal hyperplasia. Modern versions include other circulating blood cells and plasma factors that have abnormal interactions with the endothelium, in addition to erythrocytes undergoing intracellular HbS polymerisation upon deoxygenation. This multi-step and multi-cellular process leads to short-term tissue hypoxia, long-term inflammation, and endothelial vasculopathy. VWF=von Willebrand factor.

that are not further discussed here. Table 1 lists common genotypes observed in people with haemoglobinopathy traits and forms of sickle cell disease, with a brief summary of laboratory values and clinical course.

The diagnosis of sickle cell disease is relatively simple, since haemoglobin is abundant in blood, and numerous techniques can identify HbS and variant haemoglobins. Electrophoresis separating normal from abnormal haemoglobins is the most common technique, using standard alkaline gel, isoelectric focusing, capillary electrophoresis, or high performance liquid chromatography. Chemical and solubility tests that identify HbS are prone to error and should not be used in isolation to establish the diagnosis of sickle cell disease. Newer methods including DNA-based or antibody-based tests offer the possibility of accurate point-of-care diagnostics.

Pathophysiology

In sickle cell disease, erythrocytes undergo rapid but reversible shape change on deoxygenation, and intracellular polymerisation of the abnormal HbS molecule stretches the normal flexible biconcave shape into an elongated rigid form. Sickled erythrocytes cause vaso-occlusion together with many other cellular and plasma factors and abnormal endothelial interactions (figure 1), leading to a broad range of acute and chronic clinical complications caused by repeated ischaemia and inflammation. Re-oxygenation of erythrocytes breaks down the HbS polymer and restores the normal shape. This process of sickling and unsickling continues until the erythrocyte membrane is no longer flexible, and irreversibly sickled cells with dense intracellular polymers undergo intravascular haemolysis or extravascular removal by the reticuloendothelial system. Plasma free haem and plasma free haemoglobin might contribute to vascular damage.

Acute clinical complications

A wide variety of acute complications occur in sickle cell disease reflecting the complex pathophysiology of vaso-occlusion, infection, anaemia, and infarction (table 2). In well-resourced countries, acute complications are rarely fatal for children[5] but can be lethal for adults with chronic organ complications, including renal, cardiopulmonary, and vascular dysfunction.[6,7] Acute chest syndrome and stroke are potentially life-threatening and require prompt and well-structured organisation to deliver emergent and specialised care.

Acute pain, the hallmark clinical feature of sickle cell disease, reflects vaso-occlusion and impaired oxygen supply, but also infarction-reperfusion injury.[8] Infants develop dactylitis, and both children and adults can have pain affecting bones in the extremities, chest, and back. Non-steroidal anti-inflammatory drugs and opioids provide effective relief, and guidelines describe appropriate management of sickle-related pain.[4] Pain in sickle cell disease involves peripheral nociceptor activation and hyperalgesia,[9] possibly with mast cell activation,[10] suggesting novel paradigms and therapies.

Bacterial infections are serious because the spleen is damaged during infancy from intraparenchymal sickling. Functional asplenia leaves children at risk for life-threatening pneumonia, sepsis, and meningitis,

especially from encapsulated organisms like *Streptococcus pneumoniae* and *Haemophilus influenzae* type b. Pneumococcal conjugate vaccines have dramatically reduced the incidence of invasive pneumococcal disease in sickle cell disease, but non-vaccine serotypes could emerge.[11] Prophylactic penicillin is required for young children, along with education to seek emergent medical care for fever and receive blood culture and broad-spectrum antibiotics.[4] Acute chest syndrome, defined as a new pulmonary infiltrate with chest pain, fever, tachypnea, wheezing, or cough, is a leading cause of death in adults.[6] Antibiotics covering pneumococcus, chlamydia, and mycoplasma should be provided, and oxygen and transfusions should be available as needed. Respiratory deterioration with worsening hypoxaemia necessitates urgent exchange transfusion.[6,12]

Worsening of anaemia can occur with acute splenic sequestration crisis,[13] transient red cell aplasia, or increased haemolysis especially following transfusions.[14,15] Simple transfusion should correct haemoglobin concentrations only to the baseline value, avoiding cardiovascular compromise and hyperviscosity. Transfusions are warranted for severe anaemia, with clinical indications and complications summarised in figure 2.

Stroke occurs primarily in childhood, with acute onset of neurological dysfunction from ischaemia and infarction, but cerebral haemorrhage occurs primarily in adults.[16] Before transcranial Doppler (TCD) screening, stroke occurred in 5–10% of children,[16,17] leaving permanent sequelae with motor, cognitive, and psychological deficits. The sudden onset of neurological abnormalities must be presumed stroke, warranting prompt brain MRI and magnetic resonance angiography (MRA) including diffusion-weighted, perfusion, and vascular sequences if available, or else the use of CT scans can be substituted. For acute stroke, exchange transfusion should be done promptly, followed by chronic transfusions to prevent stroke recurrence.[18]

	Manifestations	Pathophysiology	Treatment
Painful event	Dactylitis; pain in the sternum/ribs; pain in the long bones; priapism	Vaso-occlusion; hypoxia; ischaemia-reperfusion	Hydration; analgesia
Infection	Bacteraemia/sepsis; meningitis; osteomyelitis; pneumonia; malaria	Splenic dysfunction; inflammation; necrotic bone	Antibiotics; surgery
Anaemia	Splenic sequestration; transient aplastic crisis; transfusion reaction; papillary necrosis	Erythrocyte sickling; infection; sequestration; hyperhaemolysis	Transfusion
Organ damage	Stroke; acute chest syndrome; splenic infarction; papillary necrosis	Ischaemia; infarction; haemorrhage	Hydration; transfusion

This table includes the major categories and examples but is not an exhaustive listing.

Table 2: **Acute clinical complications of sickle cell disease, including the common manifestations, presumed pathophysiology, and treatment options**

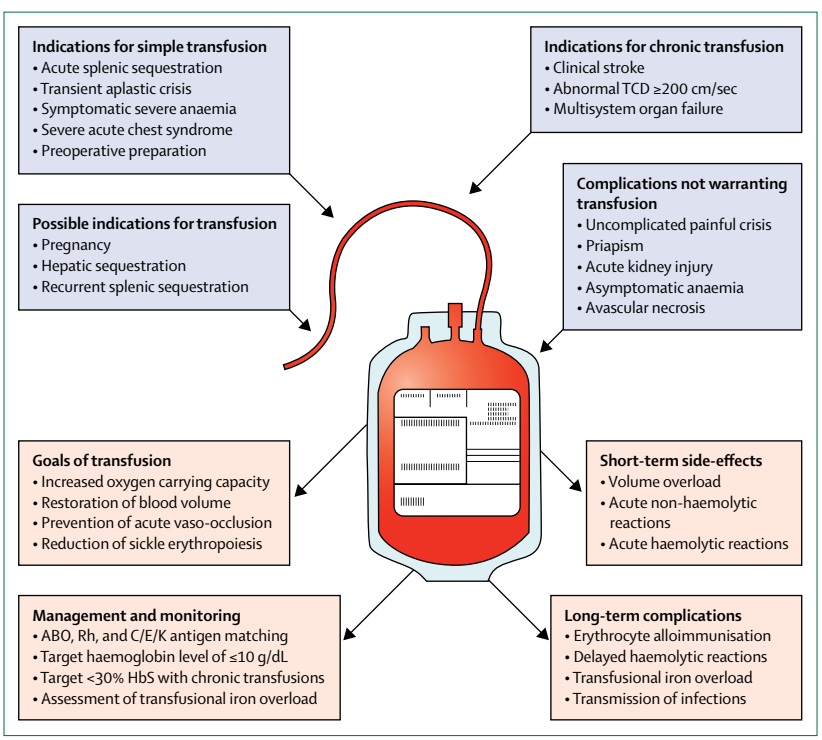

Figure 2: **Clinical indications and goals that influence the decision to transfuse a patient with sickle cell disease**
Management and monitoring, and short-term and long-term complications are described. TCD=transcranial Doppler.

Chronic organ complications

Compared with acute sickling events, less is known about the pathogenesis of organ damage resulting from repeated vaso-occlusion, infarction, and chronic haemolytic anaemia. A wide variety of organ dysfunction and failure is recognised, leading to high use of health care facilities, poor quality of life, and shortened survival. Most adult patients with sickle cell disease reach a stage, usually in the third decade, when chronic organ complications become the main cause of morbidity and mortality.

Parenchymal damage develops through different mechanisms and almost every organ system can be affected (table 3). Complications such as retinopathy, avascular necrosis, neurological decline, leg ulcers, and recurrent priapism are associated with morbidly and impaired quality of life, but renal dysfunction and cardiopulmonary disease are the most lethal.[19–21] Sickle nephropathy starts in childhood with loss of urine concentrating ability and glomerular hyperfiltration. Frequently, proteinuria develops with glomerulosclerosis, decreased glomerular filtration rate, and eventual end-stage renal disease.[22] Microalbuminuria is an early sign, occurring in 30% of patients by adulthood and progressing to nephrotic proteinuria.[23] Angiotensin converting enzyme (ACE) inhibitors can decrease proteinuria; ten patients with biopsy-proven glomerular enlargement and focal segmental glomerulosclerosis received enalapril, resulting in a 57% reduction in 24-hour urinary protein excretion ($p<0·001$).[24] ACE inhibitors are commonly prescribed for albuminuria,[22,25] but hydroxycarbamide is also effective for treatment and prevention of

	Manifestations	Treatment
Kidneys	Hyposthenuria; glomerular hyperfiltration; glomerulosclerosis; albuminuria; end-stage renal disease	ACE inhibitors; hydroxycarbamide; dialysis; renal transplant
Heart/lungs	Restrictive lung disease; elevated tricuspid jet velocity; pulmonary hypertension; restrictive cardiomyopathy	Bronchodilators; hydroxycarbamide*; transfusions*
Brain	Ischaemic stroke; haemorrhagic stroke; silent infarction; neurological decline	Transfusion; hydroxycarbamide
Liver	Jaundice; pigmented gallstones	Cholecystectomy
Spleen	Infarction; hypersplenism	Splenectomy
Bones/skin	Avascular necrosis; leg ulcers	Physical therapy; core decompression; wound care; surgery
Eyes	Retinopathy	Laser therapy
Penis	Impotence; infertility	Surgery (if needed)

Major categories and examples are included but this table does not provide an exhaustive listing. *=unproven treatment benefit. ACE=angiotensin converting enzyme.

Table 3: **Chronic clinical complications of sickle cell disease including manifestations and treatment options, by organ dysfunction**

proteinuria.[26-28] Fetal haemoglobin (HbF) and α thalassaemia trait protect against renal dysfunction, partly because haemolysis might contribute to development of sickle nephropathy.[29,30]

Cardiac and pulmonary complications were responsible for 45% of deaths in a large retrospective study of adults with sickle cell disease.[20] Pulmonary hypertension and diastolic cardiac dysfunction are associated with increased mortality in adult patients, as are increased tricuspid regurgitant velocities.[31–33] Pulmonary hypertension should be confirmed by right heart catheterisation, yet increased tricuspid regurgitant velocities predict severity and early mortality regardless of pulmonary pressures. The roles of chronic haemolysis and anaemia in this setting have been reviewed,[34] but are not universally accepted. A recent prospective study and meta-analysis[35] identified an alternative explanation of restrictive cardiomyopathy, which might bridge the gap between current hypotheses and clinical findings. Current interventions include hydroxycarbamide therapy, and in some cases, chronic transfusions and specific drugs such as bosentan or riociguat for pulmonary hypertension, but these therapies do not yet have clear evidence of efficacy.[34,36]

Established and future therapies

Transfusions

The mainstay of treatment for sickle cell disease is erythrocyte transfusions, with more than 90% of adults receiving at least one transfusion in their lifetimes.[14] Transfusions are given acutely for immediate benefits, such as increased oxygen-carrying capacity and improved blood flow, using the simple transfusion technique. Chronic transfusions help prevent long-term complications by replacing rigid sickled erythrocytes with normal deformable cells and by suppressing formation of sickled erythrocytes, using monthly simple or exchange transfusions.

Figure 2 shows the clinical indications for simple transfusions including severe anaemia, acute organ damage, and preoperative prophylaxis. Sickle cell disease always features ongoing haemolytic anaemia, but acute symptomatic exacerbation of chronic anaemia can cause symptoms of hypoxia and hypoperfusion. Examples include acute splenic sequestration,[13] transient red cell aplasia from parvovirus B19 infection, and hyperhaemolysis.[37] To avoid hyperviscosity, the post-transfusion haemoglobin concentration should not exceed the baseline amount.

Stroke and acute chest syndrome represent acute organ damage that benefits from transfusion; for such serious clinical events the target is less than 30% HbS, a threshold based on expert consensus rather than findings from randomised trials.[4] This goal is achieved most easily using exchange transfusion procedures, such as manual or automated (erythrocytapheresis) techniques, which remove sickled erythrocytes but maintain the baseline haematocrit.[37] Preoperative preparation also benefits from acute transfusion. A multicentre US-based study[38] randomly assigned patients to aggressive versus conservative transfusions; perioperative and postoperative complications were similar for both groups. The recent UK multicentre Transfusion Alternatives Preoperatively in Sickle Cell Disease (TAPS) trial randomly assigned patients to preoperative transfusion versus standard care, but was halted for increased clinical complications (risk ratio 3·8, 95% CI 1·2–12·2, p=0·027) with standard care, especially acute chest syndrome.[39]

Indications for chronic transfusion most frequently relate to stroke prevention, given that children and adults with overt stroke have high risk for recurrence.[40] Transfusions help prevent secondary stroke and discontinuation of prophylaxis is associated with stroke recurrence and increased mortality.[41] Clinical focus has now shifted towards primary stroke prevention, to identify children at highest risk using TCD screening followed by transfusion. In the NIH-sponsored phase 3 multicentre STOP trial,[42] children with abnormal TCD velocities (≥200 cm/s) randomly assigned to receive transfusions had fewer strokes than those assigned to standard care, with a 92% reduction in stroke risk, (1 [2%] of 63 transfusion group versus 11 [16%] of 67 standard care, p<0·001). The STOP2 trial[43] then showed that transfusions were required indefinitely, since children randomised to discontinue transfusions had more reversions to abnormal velocities and stroke than those continuing regular transfusions (16 [39%] of 41 discontinued transfusion group *vs* 0 of 38 continued transfusion group, p<0·001). The SIT trial[44] compared monthly transfusions with standard care for children

with silent (subclinical) cerebral infarction but without abnormal TCD velocities. In this trial, a lower incidence of recurrent infarcts occurred with transfusions (incidence rate ratio 0·41, 95% CI 0·12–0·99, p=0·04), although benefits were primarily for overt stroke (1 [1%] of 99 in transfusion group versus 7 [7%] of 97 in observation group) with similar progression of subclinical strokes (5 [5%] of 99 versus 7 [7%] of 97).

Despite their proven benefits, transfusions have complications that can limit long-term use. Erythrocyte alloimmunisation, reflecting discrepancies in blood group antigens between donors and recipients,[14] is especially relevant for sickle cell disease, in which delayed haemolytic transfusion reactions can be life-threatening.[15] In practice, serological matching for ABO blood group antigens, Rhesus antigens (RhD, RhC, and RhE), and Kell (K) antigens does not fully prevent alloimmunisation, because of *RH* alleles with reduced or altered antigen expression.[45] Extended, and even molecular genotyping, of blood group antigens is now implemented in some centres.[46]

Regularly transfused patients develop haemosiderosis with parenchymal iron deposition,[47] and can experience fatal complications.[48] Iron chelation by oral or parenteral administration is therefore mandatory when affordable. Assessment of iron overload is difficult using serum markers because chronic inflammation increases ferritin levels; MRI quantification of liver iron content is novel and proving helpful. Transfusion-acquired iron overload in the heart is rare in sickle cell disease, probably because iron released by transfusion and haemolysis is efficiently handled by the effective erythropoiesis of sickle cell disease, but not as well by the ineffective erythropoiesis in thalassaemia.[49] Erythrocytapheresis reduces iron loading from chronic transfusion programmes and reduces the need for chelation.[37]

An ongoing concern regarding transfusions relates to transmission of blood-borne organisms. Transfusion-transmitted viral infections have been greatly reduced as a result of the use of screening strategies,[50] but transmission of hepatitis B and hepatitis C, as well as HIV, still occurs with disappointing frequency in low-resource settings, where systematic screening is not universal.[51] In Senegal[52] more than 7% of accepted blood donors carry hepatitis B surface antigen, 0·71% have hepatitis C antibodies, 0·34% have syphilis, and 0·05% have HIV. In Africa, additional concerns exist about blood availability and cost.

Hydroxycarbamide

Recognising that HbF inhibits intracellular HbS polymerisation, and that higher HbF is associated with reduced morbidity and mortality,[53] pharmacological induction of HbF is a logical goal for treating sickle cell disease. Numerous treatments can increase HbF concentrations, but most are toxic and not feasible for long-term therapy. Hydroxycarbamide was shown to induce HbF in sickle cell disease over 30 years ago,[54] and subsequent studies[55–67] have proven its laboratory and clinical efficacy.

As a potent ribonucleotide reductase inhibitor, hydroxycarbamide depletes intracellular deoxynucleotide pools required for DNA synthesis and repair; this cytostatic activity is used therapeutically for malignancy,[68] HIV,[69] and myeloproliferative neoplasms.[70] HbF induction also involves other metabolic pathways including guanylyl cyclase[71] and SAR1 (a guanosine triphosphate-binding protein),[72] but altered cell cycle kinetics with stress erythropoiesis might also be important.[73]

Hydroxycarbamide is well-tolerated in sickle cell disease when given once daily orally, with few short-term side effects. After a phase 1/2 trial,[55] a multicentre randomised phase 3 trial in adults showed a successful primary endpoint with 44% reduction in the median incidence of painful crisis per year (2·5 crises per year in hydroxycarbamide group *vs* 4·5 in control group, p<0·001).[56] Secondary endpoints had significant benefits in time to painful crisis, episodes of acute chest syndrome, and number of transfusions and admissions to hospital.[56]

Trials in children quickly followed in Europe and the USA, including a cross-over design (20 mg/kg per day *vs* placebo) with significant laboratory and clinical benefits including frequency of hospital admission (p=0·0016) and duration of hospital stay (p=0·0027).[57] After a phase 1/2 trial,[58] long-term treatment in children revealed sustained benefits without long-term toxicities.[59,60] Pilot studies showed safety of hydroxycarbamide in infants[61] and toddlers,[62] leading to BABY HUG,[63] a multicentre randomised controlled phase 3 trial in 213 infants aged 9–18 months at enrolment. This study did not meet its primary endpoint of preserved organ function: for splenic uptake (27% decrease in hydroxycarbamide group *vs* 38% in placebo group, difference –11, 95% CI –26 to 5, p=0·21) and glomerular filtration rate (increased by 23 mL/min per 1·73m² *vs* increase by 21 mL/min per 1·73m², difference +2, 95% CI –16 to 20, p=0·84). However, key secondary endpoints showed benefits of hydroxycarbamide including reduced pain, dactylitis, transfusions, and admissions to hospital (all p<0·001); hydroxycarbamide also had significant favourable effects with other quantitative measures of splenic (p=0·04) and renal (p=0·02) function as well as lower TCD velocities (p<0·001).[63]

In children with known cerebrovascular disease, early studies suggested benefits of hydroxycarbamide for primary and secondary stroke prevention.[64,65] SWiTCH,[66] a multicentre randomised phase 3 trial compared standard treatment of blood transfusions with chelation with an alternative treatment of hydroxycarbamide with phlebotomy for secondary stroke prevention and management of iron overload. The composite primary endpoint was not met: more strokes occurred in the alternative treatment group (0 of 66 standard group *vs*

seven [10%] of 67 alternative group, p=0·0086) but no difference in iron unloading (median change –2·2 *vs* –1·2 mg Fe per g dry weight liver, p=0·49).[66] SWiTCH was stopped early and concluded that transfusions with chelation remains the better way to manage children with stroke and iron overload. Subsequently, the TWiTCH[67] multicentre randomised phase 3 trial compared treatment with hydroxyurea with transfusions for primary stroke prevention. The non-inferiority primary endpoint was met with an average TCD velocity of 138 cm/s (95% CI 135–142) in the hydroxycarbamide group compared with 143 cm/s (95% CI 140–146) in the transfusion group (non-inferiority $p=8·82\times10^{-16}$, post-hoc superiority p=0·023).[67] Switching from transfusions to treatment with hydroxyurea can be considered for some children with cerebrovascular disease.

Transplantation and gene therapy

Stem cell transplantation remains the only curative modality for patients with sickle cell disease.[74–76] HLA-matched sibling transplantation procedures use marrow or cord blood stem cells, with overall survival and event-free survival approaching 90%; by contrast, peripheral blood stem cells are associated with increased mortality.[76] Despite its success, stem cell transplantation has a restricted application, because only 10–20% of patients have unaffected matched sibling donors and substantial concerns remain about transplant-related mortality and long-term toxicities, particularly with regard to infertility.[77,78] Transplantation outcomes must also be compared with hydroxycarbamide therapy, for which long-term treatment appears to be safe and effective.[77]

Most successful stem cell transplantations for sickle cell disease have been limited to procedures in children, through the use of myeloablative conditioning and fully matched sibling donors. These regimens can be toxic, so are typically available only for those with severe disease but no end organ dysfunction.[79] However, transplantations are effective in preventing future clinical complications such as painful vaso-occlusive crises, acute chest syndrome, stroke, and progression of cerebrovascular disease.[74,75] Reduced toxicity conditioning regimens have allowed successful transplantation in adults, by using matched sibling or family donors and these procedures have reported 87% event-free survival.[80]

Attempts to make stem cell transplants available to more patients by expanding the donor pool using matched unrelated donors and unrelated cord blood units have not been successful. In a US multi-centre trial[81] investigating matched, unrelated transplants, the unrelated cord blood group was terminated from the study early because of increased graft rejection, with event-free survival of only 37·5% including one death from chronic graft-versus-host disease. Similar results were observed in 29 patients with matched unrelated donors with 2 year event free survival of 69%; there was a 28% and 62% incidence of acute and chronic graft-versus-host disease, respectively, with 7 deaths. As a result, the regimen was deemed unsafe for widespread use.[82] Haploidentical donors potentially allow most young patients with sickle cell disease to have a stem cell transplant, but is currently experimental and high risk; reported overall survival ranges from 75% to 100% but with high rates of graft rejection and transplantation-related morbidity.[78,83] A modified protocol with promising early results is now the basis of a multi-institutional trial (STRIDE 2 [NCT02766465], unpublished).

Gene therapy approaches to cure sickle cell disease are in a rapid state of scientific discovery and clinical investigation.[84] Bone marrow is used to isolate stem cells, followed by ex vivo incubation with viruses containing an additional gene. After treatment with myeloablative chemotherapy, the patient receives re-infusion of the modified autologous stem cells, which then repopulate the marrow and express the new gene. Current approaches add genes encoding anti-sickling beta-globins (eg, AT87Q, AS3) or HbF, designed for production only in erythroid progenitors. Clinical trials are ongoing with several patients treated to date, with encouraging preliminary results.[85] By contrast to gene addition, approaches involving gene correction for HbF induction are in preclinical stages.[86] Silencing or knockdown of *BCL11A*, a transcriptional factor that potently inhibits gamma-globin expression, is another promising approach for gene editing and correction,[84,87] along with forced chromatin looping to reverse globin switching.[88]

New therapeutic agents

Excluding hydroxycarbamide, there is an unacceptable dearth of therapeutic options for sickle cell disease. For example, the US Food and Drug Administration currently has several dozen approved therapies for HIV infection but none for children with sickle cell disease. There is renewed interest in treatments that target specific pathophysiological pathways, and new compounds are entering clinical trials. Reviewed elsewhere,[89] the main treatment categories include interruption of vaso-occlusion by reducing cellular adhesion, inflammation, or hypercoagulability; prophylaxis against vaso-occlusion by changing cellular hydration, coagulation, haemolysis, oxidation, or platelet activation; and prevention of intracellular HbS polymerisation through HbF induction or haemoglobin stabilisation.

Table 4 summarises current therapeutic approaches and open clinical trials. Treatments inhibiting cell adhesion target selectin molecules on erythrocytes, leukocytes, platelets, and endothelium:[90,91] Crizanlizumab (SelG1) targets P-selectin and has achieved success in a recent phase 2 study,[92] and pan-selectin inhibitor rivipansel (GMI-1070) and non-specific poloxamer 188 are in phase 3 investigation. Heparins have anti-selectin effects plus anti-inflammatory properties;[93] sevuparin has minimal effects on coagulation. Regadenoson is an adenosine 2A receptor agonist with potent effects on the number and activation of invariant natural killer T cells that mediate inflammation.[94]

Treatments with recent negative phase 3 results include senicapoc to modify erythrocyte hydration[95] and prasugrel with anti-platelet activity.[96] Other anti-platelet agents like ticagrelor remain under investigation, while statins represent a novel means to prevent vaso-occlusion.[97]

Although hydroxycarbamide reproducibly increases HbF levels, other treatments in development include short-chain fatty acid derivatives, histone deacetylase inhibitors, and immunomodulatory drugs.[98–100] Compounds that modify the oxygen-dissociation curve could reduce deoxygenation that starts intracellular HbS polymerisation.[101]

Global burden of disease

Despite progress to optimise clinical care and expand therapy for sickle cell disease, most observations and research have originated from patients living in North America, UK, or the rest of Europe. However, the worldwide burden is much greater in lower-resource settings and especially in sub-Saharan Africa. According to a published estimate,[102] in 2010 there were 312 302 newborns born with HbSS (IQR 294 307–329 729) and over half of these were born in Nigeria, DR Congo, and India. The prevalence of sickle trait varies between 2–30% in African populations,[103] with an estimated worldwide birth rate of 5 476 407 (IQR 5 290 779–5 679 288) babies heterozygous for sickle cell disease.[102] On the basis of population estimates, by 2050 the number of affected babies could increase by 30%, especially within Africa.[104]

Without newborn screening and simple treatments like penicillin prophylaxis and pneumococcal immunisations, most children born with sickle cell disease die before 5 years of age,[105] although accurate mortality rates in Africa are not available since most children with sickle cell disease die without proper diagnosis or management. According to the World Bank, sickle cell disease is an important cause of disability within sub-Saharan Africa and a leading cause in Nigeria.[106] The 2015 Global Burden of Disease report[107] includes sickle cell disease as causing more than 100 000 deaths, representing a 6·0% increase in prevalence since 2005. Strategies for early identification, control, and management of sickle cell disease could prevent thousands of childhood deaths each year. WHO estimates that sickle cell disease accounts for up to 15% of mortality in children under the age of 5 years in Africa.[108]

Neonatal screening for sickle cell disease has been implemented in many sub-Saharan countries (Nigeria, Ghana, Benin, DR Congo, Tanzania, Gabon, Cameroon, Uganda, and Angola) as pilot programmes, but not yet as systematic or sustainable national strategies.[109] The prevalence of sickle trait in central Africa is typically 10–20% for heterozygous HbAS and 1–2% for homozygous HbSS, with differences according to ethnic distribution and prevalence of malaria.[102,103] However, most sickle cell disease management programmes in Africa are located in urban areas, with services rarely available in rural areas, where most patients live. A recent economic analysis of newborn screening in Angola showed high cost-effectiveness,[110] which should encourage other African countries to adopt and expand screening services. India has begun targeted screening in high-burden states, recognising that marginalised tribal populations have the highest prevalence.[111] Screening in Jamaica has recently expanded to island-wide access, stimulated by compelling data of reduced infant mortality.[112] Immigration into the European Union has heightened the urgency for increased sickle cell disease screening and improved services for patients arriving from the Middle East and Africa.[113] Figure 3 indicates the steps required beyond screening for implementing a national sickle cell disease strategy.

From a clinical perspective, sickle cell disease in low-resource countries features variable phenotypic expression depending on genetic and environmental factors. More severe disease expression occurs with the Central African Republic (Bantu) beta-globin haplotype in central African countries, with worse anaemia and lower concentrations of HbF.[114] Central African patients also display specific clinical features: hand–foot syndrome, sepsis, and acute anaemia are early clinical signs; persistence of a large spleen occurs in 30–40% of children over age 5 years. Acute chest syndrome is common but can be challenging to diagnose and treat without proper

	Treatment	Phase	Endpoint measure	Enrolment
NCT01245179	Panobinostat	1	Safety, tolerability	Recruiting
NCT01685515	Decitabine and tetrahydrouridine	1	Safety, tolerability	Recruiting
NCT02114203	PDE9 inhibitor	1	Safety, tolerability	Recruiting
NCT02285088, NCT02567682	GBT440	1	Safety, tolerability	Recruiting
NCT02712346	Ambrisentan	1	Safety, tolerability	Recruiting
NCT01566890, NCT01788631	Regadenoson	2	Vaso-occlusion	Recruiting
NCT01891292	Enalapril and N-acetylcysteine	2	Renal	Recruiting
NCT01895361	SelG1	2	Vaso-occlusion	Completed
NCT02098993	Unfractionated heparin	2	Acute chest syndrome	Recruiting
NCT02214121, NCT02482298	Ticagrelor	2	Vaso-occlusion	Recruiting
NCT02373241	Losartan	2	Nocturnal BP	Recruiting
NCT02411708	Carboxyhemoglobin	2	Vaso-occlusion	Recruiting
NCT02515838	Sevuparin	2	Vaso-occlusion	Recruiting
NCT02536170	L-arginine	2	Pain	Recruiting
NCT02672540	Carboxyhemoglobin	2	Vaso-occlusion	Not open
NCT01737814, NCT02449616	Poloxamer 188	3	Vaso-occlusion	Completed
NCT02187003	GMI-1070	3	Vaso-occlusion	Recruiting
NCT02580773	Tinzaparin	3	Acute chest syndrome	Not open
NCT02604368	Omega-3 fatty acids	3	Vaso-occlusion	Not open

Studies that are investigating the use of analgesics, hydroxycarbamide, or transfusions were not included in this list, and transplantation-related protocols were also excluded. Trials were accessed on ClinicalTrials.gov on July 18, 2016. More study details are available from www.clinicaltrials.gov. BP=blood pressure.

Table 4: **Completed, current, or upcoming clinical trials for sickle cell disease with new investigational agents, listed by ClinicalTrials.gov reference number**

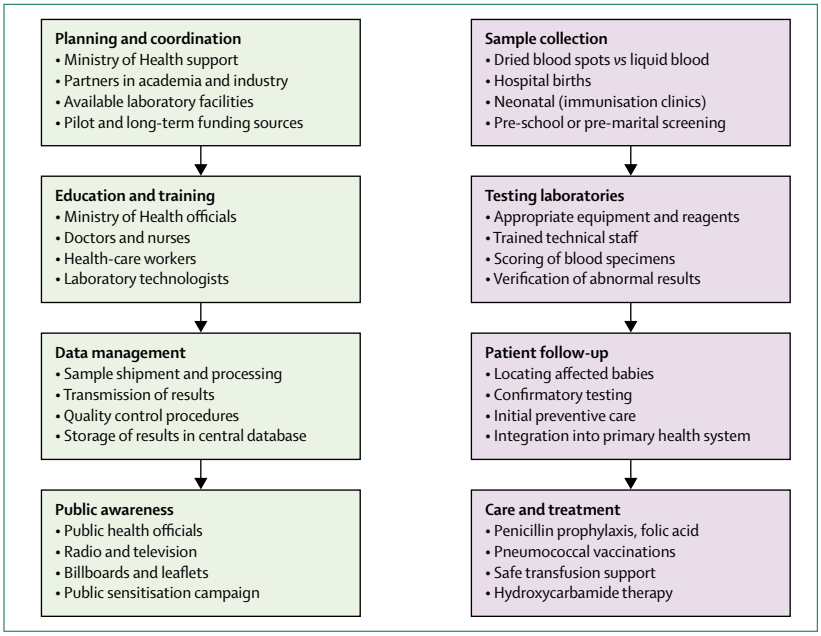

***Figure 3*: Key components of a successful neonatal haemoglobinopathy screening programme**
The left panels refer to administrative issues and the right panels focus on laboratory logistics and clinical care. Individual steps are not sequential and can be done simultaneously, indicated by pilot studies in sub-Saharan Africa. All testing laboratories should have two methods of diagnosis, although rapid point-of-care testing might serve as the first step, especially in rural areas. Sample transport works best with dried blood spots compared with liquid specimens in low-resource settings.

	Study locations	Phase	Dosage (mg/kg per day)	Primary endpoint	Enrolment target (status)
NCT01801423 (SPIN)	Nigeria	1/2	20	Adherence	40; (complete)
NCT01966731 (REACH)	Angola, DR Congo, Kenya, Uganda	1/2	20 × 1 year, then escalate to MTD	Cytopenia	600; (complete)
NCT01976416 (NOHARM)	Uganda	3	20	Malaria events	200; (complete)
NCT02149537 (Risk Stratification)	Nigeria	4	Fixed dose, low	Cytopenia	40; (recruiting)
NCT02556099 (EXTEND)	Jamaica	2	MTD	TCD velocity	50; (complete)
NCT02560935 (SPRING)	Nigeria	3	10 *vs* 20	First stroke	220; (not open)
NCT02675790 (SPRINT)	Nigeria	3	10 *vs* 20	Stroke recurrence	60; (not open)
NCT02769845 (SACRED)	Dominican Republic	2	MTD	TCD velocity	500; (recruiting)

Trials were accessed on ClinicalTrials.gov on July 18, 2016. More study details are available from www.clinicaltrials.gov. MTD=maximum tolerated dose. TCD=transcranial Doppler.

***Table 5*: Current studies investigating hydroxycarbamide treatment for patients with sickle cell disease living in low-resource settings, listed by ClinicalTrials.gov reference number**

imaging and supportive care. Torrential epistaxis, tooth decay, and hypertrophic tonsillitis are also frequent in young patients. Osteomyelitis can be severe and is often multifocal. Malaria is the primary cause of hospital admission and can be fatal; vascular complications including stroke, pulmonary hypertension, and renal failure also occur and can lead to death. Transfusions are restricted by blood availability and risk of viral infections. Hydroxycarbamide therapy is needed, but is currently unavailable because of high costs, inaccessibility, and little experience with dosing and monitoring.

Knowledge of the natural history of sickle cell disease in sub-Saharan countries is essential to promote key policies that governments are able to implement as recommended by WHO. Early diagnosis, public sensitisation, and comprehensive care programmes; rational use of safe blood transfusions; prevention of infections using oral penicillin and immunisations; use of rapid diagnostic tests particularly in rural areas; prevention of complications by accessible hydroxycarbamide are recommended and essential for improving care in resource-poor regions. Fortunately, research is now being done in high burden regions (table 5). The multi-national CADRE study[115] in west Africa follows-up thousands of patients and recently reported proteinuria in childhood, warranting early screening and treatment. Hydroxycarbamide is being introduced into sub-Saharan countries through specific prospective clinical trials;[116,117] if shown to be safe and effective, wider access with affordable pricing will be required.

Recent advances

Universal TCD screening

Stroke is a devastating clinical complication of sickle cell disease that leaves permanent motor, cognitive, and psychological deficits. TCD measures the maximum time-averaged mean velocity (TAMV) of blood flow in the intracranial arteries, and identifies children with abnormal velocities (≥200 cm/s) at highest risk of developing stroke.[118] TCD does not identify moya-moya disease or aneurysms; for this reason TCD screening in adults, using adapted TAMV thresholds, should be combined with brain MRI.[119,120] Data exist[121] that suggest alterations in the extracranial carotid artery also might increase stroke risk, even without intracranial arterial disease. In 435 stroke-free children with sickle cell disease (median age 7·9 years), increased velocities were found in 10·3% of children and were strongly associated with arterial stenosis and silent cerebral infarcts.[122,123] Taken together, compelling evidence exists for systematic and universal TCD screening to help prevent primary stroke in sickle cell disease.[124] Even in low-resource countries, TCD is robust and can be used to monitor treatment effects.[125,126]

Management of iron overload

Iron overload occurs commonly in sickle cell disease following sporadic or regular transfusions. Transfusion-acquired iron cannot be removed physiologically, and is stored in tissue macrophages until excess iron is deposited in the liver and other organs. Two oral iron chelators, deferiprone and deferasirox, represent major treatment advances because of their relative safety and efficacy profiles, and because medication adherence is improved, compared with parenteral desferrioxamine.[127,128]

Chelation therapy can reduce haemosiderosis in sickle cell disease, but serial monitoring of liver iron concentration is needed to guide dosing and effectiveness. MRI relaxation time techniques (T1, T2, and T2*) estimate liver iron concentration non-invasively, since the presence of iron in MRI shortens relaxation times, resulting in a darker image. In clinical practice, results from both T2 and T2* (reported as their reciprocal values R2 and R2*) are quantitative and the liver iron concentrations correlate well to liver biopsy results.[129] MRI-R2 is available commercially and approved by the US-Food and Drug Administration and European Medicines Agency, but the MRI-T2* technique has several advantages: liver and other organs can be assessed for iron content, estimated iron concentration correlates tightly with biopsy results, and only a single breath hold is required for each image.[47,130,131]

Point-of-care diagnostics

Haemoglobin separation techniques can establish the diagnosis of sickle cell disease accurately, but require electricity, equipment, training, and often sample transport to a specialty laboratory. The availability of an accurate point-of-care device to diagnose sickle cell disease is therefore highly desirable, particularly in low-resource settings. The time-honored method of sickle solubility (eg, SickleDex® [Streck, Omaha, NE, USA]) test, which adds buffered saponin to a blood sample to release intracellular HbS and a reducing agent (bisulfite, hydrosulfite) to generate an insoluble turbid solution, is notorious for poor accuracy and reliability. It does not distinguish sickle cell trait (HbAS) from sickle cell anemia (HbSS) reliably, and is fraught with additional caveats that reduce its predictive value, thus limiting its clinical usefulness. In the past 3 years[132–134] academia and industry have developed point-of-care devices that capitalise on biochemical, biophysical, or immunological differences between HBS and HbA to establish a true diagnosis. Most of these devices are in prototype stage but early results are encouraging;[132–134] if point-of-care diagnostic devices currently in development are found to be simple, accurate, robust, reliable, and inexpensive, then this would be a revolutionary improvement for patients with sickle cell disease living in low-resource settings.

Controversies

Role of haemolysis

Much controversy exists about the respective roles of vaso-occlusion and haemolysis in the pathophysiology of sickle cell disease complications. Two sub-phenotypes have been proposed, associating markers of haemolysis with specific clinical complications in contrast with complications related to sickling, vaso-occlusion, and viscosity.[135] The mechanism of haemolysis-associated complications is nitric oxide (NO) depletion following intravascular haemolysis, with plasma free haemoglobin directly binding and inactivating NO, while arginase released from erythrocytes depletes arginine, the precursor of NO. The most controversial element of this framework is pulmonary hypertension resulting from haemolysis and NO scavenging, detected by increased tricuspid regurgitant velocities and associated with early mortality.[33,34] Subsequent publications have questioned these findings and challenged the hypothesis,[136–138] and no consensus has yet been reached. A recent study[139] of erythrocyte survival suggests poor correlation with intravascular haemolysis in sickle cell disease.[139]

Management of pregnancy

Pregnancy in sickle cell disease is associated with increased pain, infections, pulmonary complications, and thromboembolic events. A meta-analysis[140] published in 2015 of 21 studies worldwide with 26 349 pregnant women with sickle cell disease found an increased risk of maternal mortality (relative risk (RR) 5·98, 95% CI 1·94–18·44) and stillbirth (RR 3·94, 2·60–5·96). In France, pregnant women with sickle cell disease have increased mortality, particularly post partum because of sickle-related complications.[141] Transfusions during pregnancy, reviewed in a meta-analysis[142] of 12 studies involving 1291 women, showed reductions in maternal mortality (odds ratio (OR) 0·23, 95% CI 0·06–0·91), perinatal mortality (OR 0·43, 0·19–0·99), and neonatal death (OR 0·26, 0·07–0·93). A prospective multicentre randomised trial is warranted to identify the benefits and risks of transfusions during pregnancy, and the safety of continuing hydroxycarbamide during pregnancy and lactation also needs investigation.[143]

Prevention of cerebrovascular disease

Silent and overt cerebral infarcts are common and damaging in sickle cell disease, but the benefits of prophylactic transfusions must be balanced against the concerns of transfusion complications, especially iron overload, long-term costs, and availability of antigen-matched blood supply. Another option is to treat children with hydroxycarbamide early in life, to protect against the development of cerebrovascular disease. Evidence from three phase 3 multicentre trials[63,67,125] suggests that hydroxycarbamide might provide such protective effects on the developing brain. National Institutes of Health guidelines recommend that hydroxycarbamide should be offered to infants at age 9 months,[4] but this strategy is not applied everywhere—especially in Europe—because of concerns about long-term toxicities, particularly on fertility.[144] Early stem cell transplantation, before developing cerebrovascular disease, represents another option to avoid the acute and chronic neurological morbidity associated with sickle cell disease.[145]

Conclusions

Observational studies investigating the pathophysiology of sickle cell disease and its complications have documented the serious morbidity and early mortality of

this inherited blood disorder. Interventional research trials have shown the efficacy of transfusions and hydroxycarbamide. We are now entering an exciting era when improved neonatal screening, effective infection prophylaxis, prevention of neurological complications, and early hydroxycarbamide therapy offer the chance to alter the natural history of sickle cell disease. Novel treatments hold further promise to interrupt acute vaso-occlusive complications, as well as ameliorate and even prevent chronic organ damage that reduces the quality and duration of life. Curative therapies can now be realised through stem cell transplantation, and gene therapy could allow straightforward genetic correction in the future. However, despite these exciting opportunities and advances, the global burden of sickle cell disease is enormous and remains inadequately addressed. Renewed efforts from well-resourced countries should focus on improved diagnostics and treatments that aim to lessen the crushing lethality of sickle cell disease worldwide.

Contributors

REW, MdM, LT, and MRA designed the Seminar and wrote the first draft of the manuscript, including figures and tables. All authors participated in the editing of the manuscript and approved the final version, and all fulfill authorship requirements as outlined in the ICME recommendations.

Declaration of interest

REW reports grants and non-financial support from Bristol-Myers Squibb, non-financial support and material donation from Addmedica, Grants from Biomedomics, and personal fees from Global Blood Therapeutics, NovaLaboratories, and Bayer Pharmaceuticals, outside the submitted work. MdM reports grants from Addmedica during the study, grants and personal fees from Novartis and grants from Pfizer outside the submitted work. LT is a member of the advisory board of SEBIA and Pierre Fabre Foundations. He has financial support from AFD, a French Cooperative Agency for international projects on sickle cell disease in four African countries. Other financial support was received from France Expertise/Initiative that concerns a study on malaria resistance. MRA reports grants from Eli Lilly, Pfizer, MAST therapeutics, Hemaquest, Shire, Astra Zeneca, outside the submitted work. He also reports personal fees from Pfizer and Novartis, and has participated on steering committees and advisory boards for Eli Lilly, Pfizer, and Novartis.

References

1 Lanzron S, Carroll CP, Haywood C Jr. Mortality rates and age at death from sickle cell disease: US, 1979–2005. *Public Health Rep* 2013; **128:** 110–16.

2 Heeney MM, Ware RE. Sickle cell disease. In Orkin SH, Nathan DG, Ginsburg D, Look AT, Fisher DE, Lux S, eds. Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th ed. Philadelphia, PA: Saunders, 2015. 675–714.

3 Serjeant GR. The natural history of sickle cell disease. *Cold Spring Harb Perspect Med* 2013; **3:** a011783.

4 Yawn BP, Buchanan GR, Afenyi-Annan AN, et al. Management of sickle cell disease: summary of the 2014 evidence-based report by expert panel members. *JAMA* 2014; **312:** 1033–48.

5 Couque N, Girard D, Ducrocq R, et al. Improvement of medical care in a cohort of newborns with sickle-cell disease in north Paris; impact of national guidelines. *Br J Haematol* 2016; **173:** 927–37.

6 Elmariah H, Garrett ME, De Castro LM, et al. Factors associated with survival in a contemporary adult sickle cell cohort. *Am J Hematol* 2014; **89:** 530–35.

7 Powars DR, Chan LS, Hiti A, Ramicone E, Johnson C. Outcome of sickle cell anemia: a 4-decade observational study of 1056 patients. *Medicine(Baltimore)* 2005; **84:** 363–76.

8 Eltzschiq HK, Eckle T. Ischemia and reperfusion—from mechanism to translation. *Nat Med* 2011; **17:** 1391–401.

9 Cataldo G, Raiput S, Gupta K, Simone DA. Sensitization of nociceptive spinal neurons contributes to pain in a transgenic model of sickle cell disease. *Pain* 2015; **156:** 722–30.

10 Vincent L, Vang D, Nguyen J, et al. Mast cell activation contributes to sickle cell pathobiology and pain in mice. *Blood* 2013; **122:** 1853–62.

11 McCavit TL, Quinn CT, Techasaensiri C, Rogers ZR. Increase in invasive *Streptococcus pneumoniae* infections in children with sickle cell disease since pneumococcal conjugate vaccine licensure. *J Pediatr* 2011; **158:** 505–07.

12 Gladwin MT, Vichinsky E. Pulmonary complications of sickle cell disease. *N Engl J Med* 2008; **359:** 2254–65.

13 Brousse V, Elie C, Benkerrou M, et al. Acute splenic sequestration crisis in sickle cell disease: cohort study of 190 paediatric patients. *Br J Haematol* 2012; **156:** 643–48.

14 Chou ST. Transfusion therapy for sickle cell disease: a balancing act. *Hematology Am Soc Hematol Educ Program* 2013; **2013:** 439–46.

15 De Montalembert M, Dumont MD, Heilbronner C, et al. Delayed hemolytic transfusion reaction in children with sickle cell disease. *Haematologica* 2011; **96:** 801–07.

16 Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: rate and risk factors. *Blood* 1998; **91:** 288–94.

17 Balkaran B, Char G, Morris JS, Thomas PW, Serjeant BE, Serjeant GR. Stroke in a cohort of patients with homozygous sickle cell disease. *J Pediatr* 1992; **120:** 360–66.

18 Brousse V, Kossorotoff M, de Montalembert M. How I manage cerebral vasculopathy in children with sickle cell disease. *Br J Haematol* 2015; **170:** 615–25.

19 Hamideh D, Alvarez O. Sickle cell disease related mortality in the United States (1998–2009). *Pediatr Blood Cancer* 2013; **60:** 1482–86.

20 Fitzhugh CD, Lauder N, Jonassaint JC, et al. Cardiopulmonary complications leading to premature deaths in adult patients with sickle cell disease. *Am J Hematol* 2010; **85:** 36–40.

21 Drawz P, Ayyappan S, Nouraie M, et al. Kidney disease among patients with sickle cell disease, hemoglobin SS and SC. *Clin J Am Soc Nephrol* 2016; **11:** 207–15.

22 Nath K, Hebbel R. Sickle cell disease: renal manifestations and mechanisms. *Nat Rev Nephrol*. 2015; **11:** 161–71.

23 McPhearson Yee M, Jabbar SF, Osunkwo I, et al. Chronic kidney disease and albuminuria in children with sickle cell disease. *Clin J Am Soc Nephrol* 2011; **6:** 2628–33.

24 Falk RJ, Scheinman J, Phillips G, Orringer E, Johnson A, Jennette JC. Prevalence and pathologic features of sickle cell nephropathy and response to inhibition of angiotensin-converting enzyme. *N Engl J Med* 1992; **326:** 910–15.

25 Sharpe C, Thein S. How I treat renal complications in sickle cell disease. *Blood* 2014; **123:** 3720–26.

26 Fitzhugh CD, Wigfall DR, Ware RE. Enalapril and hydroxyurea therapy for children with sickle nephropathy. *Pediatr Blood Cancer* 2005; **45:** 982–85.

27 Laurin, L. Nachman PH, Desai PC et al. Hydroxyurea is associated with lower prevalence of albuminuria in adults with sickle cell disease. *Nephrol Dial Transplant*. 2014; **29:** 1211–18.

28 Bartolucci P, Habibi A, Stehle T, et al. Six months of hydroxyurea reduces albuminuria in patients with sickle cell disease. *J Am Soc Nephrol* 2016; **27:** 1847–53.

29 Saraf SL, Zhang X, Kanias T, et al. Haemoglobinuria is associated with chronic kidney disease and its progression in patients with sickle cell anaemia. *Br J Haematol* 2013; **164:** 729–39.

30 Novelli EM, Hildesheim M, Rosano C et al. Elevated pulse pressure is associated with hemolysis, proteinuria and chronic kidney disease in sickle cell disease. *PLoS One* 2014; **9:** e114309.

31 Caughey MC, Poole C, Ataga KI, Hinderliter AL. Estimated pulmonary artery systolic pressure and sickle cell disease: a meta-analysis and systemic review. *Br J Haematol* 2015; **170:** 416–24.

32 Sachdev V, Machado RF, Shizukuda Y, et al. Diastolic dysfunction is an independent risk factor for death factor for death in patients with SCD. *J Am Coll Cardiol* 2007; **49:** 472–79.

33 Gladwin MT, Sachdev V, Jison ML, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med* 2004; **350:** 886–95.

34 Gladwin M. Cardiovascular complications and risk of death in sickle cell disease. *Lancet* 2016; **387:** 2565–74.

35 Niss O, Quinn CT, Lane A, et al. Cardiomyopathy with restrictive physiology in sickle cell disease. *JACC Cardiovasc Imaging* 2016; **9:** 243–52.

36 Gordeuk VR, Castro OL, Machado RF. Pathophysiology and treatment of pulmonary hypertension in sickle cell disease. *Blood* 2016; **127:** 820–28.

37 Chou ST, Fasano RM. Management of patients with sickle cell disease using transfusion therapy: guidelines and complications. *Hematol Oncol Clin North Am* 2016; **30:** 591–608.

38 Vinchinsky EP, Haberkern CM, Neumayr L, et al. A comparison of conservative and aggressive transfusion regimens in the perioperative management of sickle cell disease. The Preoperative Transfusion in Sickle Cell Disease Study Group. *N Engl J Med* 1995; **333:** 206–13.

39 Howard J, Malfroy M, Llewelyn C, et al. The transfusion alternative preoperatively in sickle cell disease (TAPS) study: a randomised, controlled, multicentre clinical trial. *Lancet* 2013; **381:** 930–38.

40 Powars D, Wilson B, Imbus C, Pegelow C, Allen J. The natural history of stroke in sickle cell disease. *Am J Med* 1978; **65:** 461–71.

41 McLaughlin JF, Ballas SK. High mortality among children with sickle cell anemia and overt stroke who discontinue blood transfusion after transition to an adult program. *Transfusion* 2016; **56:** 1014–21.

42 Adams RJ, McKie VC, Hsu L, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med* 1998; **339:** 5–11.

43 Adams RJ, Brambilla D, Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP2) Trial Investigators. Discontinuing prophylactic transfusions used to prevent stroke in sickle cell disease. *N Engl J Med* 2005; **353:** 2769–78.

44 DeBaun MR, Gordon M, McKinstry RC, et al. Controlled trial of transfusions for silent infarcts in sickle cell anemia. *N Engl J Med* 2014; **37:** 699–710.

45 Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DF, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood* 2013; **122:** 1062–71.

46 Casas J, Friedman DF, Jackson T, Vege S, Westhoff CM, Chou ST. Changing practice: red blood cell typing by molecular methods for patients with sickle cell disease. *Transfusion* 2015; **55:** 1388–93.

47 Wood JC, Cohen AR, Pressel SL, et al. Organ iron accumulation in chronically transfused children with sickle cell anemia: baseline results from the TWiTCH trial. *Br J Haematol* 2016; **172:** 122–30.

48 Darbari DS, Kple-Faget P, Kwagyan JK, Rana S, Gordeuk VR, Castro O. Circumstances of death in adult sickle cell disease patients. *Am J Hematol* 2006; **81:** 858–63.

49 Karafin MS, Koch KL, Rankin AB, et al. Erythropoietic drive is the strongest predictor of hepcidin level in adults with sickle cell disease. *Blood Cells Mol Dis* 2015; **55:** 304–07.

50 Dwyre DM, Fernando LP, Holland PV. Hepatitis B, hepatitis C and HIV transfusion-transmitted infections in the 21st century. *Vox Sang* 2011; **100:** 92–98.

51 Jayaraman S, Chalabi Z, Perel P, Guerriero C, Roberts I. The risk of transfusion-transmitted infections in sub-Saharan Africa. *Transfusion* 2010; **50:** 433–42.

52 Seck M, Dièye B, Guèye YB, et al. Évaluation de l'efficacité de la sélection médicale des donneurs de sang dans la prévention des agents infectieux. *Transfus Clin Biol* 2016; **23:** 98–102.

53 Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med* 1994; **330:** 1639–44.

54 Platt OS, Orkin SH, Dover G, Beardsley GP, Miller B, Nathan DG. Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. *J Clin Invest* 1984; **74:** 652–56.

55 Charache S, Dover GJ, Moore RD, et al. Hydroxyurea: effects on hemoglobin F production in patients with sickle cell anemia. *Blood* 1992; **79:** 2555–65.

56 Charache S, Terrin ML, Moore RD, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. *N Engl J Med* 1995; **332:** 1317–22.

57 Ferster A, Vermylen C, Cornu G, et al. Hydroxyurea for treatment of severe sickle cell anemia: a pediatric clinical trial. *Blood* 1996; **88:** 1960–64.

58 Kinney TR, Helms RW, O'Branksi EE, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Pediatric Hydroxyurea Group *Blood* 1999; **94:** 1550–54.

59 Zimmerman SA, Schultz WH, Davis JS, et al. Sustained long-term hematologic efficacy of hydroxyurea at maximum tolerated dose in children with sickle cell disease. *Blood* 2004; **103:** 2039–45.

60 de Montalembert M, Brousse V, Elie C, et al. Long-term hydroxyurea treatment in children with sickle cell disease: tolerance and clinical outcomes. *Haematologica* 2006; **91:** 125–28.

61 Wang WC, Wynn LW, Rogers ZR, Scott JP, Lane PA, Ware RE. A two-year pilot trial of hydroxyurea in very young children with sickle-cell anemia. *J Pediatr* 2001; **139:** 790–96.

62 Thornburg CD, Dixon N, Burgett S, et al. A pilot study of hydroxyurea to prevent chronic organ damage in young children with sickle cell anemia. *Pediatr Blood Cancer* 2009; **52:** 609–15.

63 Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). *Lancet* 2011; **377:** 1663–72.

64 Ware RE, Zimmerman SA, Sylvestre PB, et al. Prevention of secondary stroke and resolution of transfusional iron overload in children with sickle cell anemia using hydroxyurea and phlebotomy. *J Pediatr* 2004; **145:** 346–52.

65 Gulbis B, Haberman D, Dufour D, et al. Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events; the Belgian experience. *Blood* 2005; **105:** 2685–90.

66 Ware RE, Helms RW, SWiTCH Investigators. Stroke with transfusions changing to hydroxyurea (SWiTCH). *Blood* 2012; **119:** 3925–32.

67 Ware RE, Davis BR, Schultz WH, et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial Doppler flow velocities in children with sickle cell anemia–TCD with transfusion changing to hydroxyurea (TWiTCH): a multicentre, open-label, phase 3, non-inferiority trial. *Lancet* 2016; **387:** 661–70.

68 Reardon DA, Dresemann G, Taillibert S, et al. Multicentre phase II studies evaluating imatinib plus hydroxyurea in patients with progressive glioblastoma. *Br J Cancer* 2009; **101:** 1995–2004.

69 Gao WY, Johns DG, Chokekuchai S, Mitsuya H. Disparate actions of hydroxyurea in potentiation of purine and pyrimidine 2',3'-dideoxynucleoside activities against replication of human immunodeficiency virus. *Proc Natl Acad Sci USA* 1995; **92:** 8333–37.

70 Geyer HL, Mesa RA. Therapy for myeloproliferative neoplasms: when, which agent, and how? *Blood* 2014; **124:** 3529–37.

71 Cokic VP, Andric SA, Stojilkkovic SS, Noguchi CT, Schechter AN. Hydroxyurea nitrosylates and activates soluble guanylyl cyclase in human erythroid cells. *Blood* 2008; **111:** 1117–23.

72 Zhu J, Chin K, Aerbajinai W, Kumkhaek C, Li H, Rodgers GP. Hydroxyurea-inducible SAR1 gene acts through the Gia/JNK/Jun pathway to regulate g-globin expression. *Blood* 2014; **124:** 1146–56.

73 Mabaera R, West RJ, Conine SJ, et al. A cell stress signaling model of fetal hemoglobin induction: what doesn't kill red blood cells may make them stronger. *Exp Hematol* 2008; **36:** 1057–72.

74 Bernaudin F, Socie G, Kuentz M, et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. *Blood* 2007; **110:** 2749–56.

75 Walters MC, De Castro LM, Sullivan KM, et al. Indications and results of HLA-identical sibling hematopoietic cell transplantation for sickle cell disease. *Biol Blood Marrow Transplant* 2016; **22:** 207–11.

76 Gluckman E, Cappelli B, Bernaudin F, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood* 2016; published online December 13. DOI:10.1182/blood-2016-10-745711.

77 Lê PQ, Gulbis B, Dedeken L, et al. Survival among children and adults with sickle cell disease in Belgium: benefit from hydroxyurea treatment. *Pediatr Blood Cancer* 2015; **62:** 1956–61.

78 Dallas MH, Triplett B, Shook DR, et al. Long-term outcome and evaluation of organ function in pediatric patients undergoing haploidentical and matched related hematopoietic stem transplantation for sickle cell disease. *Biol Blood Marrow Transplant* 2013; **19:** 820–30.

79 Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica* 2014; **99:** 811–20.

80 Hsieh MM, Fitzhugh CD, Weitzel RP, et al. Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. *JAMA* 2014; **312:** 48–56.

81 Kamani NR, Walters MC, Carter S, et al. Unrelated donor cord blood transplantation for children with severe sickle cell disease: results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). *Biol Blood Marrow Transplant* 2012; **18:** 1265–72.

82 Shenoy S, Eapen M, Panepinto JA, et al. A trial of unrelated donor marrow transplantation for children with sickle cell disease. *Blood* 2016; **128:** 2561–67.

83 Bolaños-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood* 2012; **120:** 4285–91.

84 Hoban MD, Orkin SH, Bauer DE. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. *Blood* 2016; **127:** 839–48.

85 Cavazzana M, Ribeil J-A, Payen E, et al. Outcomes of gene therapy for severe sickle disease and beta-thalassemia major via transplantation of autologous hematopoietic stem cells transduced ex vivo with a lentiviral beta AT87Q-globin vector. American Society of Hematology 57th Annual Meeting & Exposition; Orlando, FL, USA; Dec 5–8, 2015. 202.

86 Canver MC and Orkin SH. Customizing the genome as therapy for the β-hemoglobinopathies. *Blood* 2016; **127:** 2536–45.

87 Lettre G, Bauer DE. Fetal hemoglobin in sickle-cell disease: from genetic epidemiology to new therapeutic strategies. *Lancet* 2016; **387:** 2554–64.

88 Breda L, Motta I, Lourenco S, et al. Forced chromatin looping raises fetal hemoglobin in adult sickle cells to higher levels than pharmacologic inducers. *Blood* 2016; **158:** 1139–43.

89 Telen MJ. Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease. *Blood* 2016; **127:** 810–19.

90 Kutlar A, Ataga KI, McMahon L, et al. A potent oral P-selectin blocking agent improves microcirculatory blood flow and a marker of endothelial cell injury in patients with sickle cell disease. *Am J Hematol* 2012: **87:** 536–39.

91 Telen MJ, Wun T, McCavit TL, et al. Randomized phase 2 study of GMI-1070 in SCD: reduction in time to resolution of vaso-occlusive events and decreased opioid use. *Blood* 2015; **125:** 2656–64.

92 Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Engl J Med* 2016; published online December 3. DOI: 10.1056/NEJMoa1611770.

93 Van Zuuren EJ, Fedorowicz Z. Low-molecular-weight heparins for managing vaso-occlusive crises in people with sickle cell disease. *Cochrane Database Syst Rev* 2013; **6:** CD010155.

94 Field JJ, Lin G, Okam MM, et al. Sickle cell vaso-occlusion causes activation of iNKT cells that is decreased by the adenosine A2A receptor agonist regadenoson. *Blood* 2013; **121:** 3329–34.

95 Ataga KI, Reid M, Ballas SK, et al. Improvements in haemolysis and indicators of erythrocyte survival do not correlate with acute vaso-occlusive crises in patients with sickle cell disease: a phase III randomized, placebo-controlled, double-blind study of the Gardos channel blocker senicapoc (ICA-17043). *Br J Haematol* 2011; **153:** 92–104.

96 Heeney MM, Hoppe CC, Abboud MR, et al. A multinational trial of prasugrel for sickle cell vaso-occlusive events. *N Engl J Med* 2016; **374:** 625–35.

97 Adam SS, Hoppe C. Potential role for statins in sickle cell disease. *Pediatr Blood Cancer* 2013; **60:** 550–57.

98 Reid ME, El Beshlawy A, Inati A, et al. A double-blind, placebo-controlled phase II study of the efficacy and safety of 2,2-dimethylbutyrate (HQK-1001), an oral fetal globin inducer, in sickle cell disease. *Am J Hematol* 2014; **89:** 709–13.

99 Okam MM, Esrick EB, Mandell E, Campigotto F, Neuberg DS, Ebert BL. Phase 1/2 trial of vorinostat in patients with sickle cell disease who have not benefitted from hydroxyurea. *Blood* 2015; **125:** 3668–69.

100 Dulmovits BM, Appiah-Kubi AO, Papoin J, et al. Pomalidomide reverses g-globin silencing through the transcriptional reprogramming of adult hematopoietic progenitors. *Blood* 2016; **127:** 1481–92.

101 Oksenberg D, Dufu K, Patel MP, et al. GBT440 increases haemoglobin oxygen affinity, reduces sickling and prolongs RBC half-life in a murine model of sickle cell disease. *Br J Haematol* 2016; **175:** 141–53.

102 Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet* 2013; **381:** 142–51.

103 Ndeezi G, Kiyaga C, Hernandez AG, et al. Burden of sickle cell trait and disease in the Uganda Sickle Surveillance Study (US3): a cross-sectional study. *Lancet Glob Health* 2016; **4:** e195–200.

104 Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global burden of sickle cell anaemia in children under five, 2010–2050: modelling based on demographics, excess mortality, and interventions. *PLoS Med* 2013; **10:** e1001484.

105 Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, Williams TN. Sickle cell disease in Africa: a neglected cause of early childhood mortality. *Am J Prev Med* 2011; **41** (suppl 4): S398–405.

106 IHME, Human Development Network, World Bank. The Global Burden of Disease: Generating Evidence, Guiding Policy. Sub-Saharan Africa regional edition. Seattle, WA: Institute for Health Metrics and Evaluation, 2013.

107 GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388:** 1459–544.

108 World Health Organization Regional Office for Africa. Sickle-cell disease: a strategy for the WHO African region. Geneva: WHO, 2010. Report Number AFR/FC60/8.

109 Tshilolo L, Kafando E, Sawadogo M, et al. Neonatal screening and clinical care programmes for sickle cell disorders in sub-Saharan Africa: lessons from pilot studies. *Public Health* 2008; **122:** 933–41.

110 McGann PT, Grosse SD, Santos B, et al. A cost-effectiveness analysis of a pilot neonatal screening program for sickle cell anemia in the Republic of Angola. *J Pediatr* 2015; **167:** 1314–19.

111 Colah R, Mukherjee M, Ghosh K. Sickle cell disease in India. *Curr Opin Hematol* 2014; **21:** 215–23.

112 King LG, Bortolusso-Ali S, Cunningham-Myrie CA, Reid ME. Impact of a comprehensive sickle cell center on early childhood mortality in a developing country: the Jamaican experience. *J Pediatr* 2015; **167:** 702–05.

113 Aguilar Martinez P, Angastiniotis M, Eleftheriou A, et al. Haemoglobinopathies in Europe: health & migration policy perspectives. *Orphanet J Rare Dis* 2014; **9:** 97–103.

114 Tshilolo L, Summa V, Gregorj C, et al. Foetal haemoglobin, erythrocytes containing foetal haemoglobin, and hematological features in Congolese patients with sickle cell anaemia. *Anemia* 2012; **2012:** 105349.

115 Ranque B, Menet A, Diop IB, et al. Early renal damage in patients with sickle cell disease in sub-Saharan Africa: a multinational prospective, cross-sectional study. *Lancet Haematol* 2014; **1:** e64–73.

116 McGann PT, Tshilolo L, Santos B, et al. Hydroxyurea therapy for children with sickle cell anemia in sub-Saharan Africa: rationale and design of the REACH trial. *Pediatr Blood Cancer* 2016; **63:** 98–104.

117 Anyanwu JN, Williams O, Sautter CL, et al. Novel use of hydroxyurea in an African region with malaria: protocol for a randomized controlled clinical trial. *JMIR Res Protoc* 2016; **5:** e110.

118 Adams R, McKie V, Nichols F, et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. *N Engl J Med* 1992; **326:** 605–10.

119 Valadi N, Silva GS, Bowman LS, et al. Transcranial Doppler ultrasonography in adults with sickle cell disease. *Neurology* 2006; **67:** 572–74.

120 Sampaio Silva G, Vicari P, Figueiredo MS, Filho AC, Valadi N, Massaro AR. Transcranial Doppler in adult patients with sickle cell disease. *Cerebrovasc Dis* 2006; **21:** 38–41.

121 Deane CR, Goss D, Bartram J, et al. Extracranial internal carotid arterial disease in children with sickle cell anemia. *Haematologica* 2010; **95:** 1287–92.

122 Verlhac S, Balandra S, Cussenot I, et al. Extracranial carotid arteriopathy in stroke-free children with sickle cell anemia: detection by submandibular Doppler sonography. *Pediatr Radiol* 2014; **44:** 587–96.

123 Bernaudin F, Verlhac S, Arnaud C, et al. Chronic and acute anemia and extracranial internal carotid stenosis are independent risk factors for silent cerebral infarcts in sickle cell anemia. *Blood* 2015; **125:** 1653–61.

124 Reeves SL, Madden B, Freed GL, Dombkowski KJ. Transcranial Doppler screening among children and adolescents with sickle cell anemia. *JAMA Pediatr* 2016; **170:** 550–56.

125 Hankins JS, McCarville MB, Rankine-Mullings A, et al. Prevention of conversion to abnormal transcranial Doppler with hydroxyurea in sickle cell anemia: a phase III international randomized clinical trial. *Am J Hematol* 2015; **90:** 1099–105.

126 Galadanci NA, Abdullahi SU, Tabari MA, et al. Primary stroke prevention in Nigerian children with sickle cell disease (SPIN): challenges of conducting a feasibility trial. *Pediatr Blood Cancer* 2015; **62:** 395–401.

127 Vichinsky E, Torres M, Minniti CP, et al. Efficacy and safety of deferasirox compared with deferoxamine in sickle cell disease: two-year results including pharmacokinetics and concomitant hydroxyurea. *Am J Hematol* 2013; **88:** 1068–73.

128 Hoffbrand AV, Taher A, Cappellini MD. How I treat transfusional iron overload. *Blood* 2012; **120:** 3657–69.

129 Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005; **106:** 1460–65.

130 Wood JC, Pressel SL, Rogers ZR, et al. Liver iron concentration measurements by MRI in chronically transfused children with sickle cell anemia: baseline results from the TWiTCH trial. *Am J Hematol* 2015; **90:** 806–10.

131 Hankins JS, McCarville MB, Loeffler RB, et al. R2* magnetic resonance imaging of the liver in patients with iron overload. *Blood* 2009; **113:** 4853–55.

132 Yang X, Kanter J, Piety NZ, Benton MS, Vignes SM, Shevkoplyas SS. A simple, rapid, low-cost diagnostic test for sickle cell disease. *Lab Chip* 2013; **13:** 1464–67.

133 Kumar AA, Patton MR, Hennek JW, et al. Density-based separation in multiphase systems provides a simple method to identify sickle cell disease. *Proc Natl Acad Sci USA* 2014; **111:** 14864–69.

134 McGann PT, Schaefer BA, Paniagua M, Howard TA, Ware RE. Characteristics of a rapid, point-of-care lateral flow immunoassay for the diagnosis of sickle cell disease. *Am J Hematol* 2016; **91:** 205–10.

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

136 Bunn HF, Nathan DG, Dover GJ, et al. Pulmonary hypertension and nitric oxide depletion in sickle cell disease. *Blood* 2010; **116:** 687–92.

137 Parent F, Bachir D, Inamo J, et al. A hemodynamic study of pulmonary hypertension in sickle cell disease. *N Engl J Med* 2011; **365:** 44–53.

138 Hebbel RP. Reconstructing sickle cell disease: a data-based analysis of the "hyperhemolysis paradigm" for pulmonary hypertension from the perspective of evidence-based medicine. *Am J Hematol* 2011; **86:** 123–54.

139 Quinn CT, Smith EP, Arbabi S, et al. Biochemical surrogate markers of hemolysis do not correlate with directly measured erythrocyte survival of sickle cell anemia. *Am J Hematol* 2016; **91:** 1195–1201.

140 Oteng-Ntim E, Meeks D, Seed PT, et al. Adverse maternal and perinatal outcomes in pregnant women with sickle cell disease: systematic review and meta-analysis. *Blood* 2015; **125:** 3316–25.

141 Lesage N, Deneux Tharaux C, Saucedo M, et al. Maternal mortality among women with sickle-cell disease in France, 1996–2009. *Eur J Obstet Gyn Reprod Biol* 2015; **194:** 183–88.

142 Malinowski AK, Shehata N, D'Souza R, et al. Prophylactic transfusion for pregnant women with sickle cell disease: a systematic review and meta-analysis. *Blood* 2015; **126:** 2424–35.

143 Savage WJ, Buchanan GR, Yawn BP, et al. Evidence gaps in the management of sickle cell disease: a summary of needed research. *Am J Hematol* 2015; **90:** 273–75.

144 de Montalembert M, Roberts I. Sickle cell disease: *primum non nocere*. *Haematologica* 2010; **95:** 4–5.

145 Nickel RS, Hendrickson JE, Haight AE. The ethics of a proposed study of hematopoietic stem cell transplant for children with "less severe" sickle cell disease. *Blood* 2014; **124:** 861–66.