Seminar

Sickle-cell disease

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Sickle-cell disease is one of the most common severe monogenic disorders in the world. Haemoglobin polymerisation, leading to erythrocyte rigidity and vaso-occlusion, is central to the pathophysiology of this disease, although the importance of chronic anaemia, haemolysis, and vasculopathy has been established. Clinical management is basic and few treatments have a robust evidence base. One of the main problems of sickle-cell disease in children is the development of cerebrovascular disease and cognitive impairment, and the role of blood transfusion and hydroxycarbamide for prevention of these complications is starting to be understood. Recurrent episodes of vaso-occlusion and inflammation result in progressive damage to most organs, including the brain, kidneys, lungs, bones, and cardiovascular system, which becomes apparent with increasing age. Most people with sickle-cell disease live in Africa, where little is known about this disease; however, we do know that the disorder follows a more severe clinical course in Africa than for the rest of the world and that infectious diseases have a role in causing this increased severity of sickle-cell disease. More work is needed to develop effective treatments that specifically target pathophysiological changes and clinical complications of sickle-cell disease.

Introduction

Sickle-cell disease is a multisystem disease, associated with episodes of acute illness and progressive organ damage, and is one of the most common severe monogenic disorders worldwide.[1] Herrick[2] first described the characteristic sickle-shaped erythrocytes in 1910 (figure 1), and understanding has gradually increased since then (table 1). Pauling and colleagues[5] identified electrophoretic abnormalities in sickle haemoglobin (HbS) and coined the term "molecular disease" in 1949. The haemoglobin biophysics and genetics underlying the disease have been extensively studied and have helped the understanding of other molecular diseases. However, clinical management of sickle-cell disease is still basic and, although some evidence lends support to the use of blood transfusion and hydroxycarbamide in some circumstances, no drugs have been developed that specifically target the pathophysiology of this disease.

Classification

The term sickle-cell disease is used to refer to all the different genotypes that cause the characteristic clinical syndrome, whereas sickle-cell anaemia, the most common form of sickle-cell disease, refers specifically to homozygosity for the β^s allele. In this Seminar, we mostly discuss sickle-cell anaemia, because there is little evidence for the management of other types of sickle-cell disease. In populations of African ethnic origin, sickle-cell anaemia typically accounts for 70% of cases of sickle-cell disease, with most of the remainder having haemoglobin SC disease (HbSC disease) owing to the co-inheritance of the β^s and β^c alleles.[13] The third major type of sickle-cell disease occurs when β^s is inherited with a β-thalassaemia allele, causing HbS/β-thalassaemia; this is a variable disorder dependent on the type of the β-thalassaemia mutation.[14] Apart from the many different types of HbS/β-thalassaemia, ten further genotypes that cause sickle-cell disease have been described, although most are rare (table 2).

Pathophysiology

HbS is caused by a mutation in the β-globin gene in which the 17th nucleotide is changed from thymine to adenine and the sixth aminoacid in the β-globin chain becomes valine instead of glutamic acid.[21] This mutation produces a hydrophobic motif in the deoxygenated HbS tetramer that results in binding between β1 and β2 chains of two haemoglobin molecules. This crystallisation produces a polymer nucleus, which grows and fills the erythrocyte, disrupting its architecture and flexibility and promoting cellular dehydration, with physical and oxidative cellular stress.[22] The rate and extent of HbS polymerisation is proportional to the extent and duration of haemoglobin deoxygenation, the intracellular HbS concentration (to about the 34th power), and the presence of fetal haemoglobin in the erythrocyte, which effectively reduces the concentration of HbS.[21,23] The main determinant of disease severity is the rate and extent of HbS polymerisation, which is exemplified by co-inheritance of genetic factors that modulate the intracellular HbS or fetal haemoglobin concentration, such as the protective effects of co-inherited α-thalassaemia or hereditary persistence of fetal haemoglobin. Similarly, therapeutic inhibition of the

Search strategy and selection criteria

We searched Medline and EmBase from 1960 to May, 2010, with the search term "sickle" in combination with the search terms "stroke", "pain", "infection", "malaria", "chest", "kidney", "vaso-occlusion", "haemolysis", "nitric oxide", "epidemiology", "screening", "diagnosis", "hydroxyurea", "hydroxycarbamide", "blood transfusion", "iron chelation", "gene therapy", "transplantation", "neurology", "vasculopathy", "pulmonary hypertension", "cardiac", and "treatment". We mostly selected publications from the past 5 years, but did not exclude commonly referenced and important older publications. Review articles and books are cited to provide readers with more additional details and references. Our reference list was modified on the basis of comments from peer reviewers.

cation transport channels prevents erythrocyte dehydration and effectively reduces HbS concentration, and reduces haemolysis; hydroxycarbamide increases fetal haemoglobin concentrations, reduces haemolysis, and prevents acute vaso-occlusion.[21] These manifestations are driven by two major pathophysiological processes: vaso-occlusion with ischaemia-reperfusion injury and haemolytic anaemia (figure 2).

Acute vaso-occlusive pain is thought to be caused by entrapment of erythrocytes and leucocytes in the microcirculation, causing vascular obstruction and tissue ischaemia. Although this process requires HbS polymerisation, the event that triggers the vascular obstruction by sickle erythrocytes is often inflammatory. As indicated in the microcirculation of transgenic mice expressing HbS, cycles of experimental hypoxia or treatment with inflammatory drugs increase endothelial-leucocyte-erythrocyte adhesive interactions in the postcapillary venules and start vascular occlusion.[24–27] In addition to inflammatory triggers, precapillary obstruction by rigid, deformed erythrocytes with high HbS polymer content also contributes to microvascular vaso-occlusion. Vascular occlusion is the result of a dynamic interaction between erythrocytes and the vascular endothelium, resulting in episodic microvascular occlusion and ischaemia, followed by restoration of blood flow, which further promotes tissue injury mediated by reperfusion. These cycles of ischaemia and reperfusion cause oxidant stress, with activation of vascular oxidases[28] and inflammatory stress, increasing expression of endothelial cell-adhesion molecules, increasing synthesis of inflammatory cytokines, and can cause leucocytosis.[24,26,29] Bone marrow infarction leading to fat embolisation might also contribute to vascular occlusion, particularly in the lungs, where it causes acute chest syndrome.[30]

The second pathophysiological process in sickle-cell disease is haemolytic anaemia, which is also driven by HbS polymerisation. Haemolysis has long been known to cause anaemia, fatigue, and cholelithiasis, but there is now evidence that it contributes to the development of progressive vasculopathy. As patients with sickle-cell disease age, they are at risk of vasculopathy, characterised by systemic and pulmonary hypertension, endothelial dysfunction, and proliferative changes in the intima and smooth muscle of blood vessels.[31–34] Data from epidemiological studies suggest that several complications are associated with increased rates of haemolysis; cholelithiasis, cutaneous leg ulceration, priapism, and pulmonary hypertension are associated with low steady-state haemoglobin concentrations and an increased rate of intravascular haemolysis.[33,35] An association between the development of pulmonary hypertension and the intensity of haemolytic anaemia was noted in three prospective screening studies of adults with sickle-cell disease[33,36,37] and in paediatric studies.[38,39] Pulmonary hypertension has also been reported in other forms of chronic hereditary and acquired haemolytic anaemia.[40]
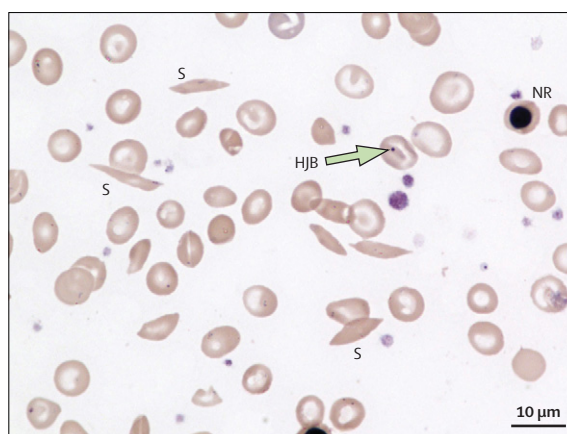
Figure 1: **Peripheral blood smear of a patient with sickle-cell anaemia**
This blood film shows irreversibly sickled cells (S), a nucleated red blood cell (NR), and a Howell-Jolly Body (HJB); these last two features are mainly associated with hyposplenism (stained with May-Grunwald-Giemsa).

	Discovery	Importance
1910	Sickled erythrocytes in Grenadan dental student[2]	First description of disease linked to abnormal erythrocytes
1924	Haemolysis in sickle-cell disease[3]	Explanation for anaemia, jaundice, and cholelithiasis
1924	Vaso-occlusion as cause of some pathological features[4]	Explanation for ischaemic tissue damage
1948	Abnormal electrophoretic mobility of sickle haemoglobin[5]	Identified pathophysiology to have a molecular basis
1948	No symptoms in infants noted[6]	Beneficial effects of high concentrations of fetal haemoglobin identified
1951	Characteristics of polymerisation of deoxygenated HbS[7]	Primary molecular mechanism identified
1980s	Value of penicillin in young children with sickle-cell anaemia[8,9]	Reduced mortality, role of neonatal screening
1984	Bone marrow transplant in child with sickle-cell anaemia and leukaemia[10]	Identified potential cure
1995	Efficacy of hydroxycarbamide[11]	Only disease-modifying drug identified
1998	Reduced stroke incidence in children with abnormal transcranial dopplers who were given blood transfusion[12]	Primary stroke prevention with fall in stroke occurrence

Table 1: **Important discoveries in the pathological and clinical features of sickle-cell disease in chronological order**

Therefore, patients with low haemoglobin concentrations and high haemolytic rates seem to form a subphenotype of patients who are more likely to develop vasculopathy than are those with higher haemoglobin concentrations who seem more prone to episodes of acute pain and, possibly, acute chest syndrome.[35] Although vaso-occlusion is important in all patients, the role of haemolysis as a pathophysiological mechanism in sickle-cell disease is more controversial and is the focus of much research.

An important disease mechanism involves the release of haemoglobin into the circulation during intravascular

	Characteristics
Severe sickle-cell disease	
HbS/S (β6Glu>Val/β6Glu>Val); sickle-cell anaemia	The most common form of sickle-cell disease
HbS/β⁰ thalassaemia	Most prevalent in the eastern Mediterranean region and India[14]
Severe HbS/β⁺ thalassaemia	Most prevalent in the eastern Mediterranean region and India; 1–5% HbA present[14]
HbS/OArab (β6Glu>Val/β121Glu>Lys)	Reported in north Africa, the Middle East, and the Balkans; relatively rare[14]
HbS/D Punjab (β6Glu>Val/β121Glu>Gln)	Predominant in northern India but occurs worldwide[14]
HbS/C Harlem (β6Glu>Val/β6Glu>Val/β, β73Asp>Asn)	Electrophoretically resembles HbSC, but clinically severe; double mutation in β-globin gene; very rare[15]
HbC/S Antilles (β6Glu>Lys/β6Glu>Val, β23Val–Ile)	Double mutation in β-globin gene results in severe sickle-cell disease when co-inherited with HbC; very rare[16]
HbS/Quebec-CHORI (β6Glu>Val/β87Thr>Ile)	Two cases described; resembles sickle-cell trait with standard analytical techniques[17]
Moderate sickle-cell disease	
HbS/C (β6Glu>Val/β6Glu>Lys)	25–30% cases of sickle-cell disease in populations of African origin[13]
Moderate HbS/β⁺ thalassaemia	Most cases in the eastern Mediterranean region; 6–15% HbA present[14]
HbA/S Oman (βᴬ/β6Glu>Val, β121Glu>Lys)	Dominant form of sickle-cell disease caused by double mutation in β-globin gene; very rare[18]
Mild sickle-cell disease	
Mild HbS/β⁺⁺ thalassaemia	Mostly in populations of African origin; 16–30% HbA present[14]
HbS/E (β6Glu>Val/β26Glu>Lys)	HbE predominates in southeast Asia and so HbSE uncommon, although frequency is increasing with population migration[19]
HbA/Jamaica Plain (βᴬ/β6Glu>Val, β68Leu/Phe)	Dominant form of sickle-cell disease; double mutation results in Hb with low oxygen affinity; one case described[20]
Very mild sickle-cell disease	
HbS/HPFH	Group of disorders caused by large deletions of the β-globin gene complex; typically 30% fetal haemoglobin[14]
HbS/other Hb variants	HbS is co-inherited with many other Hb variants, and symptoms develop only in extreme hypoxia

Genotypes that have been reported to cause sickle-cell disease are listed. All include at least one copy of the β^S allele, in combination with one or more mutations in the β-globin gene. HbS=sickle haemoglobin. HbA=haemoglobin variant A. HbE=haemoglobin variant E. Hb=haemoglobin.

Table 2: **Different types of sickle-cell disease**

haemolysis. Free plasma haemoglobin generates reactive oxygen species, such as the hydroxyl and superoxide radical,[41] which is a potent scavenger of nitric oxide.[42] Nitric oxide is normally produced by the endothelium and regulates basal vasodilator tone, and inhibits platelet and haemostatic activation and transcriptional expression of nuclear factor κB (NF κB)-dependent adhesion molecules, such as vascular cell-adhesion molecule-1, intercellular cell-adhesion molecule-1, and the selectins.[43,44] The release of haemoglobin into the plasma during haemolysis potently inhibits endothelial nitric oxide signalling, leading to endothelial cell dysfunction and nitric oxide resistance.[42,45] Haemolysis also releases erythrocyte arginase-1 into plasma. Arginase metabolises plasma arginine into ornithine, decreasing the required substrate for nitric oxide synthesis and compounding the decreased bioavailability of nitric oxide in patients with sickle-cell disease.[46]

Chronic depletion of nitric oxide and arginine might also contribute to the hypercoagulable state in haemolytic diseases. Studies have shown correlations between the rate of haemolysis and levels of platelet activation and procoagulant factors in the blood.[47] Haemolysis is also associated with the formation of erythrocyte microvesicles containing phosphatidyl serine, which is an activator of tissue factor; the numbers of microvesicles are increased further by the functional asplenia present in patients with sickle-cell disease[48] (figure 2).

Epidemiology

The global distribution of HbS is indicative of two factors: selection for carriers through their survival advantage in malaria-endemic regions and subsequent migration. Four region-specific African haplotypes (the Senegal, Benin, Bantu, and Cameroon haplotypes) and one Asian haplotype (the Arab-India haplotype) have been defined, providing support for the hypothesis that the mutation causing HbS has occurred, and been locally amplified, on at least two, and possibly several, separate occasions.[49] The evidence that malaria caused this amplification, which was first suggested more than 60 years ago,[50] is now substantial. In addition to the close geographic correlation between the frequency of the HbS gene in populations and the historic incidence of malaria[49] (figure 3), evidence for the partial resistance of carriers to all forms of *Plasmodium falciparum* malaria has been reported in many populations.[55,56] Although the mechanism of this protection is yet to be fully understood, it probably includes both innate and immune-mediated mechanisms.[57] The genetic defects that commonly combine with HbS to result in sickle-cell disease (HbC and β-thalassaemia) have also occurred through malaria selection,[49,57] with the result that such defects reach their highest frequencies in similar populations.[49] The prevalence of sickle-cell disease is highest in sub-Saharan Africa. Although the scarcity of
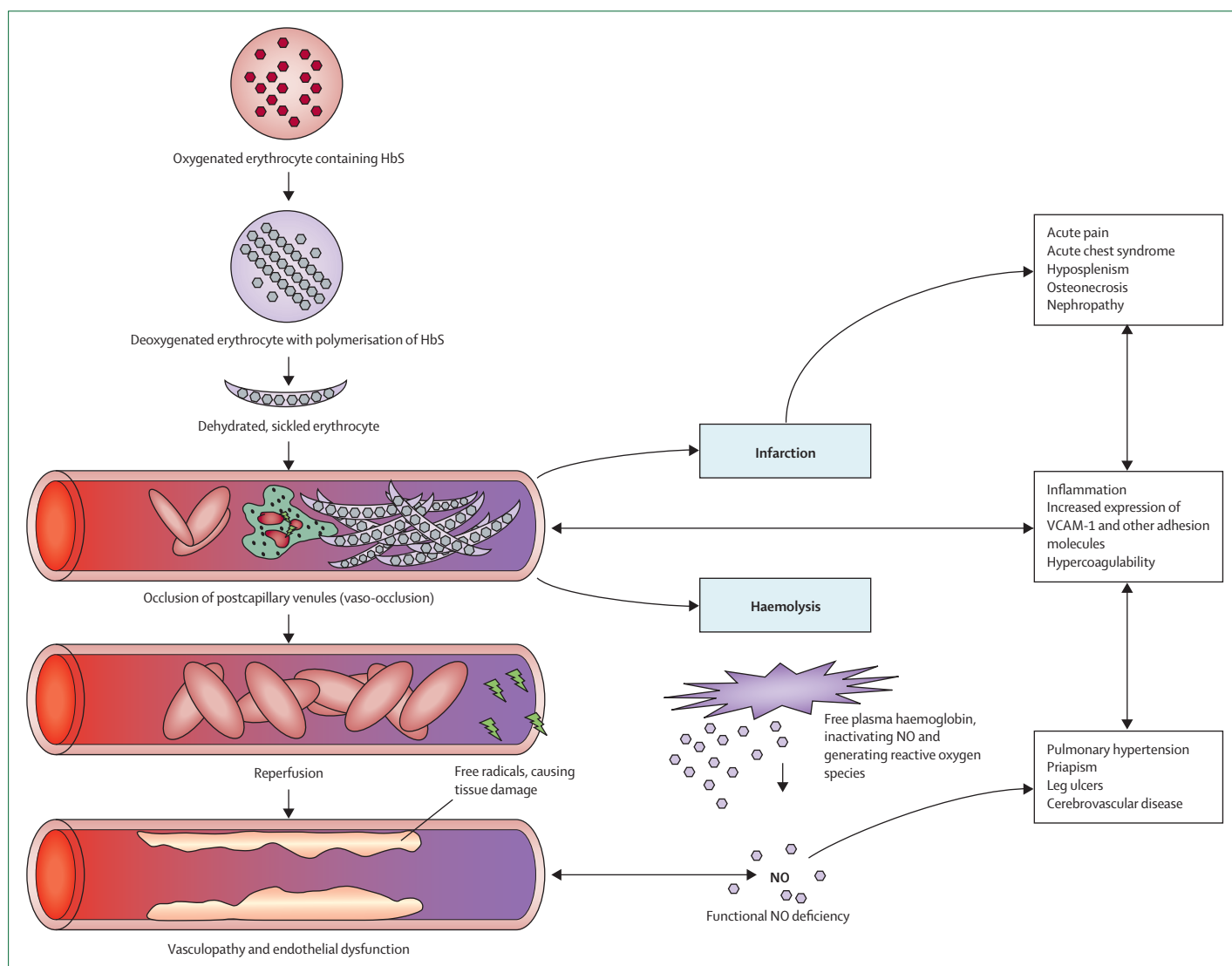
***Figure 2:*** **Pathophysiology of sickle-cell disease**
The roles of HbS polymerisation, hyperviscosity, vaso-occlusion, haemolysis, and endothelial dysfunction are shown. Deoxygenation causes HbS to polymerise, leading to sickled erythrocytes. Vaso-occlusion results from the interaction of sickled erythrocytes with leucocytes and the vascular endothelium. Vaso-occlusion then leads to infarction, haemolysis, and inflammation; inflammation enhances the expression of adhesion molecules, further increasing the tendency of sickled erythrocytes to adhere to the vascular endothelium and to worsen vaso-occlusion. Reperfusion of the ischaemic tissue generates free radicals and oxidative damage. The damaged erythrocytes release free haemoglobin in to the plasma, which strongly bind to nitric oxide, causing functional nitric oxide deficiency and contributing to the development of vasculopathy. HbS=sickle haemoglobin. NO=nitric oxide. VCAM=vascular cell-adhesion molecule.

diagnostic facilities means that precise data are not available, a recent estimate[52] suggests that more than 230 000 affected children are born in this region every year (0·74% of the births in sub-Saharan Africa), which is about 80% of the global total. By comparison, the yearly estimate of affected births in North America is 2600 and 1300 in Europe[52] (figure 3).

Little is known about sickle-cell disease in Africa.[58,59] Generally, diagnostic facilities are poor, routine screening is absent, and, despite the fact that most patients would survive if provided with a simple package of inexpensive interventions,[60] most die undiagnosed in early childhood.

Despite the high mortality associated with this disease in Africa, the causes of death in affected children are poorly documented. Although widely ascribed to two diseases—bacteraemia and malaria[14,58]—few substantial data are available to lend support to either potential cause. However, in a study undertaken in an African population,[61] both the range of organisms and the frequency of bacteraemia caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* in children with sickle-cell disease were similar to those reported in Jamaica and the USA.[8,62] This finding suggests that, with prophylaxis against both *H influenzae* and *S pneumoniae*, the occurrence of bacteraemia in children with sickle-cell
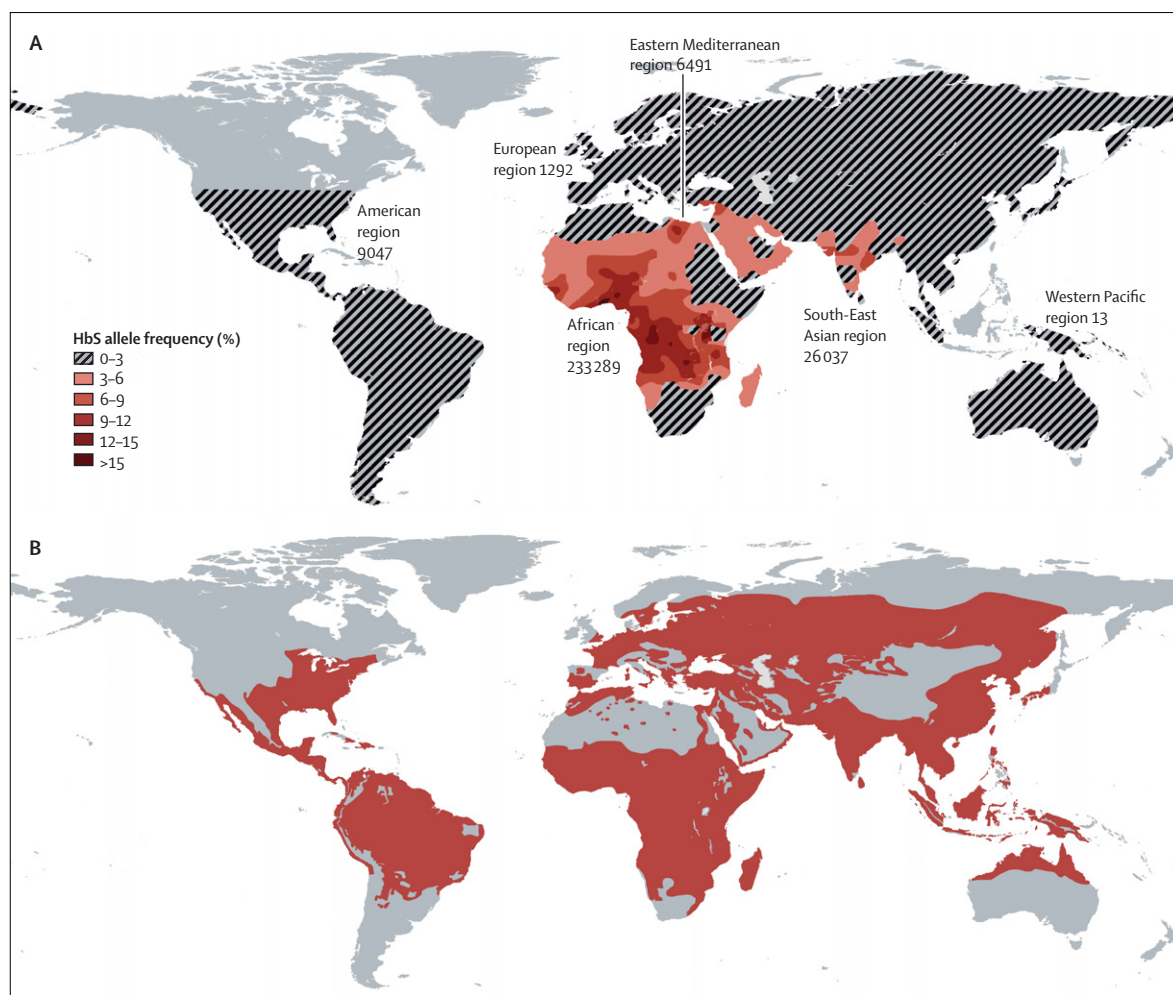
Figure 3: Global distributions of HbS and malaria
(A) This map shows the distribution of the HbS allele. It was constructed with digitised data derived from Cavalli-Sforza and colleagues.[51] The figures indicate estimates for the combined yearly total number of individuals affected by HbSS, HbSC, and HbS/β-thalassaemia by WHO region (adapted from Modell and Darlison[52]). (B) This map shows the global distribution of malaria (red) before intervention to control malaria (adapted from Lysenko and Semashko,[53] and Hay and colleagues.[54] HbS=sickle haemoglobin.

disease in Africa could be reduced by as much as 50%, providing strong support for early diagnosis.

Although malaria is commonly thought of as a major cause of death in African patients with sickle-cell disease,[58] this opinion is supported by few data. Most published reports have not had controls to enable comparisons of risk with patients without disease, and several intervention studies have not had the power to definitively assess the risk of death in African patients with sickle-cell disease.[63] In a study from Tanzania,[64] the frequency of malarial parasitaemia was lower in patients with sickle-cell anaemia than in those with normal haemoglobin; of those with sickle-cell anaemia in hospital, parasitaemia was a risk factor for both severe anaemia (haemoglobin <50 g/L), and death. Similarly, in a study in Kenya,[65] mortality from malaria was higher in children with sickle-cell anaemia than in those with normal haemoglobin, although

malarial prevalence in individuals with sickle-cell anaemia was not increased.

With a historic background of low health spending and high rates of overall child mortality, children born with sickle-cell disease in Africa are given a low priority. However, in view of the sustained declines in child mortality that are being recorded throughout much of Africa,[66] a growing proportion of overall deaths in childhood are now attributable to sickle-cell disease, and survivors will place an increasing demand on health services. A better understanding of the presenting features and natural history of this disease in Africa will therefore be essential if rational approaches to its diagnosis and management are to be developed. There have been some encouraging signs that attitudes to sickle-cell disease in Africa are changing; the disease has now been recognised as a health-care priority by both WHO and the United Nations. Early life

screening has been introduced in parts of several African countries,[67] and a network has been formed to support the acquisition of evidence-based research (the Global Sickle Cell Disease Network).

Phenotypic heterogeneity

The presentation and clinical course of sickle-cell disease shows substantial variation. For example, in the Cooperative Study of Sickle Cell Disease in the USA,[68] 39% of 3578 patients with sickle-cell anaemia had no episodes of pain but 1% had more than six per year. Such variability is characteristic of the disease and many of its complications, including cerebrovascular disease, acute chest syndrome, and premature death.

The two best established genetic modifiers are determinants of fetal haemoglobin concentrations and co-inheritance of α-thalassaemia. The maintenance of high fetal haemoglobin concentrations beyond infancy has long been recognised to ameliorate many aspects of sickle-cell disease,[6] including predicting increased life expectancy,[30] and reducing the frequency of both acute pain[68] and leg ulcers.[69] The fetal haemoglobin concentration in patients with sickle-cell anaemia varies from 1% to 30%, and is inherited as a quantitative genetic trait. Three major loci have been identified, which account for up to 50% of this variation in sickle-cell anaemia: the *Xmn1* polymorphism in the promoter region of the $^G\gamma$ globin genes,[70] the *HMIP* locus on chromosome 6q23.3,[71] and *BCL11A* on chromosome 2.[72] Genetic variation at these three loci is likely to account for some of the clinical diversity seen in sickle-cell disease, as has already been reported for acute pain,[73] and could be targeted to help to improve prenatal diagnosis and accuracy of neonatal prognostication.

α-thalassaemia trait exists in up to 30% of patients of African origin with sickle-cell disease,[74] and is present in more than 50% of patients with this disease in India[75] and Saudi Arabia.[76] α-thalassaemia reduces the concentration of haemoglobin in each erythrocyte, decreasing the tendency of HbS to polymerise, which results in increased haemoglobin concentrations and decreased rates of haemolysis. The clinical effects of α-thalassaemia are variable but generally beneficial for patients, with reduced occurrence of stroke,[77] gall stones,[78] leg ulcers,[74] and priapism,[79] although pain frequency is not reduced.[68] Data from some studies indicate an increased frequency of pain in patients with α-thalassaemia, possibly associated with the increased haematocrit and blood viscosity.[80]

Several genetic association studies have been done to try and link single nucleotide polymorphisms with particular complications of sickle-cell disease. Some associations are well described and make biological sense, such as the link between UGT1A promoter polymorphisms and gall stones.[78] Most studies have investigated large-vessel stroke, and two independent studies have suggested an association with the tumour necrosis factor promoter polymorphism at position −308.[81,82] Bayesian modelling with single nucleotide polymorphisms from 12 genes can also predict risk of stroke with 98·2% accuracy.[83] However, most genetic associations are tentative, and confirmatory studies are needed.[84]

Environmental factors are poorly characterised, but could give rise to a large amount of phenotypic variability, as indicated by the much greater severity of sickle-cell disease reported in Africa than that for other continents. In tropical countries, increased episodes of acute pain in the rainy season are reported.[85] In temperate countries, the effects of rain and cold might be important but are less well documented, although windy weather has been linked to an increased occurrence of pain.[86] The effects of air pollution are uncertain. Data from studies suggest that high ozone concentrations might be linked to acute pain; however, the importance of atmospheric concentrations of nitric oxide and carbon monoxide is unknown.[87,88]

General management

Diagnosis and screening

Diagnosis of sickle-cell disease is based on analysis of haemoglobin. Typically, this analysis involves protein electrophoresis or chromatography, which are cheap techniques and widely available worldwide, although haemoglobin mass spectrometry and DNA analysis are being increasingly used because these techniques enable high-throughput testing.[89] Antenatal screening is available to women in some countries to help to identify couples who are at risk of having a baby with sickle-cell disease, and to offer prenatal diagnosis.[89] Universal neonatal screening programmes are established in the USA and England, with other programmes being developed in Europe and Africa.[67] Some of the improvement in survival in sickle-cell disease over the past few decades has been attributed to neonatal screening, facilitating early access to prophylaxis with penicillin, comprehensive care, and parental education on the early detection of complications such as acute splenic sequestration (panel 1).[90]

Hydroxycarbamide

Many cytotoxic drugs increase fetal haemoglobin concentrations, which is potentially beneficial in patients with sickle-cell disease. Hydroxycarbamide was chosen for studies of sickle-cell disease because of its oral efficacy and low toxic effects,[91] although other beneficial effects have subsequently emerged, including increasing haemoglobin concentrations, decreasing platelet and white cell counts, changing expression of adhesion molecules,[92] and nitric oxide generation.[93] In a randomised controlled trial,[11] hydroxycarbamide decreased the frequency of painful episodes, acute chest syndrome, the need for blood transfusion, and admission to hospital in patients with sickle-cell anaemia. Many subsequent studies have shown evidence of similar benefit in both adults and children.[94,95] Hydroxycarbamide is well tolerated, with dose-dependent

Panel 1: Recommended outpatient management of sickle-cell disease

- Education for patients, parents, and carers
- Monitoring of growth, development, and nutrition
- Prescription of penicillin and monitoring of adherence
- Administration of or organisation of vaccinations specific for sickle-cell disease, including those against pneumococcus and influenza
- Monitoring of school or work attendance
- Recording of steady-state blood results and physiological measurements (oxygen saturation, blood pressure)
- Monitoring of frequency of acute complications
- Prescription and monitoring of hydroxycarbamide as appropriate
- Early detection and prevention of chronic complications, including cerebrovascular disease (transcranial doppler scanning), pulmonary hypertension, and renal disease
- Provision of psychological support

Panel 2: Indications for blood transfusion in sickle-cell disease

Indications for acute transfusions

Acute exacerbation of anaemia
Typically caused by Parvovirus B19 infection, splenic or hepatic sequestration, or severe vaso-occlusion; simple transfusion is necessary to increase haemoglobin concentrations to 80–90 g/L

Acute chest syndrome
Early simple top-up transfusion is beneficial, with exchange transfusion to reduce HbS to less than 30% if deterioration of clinical condition occurs

Stroke or acute neurological deficit
Urgent transfusion to increase haemoglobin concentrations to 100 g/L, and reduce HbS to less than 30%, which typically requires exchange transfusion

Multiorgan failure
HbS to less than 30% with haemoglobin concentration of 100 g/L

Preoperative management
Target HbS of less than 30% before major surgery (cardiothoracic, neurosurgery), typically requiring exchange transfusion; medium-risk or low-risk surgery might need simple transfusion to increase haemoglobin concentration to 100 g/L

Indications for regular, long-term transfusions

Primary and secondary stroke prevention
Regular transfusions, either simple or exchange, to keep HbS less than 30%

Recurrent acute chest syndrome not helped by hydroxycarbamide
Regular transfusions, either simple or exchange, to keep HbS less than 30%

Progressive organ failure
Including hepatic, renal, cardiac, and pulmonary failure; little evidence-based practice and transfusion strategies vary widely

Other indications
Recurrent splenic sequestration, complicated pregnancy

Controversial indications
Frequent acute pain, chronic pain, avascular joint necrosis, leg ulcers, priapism

myelosuppression being the main short-term side-effect. Concern has been raised about the possibility of hydroxycarbamide predisposing towards malignant disease, although there is little evidence to support this notion.[95] On the basis of studies in mice and several case reports, there is also concern that hydroxycarbamide might cause irreversible male subfertility, although this tenet needs to be studied prospectively.[96] Individuals who are receiving hydroxycarbamide are generally advised to avoid pregnancy and conception, although no evidence of teratogenicity was reported in a study of 94 pregnancies in which one member of the couple was taking the drug at the time of conception.[97]

Hydroxycarbamide might have other benefits, including increasing life expectancy,[98,99] protection against cerebrovascular disease,[100] and reduction of hypoxaemia[101] and proteinuria.[102] On the basis of our experience, 10–30% patients in Europe and the USA take hydroxycarbamide, although precise figures are not available. Because of fears about toxic effects, use is generally limited to patients after a severe clinical course, although emerging data, particularly for prolonged survival, suggest that this treatment is underused.[103]

Blood transfusion and iron chelation

Erythrocyte transfusion has an established role in the management of both acute and chronic complications in sickle-cell disease (panel 2). Transfusion corrects anaemia, decreases the percentage of HbS, suppresses HbS synthesis, and reduces haemolysis, all of which are of potential benefit. Erythrocytes can be given as a simple additive transfusion or by exchange, in which blood is also removed. Exchange transfusion is more likely to be necessary if the intitial haemoglobin concentration is high, or if there is a need for rapid decrease in HbS percentage without increasing the haematocrit and blood viscosity, typically in people with acute neurological symptoms. Exchange transfusion is most effectively done by use of automated apheresis machines, although use is limited by difficulties with venous access, especially in children.[104] Patients with sickle-cell disease are at risk of alloimmunisation because of differences between the ethnic origin of blood donors and patients, and blood is typically subject to an extended crossmatch for ABO, full Rhesus (Cc/D/Ee), and Kell blood groups.[105] In countries where most of the blood donors are of European origin, this procedure reduces alloimmunisation by 50% and is recommended practice. Chronic blood transfusion is inevitably associated with iron overload, although the pattern of haemosiderosis seems different to that described in thalassaemia; in particular, most iron loading occurs in the liver, with little cardiac iron deposition.[106] Iron chelation is important in chronically transfused patients with sickle-cell disease, mainly to avoid liver damage; desferrioxamine can be given parenterally, although the oral iron chelator deferasirox is increasingly used with evidence of benefit.[107]

Haemopoietic cell transplantation and stem-cell gene transfer

Haemopoietic cell transplantation was first used in sickle-cell disease 25 years ago and is the only curative treatment.[10] However, only a few hundred patients have received transplantation worldwide, and this procedure is mostly confined to children with HLA-compatible siblings, in whom the procedure is safest. Most children with sickle-cell disease have few overt complications, and haemopoietic cell transplantation is only considered when serious complications have occurred, most often in children with cerebrovascular disease who are effectively dependent on transfusions. Data from studies indicate an overall survival of 92–94%, event-free survival of 82–86%, and a transplant-related mortality of 7%.[108] Balancing of the short-term risk of death against the long-term complications of a chronic disease is difficult. In a study,[109] nine adults with sickle-cell disease received transplantation with non-myeloablative conditioning and long-term immunosuppression, resulting in stable chimerism and resolution of symptoms; further development of this approach is likely to increase the number of transplants by both decreasing toxic effects and increasing the numbers eligible for haemopoietic cell transplantation. Further studies are now being planned with haploidentical family donors using immunotolerance induction strategies.

Gene therapy continues to offer promise. Lentiviral-mediated gene transfer can correct haematological defects and organ damage in mice with sickle-cell disease,[110] and a clinical trial has started in France.[111] Future developments might include the use of induced pluripotent stem cells as a source of haemopoietic progenitors for gene therapy, and this approach recently successfully corrected sickle-cell disease in mice.[112]

Management of specific complications

Acute pain

Acute pain is the most common reason for admission to hospital for both adults and children, although it is more common in teenagers and young adults than in young children. Although acute vaso-occlusive pain is typically self-limiting and does not result in permanent organ damage, it is the most important complication from the patient's perspective, and increased frequency of pain is associated with early death in patients with sickle-cell anaemia who are older than 20 years.[68] Frequent episodes of acute pain are associated with sickle-cell anaemia (compared with HbSC disease), high haematocrit, low fetal haemoglobin concentrations,[68] sibling history of asthma,[113] and nocturnal hypoxaemia.[114] Opiate analgesia is the mainstay in the management of severe pain, with data from a randomised controlled trial indicating no additional benefit from ketoprofen.[115] Management is increasingly based on oral opiates, which seem to be equivalent to parenteral opiates in children.[116] A confidential enquiry into deaths of patients with sickle-cell disease in the UK identified opiate-related oversedation as a cause of death, emphasising the importance of careful monitoring and the involvement of specialist pain teams.[117] Corticosteroids can also shorten episodes of acute pain, although use of these drugs has mostly stopped because of a high frequency of rebound pain and hospital re-admission.[118] In trials of poloxamer 188[119] and inhaled nitric oxide,[120] marginal benefits were reported and a larger placebo-controlled trial of nitric oxide is being analysed.[121] Despite the importance of pain, no specific treatments are available that can change the natural history of an acute episode, and little research is being done in this area.

Infection

Bacterial infections are a major cause of morbidity and mortality in children with sickle-cell disease. The increased susceptibility of affected children is likely to result from several causes, including impaired splenic function, defects in complement activation, micronutrient deficiencies, and tissue ischaemia.[14] Several organisms, including *S pneumoniae*, *H influenza*, and non-typhi *Salmonella* species, have been identified as important causes of infection in developed countries,[14] where substantial improvements in prognosis have followed the introduction of penicillin prophylaxis and immunisation with conjugate vaccines directed against *S pneumoniae* and *H influenzae* type b.[8,9,122] Similar organisms are probably involved in most of the infections in patients in sub-Saharan Africa,[61] although data are less clear.[58]

Neurological complications

Sickle-cell anaemia is one of the most common causes of stroke in children. Most cases are associated with vasculopathy affecting the distal internal carotid and middle cerebral arteries, although extracranial vasculo-pathy can also be present.[123] Although the mechanisms for stroke remain uncertain, contributory factors to this vasculopathy include anaemia, leucocytois, hypoxaemia, abnormal rheology causing endothelial damage, functional nitric oxide deficiency associated with haemolysis,[77,124] and impaired regulation of blood flow causing hyperaemia.[125,126] The vasculopathy seems to start in infancy, with a first-stroke incidence of 1·02 per 100 patient-years between the ages of 2 years and 5 years, and 11% of patients with sickle-cell disease have had a stroke by the age of 20 years.[32] Vasculopathy can be detected at an early stage by use of transcranial doppler scanning. In the Stroke Prevention in Sickle Cell Anemia (STOP) study,[12] regular blood transfusion to keep HbS below 30% reduced the risk of stroke by 90% in patients with increased transcranial doppler velocities. A programme of transcranial doppler screening has been established in some countries, with evidence of a decrease in stroke incidence.[127] Studies are investigating the role of hydroxycarbamide in the prevention of cerebrovascular disease.[100] In the Stroke with Transfusions Changing to Hydroxyurea (SWiTCH)

study, the efficacy of regular blood transfusions and iron chelation was compared with hydroxyurea and phlebotomy in children with sickle-cell disease and stroke. However, the study was stopped prematurely because of the high number of strokes in the hydroxyurea group; no strokes occurred in 66 children receiving blood transfusions, but seven strokes occurred in the 67 children taking hydroxyurea.[128]

Once a stroke has occurred, the risk of recurrence is more than 60%, although this risk is substantially reduced by starting a transfusion programme.[129] Some children have progressive vasculopathy, with a moyamoya-like syndrome and further strokes despite transfusion; neurosurgical revascularisation might be helpful in these circumstances.[130]

In studies in which MRI is used, up to 20% of children with sickle-cell disease have silent brain infarcts, typically involving watershed areas in the frontal lobes.[125] These pathological changes also seem to occur in young children.[131] Silent infarcts are linked to neurocognitive problems, fits, and risk of further brain infarction.[125] The possibility of preventing progression of these infarcts with blood transfusions is being studied in a randomised controlled clinical trial.[132] Cognitive impairment also occurs in the absence of brain infarction, with a suggestion that this neurological deficit might be partly attributable to anaemia and hypoxia.[133]

Intracranial bleeds occur in patients of all ages, but are most common between the ages of 20 years and 30 years; they are typically associated with either a moyamoya-like syndrome or cerebral aneurysms. Treatment is neurosurgical and the outcome is poor, with 26% mortality at 2 weeks.[32]
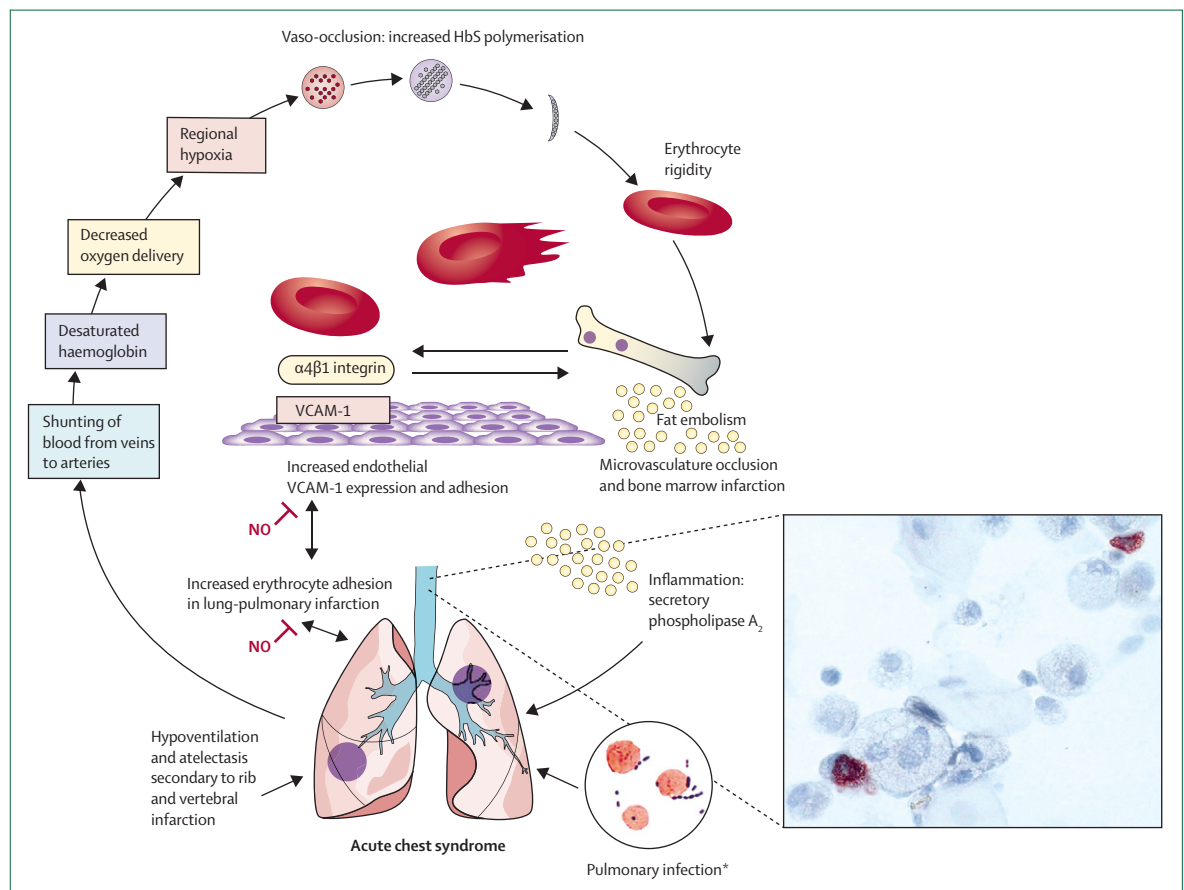
***Figure 4*: Pathophysiology of acute chest syndrome**
Infection or other inflammatory stimuli cause pulmonary hypoxia and increased expression of endothelial adhesion molecules, including α4β1 and VCAM-1; this precipitates HbS polymerisation and vaso-occlusion, causing further hypoxia and inflammation and creating a constant cycle. Vaso-occlusion causes the release of free plasma haemoglobin, which reduces NO availability, altering VCAM-1 expression. Vaso-occlusion and bone marrow infarction can cause fat embolism, further damaging the pulmonary circulation. The stain shows oil-red O staining of pulmonary alveolar macrophages, showing the characteristic red lipid inclusions that are diagnostic of fat embolism. Secretory phospholipase A2 concentrations, which increase in response to inflammation and are known to be very high in acute chest syndrome, further increase expression of adhesion molecules in the pulmonary vasculature, causing more vaso-occlusion. *29% *Chlamydia pneumonia*, 20% *Mycoplasma pneumonia*, 2% *Legionella pneumonia*, 10% respiratory syncytial virus, 4% parvovirus, 3% rhinovirus, 2% parainfluenza virus, 2% parainfluenza A virus, 2% cytomegalovirus, 1% Epstein-Barr virus, and 1% herpes simplex virus; *Staphylococcus aureus* was isolated in 5% of cases and *Streptococcus pneumoniae* in only 4% of cases. Adapted from Murray and colleagues,[137] with permission from Elsevier. VCAM-1=vascular cell-adhesion molecule-1. NO=nitric oxide.

Acute chest syndrome

Acute chest syndrome is the second most common cause of hospital admission in patients with sickle-cell disease. It is a form of acute lung injury and is defined as the development of a new alveolar pulmonary infiltrate involving at least one lung segment.[134] This syndrome is caused by a combination of infection, fat embolism, and vaso-occlusion of the pulmonary vasculature. Severity varies, but 13% of patients require mechanical ventilation and 3% die.[135] Treatment involves broad-spectrum antibiotics, bronchodilators, and oxygen. If haemoglobin concentrations decrease substantially or the patient's clinical condition deteriorates, blood transfusion is commonly given. Support is increasing for the use of early top-up transfusion, with exchange transfusion reserved for severe cases.[135] Dexamethasone can improve clinical condition and reduce the need for blood transfusion in children with acute chest syndrome, although use is limited by the occurrence of rebound pain on stopping corticosteroids and by the concern about infection[136] (figure 4).

Pulmonary hypertension

Pulmonary hypertension is an increasingly recognised complication of sickle-cell disease in teenagers and adults. In three prospective studies in adults,[33,36,37] in which echocardiography was used to measure tricuspid regurgitant jet velocity, 20% of the participants had mild elevation in estimated pulmonary artery pressures, defined by a pulmonary artery systolic pressure greater than 35 mm Hg (upper limit of normal is 32 mm Hg), and 9% had moderate-to-severe pulmonary hypertension (>45 mm Hg). Despite increases in pulmonary pressure that are much lower than those observed in patients with idiopathic or hereditable pulmonary hypertension, the prospective risk of death associated with even mild pulmonary hypertension is high in patients with sickle-cell disease.[33,36,37] In a French study,[138] despite the exclusion of patients with low creatinine clearance, reduced lung capacity, and liver disease—all additional major risk factors for the development of pulmonary hypertension—6% of 385 patients had a mean pulmonary artery pressure of greater than 25 mm Hg at right heart catheterisation. Although this prevalence is lower than in studies using echocardiography, the occurrence of pulmonary hypertension is high compared with other diseases associated with its development, and all deaths occurred in those with pulmonary hypertension.[138]

We are not aware of any robust evidence about how best to treat pulmonary hypertension in patients with sickle-cell disease. Risk factors such as hypoxaemia, sleep apnoea, pulmonary thromboembolic disease, restrictive lung disease, left ventricular systolic and diastolic dysfunction, severe anaemia, and iron overload need to be identified and treated. Treatment options include hydroxycarbamide, with regular blood transfusions if there is no response.[134] In patients with severe pulmonary hypertension (tricuspid

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Panel 3: Treatment options for some complications of sickle-cell disease

Avascular necrosis of the femoral head
Possible benefit from core decompression[144] and autologous bone marrow grafting[145]

Proliferative retinopathy
Role of laser photocoagulation uncertain;[146] case reports of benefit from intravitreal bevacizumb[147]

Sickle hepatopathy
Emerging use of liver transplantation in severe liver disease[148]

Priapism
Adrenergic agonists form mainstay of preventive treatment, with emerging use of phosphodiesterase-5 inhibitors and finasteride[149]

	Therapeutic approach	Specific examples
To reduce infective complications	Improve vaccination against *Streptococcus pneumoniae*	7-valent and 13-valent conjugated vaccines
To reduce tissue hypoxia	Treat sleep-disordered breathing, blood substitutes	Overnight oxygen, continuous positive airways pressure, pegylated haemoglobin
To reduce erythrocyte dehydration	Inhibit Gardos channels and other cation channels	Magnesium, zinc, dipyridamole, senicapoc
To improve rheology	Block surfactant, endothelin receptors	Poloxamer-188, bosentan
To improve nitric oxide availability	Increase nitric oxide availability, release, and synthesis	Inhaled nitric oxide, arginine, nitrites, sildenafil, statins
To reduce inflammation	Treat with corticosteroids, secretory phospholipase A2 inhibitors	Dexamethasone, varespladib
To reduce erythrocyte adhesion	Treat with anticoagulants, reduce endothelial activation	Heparin, sulfasalazine
To increase fetal haemoglobin production	Treat with cytotoxic drugs, short-chain fatty acids, immunomodulatory drugs	Decitabine, butyrate, pomalidomide, lenalidomide
To reduce tissue damage	Treat with antioxidants	Glutamine, N-acetyl cysteine
Empirical use	Treat with phytomedicines	Nix-0699

Further information is reviewed by Hagar and Vichinsky.[150]

Table 3: Emerging therapeutic approaches for treatment of sickle-cell disease by mechanism

regurgitant jet velocity ≥3 m/s), right heart catheterisation is necessary to confirm diagnosis and to directly assess left ventricular function. Endothelin receptor antagonists (eg, bosentan and ambrisentan), prostaglandin-based therapy (eg, epoprostenol, treprostinil, and iloprost), and the phosphodiesterase-5 inhibitors (eg, sildenafil) are all used to treat idiopathic pulmonary hypertension and might be of benefit for patients with sickle-cell disease. Treatment with oral sildenafil improved exercise tolerance and pulmonary hypertension in patients with sickle-cell disease,[139] although a multicentre, placebo-controlled trial of sildenafil for pulmonary hypertension in sickle-cell disease was stopped early because of an unexpected increase in hospital admissions for acute pain in the treatment group.

Heart disease
Left-sided heart disease occurs in about 13% of adults with sickle-cell disease and is mainly caused by diastolic dysfunction; systolic dysfunction can also occur and valvular disease is present in about 2% of patients.[33] The presence of diastolic dysfunction alone in patients with sickle-cell disease is an independent risk factor for mortality.[140] Patients with both pulmonary vascular disease and diastolic dysfunction are at a particularly high risk of death (odds ratio for death 12·0, 95% CI 3·8–38·1, p<0·001).[140] Pulmonary pressures rise acutely during vaso-occlusive pain and even more during acute chest syndrome. In a study,[141] 13% of patients manifested right heart failure with acute chest syndrome, and this subgroup had the highest risk of mechanical ventilation and death.

Renal complications
Renal damage is almost inevitable in sickle-cell disease. There is a strong tendency for HbS to polymerise in the renal medulla, because of the low partial pressure of oxygen, the low pH, and the high osmolality causing erythrocyte dehydration. The consequent vaso-occlusion causes renal infarction with papillary necrosis, and medullary fibrosis with focal segmental glomerulo-sclerosis. Glomerular hyperfiltration and tubular dysfunction also occur, and are possibly associated with anaemia and increased sensitivity to prostaglandins.[142] Renal dysfunction is apparent from an early age in patients with sickle-cell anaemia, with glomerular hyperfiltration apparent at 13 months.[143] Micro-albuminuria is common in childhood and up to 20% of adults develop nephrotic-range protein loss, with more than 3·5 g proteinuria in 24 h.[142] 30% of adults develop chronic renal failure, which is a contributory factor in many deaths.[30] Other renal manifestations include haematuria, renal medullary carcinoma, and nocturnal enuresis.[142] Treatment is beginning to focus on the early use of hydroxycarbamide and angiotensin-converting enzyme inhibitors in children with clinically significant albuminuria,[102] although there is little supportive evidence at present. In end-stage kidney failure, 10-year survival was 56% for patients after renal transplantation, compared with 14% for patients on dialysis,[142] suggesting that transplantation is the treatment of choice.

Sickle-cell disease causes multisystem problems, and panel 3 summarises new treatment approaches.

Future developments
Stem-cell transplantation and gene therapy seem likely to become more widely applicable as new techniques develop, with the use of induced pluripotent stem cells offering the most promise.[112] Data from genetic association studies should help to identify more unlinked and epigenetic factors to explain phenotypic diversity and to enable better prognosis, which might lead to the treatment of specific complications. Many drugs have given in-vitro or early benefit in sickle-cell disease without becoming clinically useful, and table 3 lists some promising pharmaceutical approaches in development. Improved understanding of sickle-cell disease in Africa would benefit the largest number of patients, and could facilitate improved management of patients worldwide, including Europe and the USA.

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