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REVIEW

Infection in sickle cell disease: A review

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Summary Infection is a significant contributor to morbidity and mortality in sickle cell disease (SCD). The sickle gene confers an increased susceptibility to infection, especially to certain bacterial pathogens, and at the same time infection provokes a cascade of SCD-specific pathophysiological changes. Historically, infection is a major cause of mortality in SCD, particularly in children, and it was implicated in 20–50% of deaths in prospective cohort studies over the last 20 years. Worldwide, it remains the leading cause of death, particularly in less developed nations. In developed countries, measures to prevent and effectively treat infection have made a substantial contribution to improvements in survival and quality of life, and are continually being developed and extended. However, progress continues to lag in less developed countries where the patterns of morbidity and mortality are less well defined and implementation of preventive care is poor. This review provides an overview of how SCD increases susceptibility to infections, the underlying mechanisms for susceptibility to specific pathogens, and how infection modifies the outcome of SCD. It also highlights the challenges in reducing the global burden of mortality in SCD.

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Introduction

Sickle cell disease (SCD) is a collective term for a number of genetic disorders in which hemoglobin is structurally abnormal, resulting in the episodic formation of sickle-shaped red blood cells (RBCs) and a wide range of clinical manifestations. It affects some 12 500 people in the UK and millions worldwide,¹ particularly those of black African and Afro-

Caribbean descent, and also those from the Mediterranean, Middle East, and parts of India.² The underlying abnormality is a single nucleotide substitution (GTG for GAG) in the gene for β -globin on chromosome 11, resulting in the replacement of a glutamic acid residue with valine on the surface of the protein (termed HbS).³ In normal adult HbA, two chains of α -globin and two of β -globin form a tetramer, stabilized by specific intramolecular points of contact, but without interactions between individual tetramers within the RBC.⁴ When the molecule binds or releases oxygen, it undergoes a conformational change. In HbS, deoxygenation exposes the abnormal valine residue on the surface of the molecule,

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which then forms hydrophobic interactions with adjacent chains. The resulting polymers align into bundles, causing distortion of the RBC into a crescent or sickle shape and reducing flexibility and deformability, which impairs passage of the cells through narrow blood vessels.³ Sickling can be precipitated by environmental factors such as hypoxia, low pH, cold, and dehydration of the RBC, as well as adhesion molecules and cytokines associated with infections.

Homozygous SS (sickle cell anemia) is generally considered the most severe form of SCD. Compound heterozygotes, in whom HbS is combined with a different mutation in the second β -globin gene, such as HbC, D, O^{Arab} or β -thalassemia (where β -globin synthesis is reduced) can also be affected, with variable phenotypes. The carrier state (HbAS) does not cause clinically significant disease (though sickling may occur under extreme conditions), so carriers are most often unaware of their genotype or their sickle gene status, and the frequency of the gene in some populations is very high: one in four among Nigerians.⁵

The clinical manifestations of SCD result from two key pathological processes: vaso-occlusion and hemolysis. Sickled cells, along with non-sickled RBCs, leukocytes, and platelets, form heterocellular aggregates, which adhere to the vascular endothelium, causing obstruction of the lumen of small blood vessels. This microcirculatory occlusion leads to acute and chronic tissue ischemia and infarction, with multisystem effects, particularly in bone, lungs, brain, kidneys, and spleen. It is responsible for acute painful episodes and crises and many of the long-term complications seen in SCD. Sickled RBCs are more readily destroyed by the reticulo-endothelial system, partly as their rigidity makes them more easily filtered in the spleen and partly due to changes in the structure of the lipid bilayer (with exposure of anionic phosphatidylserine on the RBC surface), which promotes phagocytosis.³ With sickle cell anemia (HbSS), this causes a chronic anemia (a steady state Hb of 6–8 g/dl)⁶ with a resultant increase in cardiac output and workload, which produces cardiomegaly and reduced exercise tolerance. The increased energy demands due to this and the chronically elevated rate of hematopoiesis contribute towards poor growth in children, and individuals are susceptible to any factor exacerbating the anemia, which can precipitate circulatory failure.⁷ Intravascular hemolysis also leads to release of free hemoglobin—an important scavenger of nitric oxide (NO). Reduced levels of this potent vasodilator and the hyperdynamic circulation contribute further to vascular damage and occlusion, including within larger vessels.⁴ Despite progress in therapy, SCD remains a cause of significant morbidity and mortality. Life expectancy in HbSS from a multicenter study in the USA in 1994 was estimated at 42 for men and 48 for women,⁸ and 95% of children survive to adulthood.⁹

The effect of sickle cell disease on infection

Impaired splenic function

The spleen has a key role in the increased susceptibility to certain bacterial infections seen in SCD. It functions as a phagocytic filter, removing old and damaged cells and blood-borne microorganisms, and also produces antibodies. Blood from the splenic artery first traverses the white pulp, which

contains collections of B and T lymphocytes in follicles and periarteriolar lymphatic sheaths. Activation of these cells by filtered antigenic material enables initiation and expansion of a specific acquired immune response. Blood then enters the splenic cords of the red pulp, where cells flow over a fine reticular meshwork and pass through fenestrated epithelium to enter the venous sinuses. This creates a slow flow, enabling splenic macrophages to remove defective RBCs and bacteria and to present antigen to lymphocytes.¹⁰ Some bacteria can be recognized directly by macrophages, but many first require opsonization—coating of the microbial surface by complement components (especially C3b) or other molecules, which in turn interact with receptors on phagocytes.¹¹ The spleen is the site of synthesis of tuftsin, an immunostimulatory peptide, and properdin, which participates in complement activation.¹² Opsonized bacteria are removed efficiently by macrophages in the spleen or liver, but poorly opsonized bacteria are only cleared effectively by the spleen. Such pathogens include encapsulated bacteria, in particular *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae*. Their polysaccharide capsule impedes binding of complement or prevents complement assembled on the cell wall from interacting with macrophage receptors.¹⁰ Clearance of these bacteria requires anti-polysaccharide IgM antibodies, which facilitate phagocytosis either directly or via deposition of complement over the capsule itself. A unique B cell population—IgM memory B cells—resides in the marginal zone of the spleen (adjacent to the follicles). These cells persist after an initial infection and rapidly produce antibody on subsequent exposures.¹³

Individuals with SCD typically suffer from functional hypoplasia or asplenia. The sluggish circulation through the spleen, high rates of O₂ extraction, and local acidosis cause deoxygenation of HbS, promoting sickling, which leads to congestion and engorgement of the sinusoids with sickled cells. This can cause diversion of blood via intrasplenic shunts, bypassing the normal filtering mechanisms. Macrophages engulfing the abnormally shaped cells may become 'blocked', impairing their phagocytosis of other particles. Together these effects produce a hyposplenic state that is initially reversible, for example if HbS levels are lowered to less than 50% by blood transfusion, bone marrow transplant, or hydroxyurea treatment.¹² However, over time repeated episodes of sickling and ischemic damage with progressive sclerosis of arterioles lead to multiple infarcts of spleen tissue. Unable to regenerate, the spleen becomes scarred and atrophied, culminating in 'autosplenectomy', where the organ shrinks to a small remnant and the individual is rendered effectively asplenic.¹⁴ In HbSS this sequence develops from the age of 6 months to 3 years.¹² Hyposplenic and asplenic individuals lack IgM memory B cells, suggesting a role for the spleen in their generation or function, and hence they cannot mount a rapid specific response to encapsulated organisms. Local infections can readily become systemic and this, in combination with the loss of the spleen's filtering function, can permit overwhelming sepsis to develop. The main pathogen of concern is *S. pneumoniae*, though severe and systemic infections with *H. influenzae*, *Neisseria meningitidis*, and salmonellae also occur. Before preventive measures, children with SCD were 30–600 times more likely to develop invasive pneumococcal disease (IPD), including pneumonia, meningitis, and septicemia.¹⁵ Overwhelming sepsis can develop rapidly with

no obvious primary source of infection, resulting in shock, disseminated intravascular coagulation, adrenal hemorrhage, and death within 24 to 48 hours.¹⁶ Mortality can reach 35–50% from septicemia and 10% in meningitis. The risk is confined almost exclusively to young children, with a reported incidence of 5.8 per 100 in children aged less than 3 years, 1.1 per 100 in those aged 5–9 years, and 0.6 per 100 in those aged over 10 years in the pre-treatment era.¹⁷

Curiously, recent reports^{18–20} from malaria endemic sub-Saharan Africa suggest that pneumococcal disease does not contribute significantly to the morbidity and mortality of children with SCD and it has been argued that children in this region may be succumbing to other infections, so established preventive measures used in other settings may be inappropriate.²¹ While it is theoretically conceivable that children affected by SCD in malaria hyperendemic settings may be at increased risk of death from other pathogens such as malaria and invasive salmonellosis, poor diagnostic facilities and the high childhood mortality rates call this assertion into question. Well-planned longitudinal cohort studies to define the etiologic agents that predominate in SCD morbidity and mortality in this region are urgently needed.

Defects in complement activation

A number of other mechanisms for increased susceptibility to infection in SCD have been explored. Major infections occur in early infancy when the spleen is still partially functional and some increased risk persists despite modern prophylactic measures, suggesting additional immune deficits are present.²² Patients also seem predisposed to other infections, including *Escherichia coli* urinary tract infection, *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* respiratory infections, and dental infections and cholecystitis caused by anaerobes. The complement system involves a large number of plasma proteins that are cleaved sequentially by protease enzymes to generate active fragments. These function as opsonins or chemoattractants, and the terminal components can kill some pathogens directly by creating pores in their membranes. The cascade can be activated either via the classical pathway, following binding of IgM or IgG to surface antigens, or the alternative pathway, in which C3b interacts directly with the pathogen cell surface, then recruiting further downstream components.¹¹ Studies have not consistently demonstrated any deficiencies in the amount of complement components, but early work did suggest a reduced functional activity of the alternative pathway, with lower levels of the active form of factor B (the first protein recruited by C3b) and impaired opsonization of yeast *in vitro*.²³ There have been few more recent or large scale trials to verify these findings, and it seems unwise to assume that the effect seen in highly simplified, artificial *in vitro* experiments would also apply, and be clinically relevant, *in vivo*. In the absence of extensive studies in normal populations, it is not known how much the measured activity of complement varies between healthy individuals. One small study in 1999 did show an inverse correlation between complement activity and the number of crises suffered by SCD patients,²⁴ though the mechanism can only be a matter of speculation. In some studies, reduced leukocyte function in SCD, particularly neutrophil killing ability, has been shown to

correlate with clinical severity of the disease.²⁵ However, this has not been a consistent finding.

Deficiencies in micronutrients

Zinc is known to be important for immune function, so low levels in SCD have been suggested as a contributory factor in susceptibility to infection. Zinc deficiency is associated with lymphopenia, possibly due to activation of the hypothalamo-pituitary-adrenocortical axis, causing chronic glucocorticoid production, which stimulates apoptosis of B and T cells in bone marrow and the thymus.²⁶ It has also been linked with reduced production of interleukin (IL)-2 (a cytokine needed for expansion and maintenance of thymocytes and peripheral T cells), reduced natural killer (NK) cell lytic activity, low thymulin activity, reduced CD4:CD8 ratio, and impaired Th1 cell function.²⁷ Zinc deficiency may affect 60–70% of SCD patients. High protein turnover increases requirements, while hemolysis releases zinc, which is lost via the kidneys as renal tubular damage impairs reabsorption. At the same time poor diet and inadequate intestinal absorption could reduce intake. A study in 21 zinc deficient children suggested that giving supplements increased IL-2 levels, reduced the incidence of bacterial infections and cut hospital admissions.²⁷ This was not a fully controlled trial and as changes were observed over time in a group of subjects, other factors such as altered susceptibility with age, improvements in diet or hygiene, or changes in the prevalent environmental pathogens may have been responsible for the effects. However, if such a simple measure as a mineral supplement could improve quality of life, the issue may warrant further exploration.

Genetic factors

Despite sharing the same underlying genetic mutation, the range of severity in the phenotype of SCD is striking, with some patients disabled by frequent crises and long-term complications while others live virtually normal lives. Individuals are also differently predisposed to particular pathological manifestations of the disease. This suggests that the phenotype is multigenic: since many unlinked genes are involved in the underlying pathological processes in SCD (such as destruction of sickled cells or endothelial adhesion), variation in alleles at multiple loci may modify outcome.³ Polymorphisms in a number of genes involved in the immune response have been suggested as contributing to increased susceptibility to infection in SCD. Although unlinked to the sickle gene, these variants may coincidentally occur with increased frequency in the SCD population. Particular HLAII subtypes have been shown to be predisposing or protecting factors for infectious complications,²² while certain polymorphisms of the FcR receptor (involved in clearing encapsulated bacteria), mannose-binding lectin, insulin-like growth factor 1 receptor (IGF1-R; involved in B and T cell recruitment and differentiation), and genes of the transforming growth factor β (TGF β)/bone morphogenetic protein (BMP) pathway have been associated with an increased risk of bacteremia.²⁸ These studies also highlight the need for caution in making generalizations about immune function in SCD, for example complement activation or neutrophil action, based on experiments using small

numbers of subjects in localized geographical areas. Observed differences may increase individual risk, but may not be a universal feature.²⁹ Large samples across multiple racial groups would be needed to distinguish effects due to SCD per se from those caused by other unlinked and variable alleles.

Mechanical factors

The pathological effects of SCD can themselves create an environment supporting infection. Children with SCD are predisposed to osteomyelitis. The bone marrow space is expanded to accommodate the increased hematopoiesis needed to compensate for chronic hemolysis, and oxygen demand is high. At the same time circulation is sluggish. Together these factors render bone vulnerable to vaso-occlusive episodes and infarction. Areas of necrotic bone act as foci for infection, which becomes established via hematogenous spread.³⁰ In children unaffected by SCD, *Staphylococcus aureus* is the predominant pathogen in osteomyelitis. In SCD, Salmonella is the most common agent, followed by *S. aureus* then Gram-negative enteric bacteria.³¹ In long-term retrospective reviews from the USA³² and Saudi Arabia,³³ Salmonella accounted for 57% and 41.7% of cases of acute osteomyelitis, respectively. In London, 52.3% of all bacteremia over a 15-year period in SCD patients were found to be due to Salmonella, compared to 0.4% in non-SCD individuals. Most of these infections were *Salmonella typhimurium*, a common food-borne pathogen, and one third resulted in osteomyelitis.³⁴ It may be that patchy ischemia and infarction of bowel secondary to microvascular occlusion permits gut bacteria to invade the intestinal wall and enter the bloodstream. There does not, however, seem to be any evidence of increased gastrointestinal carriage of Salmonella in asymptomatic SCD patients.³⁵

Edwardsiella tarda is another enterobacterium that has been reported with increased incidence in SCD. Increased gut permeability and biliary sludging in SCD is likely to be responsible for this association.^{36,37}

Another consequence of microvascular disease is its association with acute chest syndrome. SCD carries an increased risk of prolonged and severe respiratory infections due to Mycoplasma, Chlamydia and other pathogens, particularly in children prone to pain or microvascular sequestration, such as those with SC hemoglobinopathy. Several reports in the literature document an association between respiratory infections and acute chest syndrome,^{38,39} but definitive diagnosis remains a challenge since these are slow growing fastidious organisms and new molecular diagnostic assays are yet to be fully validated. Thus the immunopathological mechanisms leading to this increased risk are not completely clear.

Finally, SCD patients may be predisposed to certain iatrogenic infections as a result of therapeutic interventions. Blood transfusion is commonly used to treat complications, particularly aplastic crisis or splenic sequestration (when Hb falls acutely) and acute chest syndrome, priapism, or strokes (when exchange transfusion is used to reduce the proportion of HbS).⁴⁰ Increasingly, chronic transfusion therapy is being used in children to prevent strokes by keeping HbS levels below 30%. In developed countries, 5–10% of children may be involved in a chronic transfusion program at some point in

their lives.² In general such programs are potentially associated with increased risk of blood-borne infections, particularly hepatitis B and C and HIV. Although all blood products in developed countries are screened for these viruses, standards in other countries may not be so exacting, so early hepatitis B immunization is recommended as a preventive measure. Other viruses such as cytomegalovirus (CMV) and parvovirus B19 can also be transmitted. Although not a problem in immunocompetent individuals, CMV is a significant pathogen in the immunocompromised, so children who are potential candidates for bone marrow transplant should receive CMV seronegative blood.⁴⁰ Patients may also be at risk of catheter-related infections, particularly those on prolonged courses of parenteral antibiotics or with indwelling vascular devices for chronic blood transfusion. This has been reported in adults and seems to be particularly associated with bone infections.⁴¹

The effect of infection on sickle cell disease

Infection has long been recognized as one of the most common precipitants of crisis in SCD, but only as the mechanisms underlying the process of vaso-occlusion have been better elucidated have the reasons for this become clear. Vaso-occlusion, initially assumed to be due to passive mechanical blockage by sickled RBCs, is in fact a complex, dynamic process involving active interaction between adhesion molecules on the vascular endothelium (e.g., intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (V-CAM1), and $\alpha_5\beta_3$ integrin) and both RBCs and leukocytes. RBCs attach via surface ligands including $\alpha_4\beta_1$ integrin, basal cell adhesion molecule (BCAM), phosphatidylserine, and sulfated glycans.⁴² This could contribute directly to occlusion, but also acts via slowing transit of RBCs through the microvasculature. The sickling process takes time because a nucleus of ten deoxy-HbS tetramers must assemble before rapid polymerization can occur. Usually transit through the microcirculation is completed before this 'delay time' elapses, so sickling does not take place. If, however, transit time is prolonged, RBCs equilibrate at a lower O₂ tension, leading to greater concentrations of deoxy-HbS and hence sickling of many cells.³

Leukocyte adhesion may in fact be the initiating event in vaso-occlusive episodes, as microvascular occlusion occurs in post-capillary venules—the site of leukocyte attachment for passage into the extracellular space—rather than pre-capillary arterioles (to be expected from a purely mechanical effect). Steady state neutrophil count correlates with the severity of SCD, and treatment with hydroxyurea, which lowers neutrophil numbers, reduces the frequency of crises, painful episodes, and hospital admissions.⁴³ Patients with severe SCD have increased steady state expression of leukocyte adhesion molecules such as M β_2 integrin, L-selectin, and CD18.⁴⁴ These observations suggest a central role for leukocytes in the vaso-occlusive process. Narrowing of the vessel lumen by attached leukocytes may enable the accumulation of RBCs, platelets, and further leukocytes, with increasing occlusion. Local hypoxia in areas of poor flow promotes RBC sickling and propagation of the blockage, culminating in a crisis. Leukocytes produce cytokines (e.g., tumor necrosis factor (TNF)- α , IL-1 β), which induce the expression of adhesion molecules on the vascular endothelium and can cause

exposure of the underlying extracellular matrix components to which RBCs also attach.⁴

During infection with any pathogen, changes occur at a cellular level, which predispose to crises. Levels of circulating leukocytes and inflammatory cytokines increase, with elevated expression of adhesion molecules on both the vascular endothelium and leukocytes themselves. This occurs locally in infected tissues and systemically. Neutrophils, basophils, and monocytes attracted to sites of inflammation produce cytotoxic proteins such as proteases, collagenase, and elastase and generate reactive O₂ radicals, which cause oxidative damage. This promotes further endothelial activation and cell adhesion.⁴⁴ Adrenaline produced in times of stress can increase the adhesion of laminin to RBC BCAM.³ Local acidosis and hypercapnia in areas of inflammation shift the Hb oxygen dissociation curve to the right, promoting unloading of oxygen from Hb and thus increasing sickling.

The sickling process is initially reversible when HbS is reoxygenated, but dehydration of RBCs increases HbS concentration, promoting extensive polymerization and causing irreversible membrane damage. These poorly deformable dense cells contribute particularly to vaso-occlusion, adhering readily to leukocytes and endothelium, and also undergo hemolysis. RBC dehydration occurs due to activation of cation channels in the cell membrane, causing efflux of K⁺ and water. The K–Cl co-transporter is constitutively over-expressed in SCD, but is further activated by low pH. The Ca²⁺ sensitive K⁺ efflux Gardos channel is activated by cytokines, chemokines, and prostaglandin E₂ (PGE₂), further contributing to sickling, vaso-occlusion, and hemolysis in infection.^{3,4} Hemolysis releases free Hb, depleting NO and hence promoting vasoconstriction as previously described. In addition to its vasodilatory action, NO normally reduces endothelial expression of adhesion molecules and alters the activation state of leukocytes. Finally, the release of heme iron causes oxidative stress, which further stimulates adhesion molecule expression through action on redox-sensitive transcription factors (e.g., nuclear factor (NF)-κB).⁴⁴

In addition, infections can have more non-specific effects on the host physiological milieu, which increase the risk of sickling. Fever with water loss due to sweating, anorexia, and nausea with reduced oral fluid intake, diarrhea, and vomiting all contribute to dehydration. Renal impairment in SCD causes poor urinary concentrating ability, so plasma osmolarity can rise, promoting RBC dehydration. The stress and emotional response, accompanied by neural and hormonal changes, may also play a role.

Effect of specific infections on SCD

Several specific clinical conditions commonly associated with SCD are caused by particular pathogens (Table 1). Some of these specific agents are briefly discussed in this section. The mechanisms underlying the increased susceptibility to specific pathogens are summarized in Table 2.

Parvovirus B19

Parvovirus B19 is a single-strand DNA virus transmitted by respiratory droplets, occurring in outbreaks particularly in late winter and early spring.⁴⁵ It is a common childhood infection with an incidence of 11.3 per 100 patient-years. Around 26% of children are seropositive by age 5, rising to

Table 1 Common pathogens associated with infection in sickle cell anemia with underlying mechanisms for predisposition

Pathogen	Predisposing factors
Encapsulated bacteria (e.g., <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Neisseria meningitidis</i> , <i>Salmonella spp</i>)	Impaired splenic function
Salmonellae	Impaired opsonization Recurrent vaso-occlusion with intestinal infarct, necrosis and increased gut permeability Decreased neutrophil killing
Malaria	Decreased deoxyhemoglobin solubility
Parvovirus	Increased red cell turnover
Hepatitis B, C	Multiple blood transfusion
Chlamydia	Unknown
<i>Yersinia enterocolitica</i>	Iron overload
<i>Mycoplasma</i>	Unknown
<i>Edwardsiella tarda</i>	Increased intestinal permeability and biliary sludging

47% aged 10, 64% aged 15, and 73% in adulthood.⁴⁶ In normal individuals infection is often asymptomatic or gives mild flu-like symptoms, or may cause erythema infectiosum—fever and malaise followed by a characteristic ‘slapped cheek’ rash on the face, progressing to a generalized maculopapular eruption on the trunk and limbs.⁴⁵ Its significance in SCD and other hemolytic conditions is that it commonly causes aplastic crisis. Parvovirus B19 specifically infects erythroid progenitor cells in bone marrow and peripheral blood, using surface P-antigen as a receptor. This results in temporary cessation of erythropoiesis lasting 7–10 days. The viral protein NS1 is cytotoxic. It can induce cell apoptosis, has a direct lytic effect, and can cause cell cycle arrest in G1 or G2, preventing cell division and differentiation. In healthy individuals, the normal lifespan of a RBC is 120 days, considerably longer than the period of aplasia. The absence of erythroid precursors in bone marrow (occurring at peak viremia, around 8–10 days after infection) stimulates an increase in erythropoietin, promoting energetic hematopoiesis once bone marrow recovers. However, RBCs in SCD have a lifespan of just 5–15 days, so transient lack of RBC production results in a severe anemia.⁴⁷ Transient aplastic crisis occurs in 65–80% of parvovirus B19 infections. Although most children recover within two weeks, the majority require blood transfusion. Neutropenia has been reported in 18% and thrombocytopenia in 26.5%, and other complications include acute splenic or hepatic sequestration (19%), acute chest syndrome (11.8%), painful crisis, stroke, nephrotic syndrome, and meningoencephalitis. Acute infection can be confirmed by measuring parvovirus B19-specific IgM (89% sensitive and 99% specific) or using PCR to amplify viral DNA. This is important because the virus is highly contagious, with a secondary attack rate of over 50% among other household members.⁴⁵ Siblings

Table 2 Recommended immunization schedule for sickle cell disease

	Pneumococcus	Meningococcus and <i>Haemophilus influenzae</i> type b	Influenza
Under 2 years	Routine ^a	Routine ^a	Annual
Age 2–5 years (fully immunized)	Single dose PPV	Booster dose given as the Hib/MenC vaccine	Annual
Age 2–5 years (unvaccinated or partially vaccinated)	Two doses of PCV given 2 months apart, followed 2 months later by PPV	Two doses of the Hib/MenC vaccine given 2 months apart	Annual
Age >5 years (fully vaccinated)	Single dose PPV	Two doses of the Hib/MenC given 2 months apart	Annual
Age >5 years (unvaccinated)	Single dose PPV	Two doses of the Hib/MenC vaccine given 2 months apart	Annual
Reinforcing immunization	PPV every 5 years	MenC vaccine every 5 years, Hib vaccine not currently recommended	Annual

Note: Schedule summarized from Salisbury D, Ramsay M, Noakes K. Immunization against infectious diseases (The Green Book). London: Department of Health; 2006.⁷⁸ National childhood immunization in most developing countries does not currently include Hepatitis B, Hib or meningococcal vaccines. These should be strongly encouraged for children with SCD. In endemic settings consideration should also be given for immunization with typhoid vaccine.

Hib, *Haemophilus influenzae* type b; MenC, meningococcus group C; PCV, pneumococcal conjugate vaccine; PPV, pneumococcal polysaccharide vaccine.

^a Routine UK immunization schedule.

with SCD should be tested for infection and monitored for the development of aplastic crisis. After infection immunity is lifelong, leading to calls for the development of a vaccine, though aplastic crisis is uncommon after the age of 15 years.⁴⁸

Atypical bacteria and acute chest syndrome

The acute chest syndrome (ACS) in SCD is defined as the combination of chest pain, dyspnea, fever, and pulmonary infiltrates on chest X-ray.² It affects 15–43% of patients, particularly in early childhood, is responsible for 25% of deaths, and is the second most common cause of hospital admission.^{8,49} Various processes can precipitate the condition, including infection, pulmonary fat embolism from infarcted bone marrow following an acute skeletal crisis, hypoventilation (due to pain from a thoracic bony crisis, post-surgically or from excessive narcotic analgesia), in situ thrombus formation, or red cell sequestration, though often the cause is unknown. The unifying end result is sickling and vaso-occlusion in the small vessels of the lung, causing local ischemia and sometimes infarction. The ventilation–perfusion mismatch causes systemic hypoxia, predisposing to further sickling and vascular occlusion in a vicious cycle. If severe, right heart failure can occur.⁵⁰ In a large multicenter study, a cause of ACS was found in 38% of cases. There was evidence of infection in a third, with 27 different pathogens isolated, sometimes in combination. The most prevalent agents were *C. pneumoniae* (14% of all patients) and *Mycoplasma pneumoniae* (9%), the latter more common in younger patients.⁵¹ Although isolation does not confirm causation, the inflammatory response to lung infection would seem likely to provoke leukocyte and RBC adhesion and intravascular sickling. Pulmonary fat embolism was the other most frequent cause identified. Since, as previously described, non-specific infection can precipitate bone pain crises, it may be that

infection plays a greater role in ACS than studies can readily detect.

Malaria

The relationship between malaria and SCD is an intriguing one. The persistence of the sickle mutation at such high frequency in African populations in spite of the severity of SCD has been attributed to the fact that heterozygous sickle trait confers protection against severe and life-threatening malaria (in particular cerebral malaria caused by *Plasmodium falciparum*). The presence of HbS is associated with reduced parasitic invasion of erythrocytes, impaired multiplication, and accelerated clearance of parasites by the spleen, as RBC infection produces intracellular hypoxia, provoking sickling and hence splenic filtration of parasitized cells.⁵² It might be assumed that homozygous SCD would confer greater resistance to malaria, however co-existence of the two is associated with increased mortality and morbidity, and malaria is the most common precipitating cause of crisis in endemic countries.⁵³ This mainly reflects the general effects of systemic infection, including massive release of inflammatory cytokines. The metabolic activity of parasites within RBCs causes hypoxia, acidosis, and hence sickling.⁵⁴ Red cells containing schizonts adhere to the capillary endothelium even in normal individuals, causing obstruction. In SCD patients, the deleterious effects of this are magnified. The spleen plays an important role in the control of malaria, removing damaged and parasitized RBCs from the circulation, ‘pitting’ infected cells (removing parasites and returning the cells to the circulation intact), and generating specific B and T cell responses. Splenectomized individuals with *P. falciparum* have reduced clearance of parasitized RBCs, but it is unclear whether they suffer more severe malarial symptoms.⁵⁵

Malaria causes anemia via a number of mechanisms.⁵⁶ Infected RBCs undergo hemolysis as merozoites emerge after multiplying, and non-infected cells can be hemolysed due to the production of auto-antibodies against RBC surface molecules. Macrophages phagocytose both infected and non-infected cells. Malaria can cause dyserythropoiesis and splenic sequestration of RBCs (e.g., in young children who have not undergone autosplenectomy), and recurrent hemolysis can produce a folate-deficiency anemia. The anemia can be severe in normal subjects, so is particularly dangerous in SCD. In a Nigerian study, 66% of children presenting with severe anemia had malarial infection, and overall mortality was 8.7%.⁵⁷ Hyperhemolytic crisis refers to a precipitous drop in Hb associated with jaundice, reticulocytosis, unconjugated hyperbilirubinemia, and raised urine urobilinogen. In one Nigerian center this was the most common type of anemic crisis in children with SCD. Although malarial parasites were detected in the blood of only 17.6%, 70% became afebrile after transfusion and antimalarials, suggesting a role for the parasite in the majority of cases.⁵⁸ Malaria is thus a significant pathogen in SCD, and long-term prophylaxis has been shown to lower the incidence of severe anemia and the number of hospital admissions and crises, as well as reducing mortality.⁵³

HIV and SCD

Little information is available regarding the impact of co-existent HIV infection and SCD. These two conditions no doubt can present a unique challenge particularly in Africa where the incidences of both conditions are highest and resources are scarce. In a recent retrospective study in the USA, hospital discharges of children with both HIV and SCD over a 10-year period (1994–2003) were analyzed.⁵⁹ Children with both conditions were at increased risk of bacterial infection and sepsis and also had a longer average stay in hospital. However, the risk of vaso-occlusive crisis was lower and the in-patient case–fatality rate was lower than that of children with HIV infection alone. Increased risk of pneumococcal infection has also been reported in HIV-infected adults with SCD.⁶⁰

Whether SCD attenuates the clinical progression of HIV disease is an interesting concept, given the reports of high frequency of long-term non-progressors among patients with HIV and SCD.⁶¹ However, there is no proven mechanistic explanation for this observation.

Prevention

As illustrated, infection can lead to a range of complications in SCD, and these are not readily reversed simply by treating the infection. For this reason, prevention is the key strategy in management. Interventions in the last 20 years have dramatically reduced mortality, especially in children, and the recommendations continue to evolve.

Simple general measures are important in reducing the risk of infection, though the aim is to ensure as normal a lifestyle as possible. Meticulous attention to hygiene, particularly hand-washing, is vital, and to protect against *Salmonella*, patients are advised to cook food thoroughly, particularly chicken and eggs, keep items refrigerated, and avoid contamination.³⁰ Nutritional supplementation with zinc has been reported to reduce infection risk,²⁷ improve

growth rates in SCD children, and possibly improve skeletal and sexual maturation as well as having psychological benefits.⁶² Early identification of infections is another key area, enabling prompt initiation of treatment to reduce complications. Parents are encouraged to monitor their children closely at home and seek advice if they have a fever or respiratory symptoms, while maintaining good hydration. There should be a low threshold for the use of antibiotics in ill children with SCD, particularly in the presence of chest signs or symptoms, which may herald ACS. A fever of more than 38.5 °C is an indication for the empirical use of broad-spectrum antibiotics such as a third-generation cephalosporin, with a macrolide added in potential ACS. Relevant specimens (blood, urine, sputum, etc.) should be taken for culture and antibiotics later modified or stopped depending on the results.²

Antibiotic prophylaxis

As previously discussed, pneumococcus is a major threat to SCD patients and in unscreened populations the first presentation of the disease may be with sudden death due to overwhelming sepsis. The first major breakthrough came in 1986 with the pivotal PROPS trial, which showed that prophylactic oral penicillin reduced the risk of IPD by 84% in children aged less than 3 years. They reported an incidence of 9.8/100 patient-years, cut to 1.2/100 with prophylaxis.⁶³ This cheap, simple and safe intervention (hypersensitivity reactions are rare) was rapidly implemented. Current recommendations state that oral penicillin V should be commenced at 3 months, as levels of protective fetal hemoglobin (which prevents sickling by inhibiting HbS polymerization) decline and splenic hypofunction begins to develop. A dose of 62.5 mg twice daily is used for infants less than 1 year, 125 mg twice daily for those aged 1–5 years, and 250 mg twice daily in those aged above 5 years.² The duration of penicillin prophylaxis is controversial. Since the risk of IPD declines markedly with age, it may be possible to modify or stop prophylaxis without compromising outcome. The PROPS-II trial (1995) evaluated the consequences of discontinuing penicillin at 5 years and found no significant difference in the incidence of infection between the penicillin and control groups, suggesting prophylaxis could safely be stopped.⁶⁴ However, the low overall risk meant the absolute number of infections was extremely small (two in the penicillin and four in the placebo group), so any statistically significant difference would be difficult to detect. Guidelines for asplenic individuals still recommend that penicillin prophylaxis be continued lifelong,⁶⁵ and this is widely applied to SCD, although emphasis is placed on treatment in the first 5 years.

Long-term penicillin prophylaxis is not without its problems. Prolonged or intermittent antibiotic use can promote the development of resistance, so the recommendation to continue must represent a balance between the risk to the individual of pneumococcal infection versus the danger resistant organisms pose to the whole population.⁶⁶ Blood culture isolates from the general population in 2001–2 suggested that 9% of *S. pneumoniae* in the UK had reduced susceptibility to penicillin.⁶⁷ However, in those on prophylaxis, the rates may be much higher. A survey of children under 5 with SCD in East London found 20% had asymptomatic nasal carriage of *S. pneumoniae* and 25% of these were resistant to penicillin.⁶⁸

Compliance is another major issue in any long-term therapy. One study of children in New York found patients' (or parents') own reported compliance with penicillin prophylaxis was 67.5%, but when measured objectively with a urine assay the figure was 43.1%. The subgroup of under 5 s, in whom prophylaxis is most important, had better adherence (61%).⁶⁹ Similar results (approximately 50% compliance) have been found in UK children.⁷⁰ There are a variety of reasons for poor compliance. Children and indeed parents often do not understand the reason for penicillin prophylaxis or the need to take it consistently, and people are generally reluctant to take any drug in the long-term, even one whose safety is well established. In a survey in the USA, the ease of obtaining refills and the availability of transport predicted compliance.⁷⁰ Although prescriptions are free for children aged less than 16 years in the UK, there is no waiver of the standard National Health Service (NHS) prescription charge for adults with SCD, despite their need for lifelong regular drugs—penicillin, folate supplements, and painkillers. Campaigners delivered a petition to the Government in April 2008 calling for universal free prescriptions in SCD, but this met with a negative response.⁷¹ Even appreciating all of these contributory factors, interventions to increase compliance have met with very limited success. Health professionals should reinforce the reasons for and importance of penicillin prophylaxis at every clinic visit or contact with the community sickle team and suitable written material should be supplied where available. Psychological input may be of benefit in adolescents. Although these would seem sensible recommendations, there is limited evidence for their efficacy. One trial involving a formal education program for parents with intensive home follow-up failed to increase rates of compliance.⁷²

Vaccination

The other key medical strategy in the prevention of infection is vaccination. There are some 90 serotypes of pneumococcal bacteria, which vary in the molecular composition of their capsule—a strategy for immune evasion. The first pneumococcal vaccine to be developed consisted of purified capsular polysaccharide antigens. These produce immunity via a T cell independent effect, triggering B cells in the splenic marginal zone to secrete antibody.¹⁶ Early uncontrolled studies with a vaccine containing 14 antigens suggested a 50% reduction in IPD¹⁷ and so these vaccines became widely accepted, in spite of a lack of thorough controlled trials in SCD. The current vaccine (Pneumovax, PPS-23) consists of 23 purified antigens, which should in theory protect against 75% of invasive and respiratory infections, with another 14% prevented via cross-protection.⁷³ In one series, the combination of PPS-23 and penicillin halved rates of IPD to around 36.5/1000 patient-years, though this still represents a 10-fold increased risk over the general population, with a 100-fold higher risk of mortality.⁷⁴ The effectiveness of the vaccine diminishes over time, with a booster required every 5 years. Unfortunately many polysaccharides, especially those from the strains causing most infections, are either not immunogenic in children under 2 years or produce only a minimal antibody response with a lack of immunological memory (so repeat exposure to antigen does

not produce a booster antibody response).⁷³ This has prompted the search for an alternative.

The polysaccharide–protein conjugate vaccine (PCV) complexes capsular polysaccharides to protein carriers (mutant non-toxic diphtheria toxin), increasing their immunogenicity. Antibodies are produced via a T cell dependent process, involving T and B cell interactions in germinal centers. This *does* induce an effective response in infants under 2 years, with an appropriate memory response after a booster dose.⁷³ Prevnar[®], a 7-valent PCV, was licensed for use in 2000 and includes serotypes responsible for around 70% of infections in Europe and the USA. Importantly, studies suggested this vaccine would also cover 77% of isolates with resistance to penicillin, which would avert prophylaxis failure and may help to reduce the frequency of such resistant strains.⁷⁴ Routine immunization of children with PCV-7 began in the USA in 2000 and in the UK in 2006. Multiple doses are required for the most effective response: injections are given at 2, 4, 6, and 12 months in the USA and 2, 4, and 13 months in the UK. This could potentially be a factor limiting full uptake of the vaccine. There were supply problems in the early years in the USA, but by 2005 coverage had risen to 82.8%.⁷⁵ Worryingly, uptake was lower in those of black race and low household income—precisely those groups likely to be affected by SCD. In the UK, uptake in the first year was 86%—still somewhat lower than that for other routine childhood immunizations.⁷⁶ Early analysis of the effect in SCD patients has shown very encouraging results. In Tennessee, rates of IPD fell by 90.8% in children under 2 years and 93.4% in children aged less than 5 years. This brought the incidence to only 6.5 times higher than in unaffected children.¹⁵ Another study reported an overall 68% reduction in IPD in children under 10, including a significant decrease among unvaccinated children, suggesting a beneficial effect of herd immunity.⁷⁷ While vaccination reduces nasopharyngeal carriage of pneumococci, there is concern that PCV-7 could promote replacement with non-vaccine types—altering the balance of disease-causing strains, or that new pathogenic strains could emerge in the other serotypes as a result of transformation and recombination of capsular gene loci. Careful epidemiological follow-up will be required to monitor such effects. Children with SCD should continue to receive the PPS-23 vaccine once over 2 years of age, because in contrast to normal children they remain at high risk from IPD, especially up to 5 years.⁷³ Penicillin prophylaxis is still required, in part because all trials have been conducted using it. It also provides protection against non-PCV-7 pneumococcal strains.

Other vaccines important in children with SCD are *Haemophilus influenzae* (Hib) and *N. meningitidis*, hepatitis B, and influenza. Influenza, as any severe infection, can precipitate crisis, but may also predispose to bacterial pneumonia. A summary of the recommended immunization program is represented in Table 2.^{2,78} In addition, children traveling to endemic areas should be offered meningitis A and C vaccination and malaria prophylaxis.

Conclusions

In this era of effective antibiotics and vaccines, there is a tendency to see infection as a minor and treatable problem. In SCD, although these measures have been a huge step

forward in preventive care, infection remains a major cause of morbidity and mortality in the developed and even more so in the developing world. Better understanding of the mechanisms behind the increased susceptibility to infection in these patients may in future enable interventions addressing the underlying cause. Early treatment with hydroxyurea or blood transfusions to lower HbS levels could help preserve splenic function. However, these therapies are themselves associated with considerable risks, side effects, and practical difficulties, making their routine use inappropriate. More detailed elucidation of the proposed complement activation and opsonization defect could allow this to be directly targeted. If specific genetic polymorphisms conferring an increased risk of infection are identified, testing could help stratify patients, directing more intensive interventions towards high-risk groups. Since these breakthroughs probably represent distant possibilities, the most important step at present is to improve compliance with current infection prevention strategies. In developing countries, particularly Africa, which is home to the vast majority of patients with SCD and records the highest mortality rates, there is a need for early diagnosis and improved, structured patient care programs. Globally, while measures can be taken to improve understanding and education and maximize convenience for patients, altering deeply ingrained beliefs and attitudes is a much more difficult task. The challenge is to prevent both major infections, which cause considerable pathology in themselves, and minor infections, which can trigger SCD-specific effects. Reducing the frequency of crises improves quality of life and may help to delay long-term complications, ultimately extending life expectancy.

Conflict of interest: No conflict of interest to declare.

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