Role of infections in haemolysis in sickle cell disease patients

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A review of the role of infections in the aetiology of haemolysis in patients with sickle cell diseases: pathogenesis, management, and prevention

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Abstract:

Background: Sickle cell disease (SCD) is associated with chronic haemolysis, immuno-suppression and suscepti-bility to infections, which may trigger infection-associated haemolysis (IAH). SCD patients are vulnerable to anaemic effect of IAH due to vicious interaction between pre-existing 'inherited' chronic haemolysis and 'acquired' IAH. IAH in SCD manifests as febrile haemolytic crisis with clinical and laboratory features of severe anaemia or pancytopenia. Clinico-pathological perspectives of IAH in SCD are fragmented. This review presents a comprehen-sive but concise overview of pathogenesis, management and prevention of IAH in SCD.

Methodology and results: Online literature search using search terms such as 'sickle cell disease, viral, bacterial, parasitic, fungal, infections, hyperhaemolytic crisis, haemophagocytic syndrome, severe anaemia, pancytopenia' in various combinations was done on PubMed/Medline, Google, Google-Scholar and Bing. Overall, 112 relevant publications were retrieved, which included 109 peer reviewed journal articles, 2 World Health Organization (WHO) technical reports, and 1 edited text book. A range of bacterial (*Bartonella* spp, *Mycoplasma* spp., *Mycobacterium avium* complex), viral (Dengue, SARS-CoV-2, Parvovirus-B19, Cytomegalovirus, Epstein-Barr virus), parasitic (*Plasmodium* spp., *Babesia* spp.), and fungal (*Histoplasma* spp.) infections were associated with IAH in SCD. There are two broad types of IAH in patients with SCD; infection associated extra-medullary haemolysis (IAEMH) and infection associated intra-medullary haemolysis (IAIMH). While IAEMH is associated with severe anaemia due to intravascular haemolysis caused by red cell invasion, oxidative injury, auto-antibodies, and/or pathogen-haem interaction, IAIMH is associated with haemophagocytic tri-lineage destruction of haematopoietic precursors in the bone marrow.

Conclusion: Various microbial pathogens have been associated with IAH in SCD. SCD patients with fever, severe anaemia or pancytopenia should be investigated for early diagnosis and prompt treatment of IAH, which is a life-threatening haematological emergency for which transfusion therapy alone may not suffice. Prompt and sustainable termination of IAH may require therapeutic combination of transfusion, anti-microbial chemotherapy, and immune modulation therapy. SCD patients should also receive counselling on hygiene, barrier protection against vectors, routine chemoprophylaxis for locally endemic diseases, and immunization for vaccine-preventable infections as a long-term preventive strategy against IAH.

Keywords: sickle cell disease; infection; hyperhaemolytic crisis; haemophagocytic syndrome

Une revue du rôle des infections dans l'étiologie de l'hémolyse chez les patients drépanocytaires: pathogenèse, prise en charge et prévention

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Résumé:

Contexte: La drépanocytose (SCD) est associée à une hémolyse chronique, à une immunosuppression et à une susceptibilité aux infections, ce qui peut déclencher une hémolyse associée à une infection (HIA). Les patients atteints de SCD sont vulnérables à l'effet anémique de l'HIA en raison de l'interaction vicieuse entre l'hémolyse chronique "héréditaire" préexistante et l'HIA "acquise". L'HIA dans la SCD se manifeste par une crise hémolytique fébrile avec des caractéristiques cliniques et de laboratoire d'anémie sévère ou de pancytopénie. Les perspectives clinico-pathologiques de l'HIA dans la SCD sont fragmentées. Cette revue présente un aperçu complet mais concis de la pathogenèse, de la gestion et de la prévention de l'HIA dans la drépanocytose.

Méthodologie et résultats: Une recherche documentaire en ligne à l'aide de termes de recherche tels que "drépanocytose, virale, bactérienne, parasitaire, fongique, infections, crise hyperhémolytique, syndrome hémophagocytaire, anémie sévère, pancytopénie" dans diverses combinaisons a été effectuée sur PubMed/Medline, Google, Google-Scholar et Bing. Au total, 112 publications pertinentes ont été récupérées, dont 109 articles de revues à comité de lecture, 2 rapports techniques de l'Organisation mondiale de la santé (OMS) et 1 manuel édité. Une gamme bactérienne (*Bartonella* spp, *Mycoplasma* spp., *Mycobacterium avium* complex), virale (Dengue, SARS-CoV-2, Parvovirus-B19, Cytomegalovirus, Epstein-Barr virus), parasitaire (*Plasmodium* spp., *Babesia* spp.), et les infections fongiques (*Histoplasma* spp) étaient associées à l'IAH dans la SCD. Il existe deux grands types d'HIA chez les patients atteints de SCD; hémolyse extra-médullaire associée à une infection (IAEMH) et hémolyse intra-médullaire associée à une infection (IAIMH). Alors que l'IAEMH est associée à une anémie sévère due à une hémolyse intravasculaire causée par l'invasion des globules rouges, une lésion oxydative, des auto-anticorps et/ou une interaction pathogène-hème, l'IAEMH est associée à la destruction tri-lignée hémophagocytaire des précurseurs hématopoïétiques dans la moelle osseuse.

Conclusion: Divers agents pathogènes microbiens ont été associés à l'IAH dans la SCD. Les patients atteints de SCD avec de la fièvre, une anémie sévère ou une pancytopénie doivent être examinés pour un diagnostic précoce et un traitement rapide de l'HIA, qui est une urgence hématologique potentiellement mortelle pour laquelle la thérapie transfusionnelle seule peut ne pas suffire. L'arrêt rapide et durable de l'HIA peut nécessiter une combinaison thérapeutique de transfusion, de chimiothérapie antimicrobienne et de thérapie de modulation immunitaire. Les patients atteints de drépanocytose devraient également recevoir des conseils sur l'hygiène, la barrière de protection contre les vecteurs, la chimioprophylaxie de routine pour les maladies endémiques locales et la vaccination contre les infections évitables par la vaccination en tant que stratégie préventive à long terme contre l'HIA.

Mots clés: drépanocytose; infection; crise hyperhémolytique; syndrome hémophagocytaire

Introduction:

Haemoglobin-S (HbS) is a variant of the normal HbA. HbS arose as a result of GAG> GTG base transition at codon-6 of the β-globin gene on chromosome-11, which corresponds to a substitution of glutamic acid (a polar amino acid) by valine (a neutral amino acid) in the sixth position of the β-globin chain (βGlu6Val) (1,2). As a result of this substitution, HbS has less anionic potential, slower electrophoretic mobility, and reduced deoxygenated solubility that leads to polymerization and red cell sickling (1,2).

The prevalence of sickle β-gene in tropical African countries is as high as 25% - 30% (3), because sickle cell trait (SCT) protects against severe malaria (3), and confers survival advantage through natural selection (4), balanced polymorphism (5), and immunological and biochemical protective mechanisms against malaria (6). There are at least five different sickle β-gene mutation haplotypes that vary in HbF levels and disease severity. The Arab-Asian and Senegal haplotypes are associated with relatively higher HbF levels and mild sickle cell disease (SCD), while the Benin, Bantu, and Cameroon haplotypes are associated with relatively lower HbF levels and severe SCD (7).

The red cells of individuals with SCT have the HbAS phenotype, thus containing both HbS (20-40%) and HbA (60-80%) (8). The relative preponderance of HbA in the red cells of SCT prevents sickling and undue haemolysis (8). Consequently, red cells of SCT have normal life span, and SCT carriers have normal life expectancy (9). HbS gene is thus genetically recessive, and SCT carriers are essentially asymptomatic except for the occasional occurrence of haematuria due to renal papillary necrosis (8), splenic infarction at high altitude (10) or bone pain upon exposure to certain haematopoietic growth factors (11).

Sickle cell disease arises from homozygous inheritance of HbS gene or double heterozygosity of HbS gene with another haemoglobinopathy gene such as HbSC, HbSD, HbSE, HbSO and HbSβthal (1). The clinical course of SCD is characterized by painless and stable periods of relative well-being referred to as the 'steady-state', which is intermittently interrupted by painful and unstable periods referred to

as 'crisis' (12). Painful vaso-occlusive crisis (VOC) due to bone necrosis is the commonest type of crisis in SCD, and it is clinically pathognomonic of SCD (12). Clinical transition from steady state to VOC results from excessive deoxygenation of HbS and red cell sickling, which is usually triggered by several factors that vary from physiological factors (e. g., menstruation) to pathological factors (e. g., infection) on the one hand, and from psychological factors (e. g., emotional stress) to physical factors (e. g., extreme weather conditions) on the other hand (12).

Red cell sickling is a pathognomonic feature of SCD. Red cells of patients with SCD go through repeated cycles of deoxygenation (in the tissues) and re-oxygenation (in the lungs) (13). This sequence of events creates a dynamic scenario of red cell sickling and un-sickling until the red cell membrane sustains a significant degree of damage, which eventually leads to the formation of irreversibly sickled cells that are invariably prematurely haemolysed (13). Consequently, the red cell life span in SCD is shortened to less than 20 days (14), which cannot be completely compensated even at the maximum rate of erythroid hyperplasia of the most active marrow (15). Chronic haemolysis is therefore the fundamental aetio-pathogenetic cause of anaemia in SCD patients in steady state (14).

However, apart from anaemia, haemolysis is also associated with other important and serious life-threatening consequences. This is because haemolysis has dual adverse effects on patients with SCD. First, haemolysis causes anaemia thus predisposing to transfusion with concomitant risks of iron overload, transfusion transmissible infections (TTIs), immune sensitization and reactions (16). Second, haemolysis increases the availability of cell-free Hb and haem, which support bacterial growth and sepsis (17), quenches vaso-modulatory effect of nitric oxide, and causes vasculopathy with multi-organ dysfunctions such as stroke, nephropathy, and pulmonary hypertension (18). Every patient with SCD maintains a certain degree of steady state haemolysis, which can be aggravated by infections.

Infections can cause haemolysis even in persons without SCD through several mechanisms, which include red cell invasion, oxidative damage to red cell membrane, production of haemolytic toxins (haemolysins), and/or production of red cell auto-antibodies, any or all of which can cause infection associated haemolysis (IAH) (19). However, SCD patients are particularly vulnerable to the anaemic effect of IAH due to the vicious interaction between the 'acquired' IAH and pre-existing 'inherited' SCD-associated haemolysis. There is therefore the need to understand the clinico-pathophysiologic perspectives of IAH in SCD patients because of their susceptibility to infections.

Patients with SCD are susceptible to infections for two important reasons. First, SCD patients are usually managed by recurrent or chronic blood transfusion, which predisposes to acquisition of various types of TTIs (16). Second, SCD is pathophysiologically associated with immunosuppression, which predisposes to the acquisition of any locally endemic infections (20). Therefore, SCD patients are at increased risk of acquiring infections, which could potentially lead to development of IAH. Several studies had focused on the cause-and-effect relationship between infections and VOC in SCD (21), but relatively less attention has been given to the relationship between infections and haemolysis in SCD. The risk of IAH would be especially high among SCD patients living in their native tropical African countries, which carry the heaviest dual burdens of infectious diseases (22) and SCD (23).

To the best of our knowledge from literature search, the clinico-pathological perspectives of IAH in SCD are fragmented, and have not been holistically or comprehensively appraised in the literature. Nonetheless, IAH in SCD is of triple clinical significance. First, IAH tends to be persistent or recurrent as long as the infection remains active and untreated, thereby increasing the frequency of hospital visits, which would lead to high rates of school absenteeism and poor intellectual development in children with SCD (24). Second, the persistent and/or recurrent nature of IAH invariably increases patient's transfusion requirement along with its wide range of undesirable adverse effect, which includes acquisition of TTIs that may potentially worsen IAH (16). Third, IAH cannot be effectively managed by transfusion alone. Optimal management of IAH requires detection of causative pathogens, followed by synchronized application of transfusion therapy, anti-infection chemotherapy and immune modulation therapy.

These three highlighted reasons underscore the need for SCD caregivers in general and in the tropics in particular, to have thorough understanding of the clinico-pathological perspectives of IAH in patients with SCD in order to ensure that IAH is quickly diagnosed and treated, and prevented in the future. Hence, the aim of this review is to present an updated and comprehensive but concise overview of the pathogenesis, management, and

prevention of IAH in patients with SCD as accrued from the literature.

Methodology and results:

Literature search was conducted on databases using the search terms; 'sickle cell disease, viral, bacterial, parasitic, fungal, infections, hyperhaemolytic crisis, haemophagocytic syndrome, severe anaemia, and pancytopenia' in various combinations on PubMed, Medline, Google, Google-Scholar, and Bing.

Overall, 112 relevant publications were retrieved, which included 109 peer reviewed journal articles, 2 World Health Organization (WHO) technical reports, and 1 edited text book. A range of bacterial, viral, parasitic, and fungal infections were associated with IAH in SCD. The pathogenesis, management and prevention of IAH in SCD vis-à-vis the haematological features and clinical manifestations of individual causative infections are outlined in Table 1.

Table 1: Pathogenesis, management, and prevention of infection-associated haemolysis in patients with SCD

Categories	Pathogens	Possible mechanisms for haemolysis	Haematologic manifestations	Management strategy (in addition to red cell transfusion)	Preventive and avoidance strategy
Protozoa	*Plasmodium* spp.	Red cell invasion with or without autoimmune haemolysis	Anaemia	Anti-malarial chemotherapy; Immune modulation if autoimmune haemolysis present	Protection against vectors; blood donor screening; vaccination
	Babesia spp.	Red cell invasion with or without autoimmune haemolysis	Anaemia	Anti-babesia chemotherapy; Immune modulation if autoimmune haemolysis present	Protection against vectors; blood donor screening
Bacteria	*Bartonella* spp.	Red cell invasion with or without autoimmune haemolysis	Anaemia	Anti-bartonella chemotherapy; Immune modulation if autoimmune haemolysis present	Protection against vectors; blood donor screening
	Mycoplasma spp.	Autoimmune haemolysis	Anaemia	Anti-mycoplasma chemotherapy; Immune modulation for autoimmune haemolysis	Personal and environmental sanitation
	Mycobacterium avium Complex (MAC)	Haemophagocytic Lympho Histiocytosis (HLH)	Pancytopenia	Anti-MAC chemotherapy; Immune modulation therapy for HLH	Personal and environmental sanitation
Viruses	Dengue virus	Inflammation oxidative red cell injury	Anaemia	No effective anti-viral therapy; symptomatic support; platelet transfusion if severe thrombocytopenia present	Protection against vectors; vaccination
	SARS-CoV-2	Virus-haem interaction; reduced red cell deformability with or without immune haemolysis	Anaemia	Anti-viral chemotherapy; Immune modulation if autoimmune haemolysis present	Hand sanitizers; Face masks; vaccination
	Parvovirus-B19	HLH	Pancytopenia	Anti-viral immunoglobulin therapy; Immune modulation therapy for HLH	Personal and environmental sanitation
	Cytomegalovirus	HLH	Pancytopenia	Anti-viral chemotherapy; immune modulation therapy for HLH	Personal and environmental sanitation
	Epstein Barr Virus	HLH	Pancytopenia	No effective anti-viral therapy; symptomatic support; Immune modulation therapy for HLH	Personal and environmental sanitation
Fungi	*Histoplasma* spp.	HLH	Pancytopenia	Anti-fungal chemotherapy; Immune modulation therapy for HLH	Personal and environmental sanitation

HLH = haemophagocytic lympho-histiocytosis; SARS-COV-2 = Severe acute respiratory syndrome-coronavirus-2

Discussion:

There are two broad types of IAH in patients with SCD; infection associated extra-medullary haemolysis (IAEMH), and infection associated intra-medullary haemolysis (IAIMH).

Infections associated extra-medullary haemolysis in SCD: the classical acute hyperhaemolytic crisis

SCD produces a chronic uncompen-sated hemolytic anaemia in the steady state. However, an acute-on-chronic hyperhaemolytic crisis can occur and cause precipitous drop in Hb concentration in patients with SCD. The classical hyperhaemolytic crisis is characterized by the occurrence of intravascular haemolysis at a rapid rate that significantly exceeds the usually tolerated slow rate of haemolysis seen in steady state. Hyperhaemolytic crisis is there-fore associated with life threatening exacer-bation of anaemia, which can be triggered by any of the following outlined infections.

Malaria and hyperhaemolysis in SCD

Malaria is endemic in tropical countries (25), where SCD is most prevalent (26). Five mosquito transmissible *Plasmodium* species: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*, have been associated with human infections, with the first two being the most important species (25). SCD patients in tropical countries are at double risk of acquiring mala-ria through mosquito bites and blood transfu-sions because a significant proportion of tropi-cal blood donors have asymptomatic malaria (27,28). Interestingly, a recent study demons-trated that in comparison to blood donors with SCT, donors with HbAA were associated with higher risk of asymptomatic malarial parasi-taemia, which implied that HbAA blood carries higher risk of transfusion transmitted malaria (TTM) (29,30). Therefore, patients who are selectively transfused with HbAA blood, such as SCD patients, could be at greater risks of acquiring TTM, and such patients need closer post transfusion monitoring for early detection and treatment of TTM (29,30).

The malaria parasites are both erythro-cytotropic and erythrocytopathic, hence the parasites invade and replicate within the pat-ients red cells during the erythrocytic phase of their life cycles (31). Moreover, malaria is sometimes associated with development of red cell autoantibodies (32). Malaria is thus capa-ble of causing red cell haemolysis as a result of a dual concert between direct red cell invasion and autoimmune mediated haemolysis (31,32).

Consequently, malaria is strongly associated with anaemia even in non-SCD patients (33). It is therefore conceivable that malaria is an important aetiological factor in the patho-genesis of acute hyperhaemolytic crisis and severe anaemia in patients with SCD (34,35). It is nonetheless possible to minimize the risk of malaria associated hyperhaemolysis by pro-viding prompt treatment for acute malaria and continuous lifelong anti-malarial chemoprophy-laxis in the standard of care for managing SCD in malaria endemic countries (36).

Long term protection can also be achie-ved by barrier protection against mosquito vectors at home, and serological screening and deferral of malaria infected prospective blood donors at donation centres (37). However, malaria vaccine remains the ultimate strategy for sustainable and cost-effective control mea-sure against malaria in the tropical countries. Unfortunately, the RTS,S/AS01 vaccine showed only modest efficacy in preventing symptoma-tic *P. falciparum* malaria (38). In essence, the RTS,S/AS01 vaccine alone would not be suffi-cient for global or regional malaria eradication, but it can nonetheless be considered as ano-ther addition to existing list of malaria control strategies, and should not be considered as an independent malaria prevention tool (38).

Accordingly, in 2021, the RTS,S/AS01 vaccine was endorsed by the WHO for use in children in conjunction with other malaria cont-rol strategies such as the use of insecticide treated nets and environmental vector control (39). Therefore, SCD patients living in *P. falci-parum* endemic countries should be encoura-ged to receive the RTS,S/AS01 vaccine. The vaccine is a non-live recombinant protein-based vaccine (39), hence it can be given to all SCD patients including those with pre-existing HIV infections.

Babesia infection and hyperhaemolysis in SCD

Babesiosis is a zoonotic tick-borne mal-aria-like febrile illness caused species of the intra-erythrocytic protozoan parasite called *Babesia* (40). Babesiosis is particularly com-mon in mid-western and north-eastern United States, but is also seen sporadically throughout the world in parts of Europe, Asia and Africa (40). The four identified *Babesia* species that cause infection in humans are *B. microti*, *B. divergens, B. duncani,* and *B. venatorum (40)*. However, the life cycle of all four species within humans remain essentially the same (40).

Babesia parasites are intracellular obli-gate parasites that target the red blood cells (40). Besides its natural route of transmission via the infected tick vector bites, the parasite is

also transmissible by transfusion via the red blood cells of infected donors (41). Immuno-compromised persons, especially those with splenectomy or hyposplenism are at increased risk of babesiosis (42). Like malaria parasites, *Babesia* parasites are both erythrocytotropic and erythrocytopathic, hence the parasites invade and replicate within the patients red cells (40). In similarity with malaria, babesiosis is associated with the development of red cell autoantibodies (43). Babesiosis is thus capable of causing red cell haemolysis as a result of dual effects of direct red cell invasion and auto-immune mediated haemolysis (40,43). Consequently, babesiosis is an important cause of morbidity and haemolytic anaemia even in non-SCD patients (40).

Patients with SCD living in areas that are endemic for babesiosis are at high risk of infection due to the effects of auto-splenectomy, immune suppression and recurrent transfusion (41,44). In accordance with expectations, babesiosis had been shown to aggravate haemolysis, cause hyperhaemolytic crisis and severe anaemia in patients with SCD (45). Nonetheless, it is possible to minimize the risk of babesiosis associated hyperhaemolysis by providing prompt diagnosis and standard anti-babesia chemotherapy for patients with SCD (40). Long term prevention strategy is achievable through personal barrier protection against tick vectors coupled with environmental vector control programs (40), while molecular donor screening methods for *Babesia* is currently being evaluated for detection and deferral of infected donors within endemic areas (46). Unlike malaria, an effective vaccine has not yet been developed against babesiosis, but there are potential candidate vaccines in the preclinical stages of development that will hopefully be available for clinical use in the near future (47).

Bartonella infection and hyperhaemolysis in SCD

Three zoonotic species of *Bartonella* (*B. henselae, B. quintana*, and *B. bacilliformis*) are known to be responsible for the vast majority of human infections (48). While the infection caused by *B. henselae* has a worldwide distribution, *B. quintana* and *B. bacilliformis* cases are more geographically restricted infection; *B. quintana* in Europe and USA, and *B. bacilliformis* in Peru, Ecuador, and Colombia (48). *Bartonella* spp are intracellular fastidious Gram-negative bacteria that cause a wide range febrile illnesses in both immuno-competent and immuno-suppressed persons, with the latter being more severely affected (48).

Bartonella spp are spread from animals to humans by fleas, lice, sand flies, or contact with flea infested animals, and have special tropism for endothelial cells and red cells (48).

The biological ability of *Bartonella* spp to invade human red cells is of double clinical significance. First, *Bartonella* spp can be transmitted from asymptomatic blood donors to blood recipients (49). Second, *Bartonella* spp can cause significant haemolysis in persons with symptomatic infections (50,51). Moreover, *Bartonella*-induced haemolysis is sometimes aggravated by development of red cell auto-antibodies (52). Therefore, in similarity with malaria and babesiosis, *Bartonella* infection can cause red cell haemolysis as a result of the combined effects of direct red cell invasion and autoimmune mediated haemolysis (50,51,52). Consequently, bartonellosis is also an important cause of morbidity and haemolytic anaemia even in non-SCD patients (50,51).

Patients with SCD living in areas that are endemic for bartonellosis are at high risk of infection due to the effects of auto-splenectomy, immunosuppression and recurrent transfusion (44,49). Because of these risk factors, several cases of bartonellosis have been reported in SCD patients in whom the infection often run severe course (due to SCD-associated immunosuppression), causing hyperhaemolytic crisis, aggravated anaemia, and eventually increasing the risk of blood transfusion (53-55). It is therefore important for clinicians to have high index of clinic suspicion and investigate all cases of fever in SCD patients living in areas endemic for bartonellosis, for early diagnosis (56), and initiation of appropriate antibiotic therapy (57), to avert the risk of hyperhaemolytic crisis.

Long term prevention strategy is achievable through the personal barrier protection against vectors coupled with environmental vector control programs (48), while serological screening and deferral of asymptomatic infected blood donors should be enshrined in the national transfusion services of endemic countries (56). An effective vaccine has not yet been developed against bartonellosis, but there are promising candidate vaccines that are in early stages of development (58).

Mycoplasma infection and hyperhaemolysis in SCD

Patients with SCD are usually immunocompromised and thus at risk of infection with atypical bacterial species (59). One of the most important atypical causative bacterial agents of respiratory tract infection and acute chest syndrome in patients with SCD is *Mycoplasma*

pneumoniae (60). *Mycoplasma* infection is often complicated by the development of complement fixing IgM anti-I cold reacting red cell auto-antibodies that can cause haemolysis in the colder peripheral parts of the body (61).

Moreover, in rare cases, *Mycoplasma* infection may also be associated with production of warm IgG anti-Rh red cell auto-antibodies that can cause haemolysis at core body temperature of 37°C (62). Therefore, SCD patients who develop *Mycoplasma* infection are at risk of developing hyperhaemolytic crisis if the infection is associated with red cell auto-antibodies (61,62). Thus, it is important to ensure that SCD patients with respiratory infection or acute chest syndrome due to *Mycoplasma* are promptly diagnosed and treated with macrolides or other effective antibiotics against atypical bacteria (63).

Furthermore, patients infected with *Mycoplasma* should be serologically screened for cold and warm haemolytic autoantibodies, especially if any features of hyperhaemolysis are present (61,62). While keeping the patients in warm environment would largely mitigate the effect of cold autoantibodies (61), administration of steroidal immune modulation therapy may be necessary to abolish the production of both warm and cold autoantibodies in order to down regulate and eventually terminate any associated hyperhaemolysis (62).

Dengue haemorrhagic fever and hyperhaemolysis in SCD

Dengue haemorrhagic fever (DHF) is a mosquito vector (*Aedes aegypti and albopictus*) borne disease caused by the Dengue virus, which belongs to the family Filoviridae and genus Flavivirus (64). Thrombocytopaenia and hypofibrinogenemia are consistent findings in DHF (65). Hypofibrinogenemia is due to plasma leakage into pleural and peritoneal cavities (65). However, the dominant haemostatic abnormality in DHF is thrombocytopaenia, which is due to the dual effects of myelosuppression and immune mediated platelet destruction (65). Consequently, the haemorrhagic manifestation of DHF range from positive tourniquet test, to spontaneous ecchymoses, epistaxis, gum bleeding and/or severe gastrointestinal haemorrhages (65).

In addition to haemorrhagic complications, DHF has also been associated with severe acute intravascular haemolysis. Cases of Coombs negative hemolytic anemia complicating DHF have been previously reported even in non-SCD patients (66-68). These cases suggest that the virus is capable of causing direct or inflammation-induced oxidative red cell injury.

Hence, acute intravascular haemolysis with haemoglobinuria and acute renal failure are recognized, albeit rare, complications of DHF even in non-SCD patients (68).

Because, patients with SCD are usually immuno-compromised, the WHO expert guidelines on Dengue fever has considered SCD to be a risk factor for development of severe and fatal DHF (69,70). Indeed, DHF has been reported to aggravate haemolysis in patients with SCD who are already battling with pre-existing inherited haemolytic anaemia (71-73). Although low platelet count is haematological feature of DHF, severe thrombocytopenia is not often seen in SCD patients with DHF (73). This is probably due to the fact that thrombocytosis is a common finding in patients with SCD in steady state (74). Hence, the high pre-infection steady state platelet count is thought to protect SCD patients from severe thrombocytopenia and bleeding during the course of DHF (73). However, SCD patients with DHF tend to present with severe hyperhaemolysis, which aggravates the pre-existing SCD-associated anaemia and often necessitates blood transfusion (73).

It is thus important that SCD patients in DHF endemic areas who present with triad of fever, thrombocytopenia and hyperhaemolysis should be promptly screened for the infection by both serological and antigen detection methods in order to plan for transfusion and other relevant supportive therapies as there is no specific anti-viral therapy at the moment (64). It is also paramount for DHF endemic countries to control the spread of the disease through environmental hygiene and vector control programs (64). Moreover, SCD caregivers should also counsel and encourage patients with SCD to use insecticides, bed nets and other barrier protection methods, and be vaccinated with the Dengue fever vaccine, Dengvaxia (64). Dengvaxia is a live-attenuated dengue vaccine, hence it cannot be given to HIV-infected persons, including those with SCD (64). The vaccine has been shown in clinical trials to be efficacious and safe in persons who have had a previous Dengue virus infection (64).

However, persons who experience their first natural Dengue infection after receiving Dengvaxia are paradoxically at an increased risk of developing severe DHF (64). For that reason, WHO recommends that only persons aged 9-45 years with evidence of a past Dengue virus infection should receive the vaccine (64). Accordingly, non-HIV-infected patients with SCD aged 9-45 years who live in areas endemic for DHF with a past history of

Dengue infection are eligible to receive Dengvaxia (64).

Coronavirus disease-2019 and hyperhaemolysis in SCD

Coronavirus disease 2019 (COVID-19) is a viral disease caused by the severe acute respiratory syndrome – coronavirus - 2 (SARS-CoV-2) (75). Clinical manifestations of severe COVID-19 include acute respiratory distress syndrome, systemic inflammation, sepsis, thrombosis, multi-organ failure, and death (75). Although SCD is basically an inherited chronic haemolytic anaemia, it shares certain patho-physiologic and clinical manifestations with COVID-19 such as anaemia, endothelial dysfunction, chronic inflammation, hypercoagulability, ischaemic stroke, pulmonary hypertension, and acute chest syndrome (76), all of which are potential risk factors for poor COVID-19 outcomes (77-80). The clinico-pathological similarities between the two diseases suggest that SCD patients may experience more severe COVID-19, however, there is conflicting evidence on whether patients with SCD actually experience more severe COVID-19 compared with patients without SCD (81).

A critical review of the literature on COVID-19, SCD and hypercoagulability revealed that while most studies had surprisingly reported mild to moderate COVID-19-related disease course in patients with SCD, literature review suggested that SCD was associated with increased risks of acute chest syndrome, hospitalisation and death from COVID-19 (81,82). Only little is known about the tendency of SARS-CoV-2 to cause haemolysis in infected patients. However, previous studies have suggested that SARS-CoV-2 cause haemolysis via at least three possible mechanisms. First, SARS-CoV-2 has been shown to trigger the production of both IgG and/or IgM red cell autoantibodies that can cause Coombs-positive intravascular hyperhaemolysis (83-85). Second, SARS-CoV-2-associated protein ORF8 is known to bind the porphyrin part of haemoglobin at the β1 chain, thereby causing haemolysis (86). Third, SARS-COV-2-associated inflammatory and oxidative stress significantly decreases red cell deformability (86); decreased red cell deformability is known to shorten red cell survival and is major determinant of haemolysis especially in patients with SCD (76).

The second and third mechanisms were thought to be responsible for haemolysis in a reported case of severe combined vaso-occlusive and Coombs-negative intravascular hyperhaemolytic crises in COVID-19 patient with SCD in whom the haemolysis was so severe that it necessitated urgent red blood cell exchange transfusion (87). Therefore, SCD patients with COVID-19 should have their red cells preemptively grouped and their sera saved for possible cross match in anticipation of exchange transfusion if and when they develop severe hyperhaemolytic crisis. However, as anti-viral pharmacologic therapy is becoming increasingly available for COVID-19 (88), it is possible to mitigate severe manifestations of COVID-19 in SCD patients, such as acute chest syndrome (81,82) and haemolysis (87), by prompt administration of anti-COVID-19 chemotherapy.

Because patients with SCD are immuno-suppressed, they should take preventive measures against acquiring COVID-19, which includes regular use of hand sanitizers, face masks and vaccination. However, vaccine hesitancy is a significant impediment among patients with SCD (89). The hesitancy is based on two inter-related pathophysiologic perspectives (89). First, SCD is inherently associated with thrombotic tendency (90). Second, COVID-19 vaccine is also associated with thrombotic side effects (91). Hence, SCD patients, quite logically, consider themselves to be at an increased risk of vaccine-induced immune thrombotic thrombocytopenia (VIITT) (89).

While some studies consider SCD patients to be at no greater risk of post-vaccination VIITT or VOC (89), the fear for VIITT is sustained by a few incoming case reports of SCD patients who experienced significant drop in platelets count, severe VOC, or even fatal TTP-like syndrome after taking the vaccine (92-94). However, some studies have suggested that adenoviral Covid-19 vaccines are more thrombogenic than m-RNA COVID-19 vaccines (91), which we believe should be safer for patients with SCD. It is therefore the responsibility of clinicians and vaccine providers to select a less thrombogenic vaccine and render closer post-vaccination monitoring for persons with underlying pro-thrombotic disorders such as the SCD (92-94).

Infections associated intra-medullary haemolysis in SCD: the haemophagocytic syndrome

Infection associated intra-medullary haemolysis (IAIMH) is solely caused by haemophagocytic lympho-histiocytosis (HLH), which manifests as haemophagocytic syndrome that is associated with excessive and uncontrolled tri-lineage phagocytic destruction of erythroid, myeloid, and megakaryocytic haematopoietic precursors in the bone marrow. HLH is a rare

but potentially life-threatening syndrome, cau-sed by a hyper-inflammatory response leading to multi-organ damage (95,96). Hence, HLH is characterized by fever, hepato-splenomegaly, hyper-ferritinaemia, hyper-triglyceridaemia, in-tra-medullary haemophagocytosis, and periph-eral pancytopenia, all of which form the diag-nostic frame work for HLH (97).

HLH can be primary (inherited) or secondary. Primary HLH is generally seen in infancy and is associated with mutations that affect cytotoxic T-cell or inflammasome rece-ptor functions (98,99). Secondary HLH is more common, and is often triggered by infections, haematologic malignancies, autoimmune disor-ders or drugs (95). The most common form of secondary HLH is infection-associated HLH. The spectrum of infectious triggers of HLH includes a wide range of bacteria, viruses, parasites, and fungi (100,101).

Due to their susceptibility to infections (20), SCD patients could be at increased risk of HLH. However, there may be some dilemma in diagnosing HLH in patients with SCD, which should be resolved by careful consideration of the diagnostic criteria for HLH vis-à-vis SCD (102,103). For example, while severe anaemia in SCD can be caused by hyperhaemolytic or aplastic crisis, such crises are usually not asso-ciated with pancytopenia, thus severe anaemia in conjunction with pancytopenia should raise suspicion of HLH in patients with SCD (102, 103).

However, the diagnostic strength of pancytopenia should always be weighed within the context of any concurrent hydroxyurea therapy, which might also potentially cause drug-induced pancytopenia (102,104). Another diagnostic criterion of HLH that requires careful consideration in SCD is hyper-ferritinaemia, which might as well be caused by chronic hyper-transfusion in patients with SCD (16, 102,103). Moreover, it should be noted that prominent enlargement of spleen or liver might as well be caused by sequestration crisis, which must be diligently ruled. In contradistinction to HLH, acute sequestration organomegaly is usually tender and the patient is typically in hypovolaemic shock (105). Therefore, the diag-nosis of HLH in SCD requires careful interpre-tation because of the clinico-pathological over-lap between the diagnostic criteria of HLH and clinical manifestations and/or side effects of treatment for SCD (102-105).

In spite of the fact that SCD is asso-ciated with high incidence of infection (20), the literature regarding HLH in patients with SCD is surprisingly sparse (102,106-111), which would suggest a paradoxically low incidence of HLH in

SCD. It is not clear whether this paradox is due to under-reporting or under-diagnosis of HLH in patients with SCD. While it has been reported that VOC (106) and blood transfusion (107) independently trigger HLH in patients with SCD, most of the remaining few cases of HLH were reported in SCD have been associated with infections due a myriad of pathogens such as unspecified periodontal bacteria (108), Epstein-Barr virus and Cytomegalovirus (109), Parvovirus-B19 (102), *Histoplasma* spp. (110), and atypical mycobacteria (111).

Once the diagnosis of HLH is made, treatment becomes urgent. The few reported cases of HLH in SCD in the literature were treated with a combination of antimicrobials, supportive transfusion, and/or immuno-modu-latory therapy with corticosteroids, immunoglo-bulins, etoposide or interleukin-1 receptor ant-agonists (103,108). Nonetheless, systemic cor-ticosteroids must always be used judiciously in patients with SCD because of the potential risk of steroid-induced VOC (112). Documented cases of HLH in patients with SCD underscores the importance of having a high index of sus-picion for HLH in patients with SCD who pre-sent with fever, pancytopenia and multi-organ dysfunction. Such patients should be promptly screened for underlying infections and bone marrow evidence of excessive haemophagocy-tosis in order to initiate life saving transfusion with concurrent anti-microbial chemotherapy and immune modulation therapy.

Since any infection is a potential trigger of HLH, the risk of HLH in SCD should be miti-gated by ensuring that SCD patients are opti-mally immunized against all locally prevalent 'vaccine-preventable' infectious diseases, while the observance of good personal and environ-mental hygiene in conjunction with routine chemoprophylaxis should be an important def-ense against infectious diseases for which vacc-ines are not currently available.

Conclusion:

Various bacterial, viral, parasitic, and fungal infections have been aetiologically asso-ciated with IAH in SCD, which may present as classical intravascular hyperhaemolytic crisis or intra-medullary haemophagocytic syndrome. SCD caregivers should investigate all patients with fever, severe anaemia or pancytopenia for early diagnosis and prompt treatment of IAH.

IAH in SCD is a life-threatening haema-tological emergency for which transfusion the-rapy alone may not suffice. Prompt and sustai-nable termination of IAH may require therap-eutic combination of transfusion, anti-microbial

chemotherapy, and immune modulation therapy. SCD caregivers should counsel patients on personal and environmental hygiene, barrier protection against disease spreading vectors, routine chemoprophylaxis for locally endemic diseases, and immunization for vaccine-preventable infections as a long-term preventive strategy against IAH.

Contributions of authors:

SGA was involved in conceptual design, discussion and appraisal of intellectual content; UAI is involved in the literature search, selection, harmonization, tabulation of results, and manuscript draft

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References:

1. Flint, J., Harding, R. M., Boyce, A. J., et al. The population genetics of the hemoglobinopathies. Bailliere's Clin Haematol. 1993; 6: 215 - 222. doi:10.1016/S0950-3536(05)80071-X.
2. Kaul, D. K., Fabry, M. E., and Nagel, R. I. The pathophysiology of vascular obstruction in the sickle cell syndromes. Blood Rev. 1996; 10: 29-44. doi:10.1016/S0268-960X(96)90018-1.
3. Fleming, A. F., Storey, J., Molineaux, L., et al. Abnormal haemoglobins in the Sudan savanna of Nigeria: I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. Ann Trop Med Parasitol. 1979; 73: 161-72. doi:10.1080/00034983.1979.11687243.
4. Elguero, E., Délicat-Loembet, L. M., Rougeron, V., et al. Malaria continues to select for sickle cell trait in Central Africa. Proc Natl Acad Sci USA. 2015; 112: 7051-7054. doi:10.1073/pnas.1505665112.
5. Olatunji, P. O. Malaria and the sickle gene: polymorphism balance in favour of eradication. Ann Health Res. 2018; 4: 88-96. doi: 10.30442/ahr.0402-1-12.
6. Gong, L., Parikh, S., Rosenthal, P. J., and Greenhouse, B. Biochemical and immunological mechanisms by which sickle cell trait protects against malaria. Malar J. 2013; 12: 317. doi:10.1186/1475-2875-12-317.
7. Loggetto, S. R. Sickle cell anemia: clinical diversity and beta S-globin haplotypes. Rev Bras Hematol Hemoter. 2013; 35: 155-157. doi:10.5581/1516-8484.20130048.
8. Ahmed, S. G., and Ibrahim, U. A. Haemoglobin-S in sickle cell trait with papillary necrosis. Br J Haematol. 2006; 135: 415-416. doi:10.1111/j.1365-2141.2006.06318.x
9. Barbedo, M. M. R., and McCurdy, P. R. Red cell life span in sickle cell trait. Acta Haematol. 1974; 15: 339-342. doi:10.1159/000208316..
10. Fernando, C., Mendis, S., Upasena, A. P., Costa, Y. J., Williams, H. S., and Moratuwagama D. Splenic syndrome in a young man at high altitude with undetected sickle cell trait. J Patient Exp. 2018; 5: 153-155.
11. Kasi, P. M., Patnaik, M. M., and Peethambaram, P. P. Safety of pegfilgrastim (neulasta) in patients with sickle cell trait/ anemia. Case Rep Hematol. 2013; 2013: 146938.
12. Ahmed, S. G., and Ibrahim, U. A. A compendium of pathophysiologic basis of etiologic risk factors for painful vaso-occlusive crisis in sickle cell disease. Niger J Basic Clin Sci. 2017; 14:57-77.
13. Goodman, S. R. The role of the membrane skeleton in formation of the irreversibly sickled cell: A review. Cell Mol Biol Lett. 1996; 1: 105-117.
14. McCurdy, P. R., and Sherman, A. S. Irreversibly sickled cells and red cell survival in sickle cell anemia: a study with both DF32P and 51CR. Am J Med. 1978; 64: 253-258. doi:10.1016/0002-9343(78)90053-0.
15. Hillman, R.S., and Finch, C. A. Erythropoiesis: Normal and abnormal. Semin Hematol. 1967; 4: 327-336.
16. Howard, J. The role of blood transfusion in sickle cell disease. IBTS Science Series. 2013; 8: 225-228.
17. Martins, R., Maier, J., Gorki, A. D., et al. Heme drives hemolysis-induced susceptibility to infection via disruption of phagocyte functions. Nat Immunol. 2016, 17, 1361-1372. doi: 10.1038/ni.3590.
18. Taylor, J. G., Nolan, V. G., Mendelsohn, L., Kato, G. J., and Gladwin, M. T. Chronic hyper-hemolysis in sickle cell anemia: Association of vascular complications and mortality with less frequent vaso-occlusive pain. PLoS One. 2008; 3: e2095. doi:10.1371/journal.pone.0002095.
19. Berkowitz, F. E. Hemolysis and infection: categories and mechanisms of their inter-relationship. Rev Infect Dis. 1991; 13: 1151-1162. doi:10.1093/clinids/13.6.1151.
20. Cannas, G., Merazga, S., and Virot, E. Sickle cell disease and infections in high- and low-income countries. Mediterr J Hematol Infect Dis. 2019; 11: e2019042. doi:10.4084/MJHID.2019.042.
21. Ahmed, S. G. The role of infection in the pathogenesis of vaso-occlusive crisis in patients with sickle cell disease. Mediterr J Hematol Infect Dis. 2011; 3: e2011028. doi: 10.4084/MJHID.2011.028.
22. Bhutta, Z. A., Sommerfeld, J., Lassi, Z. S., et al. Global burden, distribution, and interventions for infectious diseases of poverty. Infect Dis Poverty. 2014; 3: 21. doi:10.1186/2049-9957-3-21.
23. Wastnedge, E., Waters, D., Patel, S., et al. Global burden of sickle cell disease in children under five years of age: a systemic review and meta-analysis. J Glob Health. 2018; 8: 021103. doi:10.7189/jogh.08.021103.
24. Olatunya, O. S., Oke, O. J., Kuti, B. P., et al. Factors influencing the academic performance of children with sickle cell anaemia in Ekiti, south west Nigeria. J Trop Pediatr. 2018; 64: 67-74. doi: 10.1093/tropej/fmx034.
25. Sato, S. Plasmodium-a brief introduction to the parasites causing human malaria and their basic biology. J Physiol Anthropol. 2021; 40: 1. doi: 10.1186/s40101-020-00251-9.
26. Odame, I. Developing a global agenda for sickle cell disease: report of an international symposium and workshop in Cotonou, republic of Benin. Am J Prev Med. 2010; 38: S571-S575. doi: 10.1016/j.amepre.2009.12.021.
27. Ezeonu, C. M., Adabara, N. U., Garba, S. A., et al. The risk of transfusion transmitted malaria and the need for malaria screening of blood donors in

Abuja, Nigeria. Afr J Clin Exper Microbiol. 2019; 20: 195-201. doi:10.4314/ajcem.v20i3.4.

28. Ahmed, S. G., Ibrahim, U. A., and Ibrahim, G. Prevalence and clinical significance of malaria parasitemia in donor blood in Maiduguri, Nigeria. Niger J Parasitol. 2001; 22: 29-34. doi: 10.4314/njpar.v22i1.37755.

29. Kani, K. M., Ibrahim, Z., Habeeb, A., Ibrahim, U. A., and Ahmed, S. G. Haemoglobin phenotypes and the risk of asymptomatic malaria parasitemia among blood donors in northwest Nigeria: Clinical implications in the practice of tropical transfusion medicine. Afr J Clin Exper Microbiol. 2021; 22: 179-186. doi:10.4314/ajcem.v22i2.10.

30. Ahmed, S. G., and Ibrahim, U. A. Merits and demerits of sickle cell trait donor blood in tropical transfusion medicine: Are there any indications for specific use of blood donated by carriers of sickle cell trait? Afr Sanguine. 2021; 23: 49-59. doi: 10.4314/asan.v23i1.8.

31. Venugopal, K., Hentzschel, F., Valkiūnas, G., and Marti, M. Plasmodium asexual growth and sexual development in the haematopoietic niche of the host. Nat Rev Microbiol. 2020; 18: 177-189. doi: 10.1038/s41579-019-0306-2.

32. Chamnanchanunt, S., Thungthong, P., Kudsood, S., Somwong, W., and Hirunmasuwan, M. Autoimmune hemolytic anemia and autoantibodies in a patient with Plasmodium falciparum infection: report of a rare case and review of the literature. Asian Biomed. 2017; 11: 427-432. doi: 10.1515/abm-2018-0018.

33. Sumbele, I. U. N., Sama, S. O., Kimbi, H. K., and Taiwe, G. S. Malaria, moderate to severe anaemia, and malarial anaemia in children at presentation to hospital in the Mount Cameroon Area: A cross-sectional study. Anemia. 2016; Article ID 5725634. doi:10.1155/2016/5725634.

34. Montgomery, C. P., Hoehn, K. S., and Glikman, D. Hyperhemolytic crisis caused by severe P. falciparum malaria in a boy with sickle cell anemia. Crit Care Med. 2006; 34: A164. doi: 10.1097/00003246-200612002-00569.

35. Juwah, A.I., Nlemadim, E.U., and Kaine, W. Types of anaemic crises in paediatric patients with sickle cell anaemia seen in Enugu, Nigeria. Arch Dis Child. 2004; 89: 572-576. doi: 10.1136/adc.2003.037374.

36. Oniyangi, O., and Omari, A. A. Malaria chemoprophylaxis in sickle cell disease. Cochrane Database Syst Rev. 2019; 11. doi: 10.1002/14651858.CD3489.pub2.

37. Mangano, V. D., Perandin, F., Tiberti, N., et al. Risk of transfusion-transmitted malaria: evaluation of commercial ELISA kits for the detection of anti-Plasmodium antibodies in candidate blood donors. Malar J. 2019; 18: 17. doi:10.1186/s12936-019-2650-0.

38. Arora, N. C., Anbalagan, L. and Pannu, A. K. Towards eradication of malaria: Is the WHO's RTS,S/AS01 vaccination effective enough? Risk Manag Hlth Policy 2021; 14: 1033-1039. doi: 10.2147/RMHP.S219294.

39. Drysdale, C. and Kelleher, K. WHO recommends ground breaking malaria vaccine for children at risk. Geneva: WHO; 2021. https://www.who.int/news/item/06-10-2021-who-recommends-groundbreaking-malaria-vaccine-for-children-at-risk. (Accessed: 30 July 2022).

40. Ord, R. L., and Lobo, C. A. Human babesiosis: Pathogens, prevalence, diagnosis, and treatment. Curr Clin Micro Rpt. 2015; 2: 173-181. doi: 10.1007/s40588-015-0025-z

41. Fang, D. C., and McCullough, J. Transfusion-transmitted Babesia microti. Transfus Med Rev.

2016; 30: 132-138. doi: 10.1016/j.tmrv.2016.04.002.

42. Krause, P. J., Gewurz, B. E., Hill, D., et al. Persistent and relapsing babesiosis in immuno-compromised patients. Clin Infect Dis. 2008; 46: 370-376. doi:10.1086/525852.

43. Santos, M. A., Tierney, L. M. and Manesh, R. Babesiosis-associated warm autoimmune haemo-lytic anemia. J Gen Intern Med. 2020; 35: 928-929. doi:10.1007/s11606-019-05506-5.

44. Babadoko, A. A., Ibinaye, P.O., Hassan, A., et al. Autosplenectomy of sickle cell disease in Zaria, Nigeria: an ultrasonographic assessment. Oman Med J. 2012; 27: 121-123. doi: 10.5001/omj.2012.25.

45. Karkoska, K., Louie, J., Appiah-Kubi, A. O., et al. Transfusion-transmitted babesiosis leading to severe hemolysis in two patients with sickle cell anemia. Pediatr Blood Cancer. 2018; 65. doi: 10.1002/pbc.26734.

46. Tonnetti, L., Young, C., Kessler, D. A, et al. Transcription-mediated amplification blood dona-tion screening for Babesia. Transfusion. 2020; 60: 317-325.

47. Al-Nazal, H. A, Cooper, E., Ho, M. F., et al. Pre-clinical evaluation of a whole-parasite vaccine to control human babesiosis. Cell Host Microbe. 2021; 29: 894-903.e5. doi: 10.1016/j.chom.2021.04.008.

48. Raoult, D. Infections humaines à *Bartonella* [*Bartonella* infection in humans]. Presse Med. 1999; 28: 429-434.

49. Diniz, P. P., Velho, P. E., Pitassi, L. H., et al. Risk factors for Bartonella species infection in blood donors from southeast Brazil. PLoS Negl Trop Dis. 2016; 10: e0004509. doi: 10.1371/journal.pntd.0004509.

50. Hendrix, L. R. Contact-dependent hemolytic activity distinct from deforming activity of *Bartonella bacilliformis*. FEMS Microbiol Lett. 2000; 182: 119-124. doi:10.1111/j.1574-6968.2000.tb08884.x.

51. Orf, K., and Cunnington, A. J. Infection-related hemolysis and susceptibility to Gram-negative bacterial co-infection. Front Microbiol. 2015; 6: 666. doi:10.3389/fmicb.2015.00666.

52. Van Audenhove, A., Verhoef, G., Peetermans, W. E., Boogaerts, M., and Vandenberghe. P. Autoimmune haemolytic anaemia triggered by *Bartonella henselae* infection: a case report. Br J. Haematol. 2001; 115: 924-925. doi: 10.1046/j.1365-2141.2001.03165.x.

53. Velho, P. E., Ericson, M. E., Mair, D., and Gupta, K. Sickle cell disease and bartonella spp. Infection. Mediterr J Hematol Infect Dis. 2012; 4: e2012046. doi:10.4084/MJHID.2012.046.

54. Schaiblich, S. B., Moreira, S. A. T. M., Lacet, D. F. R., Cupolilo, S. M. N. and Grunewald, S. T. F. Cat scratch disease in a child with sickle cell anemia. Residência Pediátrica. 2016; 6: 145-148.

55. Soares, T. C. B., Isaias, G. A. B., Almeida, A. R., et al. Prevalence of *Bartonella* spp infection in patients with sickle cell disease. Vector Borne Zoonotic Dis. 2020; 20: 509-512. doi:10.1089/vbz.2019.2545.

56. Łysakowska, M. E., Brzezińska, O., Szybka. M., et al. The seroprevalence of Bartonella spp. in the blood of patients with musculoskeletal complaints and blood donors, Poland: a pilot study. Clin Rheumatol. 2019; 38: 2691-2698. doi: 10.1007/s10067-019-04591-5.

57. Prutsky, G., Domecq, J. P., Mori, L., et al. Treatment outcomes of human bartonellosis: a systematic review and meta-analysis. Int J Infect Dis. 2013; 17: e811-9. doi: 10.1016/j.ijid.2013.02.016.

58. Henriquez-Camacho, C., Ventosilla, P., Minnick, M. F., Ruiz, J., and Maguiña, C. Proteins of *Bartonella bacilliformis*: Candidates for vaccine development. Int J Pept. 2015; 2015: 702784. doi: 10.1155/2015/702784.

59. Ochocinski, D., Dalal, M., Black, L. V., et al. Life-threatening infectious complications in sickle cell disease: A concise narrative review. Front Pediatr. 2020; 8:38. doi: 10.3389/fped.2020.00038.

60. Neumayr, L., Lennette, E., Kelly, D., et al. Mycoplasma disease and acute chest syndrome in sickle cell disease. Pediatrics. 2003; 112: 87-95. doi:10.1542/peds.112.1.87.

61. Inaba, H., Geiger, T. L., Lasater, O.E., and Wang, W. C. A Case of hemoglobin SC disease with cold agglutinin induced hemolysis. Am J Hematol. 2005; 78: 37-40. doi:10.1002/ajh.20244.

62. Chew, W. H, Zainal Adlishah, Z. A., Fann, R.J., Mohamad, A. Z., Ong, T. C., and Jameel, A. *Mycoplasma pneumoniae* induced warm autoimmune hemolytic anemia – A rare case report. Ann Clin Case Rep. 2020; 5: 1870.

63. Biondi, E., McCulloh, R., Alverson, B., Klein, A., Dixon, A., and Ralston, S. Treatment of *Mycoplasma* pneumonia: a systematic review. Pediatrics. 2014; 133: 1081-1090. doi: 10.1542/peds.2013-3729.

64. World Health Organization. Dengue and Severe Dengue. WHO Publication. Geneva 2021. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue (Accessed: 30 July 2022).

65. Sellahewa, K. H. Pathogenesis of Dengue haemorrhagic fever and its impact on case management. ISRN Infect Dis. 2013; Article ID 571646. doi:10.5402/2013/571646.

66. Aye, M., Cabot, J., and William, L. W. K. Severe Dengue fever with haemolytic anaemia. A case study. Trop Med Infect Dis. 2016; 1: E6.

67. Medagoda, K., Gunathilaka, S. B., and De Silva, H. J. A case of self-limiting Coomb's negative haemolytic anaemia following dengue shock syndrome. Ceylon Med J. 2003; 48: 147-148.

68. Sellahewa, K. H., Kumaratne, M. P., Halpe, S., and Marapana, K. Case Report: A case of acute intravascular hemolysis in Dengue fever. Am J Trop Med Hyg. 2020; 102: 355-358. doi: 10.4269/ajtmh.19-0743.

69. Wilder-Smith, A., and Leong, W. Y. Risk of severe dengue is higher in patients with sickle cell disease: a scoping review. J Travel Med. 2019; 2019: 1-3. doi:10.1093/jtm/tay136.

70. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control. Geneva 2009. https://apps.who.int/iris/hadle/10665/44188. (Accessed: 30 July 2022).

71. Limonta, D., González, D., Capó, V., et al. Fatal severe dengue and cell death in sickle cell disease during the 2001-2002 Havana dengue epidemic. Int J Infect Dis. 2009; 13: 77-78.

72. Moesker, F. M., Muskiet, F. D., Koeijers, J. J., et al. Fatal dengue in patients with sickle cell disease or sickle cell anemia in Curaçao: two case reports. PLoS Negl Trop Dis. 2013; 7: e2203. doi: 10.1371/journal.pntd.0002203.

73. Mosnier, E., Demar, M., Bernit, E., et al. Dengue infection in sickle cell patients in French Guiana. J Virol Retrovirol. 2015; 2: 106.

74. Akinbami, A., Dosunmu, A., Adediran, A., Oshinaike, O., Adebola, P., and Arogundade, O. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. BMC Res

Notes. 2012; 5: 396. doi:10.1186/1756-0500-5-396.

75. Wang, D., Hu, B., Hu, C., et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020; 323: 1061−1069.

76. Tanabe, P., Spratling, R., Smith, D., Grissom, P., and Hulihan, M. C. E. Understanding the complications of sickle cell disease. Am J Nurs. 2019; 119: 26-35.

77. Parra-Bracamonte, G. M., Lopez-Villalobos, N., and Parra-Bracamonte, F. E. Clinical characteristics and risk factors for mortality of patients with COVID-19 in a large data set from Mexico. Ann Epidemiol. 2020; 52: 93-98.e2.

78. Tao, Z., Xu, J., Chen, W., et al. Anemia is associated with severe illness in COVID-19: a retrospective cohort study. J Med Virol. 2021; 93: 1478−1488.

79. Evans, P. C., Rainger, G. E., Mason, J. C., et al. Endothelial dysfunction in COVID-19: a position paper of the ESC working group for atherosclerosis and vascular biology, and the ESC council of basic cardiovascular science. Cardiovasc Res. 2020; 116: 2177-2184.

80. Kichloo, A., Dettloff, K., Aljadah, M., et al. COVID-19 and hypercoagulability: a review. Clin Appl Thromb Hemost. 2020; 26: 1076029620962853. doi:10.1177/1076029620962853

81. Hoogenboom, W. S., Alamuri, T. T., McMahon, D. M., et al. Clinical outcomes of COVID-19 in patients with sickle cell disease and sickle cell trait: A critical appraisal of the literature. Blood Rev. 2022; 53: 1-12. doi: 10.1016/j.blre.2021.100911.

82. Beerkens, F., John, M., Puliafito, B., Corbett, V., Edwards, C., and Tremblay, D. COVID-19 pneumonia as a cause of acute chest syndrome in an adult sickle cell patient. Am J Hematol. 2020; 95: E154-E156. doi:10.1002/ajh.25809.

83. Lazarian, G., Quinquenel, A., Bellal, M., et al. Autoimmune haemolytic anaemia associated with COVID-19 infection. Br J Haematol. 2020, 190: 29-31. doi:10.1111/bjh.16794

84. AbouYabis, A. N., and Bell, G. T. Hemolytic anemia complicating COVID-19 infection. J Hematol. 2021, 10: 221-227. doi:10.14740/jh906

85. Narula, S., Winkle, S., Brand, K., et al. Hyperhemolysis in the Setting of Mixed-Autoimmune Hemolytic Anemia: A rare complication of COVID-19. Cureus. 2021; 13: e20356. doi:10.7759/cureus.20356.

86. Al-kuraishy, H. M., Al-Gareeb, A. A., Onohuean, H., and Batiha, G. E. COVID-19 and erythrocrine function: The roller coaster and danger. Int J Immunopathol Pharmacol. 2022; 36: 1-7. doi: 10.1177/03946320221103151.

87. Okar, L., Rezek, M., Gmeil, A., Mulikandayhil, Y., and Yassin, M. A. Severe hemolysis and vaso-occlusive crisis due to COVID-19 infection in a sickle cell disease patient improved after red blood cell exchange. Clin Case Rep. 2021; 9: 2117−2121. doi:10.1002/ccr3.3960.

88. Sanders, J. M., Monogue, M. L., Jodlowski, T. Z., and Cutrell, J. B. Pharmacologic treatments for Coronavirus disease 2019 (COVID-19): A review. JAMA. 2020; 323: 1824-1836. doi:10.1001/jama.2020.6019.

89. Jan, H., Waheeb, A., Al-Ahwal, H., et al. COVID-19 Vaccine perception and hesitancy among patients with sickle cell disease in the western region of Saudi Arabia. Cureus. 2022; 14: e21026. doi: 10.7759/cureus.21026.

90. Faes, C., Sparkenbaugh, E. M., and Pawlinski, R. Hypercoagulable state in sickle cell disease. Clin

Hemorheol Microcirc. 2018; 68: 301 - 318. doi: 10.3233/CH-189013.

91. Warkentin, T. E., and Cuker, A. COVID-19: Vaccine-induced immune thrombotic thrombo-cytopenia (VITT). UpToDate. 2022. https://www.uptodate.com/contents/covid-19-vaccine-induced-immune-thrombocytopenia-vitt (Accessed: 2 August 2022).

92. Underdown, M. J., and Nuss, R. Thrombocytopenia in a teen with sickle cell disease following COVID-19 vaccination. Pediatr Blood Cancer. 2021; 68: e29271. doi:10.1002/pbc.29271.

93. Mungmunpuntipantip, R., and Wiwanitkit, V. Comment on: Thrombocytopenia in a teen with sickle cell disease following COVID-19 vaccination. Pediatr Blood Cancer. 2022; 69: e29303. doi: 10.1002/pbc.29303.

94. Alkindi, S., Elsadek, R.A., and Pathare, A.V. Safety warning for ChAdOx1 nCov-19 vaccine in patients with sickle cell disease. Mediterr J Hematol Infect Dis. 2021; 13: e2021059. doi: 10.4084/MJHID.2021.059.

95. Ramos-Casals, M., Brito-Zeron, P., Lopez-Guillermo, A., Khamashta, M. A., and Bosch, X. Adult haemophagocytic syndrome. Lancet. 2014; 383: 1503-1516.

96. Schram, A. M., and Berliner, N. How I treat hemophagocytic lymphohistiocytosis in the adult patient. Blood. 2015; 125: 2908-2914.

97. Janka, G. E., and Lehmberg, K. Hemophagocytic syndromes-an update. Blood Rev. 2014; 28: 135-142.

98. Pachlopnik Schmid, J., Cote, M., Menager, M. M., et al. Inherited defects in lymphocyte cytotoxic activity. Immunol Rev. 2010; 235: 10-23.

99. Canna, S. W., de Jesus, A. A., Gouni, S., et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet. 2014; 46: 1140-1146.

100. Maakaroun, N. R., Moanna, A., Jacob, J. T., et al. Viral infections associated with haemophagocytic syndrome. Rev Med Virol. 2010; 20: 93-105.

101. Rouphael, N. G., Talati, N. J., Vaughan, C., et al. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. 2007; 7: 814-822.

102. Sahu, S., Agrawal, A., and Das, P. The dilemma of diagnosing hemophagocytic lymphohistiocytosis in sickle cell disease. Cureus. 2020; 12: e12255. doi:10.7759/cureus.12255.

103. Henter, J. I., Horne, A. C., Aricó, M., et al.: HLH-2004 Diagnostic and therapeutic guidelines for haemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007, 48: 124-131. doi: 10.1002/pbc.21039.

104. Vichinsky, E. P. and Lubin, B. H. A cautionary note regarding hydroxyurea in sickle cell disease. Blood. 1994, 83: 1124-1128. doi: 10.1182/blood.V83.4.1124.bloodjournal8341124.

105. Siado, J. P., and Hernandez, J. L. Acute splenic sequestration crisis. In: Munshi A, editor. Inherited haemoglobin disorders. London: IntechOpen; 2015. doi:10.5772/60811.

106. Kio, E., Onitilo, A., Lazarchick, J., Hanna, M., Brunson, C., and Chaudhary, U. Sickle cell crisis associated with hemophagocytic lymphohistio-cytosis. Am J Hematol. 2004; 77: 229-232. doi:10.1002/ajh.0198.

107. Thung, I., and Broome, H. E. Hemophagocytosis in a patient with sickle cell disease. Blood. 2016; 127: 369. doi:10.1182/blood-2015-11-680082.

108. Shoman, W., El Chazli, Y., Elsharkawy, A., et al. Hemophagocytic lymphohistiocytosis in a child with sickle cell disease. Hematol Transfus Int J. 2018; 6: 180-182. doi:10.15406/htij.2018.06.00179.

109. Leiva, O., McMahon, L., Sloan. J. M., Lee, J., and Lerner, A. Recognition of hemophagocytic lymphohistiocytosis in sickle cell vaso-occlusive crises is a potentially lifesaving diagnosis. Haematologica. 2019; 104: e167. doi: 10.3324/haematol.2018.206458.

110. Kashif, M., Tariq, H., Ijaz, M., and Gomez-Marquez, J. Disseminated histoplasmosis and secondary haemophagocytic syndrome in a non-HIV patient. Case Reports Crit Care. 2015; 2015: 295735. doi:10.1155/2015/295735.

111. Chamsi-Pasha, M. A. R., Alraies, M. C., Alraiyes, A. H., and His, E. D. Mycobacterium avium complex-associated hemophagocytic lymphohistiocytosis in a sickle cell patient: an unusual fatal association. Case Rep Hematol. 2013, 2013: 291518. doi: 10.1155/2013/291518.

112. Darbari, D. S., Castro, O., Taylor, J. G., et al. Severe vaso-occlusive episodes associated with use of systemic corticosteroids in patients with sickle cell disease. J Natl Med Assoc. 2008; 100: 948-951. doi:10.1016/S0027-9684(15)31410-3.