The Roles of Acute and Chronic Marrow Dysfunctions in the Aetiology of Anaemia in Sickle Cell Disease: Pathogenesis and Management

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ABSTRACT

Background: Majority of the literature regarding the pathophysiology of anaemia in sickle cell disease (SCD) is focused on intravascular and extravascular red cell haemolysis with relatively little reference to reduced red cell production due to a myriad of marrow dysfunctions. **Objectives:** The aim of this overview is thus to present a comprehensive but concise narrative review of the aetiopathogenesis and management of anaemia due to acute and chronic marrow dysfunctions in patients with SCD. **Methodology:** Online literature search was conducted using search terms relevant to anaemia due to acute or chronic marrow dysfunctions in patients with SCD. Only articles that examined aetiopathogenesis and/or management of marrow dysfunction vis-à-vis anaemia in SCD were selected. **Results:** Literature search revealed that in addition to ineffective erythropoiesis, SCD patients also suffer from both benign and malignant forms of acute and chronic marrow dysfunctions. Marrow dysfunctions in SCD arise from separate or combined effects of marrow ischemia, deficiencies of micronutrients, renal insufficiency, infection of marrow precursors, haemophagocytic syndrome and myelosuppressive, dysplastic, and oncogenic effects of chemotherapy, gene therapy, and stem cell transplant. **Conclusion:** Marrow dysfunction is an important cause of anaemia in SCD. While transfusion support provides general short-term management, blood and marrow analyses are often necessary for accurate aetiological diagnosis. The Long-term and definitive management of marrow dysfunctions in SCD is determined by the aetiology, and it ranges from haematinics, antimicrobials, immune modulation, differentiation therapy, chemotherapy, dialysis, and/or erythropoietin supplements.

Key Words: Ineffective Erythropoiesis, Bone Ischemia, Micronutrients Deficiencies, Renal Insufficiency, Myelosuppression, Myelodysplasia, Leukaemia

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INTRODUCTION

Haemoglobin-S (HbS) is a β-globin chain genetic variant that evolved from haemoglobin-A (HbA) as a result of a point mutation, which caused GAG>GTG base transition at codon number-6 of the β-globin gene located on chromosome-11.[1,2] The GAG>GTG base transition resulted in the substitution of glutamic acid (a polar amino acid) by valine (a neutral amino acid) at position-6 of the β-globin chain (βGlu6Val).[1,2] Because 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] The prevalence is high because sickle cell trait (SCT) protects against severe malaria,[3] and confers survival advantage through natural selection,[4] balanced polymorphism,[5] as well as immunological and biochemical protective mechanisms against the infection.[6] There are at least five different sickle β-gene mutation haplotypes that vary in haemoglobin-F (HbF) levels and disease severity. The Arab-Asian and Senegal haplotypes are associated with relatively higher HbF levels and milder SCD, while the Benin, Bantu, and Cameroon haplotypes are associated with relatively lower HbF levels and severer 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 SCT red cells prevents sickling and undue haemolysis under physiological conditions.[8] Consequently, SCT red cells have normal life span, and SCT carriers have normal life expectancy.[9] HbS gene is thus genetically recessive, and SCT is essentially asymptomatic, and is not associated with significant organ damage, except for the occasional occurrence of renal papillary necrosis,[8] splenic infarction at high altitude,[10] or marrow necrosis and bone pain upon exposure to certain haematopoietic growth factors.[11] However, SCD which arises from the homozygous inheritance of HbS gene or double heterozygosity of HbS gene with another haemoglobinopathy gene (e.g., HbSC, HbSD, HbSE, HbSO, and HbSβthal)[1] is associated with significant morbidity, mortality, and reduced life expectancy.[12,13] The pathophysiology of SCD is dominated by red cell sickling, which is a pathognomonic feature of the disease.[12] Thus red cells of patients with SCD go through repeated cycles of deoxygenation (in the tissues) and re-oxygenation (in the lungs). This sequence of events creates a dynamic scenario of sickling and un-sickling until the red cell membrane incurs a significant degree of damage, which eventually leads to the formation and accumulation of irreversibly sickled cells that lead to recurrent microvascular obstruction and tissue infarcts. Thus, the clinical course of SCD is characterized by pain-free periods of relative well-being referred to as 'steady-state', which is intermittently interrupted by painful periods of vaso-occlusive tissue infarctions and necrosis referred to as 'vaso-occlusive crisis' (VOC).[12] The clinical transition from steady-state to VOC is caused by tissue necrosis resulting from polymerization of deoxygenation of HbS and red cell sickling, and is usually triggered by several factors that vary from physiological factors (e.g., menstruation) to pathological factors (e.g., infections) 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]

Moreover, irreversibly sickled cells are invariably and prematurely haemolysed because they are fragile and they also express high levels of membrane-associated senescent neo-antigens that trigger erythro-phagocytosis.[14] Consequently, the red cell life span in SCD is shortened to less than 20 days,[15] which cannot be adequately compensated even by maximum rate of erythroid hyperplasia and erythropoiesis of the normal bone marrow.[16] Thus every patient with SCD maintains a certain degree of clinically tolerable chronic steady state haemolysis and anaemia, which can be aggravated by chronic or acute hyperhaemolytic states due to various inherited and acquired haemolytic comorbidities

or triggers.[17]

Majority of the literature regarding the pathophysiology of anaemia in SCD is focused on haemolysis with relatively little reference to aetiological factors that often lead to reduced red cell production due to a myriad of bone marrow dysfunctions. Bone marrow dysfunctions often aggravate steady state haemolytic anaemia, increase transfusion requirement, reduce performance status, and decrease the well being of patients with SCD.[18] There is thus the need to understand the clinico-pathological roles of bone marrow dysfunction in the causation of anaemia in SCD. Hence, the aim of this overview is to present a comprehensive but concise narrative review of the aetiopathogenesis and management of anaemia due to acute and chronic bone marrow dysfunctions in patients with SCD.

METHODOLOGY

Literature Search and Selection

Literature search was conducted using search terms: 'sickle cell disease, anaemia, erythropoiesis, ineffective erythropoiesis, nephropathy, renal dysfunction, erythropoietin deficiency, bone marrow ischemia, nutritional, iron, folate, vitamin B12, deficiencies, marrow precursor infection, haemophagocytosis, hydroxyurea, drug induced myelosuppression, myelodysplasia, myelodysplastic syndrome, gene therapy, stem cell therapy, myeloid malignancies, leukaemia' in various combinations in Pub Med, Medline, Bing, Google Scholar, and other online search engines. Only articles that examined aetiology, pathogenesis, and/or management of acute and chronic bone marrow dysfunction in SCD were selected. Articles that concentrated on other aspects of SCD were excluded. Literature search was conducted by both authors and the final decision to include or exclude each paper was reached through consensus. The search was 'open', unrestricted by year or place of publication, and the year of selected publications ranged from 1941 to 2023. A total of 167 relevant publications were selected, which included 108 peer reviewed full articles, 55 peer reviewed case reports and case series, 2 clinical press releases, 1 edited expert consensus statement on care of patients with SCD, and 1 edited textbook as listed in the reference section.

RESULTS

The literature revealed that in addition to ineffective erythropoiesis, SCD patients also suffer from both benign and malignant forms of acute and chronic bone marrow dysfunctions due to separate or combined effects of marrow ischemia, deficiencies of micronutrients, renal insufficiency, infection of marrow precursors, haemophagocytic syndrome, drug induced myelosuppression and dysplasia, as well as chemotherapy, gene therapy, and stem cell transplant-related dysplastic and malignant marrow disorders. The pathogenesis and management of the aforementioned aetiologic factors of marrow dysfunction in patients with SCD are outlined in Table 1 and expatiated in the discussion section.

Table 1: Aetiology, Pathogenesis, and Management Of Anaemia Due To Marr ow Dysfunctions In Patients With SCD

Aetiology Of Anaemia	Pathogenesis Of Anaemia	Haematological Manifestations	Short-Term Management	Long-Term Management
Ineffective erythropoiesis (IE)	Intramedullary deaths of HbS-containing erythroblasts	Decreased RBC production; aggravates anaemia caused by haemolysis of circulating RBCs	Transfuse if necessary	Hydroxyurea (HU) reduces IE by increasing HbF in developing erythroblasts
Ischemic Marrow Dysfunction	Extensive bone marrow necrosis	Severe leucoerythroblastic anaemia or pancytopenia	Transfuse RBCs; platelets if thrombocytopenic; antibiotics if neutropenic	Antimicrobials for infection-triggered cases; continue transfusion support till full recovery
Nutritional Marrow Dysfunction	a). Megaloblastic erythropoiesis (folate or vitB12 def) b). Micronormoblastic erythropoiesis (iron)	a). Macrocytic anaemia b). Microcytic anaemia	Transfuse RBCs if anaemia is severe	Treat specific causes: e.g. anti-parasitic drugs; offer haematinics supplements
Infective Marrow Dysfunction	a). Aplastic crisis (parvovirus) b). Haemophagocytic syndrome (various infections)	a). Aggravated anaemia b).Severe pancytopenia	Transfuse RBCs; platelets if thrombocytopenic; antibiotics if neutropenic	Antimicrobial; immune modulation; continue transfusion till full recovery.
Hydroxyurea induced Myelosuppressive Marrow Dysfunction	Trilineage suppression of myelopoiesis	Anaemia or Pancytopenia	Transfuse RBCs; platelets if thrombocytopenic; antibiotics if neutropenic	Dose suspension or adjustment
Dysplastic & Malignant Marrow Dysfunction	Low or high folate; haematopoietic stress/ageing; clonal haematopoiesis; therapy-induced mutagenesis	Myelodysplastic syndrome and/or leukaemia: anaemia, neutropenia, thrombocytopenia	Transfuse RBCs; platelets if thrombocytopenic; antibiotics if neutropenic	Differentiation therapy; chemotherapy; stem cell therapy
Nephropathic Marrow Dysfunction	a). Marrow Hypocellularity b). Marrow Hyperadiposity	a). Anaemia b). Pancytopenia	Blood transfusion	Dialysis (to remove erythro-suppressive uremic toxins); Erythropoietin (to increase erythropoiesis); Anti-sclerostin antibodies (to reduce marrow adiposity)

DISCUSSION

There are at least seven pathophysiologically distinct categories of disorders that can cause acute or chronic bone marrow dysfunction in patients with SCD as described below.

1. Ineffective erythropoiesis

The hyperplastic and relatively hypoxic marrow environment in SCD is not favorable to the HbS-containing erythroblasts. Hence, HbS in developing erythroblasts of patients with SCD polymerizes and triggers intra-medullary apoptotic death of a significant proportion of erythroblasts starting at the polychromatic stage, a phenomenon that results in ineffective erythropoiesis.[18] However, the degree of ineffective erythropoiesis is lower in SCD patients with high HbF-producing haplotypes, hereditary persistence of HbF, and in patients on hydroxyurea, because their erythroblasts have higher levels of HbF,

which inhibits intra-cytoplasmic polymerization of HbS and confers intramedullary survival advantage upon the developing erythroblasts.[19]

2. Ischaemic bone marrow dysfunction

Recurrent, random, and widespread but discrete vaso-occlusive bone infarcts are pathognomonic of SCD.[2] Such discrete bone infarcts are responsible for the characteristic generalized bone pain crisis referred to as vaso-occlusive crisis (VOC), which is not associated with pancytopenia.[12] Occasionally, bone infarcts may be more widespread, confluent, and extensive, a situation referred to as extensive bone marrow necrosis (EBMN), which presents with both pain and pancytopenia.[20] The histological picture of EBMN is characterized by extensive necrosis of the haematopoietic tissue and medullary stroma in large areas of the bone marrow with preservation of the cortical bone.[20]

EBMN is a relatively rare clinical entity with multiple aetiologies.[20] Malignancy had been identified to be the cause of EBMN in about 90% of reported cases, while the remaining cases were attributable to non-malignant causes including haemoglobinopathies, infections, drugs, anorexia nervosa, hemolytic uremic syndrome, anti-phospholipid syndrome, and disseminated intravascular coagulopathy.[20] Sickle cell disease in particular was found to be the cause of EBMN in about 2-3% of reported cases.[20] In fact, the first case of EBMN was described in an autopsy of a patient with SCD as reported by Wade and Stevenson in 1941.[21] Thereafter, several case-reports and case-series of EBMN have been described in patients with SCD.[22-33]

The pathophysiology of EBMN is not well defined, but occlusion of the bone marrow microcirculation by sickled red cells is assumed to be the initiating factor in patients with SCD.[22-33] SCD patients with EBMN usually present with fever, bone pain, fatigue, and pancytopenia with leucoerythroblastic manifestations in the peripheral blood film.[22-33] Moreover, EBMN is associated with elevated serum levels of lactate dehydrogenase, alanine transferase, alkaline phosphatase, and uric acid, while bone marrow biopsy reveals extensive necrosis of the haematopoietic tissue and medullary stroma with preservation of the cortical bone.[22-33] The risk of EBMN in SCD is increased by comorbid infections as well as living on high altitude.[29,32,33] Because pancytopenia is an important haematological correlate of EBMN, SCD patients with EBMN who present with significant thrombocytopenia could clinically mimic thrombotic thrombocytopenic purpura,[32] hence the need to exercise high index of suspicion for accurate diagnosis of EBMN in SCD. A part from causing pancytopenia, EBMN increases the risk of marrow fat detachment and embolism in SCD, and the risk is paradoxically higher in milder types of SCD (non-HbSS) than in severe type of SCD (HbSS).[29] Thus, in addition to analgesia, the management of EBMN in SCD would require supportive transfusion of red cells and other relevant blood products for patients with pancytopenia; antimicrobial agents for cases associated with infections; and oxygen therapy, anticoagulation, and red cell exchange for cases complicated by marrow fat embolism, respiratory distress, and desaturation, which is clinically indistinguishable from acute chest syndrome.[22,23,24,25,26,27,28,29,30,31,3233]

3. Nutritional bone marrow dysfunction: micronutrient deficiencies

There are two categories of nutrient deficiency-induced marrow dysfunctions that can aggravate anaemia in SCD via megaloblastic and micronormoblastic erythropoiesis as explained below.

3a. Megaloblastic bone marrow dysfunction: megaloblastic crisis, folate, vitamin B12 deficiencies

The majority of SCD patients are born and resident in poor tropical African countries,[34] where high prevalence of nutritional insufficiencies (of fruits, vegetables, poultry, and dairy products) often cause

folate and/or vitamin B12 deficiencies.[35] Consequently, a previous study of bone marrow changes in African patients with SCD who presented with anaemic crisis demonstrated megaloblastic erythropoiesis in about 25% of the subjects, a finding that was suggestive of nutritional deficiency of folate or vitamin B12 deficiencies.[36] In similarity with other hereditary haemolytic anaemia, SCD is associated with accelerated erythropoiesis resulting in increased folate utilization and requirement, which (if not met) may cause anaemic crisis due to megaloblastic erythropoiesis (often referred to as megaloblastic crisis) as previously documented in SCD.[37] Folate deficiency leads to an increase in homocysteine level.[38,39] High plasma concentration of homocysteine is a well-established risk factor for cardiovascular diseases including stroke, venous thrombosis, and arteriosclerosis in persons with folate deficiency in general.[38,39] And more specifically, folate deficiency is undesirable in patients with SCD because it does not only aggravate anaemia,[37] but it can also delay epithelialization and healing of leg ulcers,[40] retard growth,[38] and increase the risk of VOC via homocysteine-induce endothelial injury.[39] Moreover, folate deficiency may lead to poor foetal growth and development in pregnant patients with SCD, hence folic-acid supplementation is particularly essential during the time of neural-tube closure, which corresponds to the first four weeks of gestation.[41] For the aforementioned reasons, regular supplementation of folic acid had since been enshrined in the standard of care for SCD even in developed countries.[41]

There is paucity of information regarding prevalence of folate deficiency vis-à-vis benefits or harm of regular folic acid supplements in patients with SCD.[42] However, it is obvious that the global risk of folate deficiency among patients with SCD is not uniformly distributed across global regional divides. For example, despite routine prescription of folic acid, patients with SCD in low resource settings, such as Nigeria in Africa, were reported to have high prevalence of folate deficiency affecting more than one third of patients,[43,44] a situation that was attributable to non-compliance with regular folic acid intake (due to ignorance or un-affordability); and in all such cases folate deficiency was significantly associated with lower haematocrit.[43,44] Hence, folate deficient patients with SCD in such settings should be counseled to adhere to prescribed folic acid supplements in order to avert the negative outcomes of folate deficiency. In contradistinction, patients with SCD living in high resource settings, such as Canada in north America (where multi-vitamin fortification of food items is common) were reported to have no deficiency of folate.[45] Obviously folic acid supplementation in such patients would tantamount to excessive and unnecessary administration of folic acid, which had been shown to cause undesirable accumulation of potentially harmful un-metabolized folic acid (UMFA).[45] It is therefore prudent that non-deficient patients with SCD should be exempted from folic acid supplements in order to avert potential negative outcomes of high levels of UMFA such as impaired immunity and high risk of cancers (more details on folic acid and carcinogenesis in subsequent sections).[45] Thus, folic acid supplementation in SCD should be individualized and be given to only deficient patients; even then, folate deficient patients on regular folic acid supplements should be periodically assessed for evidence of accumulation of UMFA, which should prompt precautionary dose reduction or suspension.

In addition to folate deficiency, previous studies had shown that vitamin B12 deficiency (due to pernicious anaemia or poor nutrition) is not uncommon in SCD especially among adults patients in whom it can aggravate steady state anaemia.[46-48] However, diagnosis of vitamin B12 deficiency in SCD requires high index of suspicion because it can be masked by concurrent folic acid supplementation, which would also adversely aggravate the neuropsychiatric manifestations of vitamin B12 deficiency.[46-48] For the aforementioned reason, vitamin B12 deficiency must be ruled out before administering folic acid to SCD patients with megaloblastic anaemic crisis.

3b. Micronormoblastic bone marrow dysfunction: iron deficiency

As earlier mentioned, the majority of SCD patients reside in poor tropical African countries,[34] where prevalence of nutritional insufficiency of animal protein and infective causes of iron deficiency (ID) are high.[35,49,50,51] Consequently, a previous study of bone marrow changes in African patients with SCD who presented with anaemic crisis demonstrated micronormoblastic erythropoiesis with reduced or absent stainable iron in a significant proportion of the subjects, a finding that was indicative of high prevalence of nutritional deficiency of iron among the studied cohort.[36] For the aforementioned reasons, the prevalence of ID among patients with SCD living in the tropics and low resource settings should expectedly be high. But because SCD is associated with recurrent or chronic transfusion, the prevalence of ID among patients with SCD even in low resource countries is generally lower than expected, affecting only a minor fraction (0-13.3%) of studied cohorts, while the larger fraction (>80%) of patients had either normal iron status or transfusion-associated iron overload.[52,53,54,55] Hence, there is a negative correlation between frequency of transfusion and ID as the overwhelming majority of iron deficient patients with SCD belonged to the un-transfused and sparingly transfused categories.[52,53,54,55] A part from frequency of blood transfusion, the prevalence of ID in SCD is also affected by regional, dietary, and gender-associated factors. For example, from regional and dietary perspectives, previous reports suggested that the prevalence of ID among patients with SCD was generally higher in India (4.5-67.7%),[56,57,58] a situation that was attributable to the region's vegetarian dietary culture. And from gender perspectives, female patients with SCD in the reproductive age group had particularly higher prevalence of ID (6.67-83.33%) as revealed by a systematic review of studies conducted among different international cohorts of pregnant SCD patients,[59] a finding that was consistent with the cumulative effect of menstrual blood loss and mother-to-foetus transfer of iron, especially among multiparous women.

Iron deficiency is associated with reduction in red cell indices including MCHC, which is a strong determinant of HbS polymerization, red cell sickling, haemolysis, and pain crisis.[60,61] Thus, experimentally induced iron deficiency was reported to have caused a remarkable decrease in the red cell sickling, haemolysis, and number of VOC in a limited number of SCD patients,[62] but the risk-benefit profile of induced iron deficiency in SCD has not been widely evaluated. Hence, ID should neither be induced nor ignored in patients with SCD since ID does not only aggravate anaemia, but it can also worsen immune dysfunctions,[63] impair cognitive ability,[64] and retard cutaneous wound healing,[65] which is particularly important for SCD patients among whom leg ulcers are not uncommon, especially among those with vasculopathy-prone hyperhaemolytic phenotypes.[66,67] Suffices to say that normal iron balance is critical in SCD with leg ulcers since both iron deficiency and overload can independently impair wound healing,[65] hence the need for judicious use of transfusion in managing SCD. Therefore, the standard of care for ID in SCD should be to offer iron supplementation with the aim of replenishing iron stores, which will simultaneously raise steady state haematocrit to pre-deficiency level, mitigate immune dysfunction,[63] improve cognitive ability,[64] and maintain cutaneous health and integrity.[65] Moreover, patients with SCD living in the tropics should be regularly screened and treated for endemic parasitic infections such as intestinal and urinary parasites that are strongly associated with ID.[50,51,68,69]

4. Infective bone marrow dysfunction

There are basically two types of infective **bone marrow dysfunction** in SCD, viz: aplastic and haemophagocytic dysfunctions as described below.

4a. Aplastic bone marrow dysfunction: parvovirus B19 and aplastic crisis

Parvovirus B19 (PVB19) is the only member of the Parvoviridae family currently known to be pathogenic

to human beings.[70] The virus is transmissible via respiratory droplets, and it belongs to the genus Erythrovirus due to its tropism for erythroid cells.[70,71] It is a DNA virus, whose receptor is abundantly present on erythroid cells of the human bone marrow.[70,71] Infected erythroid cells reveal pathological increase in cell size coupled with intense cytoplasmic basophilia, vacuolations, and blebbing, which is followed by cell death and acute arrest of erythropoiesis.[72]

Although PVB19 is often acquired as a respiratory infection, it may also be contracted as a transfusion transmissible infection because of its tropism for erythroid cells.[73] The viral incubation period in SCD patients varies from 9 to 17 days.[74,75] Post-infection prodromal symptoms include fever and body aches coupled with mild respiratory and gastrointestinal symptoms that may last for 6-8 days.[74,75] Aplastic crisis in SCD patients infected with PVB19 is characterized by decreased haematocrit and severe reticulocytopenia that follows constitutional prodromal symptoms.[74,75] Generally, leukocytes and platelets are not affected, but mild leucopenia, thrombocytopenia, atypical lymphocytes, and eosinophilia are occasionally seen in PVB19 infected patients.[74,75] The respiratory mode of transmission of PVB19 is responsible for the occasionally observed clustering of patients and epidemic pattern of aplastic crisis in patients with SCD.[76] Although aplastic crisis is the predominant cause of severe anaemia in PVB19 infected patients with SCD, it is noteworthy that PVB19 may occasionally aggravate the anaemia of aplastic crisis by causing concurrent hypersplenism (in young children within the pre-autosplenectomy age group)[77] and/or extensive myelonecrosis.[78] Moreover, the risk and severity of PVB19 infection in SCD is also affected by nutritional status. Previous studies have reported that under-nutrition is common in patients with SCD, and is due to low socio-economic situation with insufficient access to micronutrients, including vitamins.[79,80,81] The consequences of multiple vitamin deficiencies are undesirable, as vitamins enhance immune function, and low vitamin levels are associated with poor disease outcomes in the context of several infectious diseases including respiratory viral infections.[82] A recent study has described evidence for poor immune responses and disease outcomes in vitamins A and D deficient SCD children hospitalized with PVB19 aplastic crisis, which suggest that vitamin replete diets in children with SCD may serve as prophylaxis against PVB19 infection.[83] Moreover, vitamin replete diets would also reduce disease severity and complications of established PVB19 infection.[83]

Diagnosis of PVB19 in patients with aplastic crisis can be carried out by ELISA-based serological tests for anti-viral IgM, DNA detection by PCR, and/or electron microscopic visualization of the virus in tissues and blood.[74,75] The treatment of PVB19 aplastic crisis in SCD is essentially based on supportive red cell transfusions until the patients recovers from the infection.[74,75] However, in severely immuno-suppressed patients, random donor-derived intravenous immunoglobulin can be administered as a good source of PVB19 neutralizing antibodies, since most adults donors have already been exposed to the virus and have high levels of anti-PVB19 antibodies.[74,75]

4b. Haemophagocytic bone marrow dysfunction: infection-triggered haemophagocytic syndrome

Haemophagocytic lympho-histiocytosis (HLH), which manifests as haemophagocytic syndrome, is characterized by fever, hyper-inflammation, multi-organ dysfunction, hepato-splenomegaly, hyper-ferritinemia, hyper-triglyceridemia, excessive intra-medullary haemophagocytic destruction of erythroid, myeloid, and megakaryocytic haematopoietic precursors in the bone marrow, and life-threatening peripheral pancytopenia.[84,85] 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 receptor functions.[86,87] Secondary HLH is more common in older children and adults, and is often triggered by

infections, haematologic malignancies, autoimmune disorders or drugs.[88] The most common form of secondary HLH is infection-associated HLH.[89,90] The spectrum of infectious triggers of HLH includes a wide range of bacteria, viruses, parasites, and fungi,[89,90] all of which are not uncommon in patients with SCD because of its associated immunosuppression.[91]

However, even non-infectious causes such as VOC[92] and blood transfusion[93] were reported to have triggered HLH in patients with SCD, but the majority of cases of HLH that were reported among SCD patients in the literature were triggered by infections due a myriad of pathogens such as unspecified periodontal bacteria,[94] Epstein-Barr virus,[95] Cytomegalovirus,[95] Parvovirus B19,[96] Histoplasma species,[97] and atypical mycobacteria.[98] The afore-cited literature suggest that infections, VOC, and transfusion are the most important risk factors for HLH in SCD.[92-98] Once the diagnosis of HLH is made, treatment becomes urgent. Previously reported cases of HLH in SCD in the literature[92-98] were essentially managed with a variable combination of antimicrobials, supportive transfusion, immune modulation therapy with corticosteroids, immunoglobulins, etoposide, and/or interleukin-1 receptor antagonists in accordance with standard therapeutic guidelines.[85] Nonetheless, systemic corticosteroids must always be used judiciously in patients with SCD because of the potential risk of steroid-induced VOC.[99] Health care providers for patients with SCD should apply high index of suspicion for HLH in patients who present with fever, pancytopenia and/or multi-organ dysfunction. Such patients should be evaluated vis-à-vis standard diagnostic criteria for early diagnosis and prompt initiation of concurrent transfusion therapy, anti-microbial chemotherapy and immune modulation therapy.[85,100] Since any infection is a potential trigger of HLH, the risk of HLH in SCD should be mitigated by ensuring that SCD patients are optimally immunized against all locally prevalent 'vaccine-preventable' diseases, while the application of chemoprophylaxis in combination with good personal and environmental hygiene should be an important defense against infectious diseases for which vaccines are not currently available.

5. Hydroxyurea-induced bone marrow dysfunction: tri-lineage myelosuppression

Hydroxyurea is a ribonucleotide reductase inhibitor with a potent myelosuppressive action that makes it highly efficacious in cyto-reductive management of both leukaemic and non-leukaemic chronic myeloproliferative diseases.[101] In addition to its myelosuppressive effect, hydroxyurea enhances the production of HbF (a potent inhibitor of polymerization of HbS), which makes it a cornerstone drug in the management of SCD.[102] Despite its myelosuppressive properties, hydroxyurea has been found to be well tolerated in SCD patients among whom it mainly causes only mild to moderate reversible myelosuppression during therapeutic dosing,[102] and even after apparently massive accidental overdose.[103] However, it should be appreciated that there is marked variation in the ability of individual SCD patients to respond, metabolize, and tolerate hydroxyurea.[104] Consequently, some patients maybe constitutionally more susceptible to severe hydroxyurea-induced myelosuppression[104] as severe cases of myelosuppression had been reported in some SCD patients receiving hydroxyurea.[105] Therefore, hydroxyurea is a potential cause of severe bone marrow suppression, which calls for regular monitoring of haematological parameters during therapy.[105] In order to mitigate profound hydroxyurea induced bone marrow suppression, clinicians should titrate hydroxyurea doses to the maximum tolerated dose (MTD) in individual patients. This is because the MTD is a function of a number of variables, including drug pharmacokinetic factors and so varies from one patient to the other. Thus, any haematological features of significant marrow suppression such as neutropenia (<2.5xI0-9/L), thrombocytopenia (<100xI0-9/L) and/or worsening of steady state anaemia (fall in Hb concentration of ≥20%) should warrant intervention with blood transfusion,

suspension of therapy, or de-escalation of the therapeutic dose.[105] The risk of hydroxyurea-induced myelosuppression had been shown to be higher in SCD patients with renal impairment[106] among whom low GFR and creatinine clearance correlate with poor drug elimination.[107,108] In order to mitigate the risk and incidence of severe bone marrow suppression, SCD patients scheduled for hydroxyurea therapy should have mandatory pre-therapy renal function assessment so as to identify patients with renal impairment and make necessary dose adjustments.[107,108]

6. Dysplastic and malignant bone marrow dysfunctions: combined effects of clonal haematopoiesis, folate metabolism, chemotherapy, gene therapy, and stem cell transplant

SCD is characterised by red cell sickling, VOC, haemolysis, haematopoietic stress, tissue hypoxia, ischaemia-reperfusion, generation of free radicals, and systemic inflammation, all of which promote cellular injury with accelerated ageing of haematopoiesis.[109,110] The overall effect of the aforementioned events is a general increase in the risks of dysplastic and oncogenic mutations in patients with SCD.[109,110] Although the risks are relatively low in childhood, such risks increase as SCD patients live longer and attain adulthood.[109,110] Consequently, longer life expectancy and accelerated ageing of the haematopoiesis would increase the incidence of dysplastic and malignant disorders in SCD patients among whom the disorders also occur at relatively younger ages as compared with the general population.[109,110] SCD is thus intrinsically associated with increased risks of cancers in general, and haematological dysplasia and malignancies in particular.[109,110,111,112] It can thus be surmised that the factors that make SCD intrinsically associated with haematological malignancies and dysplasia include the separate and combined effects of SCD-related immune-modulation, chronic inflammation, haematopoietic stress, and haematopoietic ageing,.[109,110,111,112] all of which increase the chances of developing potentially malignant but latent somatic mutations and cytogenetic aberrations referred to as 'clonal haematopoiesis of indeterminate potential' (CHIP).[113] The risk of malignant transformation from CHIP to overt haematological malignancies in SCD is thought to be aggravated by additional dysplastic and/or carcino-leukaemogenic effects of long term hydroxyurea, busulphan conditioning, stem cell transplant, and gene therapy, as well as low or high folate status vis-à-vis folic acid supplementation as described below.

6a. Hydroxyurea therapy as potential cause of dysplastic and malignant marrow dysfunction

As earlier noted, hydroxyurea has a favorable safety profile in SCD.[114] However, the very long term adverse effect of hydroxyurea vis-à-vis its potential mutagenic and genotoxic effects[115] when administered to young patients and continued indefinitely remains to be determined. Several studies and reports have highlighted the occurrence of clonal cytogenetic abnormalities, myelodysplastic syndrome (MDS) and/or acute leukaemia in SCD patients treated with hydroxyurea.[116,116,117,118]

6b. Stem cell transplant and gene therapy as potential causes of dysplastic and malignant marrow dysfunctions

The largest lentiviral vector (LV)-mediated β-globin replacement gene therapy trial in SCD was temporarily suspended (February to December 2021) due to a report of unexpected occurrence of MDS and acute myeloid leukemia (AML) in two patients.[119,120] One of the two patients developed post-treatment MDS, which eventually transformed to AML. However, the absence of LV and the presence of complex cytogenetic abnormalities and driver gene mutations within the blasts suggested that the AML arose from busulphan conditioning and was unrelated to the LV.[119,121] The other patient developed post-treatment AML, in which the blasts demonstrated the presence of LV, raising the possibility of insertional mutagenesis.[119,120,122] However, subsequent and

detailed analysis showed that LV integration was unlikely to have played a role in the development of the AML.[119,122,123] Although the LV insertional mutagenesis was exonerated in these two index cases of post-gene therapy AML, there is still urgent need to further investigate if the occurrence of post-treatment MDS and AML were related to other potential aetiologic factors such as the separate or combined effects of insertional mutagenesis, and/or transplant procedures such as busulphan conditioning.[124] Cases of acute leukaemia had also been reported after haematopoietic stem cell transplant in SCD,[125-127] which could have been caused by procedures such as pre-transplant chemotherapy and conditioning, persistence of host cells with radiation-induced mutations, and/or activation of pre-existing oncogenic mutations in residual host cells.[125-127]

6c. Low and high Folate levels vis-à-vis risk of cancer: how safe is routine and empirical folic acid supplementation in SCD?

Folic acid has traditionally been a regular component of the management protocol for SCD in many clinical settings with the aim of off-setting haemolysis-induced folate deficiency.[41-44] Although modest folic acid supplements have been associated with protection against cancer,[128] the potentially adverse roles of both 'low' and 'high' folate/folic acid levels in carcinogenesis calls for caution in its use among SCD patients who are known to be intrinsically associated with increased risk of cancer.[111] For example, low folate/folic acid levels, which is not uncommon in SCD patients in low resource settings,[43,44] has been associated with increased risk of cancer, including leukaemia.[129,130] Leukaemogenic effect of low folate/folic acid is thought to arise from uracil mis-incorporation into DNA, ineffective DNA synthesis, inefficient DNA repair, dys-chromosomogenesis, genetic hypo-methylation, activation of proto-oncogenes, and/or deactivation of tumor suppressor genes.[131,132] Despite the fact that folate/folic acid are water soluble vitamins that are easily excreted in urine, persistent and unwarranted supplementation in replete subjects had been shown to cause undesirably higher than normal blood levels even among patients with chronic haemolytic disorders as previously observed in among SCD patients in high resource settings.[45] High folate/folic acid levels are unsafe and have been associated with increased risk of cancer.[128] High levels of folate/folic acid are carcinogenic because they surreptitiously support the rapid growth of pre-cancerous and cancerous cells that are known to express higher number of folate receptors than normal 'benign' cells.[133] It is thus possible that both low and high folate/folic acid levels can increase the risk of leukaemia and other cancers in patients with SCD. However, the extent to which low and high folate/folic acid levels contribute to the development of leukaemia and other cancers in SCD is currently unknown. Nonetheless, it is important to ensure that only folate deficient SCD patients are given folic acid (in order to avoid low folate-associated cancer/leukaemia risk), and that folate replete patients should not be given folic acid supplements (in order to avoid high folate-associated cancer/leukaemia risk). There is thus the need to re-evaluate the safety of generalized, routine, and empirical folic acid supplementation in SCD management protocols. The need for folic acid supplementation should ideally be determined and guided by folate status of individual patients as earlier mentioned.

6d. Similarities between presenting features of dysplastic/malignant marrow diseases and SCD: negative and positive effects of some anti-dysplastic and anti-cancer drugs on VOC

In similarity with non-SCD persons, SCD patients with marrow dysplasia and/or malignancy often present with bone pain (which mimics VOC), aggravated anaemia (which mimics haemolytic crisis), and changes in leucocyte and platelet count (which mimics infection and sepsis). Furthermore, it should be appreciated that SCD patients with severe vaso-occlusive events may present with any combination of the aforementioned clinico-haematological features. However, persistence of unusually severe anaemia with transfusion

dependence, immature leucocytosis, dysplastic neutrophil changes, and/or thrombocytopenia in the absence of VOC, nephropathy, or sepsis is more likely to suggest marrow malignancy or myelodysplasia. In such cases, close clinical observation is necessary to determine the need or otherwise to conduct bone marrow aspiration biopsy for detecting quantitative, morphological, and genetic abnormalities of the haematopoietic cells in order to diagnose any comorbid leukaemia or myelodysplastic syndrome as previously reported among patients with SCD.[111,116,117,121,122,125,127] Once marrow dysplasia and/or malignancy are diagnosed in SCD with or without exposure to hydroxyurea, stem cell or gene therapy procedures, the first rational approach is to stop any offending drug and initiate transfusion and other supportive measures, and subsequently consider delivering more specific and potentially curative therapy. For example, hydroxyurea-associated MDS in SCD was reported to have been successfully treated with 5-azacytidine, which is well known for inducing DNA de-methylation,[134] cellular differentiation,[134] and HbF production.[135] 5-azacytidine cured the MDS by inducing cellular differentiation and production, and reduced the frequency of VOC by enhancing HbF production in the treated patient.[136] The dual anti-dysplastic and anti-VOC effects of 5-azacytidine makes it a very suitable and convenient drug for treating MDS in patients with SCD.[136]

A part from MDS, patients with SCD are also vulnerable to leukaemia and other malignant diseases of the marrow. However, AML is the predominant bone marrow malignancy reported in SCD with and without exposure to hydroxyurea, stem cell or gene therapy procedures.[112,116-118,122,123,125,137-139] Nonetheless, other less common marrow malignancies have also been reported in patients with SCD; these include chronic myeloid leukaemia,[140] chronic lymphoid leukaemia,[141] adult T-cell lymphoma leukaemia,[142] multiple myeloma,[143] and juvenile myelofibrosis.[144] It is therefore essential to closely observe SCD patients, especially those on hydroxyurea and those who had received stem cell transplant or gene therapy for any clinical and haematological features of AML and other malignancies in order to ensure early diagnosis and prompt chemotherapy. It is noteworthy that certain drugs that are commonly used in the management of MDS, leukaemias, or other malignant marrow disorders may have negative or positive effects on the frequency of VOC in patients with SCD. For example, steroid (such as prednisolone) and growth factors (such as G-CSF) must be used judiciously as they may trigger VOC,[11,145] which is undesirable in patients with SCD. Conversely, drugs such as Imatinib and Pomalidomide may enhance HbF levels, mitigate sickling, reduce haemolysis, improve anaemia, and prevent VOC,[146,147] all of which are desirable for patients with SCD. Nonetheless, during Pomalidomide therapy, thrombo-prophylaxis must be administered to counteract its thrombotic side effects,[148] which would otherwise aggravate the pre-existing risk of thrombo-embolism that is known to be inherently associated with SCD.[149]

7. Nephropathic marrow hypocellularity and hyperadiposity: what is the role of sickle cell nephropathy (SCN)?

Irrespective of etiology, advanced chronic kidney disease (CKD) has been shown to consistently cause marrow hypocellularity[150] and hyperadiposity.[151] CKD due SCN is a common problem occurring in about 40% in patients with SCD.[152] Thus CKD-associated marrow hypocellularity and hyperadiposity would pathophysiologically undermine red cell production and worsen steady state anaemia in SCD patients with SCN as explained below.

7a. Nephropathic marrow hypocellularity

The combined effects of erythropoietin deficiency and uremic toxins significantly contribute to erythroid hypoplasia and bone marrow hypocellularity in patients with CKD.[150] Accumulation of uremic toxins have deleterious effects on bone marrow cells[153] as shown by significant inhibition of erythropoiesis by uremic sera in an in-vitro study.[154] More specifically,

indoxyl sulfate, one of soluble uremic toxins in CKD, is known to impair erythropoiesis via a hypoxia-induced factor pathway.[155] Other uremic toxins such as the polyamines may also interfere with erythropoiesis and reduce proliferation and maturation of erythroid precursor cells.[156] CKD due to SCN is a common problem in SCD,[152] and it would certainly be associated with suppression of erythroid proliferation in the bone marrow.[150,151,152,153,154,155] However, the aforementioned hypoproliferative marrow abnormalities in patients with SCN can be mitigated by using erythropoietin (to stimulate erythroid hyperplasia) on the one hand,[157] and by using haemodialysis or haemofiltration (to remove erythro-suppressive uremic toxins) on the other hand.[158]

7b. Nephropathic marrow hyperadiposity

The bone marrow niche is a dynamic and complex microenvironment that can both 'regulate' and 'be regulated by' the bone matrix.[159] Within the bone marrow, mesenchymal stromal cell precursors reside in a multi-potent state and retain the capacity to differentiate down osteoblastic, adipogenic or chondrogenic lineages in response to numerous biochemical signals.[159] Thus, a part from erythropoietin, other biochemical agents have significant effect on the bone marrow. For example, sclerostin is an osteocyte-derived signaling inhibitor molecule that acts as a signal protein that ensures molecular communication between cortical bone matrix and marrow adipose tissue.[160] Marrow adipocytes and cortical osteoblasts are derived from the same skeletal stem cell whose preferential differentiation toward the adipocyte lineage may occur under the influence of sclerosin, which would also decrease osteoblastic bone formation and increase osteolysis.[159] Moreover, sclerostin circulates at higher levels in patients with CKD among whom it is associated with excess marrow adipose tissue formation,[161] greater cortical lysis,[162] and higher bone reabsorption,[162] all of which culminate in the development of CKD osteo-dystrophy even in non-SCD patients with CKD.[162] Despite the fact that SCN and its associated cortical osteo-dystrophy are common in SCD,[152,163] there are virtually no studies on the pathophysiologic role of sclerosin on marrow hyperadiposity in patients with SCN. Hence, the extent to which SCD-associated marrow hyperadiposity contributes to anaemia in SCD patients with SCN is currently unknown. There is therefore the need to study marrow adiposity in patients with SCN, and explore the possible benefits of monoclonal anti-sclerosin antibodies (ASA), which may produce two benefits. First, ASA would predictively improve anaemia of SCN by mitigating excess marrow adipose tissue.[160] Second, because the anti bone reabsorption effect of ASA has been proven among non-SCD patients with osteoporosis,[164-166] ASA would predictively inhibit excessive cortical bone lysis, decrease bone reabsorption, and reduce the high risk of fractures commonly seen in patients with SCD in general,[167] and especially among those with SCN in particular.[163]

CONCLUSION

In addition to ineffective erythropoiesis, SCD patients also suffer from both benign and malignant forms of acute and chronic marrow dysfunctions due to separate or combined effects of marrow ischemia, deficiencies of micronutrients, renal insufficiency, infection of marrow precursors, haemophagocytic syndrome, drug induced myelosuppression and dysplasia, as well as chemotherapy, gene therapy, and stem cell transplant-related dysplastic and malignant marrow diseases. Marrow dysfunction is thus an important cause of anaemia in SCD. While transfusion support provides general short-term management, blood and marrow analyses are often necessary for accurate diagnosis and definitive long-term management.

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