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Hemolytic anemia results when red blood cells (RBCs) are destroyed prematurely by a number of agents. Obligate intracellular parasites like the Plasmodium species proliferate by infecting RBCs, growing through different stages of their life cycles, expanding their population to unsustainable numbers and eventually rupturing the cell membranes in order to transmit and infect new RBCs. In this manner, more RBCs are infected by the parasites and destroyed together with some nonparasitized cells. Membranes of RBCs are altered and deformed by parasite antigens expressed on the surfaces of both parasitized and nonparasitized cells, which lead to their premature phagocytosis and destruction by the reticuloendothelial system. Parasites and the hemoglobin waste products produced by them are released when the RBCs burst. Activated leukocytes take up the hemoglobin waste (hemozoin which is a polymerized heme), which stimulates the innate immune system leading to the synthesis and secretion of pro- and anti-inflammatory cytokines, chemokines, growth factors and mediators. Together with the destruction of RBCs in malaria, imbalance between pro- and anti-inflammatory events results in the modification of erythroid cell proliferation leading to severe malarial anemia (SMA) and other pathophysiology of malaria. While current malarial management is targeted at the destruction of the parasite, it is the malaria-related pathophysiology (disease aspect of malaria) like severe malarial anemia that results in the high malaria morbidity and mortality. Antidisease approaches promise to be more effective at malarial management. Triterpenes with anti-oxidant, pro-oxidant, anti-inflammatory and antiparasitic effect show effects at retarding and abrogating severe malarial anemia. Asiatic acid, amongst other triterpenes like oleanolic acid, masilinic acid administered through oral or transdermal route improves severe malaria anaemia providing promise in the management of malaria pathophysiology.

Keywords: malaria, severe malarial anemia, Plasmodium falciparum, pro-inflammation, anti-inflammatory, antidisease, cytokines, chemokines, growth factors, rhoptry protein ring surface protein 2, tumor necrosis factor
1. Introduction

Anemia remains one of the most obdurate diseases affecting the general public in Africa where it contributes close to a quarter of the continent’s nutrition-related Disability Adjusted Years (DAILY’s) lost for the past decade and half [1]. There are several causes of anemia with micro-nutrient deficiencies, iron deficiency and parasitic infections contributing a major share in Sub-Saharan Africa [2]. Among the parasitic infections that contribute to global anemia, malaria, schistosomiasis and soil transmitted helminth (STH) compose a considerable disease burden in school children in developing countries [3]. There is a similar geographic distribution and polyparasitism of Plasmodium falciparum, schistosomiasis and STH infections in different epidemiologic settings in Africa that has been observed to date, with considerable contributions to anemia [4, 5]. With many factors contributing to anemia in general, establishing the relative contribution of malaria to anemia is complex, as malarial anemia is more frequently present in combination with other conditions.

The multifactorial causes of anemia make the disease a continuous and nagging problem to the human populations in different parts of the world, more so in the underdeveloped and developing world. The disease burden contributed by anemia had been projected to decrease worldwide in this century; however, signs on the ground seem to portray a different picture altogether. Hemoglobin (Hb) concentrations are used for the diagnosis of anemia and assessment of its severity.

Anemia can be defined, generally, as a decrease in Hb and related hematologic indices according to the individual’s age, gender, physiologic state and geographic location [6].

In pregnant women, premature labor with low birth weight babies may be caused by anemia [7, 8]. Small for age live birth infants, still births and high perinatal maternal and infant mortality are all common features of anemia [9]. Anemia caused by nutritional inadequacies may result in stunted growth and underweight infants that predisposes to several infectious and noninfectious diseases of childhood [10] and has a phenotypic presentation with chronic severe malarial anemia (SMA).

2. Definitions of severe malarial anemia

Severe malarial anemia (SMA) is defined by an Hb concentration of <5 g/dl or a hematocrit of <15% in children <12 years of age (<7 g/dl and <20%, respectively, in adults) together with a parasite count >10,000/μl, which distinguishes it from other diseases with similar presentations. Besides malnutrition, human immunodeficiency virus (HIV), schistosomiasis, soil transmitted helminth (STH) as causes of anemia, Plasmodium infections (malaria) contribute a major portion of the debilitating illness to the global disease burden [11]. SMA is a complication of severe malaria, which results from infection caused by the apicomplexan protozoan parasite of the genus Plasmodium. The Apicomplexa (also called Apicomplexa) are a large phylum of parasitic alveolates. Most of them possess a unique form of organelle that comprises a type of plastid called an apicoplast and an apical complex structure. The organelle is an adaptation that the apicomplexan applies in penetration of a host cell.
Any of the complications such as severe malarial anemia, significant bleeding, shock (compensated or decompensated), nonrespiratory acidosis and hypoglycemia can develop rapidly and progress to death within hours or days [12, 13].

Severe malaria is differentiated from other conditions by the demonstration of asexual forms of the malarial parasites in the blood in a patient with a potentially fatal manifestation or complication of malaria such as SMA in whom other diagnoses have been excluded. Even though the complications have been considered to be almost unique to *P. falciparum* infection, in recent years, many cases of severe malaria, including deaths, have been reported in *Plasmodium vivax* and *Plasmodium knowlesi* malaria. The case fatality of *P. falciparum* malaria is around 1%, and this accounts for more than half a million deaths per year all over the world, in which 80% of these deaths are caused by cerebral malaria. The incidence of complications and deaths due to the other two types is much lower.

Pregnancy anemia or maternal anemia is defined by an Hb of <110 g/L (11 g/dL) and a hematocrit less than 31% [14]. In malaria, these values tend to be extremely low making SMA a critical clinical emergency in pregnancy and infancy where it displays distinct physiologic and morphologic characteristics between the two groups.

SMA threatens to kill the next generation. In the pregnant women and children <5 years, malarial infection develops into a fatal SMA more often than in any other population group due to reduced immune protection. This trend is also seen in malaria of endemic areas where natural immunity is supposed to develop over time due to higher exposure to the reinfection.

There is an increased demand for RBC synthesis in pregnancy, and the intrusion by the parasite creates a dilemma from decreased efficiency of nutrient utilization. Under reduced immunity, parasitemia increases persistently, resulting in increased level of parasite toxins that inhibit bone marrow functionality. The anti-immune response of pregnancy is meant to protect the fetus from autoimmune destruction. The same scenario is observed in SMA during infancy; however, the cause of reduced immunity in this case is from immature immune system activation and reduced maternal immunoglobulins.

Compounding the disease prevalence are factors such as parasite virulence, parasite-host interactions, host characteristics, and socio-economic conditions that play out an intricate web resulting in SMA. The production of pro-and anti-inflammatory cytokines, certain genetic traits, α or β-thalassemia, Duffy (Fy) blood groups and sickle cell traits remain the most common predisposing host factors to SMA. Malarial parasite species, disease endemics, Plasmodium multiplication rates, drug resistance and antigenic polymorphism all contribute to parasite factors that lead to SMA.

3. Malarial anemia etiology

Approximately 30–40% of deaths caused by *P. falciparum* are associated with SMA development. The multifactorial causes of SMA range from increased removal of circulating parasitized and nonparasitized red blood cells (pRBCs and npRBCs) to reduced synthesis of erythrocytes in
the erythroid germinal centers. While the molecular mechanisms underlying the SMA remain obscure, malarial parasites’ ability to remodel RBC’s morphology and physiology through ligands found on both the parasite and RBC’s membrane has been investigated in the past few years. Immunologic mechanisms associated with malarial pathophysiology seem to be more effective in increasing SMA. Elicitation of immunologic and inflammatory processes by malarial parasite antigens and immunokines plays a major role in the complex milieu that results in SMA. Coinfections with other parasites may increase SMA susceptibility as they aggravate malaria-associated inflammation. A normocytic and normochromic RBCs’ morphologic appearance is a common presentation of anemia in malaria, which is characterized by the absence of spherocytes and schistocytes although there may be abundance of fragmentocytes and eliptocytes typifying increased hemolysis. High frequencies of hemoglobinopathies and iron deficiencies in areas of high malarial prevalence may change the picture to microcytosis and hypochromasia [15].

Severe malarial anemia displays inadequate reticulocytosis in the presence of the anemia signifying that there is reduced synthesis of RBCs and not just increased hemolysis. In malaria, hematocrit gradually decreases, after an apparent initial steady state even with the onset of fever, showing either an increase in reticulocytosis or an absence of hemolysis within the first 24 h after infection. The decrease in hematocrit is independent of treatment initiation and may even occur in the absence of overt parasitemia on peripheral blood films, blood transfusion and adequate antimalarial treatment. In *P. falciparum*, parasite sequestration in the microvasculature may account for the parasite-negative peripheral blood smears accompanying decreasing hematologic indices. These parasites continue to shed soluble antigens, hemoglobin metabolites and derivatives that drive various syndromes of malaria like SMA. This may explain the continued decline in hematologic indices despite the evidently low parasitemia and the malarial anemia pathogenesis, which implicates bone marrow dysfunction as displayed by low reticulocytosis [16, 17]. When Hb decreases, the normal body physiology upregulates the bone marrow erythroid progenitors and reticulocytes are increased as an indicator of this process. Failure to increase immature red blood cells in circulation during overt anemia states indicates dyserythropoiesis and/or ineffective erythropoiesis associated with the myeloid progenitors’ proliferation, which is common in malaria. Bone marrow suppression of the erythroid blast cells has been evidenced in children exposed to multiple reinfections, receiving inadequate treatment or experiencing treatment failure that tend to become asymptomatic during acute *P. falciparum* infections showing partial immunocompromised state [18] against an expected hyperimmune reactivity of an inflammatory condition like malaria. Inadequate erythropoietin production or effectiveness, the effect of the inflammasome on erythropoiesis, concomitant parasitic and bacterial infections contribute to the complex milieu culminating in SMA as well. Red blood cell membrane modifications by attached parasite ligands remodel the cells to a phenotype tagged for destruction through phagocytosis.

4. Effects of parasites on cell membranes leading to severe malarial anemia

Anemia is described as a decrease in Hb concentration, which is directly related to RBC’s mass within the circulation. Infection with *P. falciparum*, which is associated with a rapid development of SMA, is also known to influence pRBC’s sequestration in the microvasculature of
different tissues and organs that include skin, lung, gut, muscle, heart, and brain. Parasitized RBCs are commonly sequestered from circulation together with npRBCs that carry parasite antigens. Rosetting (aggregation of pRBCs into rings of four or more cells) and agglutination (combining of pRBCs and npRBCs to form clumps) are common phenomena that occur due to ligands that are found on the surfaces of pRBCs and npRBCs, which result in reduction of freely circulating RBCs and eventually on RBC’s mass and Hb concentration. Cytoadherence of pRBC’s to the microvasculature, rosetting and cell-cell agglutination are processes facilitated by several ligands of *P. falciparum* trophozoites and schizonts. These pRBC’s surface protruding molecules cause pathological cell-cell communication. These ligands such as *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) \[19, 20\] enable pRBCs to bind to endothelial cell (EC) receptors, e.g., leukocyte differentiation antigen CD36, intercellular adhesion molecule 1 (ICAM-1), integrin, chondroitin sulfate and hyaluronic acid \[21\].

Some of these ligands are necessary for the formation of the host cell-parasite connection, which allows invagination of the erythrocyte bilayer leading the parasite engulfed into the RBC. As a result, an intracellular parasite vacuole is formed and provides an environment for parasitic multiple stage growth. During the process of parasite-protein-mediated internalization as well as during the intracellular proliferation, several other parasite proteins bearing a host-targeting (HT) or plasmodial expert element (PEXEL) are also exported into RBCs \[22, 23\], providing a myriad of host cell-parasite communication paradigms.

Intracellular proliferation and parasite antigens release cause considerable reduction in RBC membrane stability and alters cell surface characteristics leading to eventual pRBC’s membrane rupture. The ability of RBCs to change shape allows them to pass through the spleen filtration mechanisms. Infection in the cell membranes causes them to be more rigid and unable to change shape when passing through capillaries and become prone to phagocytosis and hemolysis.

Some of the exported parasite ligands adhere to the membranes of npRBCs. Parasite ligand deposition on npRBC’s tags these cells and pRBCs for rapid reticuloendothelial system pooling and sequestration by the spleen, which removes them from circulation resulting in SMA. Cytoadherence and auto-agglutination, emanating from the various ligand-epitope interactions, also removes a considerable amount of cells from circulation exacerbating SMA. Reduced RBC’s flexibility occurs with a very few ligands being found on the surface of the cells reducing the half-life of such marked cells. However, such tagging only occurs on a subset of erythrocytes to account for the rapid setting of SMA encountered in malaria meaning that host cell-parasite ligand interactions along with other mechanisms play profound role in the creation of overt SMA.

5. Host cell-parasite ligand interactions and severe malarial anemia

The interaction between host cell and parasite ligands is a complex process of an inefficient invasion mechanism that may be completed in a small fraction of infection-targeted RBCs. As a result, many ligands need to be secreted and shed off into plasma resulting in many of these pRBC-adhesive proteins being present in high concentrations in plasma. These free molecules adhere to
npRBCs triggering IgG and complement binding. Subsequently, cells binding immunoglobulin (Ig) and complements are cleared from circulation through phagocytosis by macrophages and hemolysis. This results in a critical hemoglobin reduction. Furthermore, HT/PEXEL-containing-proteins are released into plasma from pRBCs and adhere to npRBCs. These processes are repeated over and over again as the parasite intracellular cycle ends, concomitantly increasing the disease. Aberrant signaling circuits are also increased in the affected cells heralding their apparent need for removal from circulation by macrophages and exacerbating SMA.

Parasite proteins in rhoptries and merozoites surface membranes are candidates associated with SMA development. Merozoites are blood asexual forms of the parasites that are released in the circulation when pRBC’s ruptures. As these parasites are intracellular, they invade new cells for the continued propagations of more asexual and sexual forms. To execute invasion, the parasite aligns its apical surface to the surface on RBCs. Rhoptries are structures protruding from the parasites. The merozoite uses the rhoptry proteins for anchoring on the surface of the RBC targeted for invasion. During the invasion processes, merozoites use rhoptries, which are also secretory structures on the merozoites apical end, to release their contents at the junction between the parasite and the erythrocyte. The P. falciparum proteins such as rhoptries secretory protein-2 (RSP-2) or rhoptries-associated protein-2 (RAP-2) have been found to be located at the surface of pRBCs as well as on npRBCs [24]. The presence of these proteins on the surface of npRBCs is possibly a result of failed invasion or when they adhere to the surface of these cells, they shed off from the merozoites into the plasma. Specific antibodies then opsonize the adhered RSP-2/RAP-2 complex accelerating complement-mediated RBC’s lysis as well as macrophage uptake of targeted npRBCs [25]. In this way, the parasite is able to facilitate hemolysis of both npRBCs and pRBCs without necessarily entering the cell (Figure 1).

5.1. Parasite rhoptry protein ring surface protein 2 (RSP-2/RAP-2) and severe malarial anemia

It has been shown that the parasite RSP-2 not only tag npRBC’s surfaces, but it extends to erythroid precursor cells in the bone marrow (BM) eliciting SMA [26] (Figure 1). The RSP-2 is transferred to the surface of the host cell around the site of contact with the merozoite. Gradually, the protein spreads over the entire surface of the cell by slow, lateral movements in both the npRBCs and pRBCs, leading to their premature identification and destruction by the reticuloendothelial system. P. falciparum-infected individuals respond to the infection by increasing the proliferation of phagocytic macrophages and their activity on tagged RSP-2 npRBCs and pRBCs.

There are other nongross RBC’s membrane abnormalities that result in RBC’s clearance in addition to adhesive-interacting ligands, RSP-2-antibody phagocytosis and complement activation. These changes are not observable by the light microscope as there are no obvious cell membrane morphologic abnormalities that have been noted. The subtle changes are due to oxidative damage of cell membranes, phosphatidylserine (PS) externalization or exposure and reduced deformability, which contribute to increased RBC’s clearance leading to SMA. The involvement of ligands in RBC’s clearance is mediated through stimulation of the inflammasome and its role in erythropoiesis in malaria [27].
Despite massive RBC’s destruction in malarial infection, there is also a delayed compensation of RBCs in overt anemic individuals due to defective erythropoiesis. Acute infection in children shows a picture of normal to small erythroid precursors in bone marrow (BM). There is considerable change noted on the erythroid cells morphology in malaria induced anaemia. These include multinucleated erythroid cells, karyorrhexis, incomplete or unequal mitotic divisions, intercytoplasmic bridges and cytoplasmic budding. A higher proportion of the erythroid progenitors are held in G₂ phase in SMA as compared with healthy individuals.
RSP-2 has been observed to be transferred to erythroid precursors only when there has been a direct contact with merozoites, in vitro. In vivo, these proteins are also known to be tagged to these cells [28]. Subsequently, erythroid precursors tagged with RSP-2 are destroyed through complement activation and cytokine oxidative stress linked apoptosis processes.

Noteworthy is that colony-forming units (CFU’s) and other stages of the erythroid lineage suffer the same fate in the presence of cytophilic antibodies accounting for their reduced numbers in SMA. The antibody-RSP-2 complex on the surface of erythroblasts triggers the decline of these cell lines through phagocytosis or morphologic alterations observed in erythroid cells in the BM and the mediators of this process are closely linked to the inflammasome in SMA.

In addition to the described BM involvement in SMA, studies in Gambian children have demonstrated that SMA was defined by erythroid hyperplasia with dyserythropoiesis and a hypercellularity with an inefficient reticulocyte production index (RPI) [29] shown as <2.0. RPI is a measure of the extent to which the reticulocyte count has risen (or not) in response to the level of anemia, which indicates that SMA is the result of erythroid suppression [30], arbitrated by inflammatory molecules. Concomitant erythroid hyperplasia and reduced RPI signifies a cellular maturation check point or bottleneck that is introduced in SMA.

6. Severe malarial anemia and the inflammasome

Inflammation is a process that tends to control the proliferation of hostile entities and foreign bodies when the physiologic aspect of the body is invaded by pathogens or when there is a physical injury at both macroscopic and microscopic levels. The inflammasome is composed, among other mediators, of both pro-inflammatory cytokines typically denoted as T-helper cells type 1 (Th1) and anti-inflammatory cytokines denoted as T-helper cells type 2 (Th2), which tend to counteract each other into a balance state under normal physiologic states. SMA in children shows a close relationship of the disease with an imbalance between the Th1 and Th2 cytokines and chemokines (Figure 1). In an attempt to control parasitemia, the host releases an array of pro-and anti-inflammatory cytokines, chemokines, growth factors, and effectors as part of a wholesome innate immune response. Depending on the timing and magnitude of the inflammatory response to the infection and release of the cytokines, parasitemia may be controlled successfully or uncontrolled parasitemia may cause imbalance of the inflammatory milieu with damage to the host and suppress the erythropoietic response [30].

Understanding the context in which inflammatory response to malarial infection culminates in SMA involves a close scrutiny of the microenvironment in which the cellular components and mediator interact. The process by which erythroid progenitors proliferate and differentiate into non-nucleated reticulocytes in the BM is called erythropoiesis. Erythroblastic islands, where erythropoiesis takes place, are specialized cellular niches composed of a central macrophage surrounded by erythroblasts in which cells proliferate, differentiate and enucleate [31]. The pro-erythroblast, which goes through four mitotic cycles, is the earliest recognizable erythroblast that gives rise to reticulocytes. Basophilic polychromatic erythroblasts give rise
to orthochromatic erythroblasts, which expel their nuclei to generate reticulocytes. The well-coordinated mechanism is characterized by a decreased cell size, more condensed chromatin material, progressive hemoglobinization and marked membrane organizational alterations. The role of various cytokines and chemokines in the regulation of erythropoiesis is revealed by the intimate interaction of the myeloid progenitors, macrophages and erythroblasts during RBC’s production.

The erythroid hyperplasia seen in SMA excludes erythropoietin deficiency as a cause of inadequate erythropoiesis of malaria intimating that the aberrant inflammatory response mounted by the cytokine milieu as the culprit. Associated with the hypercytokinemia is the ingestion by neutrophils, monocytes and macrophages of the inflammatory mediator hemozoin (Hz, a parasite-derived polymerized heme), which is known to influence the dysregulation of inflammatory responses through synthesis of a number of cytokines with subsequent induction of SMA [32]. *P. falciparum* Hz (PfHz) is a brown/black pigment that accumulates in phagocytic cells in the BM, which is formed during the intraerythrocytic asexual replication cycle when *P. falciparum* metabolizes host Hb as a source of amino acids [33]. During the formation of the insoluble PfHz, toxic iron-rich heme known as ferrirrotoporphyrin IX (FP-IX) is aggregated by heme polymerase. The engulfing of PfHz is a good indirect measure of sequestered parasite burden, recent schizogony, disease severity, decreased hematocrit and degree of erythropoiesis suppression in children with *P. falciparum*-induced SMA [34, 35].

The phagocytozed PfHz triggers the innate immune response through the toll-like receptors (TLR’s) [36] with downstream cytokine elicitation promoting RANTES suppression by a pathway involving IL-10 [37].

When and how much of interleukin 12 (IL-12), interferon gamma (INF-γ) and tumor necrosis factor alpha (TNF-α) are released is critical to minimize and preserve erythropoiesis. Activation of this pro-inflammatory cytokines elicitation should be timely abrogated by type 2 cytokine IL-10, transforming growth factor beta (TGF-β) and IL-4 to avoid host damage by the inflammatory process [38]. TNF-α is critical for parasite killing and prevention of parasite replication directly as well as through macrophage migration inhibitory factor (MIF) and through nitric oxide synthase type 2 (NOS2-inducible nitric acid synthase) and generation of nitric oxide (NO), which kills parasites directly [39]. Inflammatory responses are commonly exacerbated by the TNF-α induction of cyclooxygenase-2 (COX-2), which drives prostaglandin E synthesis (PGE) with subsequent generation of malarial symptoms such as fever, headache, nausea, vomiting, diarrhea, anorexia, myalgia and thrombocytopenia [40, 41].

During the early phases of malarial infection, natural killer cells, αβ-T cells and regulatory γδ-T cells are activated to produce IFN-γ [42], a prototypical type 1 cytokine for childhood malaria [43]. Individuals who produce IFN-γ from monocytes when immunized with asexual malarial parasites are able to resist infection by *P. falciparum* as has been seen in West Africa, Mali, Burkina Faso and Sudan as well higher Hb concentration and reduced prevalence of SMA are observed in Kenyan children challenged with pre-erythrocytic antigens [44]. Over-production of the innate inflammatory mediators is associated with anemia, and it has been observed that persistent macrophage activation is significantly greater in children with malarial complications through BM suppression, dyserthropoiesis and erythropagocytosis [45].
There is a strong alliance between the interleukins Iβ and 1α, potent endogenous pyrogens, which promotes inflammatory response against invading pathogens [46], with TNF-α in the enhancement of NO and IFN-γ production. Sustained release of IL-1β, as experienced in malaria, has the potential of inducing several hematologic and immunologic anomalies with anemia as a candidate [47, 48]. However, cytokine IL-1β has a protective role in certain haplotypes, which are predisposed to produce higher levels of this cytokine and prevents anemia development [49].

One of the cytokines, IL-6, has been demonstrated to be increased in malaria with peripheral blood mononuclear cells (PBMC) being the source of the increased cytokine production during acute malaria [50]. IL-6 mediates the protective immunity against the pre-erythrocytic phases of malaria by inducing Ilβ and TNF-α. During the erythrocytic stage, IL-6 controls parasitemia through boosting up specific immunoglobulin (Ig) G antibodies. However, lack of control over parasitemia and the resulting progression toward severe disease may explain the association between elevated levels of IL-6 and enhanced pathophysiology [27]. Macrophage migration inhibitory factor (MIF) is a ubiquitous molecule produced by T cells, monocytes-macrophages and the anterior pituitary in response to pro-inflammatory stimuli [27]. Notably, there is a rapid mobilization and expression of large concentrations of MIF during acute inflammation as the cytokine is stored in preformed vesicles only to be released without de novo gene expression.

The pro-inflammatory properties of MIF are important for both innate and adaptive immune response in both parasitic and bacterial infections [27, 51]. In animal models, elevated MIF concentrations have strong connexion with SMA while mice with MIF knockout gene have less anemia and higher survival chances when infected with Plasmodium chabaudi compared with the wild type [52]. In humans, however, there is an opposite picture of elevated MIF protein (in circulation) and MIF transcripts (in PBMC) being connected to less severe falciparum malaria [53]. Fascinating is the fact that worsening SMA is linked to decreasing circulating MIF concentrations as well as blood leukocytes MIF transcripts in Kenyan children. Remarkably, MIF concentration in peripheral blood was not significantly inter-related to reticulocyte responses in these children. Correction for age, gender and parasitemia, however, did show that elevated levels of monocyte chemotactic protein [MCP] were significantly associated with both SMA and decreased MIF production.

Phagocytosis of PfHz by PBMC causes dysregulation in MIF production in an apoptosis-independent manner. Consequently, PfHz presence in malaria suppresses peripheral blood MIF production, thus enhancing severity of anemia. The intricacy by which PfHz is involved in the malarial parasite life cycle makes it the central molecule to SMA development and other malarial pathophysiologies. Therefore, PfHz as a heme metabolism waste product deliberately synthesized by the parasite may be regarded as a long-term strategy for its survival.

Interleukin 23 is another pro-inflammatory mediator involved in conditioning the SMA pathogenesis, which is also important in mediating anemia development in autoimmune diseases [54] and chronic inflammation [55]. The subunits making up IL-23 are designated p19 and p40, and this cytokine shares a number of common properties with IL-12. Among these characteristics is the p40 subunit, ability to bind the IL-12Rβ1 receptor, release from activated
myeloid antigen presenting cells, type 1 immune response promotion, suppression by both IL-10 and IL-12 p40 homodimers. A striking feature observed with IL-23 is its sustained elevation when both IL-12 and IL-10 are suppressed [56]. In cultured PBMC, hemozoin induces sustained IL-23p19 transcript concentrations for more than 72 h, whereas IL-12p40 and IL-10 transcripts rapidly decline after reaching peak at 24 h [57]. In other words, IL-23 is important in the pathogenesis of SMA (through PfHz influence), whereas IL-12 and IL-10 play pivotal role in the regulation of IL-23 synthesis in P. falciparum infection.

Interleukin 12 is a heterodimer protein made up of 35 and 40 kDa subunits, which is a prototypical cytokine of type 1 immune response interfacing inflammation and immunity. The main IL-12 secreting cells include dendritic cells, monocytes and B-cells, which can be activated by bacterial cell wall components, intracellular pathogens and the ligation of CD40. By stimulating the synthesis of IFN-γ and TNF-α from T-cells and natural killer (NK) cells, IL-12 augments Th1 response to infection. Secretion of IL-12 can be promoted by several cytokines and chemokines to include granulocyte macrophage-colony stimulating factor (GM-CSF) and IFN-γ, while others negatively regulate its production such as IL-4, IL-10, IL-11, IL-13, monocyte chemotactic protein (MCP)-1/CCL2, and TGF-β. Reinfection with P. falciparum and development of SMA are averted through the administration of recombinant IL-12 together with the less efficacious chloroquine showing that IL-12 is crucial in the prevention of malarial anemia and dyserythropoiesis. The protective role of IL-12 in malaria lies in its ability to stimulate antibody production and its ability to act as a hematopoietic growth factor. Low concentrations of IL-12 are associated with SMA through, in part, the influence of PfHz phagocytosis that influences upregulation of monocyte-derived IL-10 which in turn suppresses the IL-12p40 subunits [58].

Cytokine involvement in SMA induction includes influencing iron trafficking. Moreover, cytokines play a critical role in the maturation of erythroid cells. Interleukin 6 (IL-6) induces hepcidin production and expression in the liver, (a master regulator of iron trafficking), resulting in decreased iron availability. Also well-known is the involvement of transforming growth factor beta (TGF-β) in the inhibition of erythroblast proliferation and the maintenance of the hematopoietic stem cells (HSC) in a state of quiescence to preserve the stem cell pool and avoid the exhaustion of the same. Neutralization of TGF-β results in the release of early HSC progenitors from quiescence [59].

Tumor necrosis factor alpha (TNF-α) is involved in the cleavage of GATA-1, which is a major erythroid transcription factor. Interferon gamma (IFN-γ) induces production of TNF-related apoptosis-inducing ligand (TRAIL) by macrophages, which inhibits erythroblast differentiation. This is suggestive of a critical role of these cytokines in SMA. Furthermore, suppression of IL-12, which is strongly associated with pediatric SMA, decreases the production of IFN-γ and IFN-α. Infection induces IL-10 synthesis which in turn suppresses IL-12 associated with hemozoin (Hz) acquisition by monocytes [58, 60].

SMA in children is, therefore, invariably associated with increased circulating concentrations of TNF, IL-6, IL-1β, interleukin-1 receptor agonist (IL-1RA), macrophage inflammatory protein 1 alpha (MIP-1α) and macrophage inflammatory protein 1beta (MIP-1β) [61, 62]. What is astonishing is the finding that prostaglandin E (PGE) and nitric oxide (NO) are suppressed in
SMA even though the increased TNF level is expected to induce higher concentration of these inflammatory mediators. However, reduction in PGE may permit over synthesis of TNF-α enhancing anemia severity. Suppression of NO in children with SMA promotes ineffective parasite eradication while suppressing erythropoiesis in the BM. In pediatric SMA, IL-12p70 and INF are associated with positive prediction of Hb (elevated Hb) whereas IL-2R, IL-13 and eotaxin predict Hb negatively (favor profound anemia development) \[62\].

7. Pro-inflammatory (Th2) cytokines involvement in severe malarial anemia pathogenesis

By preventing over-production of pro-inflammatory mediators, anti-inflammatory cytokines like IL-10 render protection against SMA development. The later stages of the innate immune response in \textit{P. falciparum} infection seek to downregulate the potentially pathogenic pro-inflammatory responses necessary for parasitemia eradication. Severity of malarial anemia can be predicted by the IL-10-TNF-α ration with low values associated low Hb concentration and hematocrit. Therefore, timing of the anti-inflammatory response relative to the proinflammatory cytokines activity and concentration has a strong influence on the malarial outcomes. Statistically significant positive association has been identified between IL-10 in the systemic circulation and malarial pigment containing leucocytes, indicating the regulatory role of \textit{Pf}Hz in the development of systemic malarial pathology when it upregulated IL-10 production (Figure 2).

![Chemical structure of asiatic acid](image)

\textit{Figure 2.} Chemical structure of asiatic acid. Formula C\textsubscript{30}H\textsubscript{48}C\textsubscript{5}. MW: 488.69912. (G/mole). Redox characteristics: hydrogen bond donor (HBD) 7.1 and hydrogen bond acceptor (HBA) 4.176 [79].
Downregulation of TNF-α and IL-10, TGF-β1 (an anti-inflammatory and growth factor) tends to protect against severe malaria in mice. Importance of TGF-β in the pathogenesis of malaria is attributable to the cytokines that can influence the erythropoiesis either positively or negatively [63]. Serum concentration of the soluble form of TGF-β1 co-receptor, endoglin (sEng or CD105/TGF-βRIII), is elevated significantly in children with severe falciparum infection showing the importance of the cytokine in malarial pathogenesis and possibly SMA [64].

8. Severe malarial anemia pathogenesis and chemokines

Chemokines or chemotactic cytokines play a critical role in immune system activation, hematopoiesis, angiogenesis and antimicrobial activities. The chemokines’ macrophage inflammatory protein 1α (MIP-1α)/CCL3 (C-C chemokine) and the C-X-C chemokine (IL-8/CXCL8) were the first to be investigated in acute *P. falciparum* malaria where a positive correlation with parasitemia was found with IL-8/CXCL3. There is a much higher concentration of IL-8 in acute malaria that is necessary for the activation of neutrophils in severe nonfatal malaria that may be associated with slow cure rate after malarial chemotherapy. Chemokine production or suppression signal transduction depends on the phagocytosis of *PfHz* through oxidative stress-dependent and oxidative stress-independent mechanisms [65]. In both humans and PBMC, MIP-1α/CCL3, MIP-1β/CCL4 proteins and transcripts tend to be increased in production by the introduction of *PfHz* [66].

Normal T-cell expression, secretion (RANTES, CCL5) and regulated activation play a critical role in SMA pathogenesis. Secreted by monocytes, macrophages, fibroblasts, NK and T-cells, CD 34+ hematopoietic progenitors, RANTES protein is sequestered in platelets granules and is released by thrombin-stimulated platelets for both innate and adaptive immune response. Furthermore, RANTES stimulates hematopoiesis, angiogenesis, cell proliferation and development [67]. Through *PfHz*-induced suppression, both RANTES protein and transcripts tend to be decreased in malaria. Higher amount of RANTES tends to be protective against SMA [68]. Suppression of RANTES is closely associated with inefficient erythropoiesis and malaria-induced thrombocytopenia, which is promoted by *PfHz* through an IL-10-dependent mechanism. In this regard, thrombocytopenia appear to be the main cause of reduced RANTES which in turn tends to suppress erythropoiesis in SMA.

9. Role of growth factors in severe malarial anemia

Growth factors play a pivotal role in the erythropoiesis cascade. A longitudinal study of malaria has shown that serum concentrations of granulocyte-colony stimulating factor (G-CSF) were elevated at day 0 in complicated malaria followed by a decline to within reference interval on day 7. Significant correlation of G-CSF with procalcitonin, parasite density and erythropoietin is a common finding at the beginning of malaria [69]. G-CSF has a negative impact on erythropoiesis, and an increased concentration of the growth factor leads to SMA development. With high parasite density associated with elevated levels of G-CSF, the
increased erythropoietin is a compensatory mechanism to protect against SMA development through a mechanism driven by both hypoxia and oxidative stress common in complicated malaria [70]. While G-CSF promotes erythropoiesis, it acts in synergy with TNF-α in increasing the eradication of parasites in erythrocytic cycle by neutrophils.

The genes/gene pathway that leads to the SMA pathogenesis has been human stem cell growth factor (SCGF, C-type lectin domain family member 11A-CLEC11A) which is up-regulated after PfHz stimulation of human PBMC’s [71]. Primarily secreted by myeloid and fibroblasts possessing burst-promoting activity for human bone marrow erythroid progenitors, SCGF is a hematopoietic growth of 323-amino acid that is cleaved to a 245-amino acid SCGF-β active form. In children with SMA, SCGF tends to be suppressed and positively correlated with Hb concentration, erythropoietic response suppression and high concentrations of naturally acquired monocytic PfHz. Genetic polymorphism of the SCGF also tends to protect against SMA development and suppression of erythropoiesis in parasitized children [72].

10. Role of effector molecules in severe malarial anemia pathogenesis

Relative expression of inflammatory mediators largely determines the various clinical outcome of malaria. The relative concentrations and timing of release of pro-and anti-inflammatory cytokines, chemokines and growth factors have a direct effect on cellular response as well as on the down-stream effector molecule production. The most notable down-stream effectors include NO, reactive oxygen species (ROS) and prostaglandins E2 (PGE2).

The toxic free radical NO has an effect on SMA development. The catalysis of l-arginine by NO synthase (NOS) produces equimolar amounts of l-citrulline and NO and in malaria the inducible NOS (iNOS or NOS2) is responsible for most of the NO production from monocytes, macrophages and neutrophils [73]. Pro-inflammatory cytokines (IL-12, IFN-γ, TNF-α) upregulates iNOS-generated NO synthesis, whereas Th2 (anti-inflammatory) cytokines (IL-10, TGF-β) downregulates NOS2 expression in malaria. NO is both protective as it has potent parasiticidal properties limiting parasitemia and pathogenic effects as it sustained high level predispose to anemia through BM suppression, dyserythropoiesis and erythrophagocytosis.

ROS appear to be both protective and pathologic in P. falciparum malaria. Increased concentrations of the free radical have observed to accompany accelerated parasitaemia clearance in Gabonese children as well as controlling of peripheral parasitaemia in children with severe malaria [74]. In Kenyan children with severe malaria, ROS damaged the RBC membranes [74]. Severe malarial cases associated with significantly elevated markers of oxidative stress like high malondialdehyde concentrations, high protein carbonyl, high nitrite, low ascorbic acid and elevated plasma copper concentrations are suspected to have SMA [74]. The arachidonic acid product, PGE$_2$, has an inverse relationship with SMA development. Cyclooxygenase (COX) enzyme (prostaglandin-H$_2$ synthase) exists in two isoforms: COX-1 (PGH synthase-1) and COX-2 (PGH synthase-2). COX-1 catalyzes the immediate formation of PGE$_2$, whereas COX-2 catalyzes the delayed formation of PGE$_2$ involved in the regulation of the inflammatory response and immunity to invading pathogens. Acquisition of intraleukocytic PfHz in placental malaria reduces
mononuclear cell PGE$_2$ production [75]. Furthermore, plasma bicycle-PGE$_2$ (stable end metabolite of PGE$_2$) and PBMC COX-2 ex vivo expression are significantly reduced in severe malaria. De novo COX-2 transcripts biosynthesis is inhibited when monocytes phagocytose PfHz [76]. Ingestion of PfHz by monocytes and the effects of antipyretics used to treat malarial fever promote overproduction of TNF-α and worsening of malarial pathophysiology like SMA when erythroid progenitors are targeted for apoptosis [76–78]. PfHz (in synergy with TNF-α) directly inhibits erythroid cell development by interfering with the erythropoietic cascade through induction of oxidative stress-driven erythroid precursor cell apoptosis and through cytokine-mediated inflammation effects on erythroid development leading to SMA.

11. Severe malarial anemia management

The only treatment that has been available for SMA has been largely blood transfusion in various forms. Erythropoietin supplementation has not been successful as most patients with SMA tend to have high concentrations of the hormone amidst a low reticulocyte count indicating a nonresponsive BM. Equally so has been the ferrous iron supplements that have proven to have worse outcomes and had to be prematurely stopped. As has been shown, SMA is a synergistic onslaught from the parasite producing PfHz, which is eventually taken up by activated leukocytes and breeds both pro- and anti-inflammatory mediators whose sustained synthesis results in malarial pathophysiology and derangements. Aiming the treatment at the parasite has been successful, to some extent. However, the continued harangue of the parasite among many populations is a tacit implication that more is required to eradicate or control the malarial pandemic. Aiming malarial treatment at the pathophysiology is an avenue currently being explored with commendable results.

Administration of the phytochemical triterpenes, asiatic acid and other triterpenoids has been shown to have antiparasitic effects and reduction of SMA development in murine malaria. Addressing the pathophysiology of malaria while eradicating parasitemia seems to provide a provocative approach that encompasses inflammation, immunoreactivity, glucose homeostasis, renal failure and other aspects, which commonly complicates malaria in humans.

11.1. Severe malarial anemia and asiatic acid administration in murine malaria

Hypothetically, drugs that may inhibit or reverse malarial pathophysiology or the disease components have a higher chance of controlling malaria even without parasite eradication. This may include targeting SMA, which is an independent malarial mortality predictor in pregnant women and children [80]. The concept of malarial pathophysiology being referred to as malarial disease and its management being denoted as antidisease in malaria is a novel term in malaria handling terminology introduced to differentiate this approach from the anti-parasitic treatment [80].

Triterpenes with antidisease properties in other conditions similar to malaria, like inflammation in sepsis and hypoxia in anemia are able to eradicate the Plasmodium parasite as well as resolve the ensuing pathophysiology. Triterpenes with pleiotropic functions, sufficient to be
antidisease as well as antiparasitic, have been reported. Betulinic acid (BA), ursolic acid (UA), maslinic acid (MA) and oleanolic acid (OA) have been shown to have moderate activity in vitro against the chloroquine-insensitive (K1) and chloroquine-sensitive (T9-96) P. falciparum parasites. MA, a possible multitargeting antimalarial, effectively inhibits proteolytic processing of the malarial merozoite surface protein (MSP1) complex, inhibits the metalloproteases and has several putative binding sites for the parasite antigens [81]. This multitarget phenomenon suppresses the parasitemia in more than one way. Furthermore, the age-old preoccupation with targeting single process of the parasite infective cycle (which is mutation prone) is avoided, and host-related responses potentiating antidisease and antiresistance outcomes are involved [80]. Asiatic acid (AA), an amphiphilic triterpene (Figure 2), has known for its antioxidant and pro-oxidant capacity, anti-inflammatory and antinociception activity in mice [82], calcium release-associated apoptosis induction [83, 84] and a potent immunomodulator. Asiatic acid shares structural and bioactivity properties with OA, MA, UA and BA. Targeting the pathophysiology of malaria, SMA, as well as the parasite provides new mechanisms for combating malaria. Noteworthy is that AA is known to attenuate, inhibit or ameliorate the above factors in other diseases, which formulate the bedrock of malarial disease and its sequelae.

11.2. Glycosylphosphatidylinositols (GPI) and severe malarial anemia

Immunity against severe malaria is partly antiparasitic and partly antitoxic (toxic effects in response to parasite factors) [85]. The majority of the adults in malarial endemic areas have resistance to severe malaria and subsequently to SMA.

The induction of innate pro-inflammatory cytokine responses is mediated by germline-encoded pattern-recognition receptors, such as toll-like receptors (TLR), which recognize conserved microbial structures, i.e., pathogen-associated molecular patterns (PAMP) [86]. Among the malarial PAMP, glycosylphosphatidylinositols (GPI) are considered the main pathogenicity factor [87]. While GPI structure is conserved among Plasmodium species, human and Plasmodium GPI differ considerably but provide potential therapeutic points [88]. Several functionally important parasite proteins, including MSP-1, MSP-2 and MSP-4, are anchored to the cell membranes through GPI moieties and are also abundantly present free of protein attachment in membranes of pathogenic protozoa [89]. P. falciparum GPI have been found to induce the production of NO, TNF, IL-1β in murine macrophages in vitro, and a synthetic malarial GPI glycan was demonstrated to be immunogenic in vivo [90].

AA modulates immunity by selective induction of mitochondria-dependent apoptosis of activated lymphocytes in the prevention of murine fulminant hepatitis [91], a mechanism that may be extendable to malaria. Using membrane DNA array technique, a wound-healing derivative of AA [2-Oxo-3, 23-isopropylidene-asiatate (AS2006A)] exerting anti-inflammatory effect was identified. The anti-inflammatory mechanism involved selective cytotoxicity to activated macrophage cell line (L-929). By upregulating the expression of apoptosis-inducing genes caspase-8, c-myc, inducible nitric oxide synthase (iNOS), mdm2, NF-kBα, I-kBα and NF-κB p105, the phytotherapeutics controlled inflammation [93]. This alludes to AA exerting anti-inflammatory effect by cytochrome c release, caspase 3 activation and poly (ADP-ribose) polymerase cleavage mechanism as did AS2006A [92].
In principle, GPI drives inflammation that leads to SMA and AA by curbing cytokine release and correcting dyserythropoiesis may be said to inhibit GPI effect.

11.3. Preservation of blood volume by asiatic acid through immune system modulation

The effect of AA in alleviating hemodynamic and metabolic alterations in metabolic syndrome is through restoration of endothelium nitric oxide synthase (eNOS)/iNOS expression [93]. Similar influence of AA in malaria may be anticipated where eNOS/iNOS ratio determines the bioavailability of NO necessary for vascular proliferation and angiogenesis. These are useful processes in the inhibition of SMA. By maintaining RBC’s concentration in malaria, besides preventing SMA, AA also contributes toward modulation of hemodynamic systems.

The hematologic indices such as Hb concentration, RBC’s volume and % hematocrit (%Hct) were depressed with increasing percentage of parasitemia, while oral administration of AA has been shown to preserve these parameters and ameliorated SMA. SMA has been shown to persist even when parasitemia has been resolved driven by an aberrant immune system and P.fHz-induced oxidative stress, and the immune system modulation of AA may correct SMA, which is also relentless even after overt infection has dissipated. By suppressing parasitemia as well as having an effect on the retardation and correcting SMA, AA is a potential antidisease agent in malaria. Although not investigated, there is a possibility that other factors (including inflammatory mediator-impaired erythropoiesis) influencing SMA development are inhibited by AA. Destruction of pRBCs occurs when the schizonts mature and merozoites rapture cell membranes. pRBC’s destruction is accompanied by the lysis of npRBCs at a ratio of 8.5 RBCs for each pRBC hemolyzed through phagocytosis and increased oxidative stress. By selectively inducing apoptosis of activated macrophages and monocytes, AA may reduce the phagocytic activity of leukocytes in malaria as has been shown by SMA retardation. Also, the antioxidant facet of AA may preserve RBC’s membrane deformability and reduce the trapping of the cells by the spleen.

SMA develops as the RBC mass is reduced rapidly without concurrent replacement as a result of ineffective erythropoiesis. This occurs when erythroid progenitor apoptosis is induced by oxidative stress. The high concentrations of EPO observed in SMA are at variance with the low degree of reticulocytosis present, indicating reduced BM response to anemia due to erythroid progenitor destruction. The antioxidant capacity of AA, by preserving erythroblast cells, corroborates with increasing EPO to alleviate SMA through increased reticulocytosis. Moreover, by reducing parasitemia, AA not only preserves erythroid precursor response in infection but also increases blood volume through normalizing reticulocytosis [94] that prevents overt SMA development.

11.4. Asiatic acid reduces oxidative stress and reduces red blood cell’s destruction

The other mechanism by which npRBCs are destroyed in malaria involves increased erythrocytic oxidative stress and parasite antigens, which cause RBC’s membrane to be less deformable and more fragile. This shortens RBC’s life spans. These cells are trapped during
splenic sequestration and destroyed through phagocytosis. AA has known for its antioxidant, anti-inflammatory and immunomodulatory properties that protects cell membranes from oxidative damage and rigidity, reduced erythrophagocytosis and reduced parasite proliferation. MA, a phytochemical similar in structure and polypharmacology with AA, has multitargeted inhibitory properties against malaria with possible blockade of parasite maturation from early ring to schizont stages [95]. BA and UA also share carbon skeleton with MA and AA. Analogs of these two triterpenes are antiplasmodial through disruption of parasite calcium homeostasis [96] and averting SMA through reduction in parasite densities. These together with AA inhibit parasitic proliferation limiting RBC’s hemolysis and preserving hematologic indices. The pleiotropic biologic effects of AA influence host control of the parasite proliferation. By modulating the immune system and removing erythropoiesis-suppressive effect of parasitic infection, hematologic indices are corrected preventing the development of SMA.

11.5. Asiatic acid, hepcidin, iron homeostasis and severe malarial anemia

The resolution of SMA (normalized hematologic indices) indirectly indicates the abrogation of the immunologic and inflammatory processes by AA. The persistence of SMA beyond parasitemia eradication is orchestrated and sustained by immunologic sequelae, which upregulate hepcidin synthesis and modulation of iron metabolism, and the fact that SMA is inhibited by AA administration, suppression of hepcidin synthesis and continuation of normal iron metabolism is a factor that is associated with the beneficial effect of triterpenes in malaria [97].

12. Conclusion

The pathogenesis of SMA is largely driven by dysregulation and imbalance between pro- and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules. Alterations in the phenotypic presentations of these innate inflammatory mediators is due, at least in part, to the phagocytosis of PfHz by monocytes, resident macrophages (including those in bone marrow), and neutrophils. The mechanisms that lead to the profoundly low Hb concentrations witnessed in children with SMA are due to hemolysis and phagocytosis of pRBCs and npRBCs, and to a large extent, by suppression of erythropoiesis that is driven by PfHz-generated dysregulation in innate inflammatory mediators.

One of the emerging novel methods for managing the malarial diseases is in aiming at ameliorating the disease aspects through utilization of antidisease initiatives. The administrations of triterpenes with known antidisease properties are indicating potential for averting SMA development through maintaining the hematologic indices in severe malaria. The use of AA, MA, OA and other phytochemicals holds potentials in eradication of SMA and pathophysiology of malaria through their antioxidant, anti-inflammatory and immunomodulation capabilities. This introduces a new era in the management of SMA.
Acknowledgements

We like to thank the National University of Science and Technology (Zimbabwe) for providing an enabling environment for research and publications (Research Board initiatives) through which this work has been possible.

Conflict of interest

The authors declare no conflict of interest in this work.

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