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Cell-Free Mitochondrial DNA as a Damage-Associated Molecular Pattern and Candidate Biomarker in Sickle Cell Disease Vaso-Occlusive Crisis: A Scoping Review

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Systematic Review

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Abstract

Background

Sickle Cell Disease (SCD) is a chronic inflammatory disease that is continuously characterized by repeated hemolysis, vaso-occlusive crises (VOC), and chronic low-grade inflammatory state which is caused by the DAMPs released from the damaged red blood cells. Cell-free Mitochondrial DNA (cf-mtDNA) is one of these DAMPs due to its capability of activating the formation of neutrophil extracellular traps for the induction of VOC. However, the inflammatory processes and molecular mechanisms of cf-mtDNA in SCD remain unclear, and it is still unexplored as a potential biomarker especially within sub-Saharan African (SSA) populations. This review tried to synthesize the current information regarding cf-mtDNA in SCD and explore its origin, inflammatory processes, and potential uses as a clinical biomarker.

Method

The scoping review was conducted according to the Guidelines of the Joanna Briggs Institute Reviewer's Manual and reported in accordance with PRISMA-ScR guidelines. Searches were done in the databases of MEDLINE/Pubmed, Google Scholar, and Scopus with the keywords "sickle cell", "DAMP", and other inflammatory-associated terms. Out of the 1584 articles searched, only 11 fulfilled the requirements for inclusion. Nine studies were conducted in the US while the remaining two studies came from the Netherlands, while no studies included participants from Africa.

Results

Evidence suggests that cf-mtDNA might come from retained mature sickle erythrocytes and trigger NET formation through the cGAS-STING-TBK1 pathway. Nevertheless, there is limited direct evidence connecting cf-mtDNA to VOC occurrence, severity and response to treatment. Studies also presented evidence about biomarkers related to whole blood mtDNA counts, deletions and heteroplasmy load being more significant than the evidence related to cf-mtDNA itself.

Conclusion

The current evidence indicates that cf-mtDNA can be regarded both as a DAMP and a biomarker nonspecifically in regard to SCD, however, clinical relevance is yet to be confirmed. Further longitudinal research is necessary to explore the predictive power of cf-mtDNA for VOCs, severity of the disease and therapy efficacy as well as surface its relation with other inflammatory DAMPs. To solve these gaps in knowledge additional studies among larger cohorts with sufficient geographic diversity especially from SSA are necessary to be conducted.

1. Introduction

1.1 Sickle Cell Disease

Sickle cell disease (SCD) is widely known to be the most common life-threatening monogenic disease presenting with diverse clinical complications and multisystemic organ damage. According to the WHO's 2025 SCD fact sheets, approximately 7.74 million people were living with sickle-cell disease globally in 2021, with an estimated 515,000 new births in sub-Saharan Africa, representing nearly 80% of SCD cases globally (Inusa et al., 2019; Piel et al., 2017; WHO, 2025; Weatherall, 2010). SCD is a hemoglobin (Hb) disorder and results from a single point mutation; that is, a single adenine-to-thymine mutation that results in an amino acid substitution, a hydrophilic Glutamic acid with a hydrophobic Valine, in the 6th position of hemoglobin's beta-globin chain, generating hemoglobin S (HbS) (Inusa et al., 2019). The abnormal HbS differs from normal adult hemoglobin (HbA) in its molecular structure. Upon exposure to deoxygenation, HbS polymerizes through binding of a hydrophobic patch created by the isopropyl group of the βVal6 side chain in one of the β^s globin chains of an HbS tetramer with the hydrophobic pocket created by βAla70, βPhe85 and βLeu88 residues of another hemoglobin tetramer, whose hydrophobic patch binds to the hydrophobic groove of a third HbS molecule. This distorts red blood cells (RBCs) into stiff, sickle-shaped cells that have reduced capacity to flow through small blood vessels (Vaso-occlusion). They are also susceptible to premature breakdown (chronic hemolytic anemia) (Kunz & Tagliaferri, 2024a). There are a variety of clinical implications of SCD among affected individuals based on the severity of the disease, which include a lifelong pattern of vaso-occlusive crises (VOC), progressive multiple organ damage, and early mortality (DuPont et al., 2026; Lilly et al., 2026).

1.2 Burden

Globally, the distribution of SCD reflects the historical distribution of *Plasmodium falciparum* malaria, in which people who possess the sickle cell trait are less likely to be severely affected (Kunz & Tagliaferri, 2024b). Because of this, SCD is overwhelmingly common in sub-Saharan Africa. According to a study conducted as part of the Global Burden of Disease (GBD) in 2023, the number of people living with SCD globally augmented by 41.4%, from 5.46 million in 2000 to 7.74 million in 2021, with total annual births of babies with SCD hitting roughly 515,000 by 2021. By this same year, SCD birth incidence in sub-Saharan Africa had exceeded 2000 cases per 100,000 live births from only six countries (Equatorial Guinea, Burkina

Faso, Togo, Nigeria, Benin and Sierra Leone), contributing up to 44% of all SCD cases globally. This increase was largely contributed to by population growth in the Caribbean and western and central sub-Saharan Africa (Adeloye et al., 2026; WHO, 2025; Thomson et al., 2023). The rate of mortality is also disproportionately high in early childhood in the above-mentioned regions. According to Osei & McGann, (2023), SCD is the most neglected global health problem, referencing persistent gaps in its prevalence, diagnosis, and access to disease-modifying therapies throughout the region.

1.3 Damage-associated molecular patterns (DAMPs) and chronic inflammation in SCD

The understanding of SCD as a chronic inflammatory disease keeps broadening, beyond the mechanical consequences of HbS. Ischemia-reperfusion injury and repeated cycles of RBC breakdown discharge a variety of endogenous molecules, including high-mobility group box 1, free haem, extracellular ATP and cell-free nucleic acids. Although these endogenous molecules are not known to be pathogens, the innate immune system recognises them as signals of tissue damage. Together, these molecules are called DAMPs (Grazioli & Pugin, 2018; Zadeh et al., 2025). For instance, free haem has been shown to trigger the signaling of Toll-like receptor 4 (TLR4) on endothelial cells and to activate neutrophil extracellular trap (NET) formation, causing a direct linkage between haemolysis and neutrophil-mediated vaso-occlusive cascade (Ramadas & Sparkenbaugh, 2025). Currently, neutrophils are identified as central effectors of this cascade. When patients report a higher neutrophil count, they are often considered to have a higher risk of severe complications and premature death. Additionally, SCD patients' neutrophils exhibit an intrinsically hyperactive characteristic even outside of acute crisis.

Over the past few years, researchers discovered that cell-free DNA (cfDNA) is a key marker of SCD plasma (Al-Humood et al., 2014; Vasavda et al., 2007). The term cfDNA is defined as the genetic material either nuclear or mitochondrial that circulates in the extracellular space. In early research works utilizing quantitative PCR (qPCR) of the beta-globin gene, it was discovered that plasma cfDNA levels were considerably elevated in patients with acute VOC as compared to those of patients at steady state or individuals with the sickle cell trait or healthy controls, strongly implying a likely role as a diagnostic or disease monitoring biomarker (Symeonidis et al., 2006; Tavazzi et al., 2018). Similarly, another study found that circulating nucleosomes, a precursor for NET formation, were increased in patients with SCD regardless of steady state and increased even further during severe crises (Schimmel et al., 2013). Despite the fact that these studies collectively defined cfDNA as an attribute of SCD pathobiology, none of them differentiated the nuclear from the mitochondrial fraction of the cfDNA nor pinpointed a mechanism by which it might significantly contribute to, rather than just cause the disease.

1.4 Cell-free mitochondrial DNA as a DAMP

Evolutionarily, mitochondria are descendants of endosymbiotic bacteria and consist of a circular genome possessing bacteria-like characteristics such as unmethylated CpG motifs. The similarities that these substances possess lead to an easy detection of mitochondrial DNA (mtDNA) in the extracellular space by the same PRRs that identify bacterial DNA, and consequently, mtDNA may serve as an endogenous trigger of inflammation (Akhter et al., 2023). This property has been illustrated in serious illness and trauma for years now. However, its significance to SCD can be found in a more recent study.

In 2021, a study from the US National Heart, Lung and Blood Institute (NHLBI) showed, for the first time, that circulating cf-mtDNA is overly increased in SCD plasma as compared to cell-free nuclear DNA (cf-nDNA). This considerable amount of cf-mtDNA is highlighted by its unusual hypomethylation state and its capability to cause the generation of neutrophil extracellular traps by the cGAS-STING-TBK1 pathway. Importantly, this research linked this cf-mtDNA with erythrocytes that retain healthy mitochondria in the course of their maturation, a process that is usually accompanied by the elimination of this organelle. Another research study published in the same issue of blood referred to this finding confirming another source of DAMPs produced by sickle erythrocytes and linking cf-mtDNA with other signaling molecules released from erythrocytes (Tumburu et al., 2021a; Zhao et al., 2025).

Apart from the above-mentioned study, in addition to a few other studies on red-cell mitochondrial retention, Toll-like receptor 9 (TLR9)-mediated mtDNA scavenging and heme-induced NETosis, no follow-up studies have been conducted to replicate the original finding in an independent cohort or assessed cf-mtDNA in the sub-Saharan African population. Also, no study has investigated whether cf-mtDNA levels predict VOC onset, severity of the condition, or therapy response.

1.5 Rationale

Considering the fact that the concept of cf-mtDNA in SCD was first mechanistically defined in 2021, it is clearly an emerging concept, and the existing literature has not been synthesized yet. A scoping review, therefore, is the suitable first move before any attempt at a systematic review or clinical validation study, which seeks to compound comparable effect estimates across a homogeneous set of studies.

1.6 Objectives & Research Questions

This review follows the JBI-recommended Population-Concept-Context (PCC) structure to address the following:

Population

Individuals with any genotype of sickle cell disease spanning

Concept

cf-mtDNA as a damage-associated molecular pattern, considering its molecular origin and pathways through which it causes inflammation and vaso-occlusion, and its clinical significance as a biomarker.

Context

Any English-published study carried out in any research setting (clinical or translational) in any country.

1.6.1 Research questions that guided synthesis of evidence

RQ1. What do we know about the biological origin and mechanism of cf-mtDNA release in SCD?

RQ2. On the molecular level, what pathways does cf-mtDNA use to contribute to inflammation and vaso-occlusion?

RQ3. Is there evidence that supports cf-mtDNA as a biomarker of disease activity or severity?

RQ4. With respect to sub-Saharan Africa, where SCD is most common, what population, geographic, and methodological gaps characterize the current evidence base?

2. Conceptual Background and Related Literature

2.1 Mitochondrial retention as a feature of sickle erythropoiesis

Eliminating all anomalies, the terminal stage of reticulocyte maturation through mitophagy; a selective autophagy process, removes mitochondria. This is so to allow the circulation of organelle and nucleus free matured erythrocytes (W. Li et al., 2021; Moras et al., 2022). This clearance process however is interrupted in SCD. Systematically, mitophagy is usually activated when PINK1 (PTEN-induced kinase 1) builds up on the outer membrane of a depolarized mitochondrion and employs the E3 ligase Parkin to ubiquitinate outer-membrane proteins, tagging the organelle for autophagosomal clearance by means of the adaptor p62/SQSTM1 (Sequestome 1). Another group of hypoxia-sensitive proteins, BNIP3 (Bcl-2/adenovirus E1B 19-kDa-interacting protein 3) and its homolog NIX (NIP3-like protein X), function together by using LC3 protein (Microtubule-associated protein 1A/1B-light chain 3) to promote mitophagy in a ubiquitination-independent manner (PSVV et al., 2026; Wang et al., 2026). Using different advanced methods including structured illumination microscopy, electron microscopy and cytometry, researchers were able to demonstrate that SCD patients exhibit the disease related abnormal metabolic retention of mitochondria. This process is closely associated with decrease of the levels of PINK1 as well as NIX proteins and increase of the content of the chaperon protein HSP90 (Martino et al., 2021). The effect of red blood cell mitochondria retention on activation of innate immune system has been demonstrated in experiments where red blood cells retaining mitochondria have been co-cultured with neutrophils and showed an effect on release of endogenous interferons (Moriconi et al., 2022).

Recent research on mouse models has highlighted that stress erythropoiesis (increased production of red blood cells occurring under conditions of chronic anemia), is itself enough to drive mitochondrial retention in reticulocytes independent of the presence of sickle hemoglobin. In comparison with SCD mice, phlebotomized anemic mice without HbS displayed levels of retained mitochondria indicating that there is a possibility that the immediate driving factor of mitochondrial retention is the anemic, high-turnover erythropoietic state common to SCD rather than the sickling process itself (Gallivan et al., 2023). The above difference is significant since mitochondrial retention is a consequence of the prolonged stress erythropoiesis, then cf-mtDNA elevation is not unique to SCD but could also be seen in other states leading to hemolysis and stress-erythropoiesis. Consequently, the significance of this phenomenon as a marker of SCD has to be determined under these various states.

2.2 The cGAS-STING pathway and its relevance to erythrocyte-derived DNA

Cytosolic cyclic GMP-AMP synthase (cGAS) detects the foreign double-stranded DNA (ds-DNA) from invading microorganisms as well as self-DNA of the host leaking from damaged cellular compartments. Upon binding double stranded DNA, its structure alters, prompting cGAS to utilize cellular ATP and GTP in order to catalyze the production of a second messenger, cGAMP, that binds to the Stimulator of Interferon Genes (STING, a receptor protein found on the endoplasmic reticulum) and subsequently activating secondary signaling proteins, TANK-binding kinase 1 (TBK 1) and NF-kB (Li et al., 2013; Yu & Liu, 2021).

The NHLBI study in relation to this review showed that, the driving factor of considerably more NET formation in healthy donor neutrophils was plasma from SCD patients, but not comparable concentrations of genomic or platelet-derived mtDNA under matched conditions. The pharmacological inhibition of the secondary signaling proteins substantially blunted this NETosis (Tumburu, Ghosh-Choudhary, et al., 2021a). Though largely in vitro, this provides direct mechanistic evidence relating cf-mtDNA specifically and not cfDNA in general to a defined inflammatory signaling cascade.

2.3 Neutrophil extracellular traps as the downstream inflammatory effector

Neutrophil extracellular traps (NETs) are structures similar to webs that are formed from chromatin and granulocyte proteases like myeloperoxidase and neutrophil elastase. Although formation of NETs is meaningful in the context of resistance to host attacks, it is commonly understood that too much NET release only causes sterile inflammation, endothelial dysfunction, tissue damage, etc. When talking about the formation of NETs associated with SCD mechanisms, studies on free haem were carried out (Chen et al., 2014; de Ligt et al., 2024). For example, in studies on humanized SCD mouse models, researchers have shown that haem infusion contributes to NET formation, which enabled the discovery of NETs in the mice's lungs and their association with hyperthermia and death. In addition, the application of DNase I therapy which disrupted NETs prevented death from the disease, suggesting that using therapy targeting NETs may result in essential changes in treatment outcomes with SCD (Chen et al., 2014). The findings from the research conducted on Dutch groups indicate the increase of levels of circulating nucleosomes for SCD patients, both during stable state of health and during cell crisis together with the neutrophil activation marker (Schimmel et al., 2013). These pathways involving haem and cf-mtDNA are not contradictory. They suggest that many DAMPs originating from erythrocytes may contribute towards NET formation, resulting in the question as to whether cf-mtDNA is just an indispensable initiator, or an emerging signal in this process.

2.4 The erythrocyte as both sources and buffer of cell-free mitochondrial DNA

An additional sophistication to this field is that RBCs seem to play a dual role when it comes to cf-mtDNA. As mentioned above, they are an abnormal source of it in SCD. Yet, under physiological conditions they are also its main endogenous scavenger. Studies outside the SCD literature particularly have demonstrated that RBCs express TLR9 and homeostatically bind circulating CpG-containing mtDNA, hence neutralizing the majority of cf-mtDNA inactive conditions. Loss of this TLR9-mediated scavenging ability in animal models intensifies lung injury succeeding a systemic inflammatory damage (Hotz et al., 2018). A related study expanded this mechanism, demonstrating that TLR9-mediated DNA binding by RBCs can itself enhance innate immune activation and anemia when dysregulated (Lam et al., 2021). None of these two studies cited took place in an SCD population, but both are of relevance in explaining cf-mtDNA dynamics in SCD, since they propose that the final plasma level of cf-mtDNA measured in any given study reveals the stability between erythrocyte-mediated release (increased in SCD as a result of mitochondrial retention) and erythrocyte-mediated clearance (arguably compromised in SCD as a result of mitochondrial retention). No study highlighted in this review, however, has directly measured RBC-bound versus free-plasma cf-mtDNA fractions in an SCD cohort, indicating an obvious conceptual and methodological gap.

2.5 Cf-mtDNA in context: comparison with other disease states

The role of Cf-mtDNA as a DAMP is not just confined to SCD alone. Increased levels of cf-mtDNA is often observed in several other inflammatory conditions and is generally associated with cellular injury and tissue damage. Analogous literature has emerged around cf-mtDNA in systemic lupus erythematosus (SLE), where erythroid mitochondrial retention has been illustrated to activate myeloid-dependent type I interferon responses, and in inflammatory bowel disease (IBD), where cfDNA in general comprising mitochondrial function, has been proposed as a mechanistic and potential diagnostic liquid biopsy marker (Chuah et al., 2023; Liu et al., 2026). These analogous literatures are enlightening for two reasons. First, they show that the general biological plausibility of cf-mtDNA as an inflammatory driver is proven outside SCD, advancing indirect support to the SCD-specific findings. Second, they demonstrate what a maturing cf-mtDNA biomarker literature looks like; disease-severity relationship, multiple independent cohorts and exploration of therapeutic modulation, against which the relatively developing SCD-specific literature can be standardized.

3. Methods

3.1 Protocol

The methodology followed in this review is based on the use of the Reviewer's manual published by the Joanne Briggs Institute (JBI) on how to conduct and report scoping reviews (Peters et al., 2024). It is also based on the principles established in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018). The protocol for the scoping review was developed and recorded on Open Science Framework (OSF) on August 7th, 2026 (Amoako et al., 2026).

3.2 Population, Concept, Context (PCC)

A PCC framework was developed to define the scope of this review. The population comprised studies of individuals with any genotype of the sickle cell disease spanning. The concept is cf-mtDNA as a damage-associated molecular pattern, considering its molecular origin and pathways through which it causes inflammation and vaso-occlusion, and its clinical significance as a biomarker. For the context, any English-published study carried out in any research setting (clinical or translational) in any country was considered. The inclusion and exclusion criteria are explained in Table 1 (see supplementary files).

3.3 Search Strategy

A structured search strategy was constructed combining three concept blocks: (i) terms for cell-free, circulating or extracellular mitochondrial DNA, (ii) terms for sickle cell disease and its alternatives, and (iii) terms for vaso-occlusive crisis, inflammation and associated mechanistic concepts (DAMP, cGAS-STRING, neutrophil extracellular traps).

The following search string was used for MEDLINE/PubMed: ("cell-free mitochondrial DNA"[tiab] OR "circulating mitochondrial DNA"[tiab] OR "mitochondrial DNA"[tiab] OR "DNA, Mitochondrial"[MeSH]) AND ("sickle cell"[tiab] OR "Anemia, Sickle Cell"[MeSH]) AND ("vaso-occlusive"[tiab] OR "vaso occlusive crisis"[tiab] OR "pain crisis"[tiab] OR "DAMP"[tiab] OR "damage-associated molecular pattern"[tiab] OR inflammation[tiab]).

Then the following was used for Scopus and Google Scholar: ("cell-free mitochondrial DNA" OR "circulating mitochondrial DNA" OR "mitochondrial DNA") AND ("sickle cell" OR "sickle cell anemia" OR "sickle cell disease") AND ("vaso-occlusive" OR "vaso occlusive crisis" OR "pain crisis" OR "DAMP" OR "damage-associated molecular pattern" OR inflammation).

3.4 Study Selection

Search results were uploaded to Rayyan software to manage screening. Eligibility criteria defined in Table 1 were used during the selection process. Two independent reviewers screened the title and abstract of each source and then the full text. A third reviewer came in to resolve disagreements.

3.5 Data Charting

A well-structured charting form was created and piloted on the first included sources before being applied to the entire set of included papers. Extracted fields comprised author(s) and year, country/setting, study design, population and sample size, exposure/measurement method for cf-mtDNA, and key findings relevant to each research question.

3.6 Collating, Summarizing and Reporting results

In accordance with scoping review methodology and the heterogeneity of study designs and outcome measure involved, the findings for this study were synthesized narratively and thematically instead of statistically. Four themes were specified during charting and are presented in Section 4.3 through to Section 4.7: (i) origin and biological source of cf-mtDNA in SCD; (ii) mechanistic pathways linking cf-mtDNA to inflammation; (iii) evidence for cf-mtDNA as a clinical biomarker; and (iv) population and geographic distribution of the evidence base.

4. Results

4.1 Sources retrieved

The searches identified 1584 results from which 11 publications met the eligibility criteria for this scoping review and were included. See Fig. 2 for the search flow, including major reasons for exclusion. Seven of these were peer-reviewed primary research articles (Moriconi et al., 2022; Morris et al., 2020; Ramasamy et al., 2025; Soupene et al., 2025; Tumburu et al., 2021; Van Avondt et al., 2023), while the remaining four (4) were peer-reviewed American Society of Hematology (ASH) annual meeting (Ahmad et al., 2020; Ray et al., 2023, Ray et al., 2025; Tumburu et al., 2021, Ahmad, et al., 2021). The publications included were predominantly from research groups in the United States (n = 9), then the Netherlands (n = 2). None were conducted in, or included participants from sub-Saharan Africa. Thus, current sickle cell research fails to represent the geographical areas where the disease is most common.

4.2 Article Characteristics

The participants in human studies included adults and children suffering from SCD, including HbSS, HbSC, HbSβ^0-thalassemia, and HbSβ^+-thalassemia genotypes, along with sickle trait individuals (HbAS), hemoglobin-C trait individuals (HbAC), and healthy controls. The studies reviewed included both non-clinical and clinical humanized Townes mouse models of sickle cell disease with HbAA, HbAS, and HbSS genotypes (Ray et al., 2023, Ray et al., 2025, Ray et al., 2026; Tumburu et al., 2021). The overview highlighted the results concerning mitochondrial retention in erythrocytes, circulating cf-mtDNA, mtDNA heteroplasmy, mtDNA-CN value, mtDNA deletions, mitochondrial functioning, oxidative stress, neutrophil extracellular traps (NETs) formation, and potential points of affecting therapy.

Table 2
Characteristics of included sources (n = 11)

References	Study design	Population	Main focus	Measurement method for ct-mtDNA	Key finding
Ahmad et al., 2020	Peer-reviewed ASH abstract, cross-sectional WGS	683 SCD patients, 621 1KG controls	mtDNA heteroplasmy burden according to SCD genotype	WGS-derived mtDNA heteroplasmy/homoplasmy calling (mitoCaller/mitoAnalyzer) from whole blood WGS	No unique homoplasmic variants in SCD vs 1KG For SCD genotypes, the heteroplasmic burden increased progressively (HbAS < HbSβ $^{+}$ thalassemia < HbSC < HbSS & HbSβ^{0}); variant 2623A > G heteroplasmy elevated with age in HbSS/HbSβ^{0}
Morris et al., 2020	Peer-reviewed primary research single-center, prospective, randomized, open-label intervention study	12 children with SCD and VOC pain	Effect of arginine on platelet mitochondrial function	Platelet-rich-plasma (PRP) mitochondrial DNA quantified to assess mitochondrial number Mitochondrial activity, and protein carbonyls and malondialdehyde (biomarkers of protein and lipid oxidation respectively) were measured spectrophotometrically	All VOC subjects experienced considerable decrease in platelet complex-V activity.
Tumburu et al., 2021a	Peer-reviewed ASH abstract, cross-sectional 3-cohort comparison	NIH SCD cohorts (n = 676), 1KG (n = 621), Jackson Heart study (n = 3,580)	Independent validation of heteroplasmy burden	WGS-derived mtDNA heteroplasmy calling (mitoCaller/mitoAnalyzer) from whole-blood WGS	Median heteroplasmic variants/individuals increased gradually, HbAA→HbAS→HbSβ+→HbSC→HbSS/HbSβ0
Tumburu et al., 2021	Peer-reviewed primary research, cross-sectional cohort study (WGS + targeted qPCR + bisulphite-seq of plasma cfDNA) accompanied by confirmatory flow cytometry	SCD patients (n = 34) at baseline, healthy controls, Townes mice	Cf-mtDNA as DAMP, mitochondrial retention, mechanism of NETosis	Actual plasma cf-mtDNA measurement by: (1) WGS of plasma cfDNA mapped to mitochondrial vs nuclear genome; (2) targeted qPCR; (3) whole-genome bisulfite sequencing of plasma cfDNA for mitochondrial DNA methylation	In SCD plasma, cf-mtDNA was disproportionately increased as compared to healthy control. cf-mtDNA fragments were short and disproportionately hypomethylated, with hypomethylation relatively higher in crisis vs steady-state. Mature circulating SCD RBCs unusually retained depolarized, high-ROS mitochondria (confirmed by flow cytometry), showing that erythrocytes are probably the source of the elevated cf-mtDNA.
Moriconi et al., 2022	Peer-reviewed primary research Cross-sectional cohort study involving *in vitro* functional assays and co-culture experiments	SCD patients, healthy controls with mature RBCs	Retention of functional mitochondria in mature RBCs	Intracellular mitochondria measured by flow cytometry in CD71 + reticulocytes and CD71- mature RBCs, confirmed by electron microscopy. mtDNA copy number measured by duplex TaqMan qPCR Mitochondrial enzymatic activities detected spectrophotometrically	SCD patients exhibited relatively higher frequencies of mitochondria-positive CD71 + reticulocytes and CD71-mature RBCs as compared to healthy donors and sickle trait

References	Study design	Population	Main focus	Measurement method for ct-mtDNA	Key finding
Van Avondt et al., 2023	Peer-reviewed primary research Cross-sectional study of paired serum/plasma samples with *in vitro* neutrophil	18 SCD patients with paired VOC/steady-state samples	Iron/heme-related NETosis and DAMP pathway	Total serum heme via colorimetric assay, serum hemopexin/Hpx via in-house ELISA, labile plasma iron/LPI via dihydrorhodamine fluorescence, and neutrophil NET.	Decreased serum hemopexin and increased cell-free heme and labile plasma iron were observed in SCD patients. When exposed to SCD sera from VOC, neutrophils discharged more NETs as compared to steady-state.
Ray et al., 2023	Peer-reviewed ASH abstract Preclinical, humanized SCD mouse model (Twones), cross-sectional tissue survey.	Townes mice (HbAA, HbAS, HbSS), 9 tissues	Tissue-specific mtDNA heteroplasmy	Genomic mtDNA obtained from tissue DNA by long-range PCR. Illumina deep sequencing, LoFreq variant calling	Out of 161 samples, 11 unique variants were found in 106 of them and HbSS showed the highest variant concentration vs HbAS and HbAA. Four variants unique to HbSS (MT:6753 COX1 Phe476Leu, MT:6794, MT:1220, MT:425)
Ray et al., 2025	Peer-reviewed ASH abstract Longitudinal, preclinical mouse model (townes)	60 Townes mice followed at 8 time points	Age-related accumulation of mtDNA heteroplasmy	Blood-obtained mtDNA deep sequencing for heteroplasmy. SIFT for deleterious-variant prediction. WGS-based mtDNA copy number quantification	HbSS mice exhibited greater mortality than HbAA/HbAS. Total heteroplasmy burden was elevated 18.38% by T3 vs baseline; HbSS increased whilst HbAA/HbAS showed minimal change.
Ramasamy et al., 2025	Peer-reviewed primary research Cross-sectional, comparative cohorts (human erythrocyte and plasma samples)	SCD erythrocytes (HbSS vs HbSC genotypes) vs healthy HbAA controls	Autophagy dysregulation, mitochondrial retention, oxidative stress	Mitochondrial retention (intracellular) measured by flow cytometry and reactive oxygen species by CM-H2DCFDA. Autophagy-related gene expression measured by RT2 Profiler PCR Array Autophagy protein levels measured via immunoblot	HbSS patients had significantly higher erythrocyte mitochondrial retention as compared to HbSC and HbAA. ROS-rich erythrocytes were also higher in HbSS (23.5 ± 3.7%) vs HbAAA (7.2 ± 1.4%) vs HbSC (3.3 ± 3.7%).
Soupene et al., 2025	Peer-reviewed primary research, cross-sectional pediatric cohort	Pediatric HbSS (n = 19) and HbSC/HbSE (n = 8)	RBC mitochondrial retention and mitochondrial load	Absolute qPCR on FACS-sorted CD45-negative RBC mtDNA Mito-positive RBC portion by flow cytometry (TMRM dye)	HbSS clearly showed higher mtDNA copies/RBC and higher mito-positive RBC fraction as compared to HbSC/HbSE copies per mito-positive RBC similar across genotypes
Ray et al., 2026	Peer-reviewed primary research Multi-cohort cross-sectional + longitudinal survival analysis (human) and cross-sectional mouse tissue study	1043 adults across 3 human cohorts, and 18 Townes mice	mtDNA copy number, heteroplasmy, mtDNA deletions, age, severity, mortality	Whole blood buffy-coat WGS-derived mtDNA and heteroplasmy (MitoHPC/LoFreq, VEP annotation, cohort-targeted VAF thresholds)	mtDNA copy number and heteroplasmy burden elevated with genotype severity in all 3 cohorts

4.3 Origin and Biological Source of cf-mtDNA in SCD

The most compelling evidence indicates that cf-mtDNA likely originates from mitochondria that remain inside mature sickle RBCs. As per Tumburu et al., 2021, mature enucleated RBCs from patients having SCD have higher amounts of mtDNA than normal controls and individuals suffering from sickle trait. The remaining mitochondria had abnormalities in its membrane characteristics and higher amounts of reactive oxygen species suggesting that inefficient clearance of mitochondria during the process of red blood cells maturation might be responsible for cf-mtDNA.

According to Soupene et al. 2025, further quantitative confirmation was provided by the flow cytometry together with standard curve qPCR. It was revealed that about 11% of the pediatric patients had red blood cells with mitochondria which had an estimated average of around 21 copies of mitochondrial DNA per RBC and 400 copies of mtDNA per each positive RBC. An inverse correlation between the number of RBCs with mitochondria and their mtDNA content was detected. It implies that RBCs with increased mitochondrial content will be cleared more intensively. Additionally, the amount of mtDNA was correlated with the concentration of hemoglobin.

According to Moriconi et al., 2022, the mature SCD RBCs were shown to have active mitochondria, whereas the work of Ramasamy et al., 2025 highlighted the changes that take place related to mitophagy and oxidative stress. Overall, both presented conclusions that suggest impaired mitochondrial clearance could lead to the maintenance of damaged and stressed mitochondria within the mature sickle RBCs leading to the release of cf-mtDNA in cells.

4.4 Mechanistic Pathways Linking cf-mtDNA to Inflammation

Tumburu et al., 2021, have found that cf-mtDNA behaves like a proinflammatory DAMP element in SCD. By means of WGS and targeted qPCR, it has been proven that the levels of mtDNA compared to nuclear DNA are elevated in the plasma of SCD patients and that the cf-mtDNA/cf-nDNA ratios increase in both steady state conditions and during VOC. Furthermore, it has been established that mtDNA is hypomethylated in higher degrees during VOC. It follows that plasma with a high level of cf-mtDNA provokes NET formation in neutrophils which are healthy. In addition, the inhibition of TBK1 reduced the described effect, while the inhibition of TLR9 and TLR4 did not cause any impact on the given event, which indicates that cf-mtDNA exerts its influence through the cGAS-STING-TBK1 pathway. Additionally, the authors confirmed that there are elevated levels of cf-mtDNA found in the case of Townes HbSS mice when compared to HbAA mice, contributing to the above-mentioned data. Thus, while the present article provided information on the underlying mechanisms of the relationship between mtDNA and NETosis, it did not present any evidence linking cf-mtDNA-induced NETosis to clinical VOCs.

van Avondt et al., 2023 indicated labile plasma iron as one of the causes of NET formation in SCD, attributed to the process of hemolysis. Although cf-mtDNA content was not analyzed in this study, there is a significant increase in NETosis related to iron during VOC, which can be inhibited with iron chelation but does not seem to be affected by hemopexin. Thus, the findings of this investigation provide some indirect information on the role of various DAMP pathways, including mtDNA and labile iron, in NET formation in SCD cases, but the study shouldn't be interpreted as making a direct conclusion about the effect of cf-mtDNA.

4.5 Evidence for cf-mtDNA as a Clinical Biomarker

The evidence of the capability of biomarkers was more extensive with mtDNA genomic indicators than with cf-mtDNA. Studies performed by Ahmad et al., 2020 and Tumburu et al., 2021, determined that the burden of heteroplasmic mtDNA variants was significantly raised in patients with more severe forms of SCD, mainly HbSS/HbSβ⁰-thalassemia. Hence, it can be asserted that mtDNA heteroplasmy correlates with cumulative stress on the mitochondria; however, it does not indicate its usability as a biomarker.

Ray et al., 2026, confirmed this finding in 1,043 adults divided into three groups. It has been shown that mtDNA copy number (mtDNA-CN) increases with the severity of genotype HbAA/HbAS < HbSβ⁺-thalassemia < HbSC < SCD (HbSS/HbSβ⁰-thalassemia). At the same time, the level of non-synonymous heteroplasmy was also increasing, measured at 0.12 for SCD versus 0.05 for other genotypes ($P = 0.001$). Among patients with sickle cell disease, non-synonymous heteroplasmy was higher in the group over 30 years old than in younger patients (0.14 vs 0.09, $P = 0.03$); however, the mtDNA-CN decreased with age. The team of scientists found an increase in mtDNA deletion burden associated with more severe cases of SCD, as well as identified mtDNA mutations leading to all-cause mortality. The lower the mtDNA-CN is, the higher the NIH phenotypic risk score is.

4.6 Population, Methodological gaps, and Geographic Distribution of the Evidence Base

One of the identified gaps in the publications under review is geographic coverage. Every research done on humans derives from research institutes based in either the USA or the Netherlands. None of the studies took into account participants from the sub-Saharan Africa region, thus limiting the extent to which the findings from the research can be generalized to populations where SCD is very common.

Several approaches, including flow cytometry, microscopy, qPCR, plasma cfDNA sequencing, WGS, and other variant-calling techniques have been used in many studies for mtDNA assessment. Soupene et al., 2025, highlighted the fact that the mtDNA normalisation method used will influence the determination of mitochondrial content in RBCs and that the time of plasma processing will determine the volume of cf-mtDNA returned. The variability in sequencing depth and variant-calling criteria has further complicated direct comparisons between heteroplasmy rates determined in the studies.

In a study conducted by Ray et al., 2026, they utilized LoFreq for variant calling in both human and mouse cohorts. Their focus was primarily on the analysis of mtDNA-CN heteroplasmy and large mtDNA deletions. The major limitation of the study is that since whole blood/buffy-coat DNA was used for human studies, it was not possible to ascertain which cells led to the detection of the different mtDNA alterations observed. This is crucial when meaning to compare findings with the findings from studies of plasma cf-mtDNA.

Evidence of longitudinal studies remains scarce. According to Ray et al., 2025, it was highlighted how mtDNA heteroplasmy increases due to aging in Townes mice. On the other hand, Ray et al., 2026, studied the correlation between mtDNA and mortality in humans related to it. However, unlike the previously mentioned studies, none of the studies focused on evaluation of how reference plasma cf-mtDNA measurements could help predict VOC, VOC severity, and treatment response.

5. Discussions

cf-mtDNA has become an effective erythrocytic DAMP in SCD, especially during VOCs. Research has shown that in SCD, patients contain abnormally elevated levels of cf-mtDNA in their plasma as a result of the abnormal retention of mitochondria in sickled erythrocytes and the destruction of the sickled cells during VOCs (Tumburu, Ahmad, et al., 2021). It is essential to note that the cf-mtDNA has a tendency to be hypomethylated and possesses incredible immunostimulatory properties and studies shows that the sickle cell patients illustrate that cf-mtDNA can activate NET formation and intensify inflammation through innate DNA-sensing mechanisms such as TLR9 and cGAS-STING (Rai & Fessler, 2025). For example, in the research conducted using cf-mtDNA plasma derived from SCD patients, it turned out that this plasma was able to cause NETosis in normal healthy neutrophils. Moreover, the experiments with co-culture of sickle red blood cells and normal neutrophils prove that sickle red blood cells contributed to inhibiting type I interferon production (Tumburu et al., 2021).

Further mechanistic analysis highlights the ways in which cf-mtDNA interacts with the host immune system. Just like bacterial DNA, cpG-motifs of unmethylated mtDNA act as powerful activators of endosomal TLR9 receptors on immune system cells, leading to stimulation of the production of NF-kB-dependent cytokines. At the same time, cytosolic cf-mtDNA activates the cGAS-STING-TBK1 cascade, inducing a type I interferon response and triggering NLRP3 inflammasome activity. Though experiments specifically highlighting cf-mtDNA-dependent activation of the cGAS-STING pathway in SCD remain rare, the link between this pathway and NETosis in several other conditions may indicate its fundamental role in perpetuation of the inflammatory cascade during VOC (Giordano et al., 2025; Vanportfliet et al., 2024). For example, research done in sterile inflammatory conditions shows that mtDNA can stimulate the cGAS-STING pathway and initiate NET formation. In SCD, neutrophils and platelets express the components of innate DNA-detection machinery, and cf-mtDNA-containing plasma in SCD can cause NETosis through a TBK1-mediated mechanism consistent with the cGAS-STING signaling pathway. Although TLR9 inhibitors have been studied in SCD and did not significantly affect cf-mtDNA-induced NETosis, direct pharmacological inhibition of STING itself has not been investigated yet (Liu et al., 2019). Thus, it remains to be established how STING contributes to cf-mtDNA-induced NETosis in VOCs.

cf-mtDNA has proved to be a potential biomarker of VOC clinically. Initial research indicated increased levels of cfDNA, mostly reflecting mtDNA, during acute pain crises. One such study provided evidence that the levels of cfDNA were higher in SCD patients during VOC than in the steady state or the control group (Al-Humood et al., 2014). Thus, an increase in cfDNA levels correlated with the severity of hemolysis, but there was no evident distinction in the processes occurring in the patients experiencing complications. Moreover, recent data demonstrate an uncommonly high amount of cf-mtDNA in SCD plasma with a greatly increased cf-mtDNA/cf-nDNA rate among patients. However, the lack of standardized tests for cf-mtDNA, for example, digital PCR for mt encoded DNA, affects the situation since tests are not performed in clinical labs (Vasavda et al., 2007). In addition, cf-mtDNA levels can be affected by the donor history and sample collection and preparation methods followed (Tumburu, Ahmad, et al., 2021).

6. Conclusions

In conclusion, there are still many significant gaps in knowledge, even though the information is compelling. Almost every mechanistic and clinical study of cf-mtDNA in SCD has been carried out outside of sub-Saharan Africa, which is the area that suffers the highest rates of morbidity and mortality caused by SCD worldwide. There have not been any large-scale studies of cf-mtDNA concentration and mtDNA inflammation in African populations suffering from SCD. There are numerous genetic and environmental factors in Africa that may determine cf-mtDNA release and immune response. For example, co-infections and diet can contribute to neutrophil stimulation. Furthermore, the existing evidence is still fragmented, as the sample sizes are small and the studies are conducted with cross-sectional designs. The major challenge is that most of the research data often comes from animal studies or observations from isolated patients. Considering that there has been no long-term observation of cf-mtDNA release from blood cells during VOC episodes of treatment (Scourfield et al., 2025). Moreover, not enough is known about the relationship between cf-mtDNA and other DAMPs like heme in VOC pathology (Nkya et al., 2026).

It is essential to solve these pressing challenges by progressing studies in African populations and including cf-mtDNA measurements in large scale clinical trials of advanced therapies for SCD (Nkya et al., 2026). If interventions aimed at neutralizing cf-mtDNA, for instance, DNase enzymes or blocking detection of cf-mtDNA occur, then new therapeutic mechanisms will be discovered. In general, the gathered data indicate that cf-mtDNA is both involved in SCD vaso-occlusion and is its marker. Understanding the role of cf-mtDNA means closing the gaps in current knowledge, especially in the context of Africa.

Abbreviations

cfDNA: Cell-free DNA

cf-mtDNA: Cell-Free Mitochondrial DNA

cf-nDNA: Cell-Free Nuclear DNA

cGAS: Cyclic GMP-AMP synthase

DAMP: Damage-Associated Molecular Patterns

ds-DNA: double-stranded DNA

GBD: Global Burden of Disease

Hb: Hemoglobin

HbA: Adult hemoglobin

HbS: Hemoglobin S

JBI: Joanne Briggs Institute

mtDNA: Mitochondrial DNA

mtDNA-CN: Mitochondrial DNA Copy Number

NET: Neutrophil Extracellular Trap

NHLBI: National Heart, Lung, and Blood Institute

PCC: Population, Concept, Context

PRISMA-ScR: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews

qPCR: Quantitative PCR

RBCs: Red Blood Cells

RQ: Research Question

SCD: Sickle Cell Disease

SSA: sub-Saharan Africa

TLR4: Toll-like receptor 4

VOC: Vaso-Occlusive Crisis

1KG: 1000 Genomes Project

Declarations

Authors' Contributions

JPA: Conceptualization, Supervision, Data curation, Investigation, Methodology, Project administration, Writing, Review and Editing.

AO: Conceptualization, Investigation, Data curation, Methodology, Writing, Review and Editing.

CPSVV: Conceptualization, Supervision, Investigation, Project administration, Writing, Review and Editing.

DEAP: Conceptualization, Investigation, Methodology, Writing, Review and Editing.

EEA: Conceptualization, Investigation, Data curation, Writing, Review and Editing.

KOA: Conceptualization, Investigation, Data curation, Writing, Review and Editing.

VM: Conceptualization, Investigation, Writing, Review and Editing.

HKA: Conceptualization, Investigation, Writing, Review and Editing.

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Figures

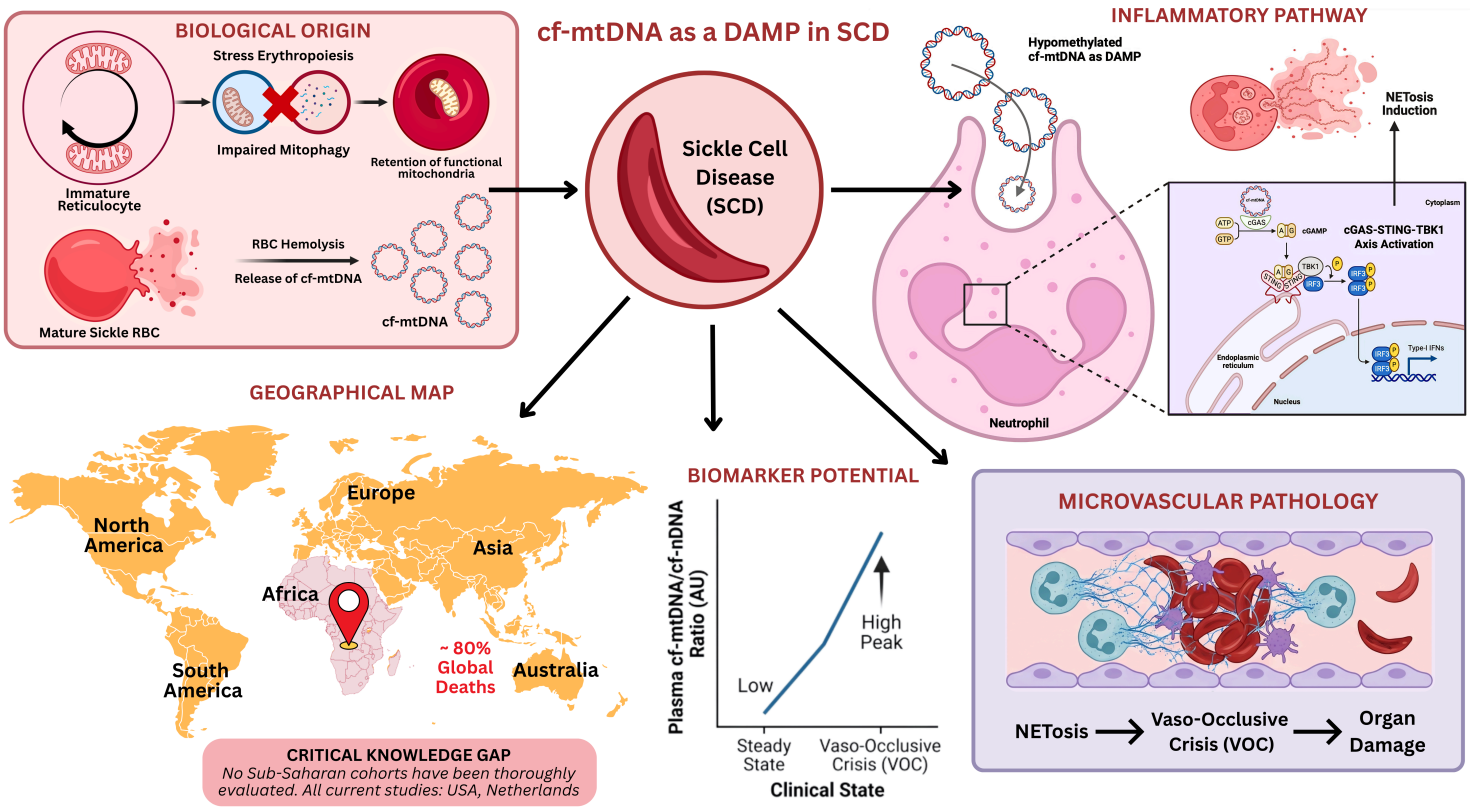


Figure 1

Graphical Abstract (Designed using BioRender and Canva software).

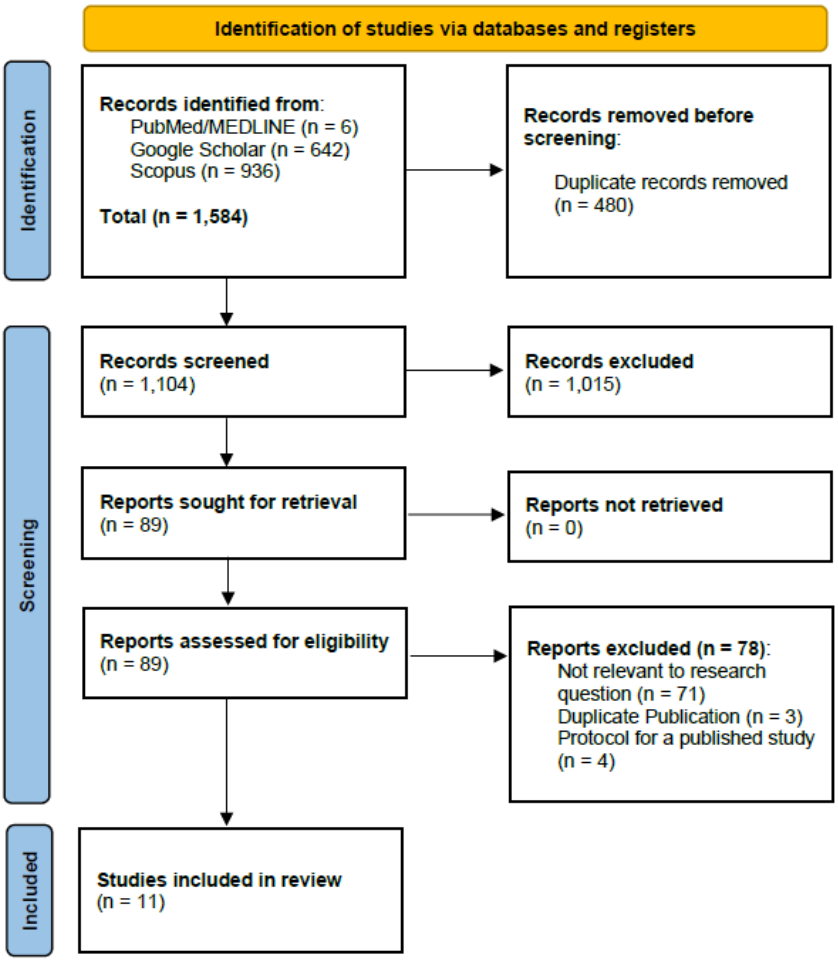


Figure 2

PRISMA Study Flow Chart

Supplementary Files

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- Table1EligibilityCriteria.pdf