

Haemorheological Biomarkers in Sickle Cell Disease: From the Viscosity–Haemolysis Paradox to Multidimensional Clinical Phenotyping

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Abstract

Sickle cell disease (SCD) is a heterogeneous inherited red blood cell disorder characterised by haemoglobin S polymerization, erythrocyte deformation, haemolysis, vaso-occlusion, inflammation, endothelial dysfunction, and progressive multiorgan injury. This narrative review is based on a structured search of PubMed, Scopus, and Web of Science for English-language literature published up to January 2026, examines the biological basis of the viscosity–haemolysis paradox, summarizes current and emerging haemorheological and haemolysis biomarkers, evaluates their relationships with clinical subphenotypes and discusses implications for precision medicine and biomarker-guided therapeutic strategies; sources were selected and synthesized qualitatively rather than by systematic meta-analysis. Although SCD results from a relatively well-defined genetic defect, its clinical expression varies considerably among individuals. Increasing evidence suggests that this heterogeneity may partly reflect the relative contribution of two interconnected pathophysiological axes: a viscosity–vaso-occlusion axis, associated with relatively preserved haemoglobin concentration, increased blood viscosity, impaired red blood cell (RBC) deformability and recurrent vaso-occlusive complications; and a haemolysis–endothelial dysfunction axis, characterised by severe anaemia, increased intravascular haemolysis, nitric oxide depletion, vascular dysfunction and complications such as pulmonary hypertension, priapism, leg ulceration and cerebrovascular disease. Recent studies have expanded this framework by identifying additional rheological, endothelial, inflammatory, oxidative and extracellular vesicle biomarkers and by demonstrating substantial overlap between traditionally defined subphenotypes. Blood viscosity, RBC deformability, RBC aggregation, point of sickling, oxygen-gradient ektacytometry parameters, haematocrit-to-viscosity ratio, lactate dehydrogenase, plasma-free haemoglobin, bilirubin, reticulocyte count, arginase activity and endothelial-derived markers may collectively provide a more informative representation of disease biology than conventional haematological indices alone. Advances in microfluidics, high-throughput deformability measurements and machine-learning approaches are further enabling multidimensional characterisation of SCD.

Keywords: Sickle cell disease; blood viscosity; haemorheology; haemolysis; red blood cell deformability; clinical subphenotypes

Introduction

Sickle cell disease (SCD) is among the most common monogenic disorders worldwide. Global Burden of Disease (GBD) 2021 analyses estimated that approximately 7.74 million people were living with SCD worldwide in 2021, while the number of affected births increased substantially between 2000 and 2021. The burden is especially concentrated in sub-Saharan Africa, where diagnostic and longitudinal disease surveillance remain limited (GBD 2021 Sickle Cell Disease Collaborators, 2023). The downstream consequence is deceptively simple to state and extraordinarily complex to treat: under deoxygenation, HbS polymerizes into rigid intracellular fibers that distort the erythrocyte, degrade its deformability, and trigger a cascade of haemolysis, adhesion, inflammation and microvascular obstruction (Eaton and Bunn, 2017; Kato *et al.*, 2017).

Critically, the pathophysiology spans spatial and temporal scales that differ by many orders of magnitude: from the nanosecond-to-second kinetics of HbS nucleation, through the millisecond mechanics of single-cell deformation in capillaries, to the years-long accrual of organ damage. The pathophysiology of sickle cell disease involves interconnected molecular, cellular, vascular, and tissue-level processes. HbS polymerization promotes erythrocyte sickling and abnormal rheological behaviour, while recurrent haemolysis contributes to nitric-oxide depletion and endothelial dysfunction. These processes converge on impaired microvascular flow, vaso-occlusion, tissue hypoxia, and progressive organ injury (Figure 1),

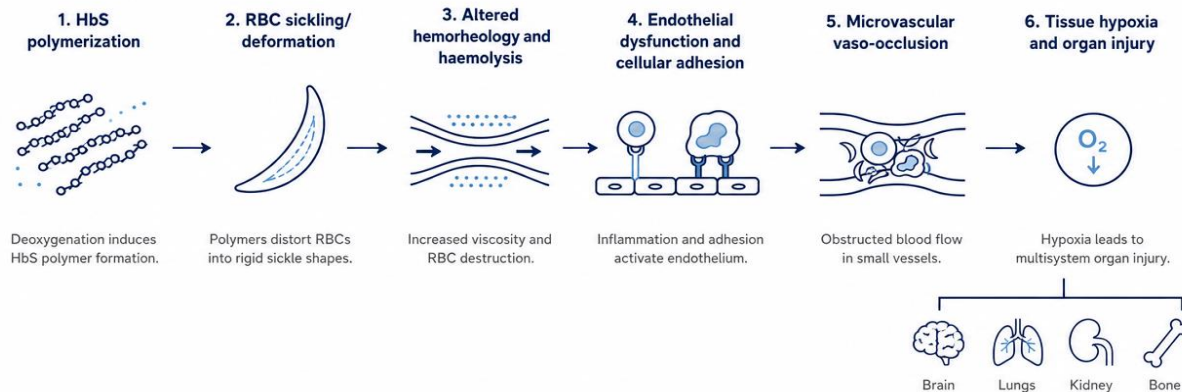


Figure 1: Pathophysiological Cascade linking HbS Polymerization to Clinical Complications in Sickle Cell Disease.

The clinical expression of SCD is remarkably heterogeneous. Some individuals experience recurrent vaso-occlusive pain, acute chest syndrome, or osteonecrosis, whereas others develop complications associated more closely with chronic haemolysis and vascular dysfunction, including pulmonary hypertension, priapism, leg ulceration, nephropathy, and cerebrovascular disease. This heterogeneity cannot be adequately explained by the presence of haemoglobin S alone and reflects the interaction of genetic modifiers, fetal haemoglobin, α -thalassaemia, RBC properties, endothelial activation, inflammation, haemolysis, and environmental factors (Connes, 2024).

Haemorheology—the study of blood flow properties and the mechanical behaviour of blood cells—provides an important framework for understanding this heterogeneity. Blood viscosity is influenced by haematocrit,

plasma viscosity, RBC deformability, RBC aggregation, cell density, and cellular interactions with the vascular endothelium. In SCD, these variables are profoundly altered. HbS polymerization produces rigid erythrocytes, while chronic haemolysis removes circulating RBCs and generates a state of anaemia that may lower whole-blood viscosity. Consequently, a patient with severe haemolysis may have relatively low blood viscosity while simultaneously experiencing substantial endothelial injury and vasculopathy (Connes, 2024).

Lactate dehydrogenase (LDH), indirect bilirubin, reticulocyte count and haptoglobin are routinely used to index haemolytic rate, and composite “haemolytic component” scores derived from them predict vasculopathic complications and mortality (Nouraie *et al.*, 2013; Kato *et al.*, 2006). However, these markers are blind to the rheological state of the circulating blood: two patients with identical LDH may differ substantially in whole-blood viscosity, RBC deformability and aggregation, and therefore in their microvascular flow reserve (Connes *et al.*, 2016; Nader *et al.*, 2019b). A haemolysis-only lens thus discards precisely the information that distinguishes vaso-occlusive from vasculopathic risk. This apparent contradiction has been described as the viscosity–haemolysis paradox. It is not, however, a true physiological contradiction. Rather, it reflects the fact that viscosity and haemolysis influence different, although interconnected, components of SCD pathophysiology. Understanding this distinction may improve clinical phenotyping and facilitate the development of composite biomarkers capable of identifying individuals at increased risk of specific complications. This review synthesizes the current evidence linking haemorheological biomarkers to clinical subphenotypes of SCD, with particular emphasis on the mechanistic basis and clinical implications of the viscosity–haemolysis paradox.

Despite considerable progress, haemorheological and haemolytic biomarkers have rarely been integrated into a single, clinically actionable framework, and prevailing classifications remain largely binary (vaso-occlusive versus hyperhaemolytic). This review addresses three questions: (i) what is the biophysical basis of the viscosity–haemolysis paradox; (ii) which haemorheological and haemolysis biomarkers map onto specific clinical subphenotypes and organ-specific complications; and (iii) how might multidimensional, computationally integrated profiling improve individualized risk stratification? We present the viscosity–haemolysis relationship explicitly as a conceptual two-axis model (Section 5) intended to organize existing evidence, not as a validated clinical classifier.

Review Methodology

This article is a narrative review. It was designed to synthesize and conceptually integrate the literature on haemorheological and haemolysis biomarkers and their relationship to clinical subphenotypes in sickle cell disease, rather than to answer a single narrowly defined question by systematic meta-analysis. We searched PubMed/MEDLINE, Scopus, and Web of Science for English-language articles published between January 2000 and January 2026, supplemented by hand-searching of the reference lists of key reviews. Search terms combined controlled vocabulary and free-text keywords for the population ("sickle cell disease," "sickle cell anaemia," "HbSS," "HbSC") with terms for the exposures and constructs of interest ("haemorheology," "blood viscosity," "red blood cell deformability," "ektacytometry," "red cell aggregation," "haematocrit," "haemolysis," "lactate dehydrogenase," "nitric oxide," "arginase," "microfluidics," "machine learning") and for clinical outcomes ("vaso-occlusion," "acute chest syndrome," "pulmonary hypertension," "leg ulcer," "priapism," "stroke," "nephropathy," "clinical phenotype").

Priority was given to primary studies, foundational biophysical and mechanistic papers, and recent (2019–2026) work introducing novel rheological or computational biomarkers. Studies were included when they addressed the biophysical basis of blood rheology or haemolysis in SCD, reported haemorheological or

haemolysis biomarkers in relation to clinical outcomes, or described relevant measurement or computational methods. Non-English publications, conference abstracts lacking sufficient methodological detail, and studies unrelated to human SCD were generally excluded, except where a landmark abstract provided otherwise unpublished data.

Because this is a narrative rather than a systematic review, we did not register a protocol, apply PRISMA screening, or perform a formal risk-of-bias or GRADE assessment; the current evidence base—dominated by cross-sectional and observational studies—does not yet support quantitative pooling. To limit selection bias, we prioritized peer-reviewed primary sources over secondary citations and, throughout the text, explicitly distinguish empirically validated findings from conceptual or hypothesis-generating proposals.

Biological Basis of Haemorheology in Sickle Cell Disease

Blood Viscosity as a Biomarker

Viscosity is the resistance of a fluid to shear deformation. For blood the operative quantity is *apparent* viscosity, because the value depends on shear rate. Its determinants are haematocrit (dominant), plasma viscosity, RBC deformability and RBC aggregation (Baskurt and Meiselman, 2003; Nader *et al.*, 2019b).

Newton's definition shear stress τ divided by shear rate $\dot{\gamma}$ frames the measurement:

$$\eta = \frac{\tau}{\dot{\gamma}}, \quad \dot{\gamma} \approx \frac{8 \times \bar{v}}{d} \quad (1)$$

where $\bar{v}$ is mean centreline velocity and d the vessel diameter, so that shear rate and hence the relevant viscosity varies systematically across the vascular tree (Connes *et al.*, 2016).

Whole-blood viscosity integrates cellular and plasma contributions; plasma viscosity isolates the protein-driven component and is itself elevated in inflammatory states relevant to SCD. Both should be reported, because a normal whole-blood viscosity can mask an abnormal plasma viscosity when haematocrit is low (Nader *et al.*, 2019b). Because blood is shear-thinning, a viscosity value is uninterpretable without its shear rate.

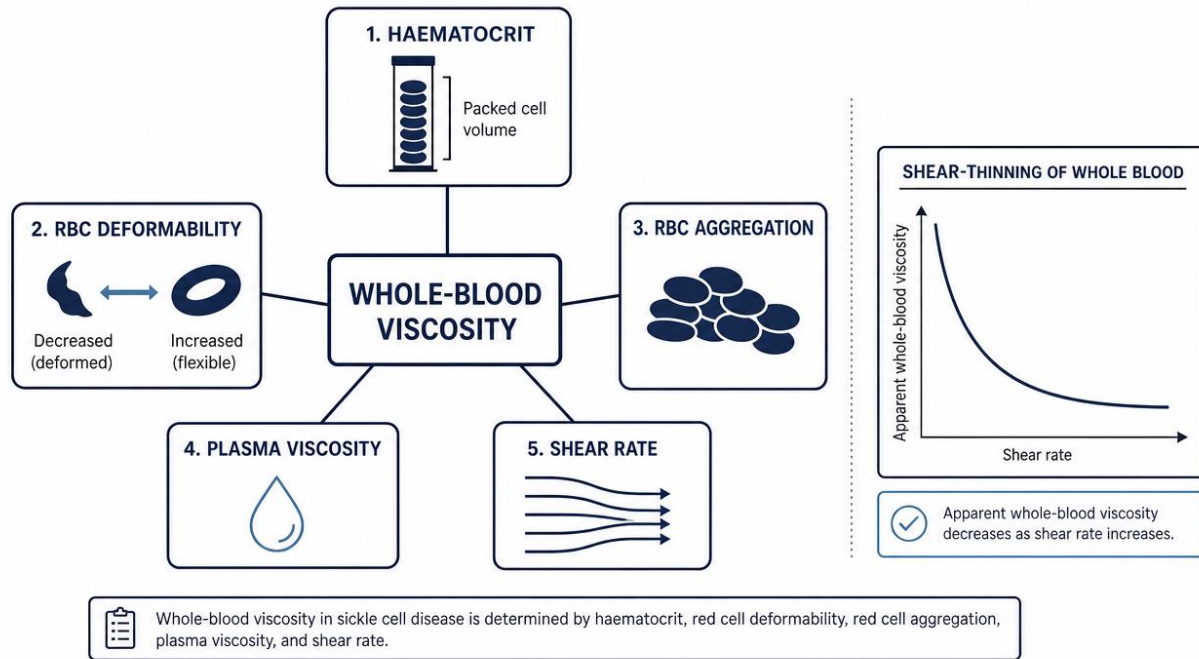


Figure 2: Major Determinants of Whole-blood Viscosity in Sickle Cell Disease and its dependence on Shear Rate.

Whole-blood viscosity in sickle cell disease reflects the combined influence of haematocrit, plasma viscosity, erythrocyte deformability, erythrocyte aggregation, and shear conditions. Haematocrit is a major determinant, while abnormal cell mechanics and aggregation become particularly important across different flow regimes. Consequently, viscosity measurements require careful consideration of the experimental shear rate and other measurement conditions.

Normal blood rheology

Blood is a concentrated suspension of deformable cells in plasma and behaves as a shear-thinning, non-Newtonian fluid: its apparent viscosity falls as shear rate rises (Baskurt and Meiselman, 2003). Three cellular phenomena dominate this behaviour. At low shear rates, RBCs form reversible face-to-face stacks (rouleaux); these aggregates raise low-shear viscosity and depend on both plasma proteins (fibrinogen) and intrinsic RBC aggregability (Nader *et al.*, 2019b). As shear rate increases, aggregates disperse and, at high shear, RBC deformability governs viscosity because deformable cells elongate and align with flow (Baskurt *et al.*, 2009). For a suspension, apparent viscosity rises steeply with the volume fraction of cells. A widely used phenomenological description is the Quemada form, in which relative viscosity depends on haematocrit ϕ and a shear-dependent structural parameter k :

$$\eta_{rel} = \frac{1}{1 - \frac{1}{2} k \phi}^{-2} \quad (2)$$

where k itself decreases from a low-shear value k_0 toward a high-shear value k_∞ as shear rate increases, capturing the transition from aggregation-dominated to deformation-dominated flow (Baskurt and Meiselman, 2003). Plasma viscosity, typically $\sim 1.2\text{--}1.3$ cP at 37°C , sets the baseline on which cellular effects are superimposed (Nader *et al.*, 2019b).

HbS polymerization and RBC deformation

The molecular engine of the disease is the polymerization of deoxygenated HbS. A defining and therapeutically pivotal feature is the *delay time* (tD): after deoxygenation, no polymer forms for a stochastic latent period, after which fibre mass rises autocatalytically (Ferrone *et al.*, 1985). The delay time is extraordinarily sensitive to intracellular HbS concentration, varying approximately with its 30th power, so that a modest reduction in HbS concentration lengthens tD by orders of magnitude:

$$t_D \propto c^{-n}, \quad n \approx 30 \quad (3)$$

This steep dependence is the biophysical rationale for anti-sickling therapy: because most erythrocytes traverse the microcirculation before tD elapses, small increases in oxygen affinity or HbF content, or small decreases in HbS concentration, disproportionately reduce in-vivo sickling (Eaton and Bunn, 2017).

Dense, dehydrated RBCs and irreversible sickling

Dense, dehydrated RBCs have a high intracellular haemoglobin concentration and generally exhibit reduced deformability. Because deformability is essential for the passage of erythrocytes through the narrow and highly regulated microcirculation, the accumulation of poorly deformable cells can increase microvascular resistance. Dehydrated cells are also more susceptible to further HbS polymerization because the concentration of HbS within the cell is increased. Consequently, cellular dehydration can simultaneously amplify the biochemical tendency to sickle and the mechanical abnormality that interferes with microvascular blood flow (Connes, 2024).

The relationship between dehydration and irreversible sickling, however, is more complex than previously assumed. Historically, irreversibly sickled cells (ISCs) have been regarded as severely dehydrated, highly dense, rigid RBCs that remain elongated following reoxygenation. This model is biologically plausible because repeated HbS polymerization can cause cumulative membrane injury and cellular dehydration, while the resulting reduction in deformability may impair microvascular transport and shorten RBC survival. Nevertheless, recent single-cell measurements have challenged the assumption that all morphologically defined ISCs are exceptionally dense or dehydrated. Reese *et al.* (2024) reported that the intracellular haemoglobin concentration distribution of ISCs was similar to that of normal discoid cells, whereas ISC volumes were, on average, larger and more heterogeneous. Their findings suggest that the relationship between irreversible morphology, cell density, and dehydration is heterogeneous and may depend on the precise cellular phenotype being measured. This distinction is important when interpreting haemorheological biomarkers in SCD. Measurements of RBC density, deformability, morphology, and hydration should not necessarily be considered interchangeable indicators of the same biological process. A patient may have a substantial population of poorly deformable cells without all of those cells being maximally dehydrated, while polymer-containing sickled cells may exhibit increased haemoglobin concentration and altered volume. Accordingly, single-cell approaches may provide greater biological resolution than bulk measurements when attempting to distinguish dense cells, dehydrated cells, polymer-containing cells, and truly irreversible sickle phenotypes (Reese *et al.*, 2024).

RBC deformability: measurement and significance

Red blood cell (RBC) deformability refers to the capacity of erythrocytes to undergo reversible changes in shape in response to mechanical forces without membrane rupture. This property is essential for maintaining microvascular perfusion because mature erythrocytes must repeatedly traverse capillaries and microvascular channels that are substantially narrower than their resting diameter. RBC deformability is determined principally by the surface-area-to-volume ratio, membrane viscoelasticity, cytoplasmic viscosity, and intracellular haemoglobin concentration. Alterations in any of these determinants can compromise microvascular transit and oxygen delivery (Liang *et al.*, 2025). Several complementary techniques are available for assessing RBC deformability. Ektacytometry is among the most extensively validated methods for evaluating RBC mechanical properties in SCD. In conventional laser-assisted optical rotational cell analysis, a dilute RBC suspension is subjected to controlled shear stress and the deformation of cells is detected through laser diffraction.

A major advance in SCD research has been oxygen-gradient ektacytometry (oxygenscan), which measures RBC deformability while oxygen tension is progressively reduced. More recent evidence supports the ability of oxygen-gradient ektacytometry to distinguish haemorheological phenotypes across SCD genotypes. In a 2025 study by Connes *et al.* (2025) involving individuals with HbSS and HbSC disease, patients with HbSS without hydroxyurea had lower RBC deformability under both normoxic and deoxygenated conditions than several comparison groups. The study also demonstrated genotype-related differences in the propensity of RBCs to sickle and in recovery of deformability after reoxygenation, highlighting the potential of oxygenscan parameters to characterize biological heterogeneity within SCD (Connes *et al.*, 2025).

Despite its utility, conventional ektacytometry has an important limitation: it produces a population-averaged measurement and may therefore underestimate the contribution of small subpopulations of highly rigid RBCs. This limitation is particularly relevant in SCD, where a relatively small fraction of severely abnormal cells may have substantial effects on microvascular flow. Microfluidic approaches such as the **OcclusionChip** have consequently been developed to assess the ability of individual RBCs or small RBC populations to traverse progressively narrower microchannels. These assays may detect abnormal subpopulations that are not readily identified by bulk ektacytometry (Man *et al.*, 2022). The **microfluidic impedance red cell assay (MIRCA)** represents another emerging approach. Rather than measuring cell elongation directly, MIRCA evaluates the ability of RBCs to pass through progressively narrowing microfluidic structures, generating indices related to the propensity of RBCs to cause microvascular obstruction. Recent clinical data involving adults and children with SCD found that MIRCA-derived deformability/occlusion indices were associated with RBC density, haemolysis, inflammation, age, disease genotype, and clinical complications. These findings support the potential utility of functional microfluidic assays as complementary biomarkers of RBC rheology in SCD (Patwardhan *et al.*, 2025).

Other emerging approaches include quantitative phase imaging, automated image analysis, optical trapping, microfluidic deformation assays, and machine-learning-based morphological classification (Ansong-Ansongton and Adamson, 2024). An additional emerging development is the assessment of RBC deformability under **oxidative stress**. Because oxidative injury is an important component of SCD pathophysiology, measurements performed under oxidative challenge may capture clinically relevant RBC vulnerability that is not apparent under standard testing conditions. Recent work using an "oxidantscan" demonstrated a shorter time to oxidative-stress-induced changes in deformability in HbSS and HbSC RBCs compared with healthy RBCs, suggesting that stress-dependent deformability measurements may provide additional information about RBC resilience and membrane pathology (Hernández *et al.*, 2025). Reduced RBC deformability has direct implications for microvascular blood flow. Rigid erythrocytes are less capable

of adapting their shape to the dimensions of the microcirculation and may therefore increase vascular resistance and promote microvascular obstruction. In SCD, these effects occur alongside abnormal RBC adhesion, leukocyte activation, endothelial dysfunction, increased blood viscosity, and HbS polymerization. Thus, impaired deformability should be understood as one component of a broader haemorheological network rather than as an isolated determinant of vaso-occlusion.

RBC deformability is relevant to the **viscosity–haemolysis paradox**. Highly rigid and dehydrated cells can increase the resistance of blood to flow and thereby contribute to hyperviscosity, whereas severely damaged RBCs have shortened survival and contribute to haemolysis. Consequently, a patient's clinical phenotype may reflect the balance between RBC number, haemoglobin concentration, cellular rigidity, blood viscosity, and haemolytic rate rather than any single biomarker. This reinforces the value of combining deformability measurements with whole-blood viscosity, haematological indices, markers of haemolysis, and clinical outcomes when defining haemorheological subphenotypes. Recent evidence also suggests that deformability measurements may have value in monitoring therapeutic response. Hydroxyurea can modify several determinants of RBC rheology, including fetal haemoglobin expression, cellular hydration, RBC turnover, and the propensity to sickle. In the 2025 comparison of HbSS and HbSC patients, hydroxyurea-treated HbSS patients demonstrated better RBC deformability than untreated HbSS patients, supporting the potential of oxygen-gradient ektacytometry to capture treatment-associated changes in RBC phenotype (Connes *et al.*, 2025).

RBC aggregation and plasma-protein effects

Red blood cell (RBC) aggregation refers to the reversible formation of RBC rouleaux or three-dimensional aggregates, particularly under low-shear or stasis conditions. In sickle cell disease (SCD), aggregation is clinically relevant because it can increase whole-blood viscosity, impair microvascular flow, and interact with RBC deformability and haematocrit to influence tissue perfusion. Unlike RBC deformability, which is largely determined by cellular properties, aggregation is strongly modulated by the plasma environment, especially by fibrinogen and other macromolecular plasma proteins. The concentration and molecular properties of fibrinogen promote RBC–RBC bridging and increase aggregate formation. Elevated fibrinogen is also a marker of inflammation, and inflammatory states may therefore amplify haemorheological abnormalities through simultaneous increases in aggregation and plasma viscosity. Other macromolecules, including immunoglobulins and high-molecular-weight proteins, can contribute to aggregation, although their effects depend on concentration, molecular size, and interactions with the RBC membrane (Nader *et al.*, 2019a; Condon *et al.*, 2023).

Haematocrit, Cell Density and Viscosity

Haematocrit (Hct), red blood cell (RBC) density and whole-blood viscosity are closely interconnected determinants of the haemorheological phenotype in sickle cell disease (SCD). Their relationships are particularly important because SCD combines chronic anaemia with marked heterogeneity in RBC hydration, density, deformability and survival. Consequently, a lower haematocrit does not necessarily imply lower blood viscosity, and patients with similar haemoglobin concentrations may exhibit substantially

different rheological profiles (Connes, 2024; Nader *et al.*, 2019b). Haematocrit is one of the major determinants of whole-blood viscosity. In general, increasing the fraction of RBCs within blood increases viscosity, with the effect becoming particularly pronounced at lower shear rates. However, the relationship is not simply linear across all physiological conditions because viscosity is also determined by RBC deformability, aggregation, plasma viscosity and shear rate. Thus, interpretation of Hct in SCD should be integrated with the mechanical properties of the RBC population rather than considered as an isolated parameter (Nader *et al.*, 2019b).

In SCD, chronic haemolysis produces anaemia and therefore tends to reduce Hct and whole-blood viscosity. This reduction in viscosity may partially compensate for the profound abnormalities in RBC deformability. However, this apparent rheological advantage can be offset when RBCs become dehydrated, dense and poorly deformable. The resulting phenotype illustrates an important component of the **viscosity–haemolysis paradox**: patients with greater haemolysis often have lower Hct and viscosity but may simultaneously have more severe vascular complications associated with haemolysis and endothelial dysfunction. Conversely, patients with relatively preserved Hct and more deformable RBCs can have higher blood viscosity and may be particularly susceptible to vaso-occlusive complications (Connes, 2024).

Microvascular flow and oxygen delivery

Oxygen delivery to tissue is the product of arterial oxygen content and blood flow, and flow is inversely related to viscosity. This yields the central trade-off of SCD physiology: raising haemoglobin raises oxygen content but also viscosity, so there exists an optimal haematocrit that maximizes oxygen transport, classically approximated by:

$$D_{O_2} \propto \frac{Hct \times SaO_2 \times k}{\eta(Hct, \gamma)} \quad (4)$$

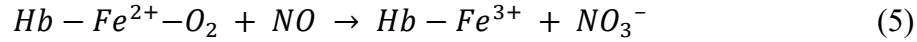
where DO_2 is oxygen delivery, Hct is haematocrit, and $\eta(Hct, \gamma)$ is the shear-dependent viscosity. In SCD, the numerator and denominator are coupled through the same variable, which is exactly why haemoglobin-raising therapies must be evaluated for viscosity effects, not oxygen content alone (Dufu *et al.*, 2018).

Haemolysis in Sickle Cell Disease

Haemolysis in SCD proceeds by two routes with distinct downstream biology. Extravascular haemolysis macrophage clearance of senescent or damaged cells in the spleen and liver drives anaemia and hyperbilirubinaemia. Intravascular haemolysis rupture within the circulation additionally decompartmentalizes haemoglobin and arginase into plasma, and it is this fraction that most directly links haemolytic rate to vasculopathy (Kato, Steinberg and Gladwin, 2017). Routine indices include LDH, indirect bilirubin, reticulocyte count, haptoglobin and plasma-free haemoglobin. Because no single marker is specific, several groups derive a composite “haemolytic component” by principal-component analysis of LDH, bilirubin, reticulocytes and aspartate aminotransferase; this composite associates with vasculopathic complications and with mortality (Kato *et al.*, 2006; Nouraie *et al.*, 2013). Table 3 summarizes the individual markers and their complication associations.

Cell-free plasma haemoglobin is a potent Nitric-oxide (NO) scavenger, and heme released from it is a damage-associated molecular pattern that activates endothelium and innate immunity. Simultaneously, erythrocyte arginase-1, liberated during haemolysis, depletes L-arginine the substrate for NO synthase compounding NO deficiency from the substrate side (Reiter *et al.*, 2002; Morris *et al.*, 2005). The

consequence is a shift of the vasomotor balance toward vasoconstriction and a proliferative, dysregulated endothelium. The dioxygenation reaction by which oxyhaemoglobin consumes NO is fast and essentially irreversible in the relevant regime:



Plasma haemoglobin thereby lowers NO bioavailability far more efficiently than intra-erythrocytic haemoglobin, providing a mechanistic bridge from haemolytic rate to pulmonary hypertension, priapism and leg ulceration (Kato *et al.*, 2017). Heme-driven oxidative stress, reperfusion injury and chronic inflammation upregulate endothelial and platelet adhesion molecules including P-selectin that tether sickle erythrocytes and leukocytes to the vessel wall, initiating the two-step model of vaso-occlusion in which adhesion in venules is followed by a rigid-cell logjam (Kato *et al.*, 2018). Adhesion biology is the point at which haemolytic and vaso-occlusive axes converge, and it is the target of P-selectin blockade (Ataga *et al.*, 2017).

The Viscosity–Haemolysis Paradox

An influential reframing of SCD proposed that haemolytic rate rather than vaso-occlusion per se drives an identifiable set of complications through NO depletion and endothelial dysfunction (Kato *et al.*, 2007). In this model, high LDH, high reticulocytes, low haptoglobin and low haemoglobin mark patients prone to pulmonary hypertension, leg ulcers, priapism and non-haemorrhagic stroke. The complementary rheological view emphasizes that patients with *higher* haemoglobin and haematocrit often those with co-inherited α -thalassaemia carry higher whole-blood viscosity and are prone to the viscosity–vaso-occlusive complications of painful crisis, acute chest syndrome and osteonecrosis (Kato and Gladwin, 2008; Kato *et al.*, 2009). Here the pathogenic variable is flow resistance, not NO scavenging. The paradox arises because a single upstream process, intravascular haemolysis has opposite effects on the two axes. By destroying red cells, it lowers haematocrit and therefore whole-blood viscosity (seemingly protective against vaso-occlusion), while simultaneously releasing free haemoglobin and arginase that scavenge NO and remodel the vasculature (frankly harmful) (Kato, Steinberg and Gladwin, 2017; Reiter *et al.*, 2002).

As formalized in Equation (4), oxygen delivery is maximized at an intermediate haematocrit. Raising haemoglobin improves arterial oxygen content but increases viscosity; the net effect on tissue oxygenation depends on where the patient sits on the curve and on the concurrent state of deformability. Anti-sickling agents that raise haemoglobin while improving deformability may raise oxygen content without the expected viscosity penalty, a possibility supported by pre-clinical rheology of HbS-polymerization inhibitors (Dufu *et al.*, 2018).

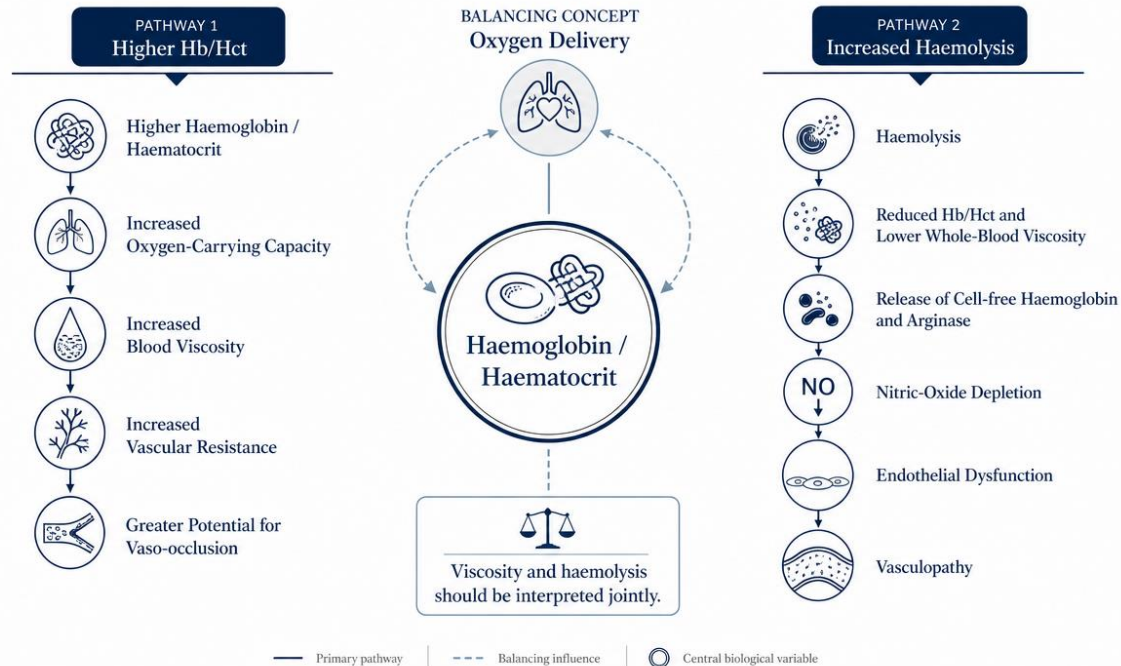


Figure 3: The Viscosity–Haemolysis Paradox and its Competing Effects on Vascular Physiology in Sickle Cell Disease.

The viscosity–haemolysis paradox (Figure 3) reflects the opposing consequences of haemoglobin concentration and haemolysis. Increasing haemoglobin may improve oxygen-carrying capacity while simultaneously increasing blood viscosity and flow resistance. Conversely, haemolysis can reduce haematocrit and whole-blood viscosity while releasing cell-free haemoglobin and arginase that promote nitric-oxide depletion and vasculopathy. Joint consideration of these processes provides a more complete representation of disease phenotype. The two models are not rivals but orthogonal axes. A given patient occupies a position in a two-dimensional space defined by viscosity and haemolysis; the classical debate reflects projection of that two-dimensional reality onto a single axis. Measuring both dimensions dissolves the apparent contradiction. We present this explicitly as a conceptual two-axis model that organizes biological heterogeneity along partly independent dimensions; it is not, in itself, a validated diagnostic classification.

Modifiers act on the two axes differently. HbF and α -thalassaemia reduce polymerization and haemolysis but, by raising haematocrit, can increase viscosity and vaso-occlusive risk; hydroxyurea raises HbF and mean cell volume, lowering both haemolysis and (through improved deformability) the tendency to vaso-occlusion (Kato *et al.*, 2009). The clinical trajectory reflects the net vector, not any single input. Studies that report protective effects of higher haemoglobin (fewer vasculopathic events) and those that report harmful effects (more vaso-occlusion) can both be correct if they sampled populations at different positions on the viscosity–haemolysis plane and measured different endpoints. The methodological corollary is that future studies should report viscosity and haemolysis jointly and stratify outcomes by complication class.

The core conceptual relationship of this review can be written as a multiplicative interaction among partly independent axes that jointly determine phenotype:

$$\text{Phenotype} = f(H \times V \times D \times A \times E) \quad (6)$$

where H = haemolysis, V = viscosity, D = deformability, A = aggregation and E = endothelial dysfunction. Equation (6) is deliberately schae-matic, its purpose is to insist that these terms interact rather than sum, and that setting any one to a “normal” value while ignoring the others discards clinically decisive information.

Clinical Subphenotypes Associated with Haemorheological Biology

Haemorheological subphenotypes and their characteristic profiles are explained below and presented in Table 1. These subphenotypes are proposed conceptual categories synthesized from the cited studies rather than empirically validated, mutually exclusive clusters; consistent with this, the BIOCADRE cohort identified no single dominant complication cluster.

Vaso-Occlusive Phenotype

Patients with relatively higher haemoglobin concentration and preserved RBC mass may develop higher blood viscosity. Increased viscosity can slow microvascular flow and interact with abnormal RBC adhesion, leukocyte activation, platelet activation, and endothelial dysfunction. This phenotype has historically been associated with recurrent pain, acute chest syndrome, and osteonecrosis. However, these complications are multifactorial and cannot be attributed to viscosity alone. Contemporary models emphasize interactions between rheology, inflammation, cellular adhesion, coagulation, and endothelial biology (Connes, 2024).

Hyperhaemolytic/Vasculopathic Phenotype

The hyperhaemolytic phenotype is characterized by lower haemoglobin, elevated LDH, reticulocytosis, increased bilirubin and plasma-free haemoglobin, and evidence of impaired NO biology. It has been associated with pulmonary hypertension, leg ulceration, priapism, and other forms of vascular disease (Kato *et al.*, 2017). Pulmonary vascular disease is particularly relevant because haemolysis, anaemia, hypoxia, inflammation, thrombosis, and high-output physiology may converge on the pulmonary circulation. Contemporary frameworks increasingly regard SCD-associated pulmonary hypertension as biologically heterogeneous rather than attributable to a single mechanism (Vallelian *et al.*, 2026).

Mixed phenotype

Increasing evidence indicates that many patients cannot be cleanly assigned to one of the two classical phenotypes. A patient may have substantial haemolysis and simultaneously exhibit abnormal RBC deformability, inflammation, adhesion, and episodic increases in viscosity. The BIOCADRE study is particularly informative in this regard. In 235 adults with SCA from Senegal and Mali, no single cluster of complications emerged despite extensive clinical, haemolytic, microcirculatory, and viscosity phenotyping. LDH was associated with elevated tricuspid regurgitant velocity, whereas blood viscosity was associated with retinopathy and the absence of macroalbuminuria. These findings argue against an overly simplistic binary classification and highlight the importance of studying distinct organ-specific phenotypes (Ranque *et al.*, 2023).

Table 1: Proposed Conceptual Haemorheological Subphenotypes of SCD

Subphenotype	Hct / viscosity	Deformability	Haemolysis	Characteristic complications
Vaso-occlusive	High / high	Relatively preserved	Lower	Pain crisis, ACS, osteonecrosis

Haemolytic–vasculopathic	Low / low	Low	High	PH, leg ulcers, priapism, some strokes
Hyperviscosity	Raised / high	Variable	Variable	Occlusion after Hct rise
Anaemic–haemolytic	Very low / low	Low	High	Cardiopulmonary, CNS injury
Mixed	Variable	Variable	High	Overlapping features

Note. ACS: acute chest syndrome; PH: pulmonary hypertension. Synthesized from Nouraie et al. (2013); Connes et al. (2016).

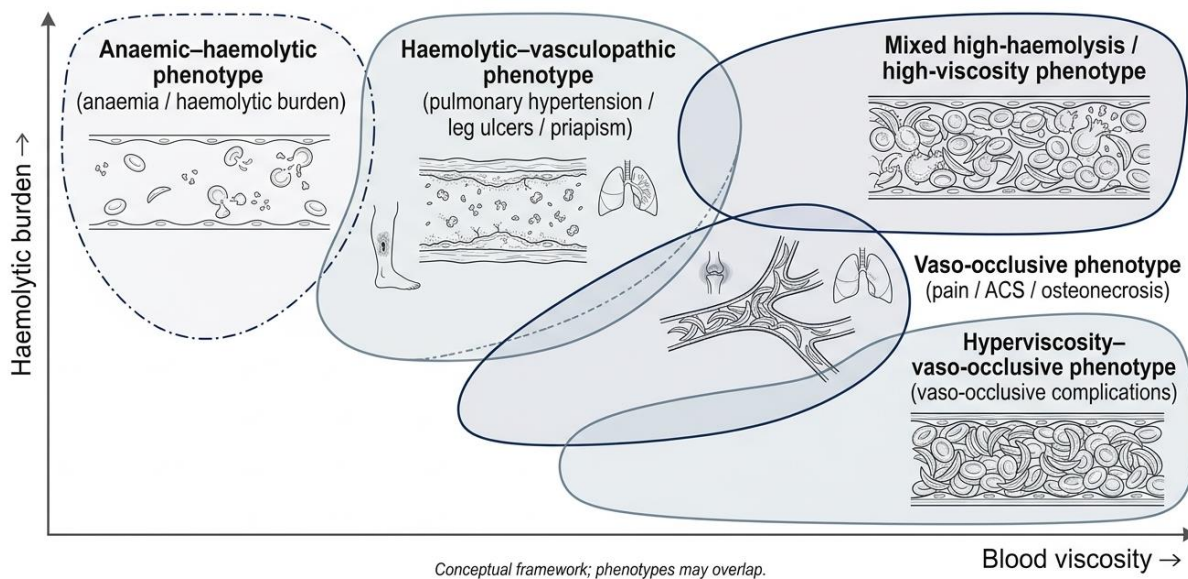


Figure 4. Conceptual Clinical Subphenotypes positioned according to Viscosity and Haemolytic Burden.

The viscosity–haemolysis plane provides a conceptual framework for understanding the heterogeneity of sickle cell disease. Patients may occupy predominantly vaso-occlusive, haemolytic–vasculopathic, hyperviscous, anemic, or mixed phenotypic regions, although substantial overlap is expected. The figure (Figure 4) is intended as a conceptual representation rather than a validated system of clinical thresholds.

Genetic and Haematological Modifiers of Haemorheology

The relationship between haemolysis and viscosity is modified by genetic and haematological factors.

α -thalassaemia: Co-inheritance of α -thalassaemia reduces intracellular haemoglobin concentration and can alter RBC density, haemolysis, and rheological properties. The resulting phenotype may involve less haemolysis but relatively greater blood viscosity, illustrating how a genetic modifier can shift the balance between the two major biological pathways (Connes, 2024).

Fetal haemoglobin: Higher fetal haemoglobin (HbF) reduces HbS polymerization and modifies RBC sickling behaviour. HbF is consequently relevant to both haemolysis and RBC deformability. Recent mechanical biomarker research has demonstrated relationships between HbF and dynamic RBC deformability measures, supporting the view that HbF influences the rheological phenotype as well as the molecular tendency toward sickling (Sahun *et al.*, 2025).

Reticulocytosis: Reticulocytosis is a physiological response to haemolysis, but reticulocytes are larger, more adhesive, and mechanically distinct from mature RBCs. Consequently, the reticulocyte count is not simply a marker of haemolysis; it may also contribute to the rheological and adhesive environment of the circulation.

RBC density and dehydration: Dense, dehydrated RBCs are particularly prone to HbS polymerization and reduced deformability. Cellular dehydration therefore represents a bridge between molecular sickling, RBC mechanics, haemolysis, and vaso-occlusion. The heterogeneity of RBC density within a single patient is an important reason why average rheological measurements may incompletely describe clinical risk (Connes, 2024; Sahun *et al.*, 2025).

From Single Biomarkers to Multidimensional Phenotyping

The evidence reviewed here suggests that the future of haemorheological biomarker research lies in integrating complementary domains. A useful conceptual framework is:

RBC production and destruction → RBC mechanical phenotype → whole-blood rheology → endothelial interaction → microvascular dysfunction → organ-specific clinical phenotype.

Within this framework, haemoglobin and haematocrit provide information about RBC mass; reticulocytes and LDH provide information about RBC turnover; plasma-free haemoglobin and haptoglobin provide additional information about intravascular haemolysis; ektacytometry or related technologies describe RBC deformability; aggregation assays characterize cellular interactions; and whole-blood viscosity integrates several of these properties. Such a multidimensional approach may be particularly important because conventional biomarkers can be discordant. For example, a patient may have high LDH but low viscosity because of severe anaemia, while another may have modest haemolysis but high viscosity because of relatively preserved haemoglobin and less severe anaemia. The absence of a single dominant phenotype in the BIOCADRE cohort supports this multidimensional interpretation (Ranque *et al.*, 2023).

Clinical and Translational Implications

The practical value of haemorheological biomarkers depends on whether they can improve clinical prediction or therapeutic decision-making. At present, routine clinical care relies predominantly on conventional laboratory variables rather than direct rheological measurements. This reflects the technical complexity, cost, limited standardization, and variable availability of viscosity and deformability assays. Nevertheless, several developments are changing this landscape. Novel microfluidic and single-cell technologies may permit more detailed characterization of RBC mechanics, while longitudinal monitoring may allow detection of changes preceding clinical events. The 2025 study by Sahun *et al.* provides an example of how dynamic deformability measurements could potentially complement conventional

biomarkers, although the small cohort means that the findings require independent validation before clinical implementation.

A second priority is standardization. Blood viscosity is highly dependent on temperature, haematocrit, shear rate, oxygenation status, and measurement methodology. Deformability assays similarly differ in their physical principles and analytical outputs. Without harmonized protocols, comparisons between studies and clinical translation will remain difficult. Third, future studies should move beyond cross-sectional associations. A clinically useful biomarker should ideally demonstrate reproducibility, temporal stability, biological plausibility, association with prospectively defined outcomes, and incremental predictive value beyond conventional laboratory and clinical variables.

Finally, biomarkers should be evaluated across diverse populations. The African SCD population represents a particularly important research setting because the majority of individuals with SCD globally live in sub-Saharan Africa, yet many biomarker datasets originate from high-resource settings. The BIOCADRE study demonstrates both the feasibility and the complexity of multidimensional haemorheological phenotyping in African populations (Ranque *et al.*, 2023).

Disease Complications through the Haemorheological Lens

For each complication we note the dominant rheological mechanism, the biomarker evidence, and the principal unresolved question. Table 4 provides a consolidated mapping.

1. Vaso-occlusive crisis (VOC): Mechanistically a two-step process: adhesion of sickle cells and leukocytes to activated venular endothelium, followed by entrapment of rigid, poorly deformable cells before the polymerization delay time elapses (Villagra et al., 2007). Higher viscosity and lower deformability associate with more frequent VOC; haemorheological risk factors have been identified prospectively in children (Lamarre et al., 2012). Unresolved: what threshold of viscosity or deformability, if any, is clinically actionable?
2. Acute chest syndrome (ACS): Shares the viscosity–vaso-occlusive mechanism, with pulmonary microvascular occlusion, infarction and fat embolism. Associated with higher haematocrit states and, prospectively, with haemorheological risk profiles (Lamarre et al., 2012).
3. Stroke and cerebrovascular disease: Heterogeneous: large-artery ischaemic stroke is linked to the haemolytic–vasculopathic axis and to markers of haemolysis and abnormal transcranial Doppler velocities, whereas other cerebrovascular events may reflect viscosity and adhesion (Kato, Steinberg and Gladwin, 2017; Bernaudin et al., 2008).
4. Pulmonary hypertension (PH): A paradigm haemolytic–vasculopathic complication. Elevated tricuspid regurgitant jet velocity marks increased mortality risk, and PH associates with haemolytic markers and NO depletion (Gladwin et al., 2004; Nouraie et al., 2013). Unresolved: the relative contributions of intravascular haemolysis, left-heart disease and thrombotic remodelling.
5. Renal disease: Sickle glomerulopathy and albuminuria track with haemolytic rate; a novel haemolysis marker associates strongly with glomerulopathy (Maier-Redelsperger et al., 2010, as reviewed in Kato et al., 2018). Medullary hypertonicity and hypoxia make the renal microcirculation especially vulnerable to sickling.
6. Leg ulcers: Strongly associated with the haemolytic–vasculopathic axis: low haemoglobin, high LDH, low deformability and NO depletion, with impaired skin microcirculation (Kato, Gladwin and Steinberg, 2007; Nader et al., 2019b).
7. Priapism: Men with priapism show increased haemolytic rate, decreased RBC deformability and increased aggregate strength, placing priapism partly on the haemolytic axis while implicating specific rheological abnormalities (Nader et al., 2019b).
8. Retinopathy: Proliferative sickle retinopathy reflects peripheral retinal capillary occlusion and ischaemia-driven neovascularization; rheological contributions (viscosity, deformability) are plausible but less quantified than in other organs.
9. Chronic pain and systemic manifestations: Chronic pain in adults reflects accumulated tissue and nerve injury superimposed on recurrent acute events, and is increasingly modeled with physiological and behavioural data streams (Section 12).

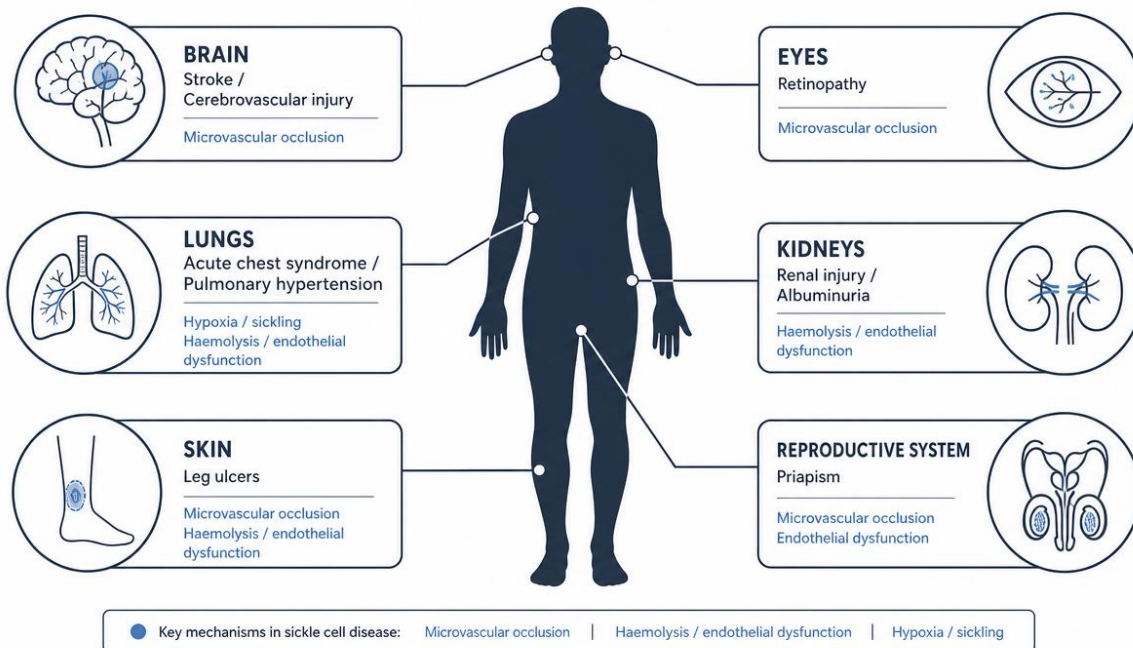


Figure 5: Organ-specific consequences associated with haemorheological and haemolytic abnormalities in sickle cell disease

Haemorheological abnormalities contribute to organ injury through mechanisms that vary across tissues. Viscosity and vaso-occlusion are particularly relevant to complications such as acute chest syndrome, whereas haemolysis, nitric-oxide depletion, and endothelial dysfunction are prominent in pulmonary hypertension, leg ulceration, and priapism. Cerebrovascular and renal complications may involve overlapping rheological and haemolytic mechanisms.

Limitations of the Current Evidence

Several limitations constrain the current clinical application of haemorheological biomarkers. First, much of the literature is observational. Associations between LDH, viscosity, deformability, and clinical complications do not establish that any individual biomarker is causally responsible for the complication. Second, SCD is genetically and phenotypically heterogeneous. Findings derived predominantly from HbSS populations may not be directly applicable to HbSC disease, HbS β -thalassaemia, or other genotypes. Third, transfusion, hydroxyurea, voxelotor or other disease-modifying therapies, renal dysfunction, inflammation, and acute illness can substantially alter haemoglobin concentration and RBC properties. Biomarkers measured during steady state therefore cannot necessarily be extrapolated to acute vaso-occlusive episodes.

Fourth, conventional haemolysis biomarkers are imperfect. LDH is not specific to erythrocyte destruction, bilirubin is affected by hepatic and genetic factors, and reticulocyte counts may be altered by marrow suppression or transfusion. Plasma-free haemoglobin may be more directly informative about intravascular haemolysis but remains vulnerable to preanalytical variation. Finally, recent systematic evidence concerning clinical complications such as priapism demonstrates that candidate biomarkers frequently show inconsistent associations between studies. A 2025 systematic review of priapism-related biomarkers concluded that although several biochemical, haematological, genetic, and rheological variables have been

investigated, the evidence remains insufficient to support clinical application of any individual candidate biomarker (Adesanya and Burnett, 2025).

Artificial Intelligence and Computational Phenotyping

1. Multivariate biomarker integration: The high-dimensional, interacting nature of the biomarker set (Equation 6) is precisely the setting in which multivariate and machine-learning methods add value over univariate association testing.
2. Unsupervised structure discovery: Principal-component analysis, k-means and hierarchical clustering, Gaussian mixture models, latent-class analysis and multiple correspondence analysis have all been applied to derive the haemolytic component and to discover complication-based subphenotypes in large SCD datasets (Kato *et al.*, 2006; Nouraie *et al.*, 2013).
3. Supervised prediction of complications: Neural networks and gradient-boosting models have been trained to predict vaso-occlusive crises from wearable physiology and patient-reported outcomes, and photoplethysmography during sleep predicts future pain-crisis frequency illustrating that the microvascular signal is detectable non-invasively (Summers *et al.*, 2024). Deep learning applied to microfluidic images can classify sickle erythrocytes by biophysical state (Praljak *et al.*, 2021).
4. Explainable AI: For clinical adoption, feature-importance and SHAP-type attribution are needed so that model outputs map back onto interpretable biology (deformability, viscosity, haemolysis) rather than opaque scores.
5. External validation, calibration and clinical utility: The recurring caution across this literature is that internal performance does not guarantee external validity; models must be validated on independent cohorts, calibrated, and evaluated for decision-analytic utility before deployment, particularly across the diverse populations affected by SCD.

Future Research Priorities

Future investigations should address five priorities. First, standardized measurement. International consensus protocols are needed for whole-blood viscosity, RBC deformability, aggregation, and related

measurements. Second, longitudinal phenotyping. Serial measurement may be more informative than a single steady-state value because haemorheological properties fluctuate with hydration, oxygenation, inflammation, treatment, transfusion, and disease activity. Third, composite biomarkers. Multivariable models integrating haemoglobin, haematocrit, LDH, bilirubin, reticulocytes, plasma-free haemoglobin, HbF, RBC deformability, and viscosity may better capture biological heterogeneity than any individual variable. Fourth, organ-specific validation. Biomarkers should be evaluated against specific outcomes rather than an undifferentiated concept of “disease severity.” Pulmonary hypertension, nephropathy, retinopathy, priapism, leg ulceration, and vaso-occlusive pain may have partially distinct biological signatures. Fifth, equitable validation. Studies should include populations from Africa, the Caribbean, the Middle East, Europe, and the Americas to establish whether proposed biomarkers have consistent performance across genetic backgrounds, healthcare environments, and disease-modifying treatment patterns.

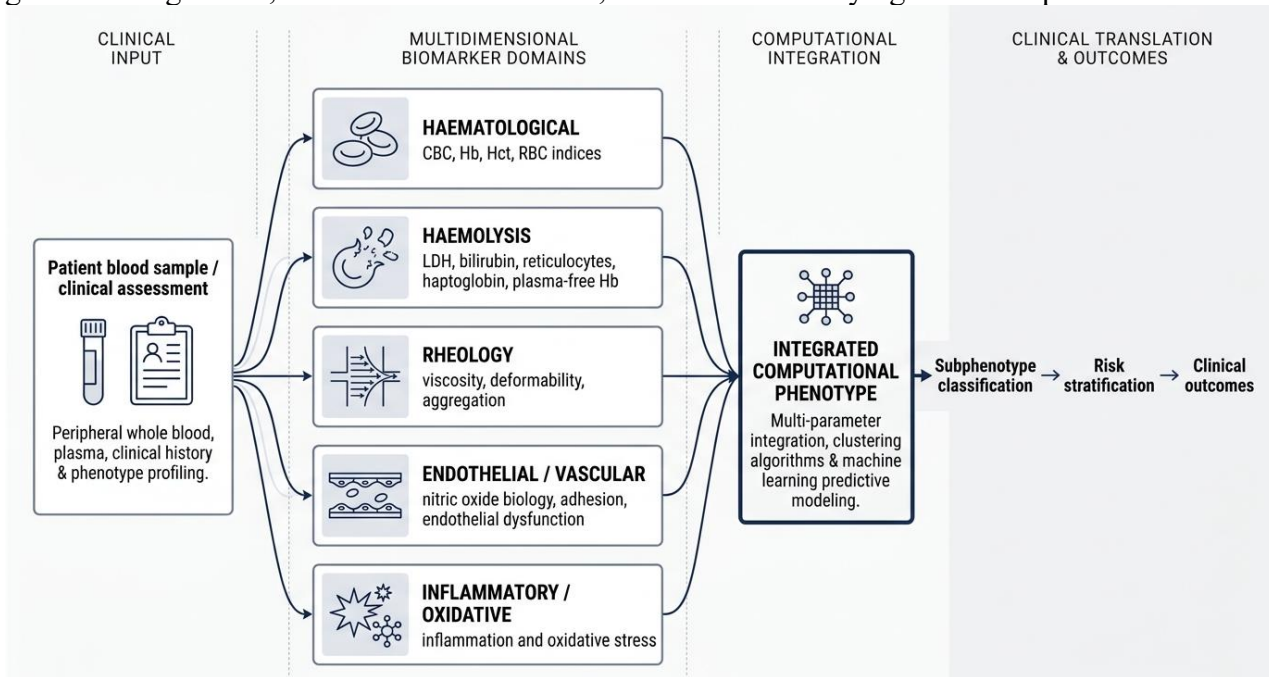


Figure 6: Integrated multidimensional haemorheological biomarker framework for clinical phenotyping in sickle cell disease.

Conclusion

The viscosity–haemolysis paradox provides a useful framework for understanding the biological heterogeneity of sickle cell disease. Haemolysis and viscosity should not be considered competing explanations for SCD complications; rather, they represent interconnected dimensions of disease biology. High haemolytic activity can reduce RBC mass and therefore lower blood viscosity while simultaneously releasing cell-free haemoglobin, heme, and arginase that impair nitric oxide signaling and promote endothelial dysfunction. Conversely, patients with relatively preserved haemoglobin may develop higher blood viscosity and greater susceptibility to viscosity-dependent microvascular impairment. Between these extremes lies a large population with mixed biological characteristics in whom haemolysis, abnormal RBC mechanics, inflammation, adhesion, and viscosity interact.

The emerging evidence therefore supports replacing rigid binary classifications with multidimensional clinical phenotyping. Conventional biomarkers such as haemoglobin, LDH, bilirubin, reticulocytes, and haptoglobin remain clinically useful, but direct measurements of viscosity and RBC deformability may

provide complementary information. Recent work on novel deformability biomarkers further suggests that dynamic mechanical measurements could eventually contribute to personalized monitoring, although validation in larger and diverse cohorts remains essential (Sahun *et al.*, 2025). Ultimately, the most promising biomarker strategy is unlikely to be a single “master” marker. Instead, clinically meaningful risk stratification may emerge from integrated profiles linking haemolysis, RBC mechanics, blood viscosity, endothelial dysfunction, and organ-specific manifestations. Such an approach could move SCD management toward biologically informed subphenotyping and more individualized prediction of complications. Multidimensional haemorheological profiling, integrated with computational phenotyping, is a promising route to individualized risk stratification. Its realization depends on three commitments the field can act on now: standardized, ideally point-of-care measurement; longitudinal, geographically representative datasets; and prospective validation with pre-specified, clinically meaningful thresholds. The viscosity–haemolysis paradox is not a contradiction to be argued away but a two-dimensional reality to be measured.

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