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Chapter 8

Sickle Cell Nephropathy: Current Understanding of the Presentation, Diagnostic and Therapeutic Challenges

Baba P.D. Inusa, Lodi Mariachiara, Palazzi Giovanni and Kenneth I. Ataga

Abstract

Sickle cell nephropathy (SCN) begins early in childhood from failure of urinary concentration (hyposthenuria), albuminuria to hyperfiltration, hematuria and progression to falling glomerular filtration to end-stage renal disease and increased mortality. Renal involvement is more severe in homozygous individuals (HbSS) than in compound heterozygous patients (HbSC). The pathogenesis of SCN is multifactorial from hypoxia, acidosis, hemolysis, ischemia-reperfusion injury and albuminuria. The clinical manifestations depend on whether the main pathology is tubular, glomerular or a mixture of both abnormalities. This chapter offers a critical review of the recent literature and will highlight the pathophysiology, epidemiology, clinical manifestations and management of sickle cell nephropathy with particular focus on the major advance in the early diagnosis. **Learning points: For SCN, the onset of hyperfiltration and albuminuria in infants and childhood is an opportunity to intervene early. There is no diagnostic markertest capable of detecting the onset of these changes. Moreover there is no reliable therapeutic agent to prevent or halt early changes due to SCN. The development of a marker of renal impairment in SCD such as such as Cystatin C assay if validated may be appropriate for wider clinical application.**

**Keywords:** sickle cell disease, sickle cell nephropathy, acute kidney injury, biomarkers

1. Introduction

Sickle cell disease (SCD) is one of the most frequent genetic disorders in the world. It predominantly affects people of African descent as well as individuals from the Middle East, India and...
Mediterranean regions. Recent estimates report about 305,800 babies with SCD are born every year in the word and over two-thirds are in sub-Saharan Africa rising to over 404,200 by 2050 [1, 2]. The disease is associated with a high lifetime morbidity and premature mortality [3], as described in the 2013 Global Burden of Disease Study [4]. The age-standardized death rate in sickle cell anemia increased from 1990 to 2013 (median change 28) [5]. The World Health Organization (WHO) has addressed the significant public health implication of sickle cell anemia, urging implementation of equitable and effective programs for the prevention and management of SCD [6]. Furthermore, encouragement was provided for the promotion, support and coordination of much needed research in SCD [6].

The term ‘sickle cell disease’ refers to all genotypes that cause the clinical syndrome. It occurs due to the inheritance of abnormal beta globin S (βS) alleles with the substitution of valine for glutamic acid in position 6 of the beta globin; the most common phenotype is homozygous βS/βS which is referred to as sickle cell anemia (SCA). The second most common phenotype, hemoglobin SC disease (HbSC), occurs due to co-inheritance of the βS and βC alleles, and presents a more moderate phenotype. HbS/β-thalassemia is the co-inheritance of βS with a β-thalassemia allele [7], those with a thalassemia null mutation (HbSβ0) presenting with a phenotype that is clinically indistinguishable from SCA, whereas individuals with HbSβ-thalassemia have a milder disorder [8]. The resulting sickle hemoglobin (HbS) polymerizes when the concentration of its deoxygenated form (deoxyHbS) exceeds a critical threshold. Low oxygen levels, increased acidity and cellular dehydration facilitate the polymerization of HbS and the distortion of the red blood cells leading to sickle-shaped erythrocytes [9]. The co-inheritance of genetic factors such as α-thalassemia or hereditary persistence of fetal hemoglobin are known to reduce the rate of HbS polymerization [10]. Sickling of red blood cells results in both obstruction of blood flow leading to organ and tissue ischemia, and hemolytic anemia [2, 11]. Reduced blood flow is mediated via a dynamic interaction between sticky HbS-containing red blood cells, white blood cells and the vessel wall [2]. Chronic intravascular hemolysis leads to the release of free hemoglobin that sequesters nitric oxide, a potent vasodilator and anti-inflammatory molecule, leading to vasoconstriction in different organs. Stroke and pulmonary hypertension are thought to be consequences of the diminished vascular relaxation caused by nitric oxide deficiency [12]. In addition, intravascular hemolysis in SCD leads to high plasma levels of cell-free heme and hemoglobin (Hb), sources of redox active iron. Iron-derived reactive oxygen species are implicated in the pathogenesis of numerous vascular disorders including atherosclerosis, microangiopathic hemolytic anemia, vasculitis and reperfusion injury [13]. Exposure of endothelium to heme greatly potentiates cell death. Recurrent cycles of ischemia-reperfusion injury in the microvasculature might amplify endothelial dysfunction and further organ injury including the stroke, pulmonary hypertension and kidney injury.

2. Sickle cell nephropathy

Renal involvement in SCD is a complex phenomenon resulting from an increased tendency of sickling in the renal medulla due to hypoxia, acidosis and hyperosmolar conditions [13]. Abnormally, high hemodynamic renal blood flow leads to early onset hypertrophic and impaired urinary concentrating ability, distal nephron dysfunction and progressive glomerulopathy. The combination of cortical hyperperfusion, medullary hypoperfusion and
vasoconstriction leads to further vasculopathy in the kidney. Sickle cell nephropathy (SCN) is a spectrum of changes resulting from a cascade of events occurring in the kidney. This is triggered by RBC vascular occlusion, infarction and reperfusion injury occurring within the renal medullar, cortex and collecting system. These may present as hyperfiltration, microalbuminuria, impaired urinary concentrating ability complicated by episodes of acute kidney disease (AKD) features early in childhood. In young adults, there is progressive increase in albuminuria and regression of the glomerular filtration rate (GFR). Further deterioration of renal function with the development of chronic kidney disease (CKD) (defined as estimated GFR of less than 90 ml/min/1.73 m²) eventually leads to end-stage renal disease (ESRD) in adulthood (Figure 1).

2.1. Pathobiology/histology

Early stages of SCN are characterized by glomerular hypertrophy, hemosiderin deposits with focal areas of hemorrhage or necrosis. This is followed by interstitial inflammation, edema, fibrosis, tubular atrophy and papillary infarcts [14–16]. Some of these features were reported in a multi-center, retrospective analysis of renal biopsies of 18 SCD patients (16-HbSS, 1-HbSC, 1 HbSβ thalassemia) who presented with proteinuria, acute or progressive impairment of renal function [17]. The study reported focal segmental glomerulosclerosis (FSGS) in seven cases, membranoproliferative glomerulonephritis (MPGN) in five and thrombotic microangiopathic glomerulopathy in three; while glomerular hypertrophy with or without mesangial hypercellularity was reported in three cases. Furthermore immunofluorescence microscopy
in the patients with FSGS-type lesions showed irregular staining for IgM and C3 in areas of sclerosis [15, 16, 18]. Complement deposition occur in the glomeruli coinciding with various degrees of proteinuria including nephrotic syndrome [19].

2.2. Risk factors for renal impairment in sickle cell disease

2.2.1. Hemolysis and vasculopathy

Hemolysis in SCD leads to release of arginase 1, asymmetric dimethylarginine and adenine nucleotides, these promote vasomotor dysfunction and proliferative vasculopathy. Circulating hemoglobin and heme both referred to as erythrocytic danger-associated molecular pattern (eDAMP) molecules activate endothelial inflammatory and angiogenesis. Hemolysis in SCD therefore leads to anemia, increased superoxide anion and reactive oxygen species (ROS) production and low ROS scavenging enzymes activity promote oxidative stress-induced vascular complications. Ikotua et al. reported in their study [20] that albuminuria was associated with increased white blood cell (WBC) count and LDH enzyme levels. Oxidative damage may alter both the structure and the function of the glomerulus due to its effects on mesangial and endothelial cells. Activated circulating white blood cells and platelets express adhesion glycoproteins leading to endothelial cell adhesion molecules and endothelial dysfunction.

2.2.2. Endothelin-A receptor antagonismo retards the progression of sickle cell nephopathy

Endothelin-1 (ET-1) is a signaling peptide produced by diverse cell types that exerts its physiologic and pathophysiologic actions by binding to two receptor subtypes, ETA and ETB. ETA receptor activation induces vasoconstriction, inflammation and nociception which is abolished by ETB activation in some tissues. ETA receptor signaling produces oxidant stress and the release of cytokines such as NF-kB activate and promote the production of ET-1, the agonist for the ETA receptor. A number of studies have reported increased production of ET-1 in SCD thus promoting sickling and tissue injury. Kasztan et al. reported that by blocking ETA receptor, the progression of SCN in a murine model of SCD was abolished [21]. The introduction of sickle RBCs into murine endothelial cells induces ET-1, leading to ETA-dependent vasoconstriction [22, 23]. It has also been shown that plasma ET-1 levels are elevated in patients with SCD during steady state periods as well as during acute vaso-occlusive crisis. Conversely, plasma ET-1 levels are decreased in SCD patients treated with hydroxyurea [24]. Elevated ET-1 level in SCD is associated with endothelial dysfunction and albuminuria in patients with SCD [25]. Bosentan, a dual ETA/ETB receptor antagonist used in murine models, decreases hypoxia-related injury to renal vessels and lung inflammation [26]. As it has been shown by Kasztan’ s studies, administration of ambrisentan (an ETA receptor antagonist), at the time of weaning and continued for 10 weeks, prevented glomerular dysfunction, tubulointerstitial inflammation and fibrosis [24]. The observations of Kasztan et al. support this speculation as the markedly elevated plasma ET-1 levels that occur in this model are normalized by chronic administration of ambrisentan [21]. Long-term administration of ambrisentan significantly reduced the degree of iron deposit in renal tubules. The reduction in tubular iron deposits suggests that ambrisentan reduces hemolysis in murine models. Free heme is a well-known promoter of oxidative stress and the generation of proinflammatory species, for example, ROS. Free heme also stimulates the production of placenta growth factor (PIGF), an angiogenic growth factor that is implicated in the
pathogenesis of tissue injury in SCD as well as the production of ET-1 [27–29]. NO is a suppressor of ET-1 synthesis and vascular ET-1 production may also increase when the vascular system is depleted due to NO binding to HbS plasma. ET-1 causes RBC dehydration by activating the Gardos channel present in the plasma membrane of RBCs [30, 31].

2.2.3. Mouse models

Mouse models provide opportunities to explore the mechanisms of globin gene regulation and the feasibility of gene therapy for this condition and the molecular basis of end-organ damage, including SCN [32]. The use of established mouse models is of invaluable help to investigate the pathogenesis of SCD-associated multiple organ complications and to identify targets for prevention and therapy.

Several murine models have been developed to mimic human SCD. Of these, the Berkeley model (BERK mice) has targeted deletions of murine α and β globins (α−/−, β−/−) with a transgene containing human α, βγ, γγ and β globins (Hba0/0 Hbb0/0 TG (Hu-miniLCreγGγAγδβS) (α−/−, β−/−, transgene +); thus, these mice almost exclusively express human sickle hemoglobin [33]. The BERK mouse model exhibits a wide spectrum of hematologic and histopathologic findings that are similar to those found in humans with SCD. Erythrocyte sickling is significant in BERK mice, and erythrocyte survival is very short resulting in massive amounts of heme being released into the plasma. As seen in humans with SCD, BERK mice showed a wide spectrum of kidney pathologies such as increased cortical hypertrophy, gross and microscopic infarcts, iron deposition, enlarged glomeruli associated with mesangial cell and mononuclear cell hypercellularity are observed in kidneys from BERK mice [34].

Another mouse model of SCD, the transgenic SAD mouse bears the human α-globin gene and the HbS mutation, βS, as well as βAntilles and βD−Punjab which greatly enhance the tendency of its hemoglobin to polymerize [35]. The SAD mice display renal hemosiderosis, microvascular occlusions, vascular thrombosis, cortical infarcts and papillary necrosis. Most mice show glomerular hypertrophy and mesangial sclerosis. The glomerular damage is associated with abnormal function, characterized by increased blood urea nitrogen levels and proteinuria [35]. The glomerular lesions of SAD mice faithfully mimic sickle cell glomerulosclerosis, the most severe renal complication observed in individuals with SCD. Therefore, the SAD mouse constitutes a valuable model to investigate the pathophysiology of the thrombotic and glomerulosclerotic complications of human SCD. Ischemic injury contributes to end-organ damage and other complications of SCD. Increased sensitivity of tissues in SCD to ischemic insults has been demonstrated in SCD mice. As it has been showed by Nath et al., after induction of bilateral renal ischemia, transgenic SCD mice exhibited massive vascular congestion, sickling of red blood cells and more prominent capillary congestion in the lungs and heart compared to control mice [36]. These results demonstrated increased susceptibility to vascular congestion and to ischemia in tissues from SCD mice, suggesting that ischemic episodes may contribute to the renal complications observed in SCD. Abnormal leukocyte-endothelium attachment associated with endothelial activation was observed in SCD mice, showing interesting parallels between the vascular injury after reperfusion and kidney damage. In addition, this study suggested that allopurinol, that prevents ischemia-reperfusion generation of reactive oxygen species, might be a potential therapy for SCD [37]. The anti-sickling property of fetal hemoglobin was also demonstrated in SCD mice [38]. Patients with SCD suffer from
painful crises associated with vaso-occlusion. Increased circulating erythrocyte membrane microparticles (MPs) have been associated with occlusion of capillaries. Interestingly, MPs triggered immediate renal vaso-occlusion in mice. In vitro studies showed that MPs stimulate the production of reactive oxygen species by endothelial cells, stimulate RBC adhesion and induce endothelial apoptosis. This work introduced a novel concept that associates the shedding of MPs from sickled RBC with vascular disease [39]. An interaction of free heme with TLR4 receptor was shown to mediate the nephrotoxicity of heme, in particular, the effects of heme on renal blood flow and inflammatory responses [40].

2.2.4. Development of sickle cell nephropathy from infancy to adulthood

Glomerular changes in SCD occur early in the first decade of life even though SCD patients remain asymptomatic. These are characterized by high renal blood flow, hyperfiltration and hypertrophy. Current data suggest that infants with SCD develop a hyperfiltration phase, which plateaus during early childhood. As early as the first year, renal enlargement is observed in correlation to hyperfiltration. Hyperfiltration is a well-known phenomenon in SCD even though the pathogenesis and pathophysiology is less well understood. As a result of hyperperfusion, increased amount of fluids is presented to the proximal tubule triggering more tubular reabsorption of sodium and water in order to restore glomerulotubular balance. Increased proximal tubular sodium reabsorption is associated with high metabolism and adaptive cellular response leading to overall renal enlargement. This complex phenomenon might be relevant to the glomerular hypertrophy that occurs in SCD [13]. Some studies show that for children with HbSS, there is an age-related increase in the estimated creatinine clearance in the first decade of life, with a decline toward normal values in the second decade [41–43]. In the study by Etteldorf and colleagues, children with SCD aged 4–11 years had a significantly higher mean measured glomerular filtration rate (mGFR) (169 mL/min/1.73 m²) than normal controls (128 mL/min/1.73 m²) [44]. In the BABY HUG trial, 176 children aged 9–19 months had a measured GFR at baseline of 125 mL/min/1.73 m² [45], which was significantly higher than published normal values for the same age group [46]. In a cross-sectional study of 410 patients with SCD aged 2–21 (mean age 11) years, 23% of HbSS patients showed elevated urinary albumin excretion (≥30 mg/g), while other investigators have reported a HbSS prevalence of 16–27% in the childhood SCD population [47–49].

Further progressive kidney injury and CKD is reflected in a declining and abnormally low GFR. During adolescence, estimated glomerular filtration rate (eGFR) begins to decline in some patients, and around 10% of adolescent patients with SCD develop a GFR of <90 mL/min/1.73 m² [50]. Similarly, Bodas et al. recently reported a CKD prevalence of 8% in a cohort of patients with SCD aged 3–17 years [51].

The prevalence of end-stage renal disease (ESRD) in the pediatric SCD population is also not well described; however, childhood SCD accounts for only 0.3% of incident pediatric ESRD [52]. Eventually, renal failure develops in early adulthood (median age 23–37 years) in SCA and in mid-life (median age 50 years) in HbSC disease.
2.2.5. Proteinuria and chronic kidney disease

The prevalence of albuminuria in SCD is age dependent. It may be classified as moderately increased albuminuria (previous called microalbuminuria)—urine albumin concentration of 30–300 mg/g creatinine and severely increased albuminuria (macroalbuminuria)—urine albumin concentration of 300 mg/g creatinine. The prevalence of albuminuria in the first three decades of life is up to 27% increasing to 68% in older SCD patients [13]. The understanding of the evolution of CKD in SCD is evolving the extent to which moderate albuminuria progresses to severely increased albuminuria and the relationship with SCN. The development of SCN is likely due to complex interactions between SCD-related risk factors and non-SCD phenotype characteristics. Albuminuria is more likely to occur in patients who express specific single-nucleotide polymorphisms in the MYH9 and APOL1 genes, which are associated with an increased risk of CKD in African Americans [53]. On the other hand, microdeletions in the gene that encodes α-globin (reflecting a form of α-thalassemia trait) leads to a lower prevalence albuminuria [54]. Genetic polymorphisms of bone morphogenetic protein receptor 1B also influence GFR in SCD [55, 56]. SCD patients with albuminuria have increased levels of urinary excretion of markers of tubular injury (KIM-1 and NAG) [57]. The individual contribution of these phenomena to SCN is not clear.

2.2.6. Chronic kidney disease and end-stage renal disease

The reported prevalence of ESRD in SCD varies from 5 to 18% depending on the age of the cohort but remains a significant cause of mortality [58, 59]. Similarly, CKD (defined based on eGFR) which is usually diagnosed between 30 and 40 years is also a risk factor death [47, 60]. In a recent study in Rio de Janeiro, Brazil, 4.3% of patients admitted with SCD had CKD [61]. A lower incidence was observed in a study from Senegal, where CKD was identified in 2.6% of 229 adults with SCD [62]. The manifestations of CKD in SCD include hypertension, proteinuria and anemia. Vaso-occlusive history, legs ulcers, osteonecrosis, retinopathy, proteinuria, hematuria, hypertension and severe anemia were all identified as predictive factors for CKD in SCD [58, 61, 63]. In a recent study from Nigeria, 50% of SCD patients with proteinuria had CKD [64]. Risk factors associated with progression of CKD to ESRD (Table 1) include increased blood pressure, low hemoglobin levels, haemolysis, leukocytosis, hematuria, prior vaso-occlusive crisis, the βS Central African Republic (CAR) haplotype, pulmonary hypertension, stroke, acute chest syndrome and infection with parvovirus B19 [65–78]. The mean survival of patients with ESRD and SCD is estimated to be 4 years, even with dialytic treatment [79].

2.2.7. Urinary concentration abnormalities

The onset of urinary concentration defects begins in early infancy (6–12 months) and may account for nocturia, polyuria and enuresis in later childhood. The defect in urinary concentration does not respond to vasopressin but it is reported to improve with chronic blood transfusions in young children [47, 63, 64, 80–83]. Further deterioration of the defect in urinary concentration is observed from the second decade of life due to the onset of medullary fibrosis and the loss of the collecting ducts system. High HBF levels are associated with better urinary concentration [84–86]. There may be a role for drugs therapy that enhance the production of HbF such as hydroxyurea and decitabine.
2.2.8. Urinary acidification deficit

The defect in urinary acidification may be a combination of ischemic changes in the medulla and reduced capacity of the collecting duct to maintain hydrogen gradient. It is not clear what is responsible for this defect but it has been suggested a resistance of the distal nephron to aldosterone may exist [81, 87].

2.2.9. Hematuria

One of the most frequent features of SCN is hematuria which is also found in individuals with sickle trait [88], it may be micro or macroscopic and it is usually painless [63, 81]. The mechanism is not well defined but capillary congestion due to vaso-occlusive and ischemic injury may account for it. It is reported that the left kidney is more likely to be involved due to the fact that the left renal vein is compressed between the aorta and the superior mesenteric artery; also referred to as a “nutcracker-like” phenomenon [37]. Hematuria might also be due to renal papillary necrosis from vaso-occlusion of vasa recta but rarely from renal medullary carcinoma [63]. Nevertheless it is important to exclude other causes such as urinary tract infections, neoplasms, vascular malformations, vasculitis, glomerulonephritis and coagulation disturbances [61, 88].

2.2.10. Sickle cell nephropathy and acute kidney injury

The prevalence of acute kidney injury (AKI) in children with SCD presenting to the hospital emergency room may be as high as 17% [89], AKI is underreported in pediatric SCD patients. In adults, AKI is reported in 4–10% of patients, and in up to 14% of adults with acute chest syndrome. AKI in SCD may also reflect the frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) to treat painful crises in this patient population [52]. A recent retrospective analysis reported 8% of AKI in 149 pediatric patients admitted for acute chest syndrome using the Kidney Disease Improving Global Outcomes (KDIGO) and higher with increased hospital

<table>
<thead>
<tr>
<th>Risk factors associated with progression of CKD to ESRD</th>
<th>Protective factors for the progression of CKD to ESRD</th>
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<tbody>
<tr>
<td>Hypertension</td>
<td>Co-inheritance with α-thalassemia</td>
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<tr>
<td>Nephrotic range proteinuria</td>
<td>Higher fetal hemoglobin level</td>
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<tr>
<td>Severe anemia</td>
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<tr>
<td>Vaso-occlusive crisis</td>
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<tr>
<td>Acute chest syndrome</td>
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<tr>
<td>Stroke</td>
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<tr>
<td>β5 CRX haplotype</td>
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<tr>
<td>Genetic variants of MYH9 and APLC1</td>
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<tr>
<td>Pulmonary hypertension</td>
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<tr>
<td>Parvovirus B19 infection</td>
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Table 1. Risk and protective factors associated with progression of CKD to ESRD.
length of stay [90]. The true incidence of AKI in pediatric SCD patients may be underestimated in retrospective studies [89, 90]. In addition, serum creatinine may be an inaccurate marker of renal function in SCD due to the relatively high proximal tubular secretion of creatinine found in this population [91]. Interestingly, a recent adult study showed that even in patients with a normal creatinine level during a pain crisis, acute tubular injury likely occurs, as evidenced by a more than twofold rise in urinary neutrophil gelatinase-associated lipoprotein excretion [92]. NSAID use is common in children with SCD [93], without evidence to support its benefit compared to other less nephrotoxic options. Similarly, the use of non-steroid anti-inflammatory agents (NSAIDS) in children with SCD hospitalized for various indications, including dehydration due to gastroenteritis, was associated with a significant increase in the incidence of AKI [94, 95]. Therefore hemodynamic changes may increase the risk of AKI secondary to NSAIDs. Another contributing factor includes potential toxic tubular effects of free hemoglobin during a sickle crisis. Some SCD patients with CYP2C9 allele variants that alter NSAID metabolism may be at increased risk of toxicity. During vaso-occlusive pain crises and acute chest syndrome, the risk of AKI is increased by the drop in hemoglobin leading to hypoxic-ischemic events, hemolysis or inflammation. In murine SCD models, brief episodes of hypoxic-ischemic events produce profound acute renal injury [36, 96]. The murine model of SCD has shown that an increase in hemolysis or exposure to excess cell-free hemoglobin can also lead to renal injury [97].

3. Assessment of kidney function

The gold standard for assessing how well the kidneys are working is direct measurement of the GFR. CKD is classified based on the eGFR and the level of proteinuria and helps to risk stratify patients (Table 2A and B). In individuals with SCD, a GFR greater than 120 mL/min/1.73 m² is an additional indicator of abnormal kidney function. However, direct measurement of GFR is invasive and time consuming and so estimations of GFR based on the serum creatinine are more commonly used. A number of equations exist, including the ‘Modification of Diet in Renal Disease’ (MDRD), CKD-EPI and Cockcroft-Gault equations [98–100]. Different estimated GFR calculations have been compared to the measured GFR in people with HbSS from the Caribbean and sub-Saharan Africa, the CKD-EPI equation was found to provide the most accurate estimate in two small studies [101, 102]. In individuals with SCD, increased proximal tubule secretion of creatinine results in the serum creatinine level being a poor estimate of GFR [103]. The diagnostic performance of cystatin C in comparison to serum creatinine was analyzed in a meta-analysis of 46 studies, including children and adults. The data compared correlation coefficients between GFR and the reciprocals of serum creatinine and cystatin C in 3703 participants and showed significantly better correlations for cystatin C, suggesting that cystatin is superior to serum creatinine for the detection of impaired GFR in cross-sectional studies [104]. Proteinuria, albumin-to-creatinine ratio (ACR) is greater than 2.5 mg/mmol in men or 3.5 mg/mmol in women, or a protein-to-creatinine ratio (PCR) is greater than 15 mg/mmol is sufficient for a diagnosis of CKD. Proteinuria may be classified as moderately increased albuminuria (3–30 mg/mmol creatinine) or severely increased albuminuria (greater than 30 mg/mmol creatinine) [105].
4. Novel diagnostic and predictive biomarkers of AKI in children

4.1. Biomarkers used in sickle cell disease

New biomarkers are promising for the early detection of renal function loss in patients with SCD (Table 3), including cystatin C, plasma neutrophil gelatinase-associated lipocalin (NGAL), serum liver fatty acid-binding protein (L-FABP), serum kidney injury marker 1 (KIM-1), serum intereleukin 18 (IL-18), soluble FMS-like tyrosine kinase-1 (sFLT-1) and N-acetyl-b-d glucosaminidase (NAG) [106].

4.1.1. Serum cystatin C

Cystatin C (CysC) or cystatin 3, a protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function. Recently, it has been studied for its role in predicting new-onset or deteriorating cardiovascular disease (CVD). CysC is a nonglycosylated low molecular weight (13 kDa) basic protein that inhibits cysteine proteases and it has been demonstrated its closely correlation to glomerular filtration rate (GFR) in children [107]. CysC crosses the glomerular membrane, and it is reabsorbed and metabolized in the renal tubules and not returned to the bloodstream. It is not secreted by the tubules, even in cases of reduced GFR, and is not affected by muscle mass, protein intake, metabolic factors, drugs and inflammatory stimuli. It has also been reported that serum CysC correlates with the level of albuminuria [108, 109] and its levels well perform as marker of renal function to detect cardiovascular outcome both in

<table>
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<tr>
<th>GFR categories (mL/min/1.73 m²)</th>
<th>ACR categories (mg/mmol)</th>
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<tbody>
<tr>
<td>Stage 1: Kidney damage with normal or increased GFR (&gt;90 mL/min/1.73 m²)</td>
<td>A1 ACR of less than 3mg/mmol</td>
</tr>
<tr>
<td>Stage 2: Mild reduction in GFR (60-89 mL/min/1.73 m²)</td>
<td>A2 ACR of 3-30mg/mmol</td>
</tr>
<tr>
<td>Stage 3a: Moderate reduction in GFR (45-59 mL/min/1.73 m²)</td>
<td>A3 ACR of more than 30mg/mmol</td>
</tr>
<tr>
<td>Stage 3b: Moderate reduction in GFR (30-44 mL/min/1.73 m²)</td>
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</tr>
<tr>
<td>Stage 4: Severe reduction in GFR (15-29 mL/min/1.73 m²)</td>
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<tr>
<td>Stage 5: Kidney failure (GFR &lt;15 mL/min/1.73 m² or dialysis)</td>
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Table 2. (A) GFR categories in CKD; (B) ACR categories (KDIGO 2012) [105].
population-based studies and in patients with non-ST-elevation acute coronary syndrome [110]. Therefore CysC is an ideal biomarker for study the renal function in SCD patients which is undergoing hemolysis and inflammation secondary to the sickling phenomenon and in which creatinine clearance is generally increased and serum creatinine is low. Tantawy et al. [111] have found significantly higher serum CysC in patients with SCD compared to healthy controls. In particular, patients of their cohort of study with nephropathy had higher cystatin levels than those without, and a significant positive correlation was found with ACR. The authors have demonstrated also that patients with SCD treated with hydroxyurea had lower CysC levels than untreated patients, possibly due to the role of hydroxyurea in decreasing inflammation [111]. In agreement with Tantawy’s results, Alvarez et al. [108] examined the value of serum CysC as a marker for GFR in small cohort of 20 children with SCD with and without albuminuria, compared to serum creatinine and creatinine clearance. The mean GFR derived from serum cystatin was significantly different among these subgroups, becoming abnormal in the proteinuric cohort (63 mL/min per 1.73 m²), compared to 94 mL/min per 1.73 m² for the microalbuminuric and 103 mL/min per 1.73 m² for the normal subgroups. Serum creatinine or creatinine clearance did not change significantly with the level of albuminuria. The authors concluded that serum CysC was higher than serum creatinine in SCD, and this probably relates to the fact that serum CysC is not secreted by the kidney, as creatinine. Moreover other studies have demonstrated the utility of CysC in patients with SCD. Asnani and Reid [109] have proved in 98 adults with SCD that CysC levels were significantly correlated with measured GFR, hemoglobin, serum creatinine, urinary albumin-creatinine ratio (UACR) and systolic blood pressure. In addition to urine, screening for albuminuria may help in the diagnosis of early renal impairment in the patients with SCD before a significant rise in serum

<table>
<thead>
<tr>
<th>Biomarker</th>
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<tr>
<td>Cystatin C</td>
<td>All nucleated cells</td>
</tr>
<tr>
<td>NGAL</td>
<td>Distal tubule and collecting duct</td>
</tr>
<tr>
<td>KIM-I</td>
<td>Proximal tubule</td>
</tr>
<tr>
<td>L-FABP</td>
<td>Proximal tubule</td>
</tr>
<tr>
<td>IL-18</td>
<td>Proximal tubule</td>
</tr>
<tr>
<td>sFLT-1</td>
<td>Proximal tubular cells</td>
</tr>
<tr>
<td>NAG</td>
<td>Proximal tubular</td>
</tr>
</tbody>
</table>

Table 3. Novel urinary biomarkers.
creatinine is observed. Receiver-operating characteristic curve (ROC) analysis has revealed that the cut-off value of CysC at 580 ng/mL could differentiate patients having SCD with and without nephropathy with 87.8% sensitivity and 84.6% specificity. Further prospective studies are needed to validate this threshold. On the other hand, Cho et al. [112] have evaluated the significance of serum cystatin C levels in pediatric patients with chronic kidney disease diagnosed by renal biopsy and showed normal serum creatinine levels. The authors have found that 95% of the patients showed only slightly increased cystatin C levels from the upper normal limit of the reference range and suggested that mildly increased cystatin C without increased creatinine might not have clinical significance.

4.1.2. Urine neutrophil gelatinase-associated lipocalin

As it has been showed in several preclinical gene expression analyses performed in AKI murine and human models, neutrophil gelatinase-associated lipocalin (NGAL) gene has been revealed to be one of the most upregulated genes in the kidney soon after an ischemic or a nephrotoxic insult [113, 114]. NGAL is filtered across the glomerulus, is reabsorbed in proximal tubules and its urinary concentration increases early during ischemic insults [115, 116]. The NGAL protein is also highly induced in regenerating and recovering kidney tubule cells. NGAL binds iron; chelation of toxic iron is an important mechanism that protects the kidney tubules from worsening injury. Thus, the biological role of NGAL in AKI is one of enhanced tubule cell proliferation and recovery [117]. Measurement of urinary NGAL (uNGAL) has been demonstrated to be an early, non-invasive marker of AKI due to a variety of etiologies, such as cardiac surgery [118], intravenous contrast administration [119], critical care settings [120] and kidney transplantation [121]. NAGL has an enormous dynamic range, responds in a dose-dependent fashion to injury, responds within 3 h of injury, and responds to a wide range of injuries, easy to measure due to the recent availability of clinical platforms including a new NGAL dipstick. Thus, uNGAL values may then be used to initiate AKI patient care algorithms earlier than serum creatinine alone. Although multiple investigations have demonstrated that uNGAL is a promising AKI biomarkers, a study by Sundaram et al. [57] has not showed any relationship with albuminuria in patients with SCD. This study has also showed that uNGAL levels were significantly subnormal (<50 ng/mL) in most patients with SCA and the overall uNGAL in most patients were well below levels usually seen in patients with acute or chronic renal injury. The authors have explained the results obtained based on the fact that proximal tubular function is supra-normal in SCA, and it is likely that any filtered NGAL may be reabsorbed much more efficiently, resulting in subnormal urinary NGAL levels in SN.

4.1.3. Soluble FMS-like tyrosine kinase-1

Soluble FMS-like tyrosine kinase-1 (sFLT-1) is a member of the vascular endothelial growth factor receptor family (VEGFR) and has an antiangiogenic effect. Soluble FLT-1 is increased in SCD due to its over-expression by vascular endothelial cells, vascular smooth muscles, activated blood monocytes and proximal tubular cells of the renal epithelia [122]. A recent study by Youssry et al. [123] investigated the relationship between serum levels of sFLT-1 and other conventional biomarkers of renal damage. The serum level of sFLT-1 in SCD patients was significantly higher than controls and its median level showed no significant difference when comparing patients with SS and Sβ genotypes, hydroxyurea therapy and iron chelation. On
the other hand, the authors have found significant positive correlations between serum levels of sFLT-1 and microalbuminuria, LDH and indirect bilirubin. Meanwhile, there were no significant correlations between serum levels of sFLT-1 and creatinine, eGFR, serum ferritin and erythrocyte sedimentation rate (ESR). This association between sFLT-1 levels and microalbuminuria combined with the association of sFLT-1 with soluble vascular cell adhesion molecule (VCAM) in prior studies [124], suggests that sFLT-1 may contribute to the pathogenesis of albuminuria in SCD by promoting endothelial dysfunction.

4.1.4. Serum liver-type fatty acid-binding protein

Serum liver-type fatty acid-binding protein (L-FABP) is an anti-oxidant, renoprotective molecule induced in the proximal tubule early after experimental AKI. It has been reported in children undergoing cardiopulmonary bypass (CPB), the increase in urinary L-FABP maybe occur within 4 h of initiating CPB [125]. However, there are conflicting data about L-FABP, such as a prospective multi-center study of 311 children undergoing cardiac surgery did not show similar increase in L-FABP [106]. In SCD patients, Sundaram et al. [57] reported that urine L-FABP level was highest in the youngest group (6–12 years old) even with little evidence of renal injury. In fact urine L-FABP levels are reduced with increasing albuminuria.

4.1.5. Kidney injury molecule-I

Preclinical studies have identified the kidney injury molecule-I (KIM-1) gene to be induced in the proximal tubule cells of ischemic rat kidneys [114]. KIM-1 protein regulates phagocytosis of damaged cells and thereby limits injury. An extracellular domain of KIM-1 can be detected by enzyme-linked immunosorbent assays and is useful as a urinary biomarker in patients with AKI. In fact, in a study conducted by Han WK et al., it was shown that in 40 children undergoing CPB, urinary KIM-1 levels were markedly increased in those who have developed AKI [126]. On the other hand, a prospective multi-center study of 311 children undergoing cardiac surgery confirmed the delay in upregulation of urinary KIM-1 in AKI patients and showed that KIM-1 was not significantly associated with AKI after adjusting for other injury biomarkers [127]. This data is in contrast with the results published by Sundaram et al. [57]. Indeed when KIM-1 levels, detected in all SCA urine samples, were compared within the different albuminuria groups, they were detected at lowest levels in patients with normal albuminuria, significantly increased in patients with moderately increased albuminuria and further increased in the severely increased albuminuria group, suggesting this may be another biomarker of relevance in sickle nephropathy that needs to be confirmed in longitudinal studies.

4.1.6. Interleukin-18

Interleukin-18 (IL-18) represents a proinflammatory cytokine that might worsen the degree of AKI. Animal studies have shown that IL-18 is induced in the proximal tubule and detectable in the urine following ischemic AKI. Numerous pediatric studies have proved that urine IL-18 obtained 6- to 12-h post-CPB moderately predicts AKI [128–131]. Cerqueira et al. [131] performed a cross-sectional study composed of 45 SCA patients. They found that IL-18 levels were correlated closely with markers of hemolysis, endothelial dysfunction and others cytokines levels. These findings suggest probable influences of IL-18 in the pathophysiology...
of vascular occlusion in SCA. In a recent article published by Duarte et al. [132], the author demonstrated that IL18 is associated with diastolic function in SCD patients, and may be involved in the pathogenesis of the disease. Genetic polymorphisms within the IL-18 gene regions are also associated with diastolic function in SCD, likely by affecting expression levels of the genes [132].

4.1.7. N-acetyl-b-p glucosaminidase

N-acetyl-b-p glucosaminidase (NAG) is a lysosomal enzyme produced in proximal tubular epithelial cells. The levels increased during kidney injury, a marker of proteinuria both in patients with diabetes and in patients with SCD [115, 133]. Sundaram et al. [57] showed that urine NAG activity was higher than baseline levels (>2 U/l) and worse in the presence of albuminuria. The elevations in NAG may precede moderately increased albuminuria, a likely diagnostic tool for early renal damage.

4.1.8. Transforming growth factor-b1

Transforming growth factor-b1 (TGF-b1) is a potent fibrogenic growth factor that may play a significant role in pathogenesis of SN [134]. It is a peptide of low molecular weight and has pleiotropic action. In the kidneys, it stimulates fibrogenesis through enhanced production of extracellular matrix proteins and nephron loss by various mechanisms, such as apoptosis of endothelial cells and podocytes [135]. Whether the urinary levels of TGF-b1 has a diagnostic significance in the early prediction of SN in children with SCD is still to be determined [136]. However in the article published by Sundaram et al. [57], urinary TGF-β was present at very low to undetectable levels in their patient population and showed no association with the degree of albuminuria. These data are in contrast with those published by Ghobrial et al. [137]. In their study, the authors have found a strong positive correlation between urinary TGF-b1 and urinary proteins and eGFR in all groups of SCD patients studied.

4.1.9. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio

Neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) ratios, as indicators of subclinical inflammation, rarely were been investigated in SCD patients. Emokpae et al. [138] recently reported a positive association between NLR, PLR and the increase of inflammatory markers in SCD patients such as C-reactive protein (CRP) and fibrinogen. The highest values of NLR and PLR were detected in patients with proteinuria and altered renal function. This data suggest that these markers may be predictive of a proinflammatory state with underlying renal damage.

4.2. Biomarkers not been investigated in sickle cell disease

4.2.1. Markers of cell-cycle arrest

Mallloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) have been identified as a markers of cell-cycle arrest and are induced in renal tubules following AKI. It is supposed that the resulting cell-cycle arrest then limits proliferation of damaged tubule cells. The metabolites of TIMP-2 and IGFBP7 can be measured in the urine. In a small study of children undergoing CPB, the urinary TIMP-1/IGFBP7 product was increased 4 h post-CPB in children who developed AKI [139].
4.2.2. Urinary excretion of uromodulin

Urinary excretion of uromodulin (UMOD), also known as Tamm-Horsfall Protein (THP), is the most abundant protein excreted in urine. It is a glycoprotein that is expressed in the thick ascending limb (TAL) of the loop of Henle [140–143]. It has also several roles in salt transport in the tubules, in the innate immunity and in the protection against kidney stones [144]. Recently, UMOD has been studied as a marker of acute renal injury both in mouse models of ischemia-reperfusion injury and in studies conducted in adult and pediatric patients prior to CPB [140, 143, 145]. These encouraging results suggest the possibility of studying UMOD also for the early determination of renal damage in SCD patients.

5. Current treatment

The treatment of renal complication in SCD patients should include an adequate fluid intake in order to avoid dehydration due to hyposthenuria. The chronic use of drugs toxic to the kidneys, such as non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided due to the potential for adverse hemodynamic-related renal function deterioration, precipitation of papillary necrosis, and the development of NSAID-associated interstitial nephritis and glomerulonephropathies.

5.1. Treatment of hematuria

Hematuria in SCD is typically self-limited. Patients with hematuria should be advised to maintain a high urine output by oral hydration and remain at rest. However, in cases of massive hematuria, a high urine output should be maintained with combination of isotonic fluids and loop diuretics, and adopt measures to alkalinate urine, with sodium bicarbonate or acetazolamide. These measures modify the acid and hypertonic environment of the medullar region, which favors erythrocyte dehydration, Hbs concentration and its polymerization [80]. Patients are advised to maintain a urinary volume of 2–4 L/day. Also blood transfusion may be necessary in order to reduce Hbs level and sickling [146].

In cases of refractory hematuria, high doses of oral urea may be required to achieve blood urea nitrogen levels greater than 100 mg/dL, or treatment with vasopressin or epsilon-amino-caproic acid (EACA) to promote clotting [147].

5.2. Treatment of proteinuria

The benefits of angiotensin-converting enzyme (ACE) inhibitors and angiotensin-II receptor blockers (ARB) in slowing kidney disease progression in many situations are well-known. Improving nocturia has been reported to be an additional beneficial effect of ACE, presumably as a result of reduction in GFR [148]. A recent Cochrane database review, in 2015, reported the potential for reduction in albuminuria and proteinuria with the use of captopril in patients with SCD compared with those without the disease [148]. However, the administration of ACE and ARB should be carried out carefully due to the risk of hypotension and hyperkalemia, the latter condition often present in SCD patients.

The use of hydroxyurea (HU) has been suggested to reduce proteinuria and hyperfiltration as suggested in one prospective study consisting of 26 patients with SCD. However, no
effect on microalbuminuria was found [77, 149]. A cross-sectional study of 149 adult patients showed that those using hydroxyurea were less likely to exhibit albuminuria (defined as urinary creatinine ratios ≥30 mg/g) [149]. A multi-center trial in infants (mean age 13.8 months) demonstrated that treatment with hydroxyurea for 24 months did not influence the GFR. However, it was associated with better urine-concentrating ability and less renal enlargement, suggesting a possible renoprotective effect [41]. In a non-randomized study of children with SCD requiring hydroxyurea for standard indications, treatment for 3 years led to a mean (standard deviation (SD)) decrease in GFR from 167 (SD 46) mL/min/1.73 m$^2$ to 145 (SD 27) mL/min/1.73 m$^2$, indicating an improvement in the hyperfiltration [150].

Dietary protein restriction is not recommended, because of the underlying growth failure and decreased energy state in most patients with SCD [151].

5.3. Treatment of anemia

The use of multiple blood transfusions demonstrated to restore the urinary concentrating ability in children with SCD [152, 153]. One study of 120 children with sickle hemoglobinopathies found that chronic red blood cell transfusions before the age of 9 years was protective against the onset of microalbuminuria [154]. Blood transfusion receives HbS, prevent direct sickling in the kidney and vaso-occlusion, reducing glomerular and tubular ischemia damage to the kidney. However, the benefits of transfusion therapy must be balanced against risks including infections, iron overload, acute or delayed hemolytic transfusion reactions [48, 155–158].

5.4. Treatment of end-stage renal disease

Hemodialysis is reportedly the leading form of renal replacement therapy for SCD-ESRD patients, as well as peritoneal dialysis and kidney transplantation. Mortality in SCD patients is approximately 26% during the first year of therapy for ESRD, nearly threefold higher than in ESRD patients without SCD. However, SCD patients who received pre-dialysis nephrology care had a lower death rate than those who did not receive such care [159].

Kidney transplantation may offer survival advantage over dialysis in ESRD. As in the general population, allograft survival for patients with ESRD is greater in those with a living donor than in those with a deceased donor. The post-transplantation one-year graft survival exceeds 60–80% [160]. Complications specific to the SCD population include higher infection risk due to autosplenectomy and precipitation of sickle cell crises with anemia correction following a successful transplant. Kidney transplant may be also complicated by allograft venous thrombosis, deep vein thrombosis, and vaso-occlusive crises [63, 161, 162]. Suggested maneuvers to decrease the incidence of post-transplant complications in these patients include [63, 163] preoperative blood transfusions to decrease hemoglobin S levels, preoperative oxygen supplementation with 40% oxygen, pretransplantation warming of the kidney allograft using 37°C saline, intraoperative and postoperative dopamine infusion at 4 μg/kg/min stem cell transplantation remain as the only curative treatment with good result and survival rates around 90% in 4 years [164, 165].
6. Conclusions

SCN represents a new challenge in the treatment of acute and chronic complications in SCD. The underlying pathophysiology is not completely understood, but it is already known that kidney damage occurs since the first months of life. The onset of hyperfiltration and albuminuria is an opportunity to intervene. The lack of diagnostic test capable of detecting the onset of symptoms remains a barrier to institute therapy. Furthermore, the absence of therapeutic strategy compounds the management of SCN. New markers of renal impairment in SCD such as the use of cystatin C assays may become available for community-based screening in order to identify patients at risk, to treat them and to improve their survival and quality of life.

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Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

BI designed the draft of the chapter, BI and ML reviewed the literature and wrote the manuscript, BI, KA and GP reviewed and made the substantial changes the manuscript. All authors discussed, read and approved the manuscript.

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