Biomarkers of impaired renal function

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Purpose of review

Renal disease is increasingly common as life expectancy of HIV-infected persons continues to improve. Several biomarkers are available for monitoring renal function, although no consensus exists on how best to apply these tools in HIV infection. This review describes recent findings for the more common renal biomarkers.

Recent findings

Although widely used in clinical practice, creatinine-based estimates of glomerular filtration rate have not been validated in HIV infection. Serum cystatin C has been proposed as a more sensitive marker of renal dysfunction in HIV infection, although it may also reflect systemic inflammation. Screening for proteinuria and albuminuria allows identification of patients at higher risk of kidney disease and other adverse outcomes. Fanconi syndrome, which has been associated with tenofovir use, is associated with severe tubular proteinuria, and several low molecular weight proteins, including retinol-binding protein, β_2-microglobulin, and neutrophil gelatinase-associated lipocalin have been studied as markers of tubular dysfunction. Studies have reported a high prevalence of subclinical proximal tubular dysfunction in patients receiving antiretroviral therapy.

Summary

Future studies are needed to determine the optimal biomarkers for the detection and monitoring of renal disease in HIV.

Keywords

biomarkers, estimated glomerular filtration rate, proteinuria, renal disease, tubular dysfunction

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Introduction

Combination antiretroviral therapy (cART) has resulted in profound improvements in immunodeficiency-associated morbidity and mortality [1–3]. As patients with HIV live longer, noninfectious comorbidities have become an important area of research, with numerous studies highlighting increased susceptibility and premature manifestations in HIV-infected patients. Several recent studies have focused on acute, chronic, and end-stage kidney disease, changes in glomerular function over time, and tubular dysfunction. Serum and urinary biomarkers are increasingly used in clinical practice to detect early stages of renal dysfunction, even though most have not been validated in HIV-infected patients.

Serum biomarkers

Accurate estimation of glomerular filtration rate (GFR) is essential for the detection and management of acute and chronic kidney disease (CKD) and for dosing and monitoring of cART. The ideal biomarker for GFR would be produced at a constant rate, freely filtered at the glomerulus, and neither reabsorbed nor secreted by the renal tubule. The most widely used biomarker, serum creatinine, is freely filtered but does not fulfil the other criteria. Creatinine is a by-product of skeletal muscle metabolism, and the rate of production depends primarily on muscle mass, with some variability with increased muscle catabolism or dietary intake of animal protein.

Creatinine-based GFR estimates incorporate demographic and anthropomorphic data to adjust for the relationship between muscle mass and serum creatinine, although they do not account for factors that influence tubular secretion. The Cockcroft–Gault equation was derived in 249 hospitalized Canadian men using 24-h urine creatinine clearance as the gold standard [4], whereas the Modification of Diet in Renal Disease (MDRD) equation was derived in more than 1600 men and women with CKD who underwent GFR measurement by iothalamate clearance [5]. More recently, the CKD Epidemiology Collaboration (CKD-EPI) developed a new estimate by combining data from 8254

patients with measured GFR enrolled in 10 observational and investigational studies. The CKD-EPI equation was tested in an external validation sample of 3896 patients and was found to be more accurate than the other estimates in the normal range [6•].

Despite the importance of accurate GFR estimation in HIV-infected individuals, available estimates have not been validated, and most comparative studies have included white men with relatively preserved kidney function. In a cross-sectional study of 90 HIV-infected adults without CKD, Cockcroft–Gault and four-variable MDRD eGFR were highly correlated, but both underestimated the 24-h urine creatinine clearance [7]. In a study of 27 HIV-infected adults in which directly measured GFR was used as the gold standard to compare the performance of several GFR estimates, the relative accuracy was highest for the four-variable MDRD eGFR and 24-h urine creatinine clearance, and lowest for eGFR based on the alternative biomarker cystatin C (CysC) [8]. Other, typically small studies have suggested the Cockcroft–Gault to be the closest to gold standard in HIV-infected patients [9], especially in younger patients [10].

Because creatinine is also influenced by muscle mass and tubular secretion, CysC has been proposed as an alternative biomarker for GFR [11]. CysC is a 13 kDa cysteine proteinase inhibitor produced by all nucleated cells, freely filtered by the glomerulus, and largely reabsorbed and catabolized by the renal tubule. In 825 participants from the MDRD study, the inverse of serum CysC was highly correlated with measured GFR and was a stronger predictor of mortality than either measured GFR or inverse creatinine [11]. CysC has been proposed as a more sensitive marker of kidney disease in HIV-infected individuals [12], although CysC did not perform as well as creatinine-based estimates in one published study that has compared CysC to measured GFR in this population [8]. Cross-sectional analyses have demonstrated higher CysC levels in HIV-infected individuals compared to HIV-negative controls, despite similar creatinine-based eGFR [13–15]. The largest of these studies compared 518 participants in the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) cohort to 290 well characterized HIV-negative controls [14]. HIV-infected individuals were nearly 10 times as likely to have a serum CysC more than 1.0 mg/l, a level that has been associated with adverse outcomes in the general population. In a subsequent analysis, FRAM participants were also more likely to have either a decline or an improvement in CysC-based eGFR during 5 years of follow-up [16•], and changes in CysC mirrored changes in virologic control, a finding consistent with other longitudinal studies [15,17•]. Although virologic suppression with cART has also been associated with improvements in creatinine-based eGFR [18], longitudinal data on serum creatinine were not provided in the FRAM analysis [16•]. In a secondary analysis of data from the Strategies for Management of Antiretroviral Therapy (SMART) trial, interruption of cART was associated with an increase in plasma CysC but no change in MDRD eGFR [17•]. Similar results were noted in an observational cohort of 92 HIV-infected patients initiating cART [15]. Although changes in virologic control could plausibly influence kidney function, it is also possible that changes in CysC reflect the influence of viral replication on systemic inflammation [15,17•]. Until the results of larger studies comparing creatinine and CysC-based GFR estimates to a gold standard in HIV-infected individuals become available, the optimal GFR estimate for use in clinical practice remains unclear.

Traditional urine biomarkers

The most widely available urine biomarker for the detection of kidney disease is urinalysis protein. The sensitivity and specificity of routine urinalysis are limited by dependence on urinary concentration, leading some experts to recommend the use of urine protein-to-creatinine (P:C) or albumin-to-creatinine (A:C) ratios for CKD screening in HIV-infected individuals [19]. Regardless of the assay used, proteinuria and microalbuminuria have been associated with mortality, cardiovascular morbidity, and CKD progression in the general population [20]. Overt proteinuria as measured by urinalysis has been associated with mortality in HIV-infected women enrolled in the Women's Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS) cohorts [21,22]. More recently, urinalysis protein has also been associated with cardiovascular morbidity in HIV-infected individuals, based on data from a nested case–control study including 315 men and women enrolled in the Johns Hopkins Clinical cohort [23•] and a retrospective cohort of 17 264 largely male patients receiving care through the Veterans Health Administration [24•]. Although these studies adjusted for many important patient characteristics, HIV-infected individuals with proteinuria are more likely to have other risk factors for adverse outcomes, including more advanced HIV disease and a higher prevalence of comorbid conditions [21,22,25]. An analysis of data from two studies coordinated by the AIDS Clinical Trials Group (ACTG) suggested that proteinuria may also be a marker of systemic immune activation, which has been linked to poor outcomes in HIV infection [26].

Lower levels of urinary albumin excretion have also been associated with adverse outcomes, and several studies have demonstrated an increased prevalence of microalbuminuria in HIV-infected populations [27,28]. In the WIHS cohort, confirmed microalbuminuria was

associated with an increase in all-cause and AIDS mortality among HIV-infected women prior to the widespread use of cART [29]. In a separate analysis, the initiation of cART was associated with stabilization of urine A:C, compared with an increase in urine A:C among matched controls who did not receive cART [30].

Although proteinuria and microalbuminuria have both been associated with mortality in HIV-infected individuals, fewer studies have investigated the association of proteinuria or microalbuminuria with kidney disease outcomes in this population. Proteinuria was strongly associated with doubling of serum creatinine in HIV-infected women enrolled in the WIHS cohort [31]. Both proteinuria and microalbuminuria were associated with biopsy findings of HIV-associated nephropathy (HIVAN) in a cross-sectional study conducted in South Africa [32], and microalbuminuria was detected in banked urine from several participants in the Manhattan HIV Brain Bank cohort with subclinical renal disorder identified at autopsy [33]. More recently, investigators have considered the utility of proteinuria and albuminuria for the detection of medication toxicity. Because albuminuria is a more specific indicator of glomerular injury, the ratio of A:C to P:C has been proposed as a screen for 'tubular proteinuria' [34]. The utility of this approach for the detection of tenofovir-associated proximal tubular dysfunction should be evaluated prospectively before being adopted into clinical practice.

Fanconi syndrome, tubular dysfunction, and tubular biomarkers in HIV infection

Fanconi syndrome is a rare disorder of proximal tubular function and may be inherited or acquired in HIV infection most commonly as a result of exposure to tenofovir. Tenofovir-induced Fanconi syndrome is characterized by urinary phosphate wasting, normoglycaemic glycosuria, mild-moderate proteinuria, hypokalaemia, hypouricaemia, and metabolic acidosis with normal anion gap, although not all features are invariably present. Renal failure and urinary concentration defects, including nephrogenic diabetes insipidus have also been reported [35–37]. In published reports, most patients have developed Fanconi syndrome while receiving tenofovir in combination with ritonavir-boosted protease inhibitors (PI/r), and many had longstanding, advanced HIV infection and extensive antiretroviral treatment histories. The duration of tenofovir exposure ranged from 0.2 to 4.2 years in a recent case series [37]. Renal biopsy may reveal acute tubular necrosis affecting primarily proximal tubules, with cellular necrosis, fading of the brush border, and mitochondrial abnormalities. The tubular dysfunction in patients with tenofovir-associated Fanconi syndrome may be associated with reduced GFR and appears largely reversible following drug discontinuation. Bone pain, osteomalacia, or pathological fractures have also been reported in the setting of tenofovir-associated Fanconi syndrome [37]. Of note, Fanconi syndrome may occur without changes in GFR and may affect those with previously normal renal function. Although guidelines for the management of HIV-infected patients recommend monitoring of renal function [38,39], the optimal strategy for early detection of Fanconi syndrome remains to be defined.

Tubular dysfunction

Several studies have reported a high prevalence of subclinical proximal tubular dysfunction in HIV-infected patients. When defined as the presence of at least two of nondiabetic glycosuria, urine phosphate wasting, hyperaminoaciduria, β_2-microglobulinuria, and increased fractional excretion of uric acid, tubular dysfunction was present in 15% of 284 patients and associated with older age; tenofovir exposure was associated with 21-fold increased odds [40•]. Among 115 patients receiving tenofovir, older age, lower body weight, and the CC genotype at *ABCC2* −24 (the gene encoding the tubular transport protein multidrug resistance protein 2) were risk factors for tubular dysfunction [41•].

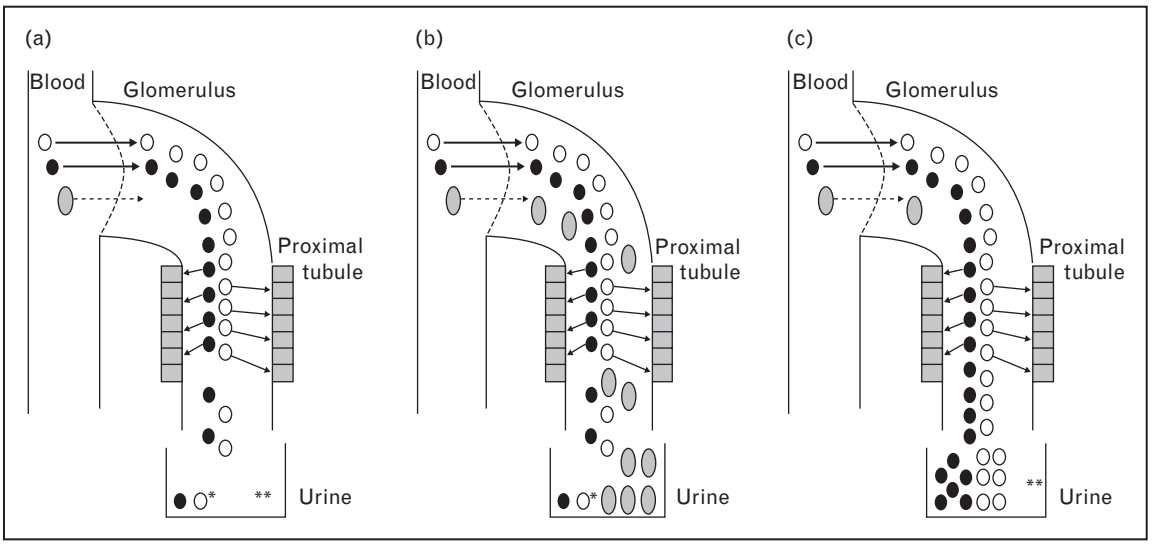
In the Swiss cohort, tubular dysfunction – defined as the presence of at least three of proteinuria, normoglycaemic glycosuria, and increased fractional excretion of phosphate or urate – was present in 6.5% of 1202 patients. Tenofovir-PI/r coadministration was associated with seven-fold increased odds of tubular dysfunction. Increased fractional excretion of phosphate, possibly the most sensitive single marker for tubular dysfunction, was present in 42–50% of patients who received tenofovir, compared to approximately 25% of patients taking tenofovir-sparing cART regimens and 4% of untreated individuals [42••].

The clinical significance of isolated tubular dysfunction remains unclear. In particular, it is unknown whether tubular dysfunction identifies patients at increased risk of Fanconi syndrome, osteomalacia, or reduced bone mineral density (BMD). Preliminary data from cross-sectional studies reported an association between tubular dysfunction and markers of increased bone turnover (serum alkaline phosphatase and β cross-laps) [43], but could not confirm an association with reduced BMD [44]. In addition, the clinical utility of tubular function tests remains limited by the requirement for fasting phosphate measurements.

Tubular biomarkers

Low molecular weight proteins (LMWPs) are small molecules that are freely filtered through the glomerulus and almost entirely removed from the ultrafiltrate and

Figure 1 Biomarkers of renal dysfunction in HIV infection



Schematic overview of phosphate and protein handling by the kidney in the context of normal kidney function (a), glomerular disease (b), and tubular disease (c). HIV infection is associated with mild glomerular and tubular dysfunction and may cause glomerular disease (HIV-associated nephropathy), whereas tenofovir may cause tubular disease (Fanconi syndrome). Phosphate is freely filtered at the glomerulus and actively reabsorbed from the ultrafiltrate by proximal tubular cells. Phosphate reabsorption is tightly regulated; excess dietary phosphate is excreted in the urine, resulting in variable urinary phosphate concentrations. Low molecular weight proteins (LMWPs) are also freely filtered and largely reabsorbed in the proximal tubule. With normal renal tubular function, very small amounts of LMWP appear in the urine. Albumin and HMWPs are not freely filtered; very small amounts of albumin may be present in the urine in the absence of glomerular disease. NB, kidney disease may affect both glomerular and tubular function, resulting in increased amounts of LMWP and albumin in urine. HMWP, high molecular weight proteins. * Urinary phosphate concentration varies with dietary phosphate intake. ** Small amounts of albumin may be present in patients with glomerular disease. ○, phosphate; ●, LMWP; ⬮, albumin/HMWP.

catabolized by the proximal tubule. Consequently, LMWPs are present in minimal amounts in the urine of individuals with normal tubular function (Fig. 1). Increased excretion of these 'tubular proteins' indicates tubular dysfunction, and their urinary concentration is a measure of the severity of tubular dysfunction. As for proteinuria of glomerular origin, tubular proteinuria is best expressed as a ratio over urinary creatinine to allow for differences in urinary concentration.

Several LMWPs, including retinol-binding protein (RBP), CysC, β_2-microglobulin (B2M), and neutrophil gelatinase-associated lipocalin (NGAL) have been studied as markers of tubular dysfunction. RBP is a 21-kDa protein and circulates in plasma bound to transthyretin; the unbound fraction (~10%) is freely filtered and subsequently reabsorbed by the proximal tubule [45]. B2M is a 12-kDa protein and a component of major histocompatibility complex (MHC) class I molecules; it is present on all nucleated cells. NGAL is a 25-kDa protein, produced in many tissues, and highly induced during inflammation. NGAL has been extensively studied as a sensitive, early marker of acute kidney injury [46]. In addition to the LMWP, *N*-acetyl-beta-D-glucosaminidase (NAG) is a high molecular weight (150 kDa) lysosomal protein present in tubular epithelial cells. Whereas increased urinary levels of the LMWP could indicate either tubular dysfunction or tubular injury, the presence of NAG in increased amounts in urine is considered indicative of proximal tubular cell damage [47].

A study from the pre-cART era suggested that HIV-infected patients have 3–10-fold higher urinary concentrations of LMWP (B2M and RBP, but not CysC) and NAG compared to HIV-uninfected controls [48]. Patients with tenofovir-induced Fanconi syndrome have very high levels of urinary LMWP (RBP and CysC), and these markers may prove to be useful to diagnose Fanconi syndrome or monitor patients on tenofovir for severe tubular dysfunction or Fanconi syndrome [37,49]. Several studies have evaluated tubular function by quantifying LMWP in asymptomatic patients who received tenofovir. In cross-sectional studies, higher levels of urinary RBP or B2M were noted in participants exposed to tenofovir compared to those on other cART or no cART [38, 50•]. Increased levels of urinary RBP and B2M were also observed in patients who, as part of a randomized clinical trial, had initiated tenofovir/emtricitabine, as compared to those on abacavir/lamivudine, with efavirenz [51•]. In an observational cohort study, exposure to tenofovir was associated with reduced phosphate reabsorption and a five-fold increase in urinary B2M excretion at 12 weeks [52]. In a cross-sectional study of 317 HIV-infected patients, exposure to tenofovir-PI/r was associated with

increased odds of RBP-defined tubular proteinuria (two-fold increased odds of RBP >17 μg/mmol creatinine and three-fold increased odds of RBP >38.8 μg/mmol creatinine); other factors associated with tubular proteinuria in this study were nonblack ethnicity and eGFR less than 75 ml/min [53]. An association between B2M-defined tubular proteinuria and tenofovir-PI/r has also been described for lopinavir, with low body weight identified as an additional risk factor [54]. Taken together, these data suggest that tenofovir exposure is associated with tubular dysfunction, and coadministration of tenofovir with PI/r possibly with more severe tubular dysfunction. However, the majority of patients have normal or mild-moderate elevations of urinary LMWP, which are not progressive over time and are associated with only modest changes in tubular phosphate handling. Furthermore, the two studies that evaluated urinary NAG excretion observed no difference between patients exposed to tenofovir and those taking cART without tenofovir [50•,51•], suggesting the absence of structural tubular damage in most patients with mild-moderate tubular dysfunction.

Clinical implications, management, and areas for further research

Measurement of serum creatinine (and use of creatinine-based estimations of renal function) and quantification of proteinuria or albuminuria should be performed at baseline, at cART initiation, and at regular (3–12 months) intervals to allow the identification of patients at greatest risk of further decline in renal function, end-stage kidney disease (ESKD), cardiovascular morbidity, and death [24•,38–39,55]. Depending on the severity of renal failure, the amount of proteinuria, and the rate of decline in renal function, patients should be considered for further investigations, including renal ultrasound and biopsy, and management should focus on avoidance of nephrotoxic drugs, management of dyslipidaemia, and optimization of blood pressure control. Depending on renal disease aetiology, patients may benefit from suppression of HIV replication, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), and possibly immunosuppressive therapy [38,39]. Although the use of ACE inhibitors and ARB has not been extensively studied in HIV-infected patients, their use in HIV-negative patients with kidney disease is associated with a reduction in the amount of proteinuria and the incidence of ESKD and cardiovascular events [56–58]. The effects of mild-moderate proteinuria on renal disease progression in HIV infection and the effects of antiretroviral therapy, CD4 cell count, and HIV RNA levels on renal function should be investigated in large observational cohort studies. Furthermore, the potential benefits of ACE inhibitors and ARB in HIV-infected patients with proteinuria deserve to be investigated in prospective, randomized, controlled clinical trials.

It is premature to incorporate tubular biomarkers in routine clinical practice. It remains to be determined whether the presence of mild-to-moderate tubular proteinuria allows the early identification of patients at increased risk of Fanconi syndrome and whether mild-to-moderate tubular dysfunction is detrimental to bone health. Further research into the (genetic) risk factors for tubular proteinuria, the effects of tubular dysfunction on bone, and the usefulness of LMWP quantification as a screening tool for Fanconi syndrome and osteomalacia is warranted.

Table 1 Biomarkers of renal dysfunction in HIV infection: advantages and disadvantages

	Advantages	Disadvantages	Comment
Serum			
Creatinine	Widely available, inexpensive	Serum concentration varies with muscle mass, affected by changes in tubular creatinine secretion	Best converted into creatinine clearance (CrCl) or estimated glomerular filtration rate (eGFR)
Cystatin C	Serum concentration is independent of muscle mass	Affected by inflammation (including HIV infection)	May be converted to eGFR
Urine			
Protein	Widely available, inexpensive	Does not distinguish between glomerular and tubular proteinuria	Proteinuria varies with urinary concentration and is best expressed as protein/creatinine ratio (PCR)
Albumin	Widely available, inexpensive	Does not measure tubular proteinuria	Albuminuria varies with urinary concentration and is best expressed as albumin/creatinine ratio (ACR)
Low-molecular weight proteins (LMWPs)			
Retinol-binding protein (RBP) Cystatin C Beta-2 microglobulin (B2M) Neutrophil gelatinase-associated lipocalin (NGAL)	More specific for tubular dysfunction	Not routinely available, expensive	Best expressed as creatinine ratio
N-acetyl beta-D glucosaminidase (NAG)	Suggestive of tubular damage	Not routinely available, expensive	Best expressed as creatinine ratio

Conclusion

We have described a range of biomarkers that are currently used or being investigated for the assessment of renal damage in HIV-infected patients, summarized in Fig. 1 and Table 1. Serum biomarkers are used primarily to estimate GFR; however, a consensus on which GFR estimates are the most accurate and how best to define clinically meaningful CKD is needed to monitor changes in this endpoint and also to compare findings from different studies. Tubular biomarkers deserve further evaluation in the management of HIV-infected patients. Increased urinary excretion of these tubular markers may assist the diagnosis of Fanconi syndrome [37,49], potentially allow early identification of patients at risk of Fanconi syndrome or severe tubular dysfunction, and possibly have a role in the early diagnosis of HIVAN [59], the most severe form of CKD affecting predominantly black HIV-infected persons.

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