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Proximal Tubular Renal Dysfunction or Damage in HIV-Infected Patients

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Abstract

Antiretroviral-associated toxicity, especially in the case of tenofovir plus boosted protease inhibitors, could affect different functions of the proximal renal tubule. Considering the long-term use of antiretroviral therapy and the concomitant presence of other risk factors, several degrees of proximal tubular toxicity, from chronic subclinical renal dysfunction to Fanconi syndrome, could be observed in HIV-infected patients. However, the clinical significance of isolated tubular dysfunction, in the short and long term, remains unclear. In addition, primary tubular abnormalities, even severe, may be missed until they affect the glomerular function. Therefore, there is a need for new biomarkers, not only based in serum creatinine and estimated glomerular filtration rates, that might help to identify tubular cell toxicity and predict the clinical outcome in HIV-infected patients. Increased values of urinary beta-2-microglobulin and retinol-binding protein, observed in up to 70% of patients, have been associated to tenofovir-associated mitochondrial dysfunction. Together with other tubular parameters or in isolation, both biomarkers could be useful for diagnosing proximal tubular toxicity. Other molecules, such as urinary kidney injury molecule- 1, neutrophil gelatinase associated lipocalin, or N-acetyl-β-D-glucosaminidase, could help to distinguish between tubular cell damage and dysfunction. Here, we review the current knowledge on tubular toxicity in HIV-infected patients on antiretroviral therapy. **(AIDS Rev. 2012;14:179-87)**

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Key words

Biomarkers. Tenofovir. Tubular dysfunction. Beta-2-microglobulin. Retinol binding protein.

Introduction

HIV-infected patients are at increased risk of developing kidney disease in the course of their lifetime. Different studies have found a prevalence of chronic kidney disease in this population ranging from 5 to 15%[1-3], although in some cohorts proteinuria has been detected in up to 30% of patients[4,5]. Both isolated proteinuria and impaired renal function have been associated with a faster progression towards AIDS and death[1,6].

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In the era before the widespread use of effective antiretroviral therapy (ART), HIV-associated nephropathy, attributed to the direct toxic effects of the virus on kidney cells, was the most common kidney complication encountered by nephrologists[7]. However, as treatments for HIV infection has improved, the nature of kidney disease associated with HIV has changed, with the direct harmful effects of the virus seen less frequently, whereas the nephrotoxic side effects of ART are becoming more common.

Kidneys can be susceptible to drug toxicity because of their layout and function. As the filtrate moves along the kidney's complex network of tubes in each nephron, levels of substances in the filtrate, particularly drugs, rise. Initially, this increased concentration of drugs is about three-times greater than that found in the blood. But as the filtrate moves away to more distant parts of the tubules, the concentration of drugs can increase to more than 100-times greater than in the blood.

Furthermore, drugs that are processed by the kidneys can produce toxic substances as they are broken down that can injure kidney cells. Thus, kidney toxicity produced by drugs may lead to acute kidney injury, chronic kidney disease, and features of proximal tubular injury[8-10].

In this context, there have been increased reports of isolated proximal tubular involvement as a form of presentation of renal toxicity in HIV-infected patients in the last years[11,12]. Different *in vitro* studies have shown mitochondrial toxicity as the main pathogenic mechanism of tubular cell damage[13]. The proximal tubule is intrinsically vulnerable to mitochondrial dysfunction because of limited anaerobic ATP-generating capacity. The consequences of tubular dysfunction, in turn, can be simple or complex, depending on whether the tubular transport of one or more substances is affected. As an example of antiretroviral drug susceptible to causing toxicity, tenofovir, an acyclic nucleotide analogue reverse transcriptase inhibitor structurally similar to adefovir and cidofovir, is eliminated unchanged in the urine by a combination of glomerular filtration and proximal tubular secretion[14]. About 20-30% of the drug is actively transported into renal proximal tubule cells by the basolateral membrane organic anion transporters (hOAT1, and to a lesser extent, hOAT3)[15,16]. Subsequently, the drug is secreted to the tubular lumen by the apical membrane transporters multidrug resistance proteins (MRP-4 and MRP-2), which are encoded by the adenosine triphosphate-binding cassette (ABC) genes *ABCC4* and *ABCC2,* respectively[17]. A number of drugs interact with these transporters and may cause excessive entry or reduced outflow of the drug, favoring intracellular accumulation and increasing renal toxicity through a mechanism of mitochondrial disruption. But, since tenofovir-based therapies are taken for long periods of time, and other toxicity-related factors may be associated, several degrees of toxicity could be observed, from chronic subclinical dysfunction to severe renal impairment.

Clinical manifestations of proximal tubular toxicity

Fanconi syndrome is the most important clinical consequence of tubular toxicity, although the isolated presentation of hypophosphatemia, normoglycemic glycosuria, and/or a decrease in phosphate reabsorption has been associated with tubular cell alteration. Although with a deceptively low incidence (0.3-2%)[18], tenofovir has been the drug most often associated with Fanconi syndrome[19], the consequences of which include calcium and phosphorus dysregulation and osteomalacia[20]. In a retrospective review of the Food and Drug Administration´s (FDA) adverse event reporting system from 2001 to 2006, 164 individuals met the definition for Fanconi syndrome. The majority (83%) received protease inhibitors in combination with tenofovir; specifically, 74% received ritonavir-boosted protease inhibitors. Nearly half (46%) of patients were hospitalized and it was a contributing factor in the death of four patients (2%)[21]. Hypophosphatemia has been reported in up to 30% of HIV-infected patients, although multiple factors are often implicated[11,22]. It has been debated whether the determination of serum phosphorus is useful for monitoring the appearance or severity of tubulopathy. However, cross sectional studies have not shown significant differences in serum phosphate concentrations between tenofovir-experienced patients and other treatment groups[12,23,24]. Moreover, an increased urinary phosphate excretion has also been associated with tenofovir use[12,23], and there is a theoretical concern regarding the potential effect of this on long-term bone density. Decreased phosphate tubular reabsorption has been described as an early marker of tubular alteration, as several studies reported a fast decline after tenofovir introduction[25,26]. Normoglycemic glycosuria has been described in 2% of tenofovir-treated patients with normal glomerular filtration rate (GFR), and it was found in five out of seven nondiabetic patients biopsied for tenofovir nephrotoxicity with increased serum creatinine and residual diuresis[27]. Although albuminuria could be more prevalent, tubular proteinuria has been reported previously in HIV-infected patients even before the use of tenofovir[28], probably reflecting the toxicity of both the virus and other toxic drugs[29].

The limited value of these alterations in identifying tubular toxicity leads to considering meaningful proximal tubular renal dysfunction when two or more of these parameters are present, with at least one of them being any of the Fanconi syndrome-defining alterations (glycosuria in nondiabetic patients, hyperphosphaturia, or hyperaminoaciduria). Sodium, water, potassium[30], and calcium wasting are excluded because these features are not specific of proximal tubular renal dysfunction. In this way, a Spanish study[12] recorded significant proximal tubular renal dysfunction in 15% of 284 patients, defined by the presence of at least two of the following: glycosuria, hyperaminoaciduria, hyperphosphaturia, hyperuricosuria, and beta-2-microglobulinuria – these findings being more frequent in patients treated with tenofovir (22%). By contrast, this pattern of abnormalities was observed in only 6% of patients treated with non-tenofovir-containing ART regimens ($p = 0.01$) and in 12% of ART-naive patients ($p = 0.06$). Also, in the

Swiss cohort, tubular dysfunction, defined by the presence of at least three alterations (proteinuria, normoglycemic glycosuria, and increased urinary excretion of phosphate or urate) was found to be present in 6.5% of 1,202 patients[11], ranging from 0% in ART-naive patients to 12% in patients with tenofovir plus a protease inhibitor. An increased urinary excretion of phosphate, possibly the most sensitive marker of tubular dysfunction, was present in 42-50% of the patients under tenofovir, versus 25% of patients receiving other antiretrovirals, and 4% of untreated patients[11]. Similarly, in a French study, the prevalence of proximal tubulopathy was found to be 6.5%, correlated to advanced age and exposure to atazanavir and tenofovir[31].

These data suggest that significant tubular cell dysfunction or damage, as defined by strict criteria, is relatively frequent, ranging from 2-15%, but a lower degree of tubular cell alteration is more usual. On the other hand, the clinical significance of isolated parameters of tubular dysfunction, in the short and long term, remains unclear. In particular, it is unknown whether tubular dysfunction identifies patients at increased risk of Fanconi syndrome, progressive alteration of GFR, osteomalacia, or reduced bone mineral density. Primary tubular abnormalities, even severe, may be missed until they affect the glomerular function. In other cases, kidney tubular dysfunction is usually an initial sign of kidney disease, particularly that related to toxic drugs and ischemia, and the resulting kidney disease can be thus restricted to tubular cells or a specific region of the nephron or, to an extent, to renal function[32]. Ando, et al. suggest that with progression, this damage becomes more complex, as reflected by the development of proteinuria and a decrease in glomerular function in HIV-infected patients on effective ART[33]. In some severe cases, patients could progress to develop Fanconi syndrome or acute kidney injury, but this seems to occur only in a minority of individuals, so we can hypothesize that Fanconi syndrome or significantly decreased GFR are the top of the iceberg (Table 1). Subclinical tubular dysfunction is much more common, and it can manifest as decreased phosphate tubular reabsorption with or without hypophosphatemia, and/or tubular proteinuria.

Table 1. Prevalence of proximal tubular alterations in patients receiving tenofovir

Tubular alterations	Prevalence*
Fanconi syndrome	0.3-2%[18,19,21]
Proximal tubular renal dysfunction defined as two or more tubular alterations	6-15%[11,12,31,59] (22% if TDF)
Serum hypophosphatemia	4-31%[11,22,60,61]
Decreased phosphate tubular reabsorption	4-50%[11,25] (naive vs. TDF-treated, 22% if non-TDF)
Increase in biomarkers (B2M, RBP)	9-71%[12,25,26,31] (naive vs. TDF-treated)

*Variations in relation to age, type of antiretroviral therapy (protease inhibitors plus TDF, non-TDF), genetic susceptibility, and the presence of other classical risk factors for renal damage.
B2M: B2-microglobulin; RBP: retinol-binding protein; TDF: tenofovir.

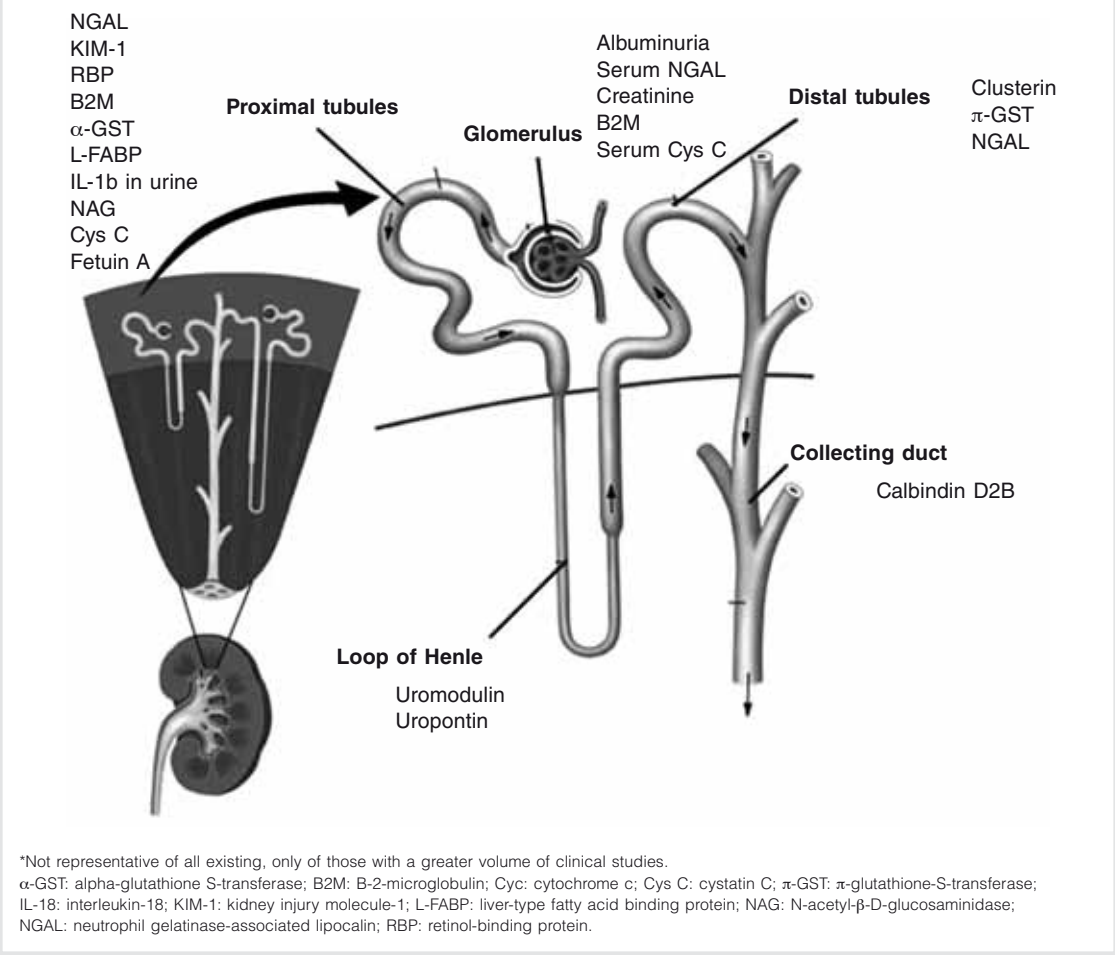
The need for renal biomarkers to identify tubular cell alteration

The term "biomarker" refers to any biological characteristic or parameter that can be measured and evaluated objectively as an indicator of some biological process (whether pathological or otherwise), or of response to some therapeutic interventions. An ideal renal biomarker should be able to detect the alteration as early as possible, with high sensitivity and specificity, and should be able to distinguish between different types of damage referred to both location (glomerulus, proximal tubule, Henle's loop, distal tubule) and the presence or absence of structural damage (progressive increase, decrease with changes in toxicity or treatment, etc.)[34,35]. Likewise, as a surrogate marker of renal alteration, a biomarker may be expected to offer information on mortality risk or, in the case of the kidney, the need for dialysis (Table 2).

Several biomarkers have been studied that could be classified according to the affected region of the kidney. Accordingly, the combination of different biomarkers

Table 2. Characteristics of an "ideal" renal biomarker

A substance of known and defined origin:
- Measurable in accessible fluids (blood and/or urine)
- Applicable across a variety of populations or subgroups of patients
- High sensitivity and specificity to indicate renal injury or the kidney response to therapy
- Gives information that is additive to that of clinical factors or other parameters
- Early manifestation in cases of dysfunction/damage, or it can identify different types of renal injury
- Correlation to the histological findings
- Usefulness as a measure of evolution over time



*Not representative of all existing, only of those with a greater volume of clinical studies.
α-GST: alpha-glutathione S-transferase; B2M: B-2-microglobulin; Cyc: cytochrome c; Cys C: cystatin C; π-GST: π-glutathione-S-transferase; IL-18: interleukin-18; KIM-1: kidney injury molecule-1; L-FABP: liver-type fatty acid binding protein; NAG: N-acetyl-β-D-glucosaminidase; NGAL: neutrophil gelatinase-associated lipocalin; RBP: retinol-binding protein.

Figure 1. *Renal biomarkers and localization.*

can allow us to define renal damage, its degree, location, and course or evolution (Fig. 1).

Therefore, the evaluation of biomarkers could improve the course of HIV-infected patients at risk of suffering tubular toxicity. This would imply that these biomarkers may be integrated into clinical decision algorithms and could improve our current ability to identify tubular cell alteration, and to distinguish between dysfunction and damage, and predict worsening toxicity and the need for treatment withdrawal.

However, further knowledge is required[36], especially in HIV-infected patients in whom several biomarkers are being included in studies of ART, although no consensus exists on how best to apply these tools. In addition, published studies have recognized many limitations, which preclude our ability to adapt their findings into clinical practice today[37].

Biomarkers assessing tubular toxicity in HIV infection

Several biomarkers have been included in studies of ART in order to evaluate the possibility of renal or/and tubular toxicity (Table 3).

Low-molecular weight proteins

Low-molecular weight proteins are small molecules freely filtered through the glomerulus, are almost completely recovered from the ultrafiltrate, and are catabolized by the proximal tubules. Therefore, low-molecular weight proteins are present in minimal amounts in the urine of individuals with normal tubular function. Increased excretion of these "tubular proteins"

Table 3. Main characteristics of urinary biomarkers assessing tubular toxicity

Biomarker	Clinical use	Sensitivity/specificity/values	Studies on HIV
B2M	Early marker of tubular dysfunction, but influenced by serum B2M and inflammation[24]	> 200-500 mcg/l UB2-M after initiating TDF[25,40]. No data on S/S	Can precede decrease in PTR. Values > 5,000 mcg/l in Fanconi syndrome[25,40]
Cystatin C	Urinary levels may predict adverse outcome (renal replacement therapy requirement), but in HIV+ can be influenced by inflammation	UCys/UCr ratio < 14 mcg/mmol showed a negative predictive value of 95.8% and a positive predictive value of 76.9% to rule out Fanconi syndrome[42]	One small study with 37 HIV+ on ART[42]
RBP	Full reabsorption in the proximal tubule via an ATP-dependent mechanism. ↑ URBP/UCr ratio is a marker of tubular transport alteration[43]	URBP/UCr ratio < 159 mcg/g[24]. No data on S/S	↑ URBP/UCr ratio on TDF-exposed patients and with tubular toxicity[24,51]
NGAL	Tubular and glomerular damage. Early predictor of AKI, as an indirect diagnostic method of HIVAN	UNGAL/UCr cutoff point of 121.5 mcg/g[46, 49]. No data on S/S	Weakly associated to RBP increase in urine in patients on TDF[46,48,62]
NAG	Derived from proximal tubular cells. Indicate tubular cell damage, severe cases of toxicity	UNAG/UCr ratio < 248 µmol/h/g[24]. No data on S/S	Increase in HIV+, not associated to TDF in several studies[28,33,51]
GST	α-GST specific for proximal tubule. Correlated to ATN	Not defined. No data on S/S. Urine GST higher in the tubular damage group[33]	Cytochrome C excretion was higher in TDF-treated patients[52]
L-FABP	Urine L-FABP is a sensitive indicator of acute and chronic tubulointerstitial injury[54]	Not defined. No data on S/S	No study
KIM-1	Marker of injury associated with renal tubular cell differentiation	Not defined. No data on S/S	One small study in a conference[55]
VCAM-1	Expressed during inflammation	Not defined. No data on S/S	No study
IL-18	Urine IL-18 is a sensitive and specific marker of ATN and delayed graft function in the post-ischemic kidney	Not defined. No data on S/S	No study
TNFR-1	Increases during renal inflammation, correlates with progression of AKI	Not defined. No data on S/S	

AKI: acute kidney injury; ART: antiretroviral therapy; ATN: acute tubular necrosis; B2M: B-2-microglobulin; GST: glutathione S-transferase; HIV+: HIV-infected patients; HIVAN: HIV-associated nephropathy; IL-18: interleukin-18; KIM-1: kidney injury molecule-1; L-FABP: liver-type fatty acid binding protein; NGAL: neutrophil gelatinase-associated lipocalin; NAG: N-acetyl-β-D-glucosaminidase; S/S: sensitivity/specificity; TDF: tenofovir; TNFR-1: tumor necrosis factor receptor-1; UB2-M: urine B2M; URBP/UCr: urine RBP:creatinine ratio; UCys/UCr: urine cystatin C:creatinine ratio; UNGAL/UCr: urine NGAL:urine creatinine ratio; UNAG/UCr: urine NAG:urine creatinine ratio; VCAM-1: vascular cell adhesion molecule-1.

is indicative of tubular alteration, and their urine concentration constitutes a measure of the severity of such dysfunction. As occurs with proteinuria of glomerular origin, proteinuria of tubular origin is best expressed as a ratio versus creatinine in urine in order to avoid differences due to concentration in urine. Several low-molecular weight proteins, including retinol-binding protein (RBP), cystatin C (Cys C), B2-microglobulin (B2M), and neutrophil gelatinase-associated lipocalin (NGAL) have been studied as markers of tubular dysfunction.

Beta-2-microglobulin

Beta-2-microglobulin is a 12 kDa protein forming part of the HLA histocompatibility antigen. It is found on the surface of most nucleated cells, passes through the glomerular filter, and is almost completely reabsorbed (99.9%) and degraded in the proximal tubule. Elevated urine B2M is typically the result of proximal tubular dysfunction, and this circumstance is accepted as a good indicator of proteinuria of tubular origin[38]. Some cross-sectional studies have shown an association

between tenofovir exposure and increased urinary excretion of B2M in both children[39] and adults[40]. Gatanaga, et al. reported on 30 out of 70 patients receiving tenofovir having high urine B2M values (high values considered if ≥ 500 μg/l, as lower values have been found in healthy volunteers), while only three of them showed creatinine alterations and 11 presented a diminished GFR. Moreover, B2M levels decreased $0.86\text{-}2.15_{log}$ 5-8 months after withdrawal of tenofovir[40], suggesting a reversibility of tubular dysfunction. In two cohort studies, an increase in B2M (from 188 mcg/l at baseline to 555 mcg/l in week 96) was observed after tenofovir initiation, while the median of phosphate tubular reabsorption decreased from 94% at baseline to 90% at week 96[25,26]. This observation led to evaluation of whether B2M is a sensitive marker of tubular dysfunction, and whether the increase in its excretion precedes deterioration of phosphate tubular reabsorption or *viceversa*. A recent study published by Dauchy, et al.[31] describes alteration in phosphate tubular reabsorption in 7% of the patients, while B2M elevation was documented in 16.7%. On the other hand, it has been suggested that although B2M could be a useful tubular dysfunction marker, its detection in urine is interfered by dependency upon the pH value and enzymatic reaction[24].

Cystatin C

Cystatin C, a low-molecular weight protein, has recently emerged as a reliable alternative biomarker of renal function. Cystatin C is a cysteine protease inhibitor that is constantly produced by nucleated cells and released into the blood, where it is normally reabsorbed and catabolized by kidney tubules without re-entering the blood stream[41]. It has been widely investigated as a glomerular filtration marker. Conversely, cystatin C has been little studied as a tubular marker. In a recent study, the urinary cystatin C to urinary creatinine ratio (UCys/UCrea) has demonstrated a negative predictive value of 95.8% and a positive predictive value of 76.9% to rule out a Fanconi syndrome. The highest accuracy was obtained with a threshold of 14 μg/mmol[42]. Urinary levels may predict adverse outcome (renal replacement therapy requirement), but in HIV-infected patients can be influenced by inflammation.

Retinol-binding protein

Retinol-binding protein is a 21 kDa protein that circulates in plasma bound to transthyretin; the free fraction (about 10%) is freely filtered through the glomerulus and is reabsorbed in the proximal tubule via an ATP-dependent endocytic mechanism. As a result, an increase in urine of the RBP-creatinine ratio is a marker of tubular transport alteration[43]. The RBP-creatinine ratio could be more specific to identify tenofovir tubular toxicity, presumably due to the inhibition of proximal tubule uptake and/or transport of tubular proteins, or a low threshold effect of proximal tubule cellular ATP depletion[24]. Decreased sensitivity may be observed in vitamin A deficient states.

Neutrophil gelatinase associated lipocalin

Neutrophil gelatinase-associated lipocalin, also called lipocalin 2 or human neutrophil lipocalin, is a 25 kDa protein produced by neutrophils and epithelial cells, such as the renal tubular cells, and is induced by inflammatory conditions. It has been extensively studied as a sensitive renal damage marker and indicator of the progression of damage. NGAL is correlated to the severity of proteinuria and is inversely correlated to GFR. Unfortunately, substantial extrarenal NGAL generation in response to systemic stress can increase urinary NGAL excretion in the absence of acute kidney injury as well, and this may also arise from chronic and not just acute, renal disease[44]. In a study of 96 patients, the serum and urine values of NGAL predicted reductions in GFR over 15 months of follow-up[45]. Data on the usefulness of NGAL in HIV-infected patients are limited. Decreased levels have been recorded in serum, followed by elevations with ART[46,47]. However, high urine levels have been correlated to HIV-associated nephropathy (387 ± 338 vs. 94 ± 101 μg/g creatinine). A cutoff point of 121.5 μg NGAL/g creatinine has been proposed for the diagnosis of HIV-associated nephropathy (sensitivity 94%, specificity 71%; AUC: 0.88)[48], but no cutoff values have been published for renal toxicity.

N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) is found in the cells of the proximal tubules as a hydrolytic enzyme basically located in the liposomal fraction, with a molecular weight of 130-140 kDa. Due to its large molecular size, NAG is not filtered and its appearance in urine may therefore reflect a loss of lysosomal integrity. It is therefore more indicative of tubular damage than of tubular cell dysfunction. In the course of active kidney disease, urinary NAG levels remain persistently elevated. The increase in urinary NAG activity indicates damage to tubular cells, although it can also reflect

increased lysosomal activity without cellular damage[49]. However, the use of NAG remains limited by the fact that urinary excretion of the enzyme is also elevated in glomerular diseases such as diabetic nephropathy[50].

In combination, a study of the pre-HAART era found HIV-infected patients to have 3- to 10-fold higher urinary concentrations of B2M, RBP, and NAG than the controls without HIV infection[28]. Recently, a study of 99 patients without proteinuria or altered serum creatinine levels recorded higher urine RBP levels in the subjects receiving tenofovir, with no differences in the NAG values according to whether tenofovir was administered or not[24]. Retinol-binding protein is believed to be more sensitive and specific in this respect, with a lesser tendency to show variations. The ASSERT study reported a greater change in the RBP-creatinine and B2M-creatinine ratios in the tenofovir/emtricitabine treatment arm (+50, +24%, respectively) in comparison with abacavir/lamivudine (no change; –47%), after 48 weeks of therapy. Excretion of NAG proved similar in both arms, suggesting the existence of functional alteration rather than established damage[51].

Others biomarkers

Glutathione S-transferases

Glutathione S-transferases (GST) are the most specific and sensitive biomarkers to detect tubular damage (a-GST is specific for proximal tubule). They are found in high concentrations in tubular cells and are easily delivered to urine. They have been correlated to acute tubular injury in cases of nephrotoxicity, ischemia, transplant rejection, and diabetic nephropathy. However, in a recent study, markers of mitochondrial toxicity (cytochrome c) or cytosolic (α-GST) together with common indicators of renal damage were assessed after including tenofovir/emtricitabine or abacavir/lamivudine into the regimen, without significant variation in both groups, while cytochrome c excretion was significantly higher in tenofovir-treated patients[52]. These data suggest more usefulness for tubular cell damage than for identifying dysfunction.

Liver-type fatty acid binding protein

Liver-type fatty acid binding protein (L-FABP) is a marker that is shed by proximal tubular cells in response to hypoxia from decreased peritubular capillary flow. Urine levels of L-FABP are a sensitive indicator of acute and chronic tubulointerstitial injury[53,54]. Increased urinary levels in acute liver injury may limit specificity[35]. Among 120 HIV-negative individuals with nondiabetic chronic kidney disease, urinary L-FABP concentrations correlated with proteinuria and serum creatinine levels[54].

Kidney injury molecule 1

Kidney injury molecule 1 (KIM-1) is a transmembrane protein present in adult proximal tubular epithelial cells only after injury, when urinary concentrations rise to very high levels. KIM-1 appears to be a marker of injury associated with renal tubular cell differentiation. Only one small study in a conference mentioned KIM-1 as a biomarker in HIV-infected patients[55].

Vascular cell adhesion molecule-1

Vascular cell adhesion molecule-1 (VCAM-1) is expressed by renal cells during inflammation and is practical for lupus nephritis and renal transplant rejection.

Interleukin-18

Interleukin-18 (IL-18) is a proinflammatory cytokine that is produced by leukocytes, vessels, and kidney tubules. During acute kidney injury, there is a substantial increase in IL-18 production by tubules. Elevated urine levels of IL-18 are a relatively sensitive and specific marker of acute tubular necrosis and delayed graft function in the postischemic kidney[56]; however, IL-18 measurement may also be influenced by endotoxemia, inflammatory, and autoimmune diseases. Interleukin-18 increases in tandem with NGAL, and continues to increase after NGAL concentrations start to fall.

Tumor necrosis factor receptor-1

Tumor necrosis factor receptor-1 (TNFR-1) is one of the major receptors for the proinflammatory cytokine TNF-α, which is expressed on infiltrating leukocytes and some resident kidney cells during renal inflammation. Serum and urine levels of soluble TNFR-1 are increased during acute and chronic renal inflammation and correlate with the progression of acute renal failure, lupus nephritis, and diabetic nephropathy[57].

There are other recently emerging biomarkers such as Netrin-1, a molecule that is not or barely expressed in tubular epithelial cells of normal kidneys. However, it is highly expressed and excreted in the urine after acute kidney injury in animals. Monocyte chemotactic peptide-1 (MCP-1) is considered a biomarker of the

mononuclear inflammatory processes that occur after ischemia-induced acute kidney injury. Urinary MCP-1 may be a useful biomarker of acute kidney injury, possibly providing complementary information to that derived from NGAL analysis[35].

In summary, both B2M and RBP are indicators of tubular dysfunction, and appear to offer high sensitivity in patients receiving tenofovir, while NAG, which indicates proximal tubular damage, is not significantly altered except in severe cases of toxicity. As mentioned, tenofovir could have a more specific effect in the RBP-creatinine ratio, though it appears to be related to the inhibition of tubular protein transport and/or increased sensitivity in ATP depletion in the proximal tubule[43]. In turn, NAG appears to indicate both glomerular and proximal or distal tubular damage. As a result, its potential role as an isolated biomarker in the absence of other information is limited[51]. There are a number of characteristics that might make KIM-1 attractive as a biomarker of kidney injury; absence of KIM-1 expression in the normal kidney, its marked upregulation and insertion into the apical membrane of the proximal tubule, and its persistence in the epithelial cell until the cell has completely recovered[55]. Other additional biomarkers may be required to improve the specificity for the diagnosis of tubular injury, such as combinations of biomarkers like NGAL and IL-18 which has been observed to increase in tandem in some cases[58].

Conclusions

Antiretroviral-associated toxicity, especially in the case of tenofovir plus protease inhibitors boosted with ritonavir, could be focused on the proximal renal tubule, producing features of minimal dysfunction, damage, or even Fanconi syndrome and decreased renal function. Data of cross-sectional studies and clinical trials suggest that dysfunction could be very frequent, whereas extensive damage is rarely observed. In any case, the clinical significance of isolated tubular dysfunction remains unclear. The presence of tubular proteinuria is thought to be the most sensitive test for proximal tubule dysfunction. Several studies have shown that urinary RBP-creatinine ratio is a useful and specific marker of tenofovir-associated tubular dysfunction, whereas the sensitivity of B2M is high. An increase in these biomarkers could be the only manifestation of tubular cell dysfunction, in some cases associated to a lower reabsorption of phosphate. NAG, NGAL, or even GST could be useful to identify those patients with more extensive lesion of cells, in whom glycosuria or other parameters associated to Fanconi could appear. In addition, KIM-1 seems an attractive biomarker of kidney injury. The use of these biomarkers in combination could permit to distinguish between dysfunction, damage, or tubular necrosis, and even the possibility of recovery after treatment withdrawal. In any case, screening of subclinical tubulopathy using these urinary biomarkers would be a simple and non-invasive means of identifying HIV-infected patients on ART at risk of Fanconi syndrome[23,42]. Moreover, the use of these biomarkers might possibly allow early identification of patients at risk of progressive tubular dysfunction and a decline in kidney function. Further studies should clarify the specific role of these biomarkers as useful predicting tools for antiretroviral associated renal toxicity.

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