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Urinary Biomarkers of Kidney Diseases in HIV-infected children

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Abstract

A significant number of children infected with the HIV-1 virus all over the world are at risk of developing renal diseases that could have a significant impact on their treatment and quality of life. It is necessary to identify children undergoing the early stages of these renal diseases, as well as the potential renal toxicity that could be caused by antiretroviral drugs, in order to prevent the development of cardiovascular complications and chronic renal failure. This article describes the most common renal diseases seen in HIV-infected children, as well as the value and limitations of the clinical markers that are currently being used to monitor their renal function and histological damage in a non-invasive manner. In addition, we discuss the progress made during the last 10 years in the discovery and validation of new renal biomarkers for HIV-infected children and young adults. Although significant progress has been made during the early phases of the biomarkers discovery, more work remains to be done to validate the new biomarkers in a large number of patients. The future looks promising, however, the new knowledge needs to be integrated and validated in the context of the clinical environment where these children are living.

Keywords

HIV associated nephropathy; renal urinary biomarkers; Hemolytic Uremic syndrome; acute kidney injury; proteinuria

The Joint United Nations Program on HIV/Acquired Immune Deficiency Syndrome (UNAIDS) estimated that there were approximately 3.2 million children under 15 years of age living with HIV around the world at the end of 2013, and 240,000 children became newly infected in 2013 (Gap Report, July 2014). Approximately 90% of these children live in Sub-Saharan Africa, and the majority of them (~70%) are not receiving appropriate antiretroviral therapy (ART). During the early years of the AIDS epidemic, approximately 40% of all HIV infected children experienced renal complications leading to poor growth, accelerated progression of AIDS, and/or premature death [1, 2]. Therefore a large number of HIV-infected children are at high risk of developing renal diseases, and it is necessary to identify these patients during the early stages of renal injury to prevent the development of

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chronic renal failure [1, 2]. This article discusses why it is important to develop biomarkers of renal diseases specifically for HIV-infected children, as well as the progress made in the renal biomarker field to assess the outcome of renal injury in HIV-infected children and young adults.

Epidemiology of HIV-related kidney diseases, risk factors, and biomarkers

According to the World Health Organization (WHO), more than 39 million HIV-infected people have died of AIDS, and there were approximately 35 million people infected with HIV all over the world at the end of 2013 (HIV/AIDS Fact sheet N° 360, WHO, November 2014). With the widespread introduction of effective antiretroviral therapy (ART), renal-cardiovascular diseases have surpassed opportunistic infections as the major cause of chronic morbidity in these patients [3]. As discussed in the recent clinical guideline for the management of renal diseases in HIV-infected patients [3], HIV infection is a major risk factor for the development of chronic kidney disease (CKD). Furthermore, compared with HIV-negative people, the prevalence of albuminuria (>30 g/day) has been reported to be 2- to 5-fold higher in HIV-infected individuals [4, 5]. Several risk factors are associated with an increased risk of developing CKD in HIV-infected people. These factors include, a family history of end stage renal disease (ESRD), African ancestry, genetic predisposition (e.g. *APOL-1* renal risk variants), lower CD4 cell count, advanced immunosuppression, history of acute kidney injury, injection of drugs of abuse, proteinuria, hypertension, diabetes, hepatitis C infection, some antiretroviral drugs, and higher HIV RNA levels [3, 6, 7]. Taken together, these findings suggest that HIV-1 per se can worsen the outcome of many renal diseases that are frequently seen in HIV-negative people. Therefore, it is important to develop new biomarkers for HIV-infected people, in order to identify the early stages of these renal diseases and initiate the corresponding treatment as soon as possible. Alternatively, other renal diseases are only seen in HIV-infected people, including HIVAN [6], HIV-immune complex renal diseases [8, 9], renal nephrotoxicity syndromes associated with the use of antiretroviral therapy, including indinavir [10] and several nucleotide analogs [11, 12], HIV-associated Hemolytic Uremic Syndrome [13], and other thrombotic microangiopathies (TMA) [14]. It is worth mentioning here that although the diagnosis of HIV-HUS or HIV-TMA diseases appears to be obvious on clinical grounds, this is not always the case. HIV-HUS usually has an insidious clinical onset in children, without a diarrhea prodrome, oliguria, or hypertension [2, 13], and these patients may also have pre-existing hematological abnormalities, including thrombocytopenia and anemia. For example, recently we reported a case of an adolescent girl with HIV-TTP who presented with a clinical history of idiopathic thrombocytopenic purpura (ITP), masking the early diagnosis of TTP [14]. Overall, we need new biomarkers to identify the early stages of all HIV-TMA diseases, determine the severity of each case, and predict the risk of long-term clinical outcome. In summary, all HIV-renal diseases need to be approached with special clinical considerations, and the selection of the most appropriate biomarkers for each HIV-renal disease is important.

Renal diseases in HIV-infected children

There is a wide spectrum of pediatric renal diseases that occur in the setting of HIV-infection. HIV-infected children may present either with exacerbations of common renal conditions, or with renal diseases that are specifically induced by the viral activity of HIV-1 in the kidney. A summary of the most frequent renal diseases that affect HIV-infected children is shown in Table 1. Here, we will discuss three renal diseases that represent the most frequent glomerular and tubular lesions seen in HIV-1 infected children, and that had been studied in order to generate new candidate biomarkers for these patients.

HIV-associated nephropathy (HIVAN)

HIVAN is a renal disease induced directly by HIV-1 almost exclusively seen in people of African ancestry [15–17]. It is the most frequent chronic renal disease seen in children of African ancestry infected with HIV-1 [1, 2, 18]. From the clinical point of view, children with HIVAN can present either with isolated proteinuria or the classic features of nephrotic syndrome, which include heavy proteinuria, edema, and hypoalbuminemia. Those who present with isolated proteinuria can sustain normal serum creatinine values for a long period of time [1]. The renal histological lesions of HIVAN are characterized by the presence of focal and segmental collapsing glomerulosclerosis (FSGS) and microcystic transformation of renal tubules (Fig. 1A) leading to renal enlargement and chronic kidney failure [17, 19–21]. The latter changes are the most consistent renal histological findings in children with HIVAN [18]. HIVAN is triggered by the infection of podocytes and renal tubular epithelial cells [22–24], yet the mechanism is unclear. A genetic predisposition associated with two genetic risk variants of apolipoprotein L-1 (APOL1), appears to play a critical role in the pathogenesis of HIVAN [25–27]. At the present time, a renal biopsy is the only way to establish a definitive diagnosis of HIVAN, and new renal biomarkers are needed to avoid this invasive procedure in children. The early institution of ART has improved the clinical outcome and survival of these patients [15, 16, 28], but the long-term consequences of ART in children are unknown. Moreover, it is unclear whether ART can cure or completely prevent the progression of HIVAN, since patients with a very low viral load, including those receiving kidney transplants, can develop HIVAN [29–31].

HIV-associated Hemolytic Uremic Syndrome

HIV-infected children can develop an atypical and often lethal form of hemolytic uremic syndrome (HUS) which is defined by the presence of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure [13]. The prevalence of HIV-HUS and other HIV-TMA disease has decreased in a significant manner in children treated with ART [1]. Nonetheless, HIV-HUS is often a lethal disease that needs to be treated as soon as possible to prevent major systemic bleeding complications [1, 2, 13]. As discussed above, performing an early diagnosis of HIV-HUS based on clinical grounds is not always easy and new markers are needed to identify children undergoing the early stages of HIV-HUS, and predict the risk of development lethal systemic vascular complications or CKD [1, 13]. HIV-HUS is characterized by an insidious clinical onset, preserved urine output, and the absence of preceding diarrhea [13, 32, 33]. Rapid progression to end stage renal disease, or death from infectious or bleeding complications is common [2]. The presence of proteinuria and

high viral load in HIV-infected children are risk factors for the development of HIV-HUS, which is triggered by infectious agents that induce renal endothelial injury and thrombotic microangiopathy (TMA) [13, 32–34]. From the histological point of view, HIV-HUS show TMA lesions characterized by the accumulation of fibrin, dilated capillaries, and tubular micro-cysts (Fig 1B) [13]. It should be noted however, that HIV-infected patients can develop asymptomatic TMA or TMA associated with other diseases, including Thrombotic Thrombocytopenic Purpura (HIV-TTP) [35]. Thus, it is necessary to develop new biomarkers to identify the early stages of HIV-HUS, and other diseases associated with TMA lesions in HIV-infected children.

Acute Kidney Injury (AKI)

In addition to HIV-HUS, HIV-infected children can develop AKI due to severe dehydration, poor renal perfusion, sepsis, and nephrotoxic drugs [2]. AKI is the most frequent renal disease seen in all HIV-infected children [1, 2]. AKI is defined as an abrupt decrease in GFR (“within 48”), which is manifested by an elevated serum creatinine [36]. However, changes in serum creatinine may not accurately estimate the GFR in a patient whose renal function is changing fast. Despite these limitations, a rise in serum creatinine continues to be the most widely used laboratory marker to diagnose AKI. Years ago, a consensus definition called the RIFLE classification of AKI was developed which consists of three graded levels of renal injury (Risk, Injury, and Failure) and two outcome measures (Loss and End-stage renal disease) [37]. This definition was adapted for pediatric patients (pRIFLE) using the estimated creatinine clearance (eCrCl) and the urine output [38] (Table 2). Nonetheless, since the assessment of the eCrCl is based on the serum creatinine values, newer biomarkers are needed to identify children undergoing the early stages of AKI.

Assessment of renal injury in HIV-infected children

The following renal findings are used in the clinical setting of HIV-infected children to define CKD: (1) A random urinary protein-to creatinine ratio > 0.2 (mg/mg) [39]. Urine protein to creatinine ratios > 1.0 (mg/mg) are considered nephrotic range proteinuria (> 100 mg/m²/d or 40 mg/m²/h) [39, 40]; (2) Urine sediment abnormalities, including the presence of cast and renal epithelial cells [1]; (3) Fluids-electrolytes abnormalities due to tubular disorders; (4) abnormal renal histology; (5) Structural abnormalities detected by imaging, including enlarged echogenic kidneys; (6) Decreased glomerular filtration rate (GFR) < 60 ml/min per 1.73 m², assessed by estimating the creatinine clearance (CrCl); (7) Increase serum creatinine values according to age. In younger children (< 2 years of age), the urinary protein excretion and GFR values are adjusted according to the normal values for the corresponding age. Although the serum creatinine concentration performs well for assessing kidney function in patients with stable CKD [41, 42], it has limitations in HIV-infected children, because these values are influenced by the total protein intake, body weight, nutritional status, muscle mass or breakdown, and the tubular secretion of creatinine in the context of ongoing renal injury and multiple medications. Unfortunately, all these factors are usually affected in HIV-infected children.

Previous studies have suggested that cystatin C could become a valuable renal biomarker in HIV-infected patients [43–46]. Cystatin C is a nonglycosylated low molecular weight protein

that is synthesized and secreted at a nearly constant rate by almost all nucleated cells, and is freely filtered by the glomeruli [36, 47]. In contrast to creatinine, cystatin C is not excreted in the urine, and it is reabsorbed and metabolized by proximal tubular cells [47]. Several studies done in HIV-negative people suggest that cystatin C may be superior to serum creatinine [47, 48]. Furthermore, cystatin C has been proposed as a more sensitive marker of kidney disease in HIV-infected adults [43–46]. However, other studies [44, 45] have questioned this notion arguing that these studies lack gold standard measurements of GFR, or that the serum cystatin C levels are affected by changes in viral load and systemic inflammation [44, 49, 50]. Thus the value of cystatin C as marker of GFR in HIV-infected children is unclear at the present time. We have found elevated levels of cystatin C in the urine of HIV-infected children with proteinuria [51]. These findings suggest an impaired ability of the proximal tubular epithelial cells to reabsorb and metabolize cystatin C in these patients. Overall, given the inherent limitations of proteinuria assessments and GFR estimations, there is a need to develop new biomarkers to assess both the GFR and renal histological damage in HIV-infected children.

Biomarkers for HIV-associated renal diseases

Iron binding proteins as biomarkers for childhood HIV-associated renal diseases

As discussed above, the identification of new biomarkers to facilitate the early diagnosis of HIVAN, HIV-HUS, AKI, and other HIV-associated renal diseases, could have significant impact in the clinical care of HIV-infected children. To explore this issue, we assessed the value of eleven urinary proteins that are considered excellent biomarkers of glomerular diseases in HIV-negative adult patients [52] in children with HIVAN and HIV-HUS [53]. These proteins are: orosomucoid, transferrin, α -1 microglobulin, zinc- α 2-glycoprotein, α 1-antitrypsin, complement factor B, haptoglobin, transyltin, retinol binding protein, albumin, and hemopexin [52]. Using two dimensional Gel Electrophoresis (2-DE) and mass spectrometry analysis we found that all children with HIVAN and HIV-HUS showed high urinary levels of all these proteins, when compared to samples collected from HIV-infected children without renal disease [53]. However, we were unable to identify a single urine protein biomarker to distinguish children with HIVAN vs. HIV-HUS. The more relevant changes were noted in the urinary excretion of the iron related proteins haptoglobin, hemopexin, and transferrin. Haptoglobin, is a plasma protein that participates in the recycling of heme iron [54]. Hemopexin, is a heme binding plasma glycoprotein, that protects against hemoglobin-mediated oxidative damage during intravascular hemolysis [55], and transferrin is a key protein involved in the transport of iron [56]. Moreover, we found elevated levels of iron, lactoferrin, and neutrophil gelatinase-associated lipocalin (NGAL) in the urine of children with HIVAN and HIV-HUS [53]. Lactoferrin is an antioxidant iron-binding protein [57], and NGAL is an iron trafficking protein [58], which is also an established biomarker of AKI [59, 60]. In agreement with these findings, HIV-Tg rats with renal disease and TMA lesions also showed elevated urinary levels of iron and NGAL [53]. In addition, since endothelial injury plays a critical role in the pathogenesis of TMA lesions, and injured endothelial cells release FGF-2 [61], we measured and detected high levels of FGF-2 in the circulation and urine of children with Stx-HUS and HIV-HUS [33, 34]. In summary, these findings identified FGF-2, iron, NGAL, and other iron-related

proteins as potential candidate urinary biomarkers for children with HIV-HUS. Considering that iron can induce renal cytotoxic effects [62], it is tempting to speculate that the accumulation of iron may play a role in the pathogenesis of these diseases as well.

NGAL and HIV-infection

NGAL is produced by distal renal tubular cells, but is also produced by other cell types including neutrophils [63], and is a critical component of the immunity to bacterial infections [64]. In agreement with this notion, a previous study in HIV-infected patients showed decreased NGAL serum levels, potentially reflecting decreased number and function of neutrophils as well as impaired bone marrow myelopoiesis, and these changes were reversed with ART [65]. Therefore, in patients undergoing systemic infections or multi-organ failure, the serum levels of NGAL are a less specific biomarker of renal injury [64, 66]. Nevertheless, in support of the notion that NGAL could become a reliable biomarker for children with HIVAN, studies in adults demonstrated that the expression of NGAL was much higher in patients with biopsy proven HIVAN than in HIV-positive and HIV-negative patients with other forms of chronic kidney disease [67]. Furthermore, in an HIV-transgenic mouse model, NGAL mRNA was increased in dilated microcystic segments of the nephron [67]. In contrast urinary NGAL did not correlate with proteinuria in human or mouse models. Based on these findings, the authors concluded that urinary NGAL could become an excellent biomarker to screen for HIVAN-related tubular damage [67]. A follow up study comprising 25 HIV-infected adults, including 18 patients with HIVAN and 7 with other glomerular diseases, demonstrated that NGAL had 94% sensitivity and 71% specificity for the diagnosis of HIVAN with an area under the receiver operator characteristic (ROC) curve of 0.88 [68]. Thus, further studies are warranted in a larger cohort to determine the value of NGAL for the noninvasive diagnosis of HIVAN.

Biomarkers for childhood HIVAN

Using the proteomic techniques discussed above, we were unable to identify a biomarker profile that could distinguish children with HIVAN from those with other glomerular diseases. Therefore, we used an alternative approach based on our knowledge of the pathogenesis of childhood HIVAN. Briefly, previous studies documented a progressive renal accumulation of FGF-2 in HIVAN [34, 69–71] in correlation with the progression of the microcystic tubular lesions. As discussed above, these lesions are the most consistent renal histological findings in children with HIVAN [72]. Therefore, we measured the urinary levels of FGF-2 in HIV-infected children with and without renal diseases, in combination with Epidermal Growth Factor (EGF) and Matrix Metalloproteinase-2 (MMP-2) [51]. FGF-2 plays an important role in the regeneration of renal tubules [73, 74] and can induce the formation of renal cysts [75, 76]. Moreover, FGF-2 is accumulated in the kidney of children with HIVAN bound to an increased number of heparan sulfate proteoglycans (HSPG) [70, 71]. The activity of MMP-2 is enhanced during the process of renal tubular regeneration [77, 78], and MMP-2 facilitates the release and activation of FGF-2 bound to HSPG [79]. EGF is a powerful mitogen which is predominately synthesized by renal tubular epithelial cells [80, 81]. However, its synthesis and release into the tubular lumen is decreased in injured renal tubular epithelial cells [80–83]. In agreement with these observations, we found elevated urine levels of FGF-2 and MMP-2, in association with

reduced urine levels of EGF in children with HIVAN (Fig 1C). In summary, these findings suggest that the urinary biomarker profile comprised of high levels of FGF-2 and MMP-2, and low levels of EGF, could become a useful biomarker tool to identify children with HIVAN [51, 84].

A new biomarker profile for AKI in critically ill children

Based on the findings described above, and considering that HIV-infected children frequently develop AKI due to changes in renal perfusion or sepsis, we tested the value of a urinary biomarker profile comprised of elevated urinary levels of NGAL, FGF-2, and decreased urinary levels of EGF, in critically ill children [85]. NGAL was selected as a marker of renal tubular injury because it is considered an established biomarker of AKI [59, 60]. FGF-2 was selected as marker of renal epithelial and endothelial injury and regeneration, because these processes play a critical role in the pathogenesis of AKI induced by poor renal perfusion and septic shock, and because injured endothelial cell release FGF-2 [34, 85, 86]. Finally, EGF was selected because previous studies show that the urinary levels of EGF could be used to monitor the outcome of AKI in children [82, 87, 88] (Fig. 1D). Using selected cutoff points for NGAL, FGF-2 and EGF, this biomarker profile demonstrated a 89% sensitivity and 89% specificity for the diagnosis of AKI in critically ill children admitted to a Pediatric Intensive Care Unit, with an area under the ROC curve of 0.94 [85]. The positive predictive value of this test was 0.85 (95% CI 0.65 to 0.98) and the negative predictive value was 0.93 (95% CI 0.71–0.97) [85]. This biomarker profile can be used in both HIV-positive and HIV-negative children, although further studies in larger cohort of children are warranted to validate these data. Several other AKI biomarkers have been identified in HIV-negative patients, as described in detail in previous publications [36, 89]. More studies are needed to define their role in HIV-infected children.

Biomarkers of renal tubular dysfunction and injury

Previous reports described the performance of well established biochemical markers to assess the tubular function of HIV-infected patients [90]. These markers included hyperaminoaciduria, phosphaturia, glucosuria in patients without diabetes, and increased fractional excretion of uric acid. In addition, hypercalciuria and acidosis were the most relevant complications seen in HIV-infected children followed in Venezuela [91]. Similar renal tubular features were seen in HIV-infected children treated in the US during the early years of the AIDS epidemic [1].

Alternatively, other studies have explored the role of low molecular weight proteins (LMWPs) as tubular biomarkers in HIV-infected patients [44]. These proteins are freely filtered through the glomerulus, and are subsequently reabsorbed and catabolized by proximal tubular cells. Therefore, in patients with normal tubular function the urine levels of LMWPs are very low. As reported before [53], using 2-DE we identified three LMWPs that are established biomarkers of proximal tubular dysfunction in HIV-infected children with proteinuria. These markers are, α -1 microglobulin (α -1M), β 2- microglobulin (β -2M) [92] and retinol binding protein (RBP) [53]. α -1M is glycosylated protein of ~ 26–33 kDa produced mainly in the liver [93]. β -M2 is a 12 kDa protein that is a component of the major histocompatibility complex (MHC) class I molecules, and is present in all nucleated cells

[94]. RBP is a 21 kDa protein that circulates in plasma bound to transthyretin, although its unbound fraction is freely filtered and reabsorbed by the proximal tubule via an ATP dependent endocytic mechanism [94]. Therefore, an increased RBP-creatinine ratio can be used to identify a tubular transport defect [95]. Taken together, high urinary levels of α -1M, β -M2, and RBP indicate the presence of tubular dysfunction or tubular injury, while other biomarkers discussed above (e.g. NGAL or EGF), could further confirm the presence of tubular injury. The elevated urinary levels of β -M2, and RBP in our cohort of HIV-infected children, were also associated with elevated urinary cystatin C levels [51], further documenting the failure of proximal tubular cells to reabsorb and/or metabolize cystatin C in these patients. HIV-infected children with trace proteinuria measured by dipstick, also show elevated levels of β -M2, and RBP, and these findings suggest that proximal tubular injury is an early event in the pathogenesis of many HIV-associated glomerular diseases, including HIVAN [53]. These findings are in agreement with the results reported in HIV-Transgenic mice [71]. Unfortunately, it is not always possible to determine whether the elevated urinary levels of LMWPs are due to the presence of isolated tubular injury or the early stages of HIV-glomerular diseases. In agreement with this notion, we found elevated levels of γ -glutamyl transpeptidase (γ -GT) and lysozyme, two enzymes primarily located in the brush border of proximal tubular cells [96], in a group of HIV-infected children with proteinuria. The urinary lysozyme levels were elevated in HIV-infected children with trace proteinuria, suggesting an early involvement of the brush border of proximal tubular cells in several glomerular renal diseases [51]. Taken together, these studies suggest that LMWPs are sensitive biomarkers to detect renal tubular dysfunction in HIV-1 infected children, but that they should be used in combination with other biomarkers to rule out the presence/absence of glomerular and tubular injury.

Biomarkers of renal toxicity induced by ART

Although ART has dramatically reduced the mortality and improved the renal outcome of all HIV-infected children, most ART drugs have some toxicity. Several antiretroviral drugs have been linked to renal toxicity, including tenofovir disoproxil fumarate (TDF) and nucleotide reverse transcriptase inhibitors (NRTI). TDF is a nucleotide analogue of adenosine 5'-monophosphate that is eliminated unchanged in the urine by a combination of glomerular filtration and proximal tubular secretion [97, 98]. Two other nucleotide analogues with structural similarities to TDF, cidofovir and adefovir, can also cause nephrotoxicity [99]. These drugs are actively transported into renal proximal tubular cells and secreted into the tubular lumen. A number of mechanisms and drugs given to HIV-infected patients can interact with the transport mechanisms and facilitate the accumulation of TDF inside renal tubular cells, causing cytotoxicity through mitochondrial disruption [100].

For this reason, current clinical practice guidelines from the Infectious Diseases Society of America, recommend avoiding TDF HIV-infected patients who have a GFR < 60 ml/minute/1.73 m², whenever feasible [3]. These guidelines also suggest avoiding TDF as part of first-line therapy in prepubertal children (Tanner stages 1–3), because TDF is associated with increased renal tubular abnormalities and bone mineral density loss in this age group. Fanconi's syndrome is the most important clinical consequence of tubular toxicity in HIV-

infected children, because it affects the regulation of calcium and phosphorus balance, inducing growth retardation and bone problems [101]. TDF has been the drug most often associated with the development of Fanconi's syndrome, albeit at a very low incidence (0.2–2%) [100]. Alternatively, TDF can cause isolated hypophosphatemia, normoglycemic glycosuria, and/or phosphaturia [90, 102]. In a large pediatric study in older children, the prolonged use of TDF was associated with low incidence of hypophosphatemia (4%), which was reversible following the TDF withdrawal [98]. The urinary levels of β 2M and RBP are considered sensitive biomarkers to detect the TDF-induced tubular injury in children and adults [92, 100, 103, 104]. However, as discussed above, these biomarkers are also elevated in children with proteinuria who are not treated with TDF [51]. Finally, one study conducted in HIV-infected adults without proteinuria or impaired glomerular function who were treated with highly active antiretroviral therapy (HAART), found that ~ 25 % of these patients developed subclinical tubular damage, which was diagnosed when at least three of the following urinary biomarkers were elevated: N-acetyl- β -D-glucosaminidase, γ -glutamyl transpeptidase, β -2M, and α -1 microglobulin [105]. Overall, these studies suggest that many biomarkers are available to assess the magnitude of ART-induced tubular dysfunction and injury in HIV-1 infected children. However, these biomarkers are also affected in many glomerular diseases seen in HIV-infected children.

Future needs and directions

The early identification of kidney injury induced by HIV-1 or ART should be an important clinical goal in the clinical follow up of HIV-infected children. The studies discussed above have identified a significant number of new candidate biomarkers that need to be tested and validated in a large group of HIV-infected children.

Five phases of biomarker development has been described for the early detection of cancer [106], and these phases have been discussed in the context of acute kidney injury [41]. Briefly, phase 1 includes preclinical laboratory studies that identify genes and proteins that are over-or under-expressed. The availability of HIV-Tg mice and rats, that mimic the renal diseases seen in patients with HIVAN, has facilitated the discovery of NGAL, FGF-2, EGF, MMP-9, and several iron binding proteins as potential candidate renal biomarkers for HIV-infected children [18, 72]. Phase 2 studies involve the translational phase of biomarker development, and include testing new clinical assays to assess the presence or absence of renal disease in samples that are collected from patients. These studies should distinguish patients with renal diseases from those without renal diseases. A few pilot studies have been done in HIV-infected children to test new candidate biomarkers for HIVAN [51, 53]. Phase 2 studies in general do not allow determination of whether the disease can be detected earlier using the proposed biomarkers, but include the estimation of true-positive and false positive rates, and the generation of receiver operating curves (ROC) and cut-off values, to assess the performance of the selected candidate biomarkers, as described for NGAL in adult patients with HIVAN [68]. Phase 3 studies are similar to Phase 2 studies, except that the biomarkers are then evaluated for their capacity to detect pre-clinical disease at earlier time points. The identification of patients carrying the APOL1 genetic risk variants that predispose to the development of HIVAN [25–27], in combination with very sensitive and specific biomarkers, may allow the identification of these patients. However, these studies have not

been done in children with HIVAN due the lack of clinical specimens collected before and after these patients develop HIVAN, and the limited number of patients available in single centers. Finally, phases 4 and 5 involve large-scale validation studies of the selected biomarkers in a prospective manner. The primary questions addressed in the phase 4 studies include the understanding of the performance of the biomarker as a screening test [41, 106]. These studies can also help understand the cost of using the new biomarkers in standard clinical care. Because the prevalence for HIVAN is low, these studies will require a large sample size, and the participation of multiple clinical centers to recruit these patients. Phase 5 studies usually explore the impact of using the selected biomarker tests in relationship with the outcome of the disease [41, 106].

In summary, significant progress has been made during the last years in the identification of renal biomarkers for HIV-infected children. Nevertheless, a great challenge remains ahead, if we intent to validate many of the biomarkers discussed above in these children. In addition, we hope that the availability of more sensitive proteomic technologies will facilitate the discovery of new biomarkers to measure the GFR in a more accurately manner, and to distinguish the different stages of CKD and AKI in HIV-infected children and young adults. Particular emphasis should be placed in using new proteomic techniques to assess the GFR and status of CKD in children < 2 years of age, since this is a unique period of kidney growth and maturation that may require a unique set of biomarkers to assess the renal function and injury.

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Abbreviations

KDIGO	Kidney Disease improving global outcomes
HIV-1	Human immunodeficiency virus -1
ACR	albumin-to-creatinine ratio
GFR	Glomerular Filtration Rate
CKD	Chronic Kidney Disease
ART	Antiretroviral therapy
TMA	thrombotic microangiopathy
FGF-2	Fibroblast Growth Factor-2
EGF	Epidermal Growth Factor
Matrix	metalloproteinases-2
HIVAN	HIV-associated nephropathy
HIV-HUS	HIV-associated hemolytic uremic syndrome

AKI**Acute Kidney Injury****References**

1. Ray PE, Rakusan T, Loechele BJ, Selby DM, et al. area: 12 years' experience. *Seminars in nephrology*. 1998; 18:396–405. [PubMed: 9692352]
2. McCulloch MI, Ray PE. Kidney disease in HIV-positive children. *Seminars in nephrology*. 2008; 28:585–594. [PubMed: 19013330]
3. Lucas GM, Ross MJ, Stock PG, Shlipak MG, et al. Clinical Practice Guideline for the Management of Chronic Kidney Disease in Patients Infected With HIV: 2014 Update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 2014; 59:e96–e138. [PubMed: 25234519]
4. Szczech LA, Grunfeld C, Scherzer R, Canchola JA, et al. Microalbuminuria in HIV infection. *Aids*. 2007; 21:1003–1009. [PubMed: 17457094]
5. Baekken M, Os I, Sandvik L, Oektedalen O. Microalbuminuria associated with indicators of inflammatory activity in an HIV-positive population. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2008; 23:3130–3137.
6. Berliner AR, Fine DM, Lucas GM, Rahman MH, et al. Observations on a cohort of HIV-infected patients undergoing native renal biopsy. *American journal of nephrology*. 2008; 28:478–486. [PubMed: 18176076]
7. Cohen SD, Chawla LS, Kimmel PL. Acute kidney injury in patients with human immunodeficiency virus infection. *Current opinion in critical care*. 2008; 14:647–653. [PubMed: 19005305]
8. Cohen SD, Kimmel PL. Immune complex renal disease and human immunodeficiency virus infection. *Seminars in nephrology*. 2008; 28:535–544. [PubMed: 19013324]
9. Kimmel PL, Phillips TM, Ferreira-Centeno A, Farkas-Szallasi T, et al. Brief report: idiopathic IgA nephropathy in patients with human immunodeficiency virus infection. *The New England journal of medicine*. 1992; 327:702–706. [PubMed: 1495523]
10. Martinez F, Mommeja-Marin H, Estepa-Maurice L, Beaufils H, et al. Indinavir crystal deposits associated with tubulointerstitial nephropathy. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 1998; 13:750–753.
11. Barrios A, Garcia-Benayas T, Gonzalez-Lahoz J, Soriano V. Tenofovir-related nephrotoxicity in HIV-infected patients. *Aids*. 2004; 18:960–963. [PubMed: 15060449]
12. Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. *Nature medicine*. 1995; 1:417–422.
13. Turner ME, Kher K, Rakusan T, D'Angelo L, et al. A typical hemolytic uremic syndrome in human immunodeficiency virus-1-infected children. *Pediatr Nephrol*. 1997; 11:161–163. [PubMed: 9090654]
14. Rakhmanina N, Wong EC, Davis JC, Ray PE. Hemorrhagic Stroke in an Adolescent Female with HIV-Associated Thrombotic Thrombocytopenic Purpura. *Journal of AIDS & clinical research*. 2014; 5
15. Szczech LA, Gupta SK, Habash R, Guasch A, et al. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney international*. 2004; 66:1145–1152. [PubMed: 15327410]
16. Wyatt CM, Morgello S, Katz-Malamed R, Wei C, et al. The spectrum of kidney disease in patients with AIDS in the era of antiretroviral therapy. *Kidney international*. 2009; 75:428–434. [PubMed: 19052538]
17. Ray PE. HIV-associated nephropathy: a diagnosis in evolution. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2012; 27:3969–3972.

18. Ray PE. Taking a hard look at the pathogenesis of childhood HIV-associated nephropathy. *Pediatr Nephrol.* 2009; 24:2109–2119. [PubMed: 19288142]
19. Cohen AH. HIV-associated nephropathy: current concepts. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association. 1998; 13:540–542.
20. D'Agati V, Suh JI, Carbone L, Cheng JT, Appel G. Pathology of HIV-associated nephropathy: a detailed morphologic and comparative study. *Kidney international.* 1989; 35:1358–1370. [PubMed: 2770114]
21. Strauss J, Abitbol C, Zilleruelo G, Scott G, et al. Renal disease in children with the acquired immunodeficiency syndrome. *The New England journal of medicine.* 1989; 321:625–630. [PubMed: 2770791]
22. Bruggeman LA, Nelson PJ. Controversies in the pathogenesis of HIV-associated renal diseases. *Nat Rev Nephrol.* 2009; 5:574–581. [PubMed: 19776779]
23. Marras D, Bruggeman LA, Gao F, Tanji N, et al. Replication and compartmentalization of HIV-1 in kidney epithelium of patients with HIV-associated nephropathy. *Nature medicine.* 2002; 8:522–526.
24. Bruggeman LA, Ross MD, Tanji N, Cara A, et al. Renal epithelium is a previously unrecognized site of HIV-1 infection. *Journal of the American Society of Nephrology: JASN.* 2000; 11:2079–2087. [PubMed: 11053484]
25. Genovese G, Friedman DJ, Ross MD, Lecordier L, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science.* 2010; 329:841–845. [PubMed: 20647424]
26. Kopp JB, Nelson GW, Sampath K, Johnson RC, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *Journal of the American Society of Nephrology: JASN.* 2011; 22:2129–2137. [PubMed: 21997394]
27. Freedman BI, Langefeld CD, Lu L, Divers J, et al. Differential effects of MYH9 and APOL1 risk variants on FRMD3 Association with Diabetic ESRD in African Americans. *PLoS Genet.* 2011; 7:e1002150. [PubMed: 21698141]
28. Lucas GM, Eustace JA, Sozio S, Mentari EK, et al. Highly active antiretroviral therapy and the incidence of HIV-1-associated nephropathy: a 12-year cohort study. *Aids.* 2004; 18:541–546. [PubMed: 15090808]
29. Canaud G, Dejucq-Rainsford N, Avettand-Fenoel V, Viard JP, et al. The kidney as a reservoir for HIV-1 after renal transplantation. *Journal of the American Society of Nephrology: JASN.* 2014; 25:407–419. [PubMed: 24309185]
30. Winston JA, Bruggeman LA, Ross MD, Jacobson J, et al. Nephropathy and establishment of a renal reservoir of HIV type 1 during primary infection. *The New England journal of medicine.* 2001; 344:1979–1984. [PubMed: 11430327]
31. Izzedine H, Wirlden M, Launay-Vacher V. Viral load and HIV-associated nephropathy. *The New England journal of medicine.* 2005; 353:1072–1074. [PubMed: 16148301]
32. Ray PE, Liu XH. Pathogenesis of Shiga toxin-induced hemolytic uremic syndrome. *Pediatr Nephrol.* 2001; 16:823–839. [PubMed: 11605791]
33. Ray P, Acheson D, Chitrakar R, Cnaan A, et al. Basic fibroblast growth factor among children with diarrhea-associated hemolytic uremic syndrome. *Journal of the American Society of Nephrology: JASN.* 2002; 13:699–707. [PubMed: 11856774]
34. Ray PE, Liu XH, Xu L, Rakusan T. Basic fibroblast growth factor in HIV-associated hemolytic uremic syndrome. *Pediatr Nephrol.* 1999; 13:586–593. [PubMed: 10460507]
35. Alpers CE. Light at the end of the TUNEL: HIV-associated thrombotic microangiopathy. *Kidney international.* 2003; 63:385–396. [PubMed: 12472814]
36. Waikar SS, Liu KD, Chertow GM. Diagnosis, epidemiology and outcomes of acute kidney injury. *Clinical journal of the American Society of Nephrology: CJASN.* 2008; 3:844–861. [PubMed: 18337550]
37. Bellomo R, Ronco C, Kellum JA, Mehta RL, et al. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second

- International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Critical care. 2004; 8:R204–212. [PubMed: 15312219]
38. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, et al. Modified RIFLE criteria in critically ill children with acute kidney injury. *Kidney international*. 2007; 71:1028–1035. [PubMed: 17396113]
 39. Chaparro AI, Mitchell CD, Abitbol CL, Wilkinson JD, et al. Proteinuria in children infected with the human immunodeficiency virus. *J Pediatr*. 2008; 152:844–849. [PubMed: 18492529]
 40. Abitbol CL, Strauss J, Zilleruelo G, Montane B, Rodriguez E. Validity of random urines to quantitate proteinuria in children with human immunodeficiency virus nephropathy. *Pediatr Nephrol*. 1996; 10:598–601. [PubMed: 8897564]
 41. Coca SG, Parikh CR. Urinary biomarkers for acute kidney injury: perspectives on translation. *Clinical journal of the American Society of Nephrology: CJASN*. 2008; 3:481–490. [PubMed: 18256377]
 42. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney international*. 2014; 85:49–61. [PubMed: 24284513]
 43. Jones CY, Jones CA, Wilson IB, Knox TA, et al. Cystatin C and creatinine in an HIV cohort: the nutrition for healthy living study. *American journal of kidney diseases: the official journal of the National Kidney Foundation*. 2008; 51:914–924. [PubMed: 18455851]
 44. Post FA, Wyatt CM, Mocroft A. Biomarkers of impaired renal function. *Curr Opin HIV AIDS*. 2010; 5:524–530. [PubMed: 20978396]
 45. Estrella MM, Fine DM. Screening for chronic kidney disease in HIV-infected patients. *Adv Chronic Kidney Dis*. 2010; 17:26–35. [PubMed: 20005486]
 46. Barraclough K, Er L, Ng F, Harris M, et al. A comparison of the predictive performance of different methods of kidney function estimation in a well-characterized HIV-infected population. *Nephron Clin Pract*. 2009; 111:c39–48. [PubMed: 19052469]
 47. Ingelfinger JR, Marsden PA. Estimated GFR and risk of death--is cystatin C useful? *The New England journal of medicine*. 2013; 369:974–975. [PubMed: 24004125]
 48. Shlipak MG, Matsushita K, Arnlov J, Inker LA, et al. Cystatin C versus creatinine in determining risk based on kidney function. *The New England journal of medicine*. 2013; 369:932–943. [PubMed: 24004120]
 49. Mauss S, Berger F, Kuschak D, Henke J, et al. Cystatin C as a marker of renal function is affected by HIV replication leading to an underestimation of kidney function in HIV patients. *Antivir Ther*. 2008; 13:1091–1095. [PubMed: 19195336]
 50. Mocroft A, Wyatt C, Szczech L, Neuhaus J, et al. Interruption of antiretroviral therapy is associated with increased plasma cystatin C. *Aids*. 2009; 23:71–82. [PubMed: 19050388]
 51. Soler-Garcia AA, Rakhmanina NY, Mattison PC, Ray PE. A urinary biomarker profile for children with HIV-associated renal diseases. *Kidney international*. 2009; 76:207–214. [PubMed: 19357719]
 52. Varghese SA, Powell TB, Budisavljevic MN, Oates JC, et al. Urine biomarkers predict the cause of glomerular disease. *Journal of the American Society of Nephrology: JASN*. 2007; 18:913–922. [PubMed: 17301191]
 53. Soler-Garcia AA, Johnson D, Hathout Y, Ray PE. Iron-related proteins: candidate urine biomarkers in childhood HIV-associated renal diseases. *Clinical journal of the American Society of Nephrology: CJASN*. 2009; 4:763–771. [PubMed: 19279121]
 54. Fagoonee S, Gburek J, Hirsch E, Marro S, et al. Plasma protein haptoglobin modulates renal iron loading. *Am J Pathol*. 2005; 166:973–983. [PubMed: 15793279]
 55. Delanghe JR, Langlois MR. Hemopexin: a review of biological aspects and the role in laboratory medicine. *Clin Chim Acta*. 2001; 312:13–23. [PubMed: 11580905]
 56. Prinsen BH, de Sain-van der Velden MG, Kaysen GA, Straver HW, et al. Transferrin synthesis is increased in nephrotic patients insufficiently to replace urinary losses. *Journal of the American Society of Nephrology: JASN*. 2001; 12:1017–1025. [PubMed: 11316861]
 57. Yamauchi K, Tomita M, Giehl TJ, Ellison RT. 3rd, Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infect Immun*. 1993; 61:719–728. [PubMed: 8423097]

58. Kjeldsen L, Koch C, Arnljots K, Borregaard N. Characterization of two ELISAs for NGAL, a newly described lipocalin in human neutrophils. *J Immunol Methods*. 1996; 198:155–164. [PubMed: 8946011]
59. Mishra J, Ma Q, Prada A, Mitsnefes M, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *Journal of the American Society of Nephrology: JASN*. 2003; 14:2534–2543. [PubMed: 14514731]
60. Bennett M, Dent CL, Ma Q, Dastrala S, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clinical journal of the American Society of Nephrology: CJASN*. 2008; 3:665–673. [PubMed: 18337554]
61. Ku PT, D'Amore PA. Regulation of basic fibroblast growth factor (bFGF) gene and protein expression following its release from sublethally injured endothelial cells. *J Cell Biochem*. 1995; 58:328–343. [PubMed: 7593255]
62. Alfrey AC. Toxicity of tubule fluid iron in the nephrotic syndrome. *Am J Physiol*. 1992; 263:F637–641. [PubMed: 1384359]
63. Yang J, Mori K, Li JY, Barasch J. Iron, lipocalin, and kidney epithelia. *Am J Physiol Renal Physiol*. 2003; 285:F9–18. [PubMed: 12788784]
64. Parravicini E. The clinical utility of urinary neutrophil gelatinase-associated lipocalin in the neonatal ICU. *Curr Opin Pediatr*. 2010; 22:146–150. [PubMed: 20061951]
65. Landro L, Damas JK, Flo TH, Heggelund L, et al. Decreased serum lipocalin-2 levels in human immunodeficiency virus-infected patients: increase during highly active anti-retroviral therapy. *Clin Exp Immunol*. 2008; 152:57–63. [PubMed: 18241231]
66. Hoffman SB, Massaro AN, Soler-Garcia AA, Perazzo S, Ray PE. A novel urinary biomarker profile to identify acute kidney injury (AKI) in critically ill neonates: a pilot study. *Pediatr Nephrol*. 2013; 28:2179–2188. [PubMed: 23783654]
67. Paragas N, Nickolas TL, Wyatt C, Forster CS, et al. Urinary NGAL marks cystic disease in HIV-associated nephropathy. *Journal of the American Society of Nephrology: JASN*. 2009; 20:1687–1692. [PubMed: 19628667]
68. Sola-Del Valle DA, Mohan S, Cheng JT, Paragas NA, et al. Urinary NGAL is a useful clinical biomarker of HIV-associated nephropathy. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2011; 26:2387–2390.
69. Liu XH, Aigner A, Wellstein A, Ray PE. Up-regulation of a fibroblast growth factor binding protein in children with renal diseases. *Kidney international*. 2001; 59:1717–1728. [PubMed: 11318942]
70. Ray PE, Tassi E, Liu XH, Wellstein A. Role of fibroblast growth factor-binding protein in the pathogenesis of HIV-associated hemolytic uremic syndrome. *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R105–113. [PubMed: 16352855]
71. Ray PE, Bruggeman LA, Weeks BS, Kopp JB, et al. bFGF and its low affinity receptors in the pathogenesis of HIV-associated nephropathy in transgenic mice. *Kidney international*. 1994; 46:759–772. [PubMed: 7996798]
72. Ray PE, Hu CA. Advances in our understanding of the pathogenesis of HIV-1 associated nephropathy in children. *Future Virol*. 2011; 6:883–894. [PubMed: 22162721]
73. Villanueva S, Cespedes C, Gonzalez A, Vio CP. bFGF induces an earlier expression of nephrogenic proteins after ischemic acute renal failure. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291:R1677–1687. [PubMed: 16873559]
74. Villanueva S, Cespedes C, Gonzalez AA, Roessler E, Vio CP. Inhibition of bFGF-receptor type 2 increases kidney damage and suppresses nephrogenic protein expression after ischemic acute renal failure. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294:R819–828. [PubMed: 18184769]
75. Gupta GK, Milner L, Linshaw MA, McCauley RG, et al. Urinary basic fibroblast growth factor: a noninvasive marker of progressive cystic renal disease in a child. *Am J Med Genet*. 2000; 93:132–135. [PubMed: 10869116]
76. Li Z, Jerebtsova M, Liu XH, Tang P, Ray PE. Novel cystogenic role of basic fibroblast growth factor in developing rodent kidneys. *Am J Physiol Renal Physiol*. 2006; 291:F289–296. [PubMed: 16597610]

77. Harada H, Furuya M, Ishikura H, Shindo J, et al. Expression of matrix metalloproteinase in the fluids of renal cystic lesions. *J Urol*. 2002; 168:19–22. [PubMed: 12050483]
78. Rankin CA, Itoh Y, Tian C, Ziemer DM, et al. Matrix metalloproteinase-2 in a murine model of infantile-type polycystic kidney disease. *Journal of the American Society of Nephrology: JASN*. 1999; 10:210–217. [PubMed: 10215319]
79. Bello-Reuss E, Holubec K, Rajaraman S. Angiogenesis in autosomal-dominant polycystic kidney disease. *Kidney international*. 2001; 60:37–45. [PubMed: 11422734]
80. Horikoshi S, Kubota S, Martin GR, Yamada Y, Klotman PE. Epidermal growth factor (EGF) expression in the congenital polycystic mouse kidney. *Kidney international*. 1991; 39:57–62. [PubMed: 2002633]
81. Comperat E, Ferlicot S, Camparo P, Eschwege P, et al. Expression of epidermal growth factor receptor and proliferative activity of cyst epithelium in human renal cystic diseases. *Urol Int*. 2006; 76:269–273. [PubMed: 16601392]
82. Tsau YK, Sheu JN, Chen CH, Teng RJ, Chen HC. Decreased urinary epidermal growth factor in children with acute renal failure: epidermal growth factor/creatinine ratio not a reliable parameter for urinary epidermal growth factor excretion. *Pediatr Res*. 1996; 39:20–24. [PubMed: 8825381]
83. Grandaliano G, Gesualdo L, Bartoli F, Ranieri E, et al. MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. *Kidney international*. 2000; 58:182–192. [PubMed: 10886563]
84. Kiley SC, Chevalier RL. Urinary biomarkers: the future looks promising. *Kidney international*. 2009; 76:133–134. [PubMed: 19564854]
85. Wai K, Soler-Garcia AA, Perazzo S, Mattison P, Ray PE. A pilot study of urinary fibroblast growth factor-2 and epithelial growth factor as potential biomarkers of acute kidney injury in critically ill children. *Pediatr Nephrol*. 2013; 28:2189–2198. [PubMed: 23872928]
86. Molitoris BA, Sutton TA. Endothelial injury and dysfunction: role in the extension phase of acute renal failure. *Kidney international*. 2004; 66:496–499. [PubMed: 15253696]
87. Chen L, Liu W. Effect of asphyxia on urinary epidermal growth factor levels in newborns. *J Tongji Med Univ*. 1997; 17:144–146. [PubMed: 9812765]
88. Askenazi DJ, Koralkar R, Hundley HE, Montesanti A, et al. Urine biomarkers predict acute kidney injury in newborns. *J Pediatr*. 2012; 161:270–275. e271. [PubMed: 22424940]
89. Coca SG, Yalavarth R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney international*. 2008; 73:1008–1016. [PubMed: 18094679]
90. Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *Aids*. 2009; 23:689–696. [PubMed: 19262355]
91. Gonzalez C, Ariceta G, Langman CB, Zibaoui P, et al. Hypercalciuria is the main renal abnormality finding in Human Immunodeficiency Virus-infected children in Venezuela. *Eur J Pediatr*. 2008; 167:509–515. [PubMed: 17593389]
92. Papaleo A, Warszawski J, Salomon R, Jullien V, et al. Increased beta-2 microglobulinuria in human immunodeficiency virus-1-infected children and adolescents treated with tenofovir. *Pediatr Infect Dis J*. 2007; 26:949–951. [PubMed: 17901802]
93. Yu H, Yanagisawa Y, Forbes MA, Cooper EH, et al. Alpha-1-microglobulin: an indicator protein for renal tubular function. *J Clin Pathol*. 1983; 36:253–259. [PubMed: 6186698]
94. Bernard AM, Moreau D, Lauwerys R. Comparison of retinol-binding protein and beta 2-microglobulin determination in urine for the early detection of tubular proteinuria. *Clin Chim Acta*. 1982; 126:1–7. [PubMed: 6184185]
95. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. *American journal of kidney diseases: the official journal of the National Kidney Foundation*. 2011; 57:773–780. [PubMed: 21435764]
96. Ward JP. Gamma-glutamyl transpeptidase. A sensitive indicator of renal ischaemic injury in experimental animals and renal homograft rejection in man *Ann R Coll Surg Engl*. 1975; 57:248–261. [PubMed: 949]

97. Roling J, Schmid H, Fischereder M, Draenert R, Goebel FD. HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*. 2006; 42:1488–1495. [PubMed: 16619164]
98. Judd A, Boyd KL, Stohr W, Dunn D, et al. Effect of tenofovir disoproxil fumarate on risk of renal abnormality in HIV-1-infected children on antiretroviral therapy: a nested case-control study. *Aids*. 2010; 24:525–534. [PubMed: 20139752]
99. Tanji N, Tanji K, Kambham N, Markowitz GS, et al. Adefovir nephrotoxicity: possible role of mitochondrial DNA depletion. *Hum Pathol*. 2001; 32:734–740. [PubMed: 11486172]
100. Del Palacio M, Romero S, Casado JL. Proximal tubular renal dysfunction or damage in HIV-infected patients. *AIDS Rev*. 2012; 14:179–187. [PubMed: 22833061]
101. Earle KE, Seneviratne T, Shaker J, Shoback D. Fanconi's syndrome in HIV+ adults: report of three cases and literature review. *J Bone Miner Res*. 2004; 19:714–721. [PubMed: 15068493]
102. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, et al. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Lab Invest*. 2009; 89:513–519. [PubMed: 19274046]
103. Gatanaga H, Tachikawa N, Kikuchi Y, Teruya K, et al. Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. *AIDS Res Hum Retroviruses*. 2006; 22:744–748. [PubMed: 16910829]
104. Kinai E, Hanabusa H. Renal tubular toxicity associated with tenofovir assessed using urine-beta 2 microglobulin, percentage of tubular reabsorption of phosphate and alkaline phosphatase levels. *Aids*. 2005; 19:2031–2033. [PubMed: 16260911]
105. Ando M, Yanagisawa N, Ajisawa A, Tsuchiya K, Nitta K. Kidney tubular damage in the absence of glomerular defects in HIV-infected patients on highly active antiretroviral therapy. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2011; 26:3224–3229.
106. Pepe MS, Etzioni R, Feng Z, Potter JD, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst*. 2001; 93:1054–1061. [PubMed: 11459866]

Figure 1.

Panel A shows a light microscopy picture of a renal section derived from a child with HIVAN. The black arrows point to a collapsed glomerulus and a tubular microcyst (Jones methenamine silver stain). Panel B shows a renal section from a child with HIV-HUS. The picture shows dilated capillary loops with red blood cell fragments, and tubular microcysts (Hematoxylin-eosin stain). The black arrow points to a renal arteriole with luminal narrowing due to red cell fragments and intraluminal thrombosis (A and B original magnification x 200). Pictures A and B are reproduced with permission from “Kidney disease in HIV-positive children” McCulloch, M.I., Ray P.E. *Seminars in Nephrology* (2008) 28, 585–594. Panel C, shows representative changes in urinary EGF, MMP-2, FGF-2, and serum creatinine (SCr), seen during the different stages of childhood HIVAN [16]. Panel D shows representative changes in urinary EGF, NGAL, FGF-2, and SCr, seen during different stages of acute kidney injury in HIV-infected children [37,42, 74].

Table 1

Renal Disease in HIV-infected children.

Glomerular diseases: HIV associated nephropathy (HIVAN) [*] ; HIV-associated immune complex diseases (HIVCK) [*] ; Post-infectious glomerulonephritis; IgA-nephropathy, membranous and membranous-proliferative glomerulonephritis, and lupus-like nephritis)
Thrombotic Microangiopathies. HIV- associated Hemolytic Uremic Syndrome (HIV-HUS) [*] , Thrombotic thrombocytopenic purpura (TTP), and multiple organ failure with disseminated intravascular coagulation (DIC).
Acute Kidney Injury, sepsis, infections, drugs.
Infectious: Urinary tract infections, Hepatitis, Opportunistic infections, Tuberculosis
Tubulopathies. Renal tubular acidosis, hypercalciuria, Fanconi syndrome
Nephrotoxic drugs. Tenofovir disoproxil fumarate (TDF) [*] , nucleotide reverse transcriptase inhibitors (NRTI) [*] , Protease inhibitors (PI) (e.g., indinavir, stones) [*] , antibiotics, other antiviral treatments.

^{*} Renal diseases that are seen only in HIV-infected patients, unless the nephrotoxic drugs TDF, NRTI, and indinavir are used to treat other diseases.

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Table 2

Urinary candidate biomarkers for HIV-renal diseases

Renal Disease	Increased (> cut-off values).	Decreased (< cut-off values).	No changes (> or < cut-off values)
HIVAN	FGF-2, MMP-2 , NGAL, gross proteinuria, RBP, β 2-M, cystatin C	EGF	
HIV-HUS	FGF-2, NGAL , iron, haptoglobin, hemopexin, lactoferrin.	EGF	
Sepsis-AKI	FGF-2, NGAL , RBP, γ -GTP, β 2-M,	EGF	
ART-induced toxicity.	RBP, β2-M, γ-GTP N-A β -D-glucosamine, α -1-M.		FGF-2, EGF

Abbreviations. Fibroblast Growth Factor-2 (FGF-2); Matrix Metalloproteinase-2 (MMP-2); Epidermal Growth Factor (EGF); Neutrophil Gelatinase Associated Lipocalin (NGAL); Retinol binding protein (RBP); β -2 Microglobulin (β -2M); γ -glutamyl-transpeptidase (γ -GTP); N-acetyl β -D-glucosamine (N-A β -D-glucosamine); α -1 microglobulin (α -1-M).