

Editorial Reviews

Cystatin C in HIV-infected patients: promising but not yet ready for prime time

Amandine Gagneux-Brunon^{1,2}, Christophe Mariat^{1,2} and Pierre Delanaye³

¹Department of Nephrology, Dialysis, Hypertension, Transplantation, CHU de Saint-Etienne, Saint-Etienne, France, ²Groupe Immunité des Muqueuses et Agents Pathogènes, EA 3064, University Jean Monnet, Saint-Etienne, PRES University of Lyon, Saint-Etienne, France and ³Department of Nephrology, Dialysis, Hypertension, Transplantation, University of Liège, CHU Sart Tilman, Liège, Belgium

Correspondence and offprint requests to: Pierre Delanaye; E-mail: pierre_delanaye@yahoo.fr

Abstract

With the development of highly active antiretroviral therapy, chronic kidney disease has become a prominent cause of morbidity in individuals infected by HIV. Because serum creatinine has significant limitations in this specific population, cystatin C is emerging as a promising biomarker for both the evaluation of glomerular filtration rate (GFR) and the detection of drug-induced kidney injury. Along with renal function, serum cystatin C concentration is associated with several biological parameters such as C-reactive protein, HIV viral load and CD4+ cells count. All these determinants of cystatin C are, however, more or less independent of GFR. Studies evaluating the accuracy of cystatin C for estimating GFR in the setting of HIV infection are scarce and methodology is often questionable (lack of reference method or inadequate statistical analyses). Thus far, data are insufficient to encourage the use of cystatin C or cystatin C-based equations to estimate GFR in the HIV-infected population. Further research is needed to explore the clinical utility of cystatin C in this setting. Beyond the use of cystatin C as a GFR marker, future studies will have to evaluate its role as a predictor of patient outcome, particularly in regard to cardiovascular morbi-mortality.

Keywords: cystatin C; glomerular filtration rate; HIV

Introduction

The United Nations Programme on HIV/AIDS estimated that 33.3 million people were living with a HIV infection in 2009 [1]. The development of highly active antiretroviral therapy (HAART) has resulted in improved survival among HIV-seropositive individuals. With advancing age and HAART-related metabolic effects (diabetes [2], hypertension [3], and dyslipidaemia [4]), chronic kidney disease (CKD) has become one of the major comorbidities in HIV-seropositive individuals [5]. The real prevalence of CKD in HIV patients is still, however, questioned. In a

retrospective chart review, Fernando *et al.* [6] found evidence of CKD in 24% of the patients and CKD Stage 3 [estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m²] or higher in 10% of the patients. Overton *et al.* [7] found a prevalence of Stage 3 CKD of 7.4% in HIV-infected subjects versus 2.1% in controls. Crum-Cianflone *et al.* [8] found a lower prevalence of CKD Stage 3 (3%). Importantly, in the latter study, patients were relatively young, had no comorbidities and no specific evaluation for proteinuria. Campbell *et al.* [9] found similar results with a prevalence of 2.4% for Stage 3 CKD.

Risk factors for CKD in HIV-infected patients vary according to the studies but include female sex, black race [10], AIDS, low CD4+ lymphocyte count, older age, hepatitis C virus (HCV) co-infection, hypertension, diabetes mellitus [6, 7] and injecting drug use [11]. Exposure to HAART is also associated with an increased risk for CKD, tenofovir, didanosine, atazanavir, lopinavir and indinavir being the most frequently incriminated anti-retroviral drugs [12, 13].

Moreover, similar to the general population, CKD is associated with an increased risk of both mortality [14, 15] and cardiovascular events (CVE) [16] in HIV-seropositive individuals. In HIV-infected women, Szczech *et al.* [17] observed that proteinuria was associated with an increased risk of AIDS-defining illness {hazard ratio (HR) = 1.37 [95% confidence interval (95% CI) 1.01–1.81]}. Proteinuria and elevated creatinine level were also associated with an increased risk of mortality with a HR of 1.35 (95% CI 1.01–1.81) and 1.72 (95% CI 1.09–2.70), respectively. These associations were curiously stronger than the association of low CD4+ lymphocyte count with AIDS-defining illness and death in this cohort [17]. In multivariate analysis, the odds ratio for CVE was 1.2 (95% CI 1.1–1.4) for every 10 mL/min/1.73 m² decrease in eGFR. These results can, however, be criticized because it has been shown in the general population that the association between glomerular filtration rate (GFR) and CVE is a graded, but not a linear one [18]. Because of the growing prevalence and burden of CKD in HIV-infected patients,

screening, prevention and management of CKD have become a key challenge in this population. In 2005, the Infectious Diseases Society of America published guidelines for CKD screening in HIV-infected patients. According to these recommendations, kidney function should be assessed at initial diagnosis by measuring proteinuria and estimating GFR. In cases where CKD risk factors are present, kidney function should be assessed every 6 months. Utilization of creatinine-based estimates, and particularly the Modification Diet in Renal Disease (MDRD) study equation, is recommended [19, 20]. This latest recommendation has, however, to be challenged by the various limitations inherent to the use of serum creatinine as a marker of GFR. Variations in generation, secretion and extra-renal elimination of creatinine have been extensively described in different populations [21]. This holds particularly true for HIV-infected patients for whom many specific factors are likely to impact the relation between serum creatinine and GFR. As a result, serum cystatin C is being touted as an alternative to serum creatinine with the potential to more accurately account for different degrees of renal impairment [22, 23]. Cystatin C level has also been shown in the general population to be more closely associated than creatinine (and creatinine-based equations) with CVE, all-cause mortality and incident end-stage renal disease [24–26]. The mechanism of this association between cystatin C and outcomes—CVE and mortality—is not fully understood. It might, at least in part, have to do with the non-GFR determinants of cystatin C level, but this is still subject to debate. Whatever the mechanisms underlying this association, cystatin C can also be seen as a potential biomarker that may better predict outcomes in the high-risk HIV patient [27].

Serum creatinine: an imperfect marker of renal function in HIV-infected individuals

Creatinine is freely filtered by the glomerulus and secreted by the proximal tubular cells. Creatinine level can be affected at three levels: creatinine generation, tubular secretion and extra-renal elimination. Creatinine is the catabolic end product of creatine. Ninety-eight percent of creatinine is generated from muscle. Serum creatinine concentration is thus highly dependent on muscle mass [28, 29]. In HIV-infected patients, creatinine generation is expected to decrease for numerous reasons (see Table 1). HIV infection induces modifications of the body composition. For example, HIV-infected men have a lower fat-free mass compared to matched healthy controls [30, 31]. HIV infection can also lead to wasting syndromes responsible for severe depletion in lean and fat tissue [32]. Similarly,

HIV-infected subjects are more often malnourished than is the general population [33]. By further modifying body composition, lipodystrophy induced by HAART may alter the association between serum creatinine and GFR.

Additionally, creatinine production in patients with liver disease is decreased [34]. Co-infection with viral hepatitis is frequent (roughly one-third of patients are co-infected with HCV or hepatitis B virus in Western countries) [35, 36]. In HIV-infected individuals, HCV co-infection is associated with a decrease in serum creatinine level [37, 38]. In addition, liver cirrhosis is associated with malnutrition and protein depletion [39]. Given that HCV is another risk for CKD, early diagnosis remains critical in these patients. Besides the problem of creatinine generation, change in creatinine metabolism might also explain the difficulty to rely on serum creatinine for estimating GFR in HIV patients. For instance, the use of trimethoprim/sulfamethoxazole (TMP/SMX) is recommended in primary and secondary prophylaxis of infections due to *Toxoplasma gondii* and *Pneumocystis jiroveci* [40, 41]. Specifically, TMP inhibits creatinine secretion by the tubules, inducing an increase in serum creatinine independent of any decrease in GFR [42]. Serum creatinine increase after trimethoprim intake varies from 15 to 30% [43, 44].

Given the numerous factors specific to HIV infection that may influence creatinine level, two recent US cohort studies have compared serum creatinine in HIV and control non-HIV individuals. In the Fat Redistribution And Metabolic change (FRAM) study, 519 HIV-positive patients were compared with 290 healthy controls from the Coronary Artery Risk Development in Young Adults (CARDIA) study [45]. There was no significant difference in creatinine level between the two groups. However, the two groups were different for several characteristics—notably a higher prevalence of HCV infection, hypertension, diabetes and proteinuria in the HIV-infected populations—which might have masked a lower creatinine level. In the second study, 250 HIV-infected subjects from the Nutrition for Healthy Living (NFHL) cohort were compared with 2628 persons matched for age, race and sex from the National Health and Nutrition Examination Survey (NHANES) [38]. Serum creatinine level was found to be significantly lower in the NFHL cohort. In the same line, Mauss *et al.* [46] compared kidney function between 261 HIV-seropositive patients naïve to anti-retroviral therapy with 193 healthy controls. While they found no significant difference between the two groups for serum creatinine, they did observe a trend towards an association between lower serum creatinine and HIV seropositivity after adjustment for sex, age and body mass index [46]. In a retrospective study, Overton *et al.* [7] compared prevalence of kidney function

Table 1. Principal factors influencing serum creatinine level in HIV-infected subjects

	Effects on creatinine	HIV
Lean mass	Increase in creatinine production	Decrease in lean mass: AIDS wasting syndrome, mitochondrial toxicity of HAART
Dietary intake	Increase in creatinine production	Strong prevalence of malnutrition in HIV-infected subjects
Liver disease	Decrease in creatine production	Prevalence of HCV infection in HIV-infected subjects around 30%
African American ethnicity	Increase in creatinine production	
	Reduction of the tubular excretion of creatinine	
Trimethoprim	Reduction of the tubular excretion of creatinine	<i>Pneumocystis jiroveci</i> infections prophylaxis

between 845 HIV-infected subjects with 845 HIV-negative subjects from the NHANES cohort. The prevalence of chronic renal failure (eGFR <60 mL/min/1.73 m²) and serum creatinine level were significantly greater in HIV-infected subjects than in controls (7.4 versus 2.1%, 1.13 versus 0.95 mg/dL, respectively). HIV-infected subjects had more risk factors for CKD, such as hypertension and hepatitis infection and more often had a history of injecting drug use (IDU). These studies yielded different results and drawing definitive conclusion are thus difficult. Obviously, the principal limitation of these studies is the absence of GFR measurement by a gold standard method. Moreover, due to the great prevalence of viral hepatitis and IDU in the HIV-infected population, matching controls with HIV-infected subjects, remains difficult.

Serum cystatin C in HIV-positive subjects: the ideal GFR marker?

Cystatin C is a low-molecular weight protein produced by all nucleated cells, freely filtered in the glomerulus and catabolyzed by tubular cells [47]. Only small amounts are found in urine in the physiological state. Limitations of serum creatinine due to creatinine extra-renal excretion or tubular secretion are thus not present with cystatin C. However, it remains important to study muscular mass and other factors, which could influence cystatin C levels independently of GFR. Two important studies in HIV-negative subjects have examined the factors that affect

serum levels of cystatin C, independently of GFR. They both identified age, gender, and C-reactive protein (CRP) level in serum [48, 49] (see Table 2). In one, current smoking was also associated with an increase in serum cystatin C level. In another study, MacDonald *et al.* [51] demonstrated by measuring GFR by inulin clearance and evaluating body composition by dual X-ray absorptiometry that cystatin C is also affected by lean mass. However, the magnitude of the associations with height and weight is greater for serum creatinine than for serum cystatin C [49]. Cystatin C is thus clearly far less dependent on muscular mass than creatinine. Moreover, in a population-based study (Multi-Ethnic Study of Atherosclerosis, MESA Study), Kramer *et al.* showed no significant difference in mean cystatin C levels between African Americans and Caucasians [52] although serum creatinine strongly varies according to ethnicity (also probably reflecting difference in muscular mass according to ethnicity) [53]. The influence of these factors can be, once again, critical in HIV-infected subjects.

The association of cystatin C to CRP is potentially problematic in HIV patients. Markers of inflammation are indeed chronically elevated in HIV-positive adults. High-sensitivity (hs) CRP was found to be 55% higher in HIV-positive patients from the Strategies for Management of Anti Retroviral Therapy (SMART) study as compared to controls from the MESA and CARDIA studies [54]. Likewise, cystatin C was found to be 27.2% higher in the SMART study group. The association between hs-CRP and cystatin C level is, however, inconsistent. In the Jaroszewicz study [55], while CRP and cystatin C levels were significantly greater in HIV-

Table 2. Factors influencing serum cystatin C level identified in HIV-negative subjects^a

	Study population	GFR evaluation method	Mean GFR	Factors associated with cystatin C (after adjustment with GFR or 24-h creatinine clearance)	Effects on cystatin C level
Knight <i>et al.</i> 2004 [48]	8058 subjects from Groningen, the Netherlands 95% Caucasian Age from 28 to 75 years 4% of patients with diabetes	24-h urine creatinine clearance	102 ± 27 mL/min	Age Male gender, weight, height Current cigarette smoking CRP level	Increase Increase Increase Increase
Stevens <i>et al.</i> 2009 [49]	MDRD study (N = 1085) AASK (N = 1205) CSG (N = 266) NephroTest (N = 438) All patients with CKD Stages 2–5 53.5% blacks 13.9% patients with diabetes	Iothalamate urinary clearance EDTA urinary clearance	48 mL/min/1.73 m ² (15–95)	Age Female gender Height, weight, BMI Urine protein Diabetes CRP level White blood cell count Serum albumin	Decrease Decrease Increase Increase Increase Increase Increase Decrease
Mathisen <i>et al.</i> [50]	Tromsø population survey (N = 1627)	Iohexol clearance	90 mL/min/1.73 m ²	Decrease in physical activity Current smoking BMI LDL-cholesterol HDL-cholesterol Triglycerides	Increase Increase Increase Decrease Increase Increase

^aNephroTest, the NephroTest initiative is a prospective hospital-based ongoing cohort that began in 2000, enrolling patients with all diagnoses of CKD Stages 2–5 referred for extensive work-up by two nephrology departments. Data included in this study were collected between 2000 and 2004; AASK, African American Study of Kidney Diseases and Hypertension; BMI, body mass index; CSG, Collaborative Study Group: Captopril in Diabetic Nephropathy Study; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

positive subjects, no significant correlation between CRP and cystatin C was found. Moreover, Estrella *et al.* [56] published a cross-sectional study with 783 HIV-infected patients from the Multicenter AIDS Cohort Study (MACS) and 150 HIV-negative subjects. eGFR based on cystatin C was lower in the HIV+ group than in the controls, while eGFR based on creatinine was not different. eGFR based on creatinine and cystatin C were both influenced by age, AIDS condition and proteinuria. In the adjusted model, eGFR based on cystatin C was not influenced by CRP level. The precise role played by other confounders (like renal insufficiency or smoking status) in the relation between serum cystatin C and CRP requires further evaluation because CKD status, *per se*, is associated with ‘microinflammation’.

Non-GFR factors potentially influencing cystatin C levels have also been studied in the specific population of HIV patients. HIV replication may affect serum cystatin C level. In 922 patients from the FRAM study cohort, Choi *et al.* [27] observed that HIV-1 viral load was higher in the group of patients with an eGFR based on cystatin C (eGFR_{cys}) level <60 mL/min/1.73 m² than in the group of patients with a greater eGFR_{cys}. This association was not observed in another study with subjects from the same cohort or in the SMART trial study cohort [45, 54]. The SMART trial was designed to compare two strategies of anti-retroviral treatment [57]. In the first arm (drug conservation), treatment was guided by immunological response and HAART was stopped when CD4+ lymphocyte count was >350/mm³. In the second arm (viral suppression), HAART was maintained [58]. The interruption of HAART in the SMART study was associated with an increase in HIV-1 viral load and cystatin C [59]. A higher viral load at baseline and an increase in HIV-1 viral load during follow-up were associated with an increase in cystatin C level in the FRAM study [60]. In the MACS cohort, a decrease in eGFR_{cys} is associated with an increase in HIV viral load [56]. In a study of validation of estimated renal function by cystatin C-based equations, higher HIV-1 viral load was associated with higher cystatin C level independently of measured GFR. This analysis was performed in only 15 patients in whom GFR was measured by ⁵¹Cr-ethylenediaminetetraacetic acid (⁵¹Cr-EDTA) clearance [61]. Because viral load is also a strong predictor of GFR and because GFR was only estimated, not measured, in most of these studies, it cannot be concluded from these studies that the potential effect of viral load on cystatin C level is really independent of GFR variation.

Other possible factors related to HIV infection might affect cystatin C level: HIV-infected macrophages producing less cystatin C than uninfected ones [62] and CD4+ lymphocyte count (most studies showing an association between a lower count of CD4+ cells and a higher cystatin C level [27, 38, 45, 63]). Here again, some discrepancies exist across studies. In the SMART trial, cystatin C at baseline was not correlated to CD4+ cells, but an increase in cystatin C during follow-up was associated with a lower CD4+ count [59]. Two others studies did not show a significant correlation between CD4+ cells and cystatin C level [55, 64]. Once again, advanced HIV disease (high viral load and low lymphocyte CD4+ count) is associated with kidney impairment [17, 65]. Given the imprecision of

actual GFR measurement both in HIV and non-HIV studies, even adjusting to GFR and finding a residual association of risk factor with cystatin C do not automatically make it a non-GFR determinant. Although more studies are needed to evaluate the impact of HIV viral load and CD4+ count on its level, cystatin C might be a good marker of GFR in advanced HIV disease and might detect subclinical kidney impairment, which occurs in this situation.

Serum creatinine versus serum cystatin C in HIV-positive subjects: and the winner is...

Assessment of GFR in HIV-infected patients, as in other patients, had two different purposes. One is to provide the most possible early diagnosis of CKD. The second one is to detect decrease in kidney function over time (the slope of GFR) [66]. The principal cohort studies comparing cystatin C and creatinine in HIV patients and control subjects are presented in Table 3. In most of them, serum creatinine level was not different or lower in the study group than in the control groups, whereas serum cystatin C was most often higher in the study group. Using eGFR based on cystatin C, the prevalence of GFR <60 mL/min/1.73 m² is greater than using the MDRD equation, varying from 5 to 15.2% versus from 1 to 2.4% [38, 46, 56], respectively. However, all these studies have an important limitation: the absence of GFR measurement by a gold standard method that can serve as a reference (the so-called ‘true GFR’). Because of this fundamental limitation, they do not ultimately permit conclusions on the superiority of cystatin C over serum creatinine for estimating GFR.

The interest of cystatin C to follow the GFR slopes (longitudinal follow-up) and to detect a decrease in GFR is not well known in the HIV population. In other specific populations of patients, cystatin C outperformed creatinine to detect a decrease in GFR as in a cohort of diabetics [67].

Another challenge is the diagnosis of a subclinical HAART-related kidney injury. Some commonly prescribed antiretroviral therapies (e.g. tenofovir) can induce proximal tubular injury leading to Fanconi syndrome. In this situation, filtered cystatin C, which is not reabsorbed by the tubule, is excreted in the urine. For this reason, urinary cystatin C is thought to be a valuable biomarker for detecting anti-retroviral tubular toxicity [68].

Up to now, only four studies on cystatin C in HIV-infected subjects evaluated agreement between estimates of GFR based on cystatin C and measured GFR. They are summarized in Table 4.

In a cohort of 27 patients receiving HAART, Barraclough *et al.* showed that the predictive performance of cystatin C or cystatin C-based equations to estimate GFR was inferior to the most used creatinine-based methods (the MDRD study equation and the Cockcroft–Gault formula). This result must, however, be questioned since cystatin C was measured in this study by a quantitative sandwich enzyme immunoassay which is clearly not recommended for measuring cystatin C [69]. Choice of the analytical method to measure cystatin C has indeed a major impact on the accuracy of the cystatin C-based equations [73].

Table 3. Major cohort studies of cystatin C in HIV-infected subjects

	Cohort of HIV-infected subjects	Control cohort	Differences between HIV-positive cohort and controls	Results	Risk factors for higher serum cystatin C level in HIV-participants
Odden <i>et al.</i> 2007 [45]	The FRAM study (<i>n</i> = 519) Between the ages of 33 and 45 years	CARDIA study (<i>n</i> = 290)	Controls were older, more women in control cohort, BMI higher in control cohort, smoking status, hypertension, dyslipidemia, proteinuria, HCV infection higher in FRAM study cohort	Serum cystatin C higher in the FRAM study cohort The prevalence of cystatin C >1 mg/L was 31% in the HIV-infected participants and only 4% in controls No difference in serum creatinine level between HIV-infected participants and controls	Lower HDL-c level, higher uric acid level, proteinuria, hypertension, higher CRP Current smoking CD4 lymphopenia Co-infection HCV, current heroin use, longer duration of efavirenz and indinavir use
Mauss <i>et al.</i> 2008 [46]	Treatment naïve, Caucasian patients (<i>n</i> = 261)	Healthy volunteers (<i>n</i> = 193)	More males in the study group Older age, higher BMI in control group CRP level, HCV co-infection, smoking status were not reported in the patient characteristics	Mean eGFR _{creat} by MDRD higher in the study group Mean eGFR _{cys} ^a lower in the study group Prevalence of CKD (Stages 2 and more) in the study group with MDRD = 23%, with eGFR _{cys} = 41%	Positive correlation between cystatin C level and HIV viral load
Jones <i>et al.</i> 2008 [38]	The NFHL cohort (<i>n</i> = 250)	The NHANES (<i>n</i> = 2628)	More African American subjects in NFHL, greater prevalence of hypertension, diabetes, liver disease in NFHL, higher CRP level in NFHL, lower albumin level in NFHL	Serum creatinine level lower in NFHL, serum cystatin C level higher in NFHL cohort Prevalence of eGFR _{cys} ^b <60 mL/min/1.73 m ² = 15.2% Prevalence of eGFR _{creat} <60 mL/min/1.73 m ² using MDRD = 2.4%	HCV infection, liver disease, lower CD4+ lymphocyte count, HIV viral load, current injecting drug use, lower serum albumin level
Neuhaus <i>et al.</i> 2010 [54]	SMART (<i>n</i> = 494)	MESA study (<i>n</i> = 5386)	Older age, more women in control cohort, more black subjects in SMART, lower BMI in SMART study, prevalence of dyslipidemia, current smoking, diabetes, hypertension greater in SMART	Cystatin C was 27.2% higher in SMART study participants	

Continued

Table 3. Continued

	Cohort of HIV-infected subjects	Control cohort	Differences between HIV-positive cohort and controls	Results	Risk factors for higher serum cystatin C level in HIV-participants
Estrella <i>et al.</i> 2011 [56]	MACS (n = 783)	Healthy volunteers (n = 150)	Hepatitis C infection status, liver enzyme level, CRP and urine protein to creatinine ratio greater in the HIV+ group, BMI, serum albumin level greater in controls, trimethoprim use in HIV+ group	eGFRcys C was reduced in HIV+ groups, no differences for eGFRreat, discordance between eGFRcys C and eGFRreat varies with the level of kidney function	HCV infection, alkaline phosphatase level, HIV viral load
Overton <i>et al.</i> 2011 [63]	SUN ^c (n = 670)	None		Prevalence of CKD (eGFR MDRD <60 mL/min/1.73 m ²) 3.3%, prevalence of cystatin C >1 mg/L 18%	Older age, HCV infection, smoking tobacco, tenofovir use, microalbuminuria, lower cystatin C was associated with undetectable viral load and HAART duration

^aeGFRcys (mL/min) = 74 835/[cystatin C (mg/L)]^{1.333}.

^beGFRcys(mL/min/1.73 m²) = 767 (cystatin C^{-1.18}) [22].

^cStudy to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy.

In a cohort of 22 HIV patients, Beringer *et al.* [70] tested four different equations combining cystatin C and creatinine and found that they all provided a more accurate estimate as compared to the Cockcroft–Gault formula and an equation using cystatin C alone. All these equations underestimated GFR measured by iothalamate. The degree of underestimation of GFR with cystatin C was greater in patients with detectable HIV viral load than in patients with HIV viral load <400 copies/mL (−28.8 versus −14.3%). This difference was, however, not statistically significant.

Bonjoch *et al.* [61] reported that cystatin C shows the strongest correlation with measured GFR by an isotopic method (⁵¹Cr-EDTA) compared to the Cockcroft–Gault, the Chronic Kidney Disease Epidemiology group (CKD-EPI), the MDRD study equations and the 24-h urine creatinine clearance. GFR was measured in 15 patients, all received HAART and 80% had no detectable viral load for an average of 90 months.

In a South African cohort, van Deventer *et al.* [71] developed a new prediction equation based on cystatin C for estimating GFR and compared performances of these new equations to existing creatinine-based equations (MDRD, CKD-EPI equations). One hundred black Africans patients were included, 50 in the development dataset and 50 in the validation dataset. In this cohort, GFR was measured by plasma clearance of ⁵¹Cr-EDTA. Twenty patients from the cohort were HIV positive, overall this cystatin C-based equation was more precise than the creatinine-based equations, particularly in patients with GFR >60 mL/min/1.73 m². In the subgroup of HIV-positive patients, this cystatin C-based equation proved to give the most accurate estimate as well. Although they all used ‘true GFR’ as a reference, these studies have in common one major limitation. They all included a very limited number of patients and should thus be seen as studies suggesting—not proving—the better performance of cystatin C. Furthermore, most of the patients included in these studies are Caucasian (except for the South African study), HAART-treated, and had an undetectable viral load and a CD4+ lymphocyte count >200/mm³. The performance of cystatin C may vary with the stage of HIV infection. With a predictive performance of 78% for MDRD, and the hypothesis of a predictive performance of 90% for cystatin C, >250 HIV-infected patients should be included in studies comparing MDRD and equations based on cystatin C.

Of note, equations used to estimate GFR vary from one study to another. Some authors used equations exclusively based on cystatin C, while others used equations combining cystatin C and creatinine or equation including other variables as age, gender and ethnicity. Up to now, data are clearly insufficient to determine which equation must be favoured in HIV patients, even if, in the general population, interesting results have been published with the equations combining serum creatinine and cystatin C [22]. The relative inaccuracy of estimates of GFR can be due to the imprecision of a single GFR measurement [74, 75]. In these four studies, the assay for measuring cystatin C concentration was not the same and, as for serum creatinine, differences in assays and in cystatin C calibration can severely impact the accuracy of the equations. Further studies are needed with greater sample size and patients with AIDS or HAART-treated patients with low HIV viral load and high CD4+ cells

count. Subjects of different ethnicities should also be more deeply studied.

Cystatin C in HIV-infected patients: a step towards outcome prediction?

In the general population, serum cystatin C is a predictor of CVE and of mortality [76, 77]. Cystatin C is more strongly and more linearly associated than serum creatinine and creatinine-estimated GFR with all-cause mortality. In addition, cystatin C in combination with urine albumin-to-creatinine ratio and serum creatinine is an accurate predictor of mortality and CVE [25]. In patients with CKD, there is a trend for a stronger association between cystatin C and mortality than between measured GFR and mortality [26], thereby suggesting that cystatin C could predict cardiovascular outcomes, at least in part, independently of its association with GFR. In the SMART trial, hs-CRP, interleukin-6 and D-dimers were associated with all-cause mortality [78] and risk of opportunistic diseases [79]. Serum cystatin C was higher in the drug conservation arm [59] and the risk of cardiovascular disease was also higher in these patients [57]. HIV-infected subjects with high cardiovascular risk assessed by Framingham score had a higher serum cystatin C level [80]. GFR was found to be associated with all-cause mortality in HIV-infected subjects only when GFR was estimated from cystatin C and not from creatinine

[27]. In the FRAM study, five-year mortality was better predicted by cystatin C-based estimates with an increase in absolute risk of roughly 10% for patients having an eGFR <60 mL/min/1.73 m² [27]. Taken together, these data suggest that cystatin C might be a valuable biomarker of cardiovascular risk assessment in HIV-seropositive patients. The interest in cystatin C to predict outcomes in HIV-infected subjects could justify by itself the use of cystatin C in the follow-up of these patients. However, several issues regarding the interest in cystatin C in HIV patients need to be addressed first. These are summarized in Table 5.

Conclusions

Serum creatinine is far from being the ideal estimate of GFR in HIV-infected subjects. Cystatin C appears to be an alternative biomarker for GFR estimation. However, studies relying on a robust methodology and comparing estimates of GFR based on cystatin C with a reference GFR measurement are currently missing. These studies should calculate bias, precision and accuracy of the cystatin C-based equations and should determine which of the various equations available must be preferred. They should also include a sufficient number of patients, take into account the standardization of cystatin C measurement, and use appropriate statistical analysis (Bland and Altman analysis). In addition, cystatin C might serve as a predictor of all-cause mortality in HIV-infected patients. Whether this

Table 4. Comparison of the four studies about the predictive performance of cystatin C in estimation of GFR in HIV-infected subjects^a

	N patients	GFR measurement	eGFRcys C equation	eGFRcreat	Results
Barracough <i>et al.</i> 2009 [69]	27	⁹⁹ Tc-DTPA clearance	eGFR = 86.7/cysC – 4.2 ^b	MDRD, CG	eGFRcys less accurate than eGFRcreat
Beringer <i>et al.</i> 2010 [70]	22	Clearance of iothalamate	eGFR = 127.7 × cysC ^{-1.17} × age ^{-0.13} × (0.91 if female) × (1.06 if African American) eGFRcys C _{creat} = 177.6 × Creat ^{-0.65} × cysC ^{-0.57} × age ^{-0.20} × 0.82 if female and/or 1.11 if African American ^c	MDRD, CG	No statistically significant differences
Bonjoch <i>et al.</i> 2010 [61]	15	⁵¹ Cr-EDTA clearance	CysC alone, no equation	MDRD, CG, CKD-EPI	CysC well correlated with isotopic GFR
van Deventer <i>et al.</i> 2011 [71]	20	⁵¹ Cr-EDTA clearance	eGFR = 10 ^{2.35} × 10 ^(CysC × -0.33) × 10 ^(-0.003 × age)	MDRD, CKD-EPI	eGFRcys C more accurate than eGFRcreat

^aDTPA, Diethylene Triamine Pentaacetic Acid.

^bMacisaac RJ *et al.* [72].

^cStevens LA *et al.* [22].

Table 5. Critical questions about the use of cystatin C in HIV-infected patients

The cost in comparison with the dosage of serum creatinine
Could an early diagnosis of CKD-infected patients affect the outcome of the HIV infection?
No eGFRcys equations are up to now validated in this population
Should cystatin be used in combination with creatinine?
Are the associations between non-HIV-related factors such as CRP level and smoking status only dependent on GFR?
Do HIV viral load and lymphocytes CD4+ count affect cystatin C level only in association with kidney function?
Do physicians need a novel GFR estimate or a predictor of clinical outcomes?
Should physicians use the same GFR estimates for all HIV-infected patients?
Is it feasible to monitor kidney function with such an assay in developing countries where the majority of HIV-infected people live?

ability to predict patient outcome will extend to cardiovascular morbi-mortality and CKD progression still has to be evaluated. Given that HIV-infected patients are at increased risk of CVE, cystatin C might be particularly valuable in this population. Whatever the role played by cystatin C in the future for the care of HIV-infected patients, financial issues will have to be addressed. Standardized measurement of cystatin C will be very soon easily available but will remain expensive in comparison to the measurement of serum creatinine. The added value of cystatin C will have to be both clinically significant and economically acceptable for its widespread diffusion, particularly in developing countries.

Conflict of interest statement. None declared

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