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ORIGINAL RESEARCH

Medium-grade proteinuria is a risk factor for incident markers of chronic kidney disease

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Objective

Medium-grade proteinuria (100–500 mg/g creatinine) is common among people living with HIV/AIDS (PLWHA) but is often undetected or ignored. This prospective, observational cohort study examined medium-grade proteinuria as a risk factor for markers of chronic kidney disease (CKD).

Methods

Quantitative urine samples were collected from 241 PLWHA without known renal disease at baseline between January 2009 and February 2011 and at follow-up 240 weeks later. Multivariate analysis was performed to assess medium-grade proteinuria as a risk factor for incident markers of CKD (estimated glomerular filtration rate < 60 mL/min/1.73 m^2, albuminuria, phosphaturia).

Results

Incident markers of CKD were identified in 33 patients (14%), of whom 24 (74%) had medium-grade proteinuria at baseline. Of these, 22 even had proteinuria of < 200 mg/g creatinine. Multivariate analysis showed an adjusted relative risk (aRR) of 2.4 for patients with baseline medium-grade proteinuria to develop signs of CKD. Age was identified as an additional independent predictor. By testing for interaction, tenofovir disoproxil fumarate (TDF)-independent proteinuria was strongly associated with incident CKD markers (aRR = 12.1).

Conclusion

Medium-grade proteinuria of 100–500 mg/g creatinine is both frequent in PLWHA and a significant risk factor for developing markers of CKD, especially in the absence of TDF. Relevant risk seems to be associated with proteinuria levels as low as 100–200 mg/g creatinine. Current guidelines recommend specific action for proteinuria exceeding 135–200 mg/g but still will miss a relevant number of PLWHA potentially at risk for CKD. An even lower cut-off to trigger nephrological work-up and potentially renoprotective interventions appears to be indicated.

Keywords: chronic, early diagnosis, HIV, kidney, proteinuria, renal insufficiency

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Introduction

Since the introduction of antiretroviral (ARV) therapy, HIV-associated mortality has decreased and life expectancy has improved dramatically [1]. Ageing of people living with HIV/AIDS (PLWHA) leads to an increase in age-related non-communicable diseases (NCDs), such as non-AIDS-related malignancies, cardiovascular disease, diabetes mellitus, and chronic kidney disease (CKD) [2,3]. Among those comorbidities, CKD is very common [4-8]. In the HIV-uninfected population CKD is mainly associated with age, diabetes mellitus, arterial hypertension, obesity, and also coronary heart disease [9]. PLWHA are additionally exposed to potential both viral and immunologic nephrotoxicity as well as effects caused by ARV treatment, which nowadays is

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usually started early after diagnosis [10]. Especially the nucleotide reverse transcriptase inhibitor tenofovir disoproxil fumarate (TDF) is associated with kidney damage due to proximal tubular dysfunction [4-8,11-13].

People living with HIV/AIDS not only are at higher risk for CKD, but studies also show that its progress is more rapid compared with uninfected individuals [14]. As CKD is a significant cause of morbidity and mortality [15,16], early identification of patients at risk should be a key premise of physicians treating PLWHA. Signs of CKD include a decreased glomerular filtration rate (GFR) below 60 mL/min/1.73 m^2, albuminuria over 30 mg/g creatinine, urine sediment or electrolyte abnormalities, as well as abnormalities detected by histology or imaging [17].

Urinary loss of large proteins such as albumin, transferrin or immunoglobulins suggests impairment of the glomerular filter which is often caused by diabetes mellitus or arterial hypertension [18]. On the other hand, infections, nephrotoxins, or some hereditary conditions lead to damage of the tubular system. As a consequence, pathological amounts of rather smaller proteins, e.g., alpha-1 microglobulin, become detectable [19]. In most instances, these two forms of proteinuria cannot be completely distinguished. Diagnostic modality of choice is quantitative urinalysis. The updated European AIDS Clinical Society (EACS) guidelines suggest checking GFR every 3–12 months and performing a dipstick or quantitative urinalysis annually. Albuminuria > 30 mg/g creatinine as well as proteinuria > 135–150 mg/g is considered to be pathological and should result in further diagnostic and therapeutic actions [20].

Several studies have shown that medium-grade proteinuria is frequent in PLWHA [4,6], with a prevalence ranging up to > 50%, depending on the cut-off [21]. A previous study performed at our HIV outpatient clinic between 2009 and 2011 detected medium-grade proteinuria with a urinary protein/creatinine ratio (uPCR) from 100 to 500 mg/g in 56% of patients. Of those, 66% had proteinuria without albuminuria, suggesting low-molecular-weight proteinuria of tubular origin. There was a significant association with exposure to TDF, older age, lower CD4-positive T-lymphocyte-(CD4-) nadir and hepatitis C infection [22]. While proteinuria in general is a known risk factor for CKD [4-6,23], the prognostic relevance of merely medium- or even low-grade proteinuria in PLWHA has not been defined.

The objective of this follow-up study, therefore, was to examine whether medium-grade proteinuria of 100–500 mg/g creatinine is a risk factor for incident renal dysfunction in PLWHA.

Methods

Setting and patient population

This prospective observational cohort study was conducted at the HIV outpatient clinic of the University Hospital of Ludwig-Maximilians-University in Munich, Germany. The university ethics committee approved the protocol (project no. 153-14). Written informed consent was obtained from every patient entering the study. The data were collected at two time points: first during the baseline visit between January 2009 and February 2011, and then during a follow-up visit on average 240 weeks later. Inclusion criteria were age > 18 years, written consent, a confirmed diagnosis of HIV infection, as well as a visit during the period January 2009 to February 2011. On both visits, blood samples and a quantitative urine sample were collected as part of routine patient care. Exclusion criteria were any history of renal disease before the first visit, a confirmed diagnosis of CKD at the baseline visit, fever or a urinary tract infection at the time of examination, and any other known causes of proteinuria, including amyloidosis, pregnancy, or sickle cell disease.

Blood and urine samples were analysed in the context of routine laboratory testing during the quarterly routine control visits. A uPCR of 100–500 mg/g at the baseline visit was defined as medium-grade proteinuria. In addition, urinary albumin/creatinine ratio (uACR), fasting urinary phosphate/creatinine ratio (uPhoCR), and urinary α_1-microglobulin/creatine ratio were measured. Estimated GFR (eGFR) was calculated using the CKD-Epi creatinine equation [17]. Laboratory testing also included a complete blood count with differential, serum creatinine, and serum electrolytes. For all patients, current CD4 T-cell count and plasma viral load were measured.

Demographic and additional medical data, such as comorbidities, blood pressure, height, weight, and current medication, including ARV therapy, were collected from the patients' files. Hypertension was defined as current use of antihypertensive drugs or systolic or diastolic blood pressure > 140 or > 90 mmHg, respectively. Diabetes mellitus was defined as current use of oral antidiabetic medication or insulin, HbA1c > 6.5% or any blood glucose measurement > 200 mg/dL. Hepatitis B and C infection were diagnosed by serological testing (anti-HBc antibodies, HBs antigen, anti-HCV antibodies, and HCV-PCR).

The main outcome was detection of incident early markers of renal dysfunction at the 240-week follow-up, defined by at least one of the following criteria: eGFR < 60 mL/min/1.73 m^2, uACR > 30 mg/g, or uPhoCR > 1000 mg/g, markers used for definition of CKD if found to be confirmed [17].

Statistical methods

The study is a retrospectively documented, but prospectively analysed cohort study. Categorical variables are shown as count and percentage and were analysed using Pearson's χ^2-test or Fisher's exact test; continuous variables are shown as median and interquartile range (IQR) and were analysed using Wilcoxon–Mann–Whitney test or Student's *t*-test for unpaired data. To analyse the association between medium-grade proteinuria and different baseline and outcome variables measured as relative risk (RR) estimates, univariate analysis was performed: patients with uPCR < 100 mg/g at baseline were compared to those with uPCR of 100–500 mg/g.

A multivariate analysis for the main outcome variable incident CKD markers (eGFR < 60 mL/min/1.73 m^2, uACR > 30 mg/g, or uPhoCR > 1000 mg/g) was performed using a modified Poisson regression model with a robust error variance [24,25]. This allows robust estimates of adjusted relative risk (aRR) as a measure of association. Variables with statistically significant association in univariate analysis or known risk factors for CKD in the current literature were included in the primary (full) multivariate model. Collinearity was assessed and, in the case of competing variables, the monodimensional instead of the compound variables or the more strongly associated variable were entered into the model (i.e. serum creatinine and age but not eGFR; age but not duration of HIV infection). Ln-linearity of continuous variables (age, body mass index, current CD4 cell count) was ensured. Manual backward elimination was used based on the influence on the aRR for medium-grade proteinuria to construct the final model. The final model includes significant independent predictors and potential confounders. Constructing a hierarchically well-formulated model also allowed us to assess the interaction between variables [26]. To rule out subjective influence on variable selection, additional models were derived using automated algorithms (SAS 9.4, *proc hpgenselect*, forward selection, stepwise regression, backward elimination).

Analyses were performed using SPSS 24 (IBM Inc., Armonk, NY, USA) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA). A *P*-value < 0.05 was considered statistically significant, except for the analysis of interaction for which a cut-off of 0.1 was used.

Results

The initial study had enrolled 393 patients. For this follow-up analysis 84 (21%) were excluded because they already had shown signs of CKD at baseline. For 68 (22%) of the remaining 309 patients no 240-week follow-up data were available. Reasons were death, missing written consent, change of HIV care provider, or acute urinary tract infection at the time of follow-up (Fig. 1).

Characteristics of the cohort that qualified for the final analysis are shown in Tables 1 and 2: of 241 patients, 186 (77%) were male and 194 (80%) were of Caucasian descent. At baseline, median time since HIV infection was 9.3 years, median age was 44.8 years. Main HIV risk factors were homosexual (n = 120, 50%) and heterosexual (n = 60, 25%) contacts. At baseline and follow-up diabetes mellitus was diagnosed in 12 (5%) and 15 patients (6%), and hypertension in 49 (20%) and 68 patients (28%), respectively. Twenty-eight (12%) and 31 (13%) were positive for anti-HCV antibodies, and HCV-RNA was detectable in 11 (4%) and 10 (4%), respectively. Median eGFR was 105 mL/min/1.73 m^2 at baseline and decreased to a median of 88 mL/min/1.73 m^2 over the course of the study. By definition, none of the patients had significant uACR > 30 mg/g at baseline.

At baseline, 126 patients (52%) had no significant proteinuria (uPCR < 100 mg/g), while 115 (48%) had medium-grade proteinuria (uPCR 100–500 mg/g). This group was significantly older [50.5 years compared with 48.0 years at follow-up (P = 0.001)] and duration of HIV infection was longer (16.0 *vs.* 13.0 years, P = 0.012). The CD4 cell count nadir was significantly higher in the group with a uPCR < 100 mg/g, both at baseline (232 *vs.* 173 cells/μL, P = 0.009) and at follow-up (202.5 *vs.* 170 cells/μL, P = 0.015). In addition, while the current CD4 count was not significantly different between groups at baseline, at follow-up the group with uPCR of 100–500 mg/g had a significantly lower current CD4 count (616 *vs.* 643.5 cells/μL, P = 0.028). With respect to ARV therapy, patients with uPCR of 100–500 mg/g used TDF significantly more often at baseline (P = 0.012). At follow-up, TDF had been stopped significantly more often in the group with uPCR of 100–500 mg/g: 15 (22% of patients with TDF at baseline, 13% overall) compared with seven (9% of patients with TDF at baseline, 6% overall) (P = 0.044). There were no significant differences between the groups relating to other comorbidities (Tables 1 and 2).

At follow-up eGFR had dropped below 60 mL/min/1.73 m^2 in 11 patients (5%), uACR had increased to > 30 mg/g in 20 (8%), and uPhoCR to > 1000 mg/g in seven (3%). Combining these criteria, signs of incident chronic kidney disease were detected in 33 patients overall (14%) at follow-up (Tables 1 and 2; Fig. 2). In 29 patients a single criterion was pathological, in three patients new-onset albuminuria was found in addition to either phosphaturia (2) 60 mL/min/1.73 m^2 (1), and in one patient all three criteria exceeded the predefined thresholds. Within the group of patients with medium-grade proteinuria at baseline 24 (21%) developed markers for CKD: eGFR had

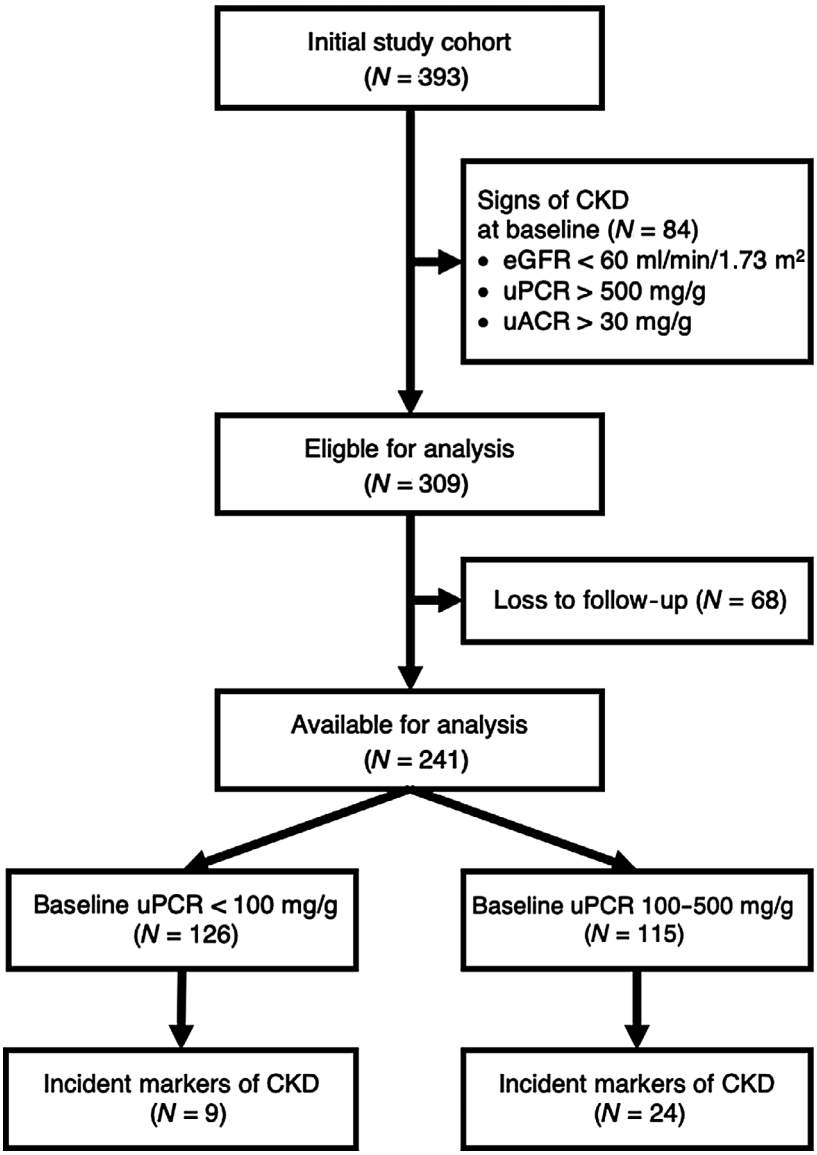

Fig. 1 Study design and patient disposition. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; uACR: urinary albumin/creatinine ratio; uPCR, urinary protein/creatinine ratio.

dropped to < 60 mL/min/1.73 m^2 in nine, uACR had increased to > 30 mg/g in 14, and uPhoCR to > 1000 mg/g in three patients. Among the 33 patients meeting the endpoint 4 (12%) had a diagnosis of diabetes mellitus and 12 (36%) of arterial hypertension at baseline. None of them had HCV-RNA detectable at baseline.

Multivariate analysis (Fig. 3) confirmed the univariate finding of a baseline uPCR of 100–500 mg/g as an independent risk factor to develop markers of CKD at follow-up, with an aRR of 2.4 ($P = 0.024$). In addition, age was an independent predictor (aRR 1.03/year, $P = 0.017$). While arterial hypertension and CD4 nadir were associated with incident signs of CKD in univariate analysis, this was no longer significant in multivariate analysis. Exposure to TDF at baseline was not a significant risk factor in the overall study population (Fig. 3). However, in addition to age, use of TDF was identified as a confounder for the association between baseline medium-grade proteinuria and incident CKD and therefore adjusted for in the multivariate model. Finally, the multivariate regression model was assessed for interaction (Table 3). There was a significant interaction between medium-grade proteinuria and exposure to TDF at baseline. Thus, the aRR for incident markers of CKD

Table 1 Patient characteristics and univariate analysis of association with level of urinary protein/creatinine ratio at baseline (categorical data)

	All patients *N* = 241 *N* (%)	No significant proteinuria (uPCR < 100 mg/g) *N* = 126 (52%) *N* (%)	Medium-grade proteinuria (uPCR 100–500 mg/g) *N* = 115 (48%) *N* (%)	*P*-value
Male gender	186 (77%)	99 (79%)	87 (76%)	0.646
Race				
Caucasian	**194 (81%)**	**95 (75%)**	**99 (86%)**	**0.036**
African American	29 (12%)	19 (15%)	10 (9%)	0.128
Other	18 (8%)	12 (10%)	6 (5%)	
Risk of infection				
Heterosexual	60 (25%)	31 (25%)	29 (25%)	0.912
Homosexual	120 (50%)	61 (48%)	59 (51%)	0.965
Intravenous drug use	11 (5%)	4 (3%)	7 (6%)	0.279
Blood products	2 (1%)	1 (1%)	1 (1 %)	1.000
Origin from endemic country	42 (17%)	25 (20%)	17 (15%)	0.301
Other/unknown	6 (2%)	4 (3%)	2 (2%)	
Baseline visit				
Diabetes mellitus	12 (5%)	5 (4%)	7 (6%)	0.450
Arterial hypertension	49 (20%)	23 (18%)	26 (23%)	0.401
Anti-HCV antibodies positive	28 (12%)	11 (9%)	17 (15%)	0.143
HCV-RNA-positive	11 (5%)	3 (2%)	8 (6%)	0.089
Current TDF exposure	**139 (58%)**	**63 (50%)**	**76 (66%)**	**0.012**
Serum creatinine > 0.8 mg/dL	**103 (43%)**	**62 (49%)**	**41 (36%)**	**0.034**
eGFR < 60 mL/min/1.73 m^2	0 (0%)	0 (0%)	0 (0%)	
uACR > 30 mg/g	0 (0%)	0 (0%)	0 (0%)	
Tubular proteinuria	115 (48%)	0 (0%)	115 (100%)	
HIV-RNA < 50 copies/mL	199 (8 %)	100 (80%)	99 (86%)	0.169
Follow-up visit				
Diabetes mellitus	15 (6%)	7 (6%)	8 (7%)	0.653
Arterial hypertension	68 (28%)	31 (25%)	37 (32%)	0.192
Anti-HCV antibodies positive	**31 (13%)**	**11 (9%)**	**20 (13%)**	**0.045**
HCV-RNA-positive	**10 (4%)**	**1 (0.8%)**	**9 (6%)**	**0.008**
HIV-RNA < 50 copies/mL	230 (95%)	120 (95%)	110 (96%)	0.878
Progress of HIV infection	33 (14%)	18 (14%)	15 (13%)	0.779
Current TDF exposure	147 (70%)	78 (62%)	69 (60%)	0.762
TDF started	**30 (12%)**	**22 (18%)**	**8 (7%)**	**0.014**
TDF stopped	**22 (9%)**	**7 (6%)**	**15 (13%)**	**0.044**
ATV started	**6 (3%)**	**5 (4%)**	**1 (1%)**	**0.106**
ATV stopped	16 (7%)	7 (6%)	9 (8%)	0.106
COBI started	11 (5%)	3 (2%)	8 (7%)	0.123
DTG started	15 (6%)	6 (5%)	9 (8%)	0.426
ACE inhibitor started	19 (8%)	7 (6%)	12 (10%)	0.160
RAS inhibitor started	22 (9%)	9 (7%)	13 (11%)	0.263
Serum creatinine > 0.8 mg/dL	165 (68%)	87 (69%)	78 (68%)	0.838
eGFR < 60 mL/min/1.73 m^2	**11 (5%)**	**2 (2%)**	**9 (8%)**	**0.020**
Cystatin C > 1.1 mg/dL	**44 (18%)**	**16 (13%)**	**28 (24%)**	**0.019**
Quantitative urinalysis				
uPCR > 100–500 mg/g	**92 (38%)**	**31 (25%)**	**61 (53%)**	**< 0.001**
uACR > 30 mg/g	**21 (9%)**	**6 (5%)**	**15 (13%)**	**0.037**
Tubular proteinuria	**71 (30%)**	**25 (20%)**	**46 (40%)**	**0.001**
α_1-microglobulin/creatinine ≥ 14 mg/g	**77 (32%)**	**20 (16%)**	**57 (50%)**	**< 0.001**
IgG/creatinine > 12 mg/g	24 (10%)	11 (9%)	13 (11%)	0.505
Incident markers of CKD	**33 (14%)**	**9 (7%)**	**24 (21%)**	**0.002**

ACE, angiotensin-converting enzyme; ATV, atazanavir; CKD, chronic kidney disease; COBI, cobicistat; DTG, dolutegravir; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IgG, immunoglobulin G; RAS, renin–angiotensin system (RAS inhibitor defined as ACE inhibitor and/or angiotensin-receptor blocker); RNA, ribonucleic acid; TDF, tenofovir disoproxil fumarate; uACR, urinary albumin/creatinine ratio; uPCR, urinary protein/creatinine ratio. Statistically significant associations shown in bold.

associated with medium-grade proteinuria was 12.1 for patients without baseline TDF exposure ($P = 0.023$), whereas the association was not statistically significant for patients exposed to TDF at baseline (aRR = 1.64, $P = 0.17$). Also, the effect of age was dependent on both serum creatinine levels and CD4 nadir. The robustness of

Table 2 Patient characteristics and univariate analysis of association with level of urinary protein/creatinine ratio at baseline (continuous data)

	All patients *N* = 241 Median (IQR)	No significant proteinuria (uPCR < 100 mg/g) *N* = 126 (52%) Median (IQR)	Medium-grade proteinuria (uPCR 100–500 mg/g) *N* = 115 (48%) Median (IQR)	*P*-value
Baseline visit				
Age (years)	**44.8 (38.8–52.2)**	**43.6 (36.9–48.9)**	**45.8 (40.4–56.2)**	**0.001**
BMI (kg/m^2)	**23.9 (22.1–26.8)**	**24.6 (22.1–27.7)**	**23.5 (22.1–25.6)**	**0.022**
Serum creatinine (mg/dL)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.16
eGFR [CKD-Epi (mL/min/1.73 m^2)]	**105.02 (96.29–115.37)**	**108.10 (97.23–117.39)**	**104.91 (92.51–114.91)**	**0.049**
uPCR (mg/g)	**96 (69.5–129.0)**	**70.0 (53.0–84.3)**	**130.0 (112.0–161.0)**	**< 0.001**
uACR (mg/g)	**10.0 (10.0–10.0)**	**10.0 (10.0–10.0)**	**10.0 (10.0–15.0)**	**< 0.001**
Time since HIV diagnosis (years)	**9.3 (3.5–15.9)**	**8.0 (3.3–14.5)**	**11.3 (4.6–17.5)**	**0.010**
CD4 nadir (cells/μL)	**196.0 (81.0–321.0)**	**232.0 (111.5–362.5)**	**173.0 (59.0–272.0)**	**0.009**
Current CD4 cell count (cells/μL)	538.0 (337.5–701.0)	558.0 (365.0–697.0)	506.0 (309.0–709.0)	0.132
Follow-up visit				
Age (years)	**49.6 (43.3–57.1)**	**48.0 (41.5–53.6)**	**50.5 (44.8–60.7)**	**0.001**
BMI (kg/m^2)	21.7 (19.6–24.6)	22.5 (19.5–24.7)	21.2 (19.8–24.3)	0.144
Tim's e since HIV diagnosis (years)	**14.0 (8.0–20.5)**	**13.0 (8.0–19.0)**	**16.0 (9.0–22.0)**	**0.012**
CD4 nadir (cells/μL)	**190.0 (78.5–305.5)**	**202.5 (102.0–324.5)**	**170.0 (59.0–250.0)**	**0.015**
Current CD4 count (cells/μL)	**626.0 (492.0–802.0)**	**643.5 (517.5–843.8)**	**616.0 (408.0–746.0)**	**0.028**
Serum creatinine (mg/dL)	1.0 (0.8–1.1)	1.0 (0.8–1.1)	1.0 (0.8–1.1)	**0.528**
eGFR [CKD-Epi (mL/min/1.73 m^2)]	**88.35 (76.53–101.83)**	**91.06 (78.66–104.34)**	**83.56 (73.95–99.11)**	**0.006**
Cystatin C (mg/dL)	**0.92 (0.81–1.04)**	**0.88 (0.79–0.99)**	**0.96 (0.87–1.11)**	**0.002**
uPCR (mg/g)	**83.5 (62.6–114.5)**	**70.0 (55.9–93.6)**	**102.0 (72.6–134.0)**	**0.000**
uACR (mg/g)	**10.0 (9.1–11.2)**	**10.0 (10.0–18.5)**	**10.0 (10.0–15.1)**	**0.008**
α_1-microglobulin/creatinine (mg/g)	**7.0 (7.0–17.7)**	**7.0 (7.0–9.0)**	**13.4 (7.0–29.5)**	**< 0.001**
IgG/creatinine (mg/g)	**5.8 (3.9–7.0)**	**4.9 (3.5–6.0)**	**6.0 (4.0–7.9)**	**0.003**

BMI, body mass index; CD4, CD4-positive T-helper lymphocytes; eGFR, estimated glomerular filtration rate; HIV, human immunodeficiency virus; IgG, immunoglobulin G; uACR, urinary albumin/creatinine ratio; uPCR, urinary protein/creatinine ratio. Statistically significant associations shown in bold.

our findings was confirmed by additional models derived using automated variable selection algorithms (data not shown).

Discussion

In our cohort of 241 PLWHA we demonstrate that despite the absence of known kidney disease at baseline more than one in five patients with merely medium-grade proteinuria developed markers of CKD after a median follow-up of 240 weeks. Thus, the presence of medium-grade proteinuria (uPCR 100–500 mg/g) was associated with a greater than two-fold risk for developing signs of CKD (aRR = 2.4, $P = 0.024$). A surprisingly high risk for incident CKD markers was identified for patients with medium-grade proteinuria and no concurrent use of TDF at baseline (aRR = 12.1, $P = 0.023$).

In accordance with our previous investigation [22] others have shown that proteinuria is highly prevalent in predominantly Caucasian PLWHA in Germany, with a uPCR > 70 mg/g in 55% of patients. Even for this low cut-off the authors were able to identify the classic risk factors of older age, diabetes mellitus, and exposure to TDF [21]. In addition to the high prevalence of proteinuria the increased risk for CKD among PLWHA has been well known for decades, and clear definitions have been used already in the pre-TDF era [27]. Already in 2004 Gupta described an association between proteinuria and the incidence of CKD in PLWHA [23]. However, in that study, proteinuria was evaluated by dipstick only. Their analysis therefore had a lower sensitivity compared with our investigation and also missed tubular proteins. Their definition of CKD also resulted in a much lower incidence of only 2%, as they and others [28] used eGFR only, while current Kidney Disease: Improving Global Outcomes (KDIGO) guidelines call for evaluation by eGFR and quantitative urinalysis [17].

Our results now not only confirm the high prevalence of proteinuria found in PLWHA [4,6,21,23] and the high risk of renal dysfunction in this population [4-7,12,14,23], but also underline the prognostic relevance of even medium-grade proteinuria of < 500 mg/g creatinine as an early indicator of increased risk for markers of CKD. The cut-off of 500 mg/g initially chosen in 2009 resulted from our clinical experience that in the absence of pre-existing renal disease specific nephrological work-up or renoprotective measures were essentially never deemed necessary for values observed in this range, despite them being reported as abnormal. In our prospective cohort, 106 (92%) out of the 115 patients with

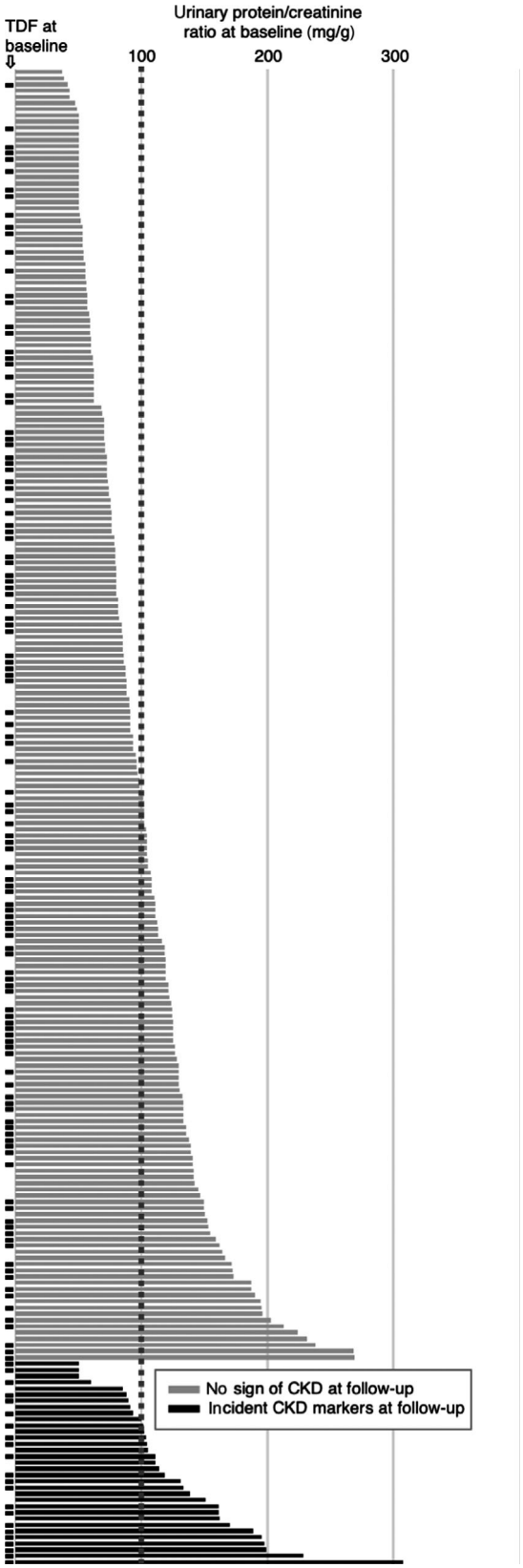

Fig. 2 Cohort overview by tenofovir disoproxil fumarate (TDF) status and level of proteinuria at baseline. Two hundred and forty-one people living with HIV/AIDS (PLWHA) without chronic kidney disease (CKD) at baseline were followed for a median of 240 weeks. Black rectangles on the left indicate PLWHA taking TDF at baseline. Bars show urinary protein/creatinine ratio (uPCR, mg/g) at baseline; levels up to 100 mg/g (dashed line) were considered normal. Black bars indicate PLWHA with incident markers of CKD at follow-up.

medium-grade proteinuria at baseline even had uPCR levels of only 100–200 mg/g. At follow-up 22 of these (21%) showed CKD markers, representing the vast majority (22/24, 92%) of persons in the medium-grade proteinuria group to do so and at a proportion basically identical to the 2/9 (22%) of patients with baseline uPCR of 200–500 mg/g. This suggests that even a level of proteinuria close to the upper limit of normal, a finding which is frequently seen in PLWHA, is a relevant predictor of renal dysfunction.

Interestingly, proteinuria independent of current TDF use at baseline was associated with a much higher risk for developing signs of CKD. TDF has long been recognized for its potential nephrotoxicity, which is reversible if identified early [13,29]. The judicious use of this ARV drug is reflected in our data, which show that in patients with medium-grade proteinuria TDF had been stopped significantly more often. Suspicion of TDF use as a potential cause of proteinuria or at least contributing to potential nephrotoxicity thus apparently led to optimization of the ARV regimen as a modifiable risk factor. Similarly, a numerically larger proportion of patients with medium-grade proteinuria at baseline were started on angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers, even though this did not reach statistical significance. Both observations demonstrate the value and effect of quantitative urinalysis in PLWHA; also, this differential treatment most likely ameliorated the overall risk associated with medium-grade proteinuria at baseline.

Experts on HIV-associated renal disease recently convened by KDIGO recommend monitoring both eGFR and urine markers including those specific for tubular dysfunction [5]. They suggest at least yearly assessments and set the uPCR cut-off for the definition of ‘high risk’ at 200 mg/g. In their 2019 update, the European AIDS Clinical Society (EACS) guidelines now recommend further diagnostic and therapeutic actions including consideration of replacing TDF for patients with albuminuria $\geq$ 3 mg/mmol or proteinuria $\geq$ 15 mg/mmol, equivalent to 135–150 mg/g creatinine [20]. This constitutes a significant change from the previous recommendation of ‘regular follow-up’ for uPCR levels up to 442 mg/g [30].

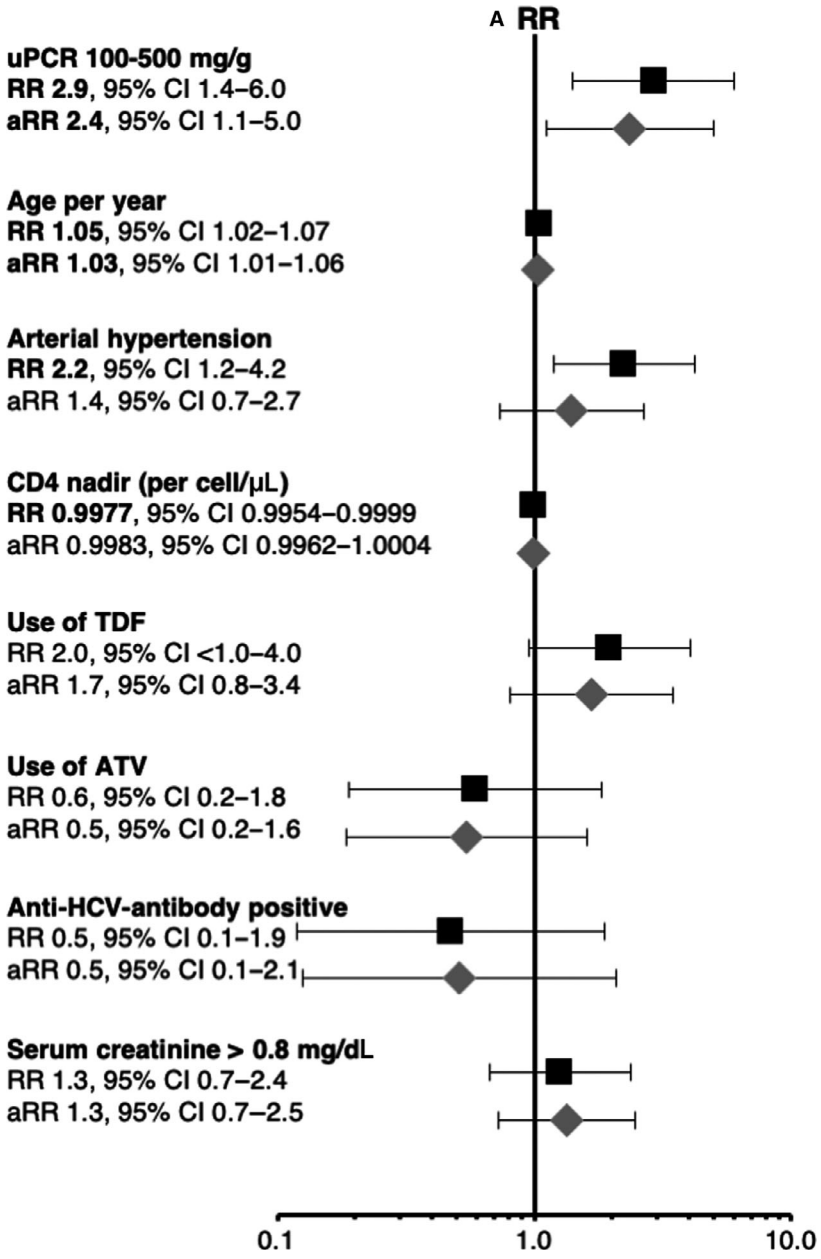

Fig. 3 Comparison of potential risk factors at baseline for developing incident markers of chronic kidney disease (CKD) after 240 weeks of follow-up by univariate and multivariate analysis. Risk ratio estimates (RR) are shown as black squares for univariate analysis, and adjusted RR estimates (aRR) are shown as grey diamonds for multivariate analysis. Error bars indicate 95% confidence intervals (CI). Numbers and graphs do not take interaction into account. ATV, atazanavir; CD4, CD4-positive T-helper lymphocytes; HCV, hepatitis C virus; TDF, tenofovir disoproxil fumarate. Statistically significant associations shown in bold.

In our study, 14% of all patients developed signs of CKD; considering only the patients with medium-grade proteinuria at baseline, the incidence was even higher, at 21%. All 33 PLWHA with CKD markers at follow-up had baseline uPCR levels well below 442 mg/g. In only two of them did uPCR exceed the level of 200 mg/g; the cut-off suggested by KDIGO experts therefore would have resulted in a sensitivity of merely 6%. Setting the cut-off for potentially pathological proteinuria to the uPCR level of 100 mg/g identified 24/33 (73%) PLWHA who eventually would show markers of CKD, resulting in a negative predictive value of 92% in our study population, while

Table 3 Identification of independent risk factors for incident markers of chronic kidney disease by a multivariate modified Poisson regression model, accounting for interaction

Variable	β-estimate	*P*-value	aRR	95% confidence interval
Predictors				
uPCR 100–500 mg/g at baseline	2.4899	0.02		
In PLWHA taking TDF		0.17	1.67	0.81–3.33
In PLWHA not taking TDF		**0.02**	**12.1**	**1.40–104**
Age per year	0.0364	0.07		
In PLWHA with:				
Serum creatinine < 0.9 mg/dL		0.16	0.96	0.91-1.01
CD4 nadir 500/μL				
In PLWHA with:				
Serum creatinine < 0.9 mg/dL		0.67	1.01	0.98-1.04
CD4 nadir 200/μL				
In PLWHA with:				
Serum creatinine > 0.8 mg/dL		0.80	1.01	0.96-1.06
CD4 nadir 500/μL				
In PLWHA with:				
Serum creatinine > 0.8 mg/dL		**0.002**	**1.05**	**1.02-1.09**
CD4 nadir 200/μL				
Serum creatinine > 0.8 mg/dL	−1.9659	0.12		
In PLWHA aged 50 years		0.49	1.26	0.66–2.39
In PLWHA aged 70 years		**0.01**	**3.02**	**1.25–7.28**
CD4 nadir per cell/μL	0.0059	0.07		
In PLWHA aged 50 years		0.14	0.999	0.997–1.001
In PLWHA aged 70 years		**0.02**	**0.996**	**0.992–0.996**
Relevant confounders adjusted for:				
Current TDF exposure	2.3083	0.03		
Current ATV exposure	0.5502	0.27		
Arterial hypertension	0.2196	0.54		
Anti-HCV antibody-positive	−0.7461	0.30		
Significant interaction terms				
uPCR 100–500 mg/g × TDF	−1.9929	0.08		
TDF × ATV	−2.0360	0.09		
Age × serum creatinine > 0.8 mg/dL	0.0439	0.05		
Age × CD4 nadir	−0.0001	0.03		

Age and CD4 count were entered into the model as continuous variables, and therefore calculation of adjusted relative risk (aRR) estimates was performed for exemplary values.
ATV, atazanavir; CD4, CD4-positive T-helper lymphocytes; HCV, hepatitis C virus; PLWHA, people living with HIV/AIDS; TDF, tenofovir disoproxil fumarate; uPCR, urinary protein/creatinine ratio. Statistically significant associations shown in bold.

such proteinuria levels were also found in 102/208 PLWHA with no apparent renal abnormalities at follow-up, resulting in a specificity of 51% and a positive predictive value of 19% in the study population. Given that the recommended measures for suspected renal dysfunction in PLWHA [5,20] – including evaluation and potential modification of medication and risk factors, renal ultrasound, and potentially referral to a nephrologist – are well defined, straightforward and primarily non-invasive, this cut-off level seems to result in a very reasonable balance of sensitivity and specificity. With CKD known to be associated with significantly increased morbidity and mortality in PLWHA [3,16], using this even lower than currently suggested cut-off therefore appears to be warranted.

This study has several limitations but also a number of strengths that merit discussion. It was performed as a monocentric study, so selection bias cannot be ruled out and the results may not be applicable to PLWHA in other settings, specifically those with different age, gender or ethnic distribution. However, a comparison with the German national epidemiological data of the Robert Koch Institute indicates that our study population adequately represents PLWHA in Germany [31]. This study is an observational study without randomization, and thus the results might be influenced by uncontrolled confounders. In order to optimally limit this source of bias inherent to all observational study designs, multivariate analysis including important potential confounders was performed by using a set of different models. All of them showed consistent results, suggesting a comfortable degree of robustness for our findings. The loss to follow-up proportion of this study was 22%, which is to be expected in this kind of outpatient setting with unrestricted choice of

provider over a 240-week interval. To confirm that the loss to follow-up was non-informative, we conducted a comparative analysis with the former publication of the initial study [22]. This comparison demonstrated that there are no significant deviations with respect to relevant characteristics between the two study populations (data not shown). We assessed kidney function by blood and urine tests only; no imaging or histological data were used. Also, only single time point data were evaluated for incident markers of CKD in order to not place additional burden on patients by exceeding the spectrum of tests at the routine check-ups. Consequently, the estimated risk for developing CKD could be both underrated due to missing histology or imaging data or overrated due to chance observations that would not have been confirmed. However, both effects would equally apply to both groups in our cohort so the estimates for the (adjusted) relative risks are not influenced by this potential misclassification bias. Finally, this being a real-life observational study, our findings may better represent the current situation of PLWHA compared with data from idealized conditions in randomized studies.

In conclusion, we identified medium-grade proteinuria in PLWHA as an independent risk factor for developing markers of CKD, more than doubling the risk. For patients not using TDF, the risk associated with medium-grade proteinuria is even significantly higher. With age being the other independent but obviously non-modifiable risk factor, quantitative urinalysis is a readily available and important tool for early risk detection and PLWHA should be checked regularly for proteinuria and signs of CKD. The recently lowered uPCR cut-off levels triggering further nephrological work-up, as now recommended by EACS, support the importance of timely identification, and potentially modification, of CKD risk factors in PLWHA. Decreasing the uPCR cut-off even further to 100 mg/g may be considered in order to increase sensitivity.

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Author contributions

SYS, AJZ, RH, JRB and US conceived the study protocol. Data acquisition and analysis were carried out by SYS, AJZ and US. Interpretation of the data was done by SYS, AJZ, RH, JRB and US. SYS and US drafted the manuscript, and SYS, AJZ, RH, JRB and US revised it. SYS, AJZ, RH, JRB and US gave final approval and are accountable for all aspects of the work.

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