Neutrophil gelatinase-associated lipocalin, a marker of tubular dysfunction, is not increased in long-term virologically controlled patients receiving a tenofovir/emtricitabine + nevirapine regimen

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Received 20 March 2013; returned 16 May 2013; revised 29 May 2013; accepted 4 June 2013

Objectives: Tenofovir may be associated with nephrotoxicity. Several studies have shown that an early increase in urinary neutrophil gelatinase-associated lipocalin (NGAL) may predict the occurrence of acute kidney injury. We investigated urine and plasma NGAL in patients on long-term treatment with nevirapine associated with either tenofovir/emtricitabine or abacavir/lamivudine.

Patients and methods: We studied 40 virologically controlled Caucasian patients on stable treatment (median >4 years) with tenofovir/emtricitabine + nevirapine (n = 20) or abacavir/lamivudine + nevirapine (n = 20), and no history of kidney disease, high blood pressure or diabetes. Plasma immunovirological parameters (NGAL and C-reactive protein) and urinary NGAL, β2-microglobulin and α1-microglobulin were measured during a routine clinical visit.

Results: Median concentrations of NGAL were in the normal range, but were significantly higher in the abacavir/lamivudine group compared with the tenofovir/emtricitabine group both in the plasma, at 74.9 and 66.0 ng/mL (P = 0.032), respectively, and in the urine, at 36.1 and 12.8 ng/mL (P = 0.017), respectively.

Conclusions: Plasma and urinary NGAL concentrations remained in the normal range in this long-term virologically controlled population without any overt renal disease. The usefulness of NGAL in detecting sub-clinical renal dysfunction appears to be very limited.

Keywords: nucleoside analogues, HIV infection, renal toxicity, NGAL

Introduction

Renal impairment is a frequent issue in the HIV population. However, the relative contributions of HIV infection, antiretroviral therapy exposure and traditional risk factors are still not completely resolved. Previous studies have indicated an increased prevalence of chronic kidney disease among HIV-infected individuals receiving a tenofovir-based therapy. Tenofovir nephrotoxicity is characterized by proximal tubular cell dysfunction that may be associated with acute kidney injury, Fanconi syndrome or chronic kidney disease. On the other hand, some antiretrovirals, such as abacavir, lamivudine and nevirapine, have been considered not to be nephrotoxic.

Screening and early diagnosis of renal dysfunction are essential to enable more suitable management of this major issue. Biological markers such as creatininemia have poor sensitivity and increase late with respect to the occurrence of renal tubular damage. Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin family, is expressed by kidney tubules that are acutely damaged. Several studies have shown that an early increase in urinary NGAL may predict the occurrence of acute kidney injury and it has recently been proved to correlate with the progression of chronic kidney disease. High urine levels of NGAL have been correlated with HIV-associated nephropathy, but association with renal toxicity in treated HIV patients is lacking. One study showed elevation of serum NGAL with anti-retroviral therapy.

The objective of our study was to evaluate whether the long-term use of tenofovir in virologically controlled patients was associated with abnormalities in renal biomarkers in a population with a low risk of renal toxicity. A group of patients on abacavir served as controls.
Patients and methods

This monocentric cross-sectional study included 40 HIV-1 infected Caucasian adults followed in the HIV reference centre of Nantes, France. Patients were receiving a triple combination of nevirapine plus either tenofovir/emtricitabine \( (n=20) \) or abacavir/lamivudine \( (n=20) \) for >6 months with an undetectable viral load (HIV-1 RNA <50 copies/mL) for >6 months. In order to minimize potential confounding factors or biases, non-Caucasian subjects, diabetes, high blood pressure >140/90 mm Hg or current antihypertensive treatment, estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m², history of an abacavir/lamivudine-based regimen if the patient currently on tenofovir/emtricitabine or history of tenofovir/emtricitabine-based regimen if patient currently on abacavir/lamivudine were non-inclusion criteria. Every patient provided written consent for biological sampling. Data were prospectively recorded via an electronic medical record (EMR). The EMR collects demographic details, clinical events, antiretroviral history, CD4 and CD8 cell count, HIV viral load and biological data, including creatinine and eGFR at intervals of 3–6 months during routine clinical assessment. Common biochemical parameters, including blood creatinine, electrolytes, C-reactive protein (CRP) and urine dipstick, urinary creatinine, electrolytes, protein, β2-microglobulin, α1-microglobulin and aminoterminal propeptide of type III procollagen (P3NP), a marker of interstitial fibrosis, were measured at the time of NGAL assessment in all patients, according to standard methods in the routine clinical laboratory. eGFR was assessed using the Modification of Diet in Renal Disease (MDRD) formula.

Plasma and urine samples were stored at −80 °C until plasma NGAL and urinary NGAL, α1-microglobulin and P3NP were assayed. Plasma and urinary NGAL levels were assessed using the immunoanalyser BioPorto Diagnostic test adapted to the P900 analyser (Roche Diagnostics, Meylan, France). Urinary P3NP levels were assessed by radioimmunometric assay (RIA-gnost®, IBA Molecular, Gif sur Yvette, France).

Characteristics of patients were described using the median and interquartile range for continuous variables and proportions for categorical variables. Differences between patient groups were assessed by Fisher’s exact and Wilcoxon tests for categorical and continuous variables, respectively. Logistic regression was used to determine possible factors associated with urinary NGAL. Factors significantly associated \( (P<0.20) \) in univariate analysis were included in multivariate analysis \( (P<0.05 \text{ as level of significance}) \). All the analyses were performed using SAS 9.2.

Results

Results are presented in Tables 1 and 2. Patients were receiving an abacavir/lamivudine- or tenofovir/emtricitabine-based regimen for a median of 46 and 50 months, respectively, with plasma HIV RNA <50 copies/mL for a median duration of 56 months, a median current eGFR-MDRD of 90 mL/min/1.73 m², a median nadir and current CD4 of 249 and 741 cells/mm³, respectively, and a CD4/CD8 ratio of 0.81. Median calcium, phosphate, Na, K, Cl and creatinine were in the normal range in both serum and urine, with no differences between the two groups. Renal function since the start of nucleoside reverse transcriptase inhibitor (NRTI) and current antiretroviral therapy was very stable, as reflected in Table 2. Urinary infection was ruled out by negativity of leucocytes and nitrates on urine dipsticks. Median concentrations of NGAL were in the normal range, at 71.1 ng/mL in plasma and 16.7 ng/mL in urine, but were significantly higher in the abacavir/lamivudine + nevirapine compared with the tenofovir/emtricitabine + nevirapine group, in both plasma, at 74.9 and 66.0 ng/mL \( (P=0.032) \), respectively, and urine, at 36.1 and 12.8 ng/mL \( (P=0.017) \), respectively. A small but significant increase in urinary NGAL/creatinine ratio was observed in the abacavir/lamivudine group compared with the tenofovir/emtricitabine group, at 35.0 and 14.4 \( (P=0.049) \), respectively. Plasma CRP was <3 mg/L in 70% of the patients, urinary β2-microglobulin was <0.2 mg/L in 72.5% of the patients and α1-microglobulin was >5.72 mg/L in 80% of the patients, with no statistically significant difference between the two groups. Median urinary P3NP/creatinine ratio was not different between the two groups, at 24.6 and 26.8 in the abacavir/lamivudine and the tenofovir/emtricitabine groups, respectively.

In univariate analysis, gender, hepatitis co-infection, duration of undetectability, nucleoside backbone (abacavir versus tenofovir),

Table 1. Characteristics of the study population at the time of measurement of renal biomarkers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abacavir/lamivudine ( (n=20) )</th>
<th>Tenofovir/emtricitabine ( (n=20) )</th>
<th>( P ) value</th>
<th>All patients ( (n=40) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (IQR)</td>
<td>50.0 (45.5–56.0)</td>
<td>46.5 (42.0–55.0)</td>
<td>0.34</td>
<td>49.0 (42.0–56.0)</td>
</tr>
<tr>
<td>Hepatitis co-infection, n (%)</td>
<td>3 (15)</td>
<td>1 (5)</td>
<td>0.61</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Gender (male/female), n/n (%/%)</td>
<td>16/4 (80/20)</td>
<td>17/3 (85/15)</td>
<td>1</td>
<td>33/7 (82.5/17.5)</td>
</tr>
<tr>
<td>BMI (kg/m²), median (IQR)</td>
<td>23.8 (21.4–25.6)</td>
<td>26.0 (23.2–30.2)</td>
<td>0.07</td>
<td>24.4 (22.7–26.7)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg), median (IQR)</td>
<td>120 (120–130)</td>
<td>130 (120–132.5)</td>
<td>0.11</td>
<td>127.5 (120.0–130.0)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg), median (IQR)</td>
<td>80 (70–80)</td>
<td>80 (80–90)</td>
<td>0.02</td>
<td>80 (75–90)</td>
</tr>
<tr>
<td>Current CD4 (cells/mm³), median (IQR)</td>
<td>876 (575–963)</td>
<td>647 (450–777)</td>
<td>0.08</td>
<td>741 (486–918)</td>
</tr>
<tr>
<td>Nadir CD4 (cells/mm³), median (IQR)</td>
<td>226 (112–288)</td>
<td>256 (191–281)</td>
<td>0.60</td>
<td>249 (134–283)</td>
</tr>
<tr>
<td>CD4/CD8, median (IQR)</td>
<td>0.77 (0.5–1.0)</td>
<td>0.81 (0.6–1.2)</td>
<td>0.31</td>
<td>0.81 (0.6–1.0)</td>
</tr>
<tr>
<td>Time from HIV diagnosis (months), median (IQR)</td>
<td>143 (109–188)</td>
<td>105 (78–153)</td>
<td>0.06</td>
<td>131 (8–162)</td>
</tr>
<tr>
<td>Duration of undetectable HIV RNA (months), median (IQR)</td>
<td>69 (33–95)</td>
<td>49 (30–69)</td>
<td>0.31</td>
<td>56 (31–85)</td>
</tr>
<tr>
<td>Duration on antiretroviral therapy (months), median (IQR)</td>
<td>114 (93–141)</td>
<td>71 (41–85)</td>
<td>0.003</td>
<td>89 (64–138)</td>
</tr>
<tr>
<td>Duration on NRTIs (months), median (IQR)</td>
<td>70 (47–112)</td>
<td>59 (40–70)</td>
<td>0.049</td>
<td>63 (45–76)</td>
</tr>
<tr>
<td>Duration on current regimen (months), median (IQR)</td>
<td></td>
<td></td>
<td>0.98</td>
<td>46.0 (35.5–65.0)</td>
</tr>
<tr>
<td>duration on abacavir/lamivudine + nevirapine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration on tenofovir/emtricitabine + nevirapine</td>
<td>46 (36–50)</td>
<td>50 (32–67)</td>
<td></td>
<td></td>
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</tbody>
</table>
Table 2. Blood and urinary data in the study population

<table>
<thead>
<tr>
<th>Blood/Urinary Parameter</th>
<th>Abacavir/lamivudine (n = 20)</th>
<th>Tenofovir/emtricitabine (n = 20)</th>
<th>P value</th>
<th>All patients (n = 40)</th>
</tr>
</thead>
</table>

**Blood**
- Calcium (mmol/L), median (IQR): 2.3 (2.3–2.4) vs. 2.2 (2.2–2.3) 0.015
- Potassium (mmol/L), median (IQR): 4.2 (4.0–4.3) vs. 4.1 (3.9–4.3) 0.70
- Sodium (mmol/L), median (IQR): 140 (139–141) vs. 141 (140–142) 0.044
- Phosphate (mmol/L), median (IQR): 0.9 (0.8–1.0) vs. 0.8 (0.7–0.9) 0.046
- Creatinine (mmol/L), median (IQR)
  - Baseline NRTIs: 76.5 (70.5–84.0) vs. 75.0 (69.0–84.0) 0.64
  - Baseline current regimen: 76.0 (70.0–83.5) vs. 76.0 (69.0–85.0) 0.85
  - Sample visit: 82.5 (73.0–85.0) vs. 79.5 (73.5–90.0) 0.82
- eGFR-MDRD (mL/min/1.73 m²), median (IQR)
  - Baseline NRTIs: 97.4 (86.0–105.9) vs. 99.5 (90.5–106.1) 0.51
  - Baseline current regimen: 98.0 (88.7–107.0) vs. 99.5 (85.7–109.4) 0.92
  - Sample visit: 90.7 (82.9–99.0) vs. 89.8 (79.9–100.0) 0.98
  - Change sample visit–baseline NRTIs: –8.8 (–14.3 to –2.0) vs. –8.0 (–17.5 to –4.4) 0.90
  - CRP <3 mg/L, n (%): 14 (70) vs. 14 (70) 1
  - NGAL (ng/mL), median (IQR): 74.9 (67.2–93.3) vs. 66.0 (60.0–72.8) 0.032

**Urine**
- Calcium (mmol/L), median (IQR): 3.3 (1.9–4.1) vs. 1.8 (1.1–2.9) 0.057
- Potassium (mmol/L), median (IQR): 71.5 (53.0–89.5) vs. 67.5 (37.5–85.5) 0.44
- Sodium (mmol/L), median (IQR): 85.0 (73.5–128.5) vs. 91.5 (68.0–121.0) 0.85
- Phosphate (mmol/L), median (IQR): 13.8 (9.9–19.7) vs. 9.3 (5.4–17.0) 0.14
- Creatinine (µmol/L), median (IQR): 7.4 (5.5–14.4) vs. 7.3 (5.2–12.9) 0.76
- α1-microglobulin <5.72 mg/L, n (%): 3 (15) vs. 5 (25) 0.69
- β2-microglobulin <0.2 mg/L, n (%): 17 (85) vs. 12 (60) 0.16
- NGAL (ng/mL), median (IQR): 36.1 (12.5–64.7) vs. 12.8 (8.6–18.2) 0.017
- NGAL/creatinine ratio, median (IQR): 35.0 (12.9–80.4) vs. 14.4 (9.0–28.7) 0.049
- P3NP (ng/mL), median (IQR): 17.4 (15.3–23.9) vs. 27.5 (10.7–76.5) 0.47
- P3NP/creatinine ratio, median (IQR): 24.6 (18.1–27.6) vs. 26.8 (18.6–47.6) 0.47

**Discussion**

In our study, plasma and urinary NGAL concentrations remained in the normal range in this long-term virologically controlled population without any overt renal disease. As several studies have shown that tenofovir was associated with impairment of renal function, with an incidence of 15–50% depending on the definition and markers used, we would have expected an abnormal urinary NGAL concentration in the group of patients on tenofovir/emtricitabine. There is an emerging literature suggesting that urinary NGAL could be a marker of chronic kidney disease, and in this population with chronic kidney disease urine NGAL concentrations are elevated and significantly correlated with increased serum creatinine, decreased eGFR and proteinuria. Several hypotheses could explain the absence of increased levels of NGAL both in the blood and in the urine in our study. Subjects with a history of renal impairment or with potential confounders that could have an independent effect on kidney function, such as non-Caucasian subjects, comorbidities, i.e. high blood pressure, pre-exposure to abacavir and tenofovir were excluded. Thus, the included population was at the lowest risk of renal impairment. In our cohort tenofovir was combined with nevirapine, a non-nucleoside inhibitor; a greater risk of renal toxicity was observed when tenofovir was combined with a boosted protease inhibitor rather than a non-nucleoside inhibitor. NGAL has been implicated in various inflammatory and infectious conditions. In a study comparing HIV-infected patients and healthy controls, serum NGAL levels were decreased in HIV patients before antiretroviral therapy initiation, and returned to normal in virological responders to antiretroviral therapy despite the persistence of elevated inflammatory markers. These findings suggest that dysregulated NGAL levels could characterize HIV populations. However, other studies have evaluated NGAL in HIV-infected populations with discordant results. In a recent study, urinary NGAL provided the best non-invasive clinical marker of HIV-associated nephropathy and could be used to distinguish it from other proteinuric glomerulopathies in the HIV-infected patient. In a study of HIV-infected women receiving or not receiving tenofovir, three urinary biomarkers of tubular impairment, including NGAL and β2-microglobulin, were measured; an increase in β2-microglobulin, but not NGAL, was observed, probably related to a baseline chronic
kidney disease rather than to the nephrotoxicity of tenofovir. In our study, urine NGAL levels were significantly lower in males than in females, independently of the nucleoside backbone, confirming the result of a previous study in an HIV-negative diabetic population; this could reflect an oestrogen-mediated difference in renal protein expression. In the SWAP study, a small increase in urine NGAL was detected among tenofovir-treated patients compared with abacavir-treated patients, but the study included patients on a ritonavir-boosted protease inhibitor and/or patients with comorbidities such as renal dysfunction and high blood pressure. In a recent study comparing HIV-infected patients who were either antiretroviral naive or receiving a tenofovir-based or tenofovir-free antiretroviral regimen, black ethnicity, but not tenofovir, was the only factor associated with high urinary NGAL. In summary, our results are consistent with these recent studies. Our study has some limitations, however, including the high degree of selection of the enrolled patients, their very low risk of renal toxicity, the absence of randomization and the lack of prospective assessment of urinary markers of renal dysfunction from the time of initiation of the tenofovir/emtricitabine- or abacavir/lamivudine-based regimen. Of note, baseline characteristics were not significantly different between the two groups. Large intra-individual variations have been observed in NGAL measurements. To minimize this variability, the ratio to creatinine was calculated, samples were stored at −80°C and a panel of renal biomarkers, i.e. α1-microglobulin, β2-microglobulin and P3NP, were measured, with coherent results. The low urinary NGAL concentration in patients on tenofovir may indeed reflect the absence of chronic tubular toxicity of this drug when combined with nevirapine. Assessing the role of NGAL as a marker of chronic tubular toxicity would need a prospective study in a population with a higher risk of renal injury. Interference between tenofovir renal metabolism and the urinary secretion of NGAL cannot be completely excluded and needs to be assessed. In conclusion, our study shows that, in a population of HIV-infected subjects with no risk of renal impairment receiving an antiretroviral regimen including nevirapine and tenofovir, plasma and urinary NGAL concentrations remained in the normal range despite long-term exposure to tenofovir. The usefulness of NGAL in detecting sub-clinical tubular dysfunction appears to be very limited.

Acknowledgements
We would like to thank the patients participating in this study and especially B. Herve and his wife.

Funding
This study was carried out as part of our routine work. UMR-S 1064 and B. Herve’s wife provided part of the financial support.

Transparency declarations
C. A. is a board member of Gilead and has received travel grants from Viiv Healthcare and Gilead. E. B. is a board member of Gilead and has received grants from Gilead, Viiv Healthcare and Boehringer-Ingelheim. F. R. has received research funding or honoraria from or consulted for Boehringer-Ingelheim, Gilead Sciences and Viiv Healthcare. All other authors: none to declare.

Author contributions
C. A., K. B.-N., F. F., F. R., V. R. and E. B. participated in the design of the study and contributed to the acquisition and interpretation of data. K. B.-N. and T. D. performed the analysis. S. S. analysed the data, C. A., F. F. and F. R. drafted and wrote the article and all of the authors approved the final submitted version.

References

