

# Long-Term Renal Safety of Tenofovir Disoproxil Fumarate in Vertically HIV-Infected Children, Adolescents and Young Adults

## A 60-Month Follow-Up Study

Alessandra Viganò,<sup>1</sup> Giorgio Bedogni,<sup>2</sup> Valeria Manfredini,<sup>1</sup> Vania Giacometti,<sup>1</sup> Chiara Cerini,<sup>1</sup> Francesca di Nello,<sup>1</sup> Francesca Penagini,<sup>1</sup> Cristiana Caprio<sup>1</sup> and Gian Vincenzo Zuccotti<sup>1</sup>

1 Department of Paediatrics, "L. Sacco" Hospital, University of Milan, Milan, Italy

2 Clinical Epidemiology Unit, Liver Research Centre, Basovizza, Trieste, Italy

### Abstract

**Background and Objective:** Sporadic cases of renal toxicity have been reported in HIV-infected children treated with tenofovir disoproxil fumarate (TDF). We assessed the long-term renal safety of TDF in a cohort of vertically HIV-infected children, adolescents and young adults.

**Methods:** We evaluated 26 HIV-infected children, adolescents and young adults, aged 4.9–17.4 years at baseline, every 6 months for 60 consecutive months. At the baseline visit, they had an undetectable viral load and a good immune reconstitution and were being treated with lamivudine, stavudine and a protease inhibitor (PI). At the same visit, stavudine was replaced with TDF and the PI with efavirenz. Serum creatinine, estimated glomerular filtration rate (GFR), urine protein to creatinine ratio, serum phosphate, ratio of the maximum rate of tubular phosphate reabsorption to the GFR ( $\text{TmPO}_4/\text{GFR}$ ), urine glucose, and urine  $\alpha_1$ -microglobulin to creatinine ratio were used as markers of renal function. The outcome-time relationships were studied using generalized estimating equations (GEEs). In addition to time (continuous, ten equally spaced intervals), sex, age at baseline and CD4+ T-cell count were used as covariates.

**Results:** A moderate reduction in GFR was observed only once in an underweight female patient. There was no occurrence of proteinuria, hypophosphataemia or glycosuria. Moreover,  $\text{TmPO}_4/\text{GFR}$  was stable and the urine  $\alpha_1$ -microglobulin to creatinine ratio was always within normal limits.

**Conclusion:** TDF had an excellent renal safety profile in HIV-infected children, adolescents and young adults regularly followed up for 60 months.

## Introduction

With the advent of combination antiretroviral therapy (CART), there has been a substantial decline in the morbidity and mortality associated with HIV infection.<sup>[1]</sup> However, HIV-infected patients are exposed to a variety of adverse effects from long-term use of antiretroviral medications, including renal toxicity.<sup>[2]</sup> Tenofovir disoproxil fumarate (TDF) is a nucleoside analogue of proven efficacy and safety, the use of which has been sporadically associated with kidney tubular dysfunction and bone disease.<sup>[3]</sup> A recent meta-analysis showed that, although TDF use is associated with a statistically significant loss of renal function, the clinical magnitude of this effect is modest and is counteracted by the positive effects of the drug.<sup>[4]</sup> Renal tubular dysfunction has been observed mainly in animals treated with doses of TDF higher than those employed in humans.<sup>[5]</sup> Sporadic cases of osteomalacia have been reported in patients treated with TDF.<sup>[6-8]</sup> However, a recent review<sup>[4]</sup> concluded that the incidence of bone fractures is similar with TDF-containing CART and other CARTs.<sup>[9,10]</sup> In addition, the longitudinal changes in bone mineral density are similar for TDF-containing CART and other CARTs.<sup>[10-13]</sup>

Two longitudinal studies have reported no evidence of TDF-related renal dysfunction in children,<sup>[14,15]</sup> but a more recent study<sup>[16]</sup> reported a worsening of tubular function attributable to TDF. In order to better evaluate the long-term renal safety of TDF, we expanded our previous 24-month follow-up<sup>[15]</sup> study to 60 months.

## Patients and Methods

### Study Design

We extended the follow-up of a previous cohort study from 24 to 60 months.<sup>[15]</sup> Twenty-seven patients treated with lamivudine/stavudine/protease inhibitor (PI) were switched to lamivudine/TDF/efavirenz and followed every 6 months for 60 months. One patient was lost to follow-up after 15 months<sup>[15]</sup> and only 26 patients were included in the present analysis. TDF was ad-

ministered once daily on the basis of body surface area: 150 mg for 0.50–0.84 m<sup>2</sup>, 225 mg for 0.85–1.29 m<sup>2</sup> and 300 mg for  $\geq 1.30$  m<sup>2</sup>. The pharmacy service of our hospital provided capsules split into subunits to make up the prescribed dose. Written informed consent was obtained from the parents or legal guardians of the patients and the study was approved by the Ethics Committee of the “L. Sacco” Hospital (Milan, Italy).

### Anthropometric Assessment

Weight was measured to the nearest 0.1 kg using a beam scale (Seca GmbH & Co. KG, Hamburg, Germany) and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Ltd, Crosswell, UK). Body mass index (BMI) was calculated as weight (kg)/height (m)<sup>2</sup>. Z-scores (also known as standard deviation scores) for anthropometric measurements were calculated using Italian reference data.<sup>[17]</sup>

### Assessment of Renal Function

Glomerular function was assessed by means of serum creatinine, estimated glomerular filtration rate (GFR) and urine protein to creatinine ratio. Increases in serum creatinine were classified as grade 1 ( $\geq 0.5$ –2.0 mg/dL vs baseline), grade 2 (2.1–3.0 mg/dL vs baseline) or grade 3 (3.1–6.0 mg/dL vs baseline).<sup>[18]</sup> GFR was estimated using Schwartz's redux equation in subjects aged <18 years and using the Modification of Diet in Renal Disease (MDRD) equation in subjects aged  $\geq 18$  years.<sup>[19,20]</sup> Renal failure was diagnosed when the estimated GFR was <60 mL/min.<sup>[19]</sup> Proteinuria was diagnosed in the presence of a urine protein to creatinine ratio  $\geq 0.2$ .<sup>[15]</sup> Tubular function was assessed by means of serum phosphate, the ratio of the maximum rate of tubular phosphate reabsorption to the GFR (TmPO<sub>4</sub>/GFR), urine glucose, and urine  $\alpha_1$ -microglobulin to creatinine ratio. Hypophosphataemia was diagnosed according to the Division of AIDS guidelines.<sup>[21]</sup> TmPO<sub>4</sub>/GFR was estimated from a random urine specimen taken simultaneously with a blood sample.<sup>[22]</sup> Glycosuria was defined as a value of urine glucose  $\geq 50$  mg/dL.<sup>[16]</sup>

### Statistical Analysis

Continuous variables are reported as median and interquartile range (IQR) because most had skewed distributions. IQR was calculated as the difference between the 75th and 25th percentiles. Categorical variables are given as the number or percentage of subjects with the characteristic of interest. Generalized estimating equations (GEEs) were used to evaluate the changes in weight (continuous, Z-score), height (continuous, Z-score), BMI (continuous, Z-score), GFR (continuous, mL/min/m<sup>2</sup>) and TmPO<sub>4</sub>/GFR (continuous, mg/dL) during the study. GEEs provide a population-average or marginal model, i.e. they quantify how much the average response would change across the population for every one-unit increase in a predictor and are robust to data missing at random (MAR).<sup>[23]</sup> When used as a dependent variable, the square root of the CD4+ T-cell count was used to achieve a normal distribution while the other outcomes of interest for GEE analysis were already normally distributed (Shapiro-Wilk test). In addition to time (continuous, ten equally-spaced intervals of 6 months), age at baseline (continuous, years), sex (dichotomous, male vs female) and CD4+ T-cell count (continuous, cells/mm<sup>3</sup>) were used as covariates. The within-subject correlation matrix of GEE was set as exchangeable and semi-robust confidence intervals (CIs) were calculated. Multivariable fractional polynomials were used to test for the existence of non-linear relationships of outcomes with time and other continuous covariates.<sup>[24]</sup> Statistical significance was set to a p-value <0.05 and all tests were two-tailed. Statistical analysis was performed using Stata 11.1 (Stata Corp, College Station, TX, USA).

### Results

Twenty-six HIV-infected children, adolescents and young adults treated with a CART including TDF were followed every 6 months for 60 consecutive months. Only two patients missed a follow-up visit, one at month 24 and the other at month 48. Missing data for the variables of interest varied from 1% to 16%, were intermittent,

and after inspection of clinical charts were assumed to be MAR and handled by GEEs. The anthropometric and clinical characteristics of the patients included at baseline and at the ten follow-up visits are reported in table I.

At baseline, the age of the study population ranged from 4.9 to 17.4 years, with a median (IQR) of 13.0 (6.7) years. Females made up 58% of the study population (n=15). HIV-RNA levels were undetectable (<50 copies/mL) in all subjects for the entire duration of the follow-up period.

As determined by GEEs, the square-root transformed CD4+ T-cell count did not change with time and was not associated with either baseline age or BMI (coefficients not shown). Likewise, the Z-scores for weight, height and BMI were stable and were also not associated with either baseline age or BMI (coefficients not shown). This is in agreement with the results of other long-term studies performed at our clinic.<sup>[25]</sup>

When GEEs were used to study the GFR-time relationship, a modest but statistically significant increase in median GFR with time was detected (0.25 mL/min/m<sup>2</sup> [95% CI 0.06, 0.45] every 6 months; p=0.01) but there were no significant associations between GFR and baseline age, sex or CD4+ T-cell count (coefficients not shown) [figure 1]. A clinically relevant reduction in GFR to 42 mL/min/m<sup>2</sup> (compared with slightly above 60 mL/min/m<sup>2</sup> at baseline) and a clinically relevant increase in serum creatinine to 1.58 mg/dL (compared with 0.9 mg/dL at baseline, i.e. a grade I increase) was detected on only one occasion (month 42) in an undernourished 19-year-old female patient (BMI Z-score -1.8). The patient continued on the same therapy and had a spontaneous return of GFR to baseline values (59 mg/dL at month 48, 75 mg/dL at month 54 and 68 mg/dL at month 60).

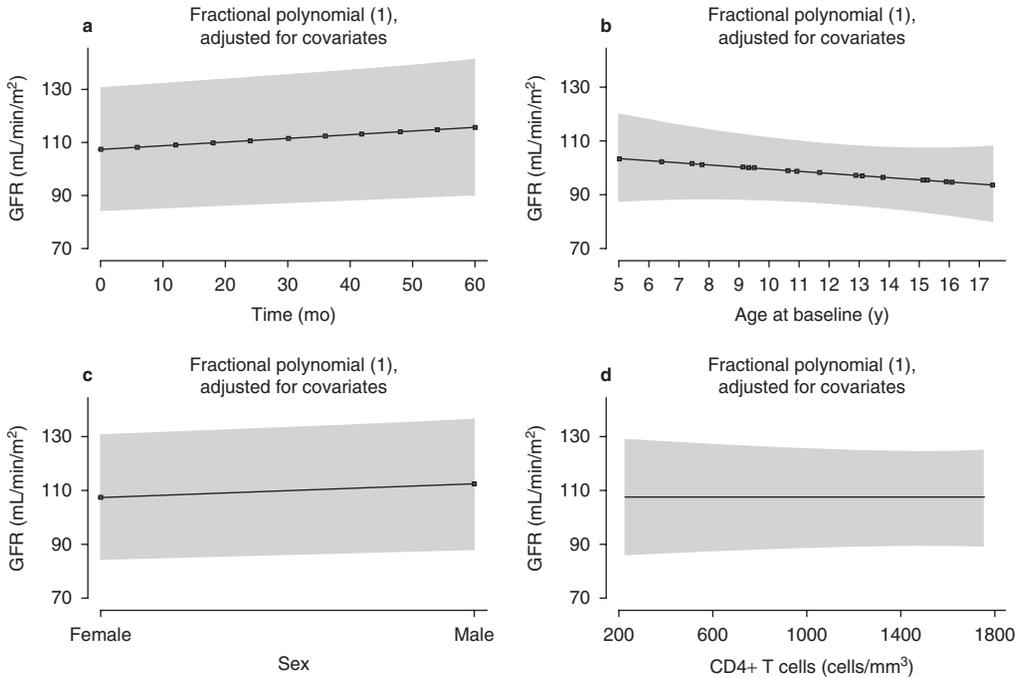
Moreover, none of the patients had hypophosphataemia and TmPO<sub>4</sub>/GFR was stable (figure 2). TmPO<sub>4</sub>/GFR was significantly associated with baseline age and CD4+ T-cell count but not sex (coefficients not shown), but these associations explained only a very modest portion of its variation over time. The relationship between TmPO<sub>4</sub>/GFR and baseline age was

**Table I.** Demographic details of the study population at baseline and at the ten follow-up visits

Variable	Follow-up visit (mo)																					
	0		6		12		18		24		30		36		42		48		54		60	
	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M	I
Age (y)	13.0	6.7	13.5	6.7	14.0	6.7	14.5	6.6	15.2	6.5	15.5	6.5	16.1	6.6	16.6	6.6	17.1	6.6	17.4	6.6	18.0	6.6
Weight (kg)	45.5	30.8	47.0	30.8	49.5	28.5	49.2	29.4	51.0	28.2	48.7	24.3	49.7	23.8	52.0	20.6	53.0	14.2	52.0	17.0	51.9	13.8
Weight (Z-score <sup>a</sup> )	-0.10	1.39	-0.19	1.39	-0.24	1.65	-0.17	1.60	-0.31	1.57	-0.25	1.68	-0.30	1.52	-0.47	1.59	-0.40	1.34	-0.45	1.39	-0.58	1.11
Height (m)	1.54	0.37	1.55	0.34	1.55	0.32	1.56	0.28	1.58	0.25	1.58	0.22	1.58	0.18	1.60	0.19	1.62	0.14	1.62	0.15	1.63	0.13
Height (Z-score <sup>a</sup> )	-0.02	1.10	-0.01	1.32	-0.10	1.38	-0.13	1.04	-0.24	1.37	-0.20	1.55	-0.11	1.50	-0.62	1.54	-0.07	1.60	-0.08	1.40	-0.05	1.21
BMI (kg/m <sup>2</sup> )	18.3	4.7	17.9	5.4	18.3	4.8	18.2	4.4	19.0	4.8	19.1	4.2	19.5	4.8	20.1	3.1	20.5	2.7	20.0	3.2	20.2	2.6
BMI (Z-score <sup>a</sup> )	-0.36	1.36	-0.31	1.31	-0.29	1.52	-0.27	1.47	-0.14	1.60	-0.16	1.64	-0.12	1.67	-0.02	1.26	-0.19	1.09	-0.26	1.57	-0.30	0.90
CD4+ T-cells (cells/mm <sup>3</sup> )	802	378	719	327	765	320	792	548	840	453	850	421	801	360	714	335	731	551	660	327	750	315
CD4+ T-cells (%)	35	9	37	9	35	6	35	9	38	9	36	12	36	10	36	11	36	13	36	9	37	8
Creatinine (mg/dL)	0.5	0.2	0.7	0.3	0.7	0.3	0.7	0.3	0.7	0.3	0.6	0.3	0.7	0.2	0.7	0.2	0.7	0.2	0.7	0.3	0.7	0.2
GFR (mL/min/m <sup>2</sup> )	100	15	98	23	99	20	99	13	104	22	109	29	104	39	104	37	102	29	110	29	103	27
Urine protein to creatinine ratio	0.10	0.09	0.10	0.07	0.09	0.12	0.00	0.00	0.13	0.07	0.12	0.10	0.10	0.05	0.10	0.09	0.11	0.06	0.10	0.11	0.11	0.09
$\alpha_1$ -microglobulin to creatinine ratio	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Serum phosphate (mg/dL)	4.6	0.8	4.7	1.0	4.6	1.2	4.3	1.3	4.4	1.3	4.3	1.2	4.0	1.3	4.4	0.9	4.2	1.0	3.9	1.0	4.0	1.2
Urine phosphate (mg/dL)	48.7	43.7	40.6	41.4	55.6	41.2	65.8	51.5	83.5	32.5	59.0	54.0	73.5	60.0	66.0	45.5	92.0	88.0	73.0	46.0	84.5	68.0
TmPO <sub>4</sub> /GFR (mg/dL)	4.4	0.9	4.4	1.0	4.1	1.3	4.0	1.5	3.8	1.3	3.8	1.1	3.7	1.2	4.1	1.1	3.8	0.9	3.7	1.0	3.5	1.3
Urine glucose (mg/dL)	2.5	19.0	3.0	5.0	6.0	16.0	20.0	0.0	20.0	0.0	20.0	0.0	20.0	12.0	5.5	9.0	7.0	4.0	5.5	7.0	7.0	5.0

<sup>a</sup> Also known as standard deviation score.

**BMI** = body mass index; **GFR** = glomerular filtration rate; **I** = interquartile range (difference between the 75th and 25th percentiles); **M** = median; **TmPO<sub>4</sub>** = maximum tubular phosphate reabsorption.



**Fig. 1.** First degree fractional polynomials for the changes in median glomerular filtration rate (GFR) over time (a) and effects of baseline age (b), sex (c) and baseline CD4+ T-cell count (d) on GFR. The values in parenthesis above each figure represent the power of the fractional polynomial; power 1 symbolizes a linear relationship. The grey bands are 95% confidence intervals.

expected from prior studies.<sup>[26]</sup> The urine protein to creatinine ratio remained within normal limits for the entire duration of the follow-up period in all patients. Lastly, the urine  $\alpha_1$ -microglobulin to creatinine ratio and glycosuria also remained within normal limits over the entire study.

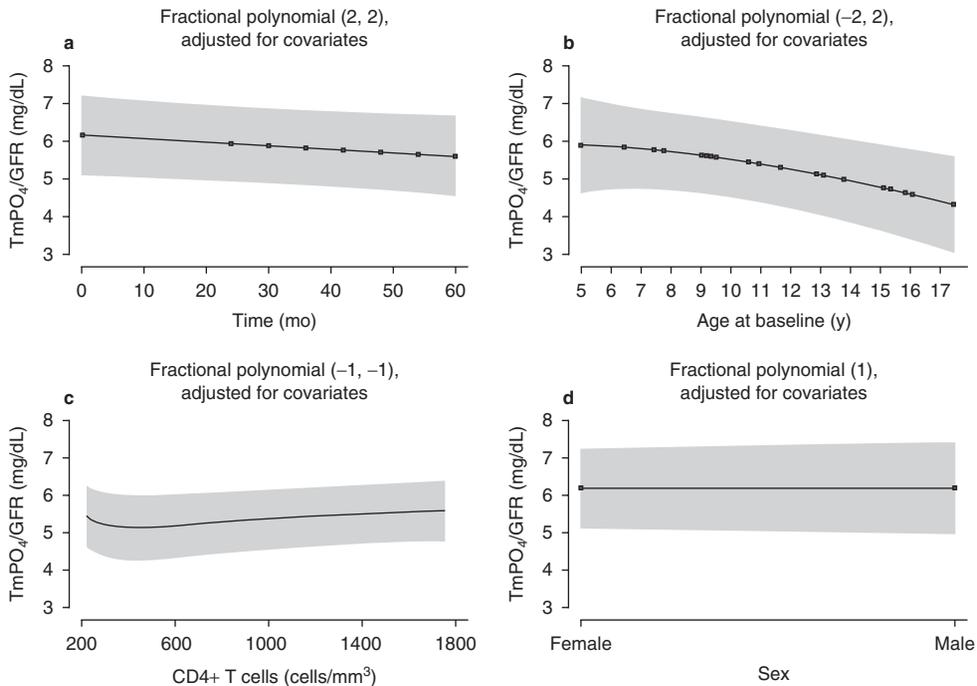
**Discussion**

We extended an earlier 24-month study<sup>[15]</sup> by evaluating the 60-month renal safety of TDF in 26 HIV-infected children, adolescents and young adults who switched from a CART based on lamivudine, stavudine and one PI to one in which the PI was replaced by efavirenz and stavudine by TDF. As before,<sup>[15]</sup> we found no evidence of renal toxicity except for a single decrease in GFR in a female patient whose GFR spontaneously returned to baseline values.

Although our study is one of the longest performed to date, it is not without limitations. First, our cohort of patients was small (n = 26), as is to

be expected from a single-centre study. However, the power to model the outcome-time relationships was substantial owing to the large number of time points at which evaluations took place. Moreover, only two patients missed a follow-up visit and the frequency and pattern of missing visits allowed us to analyse missing data under the assumption of MAR. Second, our patients were not being treated with drugs with known nephrotoxic effects such as lopinavir/ritonavir and didanosine, which meant that a direct comparison of our findings with those of other studies employing these drugs is not possible.<sup>[6,27-29]</sup> This is important as there is increasing evidence that these drugs predispose to TDF-related nephrotoxicity. Third, we studied only Caucasian subjects, which meant we were not able to test the contribution of ethnicity to TDF-associated toxicity.<sup>[30]</sup>

Sporadic cases of reduced GFR, hypophosphataemia, glycosuria and Fanconi's syndrome have been reported in HIV-infected adults



**Fig. 2.** Second degree fractional polynomials for the changes in the ratio of the maximum tubular phosphate reabsorption to the glomerular filtration rate (TmPO<sub>4</sub>/GFR) over time (a) and the effects of baseline age (b) and CD4+ T-cell count (c) and first degree fractional polynomial for the effect of sex (d) on TmPO<sub>4</sub>/GFR. The values in parenthesis above each figure represent the powers of the fractional polynomial; power 1 symbolizes a linear relationship. The grey bands are 95% confidence intervals.

treated with TDF.<sup>[2]</sup> However, clinical trials comparing CARTs including TDF with CARTs not including TDF have generally shown similar renal safety profiles.<sup>[11,18,31,32]</sup> HIV-infected adults with normal or mildly compromised renal function have a very low (1%) incidence of TDF-related nephrotoxicity.<sup>[31]</sup> Hypophosphataemia and reduced GFR are likewise infrequent in studies comparing TDF with other nucleoside analogues.<sup>[27,31]</sup> TDF-associated nephrotoxicity may be triggered by pre-existing co-morbidities, older age and concomitant use of ritonavir-boosted PIs and didanosine.<sup>[33]</sup> These data have been confirmed by a recent systematic review that concluded that, although TDF use is associated with a statistically significant loss of renal function, the clinical magnitude of this effect is modest and there is no need to restrict TDF use when the regular monitoring of renal function is not feasible.<sup>[4]</sup>

Proximal tubular dysfunction and other nephrotoxic effects have been reported in HIV-

infected children treated with TDF, especially in association with didanosine and lopinavir/ritonavir.<sup>[6,27-29]</sup> A recent study detected adverse renal events in five out of 159 children exposed to TDF,<sup>[33]</sup> with four of the events occurring in patients treated with high doses of didanosine or with an overdosage of lopinavir/ritonavir or TDF. A recent 72-month cohort study has shown the persistence of at least one renal abnormality in 22% of 2102 HIV-infected children.<sup>[30]</sup> Elevated serum creatinine (18%) was more common than proteinuria (15%), and children aged  $\geq 6$  years, Blacks and Hispanics were at greater risk of renal dysfunction. Moreover, subjects treated with TDF or indinavir had a 2-fold greater risk and children aged  $\leq 12$  years treated with TDF had a 3-fold greater risk of developing renal abnormalities. As no information on TDF dosage was provided, the possibility that the increased risk of renal dysfunction might have been due to TDF overdosage cannot be excluded.

Our patients had a urine protein to creatinine ratio within normal limits for the entire duration of the follow-up period. However, this test does not detect tubular dysfunction. Urine enzymes and low-molecular-weight proteins have been proposed as early markers of tubular dysfunction. Among the proteins that escape reabsorption when proximal tubular cells are damaged,  $\alpha_1$ -microglobulin and  $\beta_2$ -microglobulin are easily quantifiable in urine specimens and their measurement is established in clinical practice.<sup>[34]</sup> A recent study reported high values of urine  $\beta_2$ -microglobulin in HIV-infected children treated for 13 months with TDF.<sup>[35]</sup> We measured  $\alpha_1$ -microglobulin in the present study because it is more stable than  $\beta_2$ -microglobulin to changes in urine pH.<sup>[36]</sup> Using this sensitive marker, we were nonetheless unable to detect any change in proximal tubular function. Urine glucose, another marker of proximal tubular function, also remained within normal limits for the duration of the study.

Hypophosphataemia is another useful marker of proximal tubular dysfunction. In a recent study of HIV-infected adults, hypophosphataemia had a similar incidence in patients treated with stavudine/lamivudine/efavirenz as in those treated with TDF/lamivudine/efavirenz.<sup>[18]</sup> Different degrees of hypophosphataemia secondary to proximal tubular damage and Fanconi's syndrome have been reported in HIV-infected adults treated with TDF.<sup>[37]</sup> The incidence of grade 2 hypophosphataemia<sup>[21]</sup> was 4% in a recent study of HIV-infected children treated with TDF and followed up for 9 years.<sup>[38]</sup> Hypophosphataemia occurred at a median of 18 months after the initiation of TDF and was generally reversible following TDF withdrawal. However, none of our patients had hypophosphataemia during the follow-up period and no significant change in  $\text{TmPO}_4/\text{GFR}$  over time was observed.

A comparison of our study with the available paediatric studies suggests at least two reasons for our very positive findings. First, as discussed above, none of our patients was receiving lopinavir/ritonavir or didanosine.<sup>[6,27-29]</sup> Second, our patients had prolonged viral suppression and good and persistent immune reconstitution. This is important in view of the fact that HIV can invade the

kidney and replicate inside the glomeruli and tubules.<sup>[2]</sup> Moreover, a recent study reported an increased GFR in HIV-infected adults treated with TDF after achievement of viral suppression.<sup>[39]</sup> On the other hand, control of viraemia was achieved in a minority of patients in most studies of TDF toxicity. Also of interest are the results of the DART (Development of Antiretroviral Therapy in Africa) trial, performed in a cohort of HIV-infected adults with low CD4+ T-cell counts whose renal function actually improved during CART.<sup>[40]</sup>

## Conclusion

In our 60-month follow-up study of vertically HIV-infected children, adolescents and young adults, TDF, while maintaining viral suppression and stable CD4+ T-cell counts, demonstrated an excellent renal safety profile. Over the entire study period, median GFR increased slightly, although statistically significantly, but no associations between GFR and baseline age, sex or CD4+ T-cell count were observed. A moderate reduction in GFR was observed only once in an underweight 19-year-old female patient. The urine protein to creatinine ratio remained within normal limits for the entire duration of the follow-up period in all patients. Proximal tubular function, as assessed by measurement of urine glucose, urine  $\alpha_1$ -microglobulin to creatinine ratio, serum phosphate and  $\text{TmPO}_4/\text{GFR}$ , also remained stable and within normal limits during the entire study period. Longer follow-up is needed to detect whether any adverse effects, including renal dysfunction, can arise from long-term use of TDF.

## Acknowledgements

The research in this article was supported by grant 40H1 from Istituto Superiore di Sanità, Rome, Italy.

The authors have no conflicts of interest that are directly relevant to the content of this study. All authors have contributed to the entire content of the article. The authors' work was independent of the funder.

## References

1. Boyd MA. Improvements in antiretroviral therapy outcomes over calendar time. *Curr Opin HIV AIDS* 2009 May; 4 (3): 194-9

2. Atta MG, Deray G, Lucas GM. Antiretroviral nephrotoxicities. *Semin Nephrol* 2008 Nov; 28 (6): 563-75
3. Rodriguez-Nóvoa S, Alvarez E, Labarga P, et al. Renal toxicity associated with tenofovir use. *Expert Opin Drug Saf* 2010 Jul; 9 (4): 545-59
4. Cooper RD, Wiebe N, Smith N, et al. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010 Sep 1; 51 (5): 496-505
5. Van Rompay KK, Brignolo LL, Meyer DJ, et al. Biological effects of short-term or prolonged administration of 9-[2-(phosphonomethoxy)propyl]adenine (tenofovir) to newborn and infant rhesus macaques. *Antimicrob Agents Chemother* 2004 May; 48 (5): 1469-87
6. Peyrière H, Reynes J, Rouanet I, et al. Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. *J Acquir Immune Defic Syndr* 2004; 35 (3): 269-73
7. Izzedine H, Isnard-Bagnis C, Hulot JS, et al. Renal safety of tenofovir in HIV treatment-experienced patients. *AIDS* 2004; 18 (7): 1074-6
8. Parsonage MJ, Wilkins EG, Snowden N, et al. The development of hypophosphataemic osteomalacia with myopathy in two patients with HIV infection receiving tenofovir therapy. *HIV Med* 2005; 6 (5): 341-6
9. Arribas JR, Pozniak AL, Gallant JE, et al. Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naïve patients: 144-week analysis. *J Acquir Immune Defic Syndr* 2008; 47 (1): 74-8
10. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients: a 3-year randomized trial. *JAMA* 2004; 292 (2): 191-201
11. Shooley RT, Ruane P, Myers RA, et al. Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. *AIDS* 2002, Jun 14; 16 (9): 1257-63
12. Moyle GJ, Sabin CA, Cartledge J, et al., RAVE (Randomized Abacavir versus Viread Evaluation) Group UK. A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipodystrophy. *AIDS* 2006; 20 (16): 2043-50
13. Martínez E, Arranz JA, Podzamczar D, et al. A simplification trial switching from nucleoside reverse transcriptase inhibitors to once-daily fixed-dose abacavir/lamivudine or tenofovir/emtricitabine in HIV-1-infected patients with virological suppression. *J Acquir Immune Defic Syndr* 2009; 51 (3): 290-7
14. Hazra R, Gafni RI, Maldarelli F, et al. Tenofovir disoproxil fumarate and an optimized background regimen of antiretroviral agents as salvage therapy for pediatric HIV infection. *Pediatrics* 2005 Dec; 116 (6): e846-54
15. Viganò A, Zuccotti GV, Martelli L, et al. Renal safety of tenofovir in HIV-infected children: a prospective, 96-week longitudinal study. *Clin Drug Investig* 2007; 27 (8): 573-81
16. Soler-Palacín P, Melendo S, Noguera-Julian A, et al. Prospective study of renal function in HIV-infected pediatric patients receiving tenofovir-containing HAART regimens. *AIDS* 2011; 25 (2): 171-6
17. Cacciari E, Milani S, Balsamo A, et al. Italian cross-sectional growth charts for height, weight and BMI (6-20 y). *Eur J Clin Nutr* 2002 Feb; 56 (2): 171-80
18. Izzedine H, Hulot JS, Vittecoq D, et al. Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naïve HIV-1-infected patients: data from a double-blind randomized active-controlled multicentre study. *Nephrol Dial Transplant* 2005 Apr; 20 (4): 743-6
19. Hogg RJ, Furth S, Lemley KV, et al. National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. *Pediatrics* 2003 Jun; 111 (6 Pt 1): 1416-21
20. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009 Mar; 20 (3): 629-37
21. Division of AIDS. Division of AIDS table for grading the severity of adult and pediatric adverse events. Bethesda (MD): NIH, 2005
22. Alon U, Hellerstein S. Assessment and interpretation of the tubular threshold for phosphate in infants and children. *Pediatr Nephrol* 1994 Apr; 8 (2): 250-1
23. Hardin JW, Hilbe J. Generalized estimating equations. Boca Raton (FL): Chapman & Hall, 2003
24. Royston P, Sauerbrei W. Multivariable model-building: a pragmatic approach to regression analysis based on fractional polynomials for modelling continuous variables. Chichester: John Wiley, 2008
25. Viganò A, Brambilla P, Pattarino G, et al. Long-term evaluation of glucose homeostasis in a cohort of HAART-treated HIV-infected children: a longitudinal, observational cohort study. *Clin Drug Investig* 2009; 29 (2): 101-9
26. Kruse K, Kracht U, Göpfert G. Renal threshold phosphate concentration (TmPO<sub>4</sub>/GFR). *Arch Dis Child* 1982 Mar; 57 (3): 217-23
27. Zimmermann AE, Pizzoferrato T, Bedford J, et al. Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions. *Clin Infect Dis* 2006 Jan 15; 42 (2): 283-90
28. Hawkins S, Ball C. Adverse events experienced by three children taking tenofovir and didanosine in combination. *HIV Med* 2007 Sep; 8 (6): 411
29. Hussain S, Khayat A, Tolaymat A, et al. Nephrotoxicity in a child with perinatal HIV on tenofovir, didanosine and lopinavir/ritonavir. *Pediatr Nephrol* 2006 Jul; 21 (7): 1034-6
30. Andiman WA, Chernoff MC, Mitchell C, et al. Incidence of persistent renal dysfunction in human immunodeficiency virus-infected children: associations with the use of antiretrovirals, and other nephrotoxic medications and risk factors. *Pediatr Infect Dis J* 2009 Jul; 28 (7): 619-25
31. Gallant JE, Winston JA, DeJesus E, et al. The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naïve patients. *AIDS* 2008 Oct 18; 22 (16): 2155-63
32. Squires K, Pozniak AL, Pierone G, et al. Tenofovir disoproxil fumarate in nucleoside-resistant HIV-1 infection: a randomized trial. *Ann Intern Med* 2003 Sep 2; 139 (5 Pt 1): 313-20
33. Riordan A, Judd A, Boyd K, et al. Tenofovir use in human immunodeficiency virus-1-infected children in the United

- Kingdom and Ireland. *Pediatr Infect Dis J* 2009 Mar; 28 (3): 204-9
34. D'Amico G, Bazzi C. Urinary protein and enzyme excretion as markers of tubular damage. *Curr Opin Nephrol Hypertens* 2003 Nov; 12 (6): 639-43
35. Papaleo A, Warszawski J, Salomon R, et al. Increased beta-2 microglobulinuria in human immunodeficiency virus-1-infected children and adolescents treated with tenofovir. *Pediatr Infect Dis J* 2007 Oct; 26 (10): 949-51
36. Davey PG, Gosling P. Beta 2-microglobulin instability in pathological urine. *Clin Chem* 1982 Jun; 28 (6): 1330-3
37. Labarga P, Barreiro P, Martin-Carbonero L, et al. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS* 2009 Mar 27; 23 (6): 689-96
38. Judd A, Boyd KL, Stöhr W, 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 Feb 20; 24 (4): 525-34
39. Guaraldi G, Roverato A, Giovanardi C, et al. Glomerular filtration rates in HIV-infected patients treated with and without tenofovir: a prospective, observational study. *J Antimicrob Chemother* 2009 Feb; 63 (2): 374-9
40. Reid A, Stöhr W, Walker AS, et al. Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. *Clin Infect Dis* 2008 Apr 15; 46 (8): 1271-81
- 
- Correspondence: Dr *Alessandra Viganò*, Paediatric Clinic, "L. Sacco" Hospital, University of Milan, Via G.B. Grassi 74, 20100, Milan, Italy.  
E-mail: [alessandra.vigano@unimi.it](mailto:alessandra.vigano@unimi.it)