

Impact of HIV-1 tropism on the emergence of non-AIDS events in HIV-infected patients receiving fully suppressive antiretroviral therapy

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Objective: The impact of HIV-1 tropism on the emergence of non-AIDS events was evaluated in a cohort of 116 antiretroviral therapy (ART) responder patients.

Methods: The patients were followed for the emergence of hypertension, renal impairment, metabolic and bone disorders (defined as non-AIDS events) each 8 weeks at standard visits. A V3 plasma sequence genotype analysis was performed at the time of ART initiation and the geno2pheno algorithm with the results that defines the false-positive rate (FPR) was used to infer HIV tropism. The associations between the non-AIDS events and the FPR at baseline were evaluated using the χ^2 test for trend. A Cox-regression analysis using the counting process formulation of Andersen and Gill was performed to define whether the emergence of non-AIDS events was correlated to FPR.

Results: The prevalence of at least one non-AIDS event resulted higher in patients with a FPR below 10% than in patients with a R5 virus ($P=0.033$). Patients with a FPR below 5.0% most frequently developed non-AIDS events during ART ($P=0.01$). A higher prevalence of patients with at least two AIDS events was found in the group of patients with a FPR below 5.0% with respect to the others ($P<0.001$). At multivariate Cox-regression analysis, having an X4 virus and age were independently associated with a higher probability of non-AIDS event development.

Conclusion: This study shows that an X4 virus, particularly a FPR less than 5%, is related to non-AIDS events development. Further studies are warranted to understand the mechanisms underlying this phenomenon.

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Introduction

The introduction of antiretroviral therapy (ART) has dramatically modified the natural course of HIV infection, increasing life expectancy and consequently the proportion of patients ageing with HIV [1]. Hence,

age-related complications currently represent one of the most challenging concerns in addressing the management of HIV-positive patients [2–4]. Cardiovascular disease, hypertension, renal and liver pathologies and non-AIDS malignancies are now collectively considered serious non-AIDS events or diseases [5]. HIV-infected patients

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undergoing ART have been shown to be at increased risk of developing non-AIDS events, compared with people ageing without HIV infection [6–8].

In the context of an older HIV-infected population, a deeper knowledge of the factors able to accelerate and worsen ageing is warranted in order to improve both quality of life and life expectancy.

The factors related to premature ageing in HIV-infected patients are not fully understood, even if there is some evidence of the pathogenetic role of the virus itself. HIV directly affects the body along with chronic immune activation and ART toxicity, which are responsible for metabolic disorders, including diabetes mellitus, dyslipidemia and bone alterations, such as osteoporosis and osteopenia [9,10].

The chemokine receptors CXCR4 (X4) and CCR5 (R5), used to enter target cells, play a pivotal role in HIV transmission, pathogenesis and disease progression. During the early phase of HIV-infection, R5-binding viruses are more frequently involved, whereas later in the course of the disease, viral variants expressing affinity for the X4 co-receptor may be selected [11,12]. The emergence of X4 variants has been shown to be associated with a faster decline in the number of peripheral blood CD4⁺ lymphocytes, more rapid progression of the disease and a poorer prognosis for survival [13–16]. Moreover, a significantly greater decrease in CD4⁺ count and a higher number of clinical events, such as AIDS-defining illnesses or death occurring after ART initiation, were described among patients with an X4 or dual mixed X4/R5 viral tropism, compared with those harboring an R5-binding variant [17].

Although there are data that demonstrate how latent viral diversity characteristics are unlikely to be a major driver of persistent HIV-associated immune activation and ageing [18,19], until today, there have been no clinical studies that directly correlate the HIV-1 tropism isolate at the beginning of ART with the onset of non-AIDS events during treatment.

This study aimed to evaluate the impact of HIV-1 tropism on the emergence of non-AIDS events after ART initiation in a cohort of 116 patients as their first-line regimen and as full responders to ART.

Methods

One hundred and sixteen HIV-1 drug-naïve infected patients attending the Clinic of Infectious Disease of Tor Vergata University of Rome, Italy, were enrolled in the study. All patients began first-line antiretroviral treatment between 2008 and 2013, and a V3 plasma sequence was

available at the time of ART initiation for each patient. All patients received an effective ART (viral load <50/ml) and were considered for the emergence of at least one non-AIDS event during the follow-up period of their first antiretroviral treatment. To avoid any influence of active viral replication on the emergence of non-AIDS events, we enrolled only patients fully responder to ART.

The following diseases were considered non-AIDS events: hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or both, on repeated examination), metabolic disorder (high blood sugar level, excess body fat around the waist, abnormal blood cholesterol and triglyceride levels), bone disorders [osteopenia, considered as a T-score of -1 to -2.5 standard deviations with dual-energy x-ray absorptiometry (DXA), osteoporosis, considered as a T-score of less than -2.5 SD], renal impairment (confirmed glomerular filtration rate decline of $>25\%$ from baseline and to a level <60 ml/min per 1.73 m², and/or a repeated proteinuria >200 mg/day). All patients were evaluated each 8 weeks for hypertension, metabolic disorder and renal impairment. DXA evaluation was performed for each patient every 12 months. No patients developed cancer or myocardial infarction during the observation period therefore the analysis was limited to the above reported non-AIDS events. Data were extracted from a database specifically created for the collection of clinical and laboratory follow-up data of patients for the entire period of clinical observation. In particular, demographic data, risk factors for HIV acquisition, Center for Diseases Control (CDC) staging (revised 1993), blood biochemical parameters, CD4⁺ cell count, HIV tropism isolate characteristics were evaluated before the beginning of ART and plasma viral load, clinical and therapeutic history and timing of non-AIDS events were evaluated after the beginning of ART.

No specific ethics committee's consent was required due to the retrospective characteristic of the study based on information available from existing clinical documentation [Determination of the Italian Agency of Drug (AIFA) of 20 March 2008]. With respect to the privacy, all personal information was treated in a confidential manner and all clinical data were analyzed anonymously.

Determination of HIV-1 genotypic tropism testing

V3 genotype analyses were performed as previously described [20]. Briefly, HIV-1 co-receptor usage was inferred from the V3 nucleotide sequences by using the geno2pheno algorithm, set at false-positive rate (FPR) of 10%, as recommended by current guidelines [21,22], available at the following website: <http://coreceptor.bioinf.mpi-sb.mpg.de/cgi-bin/coreceptor.pl>. The system uses a support vector machine trained with a set of genotypic sequences with corresponding R5 or DM/X4 phenotypes. The tool is based on nucleotide

sequences and therefore also analyses subsequent amino-acid mixtures. The result of the interpretation is given as a quantitative value, the FPR that defines the probability of classifying an R5-virus falsely as X4.

To evaluate the impact of the burden of HIV-1 CXCR4-using species on the number and type of non-AIDS-related events, FPR values were further stratified according to the following five FPR (%) ranges: for X4 viruses, not more than five and 5–10; for R5 viruses, 10–20, 20–60, and above 60 [23,24]. In this categorization, all five ranges are left-open and right-closed (e.g. ≤ 5 ; > 5 and ≤ 10 ; > 10 and ≤ 20 ; > 20 and ≤ 60 ; and > 60).

Statistical analysis

Potential associations between the number and type of non-AIDS events that occurred during first-line ART and the FPR of V3 sequence at baseline were evaluated using the χ^2 test for trend ($P < 0.05$), after stratifying the FPR for the five categories mentioned above. Moreover, a χ^2 test was used to compare categorical variables in patients infected with viruses characterized by a FPR below 5% and 5–10% vs. patients infected with viruses characterized by a FPR above 60%. Associations between continuous variables, such as viral load, CD4⁺ cell count, age, year of diagnosis, and HIV-1 tropism, were evaluated using the Mann–Whitney test.

A uni- and multivariate Cox-regression analysis using the counting process formulation of Andersen and Gill was also performed to define whether the emergence of non-AIDS events during treatment was correlated to the baseline presence of an X4 virus (setting the FPR at 10%) and whether this association increased by decreasing the FPR. The following variables were considered for this analysis: patient's demographics, year of diagnosis, HIV-1 subtype defined on pol sequences, zenith viremia and nadir CD4⁺ cell count, CD4⁺ cell count at non-AIDS event diagnosis, years under treatment, presence of virological blips (defined as viral load detection ≥ 50 and < 1000 copies/ml), number of visits per year of ART, and first-line antiretroviral composition.

Results

Patients' characteristics

The characteristics of the study population at the time of comorbidities diagnosis are shown in Table 1. Among the 116 included patients, the majority were male (66.4%), with a median age of 45 years, heterosexual orientation and infection by HIV-1 subtype B. Only one-third of the patients were in an advanced phase (class CDC C) of infection. All patients began first-line ART and were treated for a median time of 3 years. The prevalent ART regimens included the FTC+TDF (emtricitabine +

tenofovir) combined with a protease inhibitor or non-nucleoside reverse transcriptase inhibitor (NNRTI) (56 and 25%, respectively), followed by ABC+3TC (abacavir + lamivudine) combined with a protease inhibitor (11.2%).

Virological success was reached in a median time of 16 weeks (range 8–48 weeks), and all patients maintained virological suppression during the entire observation period [median (interquartile, IQR): 3.0 years (2.0–4.0)]. Virological blips (defined as a viral load of ≥ 50 and < 1000 copies/ml, preceded and followed by undetectable values) were observed in 19.8% of patients.

During a follow-up period under effective ART, overall, 72 patients (62.1%) developed non-AIDS events: 44 patients developed one event, while 28 patients experienced more than one non-AIDS event. Among the 116 patients studied, hypertension and metabolic disorders were observed in 20 and 31 patients, respectively, kidney diseases in 37 patients, and osteoporosis in 25 patients.

By evaluating the genotypic tropism at baseline of the first antiretroviral regimen by geno2pheno algorithm set at 10%, we found that 32 out of 116 (27.6%) patients carried an X4 tropic virus, and 84 (72.4%) carried an R5 tropic virus. By further stratifying patients for the five FPR (%) ranges, 17 (14.6%) carried an X4 virus with a FPR below 5%, 15 (12.9%) carried an X4 virus with a FPR 5–10%, 12 (10.3%) carried an R5 virus with a FPR 10–20%, 37 (33.9%) carried an R5 virus with a FPR 20–60%, and 35 (30.2%) carried an R5 virus with a FPR above 60% (Table 1).

Patient characteristics according to false-positive rate

Characteristics of patients according to the five FPR (%) ranges are reported in Table 1. Patients infected by an X4 tropic virus (setting the FPR at 10%) have a significantly lower number of CD4⁺ cell counts at nadir, compared with patients infected by an R5 virus [nadir CD4⁺ cell count: 90 (23–174) vs. 181 (73–330), $P = 0.001$]. Moreover, patients infected by an X4 virus with a FPR below 5% have a significantly lower number of CD4⁺ cell counts at the first non-AIDS event development, compared with patients infected by an X4 virus with a FPR 5–10 and to patients infected by an R5 virus with a FPR 10–20, 20–60, or above 60 (CD4⁺ cell count at first comorbidity diagnosis: $P = 0.054$).

In contrast, no correlations were found between FPR ranges and viral load at the zenith point ($P = 0.832$). Furthermore, no differences were observed between patients with a different HIV tropism isolate with respect to age, year of diagnosis, ART length, ART combination and presence of virological blips during virological suppression.

Table 1. Study population characteristics at the time of comorbidities diagnosis.

Study population characteristics	Overall	X4 viruses ^a			R5 viruses ^a		
		FPR ≤ 5%	FPR = 5–10%	FPR = 10–20%	FPR = 20–60%	FPR > 60%	
Patients, N	116	17	15	12	37	35	
Sex (male), n (%)	77 (66.4)	14 (82.4)	7 (46.7)	9 (66.7)	28 (64.9)	27 (68.6)	
CDC C stage, n (%)	35 (30.2)	7 (41.2)	7 (46.7)	1 (8.3)	12 (27.0)	10 (28.6)	
Age (year), median (IQR)	45 (35–53)	48 (37–59)	36 (33–55)	46 (41–51)	47 (40–53)	40 (34–52)	
Year of diagnosis, median (IQR)	2010 (2009–2011)	2009 (2008–2011)	2009 (2009–2011)	2010 (2008–2011)	2011 (2007–2011)	2010 (2009–2011)	
Risk factor, n (%)							
Heterosexual	71 (61.2)	12 (70.6)	12 (80.0)	9 (75.0)	19 (51.4)	19 (54.3)	
Homosexual	31 (26.7)	3 (17.6)	2 (13.3)	3 (25.0)	12 (32.4)	11 (31.4)	
Drug user	14 (12.1)	2 (11.8)	1 (6.7)	0 (0.0)	6 (16.2)	5 (14.3)	
Subtype, n (%)							
B	79 (68.1)	11 (64.7)	10 (66.7)	9 (75.0)	29 (78.4)	20 (57.1)	
CRF02_AG	9 (7.8)	1 (5.9)	3 (20.0)	1 (8.3)	1 (2.7)	3 (8.6)	
G	9 (7.8)	2 (11.8)	1 (6.7)	0 (0.0)	2 (5.4)	4 (11.4)	
F	8 (6.9)	3 (17.6)	0 (0.0)	1 (8.3)	2 (5.4)	2 (5.7)	
C	6 (5.2)	0 (0.0)	1 (6.7)	1 (8.3)	0 (0.0)	4 (11.4)	
Other recombinant forms	5 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.1)	2 (5.7)	
Presence of virological blips during virological suppression, n (%)	23 (19.8)	1 (5.9)	3 (20.0)	2 (16.7)	8 (21.6)	9 (25.7)	
Co-infections, n (%)							
HBV	28 (24.1)	8 (47.1)	6 (40.0)	1 (8.3)	5 (13.5)	8 (22.9)	
HCV	11 (9.5)	2 (11.8)	0 (0.0)	1 (8.3)	6 (16.2)	2 (5.7)	
Viral load at zenith point (log ₁₀ copies/ml), median (IQR)	5.2 (4.6–5.8)	5.4 (4.7–5.9)	5.2 (4.3–5.8)	5.1 (4.9–5.6)	5.3 (4.4–5.9)	5.0 (4.2–5.7)	
CD4 ⁺ at nadir (cells/μl), median (IQR)	150 (50–313)	40 (17–117)	128 (80–220)	153 (100–283)	182 (66–338)	181 (64–315)	
CD4 ⁺ at non-AIDS-event diagnosis (cells/μl), median (IQR)	469 (304–611)	299 (215–477)	489 (391–660)	520 (404–675)	493 (317–583)	465 (254–639)	
ART length (years), median (IQR)	3 (2–4)	4 (3–5)	4 (2–5)	3 (3–4)	3 (2–3)	3 (2–4)	
Drug exposure, N (%)							
ABC + 3TC + protease inhibitor	13 (11.2)	1 (5.9)	3 (20.0)	0 (0.0)	4 (10.8)	5 (14.3)	
TDF + FTC + NNRTI	29 (25.0)	2 (11.8)	4 (26.7)	4 (33.3)	9 (24.3)	10 (28.6)	
TDF + FTC + protease inhibitor	65 (56.0)	11 (64.7)	8 (53.3)	8 (66.7)	20 (54.1)	18 (51.4)	
Regimen including ETV or MVC or RAL	9 (7.8)	3 (17.6)	0 (0.0)	1 (7.7)	4 (9.7)	2 (5.3)	
Number of visits per year, median (IQR)	5 (4–6)	5 (4–5)	5 (5–6)	5 (4–5)	5 (5–6)	5 (5–6)	

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ETV, etravirine; FPR, false-positive rate; FTC, emtricitabine; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile; MVC, maraviroc; NNRTI, non-nucleoside reverse transcriptase inhibitor; RAL, raltegravir; TDF, tenofovir.

^aHIV-1 viruses were stratified in X4 and R5 according the FPR (%) ranges: for X4 viruses not more than five; 5–10; for R5 viruses: 10–20; 20–60; above 60.

Association between false-positive rate and non-AIDS events

By analyzing the correlation between the FPR and evidence of non-AIDS events during the first-line ART treatment, we found that the prevalence of at least one non-AIDS event was higher in patients infected by an X4 virus (FPR set at 10%) than in patients infected by an R5 virus [25 (78.1%) vs. 47 (56.0%), relative risk = 1.40 (95%confidence interval, CI 1.00–1.74), $P=0.033$]. This prevalence also increased by decreasing the FPR (Fig. 1a). In particular, patients infected by an X4 virus with a FPR less than 5% most frequently developed non-AIDS events during ART than patients infected by an R5 virus with a FPR over 60% [15 (88.2%) vs. 18 (51.4%), relative risk: 1.72 (95%CI 1.08–2.09), $P=0.01$] (Fig. 1a). Moreover, patients with lower FPR (<5% and 5–10%) were more prone to develop more than one non-AIDS event with respect to patients infected by an R5 virus with a FPR over 60% [9 (52.9%) for FPR <5.0% and 8 (53.3%) for FPR 5–10 vs. 3 (8.6%) for FPR >60, relative risk:

6.18 (95%CI 1.80–26.41) and 6.22 (95%CI 1.76–26.77), respectively, $P<0.001$ each one] (Fig. 1a).

Interestingly, by analyzing the correlation between HIV-1 tropism and each specific non-AIDS event, dysmetabolic syndrome and hypertension events increased by decreasing the FPR, and their emergence was more frequently observed in patients infected by an X4 virus with a FPR less than 5.0% than in patients infected by an R5 virus with a FPR above 60% [dysmetabolic syndrome: 11 (64.7%) vs. 4 (11.4%), relative risk: 5.6 (95%CI 2.02–17.69), $P<0.001$; hypertension: 8 (47.1%) vs. 0 (0.0%), relative risk: inf.(95%CI 3.57–inf.), $P<0.001$] (Fig. 1b).

In the multivariate Cox-regression analysis based on the counting process formulation of Andersen and Gill (Table 2), the presence of an X4 virus at treatment baseline was an independent factor significantly correlated with a higher probability of non-AIDS events development during ART [adjusted hazard risk: 1.69

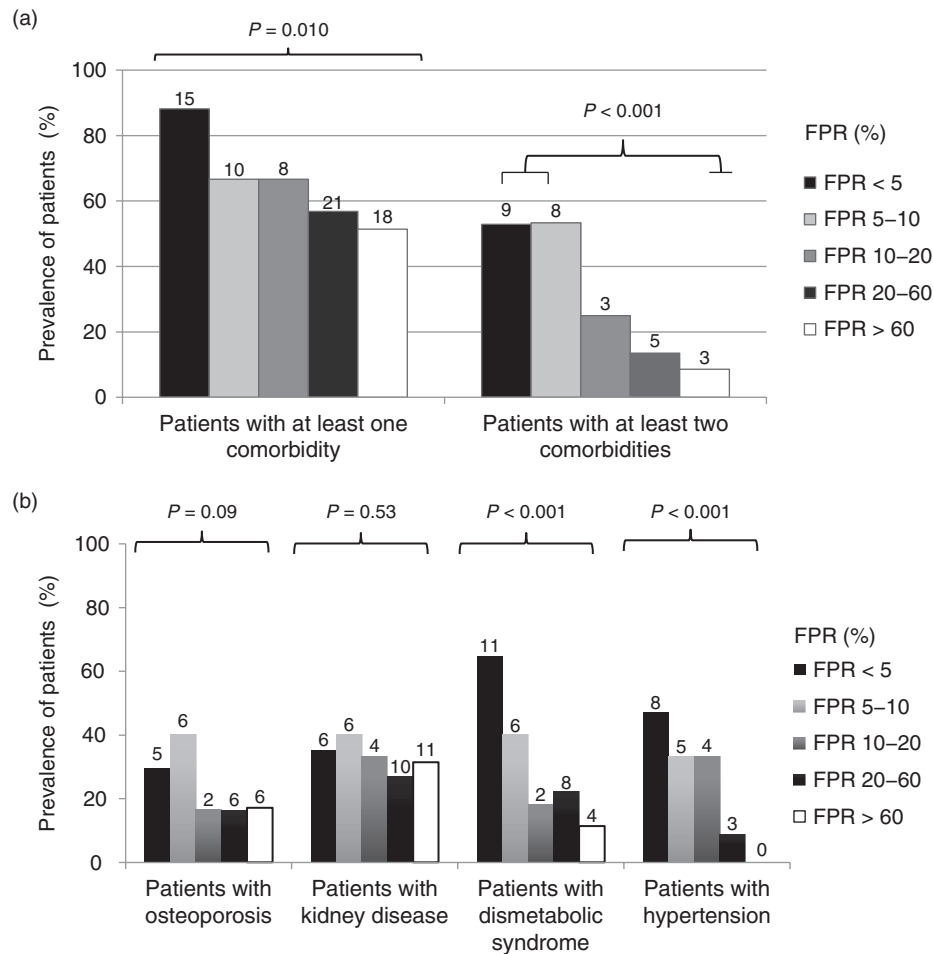


Fig. 1. Prevalence of patients with evidence of non-AIDS events stratified for FPR at baseline and number or type of non-AIDS events. (a) Significant correlation between having FPR below 5.0% and the emergence of at least one or more than one non-AIDS event. (b) Significant correlation between having FPR below 5.0% and the emergence of each non-AIDS event. The absolute number of patients for those the non-AIDS event was diagnosed was reported upper each column. P values were calculated using the χ^2 test for trend. FPR, false-positive rate.

Table 2. Hazard risks for the emergence of severe non-AIDS-related events during first line ART from fitting a Cox-regression analysis using the counting process formulation of Andersen and Gill in HIV-1-infected patients with a tropism determination at baseline.

Independent predictors of the non-AIDS-related events during first-line ART	Univariate analysis		Multivariate analysis ^a		Multivariate analysis ^b	
	Hazard risk (95% CI)	P value ^d	Hazard risk (95% CI)	P value ^d	Hazard risk (95% CI)	P value ^d
Sex (male vs. female)	1.28 (0.85–1.93)	0.230				
CDC C stage, N	1.68 (1.16–2.44)	0.006	1.46 (1.00–2.13)	0.051	1.50 (1.01–2.22)	0.044
Age (per 1 year increase)	1.04 (1.02–1.05)	<0.001	1.03 (1.02–1.05)	<0.001	1.03 (1.02–1.05)	<0.001
Year of diagnosis	1.03 (1.00–1.06)	0.056	1.03 (0.99–1.07)	0.173	1.03 (0.99–1.07)	0.169
Risk factor		0.090				
Heterosexual ^c	1					
Homosexual	0.88 (0.58–1.34)					
Drug user	0.56 (0.34–0.94)	0.591				
Subtype B	1.13 (0.73–1.74)	0.850				
Presence of virological blips during virological suppression	0.95 (0.56–1.61)					
Co-infections						
HBV	1.69 (1.17–2.44)	0.005	1.16 (0.84–1.61)	0.373	1.19 (0.85–1.66)	0.303
HCV	0.68 (0.36–1.27)	0.224				
Viral load at zenith point (per 1 log copies/ml more)	1.13 (0.88–1.45)	0.335				
CD4 ⁺ at nadir cells/ μ l (per 50 cells increase)	0.9983 (0.9971–0.9995)	0.007	1.0004 (0.9990–1.002)	0.600	1.000 (0.9991–1.002)	0.504
CD4 ⁺ at comorbidities diagnosis cells/ μ l (per 50 cells increase)	0.9997 (0.9989–1)	0.479				
ART length (per 1 year more)	0.92 (0.80–1.07)	0.281				
Number of visits per year of ART	0.99 (0.96–1.02)	0.423				
Drug exposure		0.021				
TDF + FTC + protease inhibitor ^c	1		1		1	
TDF + FTC + NNRTI	0.86 (0.53–1.37)		1.02 (0.65–1.59)		1.03 (0.66–1.60)	
ABC + 3TC + protease inhibitor	1.72 (1.11–2.65)		1.30 (0.86–1.94)		1.37 (0.91–2.07)	
Regimen including ETV or MVC or RAL	1.46 (0.83–2.60)		1.00 (0.69–1.43)		1.06 (0.70–1.60)	
X4 tropism (FPR<10%)	1.97 (1.39–2.79)	<0.001	1.69 (1.19–2.39)	0.003		0.012
Tropism prediction		0.003				
R5 FPR >60% ^c	1				1	
R5 FPR 20–60%	1.24 (0.74–2.08)				1.16 (0.73–1.84)	
R5 FPR 10–20%	1.48 (0.80–2.75)				1.66 (0.90–3.04)	
X4 FPR 5–10%	2.33 (1.34–4.04)				2.02 (1.23–3.31)	
X4 FPR <5%	2.31 (1.39–3.84)				1.89 (1.17–3.04)	

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; CI, confidence interval; ETV, etravirine; FPR, false-positive rate; FTC, emtricitabine; HBV, hepatitis B virus; HCV, hepatitis C virus; MVC, maraviroc; NNRTI, non-nucleoside RT inhibitor; RAL, raltegravir; TDF, tenofovir. The analysis was performed on 116 patients. Two multivariate models were applied for tropism prediction according

^aFPR was set at 10% to define an X4 tropic virus; in the second model.

^bFPR was stratified according to the following five FPR percentage ranges: for X4 viruses not more than five, and 5–10; for R5 viruses: 10–20, 20–60, and above 60. All independent predictors characterized by a P value not more than 0.07 in univariate model were inserted in the Cox analysis. Boldface indicates variables significantly associated with for the emergence of severe non-AIDS-related events during first line ART ($P < 0.05$).

^cDummy variable.

^dType III for interaction.

(95%CI 1.19–2.39), $P=0.003$]. By stratifying for FPR, X4 viruses with both FPR less than 5% and 5–10% were independent factors significantly correlated with a higher probability of non-AIDS events development during first-line ART, compared with R5 viruses with FPR above 60% [adjusted hazard risk: 1.89 (95%CI 1.17–3.04) and 2.02 (95%CI 1.23–3.31) $P=0.012$]. Another factor independently correlated to the development of non-AIDS events was age [per 1 year increase, adjusted hazard risk: 1.03 (95%CI 1.02–1.05), $P<0.001$]. Interestingly, CD4⁺ cell count at nadir and at the time of non-AIDS event diagnosis were not significantly correlated with non-AIDS-event development; nevertheless, there was a correlation between low CD4⁺ cell count at nadir and an X4 virus.

Due to the strong association between the presence of an X4 virus at ART initiation and the emergence of hypertension and/or metabolic syndrome, we performed a multivariate Cox-regression analysis taking into account only these two non-AIDS events (Table 3). In this analysis, the presence of an X4 virus at treatment baseline was confirmed to be an independent factor significantly correlated with a higher probability of hypertension and/or metabolic syndrome development during ART [adjusted hazard risk: 2.29 (95%CI 1.39–3.76), $P=0.001$], also when stratified for FPR [adjusted hazard risk: 3.58 (95%CI 1.41–9.10) for FPR <5.0% and 4.86 (95%CI 2.00–11.82) for FPR 5–10, $P<0.001$]. Interestingly, also R5 viruses characterized by FPR 10–20% positively correlated to the development of hypertension and/or metabolic syndrome [adjusted hazard risk: 2.87 (95%CI 1.02–8.11)], suggesting that not only X4 viruses but also the R5 ones characterized by FPR below 20% can be at risk for the development of these specific non-AIDS events.

Discussion

The results of this study demonstrate that patients infected by an X4 virus were more prone than those infected by R5 viruses to develop non-AIDS events during first-line ART and that this correlation is intensified when low FPRs were considered.

An X4 tropism was already widely associated with a faster decline in the number of peripheral blood CD4⁺ lymphocytes, a more rapid progression of the infection and poorer survival [13–15]. A significantly higher number of AIDS-defining illnesses or death after ART initiation has also been observed among patients harboring an X4 virus, compared with those infected by an R5 virus [17]. Previous findings also suggested that X4 viruses, characterized by a low geno2pheno FPR, caused poorer immunological reconstitution and lower virological response in HIV-1-infected patients starting first-line therapy, with respect to R5 viruses with high FPR [23,24]. Here, we provided evidence that an

X4 virus can also be predictive for the development of non-AIDS events.

The reasons for the rapid clinical evolution characterizing patients infected by an X4 HIV strain are not fully understood. Recently, Saracino *et al.* demonstrated a relationship between X4 tropism and the presence of surrogate markers of infection, such as high-sensitivity PCR, D-dimer, interleukin 6, interleukin 7 [18]. However, no correlation was found between the co-receptor tropism of latent virus and markers of immune activation [19], suggesting that other factors drive immune activation that persists despite effective treatment. Previous studies showed that naïve CD4⁺ T cells expressed the CXCR4 receptor more frequently than others, suggesting that these cells represented the largest pool of CD4⁺ T cells depleted after a HIV-1 X4 virus infection [25]. This finding can explain why X4 tropic HIV isolates contribute to the decrease in T-cell numbers [26]. In line with these findings, expression of the CXCR4 co-receptor on T cells has been found to be increased, compared with the expression of CCR5 among elderly donors, suggesting a specific enhancement of CXCR4 expression with age [27] that could facilitate replication of strains with a low FPR and acceleration of HIV progression in older people.

In a recent paper, Lapadula and coworkers [28] demonstrated as ART-treated patients failing to restore CD4⁺ to more than 200 cells/ μ l run a greater risk of serious non-AIDS events and how this correlates with progression to AIDS. Previously it had been suggested that low CD4⁺/CD8⁺ ratio was associated with increased risk of serious events and deaths [29]. Nevertheless, in both the papers, a correlation between CD4⁺ cell recovery or CD4⁺/CD8⁺ ratio during ART and HIV tropism is missing. In our study population, the presence of an X4-tropism isolate at the beginning of treatment was significant associated with lower nadir of CD4⁺ count, in univariate analysis, and at a lower CD4⁺ cell number at the first non-AIDS-event development. This datum should suggest that an X4 tropic virus is related with a major loss and a limited recovery of CD4⁺ cells and allows to argue that the immunological damage secondary to X4-tropism strains could be difficult to be recovered also after years of effective ART. Probably the small number of our population did not allow us to obtain a more reliable result.

Our data also confirmed the already-known association between the development of non-AIDS events and increased age. As suggested by several papers, this association can be explained by the development of mitochondrial dysfunction, the consequent poor response to oxidative stress, telomerase inhibition and the telomere shortening associated with biological senescence in HIV-positive people [30–38]. There is an abundance of evidence that oxidative stress, following

Table 3. Hazard risks for the emergence of hypertension and/or dysmetabolic syndrome events during first line ART from fitting a Cox-regression analysis using the counting process formulation of Andersen and Gill in HIV-1-infected patients with a tropism determination at baseline.

Independent predictors of hypertension and/or dysmetabolic syndrome events during first-line ART	Univariate analysis		Multivariate analysis ^a		Multivariate analysis ^b	
	Hazard risk (95% CI)	P value ^d	Hazard risk (95% CI)	P value ^d	Hazard risk (95% CI)	P value ^d
Sex (male vs. female)	2.00 (1.01–3.96)	0.047	2.00 (1.07–3.73)	0.030	2.2 (1.2–4.03)	0.010
CDC C stage, N	1.88 (1.11–3.16)	0.018	1.41 (0.85–2.33)	0.178	1.49 (0.88–2.51)	0.134
Age (per 1 year increase)	1.04 (1.02–1.06)	0.001	1.03 (1.01–1.06)	0.003	1.03 (1.01–1.06)	0.004
Year of diagnosis	1.03 (0.98–1.08)	0.288				
Risk factor		0.141				
Heterosexual ^c	1					
Homosexual	0.75 (0.40–1.40)					
Drug user	0.28 (0.07–1.08)					
Subtype B	1.21 (0.66–2.22)	0.547				
Presence of virological blips during virological suppression	0.82 (0.46–1.46)	0.498				
Co-infections						
HBV	2.38 (1.47–3.86)	<0.001	1.24 (0.77–2.02)	0.375	1.30 (0.80–2.11)	0.287
HCV	0.62 (0.16–2.44)	0.498				
Viral load at zenith point (per 1 log copies/ml more)	1.27 (0.90–1.78)	0.170				
CD4 ⁺ at nadir cells/ μ l (per 50 cells increase)	0.9665 (0.9944–0.9985)	0.001	0.9990 (0.9968–1.001)	0.394	0.9990 (0.9967–1.001)	0.396
CD4 ⁺ at comorbidities diagnosis cells/ μ l (per 50 cells increase)	0.9987 (0.9976–0.9999)	0.036	0.9998 (0.9986–1.001)	0.788	0.9997 (0.9983–1.001)	0.712
ART length (per 1 year more)	0.99 (0.85–1.15)	0.882				
Number of visits per year of ART	1.00 (0.96–1.04)	0.886				
Drug exposure		0.200				
TDF + FTC + protease inhibitor ^c	1					
TDF + FTC + NNRTI	0.44 (0.18–1.11)					
ABC + 3TC + protease inhibitor	1.18 (0.52–2.68)					
Regimen including ETV or MVC or RAL	1.36 (0.68–2.69)					
X4 tropism (FPR<10%)	3.01 (1.85–4.91)	<0.001	2.29 (1.39–3.76)	0.001		<0.001
Tropism prediction						
R5 FPR >60% ^c	1				1	
R5 FPR 20–60%	2.30 (0.88–6.04)				2.17 (0.90–5.24)	
R5 FPR 10–20%	2.87 (0.98–8.37)				2.87 (1.02–8.11)	
X4 FPR 5–10%	4.76 (1.79–12.68)				4.86 (2.00–11.82)	
X4 FPR <5%	6.20 (2.46–15.64)				3.58 (1.41–9.10)	

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; CI, confidence interval; ETV, etravirine; FPR, false-positive rate; FTC, emtricitabine; HBV, hepatitis B virus; HCV, hepatitis C virus; MVC, maraviroc; NNRTI, non-nucleoside RT inhibitor; RAL, raltegravir; TDF, tenofovir. The analysis was performed on 116 patients. Two multivariate models were applied for tropism prediction according to FPR.

^aIn the first model, FPR was set at 10% to define an X4 tropic virus.

^bIn the second model, FPR was stratified according to the following five FPR percentage ranges: for X4 viruses not more than five, and 5–10; for R5 viruses: 10–20, 20–60, and above 60. All independent predictors characterized by a *P* value not more than 0.07 in univariate model were inserted in the Cox analysis. Boldface indicates variables significantly associated with the emergence of severe non-AIDS-related events during first line ART (*P* < 0.05).

^cDummy variable.

^dType III for interaction.

the accumulation of free radicals, and mitochondrial dysfunction has a major part in the development of chronic and degenerative illnesses such as cancer, autoimmune disorders, ageing, cataracts, rheumatoid arthritis, cardiovascular and neurodegenerative diseases in HIV-negative people [39–43].

ART drugs have been implicated in the pathogenesis of renal damage, redistribution of body fat, bone remodeling, insulin resistance, diabetes, dyslipidemia and cardiovascular disease [44–46]. Nucleoside reverse transcriptase inhibitor use has been historically implicated in mitochondrial damage and telomerase inhibition [47]. The limited size of our study population did not allow to obtain any correlation between the use of specific ART regimen composition and the emergence of defined non-AIDS events.

In HIV-positive patients, a severe immune-deficiency condition and intermittent viremia were identified as risk factors for the development of certain age-related comorbidities [48–51]. Additionally, cumulative viral load exposure (defined as viremia copy-years) in treated patients has been shown to be associated with the risk of clinical events and mortality [52,53]. In our work, neither viral blips nor the zenith of HIV viremia values seems to correlate with the development of non-AIDS events, whereas, patients with more advanced HIV disease (stage CDC C) in the multivariate analysis appear to have a higher correlation with the occurrence of non-AIDS events ($P < 0.044$) (Table 2).

In contrast to the studies of other authors [54,55], in this study, no correlation was found between having a lower FPR and older age, demonstrating that X4 tropism has *per se* a direct effect on non-AIDS-event development.

Before drawing conclusions, a few limitations of our study need to be discussed. The pathogenesis of comorbidities such as hypertension and dysmetabolic syndrome is certainly multifactorial and involves genetic predisposition and environmental factors. This information is not always present in the medical records of our patient population; thus, the collection of such data was partial and insufficient to exclude a correlation with hereditary factors or lifestyle. The evaluation of viral tropism through the V3 nucleotide sequence study was performed on plasma virus before the beginning of treatment, and no other evaluation was performed on archived virus on the few occasions of viral blips during treatment; thus, we have not explored the possibility of viral tropism modification in the course of treatment. However, a tropism switch in treated patients with undetectable plasma viremia is improbable [56,57].

In conclusion, our findings show that an X4 virus and particularly a FPR below 5% defines patients at high risk of non-AIDS-events development, even in the setting of

full suppressive antiretroviral treatment. Nevertheless, further studies on larger and more homogeneous cohorts are warranted to strengthen these results and to explore the possible pathogenetic mechanisms at the base of this phenomenon.

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Conflicts of interest

The authors declare no conflict of interest related to this manuscript. However, C.F.P. and M.A. have received funds for attending symposia, speaking, grant research support, consultancy and advisory, board membership from Abbot, Bristol, Gilead, Merck, Jansenn, Cilag, Pfizer, Roche, ViiV Healthcare.

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