Non-B subtypes account for a large proportion of clustered primary HIV-1 infections in Italy

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ABSTRACT

Objectives and design Using *pol* sequences obtained for routine resistance testing, we characterised the molecular patterns of HIV-1 transmission and factors associated with being part of a transmission cluster among individuals who in 2008–2014 presented with primary HIV-1 infection (PHI) at 11 urban centres across Italy.

Methods *Pol* sequences were obtained by Sanger sequencing. Transmission clusters were identified by phylogenetic analysis (maximum likelihood method, confirmed by Bayesian analysis). Multivariable logistic regression explored factors associated with a participant being part of a transmission cluster.

Results The PHI cohort comprised 186 participants (159/186, 85.5% males) with median age 44 years, median CD4 count 464 cells/mm^3 and median plasma HIV-1 RNA 5.6 log$_{10}$ copies/mL. Drug resistance associated mutations were found in 16/186 (8.6%). A diversity of non-B subtypes accounted for 60/186 (32.3%) of all infections. A total of 17 transmission clusters were identified, including 44/186 (23.7%) participants. Each cluster comprised 2–6 sequences. Non-B subtypes accounted for seven clusters and 22/44 (50%) of clustered sequences. In multivariable logistic regression analysis, factors associated with being part of a transmission cluster comprised harbouring a non-B subtype (adjusted OR (adjOR) 2.28; 95% CI 1.03 to 5.05; p=0.04) and showing a lower plasma HIV-1 RNA (adjOR 0.80, 95% CI 0.64 to 0.99; p=0.04).

Conclusions There was a large contribution of diverse non-B subtypes to transmission clusters among people presenting with acute or recent HIV-1 infection in this cohort, illustrating the evolving dynamics of the HIV-1 epidemic in Italy, where subtype B previously dominated.

INTRODUCTION

Despite great improvements in prevention and treatment, 4.2 new HIV-1 diagnoses per 100 000 residents were made in Italy in 2019.[1] Individuals in the acute and recent phases of the infection play an important role in sustaining HIV transmission, making early diagnosis and prompt initiation of antiretroviral treatment (ART) critical.[2] Italian Network of Acute HIV Infection is a nationwide, multicentre cohort study that up to 2014 collected data and viral genome sequences from patients diagnosed with acute or early HIV-1 infection across clinical centres in Italy. Here, we performed a retrospective analysis to obtain a molecular characterisation of the infection among patients who were diagnosed in 2008–2014 and related the findings to demographic and clinical parameters. By using sequences obtained within early HIV infection, we expected to obtain a clearer snapshot of the HIV epidemic in Italy.[3 4]

METHODS

Study population

We studied 186 treatment-naïve participants with primary HIV-1 Infection (PHI), defined as detectable plasma HIV-1 RNA plus either: (1) a negative third or fourth generation HIV immunoassay or (2) an indeterminate or positive western blot or RIBA, without reactivity of p31 band. PHI was classified according to Fiebig stages I–V. Symptoms compatible with acute retroviral infection were investigated and reported.

Pol sequences (containing the full-length protease and the first 330–335 reverse transcriptase (RT) codons), obtained for routine baseline drug resistance testing, were performed at each centre by Sanger sequencing. All HIV-1 pol genotype analyses were performed on plasma samples. Transmitted drug resistance (TDR) was evaluated according to the WHO 2009 list, with the additional RT mutations K65E/N, E138G/K/Q/R, V179L, G190Q, T215N, H221Y, F227C and M230I.

Phylogenetic analysis

To determine HIV-1 subtype, pol sequences were aligned with full-length reference sequences retrieved from the LANL database (https://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html) using Clustal X and ≥ 10 reference sequences for each subtype, as previously described.[5] Transmission clusters were first deduced through the neighbour-joining method using all sequences (MEGA v6, Kimura 2-parameter model, bootstrap analysis of 1000 replicates). To avoid influence of convergent evolution, sequences were stripped at drug

resistance positions. Transmission clusters supported by a bootstrap value ≥90% and average genetic distance ≤0.020 substitution/site were selected. Robustness was further tested using the maximum likelihood method inferred through GTR+I+Γ5, and 1000 bootstrap replicates.[6 7] The tree was rooted through FigTree V.1.4.4. The GTR+I +Γ model was considered the best one (MEGA 6 model test) due to showing the lowest Bayesian Information Criterion score. Finally, a Bayesian phylogenetic tree was reconstructed with MrBayes, using GTR+I+Γ5. The Monte Carlo Markov Chain search was run for 5×10^6 generations with trees sampled every 100th generation (10% burn-in). Posterior probability of each monophyletic clade was calculated for statistical support, and a posterior consensus tree was generated after 10% burn-in. Transmission clusters showed inferred posterior probabilities ≥0.90.

Statistical analysis

Comparisons between participants belonging or not to clusters were evaluated using the Mann-Whitney test for quantitative variables and χ^2 or Fisher's exact test for categorical variables. Factors potentially associated with being part of a transmission cluster (based on p value at univariable analysis or previous scientific reports) were evaluated using multivariable logistic regression analysis. The following factors were included in the model: gender, year of diagnosis, age, Fiebig stage, transmission group, HIV-1 subtype, plasma HIV-1 RNA load and CD4 cell count at the time of diagnosis, and presence of TDR. All analyses were performed using SPSS V.23.

RESULTS

Among 186 individuals with PHI, 153 (82.3%) were from cities in the north of Italy (Milan: 54, Brescia: 38, Monza: 20, Bergamo: 16, Modena: 22, Genoa: 3); 26 (14.0%) from Rome; and 7 (3.8%) from Sardinia (Sassari 2) and Sicily (Catania 5). Their characteristics are shown in table 1.

TDR was present in 16/186 (8.6%) participants and included 2/44 (4.5%) of clustered sequences and 14/142 (9.9%) of non-cluster sequences. Molecular subtyping showed that clade B

Table 1 Characteristics of the study population stratified by clustering

Characteristic	Overall (n=186)	In cluster (n=44; 23.7%)	Out of cluster (n=142; 76.3%)	P value*
Gender, n (%)				
Male	159 (85.5)	40 (91.0)	119 (83.8)	0.33
Female	27 (14.5)	4 (9.0)	23 (16.2)	
Year of diagnosis (years), median (IQR)	2012 (2011–2014)	2012 (2011–2013)	2012 (2011–2014)	0.94
Age (years), median (IQR)	44 (36–53)	42 (34–49)	45 (37–53)	0.19
Fiebig stages				
I–IV	128 (68.8)	27 (61.4)	101 (71.1)	0.22
V	58 (31.2)	17 (38.6)	41 (28.9)	
Transmission group, n (%)				
Heterosexual	57 (30.6)	12 (27.3)	45 (31.7)	0.94
Men having sex with men	118 (63.4)	29 (65.9)	89 (62.7)	
Intravenous drug users	7 (3.8)	2 (4.5)	5 (3.5)	
Unknown	4 (2.2)	1 (2.3)	3 (2.1)	
CD4 cell count (cells/mm^3), median (IQR)	464 (335–603)	437 (316–548)	476 (340–617)	0.30
HIV-1 RNA ($\log_{10}$ copies/mL), median (IQR)	5.6 (4.9–6.4)	5.4 (4.3–6.1)	5.7 (5.0–6.4)	**0.03**
Subtype, n (%)				
B	126 (67.7)	22 (50.0)	104 (73.2)	**0.003**
Non-B	60 (32.3)	22 (50.0)	38 (26.8)	
CRF02_AG	21 (11.3)	10 (22.7)	11 (7.7)	**<0.001**
F1	17 (9.1)	9 (20.5)	8 (5.6)	
C	7 (3.8)	0 (0.0)	7 (4.9)	
CRF18_cpx	3 (1.6)	3 (6.8)	0 (0.0)	
Others†	12 (6.5)	0 (0.0)	12 (8.5)	
Country of birth				
Italian	106 (57.0)	26 (59.1)	80 (56.3)	0.66
Non-Italian‡	9 (4.8)	1 (2.3)	8 (5.6)	
Unknown	71 (38.2)	17 (38.6)	54 (38.1)	
ART initiation§				
<3 months from diagnosis	43 (24.7)	11 (28.2)	32 (23.7)	0.57
≥3 months from diagnosis	131 (75.3)	28 (71.8)	103 (76.3)	
Transmitted drug resistance, n (%)				
None	170 (91.4)	42 (95.5)	128 (90.1)	0.27
Any class	16 (8.6)	2 (4.5)	14 (9.9)	
NNRTI	9 (4.8)	1 (2.3)	8 (5.6)	0.37
NRTI	5 (2.7)	0 (0.0)	5 (3.5)	
PI	3 (1.6)	1 (2.3)	2 (1.4)	

*By Mann-Whitney test (for quantitative variables) and χ^2 test or Fisher's exact test (for categorical variables), as appropriate.
†Others: CRF01_AE, G, A1, CRF12_BF, CRF19_cpx and CRF28_BF.
‡Non-Italian area of origin was as follows: Europe for 3/9, Sub-saharan Africa for 3/9, Northern Africa for 2/9, Asia for 1/9.
§Data available for 174/186 individuals.
ART, antiretroviral treatment; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

accounted for 67.7% sequences. The most common non-B clades were CRF02_AG (21/186, 11.3%), F1 (17/186, 9.1%) and C (7/186, 3.8%). Other non-B clades were CRF18_cpx, CRF01_AE, G, A1, CRF12_BF, CRF19_cpx and CRF28_BF.

A phylogenetic tree was constructed encompassing all 186 sequences (online supplemental figure 1). A total of 17 transmission clusters were detected each including two to six sequences and encompassing 44/186 individuals (23.7%). Two clusters comprising six and four participants, respectively, involved subtype CRF02_AG, one cluster (three participants) involved CRF18_cpx and four clusters (two to three participants) involved subtype F1. The remaining 10 clusters involved two to three participants with subtype B. When comparing clustered and non-cluster participants, 22/44 (50.0%) and 38/142 (26.8%) (p=0.003), respectively, had a non-B subtype, whereas the median HIV-1 RNA load was 5.4 log10 copies/mL and 5.7 log10 copies/mL, respectively (p=0.03) (table 1). In multivariable logistic regression analysis, factors associated with being part of a transmission cluster comprised harbouring a non-B subtype (adjusted OR (adjOR) 2.28; 95% CI 1.03 to 5.05; p=0.04) and showing a lower plasma HIV-1 RNA (adjOR 0.80, 95% CI 0.64 to 0.99; p=0.04) (online supplemental table 1). In contrast, gender, year of diagnosis, age, Fiebig stage, HIV transmission group, CD4 cell count and presence of TDR were not. Individuals who were eventually started on ART within 3 months following diagnosis were equally represented in and out of MTCs. Out of 17 clusters, 9 encompassed individuals reporting to centres in distinct cities. Of those, four clusters were inter-regional.

DISCUSSION

Applying molecular epidemiology to a largely urban multicentre cohort, we showed that people with PHI harbouring non-B subtypes, whereas representing about a third of all sequences obtained between 2008 and 2014, accounted for half of all sequences occurring within transmission clusters. Our data corroborate previous reports about the spread of non-B subtypes in Italy.[8 9] There was a diversity of non-B subtypes, and most of those with known country of origin harbouring non-B subtypes were born in Italy. This suggests that non-B strains formerly associated with migration are being transmitted and have become endemic in Italy. In fact, a substantial proportion of migrants living with HIV in Europe acquired HIV, including non-B strains, postmigration.[10] Characterising subtype diversity is of epidemiological interest, but the clinical implications are not fully understood. In example, altered drug susceptibility to NRTI and NNRTI has been widely described for subtype C and a faster CD4 cell decline for subtype D, even though it is generally accepted that current antiretroviral regimens can be used reliably to treat patients with both B and non-B subtypes.[11–14] Multivariable logistic regression confirmed that harbouring a non-B subtype and showing a lower viral load were associated with being in a cluster, whereas other characteristics including HIV transmission group were not. The median viral load was lower among participants within clusters than those outside of clusters. This might indicate that most cluster participants were in the later phases of PHI and therefore having had more time to become part of transmission networks, although we did not detect an association between Fiebig stage and clustering, or early treatment and clustering. Such finding might also be a proxy for other factors, that is, lack of symptoms (neurological symptoms are hinted to correlate to higher viral loads in PHI and might hamper the opportunity of transmission) or less accurate HIV-RNA quantification for certain non-B subtypes (challenges in accuracy for CRF02_AG viral load have been described).[15 16]

In most molecular epidemiology studies, the high proportion of sequences belonging to patients with unknown duration of HIV infection poses a major limitation, hampering identification of features relevant to the time of analysis. The strength of our study is the use of sequences obtained within the first few months of infection, allowing a clearer snapshot of epidemic patterns. This is also a limitation of the study, as the methods employed only allow the detection of recent transmission clusters as the cohort does not encompass individuals with chronic infection. Other major limitations are that we could not build separate phylogenetic trees per each subtype. We also lacked important data for a proportion of participants, especially about country of origin. Nonetheless, our data show that HIV-1 diversity is increasing within the Italian epidemic and provide evidence of the indigenous transmission of non-B subtypes.

Key messages

- By phylogenetic analysis of viral *pol* sequences obtained from 186 individuals who in 2004–2018 were diagnosed with primary HIV-1 infection at centres across Italy, we found that 24% of infections occurred within transmission clusters.
- There was a diversity of non-B subtypes that accounted for half of the transmission clusters. In this population, harbouring a non-B subtype was associated with being part of a cluster.
- Non-B subtypes are being transmitted indigenously and are becoming established in Italy.

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