Research Letter

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High prevalence of subtype F in newly diagnosed HIV-1 persons in northwest Spain and evidence for impaired treatment response

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HIV-1 non-B subtype variants were found in 37.8% of 296 newly diagnosed persons in northwest Spain over the past 5 years. Subtype F was the most prevalent non-B subtype (29.6%) and displayed preferential transmission among MSM. Virologic response rates to antiretroviral therapy were lower among F subtypes compared to B subtypes at weeks 24 (31 vs. 78.3%), 48 (51.7 vs. 85.2%), and 96 (61.1 vs. 94.3%) of therapy. Subtype F was independently associated with virological response at 24 weeks.

The prevalence of HIV-1 group M non-B subtypes has been increasing in Western Europe in recent years largely as a result of population movements [1]. Specifically, in native Spaniards, the rate of non-B variants increased from 1.5% in 2000–2002 to 11.4% in 2007–2010, with the circulating recombinant form CRF02_AG (37%) being the most common non-B subtype in Spain [2]. Historically, the HIV epidemic in Galicia, a coastal region situated in northwest Spain, has been characterized by a high diversity of HIV genetic forms presumably due to international population movements through this area [3].

Natural polymorphisms at positions associated with resistance are frequently found among reverse transcriptase and protease sequences from HIV non-B variants [4]. Although the impact of these polymorphisms on treatment responses appears limited, recent studies have demonstrated their relevance in some HIV variants with specific antiretroviral agents [5,6]. Since the prevalence of circulating HIV variants is a dynamic phenomenon and new antiretroviral agents are continually being introduced into the therapeutic arsenal against HIV infection, this issue requires continuous monitoring.

Herein, we describe the characteristics of all newly diagnosed HIV-1 persons in the past 5 years at our institution in northwest Spain serving 501 526 citizens. Patient demographics (age, sex, and risk behavior), and laboratory (HIV-RNA, HIV subtypes, HIV drug resistance and $CD4^+$ cell counts) and clinical parameters at the time of diagnosis were recorded. In addition, the response to antiretroviral therapy (ART) was retrospectively analyzed.

A total of 296 newly diagnosed HIV-1 patients were identified from 2009 to 2013 at our institution. HIV subtype could be determined in 230 patients. Non-B variants were found in 37.8% of patients with the following distribution: F (29.6%), C (2.6%), A (2.2%), CRF02_AG (1.7%), G (0.9%), K (0.4%), and CRF01_AE (0.4%). Subsequent analyses were performed comparing the two most prevalent subtypes seen in our population: subtype B ($n = 143$) and subtype F ($n = 68$).

Table 1 displays the main characteristics of this population at the time of diagnosis. Persons infected with subtype F were overwhelmingly male (97.1%) with a predominance of MSM compared to subtype B patients (81.8 vs. 45.8%, respectively; $P < 0.001$). Those infected with subtype F were also significantly younger than those infected with subtype B (36 vs. 39 years, respectively; $P = 0.037$). No differences were found with regard to nationality, with the majority in both groups being Spanish. Likewise, the percentages of subtype F versus B patients with an AIDS-defining illness (24.2 vs. 35%, respectively) or $CD4^+$ cell counts below 350 cells/μl (42.6 vs. 50.3%, respectively) were similar. The mean $CD4^+$ cell count was also comparable between the groups (384.04 vs. 378.03, respectively); however, HIV-RNA levels at the time of diagnosis were higher among subtype F patients compared to subtype B patients (5.3 vs. 4.9 log copies/ml, respectively; $P = 0.002$). The rate of transmitted drug resistance during the study period was 4.2%, and resistance was only found in subtype B sequences.

After diagnosis, 80.8% of subtype F and 72.7% of subtype B patients initiated ART. The rates of virological response after ART initiation were retrospectively evaluated comparing both groups of patients (Table 1). The mean time to initiation of ART was significantly shorter among patients infected with subtype F variants (10.53 vs. 17.60 months, respectively; $P < 0.001$). No differences were observed with regard to the composition of the initial ART regimen between subtype F and B patients: two nucleoside reverse transcriptase inhibitors (NRTI) + one non-nucleoside reverse transcriptase inhibitor (NNRTI) (55.4 vs.47.5%), two NRTI + one protease inhibitor (35.7 vs. 46.5%) and two NRTI + one integrase inhibitor (INI) (8.9 vs. 6.1%).

Virologic response, defined as achievement of HIV-RNA below 50 copies/ml, was assessed at 24, 48, and 96 weeks

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Table 1. Characteristics of newly HIV diagnosed patients at the time of diagnosis and response to antiretroviral therapy (subtypes B versus F).

Characteristics	$N=211$		P
	Subtype F ($n=68$)	Subtype B ($n=143$)	
Male (%)	97.1	86	0.014
Age	36.23 ± 9.9	39.28 ± 9.7	0.037
Routes of HIV transmission (%)			<0.001
Heterosexual	16.7	47.3	
Homosexual	81.8	45.8	
Others (including IDU)	1.5	6.9	
Spanish (%)	76.5	83.2	0.263
AIDS-defining diseases (%)	24.2	35	0.121
Late diagnosis (%)	42.6	50.3	0.295
Mean $CD4^+$ at diagnosis time	384.04 ± 231.201	378.03 ± 316.800	0.512
Mean HIV-RNA (log copies/ml) at diagnosis time	5.3 ± 0.85	4.96 ± 0.81	0.002
Transmitted drug resistance (%)	0	4.2	0.180
Response to ART	$n=55$	$n=104$	
ART initiation delay after diagnosis (months)	10.53 ± 16.729	17.60 ± 22.485	<0.001
Regimens at ART initiation (%)			0.401
2 NRTI + 1 NNRTI	55.4	47.5	
2 NRTI + PI	35.7	46.5	
2 NRTI + INI	8.9	6.1	
Patients with HIV-RNA <50 copies/ml after ART initiation			
At 24 weeks on therapy (%)	31	78.3	<0.001
At 48 weeks on therapy (%)	51.7	85.2	<0.001
At 96 weeks on therapy (%)	61.1	94.3	0.005
Mean $CD4^+$ cells/μl increase after 48 weeks after ART	271.34 ± 186.333	260.71 ± 190.891	0.788
Mean $CD4^+$ cells/μl increase after 96 weeks after ART	256.35 ± 190.545	315.58 ± 275.593	0.432

ART, antiretroviral therapy; IDU, injecting drug user; INI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

after ART initiation. Interestingly, the rates of virologic response were significantly lower among patients infected with subtype F variants compared to subtype B variants at weeks 24 (31 vs. 78.3%), 48 (51.7 vs. 85.2%), and 96 (61.1 vs. 94.3%). Importantly, the virologic response did not vary based on the composition of the ART regimen (e.g. protease inhibitor vs. NNRTI). However, similar mean increases in $CD4^+$ cell counts 48 and 96 weeks after ART initiation were seen in both groups (Table 1).

In multivariate analysis, infection with a subtype F variant [odds ratio (OR) 14.8 (2.5–16.7), $P<0.001$] and a high baseline plasma HIV-RNA level [OR 11.4 (1.7–7.9), $P=0.001$] were independent predictors of a poor virologic response at 24 weeks after adjusting for ART initiation delay and baseline $CD4^+$ cell count.

Subtype F accounts for less than 1% of HIV-1 infections worldwide, primarily being found in Africa (Congo) and South America (Brazil). In Europe, subtype F has an unusually high prevalence in Rumania (>70%) due to parenteral transmission in children during the late 1980s [7]. Recently, subtype F HIV-1 infection has also been recognized among Italian heterosexual men [8]. A rapid expansion of HIV-1 subtype F among MSM in Galicia was reported in newly diagnosed patients during 2010–2011 [9]. Herein we confirm the continued spread of subtype F among MSM in northwest Spain and, for the first time, provide evidence of a suboptimal response to ART when compared with subtype B patients.

In regard to treatment response, some studies have highlighted the relevance of natural polymorphisms conferring resistance to specific antiretrovirals among ART-naïve individuals infected with non-B subtypes [5,6]. In this study, no significant differences were observed in the prevalence of major polymorphisms associated with resistance to antiretrovirals within reverse transcriptase or protease sequences from subtype F and B viruses. As expected, some differences in minor polymorphisms were found. The variant V106I, which is associated with low-level resistance to the NNRTI etravirine [10,11], was found more frequently among subtype F (compared to subtype B) sequences (83.8 vs. 3.5%, respectively; $P<0.001$). Similarly, variants L10V, M36I, and L89M at positions associated with resistance to protease inhibitors were more frequent among subtype F (compared to subtype B) sequences (91.2 vs. 6.3%; 89.1 vs. 32.2%; and 91.2 and 8.4%, respectively; $P<0.001$). The presence of these polymorphisms has been associated with a reduced susceptibility to the protease inhibitor tipranavir in subtype F specimens (fold-changes <2.7); however, no impact on darunavir susceptibility was found [5]. Since none of the patients in this study was treated with etravirine or tipranavir-based regimens, the presence of polymorphisms associated with reduced susceptibility to these drugs does not account for the poor virologic response observed among subtype F patients. Therefore, the susceptibility of subtype F variants to antiretroviral agents currently used for the treatment of HIV infection needs to be assessed.

Of note, in the study published by Poveda *et al.* [5], subtype F possessed the highest replication capacity (performed by Phenosense assay; Monogram Biosciences, San Francisco, California, USA) among the HIV subtypes tested. This finding is consistent with the significantly higher baseline plasma HIV-RNA levels observed among subtype F patients in our population. However, in the multivariate analysis accounting for baseline viral load, subtype F infection retained a significant association with delayed virologic response.

Poor adherence to ART in the group of subtype F patients is another possible explanation for the lower rates of virologic response. Although a direct measurement of adherence is not available in this study, several studies have reported a higher degree of adherence to ART and HIV care among the MSM population than in other HIV-risk groups [12]. Moreover, similar increases in $CD4^+$ cell counts were seen after ART initiation in both groups; therefore, poor adherence among subtype F patients is an unlikely explanation for the poor virologic outcomes. Finally, there was no difference between the groups in terms of the number of different ART regimens initiated during the study period.

In summary, subtype F is the most prevalent non-B subtype among newly diagnosed HIV-1-infected persons in northwest Spain, with preferential transmission among MSM. We, for the first time, identified a significantly slower virologic response to ART among subtype F patients. Subtype F and a high baseline HIV-RNA were the major determinants of a poor virologic response to ART. Factors not associated with virologic response include: the timing of ART initiation, baseline $CD4^+$ cell count, and the initial ART regimen (NNRTI vs. protease inhibitor). Given the potential clinical implications of these results, additional studies are warranted to identify the reasons for poor ART virologic response in subtype F patients.

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Conflicts of interest

None declared.

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