Cluster surveillance of French primary infections: toward a more virulent CRF02_AG?

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BACKGROUND

- HIV-1 *pol* sequences widely generated for routine genotypic resistance testing now allow characterizing transmission networks in various populations [1–3].
- HIV molecular studies can be used to identify epidemiological shifts or recent transmission clusters (RTC) allowing to describe core transmitters that fuel a large proportion of transmissions. This may help to improve our capability to track and understand pathogen transmission, leading to potential improvements in the design and implementation of population-level public health interventions [4].
- Primary infected patients (PHI) are a unique population including the most recently infected patients at a time and presenting high proportions of patients included in RTC. This population provides valuable data on recent epidemiological trends about ongoing HIV transmissions.
- We analyzed such RTC among primary infected patients diagnosed in France in 2014-2016.

METHODS

- Between January 2014 and December 2016, 1121 PHI patients diagnosed through the French network of virology laboratories (n=46) of the ANRS AC43 Resistance group were included in the study.
- Enrolment criteria were (i) a negative or indeterminate HIV ELISA associated with a positive p24 antigenemia or detectable plasma HIV RNA, (ii) a western blot profile compatible with ongoing seroconversion (incomplete western blot with absence of antibodies to pol proteins) or (iii) a negative test for HIV antibodies within 6 months before the positive HIV serology.
- For all included patients, protease and reverse transcriptase sequences were obtained from routine genotypic resistance testing and analyzed together.
- Phylogenetic trees were built by approximate maximum likelihood using FastTree 2.1 under GTR-G nucleotide substitution model. Recent transmission clusters were retrieved using ClusterPicker 1.2.3 with a maximum pairwise genetic distance at 4.5% and a minimum branch support at 95%. As ClusterPicker uses a p-distance and not genetic distances obtained with the GTR-G nucleotide substitution model, clusters were manually checked and retrieved using FigTree 1.4.3.
- Non-parametric Kruskal-Wallis test and Fisher exact test were used to compare distributions of continuous and categorical variables, respectively, of patients' characteristics according to viral subtype and clusters size. All p values were 2-sided with a significance level of 0.05. For p values below 0.05, the groups were compared 2 by 2 with a significance level of 0.01 to account for the multiplicity of tests. Analyses were performed with R 3.4.0.

RESULTS

- Overall, 1121 patients were included in our study: 355 patients in 2014, 381 in 2015 and 385 in 2016.
- The general characteristics of the study subjects are shown in Tables 1 and 2. Briefly, patients were mainly men (90%) having sex with men (MSM) (70%). Most patients (70%) were from France and 7% were from Sub-Saharan Africa. 42% of all French PHI were observed in Paris area, corresponding to Ile-de-France region and encompassing 18% of the global French population. Median CD4 cell count and plasma HIV-1 viral load measured at the time of PHI were 478 cells/µL [IQR: 329-636] and 5.51 log_{10}copies/mL [IQR: 4.71–6.46], respectively.

Molecular epidemiology

- Among included patients 56, 20 and 24% were infected with subtype B, CRF02_AG or other HIV-1 group M lineages, respectively (cf. Table 1). CRF02_AG tended to be increasingly represented across years, but this failed to reach statistical significance.
- Among other lineages, 6% (64) presented unknown recombinant motifs (herein called URFs), 4% (40) were classified as subtype F, 3% (34) subtype A, 3% (29) subtype C and 2% (26) CRF01_AE. Among the remaining strains, 15 were identified as CRF06_cpx, 9 subtype D, 8 CRF60_BC, 7 subtype G, 5 CRF22_01A1, 4 CRF25_cpx, 4 CRF37_cpx, 3 CRF12_BF, 3 CRF42_BF, 3 CRF11_cpx, 3 CRF07_BC, 3 subtype H, 2 CRF45_cpx, 2 CRF28_BF, 2 CRF18_cpx, 2 CRF56_cpx, 1 CRF33_01B, 1 CRF24_BG, and 1 CRF20_BG.
- Subtype B included more male patients (96%) than the three other lineage categories (82, 84 and 82% for CRF02_AG, URFs and the other lineages group, respectively, p<0.001 for all comparisons), more MSM (78%) than CRF02_AG (59%, p<0.001) and the other subtypes group (58%, p<0.001). Subtype B also presented a higher proportion of patients from French origins (75%) than CRF02_AG (67%, p=0.02) and the other subtypes group (60%, p<0.001). Compared to subtype B, CRF02_AG and URFs were most represented in Paris area (47 vs 39%, p=0.03, and 64 vs 39%, p<0.0001, respectively).
- Compared to subtype B, CRF02_AG presented higher viral loads (median at 5.83 log_{10} copies/mL [IQR: 4.96-6.60] vs 5.40 [4.66-6.26], p=0.004) and lower CD4 cell counts (437 cells/mm³ [294-591] vs 495 [340-650], p=0.004).
- When analyzing patients born in France separately, CRF02_AG still presented higher VL than subtype B (5.79 vs 5.42 log10 copies/mL, p=0.012).

Recent transmission clusters (RTC)

- 457 (41%) patients were infected with strains included in RTC. Among those, 214 (47%) were included in 77 and 20 small clusters of 2 and 3 sequences, respectively. The remaining 243 (53%) sequences were included in large clusters (from 4 to 12 sequences).
- Clinical and demographic analyses of patients corresponding to viral strains not included in a cluster, included in a small cluster (≤ 3 patients) or in a large cluster (> 3 patients) are depicted in Table 2.
- Patients included in RTC were more frequently born in France (74 vs 67%, p=0.009) and MSM (81 vs 63%, p<0.001) than in the non-clustering group.
- Patients included in the large cluster group were younger than those in the small cluster group (median 31 years old vs 34, p=0.01). The latter patients were also younger than non-clustering patients (34 vs 38 years old, p<0.001).
- Subtype B was more represented among the small cluster group than in other groups (72 vs 52%, p<0.001) and CRF02_AG was more represented among the large cluster group (29 vs 17%, p<0.001).
- Paris area appeared as a hub for transmission with 31/39 large RTC including ≥1 patient from this area (cf. Figure 3B).
- Only 4 of the large clusters included patients presenting resistance associated mutations: 1 with a T215S, 1 L74M, 1 K103N and 1 with an association of L76V and L90M. In none of these 4 clusters, resistance mutations were able to achieve sustainable transmissions along the whole cluster. To note, the mutation that lasted during the longer time period was the T215M, known for its low fitness cost (cf. Figure 3A).

Figure 1. Phylogenetic tree obtained for pol gene fragment (PR+RT)

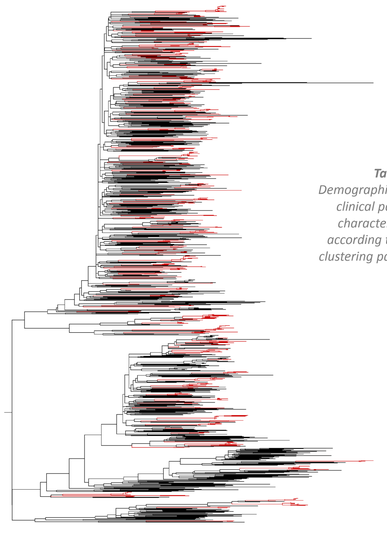

Table 1. Demographical and clinical patients' characteristics according to viral lineage

	Total	Subtype B	CRF02_AG	URFs	Other lineages	p
N (%)	1121 (100)	628 (56)	222 (20)	64 (6)	207 (18)	
Age (years) - median [IQR]	36 [28-45]	36 [28-44]	37 [30-48]	35 [28-44]	36 [28-47]	0.105
Men - n(%)	1009 (90)	604 (96)	182 (82)	54 (84)	169 (82)	<0.001
Country of birth - n(%)						<0.001
France	790 (70)	472 (75)	148 (67)	46 (72)	124 (60)	
Other European countries	37 (3)	21 (3)	5 (2)	4 (6)	7 (3)	
Sub-Saharan Africa	74 (7)	8 (1)	32 (14)	3 (5)	31 (15)	
Other/unknown	220 (20)	127 (20)	37 (17)	11 (17)	45 (22)	
Route of transmission - n(%)						<0.001
MSM	788 (70)	492 (78)	130 (59)	46 (72)	120 (58)	
HTS	199 (18)	55 (9)	65 (29)	12 (19)	67 (32)	
IDU	5 (0)	3 (0)	1 (0)	1 (2)	0 (0)	
Other/unknown	129 (12)	78 (12)	26 (12)	5 (8)	20 (10)	
Region of diagnosis - n(%)						<0.001
Paris area	476 (42)	242 (39)	105 (47)	41 (64)	88 (43)	
Other region	645 (58)	386 (61)	117 (53)	23 (36)	119 (57)	
Year of diagnosis - n(%)						0.164
2014	355 (100)	221 (62)	62 (17)	18 (5)	54 (15)	
2015	381 (100)	204 (54)	75 (20)	24 (6)	78 (20)	
2016	385 (100)	203 (53)	85 (22)	22 (6)	75 (19)	
VL (log_{10} copies/mL) - median [IQR]	5.51 [4.71-6.46]	5.40 [4.66-6.26]	5.83 [4.96-6.60]	5.45 [4.68-6.73]	5.65 [4.76-6.56]	0.004
CD4 (cells/mm³) – mean (95% CI)	478 [329-636]	495 [340-650]	437 [294-591]	491 [307-590]	459 [334-650]	0.040

Table 2. Demographic and clinical patient characteristics according to the clustering pattern

	Total	Not included in a cluster	Small cluster (≤3)	Large cluster (>3)	p
N sequences (%)	1121 (100)	664 (59)	214 (19)	243 (22)	
Age (years) - median [IQR]	36 [28-45]	38 [30-47]	34 [26-45]	31 [26-39]	<0.001
Men - n(%)	1009 (90)	568 (86)	200 (93)	241 (99)	<0.001
Country of birth - n(%)					<0.001
France	790 (70)	448 (67)	158 (74)	184 (76)	
Other European countries	37 (3)	23 (3)	5 (2)	9 (4)	
Sub-Saharan Africa	74 (7)	66 (10)	4 (2)	4 (2)	
Other/unknown	220 (20)	127 (19)	47 (22)	46 (19)	
Route of transmission - n(%)					<0.001
MSM	788 (70)	421 (63)	170 (81)	197 (81)	
HTS	199 (18)	168 (25)	19 (9)	12 (5)	
IVDU	5 (0)	4 (1)	1 (0)	0 (0)	
Other/unknown	129 (12)	77 (11)	18 (9)	31 (14)	
Region of diagnosis					0.125
Paris area	476 (42)	273 (41)	86 (40)	117 (48)	
Other region	645 (58)	391 (59)	128 (60)	126 (52)	
Year of diagnosis - n(%)					<0.001
2014	355 (32)	196 (30)	93 (43)	66 (27)	
2015	381 (34)	229 (34)	60 (28)	92 (38)	
2016	385 (34)	239 (36)	61 (29)	86 (35)	
Subtype					<0.001
B	628 (56)	365 (55)	154 (72)	109 (45)	
CRF02_AG	222 (20)	122 (18)	30 (14)	70 (29)	
URF	64 (6)	29 (4)	11 (5)	24 (10)	
Other	207 (18)	148 (22)	19 (9)	40 (16)	
VL (log_{10} copies/mL) - median [IQR]	5.51 [4.71-6.46]	5.49 [4.72-6.36]	5.72 [4.86-6.60]	5.51 [4.62-6.56]	0.322
CD4 (cells/mm³) - median [IQR]	478 [329-636]	460 [319-631]	476 [329-643]	498 [379-640]	0.120

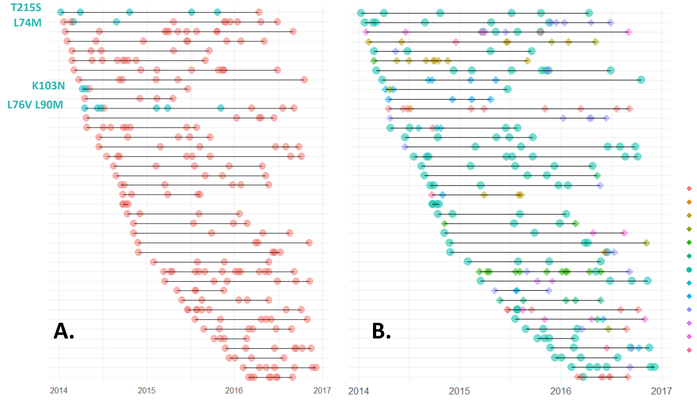

Figure 3. Time distribution of large clusters (>3 sequences included). *Each line represents a distinct large cluster of transmission while each dot depicts inclusion date of corresponding patients. (A) Turquoise dots indicate patient identified with TDRAM(s). The TDRAM or TDRAMs combination was constantly identified among those patients within the same cluster. (B) Colors indicate the sampling region, to help identifying Ile-de-France region, the main contributor to large RTC, all the other regions are presented with diamond shape.*

CONCLUSION

- Comparatively to previous similar studies conducted in France, the current work highlights the decline of subtype B in France (from 75% of PHI patients between 1999 and 2010 to 56% between 2014 and 2016) and the increasing representation of the CRF02_AG (from 14% during the 1999-2010 period to 17% and 22% in 2014 and 2016, respectively) or other non-B non-CRF02_AG lineages (from 11% during the 1999-2010 period to 20% and 25% in 2014 and 2016, respectively) [5].
- Recent transmission cluster were highly prevalent (41% of included patients) and close from those observed in the IPERGAY cohort, including patients at high risk of transmission and using PrEP (Pre-Exposure Propylaxis), or in the Montreal PHI cohort (45% and 49%, respectively) [6, Chaillon, OFID, 2019 in press].
- Patients included in transmission clusters were more frequently male, MSM and younger than other patients.
- This study also emphasizes the role of the national transmission hub represented by Paris area previously observed [3].
- CRF02_AG was more represented among large RTC, presented higher viral loads (VL) and lower CD4 cell counts. The higher viral loads were also retrieved among patients born in France to take into account variations linked to ethnicity [7]. To our knowledge, this is the first report of higher viral loads associated to CRF02_AG and may explained in part the active expansion of CRF02_AG among the largest clusters of French MSM observed in the current work.
- The possible higher virulence of CRF02_AG needs to be confirmed on further study and non-PHI populations.
- Considering the limited time frame of HIV transmission, targeted prevention strategies focusing on PHI may have a significant impact on HIV epidemic. The number of large RTC in French PHI population highlights the existence of a few number of massive localized outbreaks. Identifying growing clusters at a nationwide scale and combining social data and molecular analyses will be required to early detection of epidemiological shifts and allow valuable public health interventions, especially as PrEP is increasingly implemented.

[1] Little SJ et al. Using HIV networks to inform real time prevention interventions. PloS One. 2014;9: e98443.
[2] Grabowski MK et al. The role of viral introductions in sustaining community-based HIV epidemics in rural Uganda: evidence from spatial clustering, phylogenetics, and egocentric transmission models. PLoS Med. 2014;11: e1001610.
[3] Chaillon A et al. Spatiotemporal dynamics of HIV-1 transmission in France (1999-2014) and impact of targeted prevention strategies. Retrovirology. 2017;14: 15.
[4] Ladner JT et al. Precision epidemiology for infectious disease control. Nat Med. 2019;25: 206–211.
[5] Chaix M-L et al. Increasing HIV-1 non-B subtype primary infections in patients in France and effect of HIV subtypes on virological and immunological responses to combined antiretroviral therapy. Clin Infect Dis Off Publ Infect Dis Soc Am. 2013;56: 880–887.
[6] Brenner BG et al. Large cluster outbreaks sustain the HIV epidemic among MSM in Quebec. AIDS Lond Engl. 2017;31: 707–717.
[7] Mulu W et al. Haematological and CD4+ T cells reference ranges in healthy adult populations in Gojjam zones in Amhara region, Ethiopia. PloS One. 2017;12: e0181268.