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Coreceptor Tropism in a Moroccan HIV-1-Infected Cohort

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Abstract : *The aim of this study is to investigate predicted HIV-1 tropism in Moroccan patients. A total of 61 Moroccan HIV-1-infected patients newly diagnosed between 2008 and 2015 in Rabat were included in this study.Genotypic tropism was determined by four methods. The Mean age (SD) was 39 (9,2) years and 35 (77,8 %) were males. The WHO clinical classification indicated that 48,9 % of these patients were at stage 3 and 4. The median HIV-1 viral load and CD4 cell count were 87700 [26450-255000] copies/ml and 208 [76-379] cells/mm3, respectively. The geno2pheno algorithm with FPR of 10% predicted X4 in 2 patients (4,4%) who were infected with B subtype. For the C subtype, a total of strains showed a R5 genotype. For CRF02_AG, a total of strains showed a R5 genotype. Using PSSM tool, 1/35 (2,9%) of samples showed an X4 genotype. The net charge of V3 loop of 18/45 (40%) patients were ≥ +5. A total of strains showed a R5 genotype based on the presence of positively amino acid at position 11 and/or 25 of V3 loop The higher prevalence of R5 strains suggests that CCR5 antagonists will be promising drugs for future AIDS treatment in Morocco.*

Keywords: *Coreceptor; CCR5; CXCR4; genotypic testing; HIV-1; Morocco; tropism*

1. Introduction

The binding of HIV-1 to CD4 and a coreceptor facilitates the entry process of HIV-1 in the target cell. The two major coreceptors involved are chemokine receptors chemokine (C-C motif) receptor 5 (CCR5) and chemokine (C-X-C motif) receptor 4 (CXCR4) [1].

Coreceptor tropism (CTR) plays a crucial role in AIDS progression; in fact, the R5 viruses using CCR5 predominate in the early stages of HIV-1 infection, whereas dualtropic R5X4 and X4 variants, using both coreceptors and CXCR4 respectively, which are associated with rapid disease progression, emerge in the late chronic phase of disease in a significant proportion of patients [2]. Since the first coreceptor antagonist (Maraviroc) against HIV-1 was approved in the United States in 2007, which inhibit CCR5-tropic strain but not CXCR4-tropic strain [3], CTR testing should be performed prior to initiation of therapy with CCR5 inhibitors [4].

In Morocco, the prevalences of R5 and X4 strains are not known. Data on coreceptor usage of HIV-1 are also limited. We investigated predicted HIV-1 tropism in patients to facilitate the integration of CCR5 antagonists into clinical practice in this region.

2. Material and Methods

2.1. Study Population and Ethics Statement

A total of 61 Moroccan HIV-1-infected patients, followed-up at the Mohammed V Military Teaching Hospital in Rabat during the period 2008-2015, were included in this study. All patients were drug-Naive to treatment with plasma viral loads above 500 copies/ml.

Demographic and clinical characteristics, measurements of viral load and CD4+ T cell counts were collected for all the patients. The study was approved by the Ethics Committee for Biomedical Research in Rabat registered at the Office for Human Research Protections in US Department of Health and human Services. Written informed consent was obtained from all participants.

2.2. Genotypic Study and Data Analysis

The HIV-1 viral load (VL) was performed using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche Diagnostics, Mannheim, Germany) with a detection limit of 20 copies/ mL. The CD4 cell counts were obtained by flow cytometry using the Navios Flow Cytometer (Beckman Coulter Life Sciences, USA).

Genotypic tropism testing was performed retrospectively using stored samples from ART-naive individuals with ongoing HIV replication. Viral RNA was extracted from blood plasma samples, using the High Pure Viral RNA Kit (Roche Diagnostics Systems).

The viral RNA was used for reverse transcription polymerase chain reaction (RT-PCR) followed by a nested PCR of V3 region of gp120 env gene in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) thermal cycler and was sequenced by direct sequencing on an automated sequencer Beckman Coulter GenomeLab GeXP DNA Analyzer System using methodology recommended by the French ANRS (National Agency for AIDS Research) (hiv frenchre sista nce.org/ANRS). HIV-1 sub typing was done using HIV BLAST tools (www.hiv.lanl. gov/content/sequence/ BASIC_BLA ST/ ba sicb last. html). Genotypic tropism was determined by four methods: geno2pheno available on the web (http://corec eptor.bioinf.mpi-inf.m pg.de/ index.php), PSSM (position specific scoring matrix) available on the web (http://ub ik.micr o biol.washington.edu/ computing/pssm/), the "11/25" rule (positively charged amino acids in V3 position 11 and/or 25 predicts CXCR4 tropism) and the net charge rule (a net charge of ≥ +5 predicts CXCR4 tropism).

The Geno2pheno interpretation was made with the false-positive rates (FPRs) for prediction of X4 variants of 10% for subtype B and subtype C 5, for the recombinant form CRF02_AG, we used prediction with FPR of 5% threshold 6. The FPR indicates the probability of falsely classifying an R5 virus as X4, all FPR sequence prediction results >10% (>5% for CRF02_AG) were considered as R5-tropic, whereas FPR < 10% (<5% for CRF02_AG) were considered as X4-tropic.The statistical analysis was done on SPSS software (V13.0) and a p value less than 0.05 was considered statistically significant.

All The V3 loop nucleotide and amino acid sequences were submitted to GenBank using BankIt (www.ncbi.nlm.nih.gov/WebSub/) and are available under accession numbers MN037710 to MN037755.

3. Results

Among the 61 HIV-1-infected treatment-naive Moroccan patients, only 45 subjects could be analyzed for genotypic tropism assay. Their Mean age (SD) was 39 (9, 2) years and 35 (77, 8 %) were males. For the mode of transmission, 36 (80%) patients reported heterosexual contacts, and for 9 patients the data regarding the mode of infection were not available. The duration of HIV-1 infection was not known. The WHO clinical classification indicated that 48,9 % of these patients were at stage 3 and 4. The median HIV-1 viral load and CD4 cell count HIV-1 infected patients were 87700 [26450-255000] copies/ml and 208 [76-379] cells/mm3, respectively (Table 1).

Table1. *Baseline characteristics of patients*

Characteristic	Patients (N=45)
Gender no. (%)	
Men	35 (77,8)
Women	10 (22,2)
Age [years] Mean (SD)	39 (9,2)
Transmission risk group no. (%)	
Heterosexuals	36 (80)
Unknown	09 (20)
WHO classification no. (%)	
Class 1	14 (31,1)
Class 2	09 (20,0)
Class 3	12 (26,7)
Class 4	10 (22,2)

Plasma viral load [copies/ml] median [percentile 25–75]	87700 [26450-255000]
CD4 cell count [Cells/mm3] median [percentile 25–75]	208 [76-379]

The major subtype was B 31/45 (68, 88%) and 14/45 (31, 12%) were non-B subtypes (10 CRF02_AG and 4 subtype C). The geno2pheno algorithm predicted X4 in 2/45 (4, 44%) patients who were infected with B subtype. For the C subtype and CRF02_AG recombinant form, a total of strains showed a R5 genotype. The distribution of FPR value among the 45 patients is shown in Table 2.

The median value of geno2pheno FPR was 50, 3% [27, 6–72, 3].Using PSSM tool according to the sinsi matrix, 1/35 (2, 9%) of samples showed an X4 genotype. The net charge of V3 loop was distributed from +1 to +6, of which 18/45 (40%) were ≥ +5. A total of strains showed a R5 genotype based on the presence of positively amino acid at position 11 and/or 25 of V3 loop (table 2).

Table2. *Genetic subtype, tropism predictions using different tools for the HIV-1 isolates*

No	Sample ID	HIV-1 Subtype based on the V3 sequence	Tropism prediction method				
			G2P (10% FPR)	*G2P (5%FPR)*	*PSSM Sinsi (score)*	*Charge rule (net charge)*	*AA 11/25 position*
1	89,09	B	R5 (78,1)	R5	R5 (-8,43)	**X4** (5)	R5 (SQ)
2	942,08	B	R5 (14,7)	R5	R5 (-4,42)	**X4** (6)	R5 (SQ)
3	1516,09	B	R5 (43,3)	R5	R5 (-9,71)	**X4** (6)	R5 (SE)
4	1633,10	CRF02_AG	R5 (99,2)	R5	n/a	R5 (4)	R5 (SD)
5	1822,07	CRF02_AG	R5 (56,9)	R5	n/a	R5 (3)	R5 (SD)
6	2611,08	B	R5 (13,2)	R5	R5 (-7,93)	R5 (4)	R5 (GD)
7	3230,10	B	R5 (44,2)	R5	R5 (-7,59)	R5 (4)	R5 (GD
8	3497,07	B	**X4** (4,0)	**X4**	R5 (-5,34)	R5 (4)	R5 (GD)
9	3581,05	B	R5 (10,1)	R5	R5 (-8,09)	**X4** (6)	R5 (SD)
10	3743,07	C	R5 (72,3)	R5	R5 (-28,3)	R5 (4)	R5 (SD)
11	3935,05	B	R5 (36,9)	R5	R5 (-3,44)	**X4** (5)	R5 (G -)
12	3937,05	B	R5 (50,3)	R5	R5 (-5,50)	**X 4** (5)	R5 (S -)
13	4510,07	CRF02_AG	R5 (23,3)	R5	n/a	R5 (4)	R5 (SD
14	5044,05	B	R5 (54,5)	R5	R5 (-12,4)	**X4** (5)	R5 (SD)
15	5214,05	CRF02_AG	R5 (52,2)	R5	n/a	**X4** (5)	R5 (GD)
16	5483,05	B	R5 (78,4)	R5	R5 (-12,5)	R5 (4)	R5 (SE)
17	5575,07	B	R5 (12,1)	R5	R5 (-5,03)	**X4** (5)	R5 (GD)
18	5915,06	B	R5 (86,2)	R5	R5 (-13,7)	R5 (4)	R5 (SD)
19	5946,08	B	**X4** (6,6)	R5	**X4** (0,45)	R5 (4)	R5 (G -)
20	6264,08	B	R5 (34,5)	R5	R5 (-6,35)	R5 (4)	R5 (GE)
21	7028,05	B	R5 (60,5)	R5	R5 (-9,89)	R5 (3)	R5 (SD)
22	7091,05	C	R5 (80,8)	R5	R5 (-28,4)	R5 (4)	R5 (SD)
23	7350,07	CRF02_AG	R5 (56,1)	R5	n/a	R5 (4)	R5 (SE)
24	7437,09	B	R5 (17,1)	R5	R5 (-8,78)	**X4** (6)	R5 (GG)
25	7473,09	B	R5 (27,6)	R5	R5 (-10,7)	R5 (4)	R5 (SE)
26	7631,09	CRF02_AG	R5 (87,4)	R5	n/a	R5 (4)	R5 (SD)
27	7720,07	C	R5 (35,2)	R5	R5 (-25,1)	**X4** (5)	R5 (SE)
28	8034,08	B	R5 (76,0)	R5	R5 (-9,73)	**X4** (5)	R5 (SD)
29	8249,09	B	R5 (28,2)	R5	R5 (-5,54)	X4 (5)	R5 (SA)
30	8782,09	B	R5 (60,2)	R5	R5 (-12,9)	**X4** (5)	R5 (SD)
31	9205,08	B	R5 (31,6)	R5	R5 (-7,77)	**X4** (5)	R5 (GD)
32	10306,06	B	R5 (74,4)	R5	R5 (-13,1)	R5 (4)	R5 (SD)

33	10375,08	CRF02_AG	R5 (40,7)	R5	n/a	R5 (2)	R5 (SA)
34	10437,07	B	R5 (92,6)	R5	R5 (-13,3)	R5 (4)	R5 (SD)
35	10458,08	CRF02_AG	R5 (13,0)	R5	n/a	R5 (3)	R5 (SD)
36	10559,08	B	R5 (31,0)	R5	R5 (-8,28)	**X4** (5)	R5 (GD)
37	10621,07	B	R5 (23,0)	R5	R5 (-7,56)	**X4** (5)	R5 (GD)
38	10741,07	B	R5 (65,4)	R5	R5 (-15,1)	R5 (3)	R5 (SD)
39	10744,07	B	R5 (61,8)	R5	R5 (-12,5)	R5 (4)	R5 (SE)
40	11178,07	CRF02_AG	R5 (71,7)	R5	n/a	**X4** (6)	R5 (SG)
41	12087,06	CRF02_AG	R5 (48,0)	R5	n/a	R5 (4)	R5 (SD)
42	13253,07	B	R5 (63,0)	R5	R5 (-8,49)	R5 (3)	R5 (GD)
43	14107,06	B	R5 (19,4)	R5	R5 (-9,79)	R5 (4)	R5 (GD)
44	14988,06	B	R5 (11,4)	R5	R5 (-7,39)	R5 (3)	R5 (DD)
45	17322,09	C	R5 (77,6)	R5	R5 (-28,6)	R5 (1)	R5 (SE)

G2P: geno2pheno algorithm, PSSM_sinsi: position specific scoring matrix for syncitium inducing and non-syncitium, n/a: non applicable for non-subtype B strains

No statistical significance of differences in age, gender, routes of transmission, CD4 count and viral loads between subjects infected with X4-tropic or R5-tropic virus was observed. In this analysis we included only patients with AIDS stage, we did not find statistical differences according to coreceptor use (table 3). To avoid the possible effect of different HIV-1 subtypes when interpreting sequences, a separate analysis of each subtype was done. The results did not significantly differ from the viral subtypes obtained for all patients (table 3) (table 4).

Table3. *HIV-1 tropism predictions using different tools according to demographic, clinical, and virologic parameters in patients*

	All subtypes	**HIV-1 Subtype**			**Age (years)** Mean (SD)	**AIDS diagnosis**	**CD4 cell count** Cells/mm3 [percentile 25–75]	**Plasma viral load** copies/ml [percentile 25-75]
		B N=31 n(%)	***C N=4 n(%)***	***CRF 02_AG N=10 n(%)***				
G2p (10%) X4 R5 p value	2 (4,4) 43 (95,6)	2 (6,5) 29 (93,5) 0,62	0 (0) 4 (100) 1	0 (0) 10 (100) 1	32 (1,44) 38 (9,28) 0,27	0 (0) 22 (100) -	429 [210 - 429] 202 [72 - 378] 0,29	821950 [13900 - 821950] 87700 [26500 - 244000] 0,84
G2p (5%) X4 R5 p value	1 (2,2) 44(97,8)	1 (3,2) 30 (96,8) 0,12	0 (0) 4 (100) 1	0 (0) 10 (100) 1	33 39,2 (9,2) 0,51	0 (0) 22 (100) -	210 205 [73 - 378] 1	1630000 83400 [26425 - 237750] 0,13
Rule 11/25 X4 R5 p value	0 (0) 45 (100)	0 (0) 31 (100) -	0 (0) 4 (100) -	0 (0) 10 (100) -	- 39 (9,2) -	0 (0) 22 (100) -	0 (0) 208 [76 - 379] -	- 87700 [26450 - 255000] -
Charge rule X4 R5 p value	18 (40) 27 (60)	15 (48,4) 16 (51,6) 0,19	1 (25) 3 (75) 0,64	2 (20) 8 (80) 0,27	39 (9,7) 39,1 (89) 0,98	9 (40,9) 13 (59,1) 0,9	176 [55 - 386] 210 [81 - 378] 0,71	53450 [18425 - 253750] 105000 [26500 - 266000] 0,31
PSSM X4 R5 p value	1(2,9) 34 (97,1)	1 (3,2) 30 (96,8) 0,12	0 (0) 4 (100) 1	n/a n/a	31 38,7 (8,7) 0,39	0 (0) 17 (100) -	648 152 [64 - 360] 0,17	13900 83400 [26475 - 294250] 0,34

Table4. *FPR values for patients according to HIV-1 subtype*

	All subtypes	HIV-1 Subtype			*p value*
		B N=31	***C N=4***	***CRF02_AG N=10***	
G2p FPR median [percentile 25–75]	**48%** [23,15-72]	**36,9%** [17,1-63]	**74,95 %** [44,47-80]	**54,15 %** [26,49-75,62]	**0,139**

4. DISCUSSION

In this study, we report the first analysis of prediction HIV-1 coreceptor usage in Morocco. The sequences of the partial env gene encompassing the V3 region of sixty one samples from Morocco were analyzed. Our results, showing subtype B predominance (68, 88%) with co-circulation of non-B strains (31, 12%). These results confirm our previous publication detailing the predominance of subtype B using the protease and reverse transcriptase genes 7-10. However, the prevalence of non-B subtypes in our country increased significantly from 3% before 1997 to 26% in 2012 7-12, Morocco has strong historic links with several African countries endemic for HIV. The diversity highlighted here confirms the dynamic of HIV-1 epidemic throughout the Moroccan population. The increasing of the prevalence of non-B subtypes has been observed in many other Mediterranean countries 13-15.

The Online tool Geno2pheno system was selected as reference as it is most widely used and tested. This method has shown a similar performance to the Trofile phenotypic assay, the most often used tropism method 16. The geno2pheno algorithm allows for an adjustable cutoff and can determine HIV-1 co-receptor usage in all viral genotypes 17. Moreover, the method has been shown to achieve higher sensitivity while retaining high level of specificity when compared with the performance of different algorithms 17. The Geno2pheno interpretation was made with the FPRs of 10%, which is in accordance with the current European guidelines 4, 5. For the recombinant form CRF02_AG, we used prediction with FPR of 5% threshold 6.

In this study, we sought to estimate the prevalence of co-receptor tropism of the strains in drug-naive HIV-1 infected individuals using a total of 61 samples, the tropism could not be determined in 16 samples (26.22%) because of the low viral loads in these subjects and the great diversity of the HIV-1 envelope genes. Our data showed that a majority of viruses (95, 56%) were predicted to be R5-tropic and only a smaller percentage (4, 44%) presented X4 strains. Many studies have reported varying frequencies of X4 strains (from 3.2% to 19.4%) in antiretroviral naive patients [18, 19]. The prevalence of X4 strains in our study was lower than the prevalence determined in the previous studies in European countries, where HIV-1 subtype B predominates, reported that 80 to 90% of untreated HIV-1-infected patients harboured R5 strains [18-20]. In a study from Spain, the researchers showed that 13.4% of the 67 HIV-1 seroconverters harboured X4 viruses [18]. A French study also showed that 15.9% of 390 primary HIV subtype B infection contained X4 viruses [21]. In Italy, Meini et al showed that 26.2% of HIV infected treatment-naïve patients had X4 viruses [22]. This disparity in prevalence of co-receptor use between our study and the other studies might be due to the different patient populations, stage of HIV infection (primary vs chronic infection). In our study, the duration of HIV infection was undetermined. However, patients with HIV-1 diagnosis during long-term infection predominated and most patients were diagnosed at the AIDS stage with very low CD4+ T-cell count. This difference could also have resulted from the diverse approach to tropism prediction in these studies, triplicate sequencing or Deep sequencing of V3 coding region reported in previous studies [23-25], which are able to detect more X4 variant in comparison with analysis of single sequences used in our study.

It was demonstrated that low baseline FPR determined by the geno2pheno tool can predict tropism switch from CCR5 to CXCR4, and patients with R5 viruses predicted at diagnosis with a geno2pheno FPR of less than 50% (or <40%) were more prone to switch coreceptor over time than patients with FPR values of >50% (or >40%) [26, 27]. In our study, the median value of geno2pheno FPR was 50,3% [27,6–72,3]. The lower frequency of X4 viruses in our study may be explained by the low switch from CCR5 to CXCR4 during the HIV-1 infection among our patients.

Previous reports showed that different HIV genotypes may have specific coreceptor preferences [21, 28]. A study in ART-naïve Ugandan women showed that 64% of those infected with HIV-1 subtype D harboured R5 strains compared with 100% of those infected

with subtype A [29]. Another study from Hong Kong showed that the prevalence of X4-tropic virus in antiretroviral-naïve subtype CRF01_AE was 24%, which was significantly higher than subtype B 14% [30]. Here we found that all C and CRF02_AG strains were predicted to use CCR5 co-receptor, that agreed with previous observations of predominance of R5 viruses in the HIV-1 subtype C and CRF02_AG infections [5, 6, 21, 31, 32]. Therefore, a large study on coreceptor tropism of non-B subtypes should be performed to confirm these data in Morocco.

No statistical significance of differences in age, gender, routes of transmission between subjects infected with X4-tropic or R5-tropic virus was observed, which was in agreement with many studies [33-36]. We also did not find a association between a low CD4 count at baseline and the detection of X4 virus which was in agreement with some previous studies [34, 36].

While, other studies showed that patients with X4 viruses harboured significant lower baseline CD4+ T-cell counts than those with R5 viruses [35, 37]. Otherwise, we did not find a association between a high viral load and detection of X4 strain. That is in contrast to other studies showing that patients harbouring the X4 strain had a higher viral load [38].

This result may be explained by the relatively small case numbers in our series, and our patients were infected recently with relatively higher viral loads. Further study is clearly needed to confirm this hypothesis.

In this study, X4 tropism frequency was different depending on the prediction tool used and varied from 0% (11/25" rule), thorough 2,9% (PSSM), 4,44% (geno2pheno FPR10%) to 40% (charge rule). Geno2pheno algorithm was selected as reference as it is most widely used and tested. Comparing the three tools to geno2pheno, PSSM, 11/25 rule and the charge rule agreed with pheno2geno at FPR 10% in 97,1% (34/35), 95,5% (43/45) and 55,5% (25/45) of the cases respectively. However, in FPR ranging from 20-100%, the three tools t agreed with pheno2geno at FPR 10% in 100% (26/26), 100% (35/35) and 60% (21/35) of the cases respectively. High degree of concordance between the four tools in this range was described previously [39, 40]

However, the major limitation of our study is a relatively small sample size. Since we were able to identify tropism in samples of only 46 patients, it would be difficult to generalize our results to the country's total population and testing a larger number of specimens in this country may be needed to provide more accurate and reliable information about the use of prediction tools for HIV tropism determination. In addition, possible bias derived from high rate of PCR failure could be another limitation of our study.

Subjects who could not be tested by our genotypic tropism assay (24.5%) might have certain characteristics associated with testing results. Another limitation of our study is linked to the years of samples collection (2008–2015) and sampling considerably preceded the time of publication making the results significant for that period. Moreover, this study is limited by its retrospective nature and a degree of selection bias. Only patients not requiring early ART initiation were analysed. Therefore, these patients may not be representative of all the Moroccan antiretroviral-naïve patients. Finally, our sequencing method only detects the predominant variants, the prevalence of X4 viruses may be underestimated if they are present as minor populations of quasispecies.

5. Conclusion

Our study provides an insight of the coreceptor tropism prevalence among Moroccan antiretroviral-naive patients. We found relatively high prevalence of non-R5 strains, even in patients with advanced stages of AIDS, indicating a potential benefit of CCR5 antagonists as a therapeutic option in Morocco and will be promising drugs for use in future HIV-1 infection treatment. Tropism testing should be performed before initiating treatment even in resource-limited settings such as Morocco.

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REFERENCES

[1] Berger EA, Doms RW, Fenyo EM *et al*. A new classification for HIV-1. *Nature.* 1998; 391: 240.

[2] Shepherd JC, Jacobson LP, Qiao W *et al*. Emergence and persistence of CXCR4-tropic HIV-1 in a population of men from the multicenter AIDS cohort study. *J Infect Dis.* 2008; 198: 1104–1112.

[3] Tan Q, Zhu Y, Li J, et al. Structure of the CCR5 chemokine receptor- HIV entry inhibitor maraviroc complex. *Science.* 2013; 341:1387-90.

[4] Vandekerckhove LP, Wensing AM, Kaiser R, et al. European guidelines on the clinical management of HIV-1 tropism testing. *Lancet Infect Dis*. 2011; 11(5):394-407.

[5] Raymond S, Delobel P, Mavigner M et al. Accurate Prediction of Human Immunodeficiency Virus Type 1 Subtype C Tropism by Subtype B Genotypic Determinants. *JAIDS*. 2010; 53 (2), 167-175

[6] Soulié C, Fofana DB, Boukli N et al. Performance of genotypic algorithms for predicting tropism of HIV-1CRF02 AG subtype. *J Clin Virol*. 2016; 76:51-4.

[7] El Annaz H, Recordon-Pinson P, Tagajdid R et al. Drug resistance mutations in HIV-1 isolates from patients failing antiretroviral therapy in Morocco. *AIDS Res Hum Retroviruses. 2012 Aug*; 28(8): 944-8.

[8] El Annaz H, Recordon-Pinson P, Baba N et al. Presence of drug resistance mutations among drug-naive patients in Morocco. *AIDS Research and Human Retroviruses*. 2011; 27(8).917-920

[9] Bakhouch K, Oulad-Lahcen A, Bensghir R, et al.: The prevalence of resistance-associated mutations to protease and reverse transcriptase inhibitors in treatment-naive (HIV1)-infected individuals in Casablanca, Morocco. J Infect Dev Ctries. 2009; 3(5):380–391.

[10] El Aouad R, Diez M, and Cherkaoui I: Impact of immigration on HIV and tuberculosis epidemiology in the Euro-Mediterranean area. Euro Surveill 2009; 14(15):pii = 19173.

[11] Elharti E, Elaouad R, Amzazi S, et al.: HIV-1 diversity in Morocco. Lancet 1997; 11:1781–1783.

[12] Akrim M, Adnaoui M, Himmich H, Khattabi H, Andre´ P, and El Aouad R: Dynamic of distribution of HIV-1 subtypes in Morocco. 16th International AIDS Conference Toronto, Canada, August 2006; 16:13–18.

[13] Bouzeghoub S, Jauvin V, Recordon-Pinson P, et al.: High Diversity of HIV Type 1 in Algeria. AIDS Res Hum Retroviruses 2006; 22(4):367–372.

[14] Yebra G and Holguı´n A: Epidemiology of drug-resistant HIV-1 transmission in naive patients in Spain. Med Clin (Barc) 2010; 135(12):561–567.

[15] Bonura F, Tramuto F, Vitale F, et al.: Transmission of drug resistant HIV type 1 strains in HAART-naive patients: A 5- year retrospective study in Sicily, Italy. AIDS Res Hum Retroviruses 2010;26(9):961–965.

[16] Chueca N, Garrido C, Alvarez M, Poveda E, de Dios LJ, Zahonero N, et al. Improvement in the determination of HIV-1 tropism using the V3 gene sequence and a combination of bioinformatic tools. J Med Virol. 2009; 81(5):763–7.

[17] Raymond S, Delobel P, Mavigner M, Cazabat M, Souyris C, Sandres-Saune K, et al. Correlation between genotypic predictions based on V3 sequences and phenotypic determination of HIV-1 tropism. AIDS. 2008; 22(14):F11–6.

[18] De Mendoza C, Rodriguez C, Garcı´a F, et al. Spanish HIV Seroconverter Study Group. Prevalence of X4 tropic viruses in patients recently infected with HIV-1 and lack of association with transmission of drug resistance. J Antimicrob Chemother 2007; 59:698–704.

[19] Parisi SG, Andreoni C, Sarmati L, et al. HIV coreceptor tropism in paired plasma, peripheral blood mononuclear cell, and cerebrospinal fluid isolates from antiretroviral-naive subjects. J Clin Microb 2011; 49:1441–1445.

[20] Moyle GJ, Wildfire A, Mandalia S, et al. Epidemiology and predictive factors for chemokine receptor use in HIV-1 infection. J Infect Dis 2005; 191:866–72.

[21] Frange P, Chaix ML, Raymond S, et al., French ANRS CO 06 PRIMO Cohort Study Group. Low frequency of CXCR4-using viruses in patients at the time of primary non-subtype-B HIV-1 infection. J Clin Microbiol 2010; 48:3487–91.

[22] Meini G, Rossetti B, Bianco C, et al., on behalf of the ICONA Foundation Group. Longitudinal analysis of HIV-1 coreceptor tropism by single and triplicate HIV-1 RNA and DNA sequencing in patients undergoing successful first-line antiretroviral therapy. J Antimicrob Chemother 2014; 69:735–41.

[23] Poveda, E. et al. Update on clinical and methodological recommendations for genotypic determination of HIV tropism to guide the usage of CCR5 antagonists. AIDS Rev 2012. 14, 208–217.

[24] Sierra, S. et al. Parameters influencing baseline HIV-1 genotypic tropism testing related to clinical outcome in patients on maraviroc. PLoS One 2015. 10, e0125502.

[25] Bunnik EM, Swenson LC, Edo-Matas D, et al. Detection of inferred CCR5- and CXCR4-using HIV-1 variants and evolutionary intermediates using ultra-deep pyrosequencing. PLoS Pathog. 2011; 7(6):e1002106.

[26] Arif, M. S. et al. Pace of coreceptor tropism switch in HIV-1-infected individuals after recent infection. J. Virol 2017; 91, e00793–17.

[27] Mortier, V. et al. Frequency and predictors of HIV-1 co-receptor switch in treatment naive patients. PLoS One 2013; 8, e80259.

[28] Panos G and Watson DC. Effect of HIV-1 subtype and tropism on treatment with chemokine coreceptor entry inhibitors; overview of viral entry inhibition. Critical Reviews in Microbiology 2014; 41, 473.

[29] Huang W, Eshleman SH, Toma J et al. Coreceptor tropism in human immunodeficiency virus type 1 subtype D: high prevalence of CXCR4 tropism and heterogeneous composition of viral populations. J Virol 2007; 81: 7885–7893.

[30] To SW, Chen JH, Wong KH, et al. Determination of the high prevalence of Dual/Mixed- or X4-tropism among HIV type 1 CRF01_AE in Hong Kong by genotyping and phenotyping methods. AIDS Res Hum Retroviruses 2013; 29:1123–8.

[31] Coetzer M et al. Genetic characteristics of the V3 region associated with CXCR4 usage in HIV-1 subtype C isolates. Virology 2006; 356, 95–105.

[32] Mbondji-Wonje C, Ragupathy V, Zhao J, et al. Genotypic Prediction of Tropism of Highly Diverse HIV-1 Strains from Cameroon. PLoS ONE 2014; 9(11): e112434.

[33] Guo JL et al. Coreceptor tropism and genetic characteristics of the V3 regions in variants of antiretroviral-naive HIV-1 infected subjects. Epidemiology and Infection 2019; 147, e181, 1–7.

[34] Sierra-Enguita R et al. X4 tropic viruses are on the rise in recent HIV-1 seroconverters in Spain. AIDS (London, England) 2014; 28, 1603.

[35] Brumme ZL et al. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. The Journal of Infectious Diseases 2005; 192, 466–474.

[36] Chalmet K et al. Presence of CXCR4-using HIV-1 in patients with recently diagnosed infection: correlates and evidence for transmission. Journal of Infectious Diseases 2012; 205, 174.

[37] Ghosn J et al. CD4T cell decline following HIV seroconversion in individuals with and without CXCR4-tropic virus. Journal of Antimicrobial Chemotherapy 2017; 72, 2862.

[38] Furrer H, Wendland T, Minder C, et al. Association of syncytium inducing phenotype of HIV-1 with CD4 cell count, viral load and sociodemographic characteristics. AIDS 1998; 12:1341–6.

[39] Garrido C, Roulet V, Chueca N, et al. Evaluation of eight diff erent bioinformatics tools to predict viral tropism in diff erent human immunodefi ciency virus type 1 subtypes. J Clin Microbiol 2008; 46:887-91

[40] M. Parczewski, M. Leszczyszyn-Pynka, A. Urbańska, et al. Analysis of V3 loop sequences using various bioinformatic tools designed for genotypic HIV-1 tropism testing. hiv & aids review 2010; 9(3): 65–71

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