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HIV-1 Subtypes Circulating in Constanta

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ABSTRACT
The HIV epidemic in Constanta describes a unique pattern in the world. It has outbreak in 1986, in the pediatric population and it is of a monoclonal aspect, being determined by the F1 subtype. The evolution of the epidemic has known different stages in time: it spreads to the adult population, the transmission pathway becomes predominantly sexual and the circulating viral strains become diversified. The study proposes the characterization of the circulating HIV-1 subtypes in Constanta from the beginning of the epidemic to the present. The results indicate that subtype F1 remains dominant in patients from Constanta, mainly due to cases coming from the pediatric cohort that have now reached adulthood and are generating secondary cases through sexual transmission. As subtype B strains appeared sporadically before 1999, the strains C and B appear systematically after that moment in time. In 2004 the first subtype A strains were isolated. The year 2007 is the one with the highest biodiversity: along with the dominant subtype F1, subtypes C, A, B, G were isolated and also the circulating recombinant forms CRF02_02, CRF06_cpx and CRF01_AE. Since 2008, the viral population has remained polymorphic, with a dynamic evolution.

Keywords: HIV, HIV-1 subtypes, Constanta

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Introduction

The HIV pandemic is one of the greatest challenges humanity has faced over the last 40 years, the dynamics of this type of infection being directly related to humans as a vector in the transmission and dissemination of the disease. In Romania, the epidemiological situation is a special one. A first aspect is that it is an F1 subtype enclave surrounded by different viral subtypes, A and B ones. Also, the outbreak described in the pediatric population at the beginning of the '90s is a special feature of Romania in the context of the evolution of the HIV infection at a worldwide level. In Constanta, the level of the epidemic is ahead of the rest of the country by at least one year as shown in Figure 1 [1].

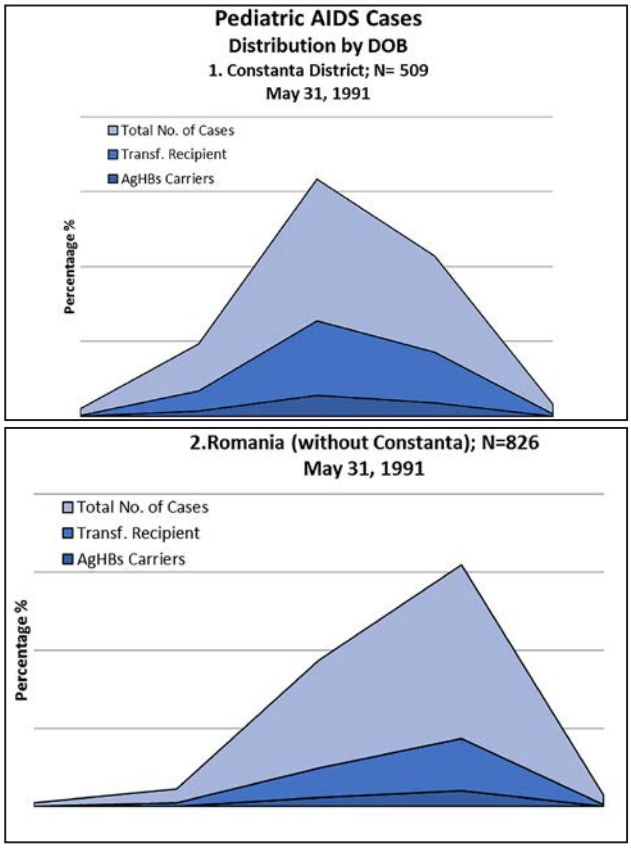

Figure 1. Distribution of HIV pediatric cases in Constanța and in Romania, 1991 (Source: Romanian Ministry of Health, May 31st, 1991) [1]

The transmission of the infection differs, percentage-wise, when comparing blood transfusion and maternal-fetal transmission in the two subgroups (Constanta and the rest of the country). Almost half of the Constanta cases (49.3%) were blood transfusion recipients and 9% were the result of maternal-fetal transmission, considering a number of 513 pediatric cases reported on 31 May 1991. In contrast, in the Romanian national subgroup, of 832 of pediatric cases, only 27.2% of children were transfused and the maternal-fetal transmission rate was only 3.1%, which explains the lesser "burden" in terms of HIV prevalence in the rest of the country. This data comes from the Ministry of Health reporting at the end of May 1991 and is expressed in Figure 2.

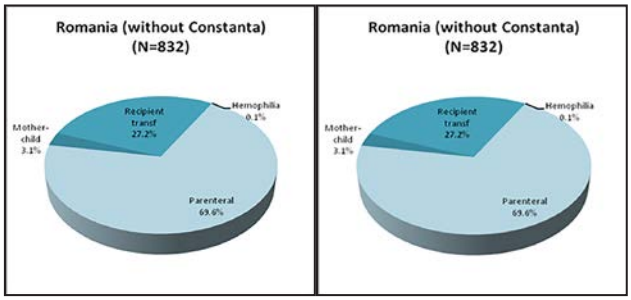

Figure 2. Route of Transmission for HIV pediatric cases (Source: Romanian Ministry of Health, May 31st, 1991)

Children infected between 1986-1990 are a local pediatric cohort, with some common features: first-year infection (either horizontally or vertically by mother-to-child transmission, between 1987 and 1990 [1]), F1 subtype in almost all cases [2], co-infection with hepatitis concomitant with HIV infection [3], almost equal sex distribution [4]. These patients were registered, treated according to national guidelines and benefited from active surveillance of the infection as well as of the associated conditions [5]. They have become long-term survivors who have experienced many therapeutic regimens and have started to show the phenomenon of "treatment fatigue" [4]. Also, they became sexually active.

Over the years, epidemic dynamics have changed, the heterosexual transmission path becoming the most important. As some of these patients come from among people working outside the country, we assume that the subtype population would have had to acquire new variants with different profiles of primary resistance. Also, in recent years, frequent cases of acute infection are detected in the MSM community, where an epidemic overlapping the global epidemic profile, described in the early 1980s, is outlined.

The main objective of this study is to determine the viral HIV-1 circulating subtypes in patients registered in the Center of Excellence Constanta, as there is little published data and much interest in this area (Constanta being a naval and airway gateway, a highly transitory tourist area, with great population mobility).

Material and Method

A number of 388 patients of the HIV Center of Excellence were included, treatment-naïve and ARV multiexperienced who have had detectable HIV viral loads (HIV-RNA 1000 copies/ml) that allowed genotyping analysis.

The genotypes were determined in the Laboratory of Molecular Genetics of the National Institute of Infectious Diseases "Prof. Dr. Matei Balş" in Bucharest, at the Institute of Virology "Ştefan S. Nicolau" in Bucharest and in the Quest Diagnostics, Monogram Bioscience, and Virco laboratories. The complete sequences that have been obtained, representing the entire PR gene and two-thirds of the RT gene, were generated in the Fasta format. HIV-1 subtyping was performed on the basis of the HIV-1 & 2 REGA algorithm.

The data was organized and mathematically structured in MySQL databases. The BASH and "make" computer applications were used to manage the project, and M.A.W.K. was used to recognize patterns in the database. For statistical calculations, the R-Project software was used. Graphic representations were performed using GNUPLOT computer application.

Results

HIV strains of 388 patients, both naïve and experienced, were interpreted. Their distribution was based on the year of diagnosis of HIV infection so that we can obtain a more accurate picture of circulating subtypes over time. The results are in Figure 3.

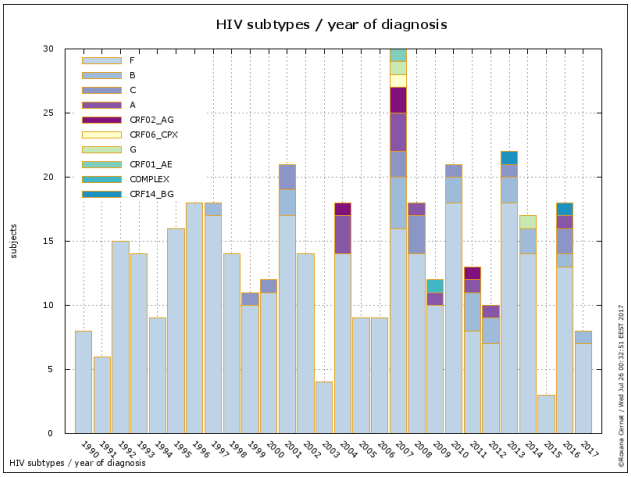

Figure 3. Characterization of HIV Sub-types in Constanta/year of diagnosis

By summing up the studied groups (the group of 153 naïve patients and the group of 235 multiexperienced patients), the dynamics of viral subtypes were analyzed from the earliest cases up to 2017. A total of 388 HIV strains were interpreted and, as expected, the dominant subtype is F1. If sporadic subtype B strains occur before 1999, these strains, C and B, appear systematically in a small number after that year. In 2004, the first subtype A strains were isolated and it is the moment when the circulating recombinant forms are introduced as CRF02_AG. The year 2007 is the year with the highest biodiversity: besides F1, there are cases with subtypes C, A, B, G, as well as the circulating recombinant forms CRF02_02, CRF06_cpx and CRF01_AE. Since 2008, the viral population remains polymorphic, but with the predominance of the F1 subtype, which predicts in the future years a more active dynamic, with the emergence of minority subtypes at the expense of the majority strain.

In the case of naïve patients identified between 2007-2017, the most common viral subtype that was isolated was F1 in 114 subjects (74.51%), non-F subtypes representing approximately one-fourth of the viral population (Figure 4). Subtype B was found in 15 patients, subtype C in 8 patients, subtype A in 7 patients, subtype G in 2 patients and the circulating recombinant forms CRF02_AG (2), CRF14_BG (2), CRF01_AE (1), CRF06_CPX (1) plus a CRF form that could not be determined (Figure 5). As most

infections were acquired in Romania, most non-F viral subtypes came from sources located in other countries. Subtype B is present in patients who worked or have traveled on the European continent (Spain 5 cases, Italy 3 cases, Germany and the Netherlands one case), subtype C is "imported" either from Africa or from Latin America (Venezuela or Brazil), as well as subtype A1. Subtype G occurs in 2 patients, the countries of origin being Nigeria and England, respectively.

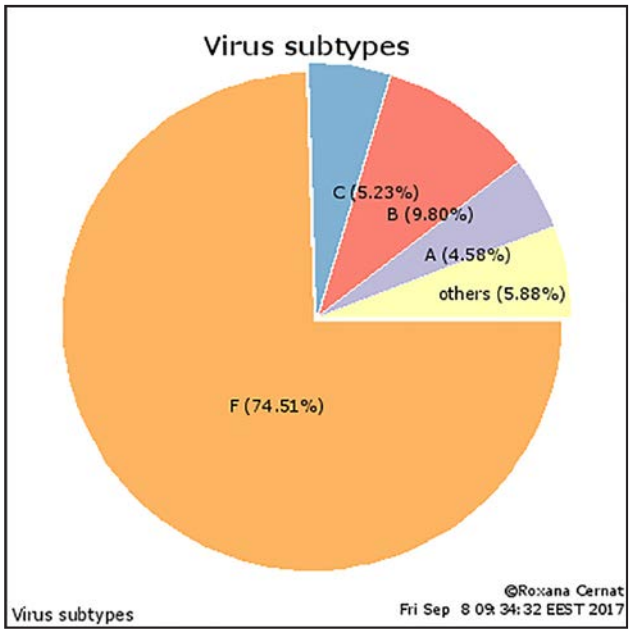

Figure 4. HIV-Subtypes in naive patients

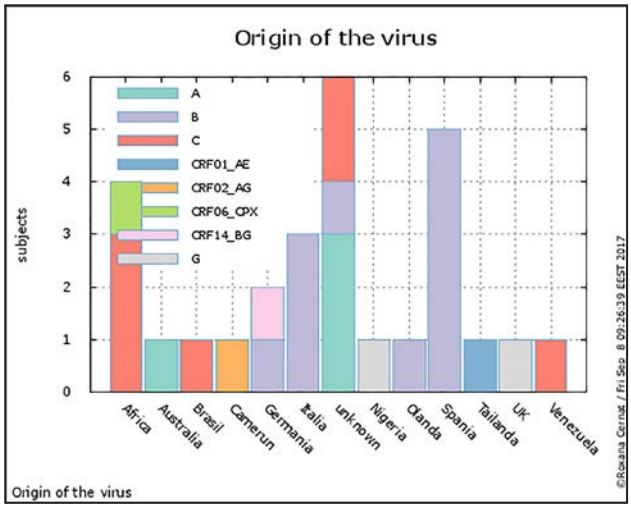

Figure 5. The origin of non-F HIV Subtypes in naïve population

It should be noted that these minority subtypes are also present in some cases of subjects who have never left Romania (A in 4 patients, B in 5 patients, C in 3 patients), hence the infection was transmitted locally, most likely from a source that has been infected outside the country. Of those who have never left the country, three cases are women, partners of people working abroad, while the rest are found in the MSM community.

Most of the naïve group of patients, diagnosed from 2007 to 2017, recognize the sexual transmission (91.5%), a small percentage coming from the pediatric cohort (8.50%). In the case of sexual transmission, the source of the infection was known to an important percentage (35.29%).

In the case of multiexperienced HIV patients, the viral population is clearly dominated by the F1 subtype. The explanation is related to the fact that these patients have had an old infection, in most cases discovered in the early 1990s. The lot includes patients from the pediatric cohort, who were either horizontally or vertically infected. As 17 cases recognize maternal-fetal transmission before the end of the 1990s, this indicates that the F1 virus was already circulating in the adult population before the onset of the epidemiological accident (Figure 6).

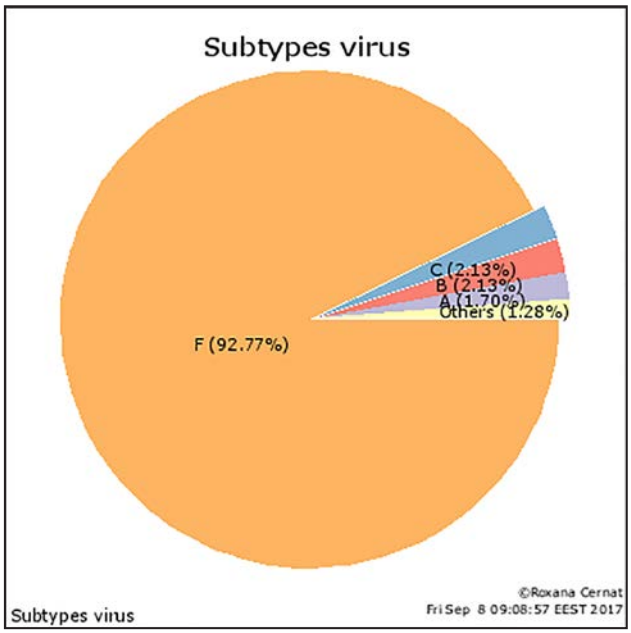

Figure 6. HIV-Subtype in multiexperienced patients

In addition to the dominant F1 subtype, other subtypes were rarely isolated: B, C (5 cases each) and A (4 cases). Also, there are 2 cases of circulating recombinant forms: CRF01_AE. Interestingly, 1 of them recognize maternal-fetal transmission.

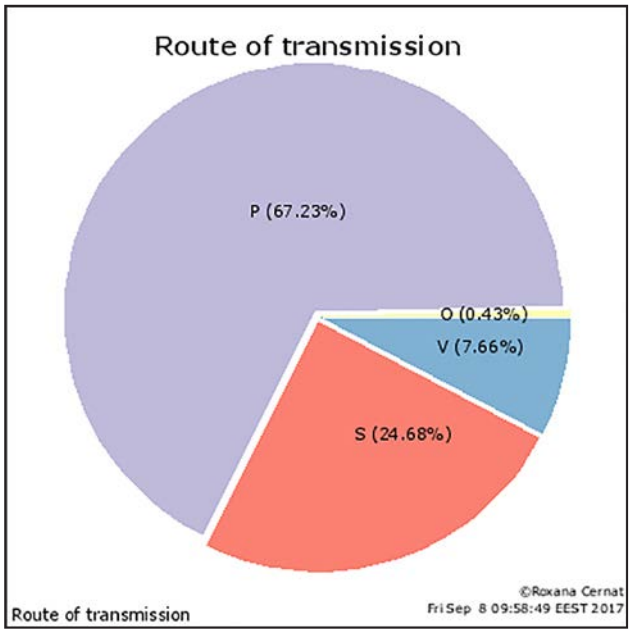

Figure 7. Infection route of transmission in multiexperienced patients

Discussion

Emerging in the late 1980s, the HIV epidemic in Constanta is staggering due to the significant number of infected children, both horizontally and vertically [6]. This epidemiological accident has generated a unique pediatric cohort in the world and in Romania, the F1 subtype being the incriminated one [7], a subtype that phylogenetically originates in Angola.

However, there is data supporting a biodiversity of subtypes in Constanta. In 1999, Apetrei et al. [8] isolated the subtypes A and B in addition to the dominant F subtype from a small group of 34 children and 8 adults.

In a retrospective study of blood donors in Constanta during 1990-1997 [9], the authors identified 25 HIV-1 cases, subtracting 18 strains. 14 of the donors having the F1 subtype, while the remaining presented the subtypes A, B, C and G (one each case).

At national level, a survey conducted by virologists in the Reference Laboratory of the National Institute of Infectious Diseases Prof. Dr. Matei Bals [9] on a number of 2132 isolated sequences between 2003-2012 from patients in different regions of Romania identified the F1 subtype as dominant (91%); the rest of the isolated strains being B (4%), C (3%) and other subtypes (2%). Among these minority subtypes there were: A, G as well as the circulating recombinant forms CRF01_AG, CRF14_BG, CRF01_AE, CRF06_cpx, CRF12_BF, and other complex forms. As most of the strains from our patients were processed in the same reference laboratory, part of these results is included in these national results.

Our study attempts to evaluate the circulation of viral subtypes of the HIV epidemic in Constanta, from the beginning of the epidemic to the present. 388 isolates were genotyped and subtyped generating an overview of the local epidemiological situation.

The "national" F1 subtype dominates the viral circulation in Constanţa (85.56%). The reason comes from the multiexperienced group analysis: a large number of patients are coming from the pediatric cohort. Given that these patients have reached adulthood, they have become potential sources, generating secondary cases of sexual transmission. Thus, the epidemic was generated in a monoclonal manner, thereafter spreading via sexual transmission.

At the beginning of the epidemic, other subtypes were found sporadically: B and C, and then A. None of these subtypes succeeded in spreading in the population compared to subtype F1.

Infections among patients diagnosed over the past 10 years have shown a dynamic of the viral population. The year of 2007 is the first where, besides pure viral subtypes, many recombinant circulating forms occurred. The explanation lies in the number of subtype strains, which is high in 2007, and as a consequence, the results brought more data on the circulation of these viruses (compared to 2003 and

2015 when the small number of genotyped strains surprised only the major strain). Another explanation would be population mobility, which is most often of occupational nature. The route of transmission in these cases is predominantly sexual. Most non-F subtypes isolated in the batch of naive patients are imported: subtype B comes from Europe, C and A from Africa or Latin America. Subtype G is rarely isolated but is to be considered, as patients infected with it show an accelerated progression of the disease. It is notable the occurrence of these subtypes in population from Constanta who have never left Romania, hence the HIV infection was acquired locally, from sources that were infected abroad.

Furthermore, the circulating recombinant subtypes are diverse, occurring in sexually transmitted cases as well as in 1 vertical case. Most are CRF02_AG, followed by CRF14_BG, CRF01_AE, and CRF06_cpx, and there is one unidentifiable subtype. They come from diverse areas: Europe (especially CRF14_BG, the form encountered in communities of intravenous drug users, CXCR4 tropic strains that imply a rapid progression of the disease), Africa, SE Asia. All of these new cases with sexual transmission point to the fact that prevention means in Constanta are inadequate and that their use is not a rule, even in couples where one of the partners has a known HIV status.

Conclusions

The HIV epidemic in Constanta started as a monoclonal extension of the F1 subtype, but in time we are witnessing a diversification of the subtypes, at this moment there are circulating alongside F1, whose prevalence is maintained, subtypes B, C, A, G, as well as various recombinant forms such as CRF01_AE, CRF 02_AG, CRF06_cpx, CRF14_BG.

The implementation of prevention programs in order to limit the spread of the epidemic and the close monitoring of the circulation of these subtypes is recommended.

References

1. Popovici, F., Zolotușca, L., Apetrei, R.C., Beldescu, N., Hersh, B. & Heymann, D. (1991). The Epidemiology of AIDS in Romania. *Seventh International Conference on AIDS*, Florence, Italy, 16 – 21 June 1991
2. Coul, E. O. D., Burg, R. V. D., Asjo, B., Goudsmit, J., Cupsa, A., Pascu, R., Usein, C. & Cornelissen, M. (2000). Genetic evidence of multiple transmissions of HIV type 1 subtype F within Romania from adult blood donors to children. *AIDS research and human retroviruses*, *16*(4), 327-336. DOI:10.1089/088922200309205
3. Ruta, S. M., Matusa, R. F., Sultana, C., Manolescu, L., Kozinetz, C. A., Kline, M. W., & Cernescu, C. (2005). High prevalence of hepatitis B virus markers in Romanian adolescents with human immunodeficiency virus infection. *Journal of the International AIDS Society*, *7*(1), 68. DOI: 10.1186/1758-2652-7-1-68
4. Streinu-Cercel, A. (2013). Specific Challenges of the HIV Epidemic in Romania. *EACS Conference Brussels.*
5. Surveillance Report: HIV/AIDS Surveillance in Europe 2013. ECDC Stockholm, http://www.ecdc.europa.eu/en/publications/Publications/hiv-aids-surveillance-report-2012-20131127.pdf
6. Guimarães, M. L., Vicente, A. C. P., Otsuki, K., da Silva, R. F. F., Francisco, M., da Silva, F. G., Serrano, D., Morgado, M.G. & Bello, G. (2009). Close phylogenetic relationship between Angolan and Romanian HIV-1 subtype F1 isolates. *Retrovirology*, *6*(1), 39. DOI:10.1186/1742-4690-6-39
7. Apetrei, C., Necula, A., Holm-Hansen, C., Loussert-Ajaka, I., Pandrea, I., Cozmei, C., Streinu-Cercel, A., Pascu, F.-R., Negut, E., Molnar, G., Duca, M., Pecec, M., Brun-Vézinet, F. & Simon, F. (1998). HIV-1 diversity in Romania. *AIDS, 12*(9), 1079-1085.

8. Cambrea, C.S., Rugină, S., Vinteanu, I., Incidența Infecției HIV la donatorii de sânge din județul Constanța [HIV Infection Prevalence in Blood Donors in the Constanta County], *Dobrogea Medicală*, Year I nr 1
9. Simona, P., Ionelia, B., Leontina, B., Mihaela, F., Angelica, D., Vizitiu, I., & Otelea, D. (2012). Recombination analysis for subtyping unclassified hiv-1 sequences from romania. *Therapeutics, Pharmacology & Clinical Toxicology*, *16*(3), 163-167.