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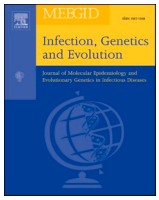

Molecular epidemiology of HIV among people who inject drugs after the HIV-outbreak in Athens, Greece: Evidence for a 'slow burn' outbreak

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ABSTRACT

Background: New diagnoses of HIV-1 infection among people who inject drugs (PWID) in Athens, Greece, saw a significant increase in 2011 and a subsequent decline after 2013. Despite this, ongoing HIV-1 transmission persisted from 2014 to 2020 within this population. Our objective was to estimate the time of infection for PWID in Athens following the HIV-1 outbreak, explore the patterns of HIV-1 dispersal over time, and determine the duration from infection to diagnosis.

Methods: Time from HIV-1 infection to diagnosis was estimated for 844 individuals infected within 4 PWID-specific clusters and for 8 PWID infected with sub-subtype A6 diagnosed during 2010–2019. Phylogeny reconstruction was performed using the maximum-likelihood method. HIV-1 infection dates were based on molecular clock calculations.

Results: In total 86 of 92 (93.5%) sequences from PWID diagnosed during 2016–2019 were either related to the previously identified PWID-specific clusters ($n = 81$) or belonged to a new A6 cluster ($n = 5$). The median time between infection and diagnosis was 0.42 years during the outbreak period and 0.70 years during 2016–2019 ($p < 0.001$). The proportion of clustered sequences from PWID was very low at 5.3% during the pre-outbreak period (1998–2009), saw an increase to 41.7% one year before the outbreak in 2010, and consistently remained high during the whole period after 2011, spanning the post-outbreak period (2016–2019) with a range from 92.9% to 100%.

Conclusions: The substantial proportion of clustered infections (93.5%) during 2016–2019 implies a persistent 'slow burn' HIV outbreak among PWID in Athens, suggesting that the outbreak was not successfully eliminated. The consistently high proportion of clustered sequences since the onset of the outbreak suggests the persistence of ongoing HIV-1 transmission attributed to injection practices. Our findings underscore the importance of targeted interventions among PWID, considering the ongoing transmission rate and prolonged time from infection to diagnosis.

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1. Introduction

It is estimated that, globally, 11.3 million people inject drugs (Harm Reduction International, 2020; United Nations, 2020). People who inject drugs (PWID) are among the key populations for HIV infection (UNAIDS, 2018). In 2021, the prevalence of HIV among PWID is estimated to be 12.6% in the European Union/European Economic Area (EU/EEA) (WHO Regional Office for Europe, European Centre for Disease Prevention and Control, 2022). In addition, the proportion of new HIV diagnoses among PWID in the EU/EEA was 3.5% (WHO Regional Office for Europe, European Centre for Disease Prevention and Control, 2022).

According to the National Public Health Organization surveillance report, the proportion of new HIV diagnoses among PWID in 2022 in Greece was approximately 11.9% (National Public Health Organization, 2023). This proportion was among the highest in Western Europe and only in Eastern European countries it was higher than in Greece (WHO Regional Office for Europe, European Centre for Disease Prevention and Control, 2022). The overall number of diagnoses among PWID has declined since 2012 in Europe, except for occasional increases associated with outbreaks in some countries. In the last 10 years, HIV-1 outbreaks in PWID have been reported for Athens (Greece) (Hatzakis et al., 2015; Paraskevis et al., 2011; Paraskevis et al., 2013; Sypsa et al., 2017), Bucharest (Romania) (Botescu et al., 2012; Hedrich et al., 2013; Paraskevis et al., 2015), Dublin (Ireland) (Giese et al., 2015), Glasgow (Scotland) (McAuley et al., 2019; Ragonnet-Cronin et al., 2018), Luxembourg (Arendt et al., 2019), and Thessaloniki, (Greece) (Sypsa et al., 2023). Additionally, a few outbreaks have been detected in North America and Israel including Saskatchewan (Canada) (DesJarlais et al., 2020), Scott County (USA) (Broz et al., 2018; Peters et al., 2016), and Tel Aviv (Israel) (Katchman et al., 2017). Molecular investigation suggested that in all cases, HIV sequences sampled from PWID had high genetic similarity suggesting that during the outbreaks HIV transmissions occur mostly among PWID (Paraskevis et al., 2011; Paraskevis et al., 2013; Paraskevis et al., 2015; Ragonnet-Cronin et al., 2018; Arendt et al., 2019; Kostaki et al., 2017).

In Greece, there was a notable surge in new diagnoses of HIV-1 infection among PWID in 2011. The trend continued, and from 2011 to 2013, Athens, the capital city of Greece, experienced a substantial HIV outbreak within the PWID community (Paraskevis et al., 2011; Paraskevis et al., 2013). This event marked the largest recent HIV outbreak in PWID in Europe and North America (DesJarlais et al., 2020). Molecular surveillance studies during the early stages of the outbreak revealed the existence of 4 major PWID-specific phylogenetic clusters of sub-subtype A1, subtype B, and circulating recombinant forms (CRFs) CRF14_BG and CRF35_AD (Paraskevis et al., 2011; Paraskevis et al., 2013). An updated molecular epidemiology study on a dense sample of PWID diagnosed during the outbreak period estimated the transmission dynamics for the 4 PWID-specific clusters and revealed a significant reduction in the number of HIV-1 infections in all of them after 2013 (Kostaki et al., 2017). HIV incidence decreased rapidly from 7.8/100 person-years (PYs) in August–December 2012 to 1.7/100 PYs in August–December 2013 (Sypsa et al., 2017). This decline could be explained by the expanded coverage of harm reduction programmes. Specifically, ARISTOTLE programme played a major role by providing HIV testing and linkage to care to a high proportion of the PWID population (Hatzakis et al., 2015; Sypsa et al., 2017; Flountzi et al., 2022). Additionally, other interventions, such as the TRIP programme (Nikolopoulos et al., 2016), took place in Athens metropolitan area for the control of the outbreak.

Although the number of newly HIV-1 diagnosed PWID in Greece declined after 2013, it has not yet reached the pre-outbreak levels (https://eody.gov.gr/). Furthermore, a recent study revealed that HIV prevalence in repeatedly tested PWID in Athens increased from 14.2% in 2012–2013 to 22.0% in 2018–2020, and that HIV incidence remained at stable moderate levels, similar to these reached in the second half of 2013 (Roussos et al., 2022). Given these findings, our objective was to estimate the HIV-1 acquisition dates of newly diagnosed PWID in Athens (2016–2019). Also, we aimed to explore the patterns of HIV-1 dispersal over time, to infer the duration from HIV-1 infection to diagnosis, and to examine potential trends in that timeframe for PWID diagnosed in the recent years (2016–2019) compared to those diagnosed during the outbreak period (2011–2014).

2. Material and methods

2.1. Study population

Our study population consisted of 777 people living with HIV (PLHIV) infected within the 4 major PWID-specific phylogenetic clusters who were diagnosed during 2010–2014, and 92 PWID diagnosed in the recent years (2016–2019) for whom nucleotide sequences were available. The latter number accounts for 23.1% (92 of 399) of all PWID diagnosed with HIV during 2016–2019 in Greece (surveillance data were available from the National Public Health Organization), although we note that for 2016 and 2019 only a few sequences were available (4 of 92, 4.4%). For 2017–2018, our data (88 of 92, 95.6%) correspond to 41.7% (88 of 211) of the total PWID cases diagnosed in Greece. The 92 PWID diagnosed between November 2016 and January 2019 in Athens were individuals who participated either in the ARISTOTLE HCV-HIV community-based programme conducted during 2018–2020 (Roussos et al., 2022) ($n = 41$, 44.6%) or in the HIV-1 genetic surveillance study of newly diagnosed cases ($n = 51$, 55.4%). The PWID-specific clusters and the 777 PLHIV infected within these clusters have been described in detail elsewhere (Kostaki et al., 2017). Briefly, previous phylogenetic analyses showed high levels of regional transmission among PWID in Athens and the existence of 4 major PWID-specific phylogenetic clusters of CRF14_BG, CRF35_AD, subtype B, and sub-subtype A1. These clusters consisted of PWID ($n = 741$, 95.4%) and a few non-PWID ($n = 36$, 4.6%) diagnosed in Athens during August 2010–December 2014.

The study was approved by the Ethics committee of the Medical School of the National and Kapodistrian University of Athens (1617006491/25-10-2016, 1718025226/24-04-2018). Eligible participants were asked to provide written informed consent. All experiments were performed in accordance with relevant guidelines and regulations.

2.2. HIV-1 subtyping and phylogenetic analysis

HIV-1 subtypes were determined for the 92 nucleotide sequences available in the *pol* gene (protease and partial reverse transcriptase regions generated as previously described (Rodger et al., 2016; Rodger et al., 2019)) of the newly diagnosed PWID by using different approaches. First, preliminary subtyping was performed by using the online automated REGA HIV-1 Subtyping Tool - Version 3.0 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/). Second, the sequences' subtype was determined by analyzing phylogenetically the 92 sequences under study along with 332 reference sequences by using the approximate maximum likelihood method (GTR + cat) as implemented in FastTree v2.1 program (Price et al., 2010). In more detail, we used as references: i) 216 global sequences representative of all pure HIV-1 subtypes, sub-subtypes and most of the CRFs (Los Alamos HIV-1 sequence database, http://www.hiv.lanl.gov), ii) 48 of the 777 sequences of the 4 major PWID-specific phylogenetic clusters of sub-subtype A1, subtype B, CRF14_BG, and CRF35_AD, which were selected randomly, and iii) 68 global sequences identified by the HIV BLAST tool (https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html) as the most genetically similar sequences to the sequences under study. As previously described, the 4 major PWID-specific clusters were defined as phylogenetic clusters consisting of sequences from PWID at a proportion >75% compared to the total number of sequences within each cluster and receiving Shimodaira-Hasegawa values higher than 0.95 (Kostaki et al., 2017). Third, a supplementary phylogenetic analysis (utilizing GTR + cat in FastTree v2.1 program)

was conducted on a subset of sequences ($n = 8$). These sequences were initially classified as sub-subtype A1 by the REGA tool, but they neither clustered within the sub-subtype A1 PWID-specific phylogenetic cluster nor displayed characteristics of recombinant forms incorporating partial genomic fragments from this cluster. In the context of this analysis, we used as references a random set of global subtype A sequences available in the Los Alamos HIV-1 sequence database ($n = 1500$), all the available subtype A sequences sampled in Greece ($n = 1992$), and the most genetically similar sequences identified by the HIV BLAST tool ($n = 18$). Finally, for all sequences remained unclassified at the previous steps of the analysis, we used the RDP4 (Martin et al., 2010) and SimPlot v3.5.1 (bootscanning approach) (Lole et al., 1999) programs to test them for the presence of recombination.

We used MEGA X program (Kumar et al., 2018) for the sequence alignment (MUSCLE algorithm) and editing, and for the estimation of the pairwise genetic distances (Tamura-Nei model) within each one of the 4 major PWID-specific phylogenetic clusters. FigTree v1.4.3 program (http://tree.bio.ed.ac.uk/software/figtree/) was used for trees' visualization and annotation.

2.3. Phylodynamic analysis

Molecular clock analysis was used for the estimation of the HIV-1 acquisition dates (infection dates) of the individuals infected within the 4 PWID-specific phylogenetic clusters and of PWID infected with sub-subtype A6. Analysis was performed separately on each subtype/CRF (sub-subtype A1, sub-subtype A6, subtype B, CRF14_BG, CRF35_AD), and it was based on the Bayesian method. Specifically, we used the GTR + G as a nucleotide substitution model, an uncorrelated lognormal relaxed clock model with TipDates, and the birth-death basic reproductive number models in BEAST v1.8.0 program (Drummond et al., 2012). To increase the sampling window of the sub-subtype A6 sequences ($n = 8$), we included in the analysis the most closely related sequences to them ($n = 16$) according to the structure of the phylogenetic tree. We did not use informative priors for the Markov chain Monte Carlo (MCMC) runs. The MCMC analysis was run for 40–50 $\times$ 10^6 generations and sampled every 4–5000 steps (burn-in: the first 10% of the samples). The program Tracer v1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/) was used to check the MCMC convergence, the time to the most recent common ancestor (t_{MRCA}) and if the Effective Samples Sizes (ESS) were > 200. The maximum clade credibility tree was produced by the TreeAnnotator v1.8.0 program (Drummond et al., 2012).

Furthermore, we estimated the time interval between the HIV-1 infection and diagnosis dates of the individuals infected within the 4 PWID-specific phylogenetic clusters (i.e., 777 PLHIV diagnosed during 2010–2014 and 67 PWID diagnosed during 2016–2019), and of the 8 PWID infected with sub-subtype A6 who were diagnosed during 2017–2018. The infection date was approximated to the median estimate of the t_{MRCA} between the most closely related sequences of the dated tree estimated by molecular clock analysis (Fig. 1). The sampling date of the first available sample for each individual was used as a proxy for the diagnosis date since it corresponds either to the first visit of the individual to an HIV/Infectious Diseases Unit in Greece or to the sampling date of the first available sample at diagnosis as part of the HIV intervention programmes on PWID.

2.4. Statistical analysis

We used median with 25th–75th or 10th–90th percentiles (continuous variables) and absolute with relative frequencies (categorical variables), to summarize the study data. The Shapiro-Wilk test was used to examine normality. Statistical analysis was carried out using the non-parametric Mann-Whitney *U* test. The level of significance was set to 0.05. Analysis was performed in Stata 14.2-StataCorp LLC software.

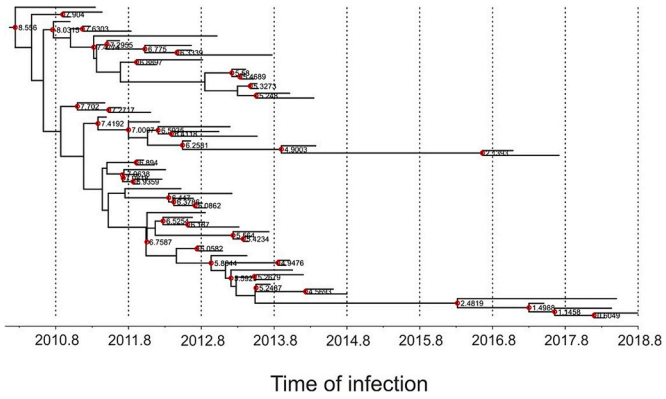

Fig. 1. Dated tree of the sub-subtype A1 PWID-specific phylogenetic cluster, estimated by molecular clock analysis that was based on the Bayesian method as implemented in BEAST v1.8.0 program.

3. Results

3.1. HIV-1 subtyping results and phylogenetic clusters

Subtyping analysis was performed for the 92 sequences of the newly diagnosed PWID (2016–2019) and showed that most of these sequences ($n = 67$, 72.8%) clustered within the 4 PWID-specific phylogenetic clusters identified previously: CRF14_BG: 39, 58.2%; CRF35_AD: 17, 25.4%; sub-subtype A1: 6, 8.9%; subtype B: 5, 7.5%. The distribution of the recently sampled sequences (2016–2019) among the PWID-specific clusters was almost the same as the distribution of sequences sampled during the outbreak period (2011–2014) (Kostaki et al., 2017). Moreover, unique recombinant forms (URFs) including partial genomic fragments from the PWID-specific clusters were detected among the 92 sequences ($n = 14$, 15.2%). Thus, 81 of 92 (88.0%) sequences of our study dataset were found to be related to the previously identified clusters of PWID. The remaining 11 of 92 (12.0%) sequences were categorized as sub-subtype A6 ($n = 8$), CRF01_AE (n = 1), CRF02_AG (n = 1), and recombinants forms of the virus (n = 1).

The additional phylogenetic analysis which resulted in the classification of 8 study sequences as sub-subtype A6, revealed the existence of a new phylogenetic cluster consisting of 5 (62.5%) A6 sequences from PWID diagnosed in 2018. Therefore, a total of 86 of 92 (93.5%) sequences from newly diagnosed PWID were either linked to the previously identified PWID-specific clusters ($n = 81$) or belonged to newly identified PWID clusters ($n = 5$). According to the phylogenetic tree, the most closely related sequences to the 5 clustered A6 sequences were 2 sequences from PWID diagnosed in Athens in 2014. Furthermore, 2 of 5 (40.0%) sequences within the new A6 cluster were from migrant PWID.

3.2. Analysis of clustering patterns among HIV-1 sequences from PWID over a span of > 20 years (1998–2019)

We conducted a more in-depth analysis of the proportion of clustered sequences from PWID, categorizing them as sequences belonging to the previously identified PWID-specific clusters, sequences categorized as URFs with partial genomic fragments from these clusters, or sequences belonging to the newly identified PWID A6 cluster. Interestingly, the percentage of clustered sequences from PWID remained consistently high throughout the period after 2011, encompassing the post-outbreak years (2016–2019) with a range from 92.9% to 100.0% (Table 1, Fig. 2). In contrast, this percentage was notably low (5.3%) during 1998–2009, increased to 41.7% one year before the outbreak (2010), and has since maintained very high levels (Table 1, Fig. 2).

Table 1
People who inject drugs (PWID) diagnosed with HIV in Greece, and available and clustered sequences from PWID per year or period of sampling.

Sampling year/period	Number of diagnosed PWID (NPHO)[1]	Number (%) of available sequences (sampling coverage)[2]	Number (%) of clustered sequences[3]
1998–2009	329	76 (23.1)	4 (5.3)
2010	28	12 (42.9)	5 (41.7)
2011	319	144 (45.1)	134 (93.1)
2012	524	312 (59.5)	292 (93.6)
2013	270	314 (>100)[4]	290 (92.4)
2014	120	104 (86.7)	92 (88.5)
2015	95	–	–
2016	100	3 (3.0)	3 (100.0)
2017	92	28 (30.4)	26 (92.9)
2018	119	60 (50.4)	56 (93.3)
2019	88	1 (1.1)	1 (100.0)
2020	86	–	–
2021	86	–	–

PWID, People Who Inject Drugs; NPHO, National Public Health Organization.
[1] This number corresponds to the total number of HIV diagnoses among PWID since the beginning of the HIV epidemic in Greece.
[2] This number ($N = 1054$) corresponds to the total number of available HIV-1 sequences from PWID during 1998–2019 in Greece and accounts for 50.6% (1054 of 2084) of all PWID diagnosed with HIV-1 during 1998–2019 in Greece.
[3] This number corresponds to the number of sequences from PWID belonging to the previously identified PWID-specific phylogenetic clusters (A1, B, CRF14_BG, CRF35_AD), being unique recombinant forms including partial genomic fragments from these clusters, or belonging to the new A6 cluster.
[4] Given that the first samples were taken a few months after HIV-1 diagnosis, the latter preceded the sampling dates and for this reason for 2013 the number of available sequences was higher than the corresponding HIV-1 cases diagnosed in that year.

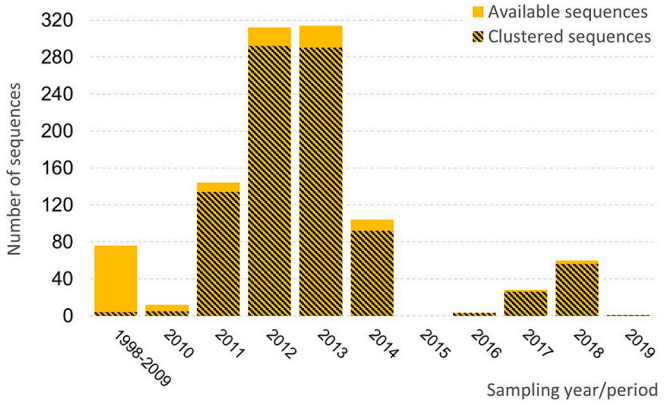

Fig. 2. Available and clustered sequences from people who inject drugs (PWID) per year or period of sampling. Clustered sequences defined as sequences belonging to the previously identified PWID-specific phylogenetic clusters (A1, B, CRF14_BG, CRF35_AD), sequences being unique recombinant forms including partial genomic fragments from these clusters, or sequences belonging to the new A6 cluster.

3.3. HIV-1 infection dates and time from infection to diagnosis

Molecular clock analysis of all sequences clustered within any of the PWID-specific phylogenetic clusters (including the newly identified A6 PWID-cluster) revealed that 734 of 852 (86.2%) individuals were infected during the outbreak period (2011–2014) and 61 of 852 (7.2%) in the recent years (2016–2019) (Fig. 3). During the outbreak, there was a significant decrease in new HIV-1 infections after 2012, with a notable decline in new diagnoses observed from 2013 onwards (Fig. 3). Although no time trend in new HIV-1 infections (incidence trend) was observed after 2015 (Fig. 3), there was an increase in the number of new HIV-1 infections (incidence) for CRF14_BG and sub-subtype A6 during 2016–2018, contrasting with other subtypes/CRFs. The distribution of new HIV-1 infections per subtype/CRF and estimated year of infection is presented in Table 2.

Furthermore, a significant increase was found for the time interval between HIV-1 infection and diagnosis during 2016–2019 versus the outbreak period. The median time from infection to diagnosis was 0.42 (25th, 75th: 0.22, 0.76) years during the outbreak period and 0.70 (25th, 75th: 0.33, 1.89) years during 2016–2019. The above difference in time from infection to diagnosis was significantly longer in 2016–2019 compared to the outbreak period ($p < 0.001$).

3.4. Analysis based on HIV-1 genetic distances

We further analyzed the pairwise genetic distances within each one of the 4 PWID-specific phylogenetic clusters to assess whether HIV-1 infections within the clusters are linked. The median genetic distances along with the 10th and 90th percentiles are presented in Table 3. The median distances for each cluster during the outbreak period for sub-subtype A1, CRF35_AD, and CRF14_BG were very low and consistently lower than 0.02 substitutions/site which is considered as a threshold for individuals who are linked (Hassan et al., 2017). The 90th percentile for these three clusters also remained lower than 0.02. In contrast, for subtype B, the distances were higher with the 90th percentile being 0.0234 in 2013. Over the period from 2011 to 2014, there was an increase in the genetic distances. The median distances remained low also in the period between 2016 and 2019 following a similar pattern with subtype B pairwise distance to be the highest compared to the other three clusters. In the recent period, all distances remained lower than 0.02 except for subtype B which slightly exceeded this threshold. Additionally, we estimated the genetic divergence between the sequences sampled in 2016–2019 and those from the outbreak period, and distances remained low. For the clusters with the highest number of infections (CRF14_BG and CRF35_AD), there was an increase in distances for the years 2013 and 2014 compared to the first two years of the outbreak (2011 and 2012).

4. Discussion

Our analysis revealed that 93.5% of HIV sequences from PWID diagnosed in Athens during 2016–2019, the period following the rapid decline in HIV incidence observed in 2013, belonged to PWID-specific phylogenetic clusters. Primarily, these infections either originated from previous outbreak clusters or were identified as recombinants with outbreak viruses. A minority of these sequences fell within a newly identified sub-subtype A6 phylogenetic cluster. Furthermore, differences in the incidence were observed among the PWID clusters, and specifically, a higher incidence was found for CRF14_BG and sub-subtype A6 during 2016–2018 compared to the other variants. Previously, there were more transmissions within sub-subtype A1 and CRF35_AD clusters at the early stages of the outbreak, and subtype B and CRF14_BG infections increased later (Kostaki et al., 2017). Sub-subtype A6 provided a new introduction, and the differences in transmissions within the clusters over time reflect, probably, variations in risk behaviours of PWID during the study period.

It was found that the proportion of clustered infections remained consistently high, exceeding 92%, during the post-outbreak period (2016–2019). In contrast, during the pre-outbreak period (1998–2009), only 5.3% of the sequences from PWID belonged to PWID clusters, suggesting that HIV infection during this period was likely attributable to sexual rather than injection practices (Paraskevis et al., 2013). The elevated proportion of clustered infections from 2016 to 2019 indicates ongoing HIV transmission among PWID, likely due to changes in injection practices, differing from the patterns observed before the outbreak. These findings indicate an ongoing ‘slow burn’ HIV outbreak among PWID, underscoring the persistence of the virus in this highly vulnerable population despite efforts to eliminate it. This occurrence might be

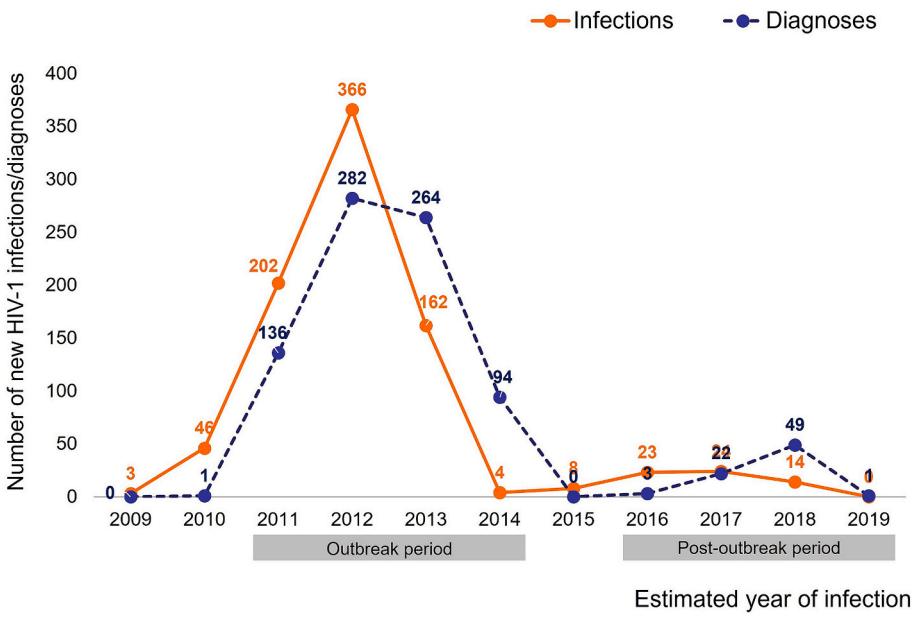

Fig. 3. Number of new HIV-1 infections and diagnoses for people who inject drugs (PWID) infected with sub-subtype A6 and individuals infected within the 4 major PWID-specific phylogenetic clusters (A1, B, CRF14_BG, CRF35_AD).

Table 2
New HIV-1 infections per estimated year of infection for people who inject drugs (PWID) infected with sub-subtype A6 and individuals infected within the 4 major PWID-specific phylogenetic clusters (A1, B, CRF14_BG, CRF35_AD).

Infection year (estimated)	HIV-1 subtype/CRF				
	A1	B	CRF14_BG	CRF35_AD	A6
2009	–	3	–	–	–
2010	5	7	8	26	–
2011	26	42	76	58	–
2012	10	54	269	33	–
2013	16	9	105	31	1
2014	2	–	2	–	–
2015	–	2	5	–	1
2016	3	1	10	7	2
2017	2	–	15	6	1
2018	1	1	5	4	3
Total	65	119	495	165	8

CRF, Circulating Recombinant Form.

Table 3
HIV-1 genetic distances at different years/periods for individuals infected within the 4 major PWID-specific phylogenetic clusters (A1, B, CRF14_BG, CRF35_AD).

	HIV-1 subtype/CRF			
	A1	B	CRF14_BG	CRF35_AD
Year/period	Median (PC 10th, 90th)	Median (PC 10th, 90th)	Median (PC 10th, 90th)	Median (PC 10th, 90th)
2011	0.0024 (0.0000, 0.0060)	0.0070 (0.0012, 0.0165)	0.0023 (0.0012, 0.0131)	0.0012 (0.0000, 0.0035)
2012	0.0035 (0.0000, 0.0059)	0.0093 (0.0012, 0.0175)	0.0035 (0.0000, 0.0117)	0.0023 (0.0011, 0.0303)
2013	0.0059 (0.0012, 0.0096)	0.0140 (0.0023, 0.0234)	0.0035 (0.0012, 0.0093)	0.0023 (0.0000, 0.0058)
2014	0.0030 (0.0012, 0.0048)	0.0141 (0.0061, 0.0189)	0.0058 (0.0023, 0.0105)	0.0035 (0.0012, 0.0058)
2016–2019	0.0059 (0.0021, 0.0122)	0.0219 (0.0052, 0.0284)	0.0093 (0.0008, 0.0155)	0.0070 (0.0023, 0.0169)
2011 & 2016–2019	0.0071 (0.0033, 0.0130)	0.0130 (0.0012, 0.0248)	0.0069 (0.0023, 0.0152)	0.0048 (0.0012, 0.0105)
2012 & 2016–2019	0.0060 (0.0024, 0.0107)	0.0139 (0.0024, 0.0235)	0.0069 (0.0023, 0.0153)	0.0058 (0.0012, 0.0187)
2013 & 2016–2019	0.0072 (0.0035, 0.0120)	0.0187 (0.0035, 0.0284)	0.0070 (0.0023, 0.0152)	0.0058 (0.0012, 0.0116)
2014 & 2016–2019	0.0066 (0.0024, 0.0119)	0.0165 (0.0061, 0.0248)	0.0081 (0.0024, 0.0153)	0.0058 (0.0023, 0.0106)

CRF, Circulating Recombinant Form; PC, Percentile.

attributed to diminished access to HIV testing, suboptimal coverage of needle and syringe programmes, and the deterioration of socioeconomic factors such as homelessness, which escalated from 16.2% in 2012–2013 to 25.6% in 2018–2020 (Roussos et al., 2022). Our observation about an ongoing 'slow burn' HIV outbreak was further supported by the low genetic distances observed within the PWID clusters during 2016–2019, suggesting that most HIV-1 transmissions are recent and genetically linked.

The sampling coverage in our study is approximately 42% for the period 2017–2018 during which most of the study sequences had been sampled. Previous analyses have shown that Sanger sequencing data can be used for the accurate estimation of HIV-1 infection dates for sequences belonging to molecular transmission clusters (Kostaki et al., 2021). Especially for PWID, it was found that the difference between the clinically estimated and the molecular clock inferred infection dates was the shortest, or the accuracy of the estimated dates was the highest compared to non-PWID data (Kostaki et al., 2021). In our study, the sampling coverage is high enough to assume that the accuracy would be similar as previously reported.

Our study offers a comprehensive analysis of HIV transmission patterns among PWID spanning more than two decades (1998–2019), utilizing HIV nucleotide sequences available from this population. Notably, the proportion of HIV sequences within PWID clusters, indicating transmission through injection drug use practices, remains persistently high even several years after the large outbreak in Athens. This finding is intriguing, especially when compared to the HIV dispersal patterns among PWID in New York City, the USA. Genetic analysis revealed that during the first three decades of the HIV epidemic in this

city, sequences from newly diagnosed PWID were more likely to be genetically linked to sequences from previously diagnosed PWID or MSM/PWID (Torian et al., 2022). This pattern changed; post-2002, the majority of transmission links for PWID were not with other PWID, and after 2011, PWID were no longer preferentially linked to their peers (Torian et al., 2022). Although there was evidence indicating a resurgence of genetic links among PWID sequences in 2018–2019, the study findings suggest a transition from parenteral to sexual transmission as the primary source of new HIV diagnoses among PWID in New York City (Torian et al., 2022). Contrastingly, in Athens, there has not been a shift in the transmission route. HIV prevalence remains elevated among PWID, and new transmissions continue to occur preferentially within this group. New York City did not experience an outbreak during the last 20 years and therefore the situation there was not similar to that in Athens; however, it provides one of the few molecular studies analyzing the patterns of HIV transmission among PWID over several decades (Torian et al., 2022). The study conducted in New York City and the present study conducted in Athens stand out as among the few that have employed molecular analyses over an extended time period. Notably, they reveal distinct patterns of transmission in the two cities.

Despite a decline in HIV incidence from 7.8 to 1.7 new cases per 100 PYs in 2012 and 2013, respectively, and subsequent stability [2014–2020: 1.94 (95% CI: 1.50–2.52) new cases per 100 PYs] (Roussos et al., 2022), our findings underscore that HIV continues to propagate within PWID-specific clusters, as during the outbreak peak (2012−2013). Specifically, we observed a nearly twofold increase in the time interval between HIV infection and diagnosis during 2016–2019 compared to the outbreak period, potentially attributed to reduced access to HIV testing. These findings were supported by the higher genetic distances within each cluster from 2016 to 2019 compared to the previous period (2011–2014), suggesting that the time interval between infection and diagnosis has increased. This delay raises concerns about an elevated risk of onward transmission. It's worth noting that from 2011 to 2013, a seek-test-treat programme (ARISTOTLE) with extensive coverage was implemented, reaching a significant proportion of the PWID population, and providing HIV testing along with linkage to HIV care (Hatzakis et al., 2015; Sypsa et al., 2017). The use of the sampling date of the first available sample for each individual as a proxy for the individual's diagnosis date might be a potential limitation of our study. On the other hand, it provides an indicator of the linkage to care, and based on our findings, the interval between infection and this specific date has increased over time.

Our findings imply a potential complacency in sustaining prevention and harm reduction programmes before eliminating the outbreak. Additionally, noteworthy figures, such as the time from infection to diagnosis - a proxy for the duration a PWID remains infectious -, indicate a worsening trend. The ongoing spread within this highly vulnerable population, coupled with the deterioration of socioeconomic characteristics such as homelessness and a notable shift in cocaine use (from 16.6% in 2012–2013 to 28.1% in 2018–2020) (Roussos et al., 2022), emphasizes the urgent need for targeted interventions in PWID.

To our knowledge, this study represents one of the few molecular epidemiology analyses dedicated to examining HIV dispersal patterns among PWID over a long timeframe. Our study uncovered an ongoing HIV transmission among PWID, resembling a ‘slow burn’ outbreak that warrants additional attention for elimination. Considering the magnitude of the Athenian outbreak as the largest recent HIV outbreak in Europe and North America, it is noteworthy that sustained prevention and harm reduction programmes are crucial. These efforts should persist until the proportion of clustered infections is significantly reduced to effectively curb the outbreak among PWID. In conclusion, our study underscores the significance of incorporating molecular epidemiology estimates, such as clustering and time to diagnosis, alongside traditional epidemiological measures like prevalence and incidence. These additional metrics offer valuable insights for public health implications and interventions.

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CRediT authorship contribution statement

Evangelia Georgia Kostaki: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Sotirios Roussos:** Writing – review & editing, Investigation. **Anastasia Maria Kefala:** Writing – review & editing, Investigation. **Stefanos Limnaios:** Writing – review & editing, Investigation. **Mina Psichogiou:** Writing – review & editing, Investigation, Conceptualization. **Eleni Papachristou:** Writing – review & editing, Investigation. **Georgios Nikolopoulos:** Writing – review & editing, Funding acquisition, Conceptualization. **Eleni Flountzi:** Writing – review & editing, Investigation. **Samuel R. Friedman:** Writing – review & editing, Funding acquisition, Conceptualization. **Pagona Lagiou:** Writing – review & editing, Resources, Conceptualization. **Angelos Hatzakis:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Vana Sypsa:** Writing – review & editing, Funding acquisition, Conceptualization. **Gkikas Magiorkinis:** Writing – review & editing, Resources, Conceptualization. **Apostolos Beloukas:** Writing – review & editing, Resources, Conceptualization. **Dimitrios Paraskevis:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

Authors do not have a commercial or other association that might pose a conflict of interest.

Data availability statement

Data available upon request.

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