

ORIGINAL ARTICLE

Estimation of the determinants for HIV late presentation using the traditional definition and molecular clock-inferred dates: Evidence that older age, heterosexual risk group and more recent diagnosis are prognostic factors

Evangelia Georgia Kostaki¹  | Stefanos Limnaios¹ | Georgios Adamis² | Georgios Xylomenos² | Maria Chini³ | Nikos Mangafas³ | Marios Lazanas³ | Stavros Patrinos⁴ | Simeon Metallidis⁵ | Olga Tsachouridou⁵ | Vasileios Papastamopoulos⁶ | Dimitrios Chatzidimitriou⁷ | Anastasia Antoniadou⁸ | Antonios Papadopoulos⁸ | Konstantinos Protopapas⁸ | Chrysa Tsiara⁴ | Mina Psychogiou⁹ | Dimitrios Basoulis⁹ | Dimitrios Pilalas⁷ | Dimitra Paraskeva¹⁰ | Georgios Chrysos¹⁰ | Vasileios Paparizos¹¹ | Sofia Kourkounti¹¹ | Helen Sambatakou¹² | Vasileios Bolanos¹² | Nikolaos V. Sipsas¹³ | Malvina Lada¹⁴ | Emmanouil Barbounakis¹⁵ | Evrikleia Kantzilaki¹⁵ | Periklis Panagopoulos¹⁶ | Vasilis Petrakis¹⁶ | Stelios Drimis¹⁰ | Ioannis Katsarolis¹⁷ | Pagona Lagiou¹ | Angelos Hatzakis¹ | Gkikas Magiorkinis¹ | Lemonia Skoura⁷ | Dimitrios Paraskevis¹ 

¹Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece

²1st Department of Internal Medicine, G. Gennimatas General Hospital, Athens, Greece

³3rd Department of Internal Medicine-Infectious Diseases Unit, "Korgialeneio-Benakeio" Red Cross General Hospital, Athens, Greece

⁴National Public Health Organization, Athens, Greece

⁵1st Department of Internal Medicine, AHEPA University Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Objectives: HIV late presentation (LP) has been increasing in recent years in Europe. Our aim was to investigate the characteristics of LP in Greece using in addition to the traditional definition for LP, the time interval between HIV infection and diagnosis.

Methods: Our nationwide sample included HIV-1 sequences generated from 6166 people living with HIV (PLWH) in Greece during the period 1999–2015. Our analysis was based on the molecularly inferred HIV-1 infection dates for PLWH infected within local molecular transmission clusters of subtypes A1 and B.

Results: Analysis of the determinants of LP was conducted using either CD4 counts or AIDS-defining condition at diagnosis or the time from infection to diagnosis. Older age, heterosexual transmission risk group and more recent

Gkikas Magiorkinis, Lemonia Skoura and Dimitrios Paraskevis are contributed equally to this study.

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⁶5th Department of Internal Medicine and Infectious Diseases, Evaggelismos General Hospital, Athens, Greece

⁷National AIDS Reference Centre of Northern Greece, Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

⁸4th Department of Medicine, Attikon General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

⁹1st Department of Medicine, Laikon General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

¹⁰Department of Internal Medicine, Tzaneio General Hospital, Piraeus, Greece

¹¹HIV/AIDS Unit, A. Syngros Hospital of Dermatology and Venereology, Athens, Greece

¹²HIV Unit, 2nd Department of Internal Medicine, Hippokration General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

¹³Department of Pathophysiology, Laikon General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

¹⁴2nd Department of Internal Medicine, Sismanogleion General Hospital, Athens, Greece

¹⁵Department of Internal Medicine, University Hospital of Heraklion "PAGNI", Medical School, University of Crete, Heraklion, Greece

¹⁶Department of Internal Medicine, University General Hospital, Democritus University of Thrace, Alexandroupolis, Greece

¹⁷Medical Affairs, Gilead Sciences Hellas, Athens, Greece

Correspondence

Dimitrios Paraskevis, Department of Hygiene, Epidemiology and Medical Statistics, Medical School National and Kapodistrian University of Athens, GR-11527, Athens, Greece.
Email: dparask@med.uoa.gr

Funding information

Gilead Sciences

diagnosis were associated with increased risk for LP. In contrast to previous studies, people who inject drugs (PWID) had a shorter median time to diagnosis (0.63 years) compared to men who have sex with men (MSM) (1.72 years) and heterosexuals (2.43 years). Using HIV infection dates that provide an unbiased marker for LP compared to CD4 counts at diagnosis, which are age-dependent, we estimated that the time to diagnosis increased gradually with age. Migrants infected regionally do not differ with respect to LP status compared to native Greeks.

Conclusions: We demonstrate that older people and heterosexuals are among those at higher risk for LP; and given the growing number of older people among newly diagnosed cases, tailored interventions are needed in these populations.

KEYWORDS

Greece, HIV, infection dates, late presentation, molecular epidemiology, risk factors

INTRODUCTION

HIV remains a major health challenge. Although HIV transmissions have declined over time, the incidence remains high (<http://www.UNAIDS.org>). The rate of HIV

diagnoses has been declining over the last decade in Europe after a peak that occurred in 2012, but large differences still exist in different areas. Specifically, the rate of HIV diagnoses was considerably higher in Eastern (32.6) than Western (3.7) or Central Europe (2.3). The proportion

of HIV diagnoses among older people (>50 years old) was much higher in Western (22.9%) than in Central (13.8%) or Eastern Europe (12.9%) [1]. The transmission risk group differs significantly with the geographic area, where sex between men (39.2%) and heterosexual transmission in women (16.9%) were the predominant transmission modes in Western Europe. Sex between men (28.0%) and heterosexual transmission in males (19.2%) were the most frequent risk groups in Central Europe, and heterosexual transmission in both males (33.1%) and females (31.0%) were dominant in Eastern Europe [1].

Late presentation (LP), defined as diagnosis with a CD4 count <350 cells/mm³, or presentation with advanced HIV disease (PAD), defined as diagnosis with a CD4 count <200 cells/mm³ or an AIDS-defining event [2], has been of increasing concern in Europe and globally due to the lower life expectancy, higher transmission rates, greater morbidity and higher healthcare costs [1]. The World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) recommend that in order to reduce HIV transmission we need to prioritize several actions, including efficient HIV counselling, and testing services to promote early diagnosis and treatment initiation, which increases life expectancy and reduces the risk for HIV transmission [1]. Half of the people (50%) in the European Union (EU)/European Economic Area (EEA) presented with CD4 counts <350 cells/mm³, with proportions varying by regions and risk groups. LP was more common among heterosexuals, people who inject drugs (PWID) and older people in the region [1]. In previous studies, major risk factors for LP included heterosexual transmission, and injection drug use, immigration status and older age [2–15].

LP also remains a challenge in Greece in common with the rest of the EU/EEA countries [16]. In our previous analyses using a nationwide sample, we investigated the patterns of regional transmission for the predominant subtypes A1 and B, and we inferred the HIV-1 infection dates for the people living with HIV (PLWH) infected in local molecular transmission clusters (MTCs) [17, 18]. Besides surveillance data, the characteristics of LP have not been investigated in detail using a nationwide sample. Our aim was to investigate LP in Greece using a combination of methods based on the traditional definition for LP (i.e., CD4 counts or AIDS-defining condition at diagnosis) and the time interval between HIV infection and diagnosis.

METHODS

Study population

The study population included 6166 PLWH in whom HIV-1 sequences were available on the protease and partial reverse transcriptase regions (*pol* gene) obtained

during the period 1999–2015 in Greece [17, 18]. This number corresponds to 57.2% of all PLWH diagnosed during the same period in Greece ($N = 10\,787$) according to the National HIV-1 surveillance system [19]. Study sequences were sampled in different parts of the country: 4790 (77.7%) sequences were obtained in Athens (Central/Southern Greece), 1298 (21.0%) in Thessaloniki (Northern Greece) and 78 (1.3%) in Crete [17, 18].

Clinical AIDS-defining conditions and CD4 cell counts at diagnosis were available from the National HIV-1 surveillance system (<https://eody.gov.gr/>).

The current study was approved by the Ethics Committee of the Medical School of the National and Kapodistrian University of Athens.

HIV-1 subtyping, phylogenetic and phylodynamic analysis

HIV-1 subtyping, identification of MTCs and estimation of HIV-1 infection dates are described in detail in Appendix S1. In brief, HIV-1 subtypes were determined by online automated subtyping tools (COMET, REGA) and confirmed by phylogenetic analysis [17]. Phylogenetic analysis using large sets of globally sampled reference sequences was performed in five replicates separately on subtype A1 ($N = 1751$) and B ($N = 2575$) sequences from Greece [17]. MTCs were defined as monophyletic clusters fulfilling the criteria that were set (i.e., geographic and phylogenetic confidence criteria) [17, 20]. Molecular clock calculation with phylodynamic analysis was performed on subtype A1 ($N = 1533$) and B ($N = 1560$) sequences belonging to large MTCs [17, 18]. The time to the most recent common ancestor (tMRCA) (median estimate) of the internal nodes within each MTC dated tree was used as a proxy of the HIV-1 infection dates [18].

Statistical analysis

Multivariable linear regression analysis and multinomial logistic regression analysis were used to identify factors associated with LP and PAD. More details about statistical analysis are provided in Appendix S1.

Definitions

- i. “*Diagnosis date*”: the date of HIV-1 diagnosis as reported to the National HIV-1 surveillance system (<https://www.eody.gov.gr/>).
- ii. “*Linkage to care date*”: the date of the first sample obtained for HIV-RNA or genotypic resistance testing according to the National HIV-1 surveillance

TABLE 1 Study population demographic and epidemiological characteristics

Characteristic	Number (n)	Percentage (%)
Sex		
Male	2708	87.5
Female	272	8.8
Unknown	113	3.7
Antiretroviral therapy (ART)		
Untreated	2429	78.5
Treated ^a	501	16.2
Unknown	163	5.3
Origin		
Greek	2527	81.7
Non-Greek	211	6.8
Unknown	355	11.5
Transmission risk group		
Heterosexuals	505	16.3
PWID	236	7.6
MSM	2110	68.2
Other	23	0.8
Unknown	219	7.1
Diagnosis date		
Available	2464	79.7
Unavailable	629	20.3

Abbreviations: MSM, men who have sex with men; PWID, people who inject drugs.

^aPeople living with HIV (PLWH) on ART at genotypic resistance testing.

system (<https://www.eody.gov.gr>). Given that the samples from our study population were obtained during the period 1999–2015, and that guidelines for treatment initiation were modified during this time period, we used the date of the first sample for laboratory testing as a proxy for the linkage to care date.

- iii. “*Antiretroviral treatment (ART) initiation*”: the date of ART initiation as reported at the National HIV-1 surveillance system (<https://www.eody.gov.gr>).
- iv. “*Molecular clock-inferred infection date*”: the infection date estimated by the molecular clock and phylogenetic analysis [18].
- v. “*Presentation with advanced HIV disease (PAD)*”: cases diagnosed with CD4 counts <200 cells/mm³ or an AIDS-defining event [2].
- vi. “*Late presentation (LP)*”: cases diagnosed with $200 \text{ cells/mm}^3 \leq \text{CD4 counts} < 350 \text{ cells/mm}^3$ [2].

TABLE 2 Multivariable multinomial logistic regression estimates using HIV presentation status (categories were based on CD4 counts and clinical AIDS-defining conditions at diagnosis) as the outcome variable (2463 complete observations)

Explanatory variable	Relative risk ratio (95% CI)	p-value
Advanced disease versus non-late presentation		
Transmission risk group (^a Heterosexuals)		
PWID	0.65 (0.39–1.09)	0.104
MSM	0.48 (0.34–0.67)	<0.001
Other	1.13 (0.31–4.03)	0.856
Unknown	1.17 (0.57–2.41)	0.667
Sex (^a Female)		
Male	1.37 (0.88–2.12)	0.159
Age (in years)	1.06 (1.05–1.07)	<0.001
Origin (^a Greek)		
Non-Greek	1.58 (1.04–2.42)	0.034
Unknown	0.12 (0.03–0.54)	0.006
Diagnosis year	0.93 (0.92–0.95)	<0.001
Late versus non-late presentation		
Transmission risk group (^a Heterosexuals)		
PWID	0.86 (0.49–1.53)	0.607
MSM	0.68 (0.46–1.02)	0.064
Other	1	-
Unknown	1.18 (0.52–2.68)	0.684
Sex (^a Female)		
Male	1.17 (0.70–1.97)	0.554
Age (in years)	1.04 (1.02–1.05)	<0.001
Origin (^a Greek)		
Non-Greek	1.11 (0.66–1.85)	0.698
Unknown	1.20 (0.51–2.84)	0.681
Diagnosis year	0.99 (0.97–1.00)	0.285

Abbreviations: CI, confidence interval; MSM, men who have sex with men; PWID, people who inject drugs.

^aReference category.

RESULTS

Our study population for the LP analysis consisted of 3093 PLWH in whom HIV-1 sequences were found within subtype A1 ($N = 1533$) and B ($N = 1560$) large MTCs. The characteristics of the study population are shown in Table 1. The majority of the study population were males (87.5%), of Greek origin (81.7%) and men who have sex with men (MSM) (68.2%). The date of diagnosis was available for 2464 PLWH (79.7%), and most samples were obtained from treatment-naïve individuals (78.5%) (Table 1).

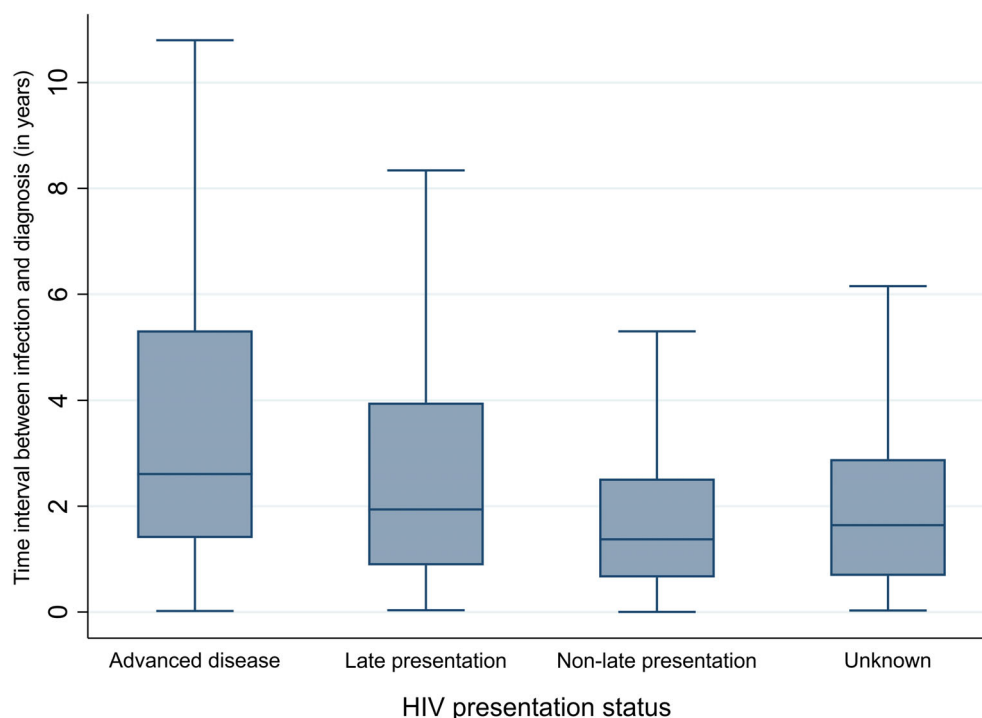


FIGURE 1 Time interval between infection and diagnosis in years (box plot presentation) for different HIV presentation statuses [late presenters with advanced disease (CD4 at diagnosis <200 cells/mm³ or presence of an AIDS-defining condition), late presenters (diagnosed with 200 cells/mm³ \leq CD4 counts <350 cells/mm³), non-late presenters (CD4 at diagnosis ≥ 350 cells/mm³) and PLWH with unknown status of presentation]

Analysis based on CD4 and clinical AIDS at diagnosis

Based on the CD4 counts and clinical AIDS-defining conditions at diagnosis, we found that 698 (22.6%), 449 (14.5%) and 1189 (38.4%) PLWH were classified as PAD, LP and non-late presentation, respectively, while 757 (24.5%) PLWH could not be classified (unknown status of presentation). PAD was more frequent among females (29.4%) than males (22.8%), while no major gender difference was found for LP (Table S1). A higher proportion of heterosexuals were diagnosed with PAD (36.8%) than MSM (21.3%). For LP, no major difference was found between the two groups (16.3% vs. 15.4% for heterosexuals and MSM, respectively) (Table S2). For PWID, lower proportions for PAD (14%) and LP (11.4%) were found. Furthermore, PWID had the highest proportion (44.9%) in the category of unknown compared to other groups (Table S2). During the study period, no difference was found in PAD between PLWH of Greek (25.0%) and non-Greek origin (26.1%), but for non-Greeks the proportion of those with unknown status of presentation was much higher (30.8%) than for Greeks (16.9%), suggesting that probably PAD was more frequent among the non-Greeks

(Table S3). Analysis of LP per age group revealed higher proportions for older (age groups: 41–50, 51–60, 61–70 years) versus younger PLWH (Table S4). Specifically, PAD was found at 34.2%, 42.0% and 46.4% versus 15.0% and 21.6% for age groups 41–50, 51–60, 61–70, and 21–30 and 31–40 years, respectively (Table S4). Except for the older group of 61–70 years, no major differences were observed for LP.

Multivariable multinomial logistic regression analysis showed that transmission risk group, age, origin, and year of diagnosis were significantly associated with PAD (Table 2). Specifically, MSM had a lower relative risk for PAD versus non-late presentation (relative risk ratio [RRR]: 0.48; 95% confidence interval [CI]: 0.34–0.67; $p < 0.001$) compared to heterosexuals. For every 1-year increase in age, the relative risk for PAD would be expected to increase by a factor of 1.06 (95% CI: 1.05–1.07; $p < 0.001$). Non-Greeks had a higher relative risk (RRR: 1.58; 95% CI: 1.04–2.42; $p = 0.034$) compared to Greeks. Finally, for more recent diagnosis the relative risk for PAD would be expected to decrease (RRR: 0.93; 95% CI: 0.92–0.95; $p < 0.001$) (Table 2). Conversely, only for older age would the relative risk for late versus non-late presentation be expected to increase (RRR: 1.04; 95% CI: 1.02–1.05; $p < 0.001$) (Table 2).

TABLE 3 Time interval between infection and diagnosis by HIV-1 subtype, sex, transmission risk group and origin

Characteristic	Time interval between infection and diagnosis (median, IQR)
HIV-1 subtype	
A1	1.88 (0.93–3.91)
B	1.61 (0.73–3.21)
Sex	
Male	1.75 (0.81–3.41)
Female	2.38 (1.06–4.64)
Transmission risk group	
MSM	1.72 (0.82–3.32)
Heterosexuals	2.43 (1.12–5.11)
PWID	0.63 (0.30–1.45)
Other	2.59 (1.44–4.76)
Unknown	2.16 (1.29–4.89)
Origin	
Greek	1.75 (0.81–3.52)
Non-Greek	1.92 (1.29–3.50)
Unknown	2.44 (1.63–5.54)

Abbreviations: IQR, interquartile range; MSM, men who have sex with men; PWID, people who inject drugs.

Analysis based on molecular clock-inferred infection dates

Additional analysis using the molecular clock-inferred infection dates revealed that the median time interval between infection and diagnosis was 2.61 (interquartile range [IQR]: 1.40–5.32), 1.94 (IQR: 0.88–3.96) and 1.37 (IQR: 0.65–2.52) years for PLWH with PAD, LP and non-late presentation, respectively (Figure 1). The corresponding interval between infection and linkage to care were 3.26 (IQR: 1.67–5.97), 2.12 (IQR: 1.09–4.28) and 1.59 (IQR: 0.77–3.01) years for the three groups, respectively. The median time between diagnosis and treatment initiation was 0.10 (IQR: 0.06–0.22), 0.24 (IQR: 0.11–0.66) and 1.53 (IQR: 0.52–3.50) years for PAD, LP and non-late presentation, respectively.

To investigate the time interval between infection and diagnosis for individuals with PAD, LP or non-late presentation based on CD4 counts or clinical AIDS at diagnosis, we estimated the proportions of PLWH with PAD, LP or non-late presentation based on the CD4 counts or clinical AIDS at diagnosis within the first 6 or 12 months after infection (Table S5). Recent diagnosis within 6 months was found for 8.0%, 11.7% and 19.2% for PAD, LP and non-late presentation, respectively. Similarly, within the first year, proportions gradually increased for

TABLE 4 Multivariable linear regression estimates using late presentation (by means of the time from infection to diagnosis) as the outcome variable (1824 complete observations)

Explanatory variable	Coefficient (95% CI)	p-value
Transmission risk group (^a Heterosexuals)		
PWID	−2.09 (−2.72, −1.46)	<0.001
MSM	−0.62 (−1.02, −0.22)	0.002
Other	0.44 (−1.31, 2.19)	0.623
Unknown	0.06 (−0.75, 0.87)	0.883
Sex (^a Female)		
Male	−0.30 (−0.83, 0.23)	0.270
Age (in years)	0.04 (0.03, 0.05)	<0.001
Origin (^a Greek)		
Non-Greek	−0.14 (−0.64, 0.36)	0.582
Unknown	0.91 (−0.55, 2.37)	0.220
Diagnosis year	0.08 (0.05, 0.10)	<0.001

^aReference category.

Abbreviations: CI, confidence interval; MSM, men who have sex with men; PWID, people who inject drugs.

PAD (16.0%), LP (27.8%) and non-late presentation (38.4%) (Table S5). The median time between infection and diagnosis was found to be slightly higher for subtype A1 (1.88 years) versus subtype B (1.61 years) (Table 3), and different across transmission risk groups and sexes. Specifically, the median time to diagnosis was shorter for MSM (1.72 years) compared to heterosexuals (2.43 years), other (2.59 years) and unknown (2.16 years) groups, but longer than PWID (0.63 years), who were found to be diagnosed earlier than the other groups. Moreover, for females, HIV status was confirmed later (median time to diagnosis: 2.38 years) than males (median time to diagnosis: 1.75 years), and similarly for non-Greeks (median time to diagnosis: 1.92 years) and those with unknown origin (median time to diagnosis: 2.44 years) compared to Greeks (median time to diagnosis: 1.75 years) (Table 3).

We further investigated the time trend in the intervals between infection and diagnosis, infection and linkage to care, diagnosis and linkage to care, and diagnosis and ART initiation over the time period 1999–2015. An increasing trend was observed for the time to diagnosis during 2009–2015 (p for trend = 0.036) (Table S6). An increasing trend was also observed for the time between infection and linkage to care during that period, but this increase was not statistically significant (p = 0.08). No trend was observed for the time interval between diagnosis and linkage to care, but notably the time from diagnosis to treatment initiation was significantly reduced during the recent years of the study period (p = 0.029)

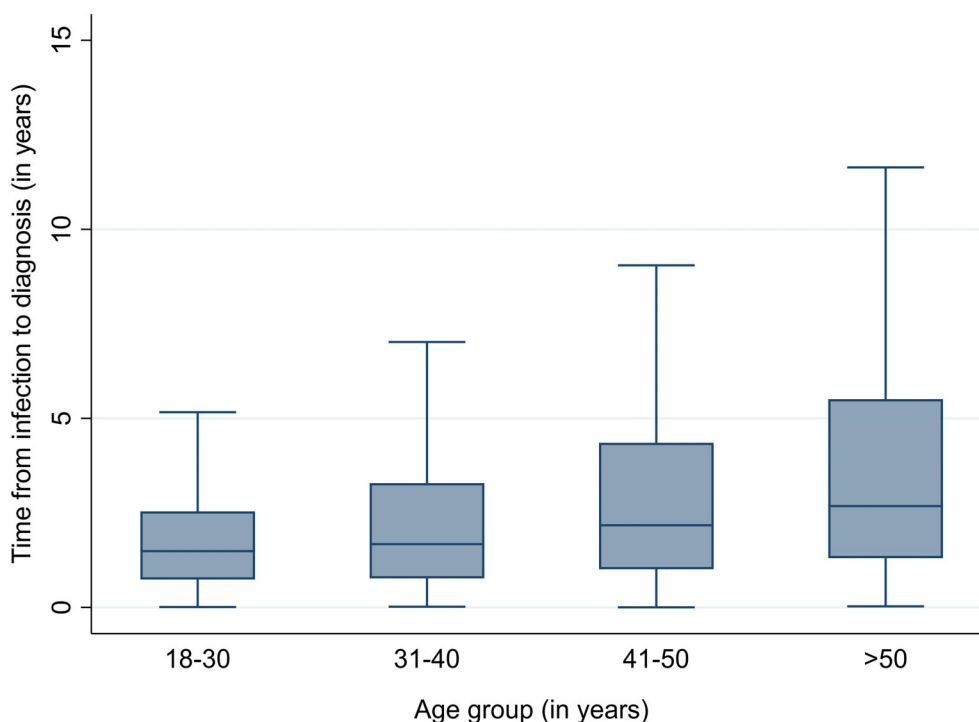


FIGURE 2 Time interval between infection and diagnosis in years (box plot presentation) for the age groups: 18–30, 31–40, 41–50 and >50 years. The time interval was estimated using the molecular clock-inferred HIV infection dates

(Table S6). A similar trend was observed for MSM and heterosexuals, with a significant increase in the time between infection and diagnosis during the period 2009–2015. For heterosexuals, a significant increasing trend was also detected for the time for linkage to care (Table S7). In addition, we investigated the time trend for PWID and all the other transmission risk groups separately. During 2009–2015, the time interval between infection and diagnosis increased for all transmission risk groups other than PWID ($p = 0.019$), for whom an increasing trend was detected only during 2011–2015, but it was not statistically significant ($p = 0.086$) (Table S8). For both groups, a non-significant increasing trend was observed for the time between infection and linkage to care ($p = 0.080$ and $p = 0.086$).

The potential association of factors, such as transmission risk group, age, sex, origin, and date of diagnosis, with LP (by means of the time from infection to diagnosis) was investigated by multivariable linear regression analysis (Table 4). Analysis revealed that MSM (coefficient: -0.62 ; 95% CI: $-1.02, -0.22$; $p = 0.002$) and PWID (coefficient: -2.09 ; 95% CI: $-2.72, -1.46$; $p < 0.001$) had a shorter time to diagnosis compared to heterosexuals (Table 4). Conversely, older age (coefficient: 0.04 ; 95% CI: $0.03, 0.05$; $p < 0.001$) and more recent diagnosis (coefficient: 0.08 ; 95% CI: $0.05, 0.10$; $p < 0.001$) were associated with an increase in the time to diagnosis (Table 4). Origin and sex were not significantly associated with LP, suggesting that

non-Greeks infected with subtypes A1 and B follow a similar pattern as PLWH with Greek origin. The time between infection and diagnosis for different age groups is shown in Figure 2.

DISCUSSION

Our study investigated the characteristics of LP using a nationwide sample of HIV-1 sequences obtained since 1999 combined with demographic and surveillance data on CD4 counts and clinical AIDS at HIV diagnosis. Our analysis provides important new information about the characteristics of LP and the time interval across the cascade of care.

We have demonstrated that using either CD4/clinical AIDS or the time interval between infection and diagnosis as a definition for LP, age, origin, transmission risk group and diagnosis date were significant prognostic factors for the time of HIV-1 diagnosis. In both analyses, transmission risk group, age and diagnosis date were associated with the characteristics of the time to diagnosis. Specifically, MSM were diagnosed earlier than heterosexuals but not earlier than PWID. For the latter group, earlier diagnosis was due to intervention programmes, including ARISTOTLE and TRIP, which focused on early diagnosis and treatment in addition to syringe provision. These programmes started in 2012 and lasted until 2013

and 2014, respectively [21, 22]. LP was positively associated with older age and more recent diagnosis, which probably suggests that stigma remains a significant barrier among older people in Greece. This is in accordance with the situation that transmission risk group remains unreported in a high percentage of males at their diagnosis (<https://www.eody.gov.gr>). Specifically, the route of HIV transmission remained undetermined in 19.6% of HIV diagnoses in 2021 and in 16.2% of the total number of diagnosed cases since the beginning of the HIV epidemic in Greece [16]. The non-disclosure of transmission risk group at diagnosis is mainly due to males' sexual orientation and related stigma. These findings show that social stigma about sexual orientation is a barrier to HIV diagnosis that is probably more profound among older males, highlighting that additional effort is needed to overcome this hurdle among this population.

LP has been also associated with more recent diagnosis in our analyses, suggesting that counselling and testing campaigns, targeting the highly vulnerable but also the general population, are urgently needed to promote timely detection of HIV-1 infection. Unfortunately, a similar trend in LP has been observed recently, with 54.5% and 31.3% of newly reported cases in 2021 diagnosed with CD4 counts below 350 and 200 cells/mm³, respectively [16]. Ethnic origin has been identified as an additional risk factor for LP using CD4 counts and clinical AIDS, but not in the subsequent analysis where molecular clock-inferred dates were used. Given the potential differences in rates of CD4 decline across diverse populations and HIV-1 subtypes [23, 24], the non-identification of origin as a risk factor for LP in the second analysis provides the most plausible hypothesis for migrants infected with subtype A1 and B in Greece. This finding is of importance since our study population consists only of PLWH infected locally. Therefore, migrants with regional infection do not differ from the local population with respect to time of infection, suggesting that this population is not marginalized from HIV testing and other services.

Our findings were similar to the determinants of LP as reported in the recent HIV surveillance report in Europe [1]. Specifically, the proportions of people diagnosed with CD4 counts <350 cells/mm³ increased gradually with age (ranging between 33% and 65% for people aged between 15 and 19 years and older than 50 years) and were higher for both heterosexual males (58%) and females (54%) than MSM (41%) or PWID (43%) [1]. Similar proportions of late presenters were reported for males (50%) and females (52%). Similar results were reported in a large European study, where older individuals (aged >56 years) and heterosexuals had a higher risk for LP (CD4 counts <350 cells/mm³) [5]. In this study, African and South American origin and non-B subtypes were

additional determinants for LP [5]. Notably, sex was not found to be a significant factor for LP. In addition, an increasing trend was observed for LP during 2017–2019 [5].

In a different setting in Portugal, where the majority of PLWH reported heterosexual contact as their risk factor (57.0%), significant factors associated with LP were older age and sub-Saharan origin [4]. In another study from Belgium, similar risk factors were reported, including older age, male sex, heterosexual risk group and sub-Saharan origin [6]. Similar findings have been reported in studies from Denmark [8], Germany [10], Italy [11, 14], Switzerland [13], France [25] and Poland [9], where age, transmission risk group and origin were reported, with minor differences across the different settings. The differences in our study were the identification of PWID as an indicator of non-late presentation and that origin was not associated with LP. The first was explained previously and was due to the intervention programmes including extensive HIV-testing among this group. Greece provides a special case due to the previous outbreak in Athens, while in most countries PWID had a higher risk for LP compared to MSM, as shown in a recent meta-analysis [12]. The non-identification of migrants as late presenters in our study was probably due to selection of PLWH infected locally. In a single study from Northern Greece before the outbreak among PWID, risk factors associated with LP were transmission risk group (i.e., PWID and heterosexuals), older age and immigrant status [15].

The effect of older age was investigated in a review analysis in which it was stated that an increasing proportion of people were diagnosed at older age (aged >50 years) presenting with lower CD4 counts than younger people [3]. In most areas, the majority of older people were late presenters [3]. This study highlights that CD4 counts decline with age among uninfected individuals [23], but the differences across age cannot explain the increasing proportions of people older than 50 years presenting late for care. We investigated the effect of age and other factors independent of CD4 counts at diagnosis, but since this marker is age-dependent, we provide evidence using molecular clock-inferred infection dates that the interval between infection and diagnosis is gradually increasing with age. This provides an unbiased estimation of the effect of age and confirms its association with LP. These findings further support the need for targeted interventions among older people to overcome the hurdle of LP.

To our knowledge our study is among a few, in addition to surveillance data for LP, that implement the molecular clock-inferred infection dates as a marker to describe the characteristics of LP in Greece. Although inferring

infection dates using molecular clock and phylodynamics has some limitations, it has been shown that this method can be accurately applied in the case of MTCs [18, 26]. In the current analysis, we provide information about the timeframe of infections as well as the time interval between infection and diagnosis or linkage to care for a nationwide sample. Based on our findings, the median time to PAD, LP and non-late presentation was 2.61, 1.94 and 1.37 years, respectively, but values ranged within wide intervals. Notably, in our analysis of the proportions of PLWH who were classified according to their diagnosis status using CD4/clinical AIDS as people with LP or PAD, 27.8% and 16.0% were found to be diagnosed within 12 months, suggesting that in some cases immunological markers or clinical status may fail to reflect the time since infection. Conversely, given the ease of use and the availability of these markers, they currently provide the best proxy for the characteristics of people at the time of HIV diagnosis. Moreover, a limitation of our study is that our population corresponds to PLWH for whom sequences were available until 2015.

In conclusion, using a combined approach of LP markers and inferred infection dates, we showed that older age, more recent diagnosis, and heterosexual risk group were associated with higher risk for LP. We found that migrants infected at a regional level were no more likely to be late presenters than native Greeks. Notably, besides the CD4 counts at diagnosis, we provide an unbiased estimation of the gradual increasing risk for LP by age. This is of concern due to the increasing age of the population and the increasing proportion of new diagnoses among older individuals. Improving the timing of HIV diagnosis is of major importance for the HIV cascade of care, for improving patients' quality of life, and for HIV prevention. Using a more detailed framework for analysis, we show that the determinants of LP can be identified in more detail, as in this case for PWID, migrants or older people, and consequently our efforts tailored according to patients' characteristics. LP remains an important hurdle in HIV cascade of care, and more effort is needed to control this problem nationally and globally.

AUTHOR CONTRIBUTIONS

EGK organized the data, performed the analysis, prepared the figures and the manuscript. SL contributed to the analysis and provided critical comments about the manuscript. GA, GX, MC, NM, ML (Marios Lazanas), SP, SM, OT, VP (Vasileios Papastamopoulos), DC, AA, AP, KP, CT, MP, DB, DP (Dimitrios Pilalas), DP (Dimitra Paraskeva), GC, VP (Vasileios Pappazotos), SK, HS, VB, NVS, ML (Malvina Lada), EB, EK, PP, VP (Vasilis Petrakis) and SD participated in the study design, performed the data collection and provided critical comments on

the manuscript. IK, PL and AH contributed to the study design and provided critical comments on the manuscript. GM and LS contributed to the study design, manuscript writing and editing. DP (Dimitrios Paraskevis, corresponding author) performed the study design, supervision and contributed to manuscript writing and editing.

ACKNOWLEDGEMENT

This study was a collaborative research project that was supported and funded by Gilead Sciences Hellas.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Evangelia Georgia Kostaki  <https://orcid.org/0000-0002-3346-0930>

Dimitrios Paraskevis  <https://orcid.org/0000-0001-6167-7152>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kostaki EG, Limnaios S, Adamis G, et al. Estimation of the determinants for HIV late presentation using the traditional definition and molecular clock-inferred dates: Evidence that older age, heterosexual risk group and more recent diagnosis are prognostic factors. *HIV Med*. 2022;23(11):1143-1152. doi:[10.1111/hiv.13415](https://doi.org/10.1111/hiv.13415)