Persistent poor clinical outcomes of people living with HIV presenting with AIDS and late for HIV diagnosis – results from the Icona cohort in Italy, 2009-2022

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HIGHLIGHTS

- A mortality gap remains for late-, particularly AIDS-presenters, in recent years
- Increased mortality for AIDS-presenters was driven by AIDS events in the 1st year
- 2-year immune-recovery is the key for long-term mortality in short-term AIDS survivors
- Late- and AIDS-presenters still shows also a higher risk treatment failure
- Urgent public health strategies are needed for emerging unknown HIV infections

Persistent poor clinical outcomes of people living with HIV presenting with AIDS and late for HIV diagnosis – results from the Icona cohort in Italy, 2009-2022

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Running Title: Poor clinical outcomes for AIDS presenters Italy

ABSTRACT

Background: Limited data are available on long-term outcomes in recent years for late HIV diagnosis (LD).

Methods: All HIV-positive subjects enrolled in Icona Cohort in 2009-2022 starting ART within 4-months from diagnosis were included and divided into:1) pre-ART CD4 count≥350/mm3 without AIDS (non-LD), 2) pre-ART CD4 count<350/mm3 without AIDS (LD-Asymptomatic), 3) with AIDS events pre-ART (LD-AIDS). Estimated probability and independent risk for mortality (all-cause and cause-specific) and treatment failure (TF) were evaluated.

Results: 6,813 participants: 2,448 non-LD, 3,198 LD-Asymptomatic, and 1,167 LD-AIDS, 161 (2.4%) died after ART initiation. At survival analysis, a higher probability of all-cause mortality has been identified for LD compared to non-LD (p<0.001), and within the former, for LD-AIDS over LD-Asymptomatic (p<0.001). After adjusting for confounders, LD showed a higher risk of all-cause mortality (vs non-LD aHR=5.51, p<0.001), and, in particular, being an AIDS presenter predicted a greater risk of all-cause (aHR=4.42, p<0.001), AIDS-related (aSHR=16.86, p<0.001) and not AIDS-related mortality (aSHR=1.74, p=0.022) compared to the rest of the late presenters. Among short-term survivors LD-AIDS, the long-term mortality was mediated by the lack of immune-recovery at 2-years. LD compared to non-LD, and particularly among the former, LD-AIDS over LD-Asymptomatic, showed also a greater risk of TF.

Conclusions: In recent years, LD subjects, particularly AIDS-presenters, remained at a higher risk of poorer outcomes. Public health strategies for early HIV diagnosis are urgently needed to constrain the mortality gap.

INTRODUCTION

Despite universal access to HIV testing and antiretroviral treatment (ART), diagnosis of HIV at a late stage of the disease is still a significant challenge, even in high-income countries [1,2]. Late HIV diagnosis (LD) has been defined as a person first diagnosed with HIV with a CD4 count below 350 cell/mm^3 or with an AIDS-defining event (ADE) regardless of the CD4 count, excluding individuals with evidence of recent HIV infection [3,4]. In 2021, according to the European and Italian HIV surveillance data, 54% of newly diagnosed HIV-positive subjects in Europe and 63% in Italy were diagnosed late [1,2]. Furthermore, more than 80% of new AIDS diagnoses still occurred within few months from HIV diagnosis [1,2].

LD has been widely associated with poor clinical outcomes both at the individual and community levels. In fact, late presenters have a greater risk of morbidity and mortality, mostly but not only due to ADEs, particularly over the first year of diagnosis [5-8], suboptimal virological control and immunological recovery and treatment discontinuation [9-12]. The role of immune-recovery after ART initiation in PLWH with late diagnosis, on clinical progression is still highly debated, a recent study from the Spanish PISCIS cohort, showed that CD4 counts nadir, do not necessarily fully explain the long-term survival in late presenters, and, after an early high-risk of mortality, LD who achieved CD4>500 cells/mm^3 2 years post-ART start had a long-term mortality comparable to the rest of naïve PLWH [13]. Conversely, another recent study reported that subjects starting ART with low CD4 cell count remained at greater risk for clinical progression and death, even after the restoration of immunocompetence [14].

Additionally, LD leads to high healthcare costs and enhances the risk of onward transmission due to the lack of awareness of HIV positivity [12,15].

Although late presentation is still a relatively frequent condition, data on long-term clinical outcomes of people living with HIV (PLWH) who have been diagnosed late in recent years, and who started ART with more potent and tolerable antiretroviral drugs, are limited. In this study, we

aimed to assess the impact of LD on mortality and treatment outcomes in a large national cohort of newly diagnosed PLWH who started ART in Italy over the last fourteen years (2009-2022).

MATERIAL AND METHODS

Study Design and Population

This is a retrospective analysis of prospectively collected data from the Icona (Italian Cohort Naïve Antiretrovirals) Foundation cohort, an Italian nationwide observational cohort, set up in 1997, including adult HIV-1-infected subjects, naïve from ART at the time of enrolment. Details of the cohort have been described elsewhere [16].

We included all consecutive HIV-infected persons enrolled in the Icona cohort who started ART within 4 months from HIV diagnosis from 1 January 2009 to 31 December 2022 having an available measure of CD4 before ART initiation and at least one-month follow-up after treatment start. Included patients were classified, according to CD4 count and clinical presentation before ART initiation, into two exposure groups: 1) subjects with CD4 count <350cell/mm^3 or a diagnosis of ADE regardless of CD4 count (late diagnosis, LD); 2) subjects with CD4 count ≥350 cell/mm^3 without history of ADE (non-late diagnosis, non-LD). We further divided the former group into two subgroups: 1) subjects with pre-ART CD4 count <350/mm^3 without a history of ADE (Asymptomatic late diagnosis, LD-Asymptomatic) and 2) subjects with diagnosis of ADE before ART start (AIDS presenters, LD-AIDS). The CD4 count considered for the classification of the exposure was the closest available pre-ART measurement.

Subjects who started treatment more than 4 months after HIV diagnosis were excluded to guarantee consistency in the definition of late diagnosis, avoiding classifying as LD someone who was diagnosed with high CD4 counts but started ART many months later. This could have frequently occurred especially prior to 2015 when ART was guided by current CD4 count. Furthermore, individuals who had a previously available CD4 count discordant with the group into which they were classified (i.e. subjects classified as either LD with a previous CD4 count≥350/mm^3 or non-

LD with a previous count<350/mm^3) were excluded from the analysis to avoid potential misclassification.

Follow-up accrued from the date of ART initiation to the achievement of the defined primary endpoint or the last follow-up visit, whichever came first.

Objectives – endpoint definitions

The primary study objective was to estimate the impact of LD on survival in ART-naïve individuals starting ART between 2009 and 2022 within four months from diagnosis. Deaths for any reason, whether AIDS-related or not, all counted as events. Secondary objectives were to evaluate the probabilities and the independent risks for the exposure groups of (i) mortality due to ADEs, (ii) mortality due to non-AIDS and (iii) treatment failure (TF), a composite outcome defined as virological failure (VF, confirmed HIV-RNA>200 copies/mL 6 months after ART start) or treatment discontinuation (TD, discontinuation of at least one drug in the initial regimen for failure or toxicity, as reported by the treating physician). As a final objective, we evaluated how much of the total effect of LD on mortality risk might be mediated by the failure to restore immune competence by 2 years from starting ART. Of note, the reasons of death were classified into 2 main groups of AIDS-ralated and non-AIDS related death and specific sub-groups based on HICDEP codes (Supplementary Materials).

Statistical Analysis

Descriptive characteristics at baseline were compared among the exposure groups using Chi-square test (Fisher's exact test when applicable) for categorical variables and Mann-Whitney or Kruskal-Wallis test, as appropriate, for continuous variables.

Cumulative probabilities of all-cause mortality and TF were estimated by Kaplan-Meier analysis and compared among the exposure groups (both non-LD vs. LD group and non-LD vs. LD-Asymptomatic vs. LD-AIDS) by log-rank test. Kaplan-Meier curves have been also used to

estimate the median survival time to reach CD4>500 among LD-Asymptomatic and LD-AIDS patients.

Crude and adjusted standard Cox regression models were used to evaluate the risks of all-cause mortality and TF associated with LD. Covariates included were identified under a set of assumptions regarding the causal relations between variables, specifically, the following time-fixed factors measured at baseline were identified as potential confounders: age, sex, mode of HIV transmission, nationality, calendar year for ART initiation, hepatitis-coinfections, type of third drug class included in the initial ART (Non-Nucleoside Reverse Transcriptase Inhibitors [NNRTIs] versus protease inhibitors [PIs] versus Integrase strand transfer inhibitors [INSTIs]).

A competing-risk analysis was also conducted after classifying the reasons of deaths into ADEs and non-AIDS, as reported by the treating physician. Cumulative probabilities of AIDS-related and not-related mortality were estimated and compared among the exposure groups (LD-AIDS vs. Asymptomatic-LD vs. non-LD) by competing-risk Kaplan-Meier curves. Fine-Gray regression models were used to assess the independent risks of death due to ADEs/non-AIDS by the three exposure groups, in which deaths due to SNAEs/non-AIDS were handled as competing events.

An interaction test between the calendar year and exposure groups was performed for the main outcomes to investigate whether the calendar year of ART initiation was an effect measure modifier of the association between LD and risk of death. In case of a significant statistical interaction, results were reported stratified by periods of ART initiation constructed using two consecutive 7-year time windows (2009-2014 and 2015-2022), the latter period reflecting the years after the introduction of treatment guidelines of universal ART initiation irrespective of CD4 count [17].

Finally, in order to expand the analysis to factors measured after ART initiation and to gain maximum insight into how much of the effect of LD and particularly AIDS presentation on long-term mortality in those surviving for the first two years post-ART might be mediated by an optimal immune recovery (reaching CD4>500 cell/mm^3 at 2-year from ART start), we used a counterfactual framework four-way decomposition method for analysis. This mediation analysis allows us to

understand the extent to which the overall effect of an exposure (e.g LD-AIDS) on an outcome (e.g. long-term mortality) in the presence of a mediator with which the exposure may interact (e.g. optimal immune recovery 2 years after ART start) is due to mediation, to interaction, to both of them or to neither [18].

All statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA). All p-values presented are two-sided and a p-value < 0.05 indicates conventional statistical significance.

Ethics Statement

The ICONA Foundation study was approved by the local Ethics Committees of participating clinical sites. All patients signed a consent form to study participation and processing of data in accordance with the ethical standards of the committee on human experimentation and the Helsinki Declaration (last amended in October 2013).

RESULTS

Baseline characteristics

Of the 13,571 Icona participants who began first-line ART between 2009 and 2022, 6,145 (45%) were excluded from the analysis because they started treatment more than four months after diagnosis. An additional 613 (4.5%) were excluded due to lack of available pre-ART CD4 count (n=65), lack of follow-up after ART initiation (n=221), or discordance between exposure group and CD4 count prior to that used for group classification (n=327) [Supplementary Figure 1].The study population consisted mostly of men (81%) with a median age of 40 years (interquartile range [IQR] 31-49) who mainly acquired HIV infection through sexual intercourse (87%). LD subjects accounted for 64.1% (n=4,365) of the included patients, with 3,198 LD-Asymptomatic (73.3% of LDs) and 1,167 LD-AIDS (26.7% of LDs). The exposure groups significantly differed for most of the baseline characteristics (Table 1). Particularly, LD subjects, compared with non-LD ones, were

more likely to be female (20.9% vs. 15.6%, p<0.001) and Italian (44.3% vs. 39.4%, p<0.001) and to have a lower education level (post-secondary school degree in 37.5% vs. 45.1%, p< 0.001). In addition, compared to non-LD, PLWH LD, and particularly AIDS presenters, were significantly older (LD-AIDS 45 years vs. LD-Asymptomatic 41 years vs. non-LD 36 years, p<0.001), heterosexuals (LD-AIDS 53.1% vs LD-Asymptomatic 47.7% vs. non-LD 30.5%, p<0.001) and with comorbidities at ART initiation (LD-AIDS vs. LD-Asymptomatic vs. non-LD: diabetes, 4.2% vs. 2.4% vs. 1.3%, p<0.001; cardiovascular disease, 1.5% vs 0.8% vs. 0.4%, p=0.003; hypertension 7.5% vs 4.7% vs. 3.6%, p<0.001). Median baseline CD4 count was 528 [IQR 435-680] cells/mm^3 for non-LD and 138 [IQR 49-247] cells/mm^3 for LD patients (p<0.001). Among these latter, as expected, AIDS presenters had a worse immunological status compared with LD-Asymptomatic (49 [IQR 21-125] cells/mm^3 and 181 [IQR 80-265] cells/mm^3, p<0.001). Median baseline HIV viral load was higher in LD, particularly AIDS-presenters, respect to non-LD subjects (Median HIV-RNA, log_{10}: LD-AIDS 5.32 copies/mL vs. LD-Asymptomatic 5.05 vs. non-LD 4.51 copies/mL, p<0.001).

Antiretroviral treatment

As to inclusion criteria, ART was started within a median of one month (IQR 0-2) from HIV diagnosis mostly with a three-drug regimen (92.3%). A detailed description of ART regimens is shown in Table 1. LD subjects were more likely to start a triple regimen (94.5% vs 88.4%, p<0.001) than non-LD ones. Among LD-patients, INSTI-based (LD-Asymptomatic 47.3%, LD-AIDS 45.4% p<0.001) and PI-based (LD-Asymptomatic 33.8%, LD-AIDS 38.9% p<0.001) triple therapies were the most prescribed regimens. Non-LD subjects preferentially started INSTI-based (53.0%) 3-drug regimens. Among LD patients boosted darunavir (DRV/b) and dolutegravir (DTG) were the most prescribed anchor drug (LD-Asymptomatic: DRV/b 22.5% and DTG 22.6%; LD-AIDS: DRV/b 28.2% and DTG 23.2%) whereas non-LD subjects received more frequently DTG as third drug (26.7%).

All cause-mortality

Over a median follow-up of 46 (IQR 17-83) months, a total of 161 patients (2.4%) died: 11 (0.4%) in the non-LD and 150 (3.4%) in the LD group, including 99 (8.4%) LD-AIDS and 51 (1.6%) LD-Asymptomatic. Kaplan-Meier curves revealed a significantly higher probability of all-cause mortality for LD compared to non-LD patients ($p<0.001$, Figure 1a) and, particularly, for AIDS presenters with respect to the rest of the study population ($p<0.001$, Figure 1b). Specifically, by 5 years from ART initiation, the estimated probabilities (95% confidence interval [CI]) for all-cause mortality were: 9.2% (7.4-11.1%) for LD-AIDS, 1.5% (1.0-2.0%) for LD-asymptomatic and 0.6% (0.2-1.1%) in the non-LD group. In the LD-AIDS group, the estimated risk was already 5.0% at 1 year, 6.6% at 2 years, 7.5% at 3 years, and 8.2% at 4 years, indicating a large increase in risk over the first year followed by a gradual increase of approximately 1% per year.

After adjusting for potential confounders, LD individuals, compared to non-LD ones, showed a significantly higher risk of death for any cause [adjusted hazard ratio (aHR) 5.51, 95% CI 2.87-10.60, $p<0.001$] [Table 2a]. Furthermore, compared to LD-Asymptomatic, being an AIDS presenter was associated with a risk more than four times greater of all-cause mortality [aHR 4.42, 95% CI 3.14-6.22, $p<0.001$] whereas being non-LD significantly reduced this risk [aHR 0.35, 95% CI 0.17-0.59, $p=0.002$] [Table 2b]. No difference in the effect of LD on mortality risk by calendar year of ART initiation was observed [Table 2a and 2b].

Mediation analysis

A total of 2,568 participants (1,697 (66%) non-LD and 871 (34%) LD-AIDS) survived for more than 2 years after treatment initiation and they had a measure of CD4 count within the time window of 21 to 27 months after starting ART. Of these, 1065 (41%) did not achieve a CD4 count >500 cells/mm^3 by 2 years. Overall 43 deaths (2%) were observed, 36 (84%) in the LD group and 7 in the non-LD group. Interestingly, in this selected subset, we found evidence that the failure of achieving

a CD4 count > 500 cells/mm^3 was both an effect measure modifier and a mediator for the effect of AIDS presentation on the long-term mortality risk. Particularly, the four-way decomposition analysis revealed that approximately 80% of the total effect of LD-AIDS (vs. non-LD) on the risk of long-term mortality was due to mediation (of which 72% was interaction) with the 2-year CD4 count gain [Supplementary Figure 3]. This suggests that among this group of short-term survivors, LD-AIDS participants who did not achieve full immune-recovery by 2 years have an even higher risk of long-term mortality when compared to non-LD (i.e. there is an interaction) and, at the same time, the LD-AIDS condition itself is needed for a CD4 count ≤500 cells/mm^3 to be present at 2-year (i.e. LD-AIDS causes a CD4 count ≤500 at 2 years which is itself necessary for LD-AIDS to have an effect on mortality). The estimated median time to full immune-recovery (CD4>500 cells/mmc) was 4.1 years (95% CI:3.7-4.5) for the LD-AIDS and 2.2 years (95% CI:2.-02.3) for the LD-asymptomatic group [Supplementary Figure 2].

AIDS-related and Non-AIDS-related mortality

Over the study period, we observed 73 (45.3%) deaths due to ADEs and 88 (54.6%) to non-AIDS. As expected, when restricting the analysis to those who died, the proportion of AIDS-related deaths gradually decreased over time accounting for 63.6% of total deaths during the first year after ART started, 35.1% between the second and fifth year, and 14.8% thereafter [Supplementary Table 1]. AIDS-related deaths occurred exclusively in LD patients, particularly AIDS presenters, for whom they accounted for 78% of all deaths during the first year, 52% between the second and the fifth year, and 37% later [Supplementary Figure 4]. Among non-AIDS-related deaths, malignancies were the leading cause of death. In contrast, AIDS-related mortality was mainly driven by infections over the first year after ART initiation and by malignancies thereafter [Supplementary Table 1].

Competing risk Kaplan-Meier curves showed a significantly higher probability of both AIDS-related and not-related mortality for LD-AIDS compared with the other exposure groups (p<0.001) [Figure 1c and 1d]. Among LD patients, LD-AIDS showed a risk of dying for ADEs approximately

17-fold greater [aSHR 16.86, 95% CI 8.24-34.46, p<0.001] and for non-AIDS about two times higher compared to LD-Asymptomatic [aSHR 1.74, 95% CI 1.08-2.78, p=0.022]. The interaction test carried no evidence that this risk might be different in recent years as opposed to earlier periods both for AIDS-related and not-related mortality [Table 2c and 2d].

Treatment failure

Overall, 1,709 (25.1%) subjects experienced TF: 453 (18.5%) in the non-LD and 1,256 (28.8%) in the LD-group, including 399 (34.2%) LD-AIDS and 857 (26.8%) LD-Asymptomatic subjects. In all exposure groups, TF was mainly driven by TD (90.7%, 91.3%, and 87.0% of non-LD, LD-Asymptomatic, and LD-AIDS patients, respectively) rather than VF. Of note, after disaggregating the composite outcome, less than 5% of the study population experienced the VF in the TF outcome (1.7%, 2.4% and 4.5% of non-LD, LD-Asymptomatic and LD-AIDS subjects, respectively) which was mainly related to rebounds after the achievement of viral suppression (particularly 39 of 42 VFs in non-LD group, 67 of 75 VFs in LD-Asymptomatic group and 48 of 52 VFs in LD-AIDS were due to viral rebound), with no evidence for a difference by exposure groups (p=0.762).

The estimated probabilities of TF significantly differed among the exposure groups (log-rank p<.001) [Figure 2a and 2b]. From fitting the multivariable Cox regression models, LD individuals compared to non-LD [aHR 1.21, 95% CI 1.08-1.36, p=0.001] as well as AIDS presenters compared to LD-Asymptomatic subjects [aHR 1.30, 95% CI 1.15-1.47, p<0.001] showed a higher risk of TF. On the contrary, non-LD patients were associated with a lower risk of TF compared to LD-Asymptomatic [aHR 0.88, 95% CI 0.78-1.00, p=0.046] (Table 3a and 3b). The analysis stratified by calendar period, conducted for the evidence of interaction with the year of ART starting (p<0.001), compared to LD-Asymptomatic, PLWH LD-AIDS had a higher risk of TF in both 2009-2014 and 2015-2022, while non-LD showed a lower risk only in most recent calendar period [ARH 0.79, 95%CI 0.66-0.94] [Supplementary Table 3].

DISCUSSION

In this observational study, including 6,813 PLWH starting ART within 4 months from diagnosis between 2009 and 2022, we showed that, even in more recent years, subjects presenting late to care, and particularly AIDS presenters, despite a prompt linkage to care after diagnosis, remained at substantially greater risk for both mortality and treatment failure.

Consistent with previous evidence [6-8,19], we found that the overall risk for all-cause mortality was approximately 5.5-fold higher in LD individuals than in those without late diagnosis, and 4.5-fold higher in AIDS presenters compared to the remaining late diagnosed patients, particularly in the first year after diagnosis (1-year mortality risk: 5%). Specifically, the increased risk of mortality for AIDS presenters was mainly driven by ADEs, responsible for more than three-quarters of deaths within the first year after diagnosis, but also by non-AIDS events. Similarly, LD-Asymptomatic subjects showed a higher risk of dying for reasons other than AIDS compared with non-LD ones.

Previous studies described an increased risk for non-AIDS in subjects with LD or AIDS presentation compared to their counterparts [8,20-23]. A previous Italian study showed that the risk of non-AIDS was specifically increased in advanced HIV patients and in those with previous ADE who failed to restore their immunocompetence despite effective ART [20]. Pre-existing immune dysfunction, immune activation, and persistent inflammation have been suggested as possible mechanisms for the development of non-AIDS in this vulnerable population [20]. In addition, a higher burden of multimorbidity has been described in late presenters' patients [15]. To the best of our knowledge, this is one of the few large cohort studies that separately analyzed mortality due to ADEs and non-AIDS.

Recently, conflicting evidence on the role of immunological recovery after ART initiation as a protective factor for long-term mortality in patients with LD have emerged [13,14]. In our study, we performed a formal mediation analysis which showed that a large part of the effect of AIDS presentation on the risk of long-term mortality in those surviving the first two years after ART start

was explained by the failure to restore immunocompetence, suggesting that a significant part of the excess mortality risk in advanced HIV patients is possibly explained by their current immunological status. Of note, despite recent evidence suggesting a beneficial role of first-line INSTI-based regimens on survival in advanced HIV subjects [13,24], in our study the increased risk of both all-cause and cause-specific mortality for LD and LD-AIDS persisted after adjustment for anchor drug class and calendar year of ART start (a proxy for change in therapeutic indications).

In our cohort, LD individuals and, particularly AIDS presenters, showed increased rates of TF, mostly driven by the discontinuation of one or more drugs in the initial regimen due to toxicity/failure. This finding is in line with a recent observation reporting a higher risk of TD due to adverse events in late presenters [12]. The choice of ART in LD subjects, especially AIDS presenters, is challenging and very few data on the optimal first-line regimen are available, as these patients are still poorly represented in clinical trials. Recently, large cohort studies described higher discontinuation rates in advanced HIV patients starting PI-based compared to INSTI-based regimens [9,24-26]. However, this finding has not been confirmed by other reports [27,28], and needs to be clarified by the results of ongoing clinical trials [29,30]. Of note, in our study, INSTI (particularly DTG)-based and PI (mostly DRV)-based regimens were the most prescribed first-line ART in LD and LD-AIDS patients and interestingly the higher risk for TF persisted after adjustment for the third-drug class.

Finally, the prevalence of LD in our cohort, despite being biased by the selection of patients who started ART within few months from diagnosis, is in line with data reported by cohort studies from other high-income countries and recent European and Italian surveillance reports in which rates of late HIV diagnosis and AIDS presentation ranged approximately between 40 to 60% and 8 to 20%, respectively [1,2,8,12,13]. In these settings, late presentation appears to disproportionally affect certain demographic groups such as women, older adults, heterosexuals, migrants, and persons with low educational levels [7,8,12,19,31], suggesting that the risk of LD is greater in groups not traditionally considered to be at high risk of acquiring HIV [32]. It is worth noting that in our study,

the characteristics of LD population mirror most of the risk factors which have been associated with delayed presentation to care.

Our study has some limitations. First, the observational nature of the study, which is subject to bias due to unmeasured confounders. In particular, we did not include in the multivariable models potentially important confounders such as the rate of access to healthcare services, fear of stigmatization and health locus of control (the degree to which individuals believe that their health is controlled by internal or external factors) which were not covered by our data collection [33]. Additionally, we did not adjust for baseline comorbidities, as they were considered mediators and not confounders for the considered outcomes. However, results were similar after repeating the analyses for the main outcomes (all-cause mortality and TF) including baseline comorbidities in the model. Second, the choice of ART initiation as the baseline for the survival analysis might have introduced immortal-time bias (only the late diagnosed who survived long enough to be able to start ART are included), which was partially mitigated by restricting the analysis to participants who started ART within 4 months from the diagnosis. On the other hand, choosing the date of enrolment in the cohort as the baseline for the survival analysis, might have led to non-proportional hazards due to the high risk of death in participants diagnosed late in absence of ART, as well as generated the issue of how to correctly control for time-varying ART initiation. If anything, immortal time bias could have conferred an advantage to the late diagnosed and therefore our estimates of the difference in risk are potentially even under-estimated.

Furthermore, always regarding the selection of study population, it needs to be noted that the inclusion of subjects with a short time from enrolment to ART initiation might have led to an artificially selected group of rapid starters in the non-LD patients enrolled before 2015 who are also likely to have better prognosis than the average non-LD patient. Nonetheless, we did not find evidence for interaction between calendar periods (2009-2014 and 2015-2022) and the main outcomes all-cause mortality and AIDS-related mortality, but with the treatment failure. In the analysis restricted after 2015 the non-LD group had lower risk of TF compared to LD-

Asymptomatic, not identified in the period (2009-2014). In addition, we might have misclassified participants presenting with a low CD4 count as an LD when he/she was a case of acute infection. This potential bias was partially mitigated by excluding subjects with an available previous CD4 count discordant with the exposure group of classification. Fifth, the change in the guidelines on recommended first-line antiretroviral drugs and indication for ART initiation during the observation period might have introduced a bias (as time from enrolment to ART initiation was shorter with more recent calendar time), only partially attenuated by adjusting the analyses by the type of regimen started and calendar year. Finally, the lack of information about the reason of death for approximately one-fifth of deaths may have limited the detailed interpretation of the mortality outcomes.

Nevertheless, our analysis has also important strengths. Firstly, the use of real-life data from a large national cohort makes our results highly representative of the situation of HIV-infected individuals in Italy. Additionally, the relevant length of the observation period and the 5-year follow-up gives a valuable representation of the changes in epidemiology and outcomes that occurred during the last fourteen years.

CONCLUSIONS

Our study showed that PLWH presenting late to care, particularly AIDS presenters, despite a rapid linkage to care and ART initiation, still presented significantly poorer outcomes in terms of both survival and treatment durability compared with the rest of ART-naïve subjects. Of note, for early-survivors, most of the long-term effect was mediated by the failure to achieve immunological recovery after ART initiation. Considering the persistent mortality gap of LD and AIDS-presenting patients, also in high-income countries, public health strategies for emerging unknown infections are urgently needed, including the extension of HIV testing beyond routine settings, the increase of indicator-conditions-guided testing in all healthcare services, the development of public campaigns

to normalize HIV testing and reduce the stigma and the decrease of structural barriers for HIV testing [31,32,34].

AUTHORS' CONTRIBUTIONS

Conception: A.M., A.C-L., A.T., A.d.M., A.A.; Study Design: A.M., A.C-L., A.T.; Statistical Analysis: A.C-L.; Acquisition of data: A.T.; A.G., G.O., G.D.G., C.P.; Interpretation of the data: A.M., A.C-L., A.T., A.G., A.S., A.B., G.M., E.G., C.M., A.d.M., A.A.; Draft of the manuscript: A.M.; Review of the article and critical revision for important intellectual content: All the Authors. Final approval of the submitted version: All Authors. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication

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DATA AVAILABILITY STATMENT

The datasets generated during the current study are not publicly available because they contain sensitive data to be treated under data protection laws and regulations. Appropriate agreement of data sharing can be arranged after a reasonable request to the corresponding author.

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Table 1. Main characteristics (A) and first-line ART regimens (B) of total population and according to the treatment group at ART initiation

(A)	Total N= 6,813	Non-late Diagnosis (non-LD) N= 2,448 (35.9%)	Late Diagnosis (LD) N= 4,365 (64.1%)	LD-Asymptomatic N=3,198 (46.9%)	LD-AIDS N=1,167 (17.1%)	p-value[a]	p-value[b]
Female Gender, n (%)	1,295 (19.0)	382 (15.6)	913 (20.9)	660 (20.6)	253 (21.7)	**<0.001**	**<0.001**
Age, years, median (IQR)	40 (31-49)	36 (29-45)	42 (34-51)	41 (33-50)	45 (37-53)	**<0.001**	**<0.001**
Mode of HIV Transmission, n (%)						**<0.001**	**<0.001**
MSM	3,040 (44.6)	1,408 (57.5)	1,632 (37.4)	1,256 (39.3)	376 (32.2)		
Heterosexual	2,892 (42.4)	746 (30.5)	2,146 (49.1)	1,526 (47.7)	620 (53.1)		
IDU	322 (4.7)	118 (4.8)	204 (4.7)	140 (4.4)	64 (5.5)		
Other/Unknown	559 (8.2)	176 (7.2)	383 (8.8)	276 (8.6)	107 (9.2)		
Not Italian Nationality, n (%)	3,916 (57.5)	1,484 (60.6)	2,432 (55.7)	1,805 (56.4)	627 (53.7)	**<0.001**	**<0.001**
HBsAg, n (%)						**<0.001**	**<0.001**
Negative	1,913 (28.1)	778 (31.8)	1,135 (26.0)	850 (26.6)	285 (24.4)		
Positive	4 (0.1)	2 (0.1)	2 (0.0)	2 (0.1)	0 (0.0)		
Not tested	4,896 (71.9)	1,668 (68.1)	3,228 (74.0)	2,346 (73.4)	882 (75.6)		
HCVAb, n (%)						**<0.001**	**<0.001**
Negative	1,784 (26.2)	724 (29.6)	1,060 (24.3)	800 (25.0)	260 (22.3)		
Positive	128 (1.9)	57 (2.3)	71 (1.6)	49 (1.5)	22 (1.9)		
Not tested	4901 (71.9)	1,667 (68.1)	3,234 (74.1)	2,349 (73.5)	885 (75.8)		
Calendar year of baseline, median (IQR)	2016 (2014-2019)	2017 (2015-2019)	2016 (2013-2019)	2016 (2013-2019)	2016 (2012-2018)	**<0.001**	**<0.001**
CD4 count nadir, cells/mm³, median (IQR)	257 (95-451)	512 (425-655)	136 (48-242)	179 (78-264)	49 (20-120)	**<0.001**	**<0.001**
BL CD4 count, cells/mm³							
Median (IQR)	258 (96-452)	528 (435-680)	138 (49-247)	181 (80-265)	49 (21-125)	**<0.001**	**<0.001**
≤200 *cells/mm³, n (%)*	2,818 (41.4)	-	2,818 (64.6)	1,792 (56.0)	1,026 (87.9)	**<0.001**	**<0.001**
HIV-RNA, log₁₀ copies/mL, median (IQR)	4.91 (4.18-5.52)	4.51 (3.73-5.14)	5.12 (4.50-5.66)	5.05 (4.45-5.57)	5.32 (4.63-5.86)	**<0.001**	**<0.001**
AIDS diagnosis, n (%)	1,167 (17.1)	-	1,167 (26.7)	-	1,167 (100.0)	**<0.001**	**<0.001**
Comorbidities and Habits							
Diabetes, n (%)	160 (2.3)	33 (1.3)	127 (2.9)	78 (2.4)	49 (4.2)	**<0.001**	**<0.001**
CDV disease, n (%)	56 (0.8)	11 (0.4)	45 (1.0)	27 (0.8)	18 (1.5)	**0.011**	**0.003**

Hypertension, n (%) *	326 (4.8)	89 (3.6)	237 (5.4)	150 (4.7)	87 (7.5)	**<0.001**	**<0.001**
Dyslipidemia, n (%) **	104 (1.5)	30 (1.2)	74 (1.7)	49 (1.5)	25 (2.1)	0.129	0.110
Smoking, n (%)	1,942 (28.5)	788 (32.2)	1,154 (26.4)	888 (27.8)	266 (22.8)	**<0.001**	**<0.001**
Social Determinants							
Education, n (%)						**<0.001**	**<0.001**
Primary school	320 (4.7)	65 (2.7)	255 (5.8)	161 (5.0)	94 (8.1)		
Secondary school	940 (13.8)	251 (10.3)	689 (15.8)	490 (15.3)	199 (17.1)		
College	1880 (27.6)	699 (28.6)	1,181 (27.1)	869 (27.2)	312 (26.7)		
University	857 (12.6)	404 (16.5)	453 (10.4)	339 (10.6)	114 (9.8)		
Other/Unknown	2816 (41.3)	1,029 (42.0)	1,787 (40.9)	1339 (41.9)	448 (38.4)		
Employment, n (%)						**<0.001**	**<0.001**
Unemployed	866 (12.7)	307 (12.5)	559 (12.8)	409 (12.8)	150 (12.9)		
Employed/Self-employed	3,489 (51.2)	1,238 (50.6)	2,251 (51.6)	1,649 (51.6)	602 (51.6)		
Occasional	168 (2.5)	42 (1.7)	126 (2.9)	86 (2.7)	40 (3.4)		
Student	214 (3.1)	138 (5.6)	76 (1.7)	64 (2.0)	12 (1.0)		
Retired/Invalid	229 (3.3)	45 (1.8)	186 (4.3)	121 (3.8)	63 (5.4)		
Housewife	112 (1.6)	31 (1.3)	81 (197)	51 (1.6)	30 (2.6)		
Other/Unknow	1,735 (25.5)	647 (26.4)	1,088 (24.9)	818 (25.6)	270 (23.1)		
Follow-up time, months, median (IQR)	46 (17- 83)	40 (16-73)	50 (19-90)	50 (18-90)	53 (20-90)	**<0.001**	**<0.001**
First-line ART regimens							
Months from HIV diagnosis to ART start, median (IQR)	1 (0-2)	1 (0-2)	1 (0-1)	1 (0-1)	1 (0- 1)	**<0.001**	**<0.001**
Type of regimen started, number of drugs, n (%)						**<0.001**	**<0.001**
2-drug regimen (3TC+DTG)	130 (1.9)	89 (3.6)	41 (0.9)	38 (1.2)	3 (0.3)		
3-drug regimen	6332 (92.9)	2171 (88.7)	4161 (95.3)	3043 (95.2)	1118 (95.8)		
- *INSTI-based*	3319 (48.7)	1293 (52.8)	2026 (46.4)	1505 (47.1)	521 (44.6)		
- *PI-based*	1931 (28.3)	409 (16.7)	1522 (34.9)	1076 (33.6)	446 (38.2)		
- *NNRTI-based*	1032 (15.1)	460 (18.8)	572 (13.1)	441 (13.8)	131 (11.2)		
- *other*	50 (0.7)	9 (0.4)	41 (0.9)	21 (0.7)	20 (1.7)		
≥4-drug regimen	351 (5.2)	188 (7.7)	163 (3.7)	117 (3.7)	46 (3.9)		
Type of drug, n (%)						-	-
DTG	1648 (24.2)	653 (26.7)	995 (22.8)	724 (22.6)	271 (23.2)		
BIC	820 (12.0)	340 (13.9)	480 (11.0)	376 (11.8)	104 (8.9)		
RAL	631 (9.3)	245 (10.0)	386 (8.8)	260 (8.1)	126 (10.8)		
DRV/b	1462 (21.5)	413 (16.9)	1049 (24.0)	720 (22.5)	329 (28.2)		
ATV/b	571 (8.4)	132 (5.4)	439 (10.1)	338 (10.6)	101 (8.7)		
EFV	490 (7.2)	110 (4.5)	380 (8.7)	266 (8.3)	114 (9.8)		

Notes: a. *comparison non-LD versus LD* ***b****. comparison non-LD versus LD-Asymptomatic versus LD-AIDS; **Use of blood pressure lowering drugs ***Use of statins*

Abbreviations: *LD: late diagnosis; non-LD= not late diagnosis; ART= Antiretroviral therapy; MSM=Men Sex with Men; IDU= Intravenous Drug User; BL= baseliene; Ag=antigen; Ab=antibody; CDV=Cardiovascular;* 3TC=lamivudine; DTG= dolutegravir; NNRTI= non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor; INSTI= integrase strand transfer inhibitor; BIC= bictegravir; RAL=raltegravir; DRV/b=boosted darunavir; ATV/b=boosted atazanavir; EFV= efavirenz; RPV=rilpivirin; DOR=doravirine; TDF=tenofovir disoproxile fumarate; TAF= tenofovir alafenamide; ABC=abacavir

Table 2: Unadjusted and Adjusted Hazard Ratio for all-cause mortality (non-LD versus LD [Table 3a] and non-LD vs LD-Asymptomatic vs LD-AIDS [Table 3b]) and Unadjusted and Adjusted Sub-Hazard Ratio for specific-cause mortality (AIDS-related [Table 3c] and not-AIDS related mortality [Table 3d]) associated with late HIV diagnosis

	HR	95% CI	p-value	aHR*	95% CI	p-value
Table 2a: all-cause mortality#						
Non-LD	1	-		1	-	
LD	6.86	3.72-12.67	**<0.001**	5.51	2.87-10.60	**<0.001**
X *interaction test exposure group and calendar year of ART start: p=0.41*						
Table 2b: all-cause mortality#						
LD-Asymptomatic	1	-		1	-	
LD-AIDS	5.19	3.70-7.28	**<0.001**	4.42	3.14-6.22	**<0.001**
Non-LD	0.31	0.16-0.60	**<0.001**	0.35	0.17-0.69	**0.002**
X *interaction test exposure group and calendar year of ART start: p=0.07*						
	SHR	**95% CI**	**p-value**	**aSHR***	**95% CI**	**p-value**
Table 2c: AIDS-related mortality&						
LD-Asymptomatic	1	-		1	-	
LD-AIDS	19.13	9.52-38.42	**<0.001**	16.86	8.24-34.46	**<0.001**
Non-LD	-	-	**-**	-	-	**-**
X *interaction test exposure group and calendar year of ART start: p=0.42*						
Table 2d: not AIDS-related mortality&						
LD-Asymptomatic	1	-		1	-	
LD-AIDS	2.09	1.32-3.33	**0.002**	1.74	1.08-2.78	**0.022**
Non-LD	0.42	0.22-0.82	**<0.001**	0.53	0.26-1.11	**0.093**
X *interaction test exposure group and calendar year of ART start: p=0.65*						

***** adjusted for age, gender, mode of HIV transmission, nationality, calendar year for ART initiation, hepatitis-coinfection and type of ART regimen

standard Cox regression model. **&**Fine-Gray Cox regression model

List of abbreviations HR Unadjusted Hazard Ratio, aHR, adjusted Hazard Ratio;CI, confidence interval; LD= late diagnosis; non-LD= non late diagnosis

Table 3: Unadjusted and Adjusted Hazard Ratio for treatment failure associated with late HIV diagnosis (non-LD versus LD, Table 3 a and non-LD vs LD-Asymptomatic vs LD-AIDS, Tab 3b) from fitting a standard Cox regression model.

Table 3a: treatment failure	**HR**	**95% CI**	**p-value**	**aHR***	**95% CI**	**p-value**
Non-LD	1	-		1	-	
LD	1.51	1.35-1.68	**<0.001**	1.21	1.08-1.36	**0.001**
[X] *interaction test exposure group and calendar year of ART start:* ***p<0.01***						
Table 3b treatment failure						
LD-Asymptomatic	1	-		1	-	
LD-AIDS	1.36	1.21-1.53	**<0.001**	1.30	1.15-1.47	**<0.001**
Non-LD	0.72	0.65-0.81		0.88	0.78-1.00	**0.046**
[X] *interaction test exposure group and calendar year of ART start:* ***p<0.01***						

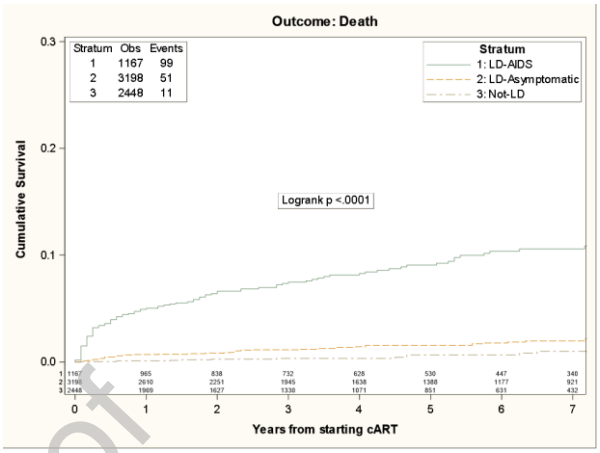
* adjusted for age, gender, mode of HIV transmission, nationality, calendar year for ART initiation, hepatitis-coinfection, type of ART regimen according to the third drug

** List of abbreviations: HR; Unadjusted Hazard Ratio, aHR, adjusted Hazard Ratio; CI, confidence interval; LD: late diagnosis; non-LD= non-late diagnosis

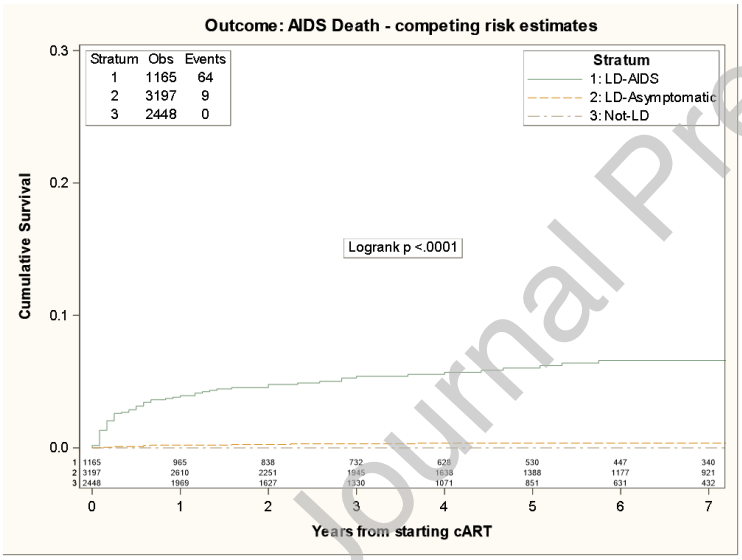
Figure 1: Kaplan-Meier curves for all-cause mortality [non-LD versus LD (Figure 1a) and non-LD versus LD-Asymptomatic versus LD-AIDS (Figure 1b)] and cause-specific mortality* [AIDS-related deaths (Figure 1c) and not-AIDS related deaths (Figure 1c) according to the exposure group.

1a

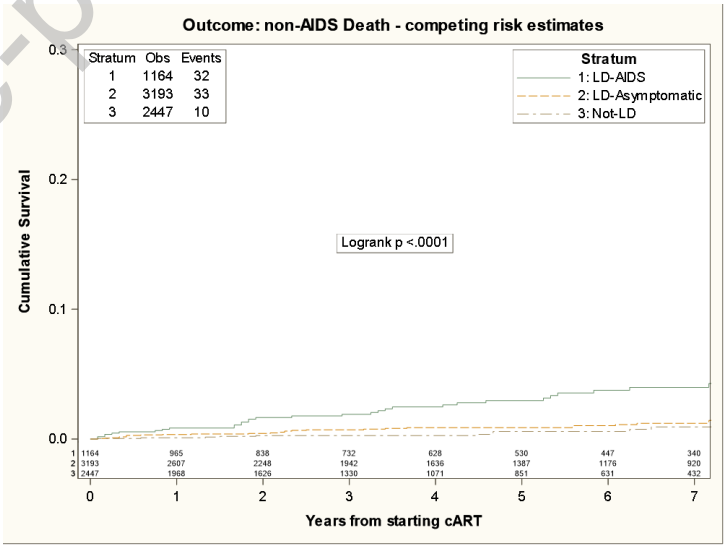
1b



1c



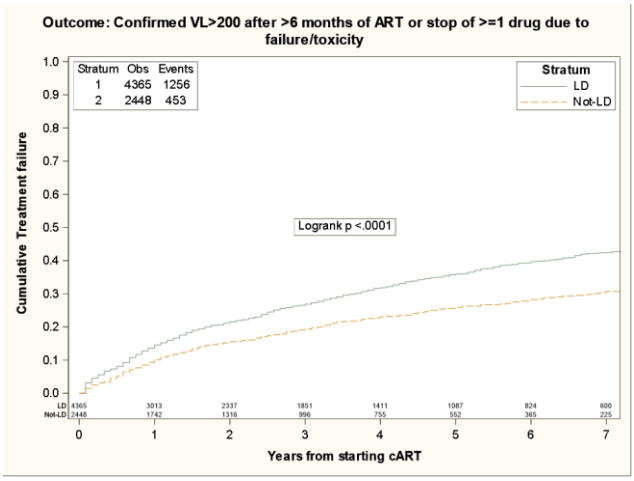
1d



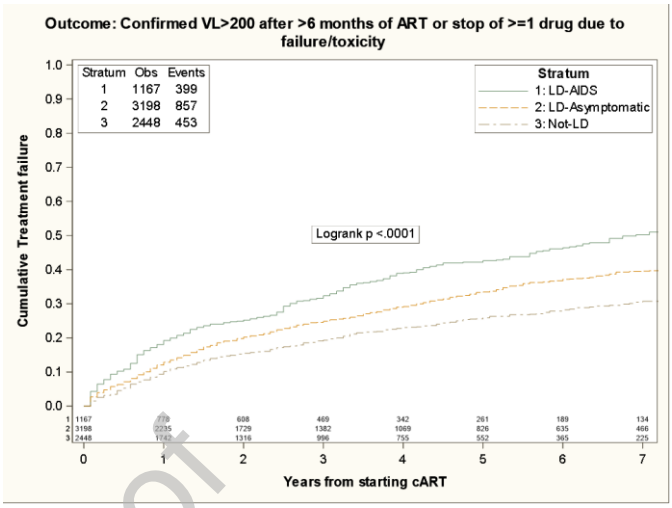
* competing-risk Kaplan-Meier curves

Figure 2: Kaplan-Meier estimates for treatment failure according to the exposure group: non-LD versus LD (Figure 2a) and non-LD versus LD-Asymptomatic versus LD-AIDS (Figure 2b)

2a



2b



DECLARATION OF INTEREST

A.M. received speakers' honoraria from Gilead Sciences and ViiV Healthcare, travel fee and participated in advisory boards sponsored by Viiv Healthcare. A.C. received funding for scientific advisory boards, travel, or speaker honoraria from Gilead Sciences, ViiV Healthcare, Janssen-Cilag, MSD. A.Giacomelli reports speakers' honoraria for ViiV Healthcare and Gilead Sciences, advisor for Janssen-Cilag and Mylan. C.P. received personal fee from Gilead Sciences for a case presentation and a travel grant and served on an advisory board for Janssen-Cilag; A.Gori received speaker's honoraria and fees for attending advisory boards from ViiV Healthcare, Gilead Sciences, Janssen-Cilag, MSD, BMS, Pfizer and Novartis and received research grants from ViiV, BMS, and Gilead Sciences. A.S. received speakers' honoraria or participated in advisory boards sponsored by Gilead Sciences, ViiV Healthcare, MSD and Janssen-Cilag. A.B. received speaker's honoraria and fees for attending advisory boards from Astra-Zeneca, BioMerieux, Janssen-Cilag, Nordic Pharma, Pfizer, Qiagen, SOBI, ViiV and received research grants from Gilead; G.M. participated to advisory boards of Gilead Sciences, ViiV Healthcare, Angelini and Janssen-Cilag, and received travel grants from ViiV Healthcare, MSD and Janssen-Cilag; E.G. received grant support from Gilead Sciences and Mylan, and speaker's honoraria from Gilead Sciences. C.M. received speakers' honoraria or participated in advisory boards sponsored by Gilead Sciences, ViiV Healthcare, MSD and Janssen-Cilag and received research grants from Gilead Sciences; A.d.M. participated in advisory board of Gilead Sciences, ViiV Healthcare, MSD, Pfizer and GSK and reports research grant from Gilead Science, ViiV Healthcare, Merck Sharp and Dohme, GSK and Janssen-Cilag; A.A. received Research grants from Gilead Sciences, AstraZeneca, ViiV Healthcare and Honoraria from Gilead Science, AstraZeneca, GSK, Pfizer, MSD, Moderna, Mylan, Janssen-Cilag, ViiV Healthcare; A.C-L., A.T., G.D.G. and G.O. have nothing to declare.