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Does syphilis increase the risk of HIV-RNA elevation >200 copies/mL in HIV positive patients under effective antiretroviral treatment? Data from the ICONA cohort

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Running head

Syphilis and risk of HIV viral rebound

Abstract

Background: To assess the impact of syphilis infection on the risk of HIV-RNA elevation in people living with HIV (PLWH) with current HIV-RNA ≤50 copies/mL.

Setting: The Italian Cohort Naïve Antiretrovirals (ICONA).

Methods: All PLWH (2009-2020) under antiretroviral treatment with at least 2 consecutive HIV-RNA values ≤50 copies/mL before the date of syphilis diagnosis and at least one HIV-RNA determination after the syphilis event were enrolled. A control group of PLWH without syphilis was matched for mode of HIV transmission. Outcomes were defined using the first HIV-RNA measure in the time window ranging between -2 and +6 months of the diagnosis/index date. The primary outcome used a single value>200 copies/mL to define HIV-RNA elevation associated with risk of transmission. The association between syphilis infection and the protocol defined outcome was evaluated using logistic regression analysis.

Results: Nine hundred and twenty-six PLWH with a syphilis event were enrolled and matched with a random sample of 1370 PLWH without syphilis. Eighteen of the 926 (1.9%) with syphilis had ≥1 HIV-RNA>200 copies/mL in the window vs. 29/1370 (2.1%) of the not exposed (p=0.77). In the multivariable analysis adjusted for age, year of diagnosis/index date and clinical site, syphilis infection was not associated with the risk of HIV-RNA >200 copies/mL [adjusted Odds Ratio 0.81; 95% confidence interval 0.43-1.52, p=0.508].

Conclusions: We did not find any evidence for an association between syphilis infection and viral elevation >200 copies/mL.

Key words: HIV, PLWH, syphilis, viral rebound, STDs, TasP.

Introduction

The rate of sexually transmitted infections (STIs) is increasing at an alarming rate especially among men who have sex with men (MSM) [1, 2] and people living with HIV (PLWH) worldwide [3].

Among STIs, syphilis has resurged as a global public health problem with 6 million new syphilis infections estimated worldwide in 2016 [1]. This increase seems more pronounced in high income countries [4,5]. A parallel trend toward an increased incidence of syphilis has been observed in MSM PLWH both in America [6], Europe [7] and China [8]. In Italy, data from the Italian Cohort Naïve Antiretrovirals (ICONA) collected between 1998 and 2012 showed a crude incidence rate of any-stage syphilis of 3.95/1000 Person Years Follow Up (95% CI 3.59–4.35/1000) [3].

Untreated syphilis can facilitate the transmission and acquisition of HIV infection [9]. In the MSM group, syphilis surveillance showed an increasing HIV and syphilis co-infection rate, ranging from 30% to 60% depending on the geographic location [10, 11].

Among STIs, syphilis has been associated with HIV-RNA increase combined with a temporary decline in CD4 T-cell count in PLWH not receiving antiretroviral treatment (ART) [12, 13]. Whether syphilis could increase the risk of viral rebound with potential implication for HIV transmission in PLWH under effective ART is still a matter of debate [14, 15].

The aim of the present analysis is to assess the impact of syphilis infection on the risk of HIV-RNA elevation >200 copies/mL in virologically suppressed PLWH.

Materials and methods

Study design

Observational study nested within the ICONA Foundation study cohort.

Setting

The ICONA is an Italian multicentric cohort of PLWH settled in 1997. The entry criteria to be enrolled in the cohort is to be antiretroviral naïve. Details of the cohort protocol for data collection have been published elsewhere [16].

Patients

The subset of the patients enrolled in ICONA from January 2009 to September 2020 who ever developed syphilis over follow-up were included in the analysis if they satisfied the following inclusion criteria:

- Had at least 2 consecutive undetectable HIV-RNA (≤50 copies/mL) while receiving ART and before the date of syphilis diagnosis (the most recent undetectable value had to be at least 3 months prior to the diagnosis).
- Had at least 1 additional HIV-RNA in the time-window [-2; +6] months of the diagnosis

All syphilis infection events were considered but only the first syphilis event which met the inclusion criteria for each PLWH was included in the analysis. This group represents the exposed group of interest.

A group of non-syphilis infected (unexposed) (at least one not exposed for every participant who developed syphilis) was randomly selected among participants of the ICONA cohort study if they satisfied the same inclusion criteria of the exposed but who, after the same duration of follow-up, were free from syphilis. Not exposed were further matched for mode of HIV transmission. The study comprised of matched sets of different sizes, ranging from n=2 (1 matched uninfected for

every PLWH infected with syphilis) to n=4 (3 matched uninfected for every PLWH infected with syphilis).

The hypothesis is that the syphilis could directly impact on the risk of HIV-RNA >200 copies/mL in virologically suppressed PLWH. The confounders, identified through a direct acyclic graph (DAG) (Figure 1), that could interact, between a direct or indirect route, with both exposure and outcomes were:

-Mode of HIV transmission (controlled by matching): MSM could be at higher risk of syphilis acquisition and different mode of transmission could affect the risk of HIV-RNA >200 copies/mL through adherence.

- Age (adjusted in the regression model): age could affect the risk of syphilis acquisition as younger people are at higher risk of STIs. In addition older subjects are generally more adherent to ART and are likely to have a longer antiretroviral exposure and a higher number of previous virological failures all factors which can potentially modify the risk of HIV-RNA >200 copies/mL.

- Calendar year of diagnosis/index date (adjusted in the regression model): in more recent years a known increase of STIs and in particular syphilis was observed whereas more effective and tolerable ART regimens are available.

- Clinical site (adjusted in the regression model): it is possible that routine syphilis screening and quality of care may vary by clinical site.

All patients signed a written informed consent to be enrolled in ICONA.

Definition and clinical classifications

For the not exposed we will refer to the date, matching the date of diagnosis for the corresponding exposed case, as the 'index date'.

Syphilis occurrence was defined as the clinical diagnosis of any-stage syphilis (primary, secondary, tertiary/neurosyphilis, early latent, late latent) [17]. PLWH syphilis cases were classified in the absence of clinical signs/symptoms as early latent, if they had a negative treponemal test within 12 months from the diagnosis or if they have a negative non treponemal test in case of previous syphilis within the previous 12 months, and the remaining as late latent syphilis [17].

Outcomes

The outcome was defined as having ≥1 HIV-RNA>200 copies/mL measured in the time window [-2; +6 months] of the diagnosis of syphilis (for exposed) or matching index date (for not exposed). This time window was chosen on the basis of the incubation period of syphilis and the potential latency in the diagnosis [14]. If there was at least one HIV-RNA was >200 copies/mL in the window the participants were defined as having a viral load elevation potentially associated with transmission (primary binary outcome) [18]. In an alternative analysis single HIV-RNA elevation was defined using the threshold of >50 copies/mL.

Statistical analysis

The association between syphilis infection and the protocol defined outcomes was evaluated using logistic regression analysis. The multivariable logistic analysis was adjusted for potential confounders chosen a priori according to our assumed underlying causal structure of the data depicted in the DAG: age, calendar year at diagnosis/index date and clinical site. In addition, mode of transmission was controlled for by matching. Besides syphilis infection, which was the main exposure of interest, we also investigated the association between the potential confounders included in the model and the risk of protocol outcomes.

The analysis was repeated after restricting only to PLWH with early syphilis (primary, secondary and early latent).

Finally, an interrupted time series analysis (ITS) was used to assess the trend of HIV-RNA in PLWH with syphilis before and after the date of diagnosis.

The statistical analysis was conducted using SAS (version 9.4, Carey NC USA). A p value <0.05 was considered statistical significant.

Results

Nine hundred and twenty-six PLWH with a syphilis event were enrolled and matched with a random sample of 1370 PLWH without syphilis.

The clinical and demographic characteristics of the study population are reported in Table 1.

We have classified the infections as follows: 29 primary syphilis (3.1%), 52 secondary syphilis (5.6%), 2 tertiary/neurosyphilis (0.2%), 155 (16.7%) early latent syphilis and 688 (74.3%) late latent syphilis. Therefore there were a total of 236 (25.5%) early and 690 (74.5%) late infections.

Eighteen of the 926 (1.9%) with syphilis had ≥1 HIV-RNA>200 copies/mL in the window vs. 29/1370 (2.1%) of the not exposed (p=0.77), whereas an HIV-RNA >50 copies/mL occurred in 51/ 926 (5.5%) exposed and 65/1370 (4.7%) non-exposed (p=0.413).

Factors associated with HIV-RNA elevation >200 copies/mL and >50 copies/mL

In the unadjusted analysis, controlled only for mode of HIV transmission matched by design, there was no evidence for an association between syphilis infection and risk of a single HIV-RNA elevation >200 copies/mL (Odds Ratio (OR) 0.92; 95% CI 0.51-1.66), p=0.744). Results were similar after further controlling in the logistic regression model for age, year of diagnosis/index date and clinical site (adjusted Odds Ratio (AOR) 0.81; 95% CI 0.43-1.52), p=0.508). Regarding the other factors included in the model, more recent years of diagnosis/index date was associated with lower odds (AOR 0.83; 95% CI 0.78-0.88, *p<0.001*].

When using the alternative outcome of single HIV-RNA elevation >50 copies/mL, in the multivariable analysis adjusted for the same factors used in the main analysis, again the data were inconclusive with a 95% confidence interval compatible with both the null hypothesis of no difference and a less than half-fold increased risk of VL>50 copies/mL associated with a diagnosis of syphilis (AOR 1.00; 95% CI 0.67-1.49), p=0.994). Again, a more recent year of diagnosis/index date was associated to lower odds of HIV-RNA >50 copies/mL [AOR 0.85; 95% CI 0.81-0.88; *p<0.001*) also in this analysis.

After restricting to events of early syphilis we included a total of 236 infections matched to 408 non-infected participants. In this subset, 2/236 (0.8%) vs. 9/408 (2.2%) had ≥1 HIV-RNA>200 copies/mL (p=0.200). The estimates provided by the logistic regression model showed inconclusive evidence for an association between early syphilis and risk of viral elevation >200 copies/mL (AOR=0.41, 95% CI:0.08-2.15, p=0.290)

No statistically significant trend towards higher population HIV-RNA levels at time of diagnosis when compared to periods preceding or following the diagnosis was observed in the ITS analysis restricted to PLWH with syphilis.

Discussion

In our cohort we observed a low rate (approximately 2%) of HIV-RNA elevation >200 copies/mL in PLWH with syphilis infection and a previous history of suppressed HIV-RNA<50 copies/mL.

In the pre-ART era, it had been reported that syphilis infection could cause elevation of HIV-RNA and reductions in CD4 cell count, findings which were confirmed by several observational studies [12, 13]. Although currently the vast majority of patients experience a stable viral suppression, it remains unclear whether syphilis infection could still determine changes in HIV-RNA under effective ART. One anecdotal report described a patient experiencing viral rebound during early

syphilis [19]. In an observational study of PLWH who received ART, a concomitant early syphilis resulted associated with higher odds of HIV-RNA elevation ≥500 copies/mL after achieving viral suppression ≤500 copies/mL [14]. Conversely, a recent report by Grewald *et al* using the data of the Ontario HIV Treatment Network cohort showed no evidence for an association between syphilis infection and risk of virological failure in MSM who were virologically suppressed [15]. Our findings are consistent with this observation in the context of HIV-RNA elevations potentially associated with the risk viral transmission.

In our analysis more recent years resulted independently associated with lower odds of both HIV-RNA above 50 copies/mL and 200 copies/mL. This finding is not unexpected considering the improved efficacy and tolerability of new ART regimens allowing a less strict adherence to maintain the target of undetectability [20,21].

The present study has some limitations. First, although STIs screening comprehensive of syphilis is performed in our cohort in accordance with current international guidelines at least once a year, we cannot be sure that all events of syphilis infections are captured so mis-classification of the exposure is possible. Second, our results are condition on our model assumptions to be correct and because of the observational nature of the study we cannot rule out the presence of residual or unmeasured confounding bias. Third, the time window chosen to investigate whether there was an HIV-RNA elevation concomitant or immediately following the diagnosis of syphilis/index date was arbitrary. In addition, because the date of diagnosis is likely to be interval censored it is possible that the chosen time window did not exactly match the time in which participants were experiencing active syphilis. However, results were similar in a sensitivity analysis censoring the window 4 months after the index date (data not shown). Last, because of the relatively small sample size and small number of viral load elevation events, the lack of association might be due to low statistical power. Indeed, although the data are compatible with the null hypothesis of no difference, a one

half-fold higher risk of HIV-RNA elevation in patients with syphilis cannot be excluded with 95% confidence.

In conclusion, in a large cohort of PLWH under effective ART our data show inconclusive evidence for an increased risk of viremia >200 copies/mL, including the potential risk of HIV transmission, associated with a concurrent syphilis infection. Nevertheless, a continuous effort to limit the global spread of STIs is warranted considering the worldwide rising trend of STIs and of syphilis in particular in PLWH.

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References

1. World Health Organization. *Report on global sexually transmitted infection surveillance, 2018*. Geneva: World Health Organization, 2018. Accessed 23 January 2021

2. Kirkcaldy RD, Weston E, Segurado AC, *et al*. Epidemiology of gonorrhoea: a global perspective. *Sex Health* 2019;16:401.

3. Cingolani A, Zona S, Girardi E, et al. Incidence and factors associated with the risk of sexually transmitted diseases in HIV-infected people seen for care in Italy: data from the Icona Foundation cohort. HIV Med. 2015;16(7):412–420.

4. Read P, Fairley CK, Chow EPF. Increasing trends of syphilis among men who have sex with men in high income countries. Sex Health 2015;12:155–63. 3

5. Centers for Disease Control and Prevention. Syphilis surveillance supplement 2013–2017. Atlanta, US: Department of Health and Human Services, 2019. Accessed 23 January 2021

6. Lang R, Read R, Krentz HB et al. Increasing incidence of syphilis among patients engaged in HIV care in Alberta, Canada: a retrospective clinic-based cohort study. BMC Infect Dis. 2018;18(1):125.

7. Jansen K, Schmidt AJ, Drewes J, et al. Increased incidence of syphilis in men who have sex with men and risk management strategies, Germany, 2015. Euro Surveill. 2016;21:30382.

8. Wu Z, Xu J, Liu E, et al. Hiv and syphilis prevalence among men who have sex with men: a cross-sectional survey of 61 cities in China. Clin Infect Dis 2013;57:298–309

9. Chesson HW, Pinkerton SD. Sexually transmitted diseases and the increased risk for HIV transmission: implications for cost-effectiveness analyses of sexually transmitted disease prevention interventions. *J Acquir Immune Defic Syndr*. 2000;24(1):48-56.

10. Zetola NM, Klausner JD. Syphilis and HIV infection: an update. Clin Infect Dis 2007;44:1222–8.

11. Wu Z, Xu J, Liu E, et al. Hiv and syphilis prevalence among men who have sex with men: a cross-sectional survey of 61 cities in China. Clin Infect Dis 2013;57:298–309

12. Buchacz K, Patel P, Taylor M et al. Syphilis increases HIV viral load and decreases CD4 cell counts in HIV-infected patients with new syphilis infections. AIDS. 2004 Oct 21;18(15):2075-9

13. Kofoed K, Gerstoft J, Mathiesen LR, Benfield T. Syphilis and human immunodeficiency virus (HIV)-1 coinfection: influence on CD4 T-cell count, HIV-1 viral load, and treatment response [published correction appears in Sex Transm Dis. 2006 May;33(5):336]. *Sex Transm Dis*. 2006;33(3):143-148.

14. Jarzebowski W, Caumes E, Dupin N et al; FHDH-ANRS CO4 Study Team. Effect of early syphilis infection on plasma viral load and CD4 cell count in human immunodeficiency virus-infected men: results from the FHDH-ANRS CO4 cohort. Arch Intern Med. 2012 Sep 10;172(16):1237-43.

15. Grewal R, Allen VG, Bayoumi AM et al; OHTN Network Cohort Study Research Team. Brief Report: Syphilis Coinfection Is Not Associated With an Increased Risk of Virologic Failure Among HIV-Positive Men Who Have Sex With Men on Antiretroviral Therapy. J Acquir Immune Defic Syndr. 2019 Apr 15;80(5):585-589.

16. d'Arminio Monforte A, Lepri AC, Rezza G, Pezzotti P, Antinori A, Phillips AN, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. AIDS. 2000;14(5):499–507.

17. 2015 Sexually Transmitted Diseases Treatment Guidelines. Syphilis. https://www.cdc.gov/std/tg2015/syphilis.htm Accessed 18 May 2021

18. LeMessurier J, Traversy G, Varsaneux O, et al. Risk of sexual transmission of human immunodeficiency virus with antiretroviral therapy, suppressed viral load and condom use: a systematic review. *CMAJ*. 2018;190(46):E1350-E1360. doi:10.1503/cmaj.180311

19. Giacomelli A, Micheli V, Cattaneo D, Mancon A, Gervasoni C. Multidrug-resistant HIV viral rebound during early syphilis: a case report. *BMC Infect Dis*. 2020;20(1):273.

20. Di Biagio A, Cozzi-Lepri A, Prinapori R, et al. Discontinuation of Initial Antiretroviral Therapy in Clinical Practice: Moving Toward Individualized Therapy. Journal of Acquired Immune Deficiency Syndromes (1999). 2016 Mar;71(3):263-271.

21. El Bouzidi K, Jose S, Phillips AN, et al. First-line HIV treatment outcomes following the introduction of integrase inhibitors in UK guidelines. *AIDS*. 2020;34(12):1823-1831.

Figure 1. Direct acyclic graph (DAG) of the underlying causal structure of the data.

List of abbreviations: VL, viral load; STI, sexually transmitted infection; ART, antiretroviral treatment; VF, virological failure.