PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A retrospective study of the clinical features of new syphilis infections in a HIV positive cohort in Alberta, Canada.
AUTHORS	Lang, Raynell; Read, Ron; Krentz, Hartmut; Peng, Mingkai; Ramazani, Soheil; Vu, Quang; Gill, M John

VERSION 1 – REVIEW

REVIEWER	Dominique L Braun Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland
REVIEW RETURNED	03-Feb-2018

GENERAL COMMENTS	Title: It is not clear to me what the impact mentioned in the title exactly refers to. I suggest rather using a descriptive title instead of the actual one. Introduction: Page 4: Line 15: Please discuss increases in condomless sex seen in HIV-infected individuals as factor (Kouyos et al, OFID 2015) Line 26: clarify “reduced legal risk” Line 34: The missing association with PrEP should rather be discussed in the method than in the introduction Line 40: severe instead of aggressive? Line 49: please clarify “prophylactic ART”; PrEP? Line 54: Please shortly discuss the higher possibility of atypical presentation of syphilis in the HIV-infected population Page 5 Line 6: there are newer guidelines (CDC, EACS, etc) and recommendations available. Please cite. Line 26: “all HIV-infected” seems a little bit strong because we do not know the exact denominator Page 8 Line 35: delete “broad study criteria” Line 38: what was the laboratory constellation of these false-positive tests? Line 54: recurrent episodes instead of repeat? Page 9 Line 6ff: it would be interesting to see a comparison of the incidences before the year 2008 compared to after 2008, i.e. the introduction of the “Swiss statement” claiming that HIV-infected persons on suppressive ART are not infectious for their sex

	partners. Maybe the authors can provide a figure showing the incidence over the time, i.e. on the y-axis the syphilis incidence, on the x-axis the time line 2006-2016? Page 11 Line 8: latent syphilis should be defined in the methods Line 17 and discussion: the frequency of diagnosis of late latent syphilis depends from the intervals and the frequency of testing; please comment. Line 38: clarify the term “bidirectional”. Was there a more pronounced decrease noted in patients with self-reported co-infection (i.e., chlamydia or gonorrhea?) Page12: Line 40ff: based on which criteria ZNS involvement was assessed by lumbar puncture? Please comment and add this information in the method section. What was the fraction of patients who underwent lumbar puncture based on the denominator (n=194)? Line 6: the order of the figures seems not logically to me, please check Page 13: Line 17ff: what was the reason that patients received oral doxycycline instead of benzathine? Please comment. What was the clinical and serological success rate among the individuals receiving doxycycline? Line 47: I do not understand how a syphilis can be assessed as “primary” and at the same time as being asymptomatic. Please comment. Please ad the definition of primary/secondray7late/late latent in the method section. Page 14 Line 47: what was the cut-off for being virologically suppressed? >50 copies/ml? Line 15: Line 15: was CNS involvement assessed systematically? General comment: The manuscript would definitively benefit from being edited by a native speaker to improve its readability. I highly recommend editing the manuscript Prior to publication.

REVIEWER	Márta Marschalkó Semmelweis University, Budapest, Hungary
REVIEW RETURNED	17-Feb-2018

GENERAL COMMENTS	Referee’ comments: Aim of this work is clear, Introduction, Discussion, Abstract chapters are concise, well written, however interpretation of the Results seems to me not always adequate. Practical result of the work: recognising of silent syphilitic cases in an HIV population is convincing. There are minor inconsistencies, miscalculation. Title of the work is not the best: The impact of incident syphilis infection on HIV-infected patients engaged in care.

	The characteristics of syphilitic infection in association with HIV is far more studied than the characteristics of HIV infection under the influence of a co-associated syphilis. „One hundred and eleven syphilis episodes were excluded; 38 were confirmed false positive screening tests,…." My comment: if in 38 episodes the screening tests were false positive, these episodes in fact were not syphilitic ones. Therefore there were not 360 syphilitic infections in the study group but 360 minus 38 episodes (How many cases? later on it turns out that 38 episodes occured in fact in 38 patients). Authors describe that 111 episods were excluded- so 249 episods remained to be analysed. Regarding the patients number the same number of patients, namely 111 patients were excluded- so 194 patients remained to be analysed. It would have been more plain phrasing that 111 episods by 111 patients were excluded. In Table 1 „ Self reported ethnicity" In syphilis positive group 2 patients are missing. Figure 2 is difficult to see through. It is to demonstrate that in symptomatic cases, in symptomatic early stage diseases RPR titer is above 4, in most secondary cases are above 32 and in the majority of the primary cases are below 32. In symptomfree cases RPR titer is low. These facts are well known, and observed in non HIV + cases, too. One of their main messages is: in cases of HIV/syphilis coinfection the number of symptomfree cases with low RPR titer is high. (However late latent syphilis cases are characterized by the same serological features, and indeed they had such cases.) These facts should be presented by a more simple way, moreover I do not see the evidence that the reason for this fact is the HIV infection exclusively. Important fact from Figure 2 is the association of neurosyphilis with high RPR titer. I am not able to interpret the phrasing: Serologic Effect of Syphilis on HIV and Serologic Effect of HIV on Syphilis Syphilitic infection, or T. pallidum can have effects on the associated infection, and reverse, but what is the serologic effect? Effect of HIV infection on syphilis serology? Effect of syphilitic infection on HIV serology? However CD4 + cell count and viral load are not serology. Neurosyphilis .."Ten patients (4%) experienced CNS involvement with…." if the number of HIV/syphilis coinfected patients is 194, then 10 patients mean 5,15 %. I think that neurosyphilis is not just a syphilitic episode, it is a case with neurosyphilis. Discussion .. In our study population,50.8% (135) syphilis episodes were asymptomatic at presentation, including 21% (10) of the primary syphilis infections." . How do the Authors make the diagnosis of primary syphilis without symptoms? A definiton of it should be required.

	…"False positive syphilis testing among nontreponemal antibody is more common in the HIV-infected patients[9, 14, 18]. A rate of approximately 11% is reported by Rompalo et al. which is very similar to our findings (10.5%),…. I do not find this number in the results: they reflect to their 38 false positive cases? Then the percentage of this number should be given in the appropriate place, and more attention should be given to this fact in the text. I miss the report on treatment of HIV cases. All of the cases were treated? with satisfactory result?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Title: It is not clear to me what the impact mentioned in the title exactly refers to. I suggest rather using a descriptive title instead of the actual one.
As above we have revised the title to better reflect this study. Thank you.

Introduction:

Page 4: Line 15: Please discuss increases in condomless sex seen in HIV-infected individuals as factor (Kouyos et al, OFID 2015)
Thank you for suggesting that we mention this interesting and relevant paper. We have referred to its findings in the introduction.

Line 26: clarify "reduced legal risk"
We agree that this phrase was vague and have reworded this paragraph which no longer includes this phrase.

Line 34: The missing association with PrEP should rather be discussed in the method than in the introduction
As suggested this has been moved to the methods section as we agree it is a better fit there.

Line 40: severe instead of aggressive?
We have changed the word throughout the manuscript as recommended.

Line 49: please clarify "prophylactic ART"; PrEP?
Yes, this was our intention. However, on reading over the sentence we realize it was redundant to specify therapeutic and prophylactic ART and therefore have simplified the sentence to just ART.

Line 54: Please shortly discuss the higher possibility of atypical presentation of syphilis in the HIV-infected population
As recommended we have briefly discussed in the introduction section the types of atypical presentations that have been previously identified in studies of syphilis infection in HIV positive individuals.

Page 5 Line 6: there are newer guidelines (CDC, EACS, etc) and recommendations available. Please cite.
Thank you, the newer guidelines have been cited and added as references.

Line 26: “all HIV-infected” seems a little bit strong because we do not know the exact denominator
We agree that we cannot ensure all HIV infected individuals are in care therefore the “all” has been removed.

Page 8 Line 35: delete “broad study criteria”
This line has been removed.

Line 38: what was the laboratory constellation of these false-positive tests?
As per following comments from reviewer 2, it was decided to remove the false positive tests from this manuscript as indeed they are not actual cases (just laboratory artifacts) and therefore should not be mentioned as such. Thank you.

Line 54: recurrent episodes instead of repeat? We agree, this is a better word choice and it has been changed in the manuscript throughout.

Page 9 Line 6ff: it would be interesting to see a comparison of the incidences before the year 2008 compared to after 2008, i.e. the introduction of the “Swiss statement” claiming that HIV-infected persons on suppressive ART are not infectious for their sex partners. Maybe the authors can provide a figure showing the incidence over the time, i.e. on the y-axis the syphilis incidence, on the x-axis the time line 2006-2016?
We agree that the global public health challenge of resurgent syphilis is of interest and have included a reference to an epidemiologic paper of ours on this area.

Page 11 Line 8: latent syphilis should be defined in the methods
As suggested this has been clarified in the manuscript to describe our staging of syphilis. All cases were evaluated by an infectious disease and STI specialist and based on both clinical presentation and laboratory findings and were staged accordingly.

Line 17 and discussion: the frequency of diagnosis of late latent syphilis depends from the intervals and the frequency of testing; please comment.
We completely agree that the diagnosis of late latent syphilis will depend upon the frequency and interval of testing but believe readers will not need to have that pointed out. The emphasis here is on regular testing of patients being seen every four months for their HIV care.

Line 38: clarify the term “bidirectional”. Was there a more pronounced decrease noted in patients with self-reported co-infection (i.e., chlamydia or gonorrhea?)
By using the term “bidirectional”, we wished to stress that while HIV can lead to a difference in disease markers of syphilis (RPR, symptomatology), syphilis can also lead to a difference in disease markers of HIV (viral load, CD4). As such we feel bidirectional is the optimal word to use.
We were unable to identify any changes in self-reported chlamydia or gonorrhea as the data we collected was patients who had ever had a history of these STIs. The manuscript has been edited to make this clearer. Thank you.

Page12: Line 40ff: based on which criteria ZNS involvement was assessed by lumbar puncture? Please comment and add this information in the method section. What was the fraction of patients who underwent lumbar puncture based on the denominator (n=194)?
Thank you, neurologic involvement was assessed by having a documented positive CSF-VDRL on lumbar puncture undertaken in the patients assessed by STI specialist to have possible CNS involvement .This has been clarified in the methods section. Regarding the fraction of patients who

underwent LP, we do not have this data readily available, however if deemed necessary by editorial staff would be able to obtain this data. Thank you.

Line 6: the order of the figures seems not logically to me, please check
Thank you. The order of the figures is chronological according to when they are discussed in the paper.

Page 13: Line 17ff: what was the reason that patients received oral doxycycline instead of benzathine? Please comment. What was the clinical and serological success rate among the individuals receiving doxycycline?
There were several reasons leading to the use of doxycycline instead of Benzathine penicillin. The most common reason was that some patients were very reluctant to return to a healthcare facility for further benzathine injections and instead requested a course of oral doxycycline. For some patients traveling or living outside of the city doxycycline was preferred. In patients describing a credible penicillin allergy, doxycycline was used as the second line agent. There were no clinical or serological treatment failures noted with doxycycline therapy as noted in the text.

Line 47: I do not understand how a syphilis can be assessed as “primary” and at the same time as being asymptomatic. Please comment. Please ad the definition of primary/secondray7late/late latent in the method section.
A diagnosis of primary syphilis was made without symptoms based on the rising RPR titers that would be more indicative of primary vs latent disease. As an example, if the most recent RPR titer was 0 and then following titer was 4 months later was 1:128 as in one of our cases, this would indicate primary infection rather than latent infection. If the patient had experienced clear symptoms of secondary syphilis in the past or remembers having symptoms of primary syphilis then this would indicate that the asymptomatic presentation would be staged then as latent disease. All cases were evaluated and staged by an STI specialist based on both clinical and laboratory findings.
This has been clarified in the manuscript in the methods section, thank you.

Page 14 Line 47: what was the cut-off for being virologically suppressed? >50 copies/ml?
As stated in the methods HIV viral suppression was defined as a plasma HIV RNA viral load <40 copies/mL.

Line 15: was CNS involvement assessed systematically?
Yes, all patients who had incident syphilis were routinely assessed by an STI specialist for CNS symptoms or signs and a LP done as required. We have clarified in the text.

Reviewer: 2
Aim of this work is clear, Introduction, Discussion, Abstract chapters are concise, well written, however interpretation of the Results seems to me not always adequate.
Thank you for your comments, the results section was edited for better readability.
Title of the work is not the best: The impact of incident syphilis infection on HIV-infected patients engaged in care. The characteristics of syphilitic infection in association with HIV is far more studied than the characteristics of HIV infection under the influence of a co-associated syphilis.
Thank you for your comment, we have revised the title to better reflect this study.

„One hundred and eleven syphilis episodes were excluded; 38 were confirmed false positive screening tests,….” My comment: if in 38 episodes the screening tests were false positive, these episodes in fact were not syphilitic ones. Therefore there were not 360 syphilitic infections in the study group but 360 minus 38 episodes (How many cases? later on it turns out that 38 episodes occured in

fact in 38 patients). Authors describe that 111 episods were excluded- so 249 episods remained to be analysed. Regarding the patients number the same number of patients, namely 111 patients were excluded- so 194 patients remained to be analysed. It would have been more plain phrasing that 111 episodes by 111 patients were excluded.
Thank you for this suggestion, we have re-worded this section to be clearer and to demonstrate that the 111 episodes were in 111 patients. After further consideration and reviewers 1 comment, we agree that the false positive cases are not incident cases of syphilis but incident cases of a false lab test, and have decided to remove these from the manuscript. This has led to the simplification and better readability of our work.

In Table 1 „ Self reported ethnicity" In syphilis positive group 2 patients are missing.
Thank you for bringing this to our attention. We have re-looked at our data and found that we missed including an ethnic group into the other category which accounts for these two missing patients. We apologize for this error.

Figure 2 is difficult to see through. It is to demonstrate that in symptomatic cases, in symptomatic early stage diseases RPR titer is above 4, in most secondary cases are above 32 and in the majority of the primary cases are below 32. In symptom free cases RPR titer is low. These facts are well known, and observed in non HIV + cases, too. One of their main messages is: in cases of HIV/syphilis coinfection the number of symptom free cases with low RPR titer is high. (However late latent syphilis cases are characterized by the same serological features, and indeed they had such cases.) These facts should be presented by a more simple way, moreover I do not see the evidence that the reason for this fact is the HIV infection exclusively. Important fact from Figure 2 is the association of neurosyphilis with high RPR titer.
Our main take home points from Figure 2 are that there is a high rate of asymptomatic cases. However, this rate decreases as RPR increases. We also wanted to demonstrate the range of different symptomatic presentations and that rash and ulcer were the most common, with patients rarely presenting with flu like illness, condylomata or lymphadenopathy. Also, noted there is an association with higher RPR in neurosyphilis and there were no neurosyphilis cases with RPR titers less then 1:32. This is what is highlighted by this figure. We have edited the figure legend to better explain the highlights of this figure. We have however decided not to edit the figure itself as we believe it demonstrates important data. Thank you for your suggestions.

I am not able to interpret the phrasing: Serologic Effect of Syphilis on HIV and Serologic Effect of HIV on Syphilis Syphilitic infection, or T. pallidum can have effects on the associated infection, and reverse, but what is the serologic effect? Effect of HIV infection on syphilis serology? Effect of syphilitic infection on HIV serology? However CD4 + cell count and viral load are not serology.
This is indeed confusing wording so we have chosen to use this wording: Effect of HIV infection on markers of syphilis and effect of syphilitic infection on markers of HIV.
Thank you for this suggestion.

Neurosyphilis .."Ten patients (4%) experienced CNS involvement with…." if the number of HIV/syphilis coinfected patients is 194, then 10 patients mean 5,15 %. I think that neurosyphilis is not just a syphilitic episode, it is a case with neurosyphilis.
We used syphilis episodes instead of patients. Therefore, it is 10/249 and 4% of episodes. This was clarified in the manuscript. Thank you

Discussion .. In our study population,50.8% (135) syphilis episodes were asymptomatic at presentation, including 21% (10) of the primary syphilis infections." . How do the Authors make the diagnosis of primary syphilis without symptoms? A definition of it should be required.

A diagnosis of primary syphilis was made without symptoms based on the rising RPR titers that would be more indicative of primary vs latent disease. As an example, if the most recent RPR titer was 0 and then following titer was 4 months later was 1:128 as in one of our cases, this would indicate primary infection rather than latent infection. If the patient had experienced clear symptoms of secondary syphilis in the past or remembers having symptoms of primary syphilis then this would indicate that the asymptomatic presentation would be staged then as latent disease. All cases were evaluated and staged by an STI specialist based on both clinical and laboratory findings.
These criteria for classification have been clarified in the manuscript in the methods section, thank you.

…"False positive syphilis testing among nontreponemal antibody is more common in the HIV-infected patients[9, 14, 18]. A rate of approximately 11% is reported by Rompalo et al. which is very similar to our findings (10.5%),…. I do not find this number in the results: they reflect to their 38 false positive cases? Then the percentage of this number should be given in the appropriate place, and more attention should be given to this fact in the text.
We agree that this is confusing, and as per our response to reviewer 1 above we have removed all mention of the false positive cases in our manuscript. Thank you.

I miss the report on treatment of HIV cases. All of the cases were treated? with satisfactory result?
This is indeed covered in the second sentence of the treatment section of the results.

VERSION 2 – REVIEW

REVIEWER	Márta Marschalkó Department of Dermatology, Venereology and Dermatooncology, Semmelweis University, Faculty of Medicine Budapest, Hungary
REVIEW RETURNED	05-Apr-2018

GENERAL COMMENTS	I accept the response and corrections of the Authors.