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The impact of incident syphilis infection on HIV-infected patients engaged in care.

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TITLE: The impact of incident syphilis infection on HIV-infected patients engaged in care.

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ABSTRACT

Objectives: Syphilis is a global health concern with an estimated twelve million infections occurring annually. Due to the increasing rates of new syphilis infections being reported in HIV-infected patients, and their higher risk for atypical and severe presentations, periodic screening has been recommended as a routine component of HIV care. We aimed to characterized incident syphilis presentation, serologic features and treatment response in a well-defined, HIV-infected population over 11 years.

Methods: Since 2006, as routine practice of both the Southern Alberta Clinic (SAC) and Calgary STI Programs (CSTI) syphilis screening has accompanied HIV viral load measures every four months. All records of patients who, while in HIV care, either converted from being syphilis seronegative to a confirmed seropositive or were re-infected as evidenced by a four-fold increase in rapid plasma reagin (RPR) after past successful treatment, were reviewed.

Results: We identified 249 incident syphilis infections in 194 different HIV-infected individuals; 72% were initial infections whereas 28% were reinfections. Half (50.8%) of the infections were asymptomatic and identified only by routine screening. Symptomatic syphilis was more common when rapid plasma reagin (RPR) titers were higher (P=0.03). In patients with repeat syphilis infection, a trend was noted favoring symptomatic presentation (62%, P=0.07). All 10 patients with CNS syphilis involvement presented with an RPR titer ≥1:32. Following syphilis infection a decline of 42 cells/mm^3 in CD4 (P=0.004) was found, but no significant changes in viral load occurred. No association was found with the stage of syphilis or symptoms at presentation and ART use, CD4 count or virologic suppression.

Conclusion: Routine screening of our HIV-infected population identified many asymptomatic syphilis infections. The interaction of HIV and syphilis infection appears to be bidirectional with effects noted on both HIV and syphilis clinical and serological markers.

ARTICLE SUMMARY

Strengths and limitations of this study

1. All HIV and STI care in our region is highly centralized and coordinated allowing for detailed analyses of our population.
2. Routine syphilis serology regardless of risk behaviors or symptomatology was obtained every four months in our HIV-infected population, allowing close monitoring of clinical characteristics, bidirectional interactions as well as inclusivity of incident syphilis infections.
3. The study population, while comprehensive and representing a Canadian perspective, is from a single regional area and may not be representative populations elsewhere that have different rates of unprotected sexual activity and both prevalent HIV and syphilis infections. In addition, access to care varies between centers and populations and our rates and identification methods may not precisely match others.
4. This study may underestimate the clinical impact of syphilis in an HIV-infected population as patients not accessing care and individuals infected but lost to follow up or moving out of Alberta were not analyzed.

INTRODUCTION:

Syphilis continues to be a major public health concern globally, with an estimated twelve million new infections annually[1]. HIV-infected individuals are eight times more likely to become infected with syphilis than the general population[2]. In 2016, in Alberta Canada, over 25% of all new syphilis infections occurred in men who have sex with men (MSM) co-infected with HIV[3]. It has been suggested that increased use of social media including websites and mobile apps targeted towards meeting sex partners as well as serosorting (finding sex partners with the same HIV serostatus for unprotected sex), may be contributing to the rebound of high risk sexual activity in this population[2, 4]. The suppression of HIV viral replication using antiretroviral therapy (ART) resulting in minimal risk for sexual transmission of HIV has received legal recognition in Canada[5]. This reduced legal risk for transmission among virologically suppressed individuals (HIV viral load <1,000 copies/mL) may also be leading to increased high risk sexual behavior and contributing to the epidemic of syphilis among the HIV-infected population[4, 5]. HIV PrEP use was not extensively used in the community during the study period and any potential role seemed unlikely.

Syphilis infection in HIV-infected patients can present in atypical or aggressive forms, such as ulcerative skin lesions, persistent chancres, gummatous disease and neurosyphilis[6-9]. Sexually transmitted infections (STIs) may increase risk of HIV acquisition via interruption of mucosal barriers and increased viral shedding[9-11]. It has also been suggested that both therapeutic and prophylactic ART may inadvertently increase the incidence of syphilis by altering innate and acquired immune responses that may enhance susceptibility to syphilis infection[12]. Due to these increasing rates of syphilis and the higher likelihood of atypical and severe presentation,

routine periodic screening (2-4 times annually) of HIV-infected persons has been recommended[4, 9, 13].

The aim of this retrospective cohort study was both to characterize syphilis presentation, serologic features and treatment response in a large cohort of HIV-infected individuals engaged in HIV care and receiving regular syphilis testing, as well as to examine the effect of incident syphilis on HIV disease markers.

METHODS

Study Population

The Southern Alberta Clinic (SAC) and Calgary STI Clinic (CSTI) provide exclusive care to all HIV-infected individuals living in southern Alberta, Canada. In a quality assurance project (approved by University of Calgary Bioethics committee) at both programs between January 1, 2006 and December 31, 2016, routine syphilis serology regardless of risk was ordered every four months accompanying HIV viral load testing. The records of all incident syphilis infections occurring in HIV-infected patients were reviewed. Every indeterminate or positive syphilis serology for a SAC patient was discussed with or referred to CSTI at the time of testing.

All individuals with at least one visit between January 1, 2006 and December 31, 2016 were studied. Patients were followed until December 31, 2016 or until they moved, died or were lost-to-follow-up. All patients, who while in HIV care, converted from being seronegative for syphilis to a confirmed positive status or were re-infected with syphilis were reviewed through the SAC database and a CSTI chart review.

Diagnosis

The syphilis screening algorithm and confirmatory testing was achieved using indirect serologic methods. Initially screening for syphilis was done with the non-treponemal rapid plasma reagin (RPR), however in 2008 the screening test was changed to an enzyme immunoassay (EIA), a treponemal test. The RPR continued to be used as a confirmatory test as well as for monitoring response to therapy[13, 14]. In Calgary, the secondary confirmatory test was either the fluorescent treponemal antibody absorption test (FTA-ABS) or the line immunoassay (INNO-LIA)[15]. Repeat syphilis episodes were identified by a four-fold increase in RPR after a prior documented successful treatment course for syphilis and were evaluated and staged by an STI specialist (RR). Neurosyphilis was documented by a positive CSF-VRDL (Venereal disease research laboratory) on lumbar puncture as well as evaluated by an STI specialist (RR).

Data Collection

Detailed standardized information was collected by one physician (RL), through a comprehensive review of both SAC and CSTI charts and databases. Multiple data sources in these records were accessed including nursing interviews, social work reports, self-administered questionnaires, laboratory reports, and physician notes.

From the SAC database, we identified the number of syphilis tests performed yearly at the clinic per patient as well as the interval between tests. Demographic data was collected at the time of HIV diagnosis and incorporated into the SAC database. These data included: gender (i.e. male, female, transgendered), self-reported ethnicity (i.e. Caucasian, Indigenous,

African/Caribbean/Black (ACB), Other) and most likely HIV exposure risk (i.e. MSM, HET-heterosexual sex, PWID (persons who inject drugs), and other).

The stage of syphilis (i.e. primary, secondary, early latent, late latent) and symptomatology at presentation (i.e. rash, ulcer/lesion, flu-like illness, condylomata, lymphadenopathy, neurological (tinnitus/ocular), asymptomatic, other) were collected via review of CSTI charts. All episodes of syphilis were staged by an STI specialist (RR). Prior history of comorbid infections including *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were self-reported and documented in CSTI charts at the time of syphilis diagnosis.

The initial RPR was documented at the time of syphilis diagnosis and recorded in CSTI charts. HIV viral load and CD4 counts were measured at the time of syphilis diagnosis and subsequently at the next routine HIV follow-up appointment. HIV viral suppression was defined as a plasma viral load <40 copies/mL. Treatment modalities (i.e. Benzathine Penicillin, Doxycycline, Penicillin G) and response to therapy were reviewed retrospectively through a comprehensive chart review. All data was anonymized prior to analysis.

Statistical Analysis

Demographic and clinical factors of patients were compared using chi-square test. Viral load and CD4 counts prior to and following episode of syphilis infection were compared using linear mixed effect model while accounting for repeated measurement and more than one episode for some patients. Subgroup analyses were performed on neurosyphilis infections and those with repeat episodes of syphilis. Patients not accessing care and individuals infected but lost to follow

up or moving out of Alberta were not analyzed. All statistical analysis was performed using R (R Development Core Team, 2005). All charts were created with Microsoft Excel and R.

RESULTS:

Demographics

Between 2006 and 2016, there were 20,203 syphilis tests done on a total of 2,448 patients who attended at least one regular SAC visit during that time. On average there were 180 days between each syphilis test per patient. The average number of syphilis screening tests that were done per patient each year over the 11-year period was 2.1. In 2006 the average number of tests per year was 1.3, whereas in 2016 this was 2.8. For high risk patients (MSM) screening rates were more frequent with the average testing over 11 years being 2.4 tests per year.

Of the 2,448 HIV-infected individuals at SAC and CSTI programs encompassing 15,175 person years of follow up between 1/1/2006 and 12/31/2016, we identified 360 incident syphilis infections, meeting our broad study criteria, occurring in 305 different patients. One hundred and eleven syphilis episodes were excluded; 38 were confirmed false positive screening tests, in 41 infections the patient, while being tested in Alberta, had moved out of province resulting in incomplete availability of their clinical data, and in 32 episodes, there was inadequate basic information available for study inclusion. We therefore analyzed 249 episodes in 194 individuals.

Of the 249 infections, 178 (72%) were first episode of a syphilis infection, whereas the remaining 71 (28%) were repeat episodes. Concurrent STI's included; 32% of cases having a

self-reported history of *Neisseria gonorrhoeae* and 24% having had *Chlamydia trachomatis* infection. The annual incidence rates of syphilis in our HIV-infected population tripled from 2011, 8.08/1000 patient-years (95% confidence interval (CI): 4.14-14.75), to 27.04 per 1000 person-years (95% confidence interval (CI): 19.45-36.76) in 2016. The characteristics of the 194 individuals included in this analysis are described in table 1.

Table 1: Characteristics of HIV+ patients regularly followed at the Southern Alberta Clinic between 1/1/2006 and 12/31/2016 comparing patients who were negative for syphilis (Syphilis Neg) to patients who ever tested positive for syphilis (Syphilis Pos).

	Syphilis Neg	**Syphilis Pos**	**P-value**
N (%)	2254 (92.1)	194 (7.9)	
Age at HIV Diagnosis (years)			
Mean (range)	35 (1-79)	35 (16-69)	0.893
<30	813 (36.1)	75 (38.7)	0.801
30-39	802 (35.6)	66 (34.0)	
40-49	438 (19.4)	37 (19.1)	
≥50	201 (8.9)	16 (8.3)	
Gender			
Male	1675 (74.3)	183 (94.3)	**<.001**
Female	572 (25.4)	11 (5.6)	
Transgendered	7 (0.3)	0 (0.0)	
Self-reported Ethnicity[1]			
Caucasian	1259 (56.0)	140 (72.2)	**<.001**
Indigenous	216 (9.6)	6 (3.1)	
ACB	536 (23.8)	24 (12.4)	
Other	243 (10.8)	22 (11.3)	
Most Likely HIV Exposure Category[2]			
MSM	915 (40.6)	145 (74.4)	**<.001**
HET	512 (22.7)	14 (7.2)	
PWID	731 (32.4)	30 (15.6)	
Other	96 (4.3)	5 (2.6)	

[1]*Indigenous people includes Aboriginal, Metis and Inuit; ACB includes African, Caribbean, Black; Other includes IndoAsian, Hispanic, East Asian, and other*
[2]*MSM=self-reported men who have sex with men identification; HET=self-reported heterosexual identification; PWID=self-reported intravenous drug use identification; Other HIV Risk factor behavior includes: blood transfusions, hemophiliac, neonatal, postnatal infection, unknown or not reported.*

Symptomatology

Asymptomatic syphilis episodes

Just over half of the episodes (50.8%) of incident syphilis infections were asymptomatic and identified by routine screening (Figure 1). RPR titers were higher in patients with symptomatic versus asymptomatic syphilis (P=0.03) (Figure 2). The majority of episodes with an initial RPR of 1:4 or less were asymptomatic (71%). Those with lower CD4 (<200 cells/mm^3) counts at syphilis diagnosis had no significant differences in symptomatology as opposed to those with CD4 counts >200 cells/mm^3 (P= 0.65). Comparing symptomatic verses asymptomatic episodes neither virologic suppression of HIV nor ART influenced the individual's likelihood to present with any of the symptoms of syphilis at diagnosis.

Symptomatic syphilis episodes

The most common presenting symptom was rash (23%), followed by skin lesion or ulceration (18%). Uncommon presentations included lymphadenopathy, flu-like illness, condylomata lata and neurological symptoms (Figure 1).

Those with primary syphilis presented most commonly with skin ulceration/lesion (57%) and in those with secondary syphilis the presentation was a rash (76%). However, 15% of those diagnosed with secondary syphilis also complained of skin ulceration or lesion in addition to a rash. Although rare overall as presenting symptoms, lymphadenopathy (86%), flu like illness (50%) and condylomata (100%) were most seen in primary syphilis.

Stage of Syphilis

Both ART and virologic suppression of HIV had no association with the individual's stage of syphilis at diagnosis. Of those diagnosed with late latent syphilis, 98% had an initial RPR of 1:16 or less. Patients with secondary syphilis tended to present with a higher RPR with 33% having an RPR of 1:256 or higher.

Since 2008, the proportion of late latent syphilis infections diagnosed among our HIV-infected patients in care had decreased from 44% to 4.4% (Figure 3). Caucasian individuals were more likely to present with primary (24%) or secondary (28%) syphilis (P=<0.001), whereas the non-Caucasian population were more likely to present with latent disease (41%) with only 26% having either primary or secondary syphilis (P=<0.001). In males, the majority of infections were early latent (34%) and the minority being late latent (18%). However, in females 77% of infections were late latent.

Serologic Effect of Syphilis on HIV

As the interaction of HIV and syphilis infection may be bidirectional we explored CD4 and viral load response to syphilis infection. A significant decrease in CD4 count of 42.2 cells/mm^3 (P=0.004) was noted in association with syphilis coinfection (Figure 4). No change in HIV viral load was noted in association with syphilis infection (P=0.47) (Figure 5).

Serologic Effect of HIV on Syphilis

Nearly half (49%) of all patients presented with RPR (non-treponemal) titers between 1:32-1:128. There were two episodes presenting with an initial RPR greater than 1:2048; both patients

were not HIV virologically suppressed (HIV plasma viral load >1,000 copies/mL) at the time of syphilis infection (Figure 2). The individuals viral load (P=0.82) or CD4 count (P=0.48) did not appear to have any correlation with the initial RPR titer. Due to the small number of patients in this group (n=48) we were unable to evaluate if absence of ART had an impact on RPR titer.

Repeat Episodes of Syphilis

In patients with repeat syphilis infection, a trend (P=0.07) was noted favoring a symptomatic presentation (62%). Rash and skin lesion/ulceration also remained the most common complaint (Figure 6). Repeat episodes of syphilis were much less likely to have late latent disease (3%) and instead more likely to have primary (28%), secondary (28%) or early latent disease (39%). Of those with a repeat syphilis episode, 29% had RPR titers over 1:256, compared to 18% of the total population in the study. Only 10% of the patients with prior syphilis exposure had an initial RPR less than 1:4 compared to 32% of the patients with initial infection, however this did not reach significance (P=0.604).

Neurosyphilis

Ten patients (4%) experienced CNS involvement with a positive CSF-VDRL on lumbar puncture. Ocular symptoms with blurred vision or painless visual loss occurred in four patients, tinnitus in three patients and three were asymptomatic. Nine patients were male and Caucasian with eight being >40 years old. Eight were initial syphilis episodes and two were reinfections. Seven of the ten patients were on ART, five were virologically suppressed with seven having a CD4 count > 500 cells mL. The RPR titer at diagnosis was ≥1:32 in all episodes of CNS involvement with five having an RPR titer of ≥1:512 and two of these episodes diagnosed with

initial RPR titers of 1:8192. These RPR titers were much higher than any other symptom presentation or stage of syphilis (P=<0.001) (Figure 2). All patients with CNS involvement were treated successfully, based on both clinical and serologic response, with intravenous penicillin G for 14 days.

Treatment

A standard three-week course of weekly intramuscular injections of Benzathine penicillin (2.4MU per dose) was used for 77% of the patients, while 10% received an oral course of doxycycline, and 10% received a combination of the two medications. Successful completion of the full course of treatment was achieved in 94% (with 5% requiring retreatment from inadequate initial adherence and 1% never completing their full course).

DISCUSSION:

Our introduction in 2006 of syphilis screening to accompany routine HIV viral load testing allowed for the identification and analysis of incident syphilis infections in the HIV population in care in Calgary, Alberta. Our results confirm prior findings that co-infection with HIV can result in atypical or aggressive syphilis presentations[6-9]. Compared to non HIV-infected populations, prior studies have found higher rates of asymptomatic primary syphilis, which may result in missed diagnosis and increased episodes of secondary syphilis[9, 17]. In our study population, 50.8% (135) syphilis episodes were asymptomatic at presentation, including 21% (10) of the primary syphilis infections. Braun et al. recently published a study evaluating symptoms of syphilis in 19 HIV-infected individuals and found the rate of asymptomatic syphilis infections in

HIV-infected individuals to be 40%[16]. Routine syphilis screening has been confirmed to be effective in detecting early asymptomatic syphilis in HIV-infected outpatients[17].

Our study demonstrated a decline in latent syphilis between 2008 (44%) and 2016 (4%). In 2008, the high numbers of latent syphilis may be reflective of a change to the testing algorithm for syphilis, from an initial RPR to enzyme immunoassay (EIA), resulting in an improved test sensitivity and the identification of latent syphilis[14, 15]. While latent episodes have been steadily declining since 2013, the number of primary syphilis diagnoses are increasing. Through regular syphilis screening in this HIV-infected population, earlier detection of syphilis in its primary stage has been achieved, leading to prompt therapy, which may decrease ongoing syphilis transmission[4].

The interaction of HIV and syphilis infection appears to be bidirectional with effects noted on both HIV and syphilis serologic markers[9]. False positive syphilis testing among non-treponemal 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%), however this study was done in 1992 and had fewer HIV-infected participants[18]. Prior studies have reported that syphilis infection may increase HIV viral load and decrease CD4 count[19-21]. We observed a statistically significant decrease in CD4 count associated with incident syphilis infections, but no change in viral load was noted. This difference in findings compared with past studies may in part, be explained by the majority of our patients being on ART, which are perhaps more potent in suppressing viral replication.

An increased prevalence of neurologic manifestations has been reported in HIV-infected individuals[2, 4]. Approximately one third of any patient with early syphilis will have treponemal invasion into their CNS regardless of their HIV status[4]. However, an increased rate of early neurosyphilis among HIV-infected individuals has been noted and may be linked to the patient's inability to control the CNS infection rather than increased invasion into the CNS[4, 22]. Our data revealed 10/249 (4%) of the syphilis episodes diagnosed in our HIV-infected cohort were neurosyphilis.

Neurosyphilis is more likely to be asymptomatic in HIV co-infected individuals and therefore a more difficult diagnosis [4]. Three of our ten neurosyphilis episodes were indeed asymptomatic. As a response to the absence of symptoms CDC guidelines recommend HIV-infected individuals who receive a diagnosis of late latent syphilis, unknown duration of disease, have neurologic symptoms or treatment failure should undergo CSF evaluation[4, 23]. It is controversial whether all HIV co-infected individuals require evaluation for neurosyphilis at the time of syphilis diagnosis[4].

Recent data suggests that there is an association with RPR titers ≥1:32 and laboratory defined neurosyphilis (sensitivity of 100%, specificity of 40%)[24, 25]. This is in keeping with our study findings, deducing that lumbar puncture could be restricted to the subgroup of patients with neurologic manifestation or a serum RPR of ≥1:32[24, 25]. Prior studies have found that patients with CD4 counts <350mm^3, may be at increased risk for neurosyphilis, however we identified no specific correlation[4, 25, 26]. We did note that five of the individuals with neurosyphilis were

not HIV virologically suppressed, suggesting that there may be a link between increased HIV viral loads and neurosyphilis, however this requires further study.

CONCLUSIONS:

Through routine screening of an HIV-infected population engaged in care, many asymptomatic syphilis episodes were identified and treated resulting in a shift in diagnostic stage of syphilis infection from latent to primary and a theoretical decrease in ongoing transmission. Individuals with symptomatic syphilis infections were more likely to have higher RPR titers and those with highest RPR titers were at greater risk of neurosyphilis. ART, CD4 count and virologic suppression of HIV had no association with the individual's stage of syphilis or symptoms at diagnosis. Syphilis infection was associated with a temporary decrease in CD4 count with no impact on HIV viral load. As the rates of syphilis rise among the HIV-infected population, ongoing vigilance in screening and treatment is required in addition to further examination of co-infection interactions.

KEY MESSAGES

1. Through routine syphilis testing of an HIV-infected population many asymptomatic syphilis episodes were detected and treated.
2. Symptomatic individuals at diagnosis were more likely to have higher RPR titers.
3. Syphilis coinfection was associated with a temporary decrease in CD4 count, but no change in viral load was noted.
4. Patients with neurosyphilis were more likely to have higher RPR titers at diagnosis with no cases occurring in patients with titers <1:32.

FIGURE LEGENDS:

Figure 1: Percentage of episodes of syphilis diagnosed based on symptoms in a HIV-infected population.

Figure 2: Percentage of syphilis episodes in individuals with HIV based on their initial RPR titer and divided by symptoms of syphilis at presentation. Individuals who had symptoms compared to those that did not were more likely to have a higher initial RPR (P=0.0339). Those with neurologic symptoms had a significant elevation of their initial RPR titers compared with all other symptoms (P=<0.001).

Figure 3: There is an increased number of incident syphilis infections among HIV positive individuals who are active in care programs from 2006-2016. There is an apparent trend of decreased proportion of late latent disease.

Figure 4: Scatter plot of CD4 count at syphilis diagnosis versus CD4 count at follow up appointment after treatment of syphilis. CD4 count was noted to decrease by an average of 42.2 cells/mm^3 (P =0.004).

Figure 5: Scatter plot of viral load (adjusted on a logarithmic scale to account for wide variation in values) at syphilis diagnosis versus viral load at follow up appointment after treatment of syphilis. Viral load was noted to increase by an average of 3.09 copies/mL in relation to syphilis co-infection (P =0.47).

Figure 6: The percentage of syphilis episodes comparing initial symptom presentation divided by repeat infections. There is a trend demonstrating that individuals with repeat syphilis infections

were more likely to be symptomatic on presentation, however this did not reach significance (P=0.0799).

DECLARATIONS:

Ethics approval and consent to participate: Ethics approval was obtained through the University of Calgary Bioethics committee as a quality assurance project through A Project Ethics Community Consensus Initiative (ARECCI). Approval was granted both verbally and written on Aug 23, 2016.

Data sharing: The datasets generated and/or analyzed during the current study are not publicly available due to patient confidentiality. The sensitive nature of this information as well as the relatively small number of patients included in this dataset may lead it to be identifying and therefore does not allow this dataset to be made public.

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Conflicts of interest: We have no relevant conflicts of interest to disclose.

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Authors' contributions: RL, RR, HK and JG were involved in study design, data extraction, data analysis, drafting and final review of this work. SR, MP, and QV were involved in data extraction, data analysis and final review of this work. All authors read and approved the final manuscript.

REFERENCES:

1. World Health Organization, Dept of Reproductive Health and Research. Global incidence and prevalence of selected curable sexually transmitted infections – 2008. 2012. Available at: http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/. Date accessed: Dec 6, 2017.
2. Karp G, Schleffer F, Jotkowitz A, *et al*. Syphilis and HIV co-infection. *Eur. J. Intern. Med* 2009;**20**:9-13.
3. Gratrix J. personal communication Dec 6, 2017. Alberta Health Services, STI Centralized Services.
4. Zetola NM, Klausner JD. Syphilis and HIV Infection: An Update. *CID* 2007;**44**:1222-8.
5. R. *v.* Mabior, 2012 SCC 47, [2012] 2 S.C.R. 584. Available at: https://scc-csc.lexum.com/scc-csc/scc-csc/en/item/10008/index.do. Date accessed: Dec 1, 2017.
6. Rompalo AM, Joesoef MR, O'Donnell JA, *et al*. Clinical Manifestations of Early Syphilis by HIV Status and Gender: Results of the Syphilis and HIV Study. *Sex Transm Dis* 2001;**28**(3): 158-65.
7. Marra CM, Tantalo LC, Sahi SK, *et al*. Reduced Treponema pallidum-Specific Opsonic Antibody Activity in HIV-Infected Patients with Syphilis. *J Infect Dis* 2016;**213**:1348-54.
8. Collis TK, Celum CL. The Clinical Manifestations and treatment of Sexually Transmitted Diseases in Human Immunodeficiency Virus-Positive Men. *Clin Infect Dis* 2001;**32**:611-22.
9. Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. *Lancet Infect Dis* 2004;**4**:456-66.

10. McCoy SI, Eron JJ, Kuruc JD, *et al*. Sexually transmitted infections among patients with acute HIV in North Carolina. *Sex Transm Dis* 2009;**36**(6):372–4.

11. Fleming DT. Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* 1999;**75**:3–17.

12. Rekart ML, Ndifon W, Brunham RC, *et al*. A double-edged sword:does highly active antiretroviral therapy contribute to syphilis incidence by impairing immunity to *Treponema pallidum*? *Sex Transm Infect* 2017;**0**:1-5.

13. Branger J, Van Der Meer JT, Van Ketel RJ, *et al*. High Incidence of Asymptomatic Syphilis in HIV-Infected MSM Justifies Routine Screening. *Sex Transm Dis* 2009;**36**(2):84-5.

14. Ratnam S. The laboratory diagnosis of syphilis. *Can J Infect Dis Med Microbiol* 2005;16(1):45-51.

15. Public Health Agency of Canada. Canadian Guidelines on Sexually Transmitted Infections: Syphilis. 2014. Available at: http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/section-5-10-eng.php. Date accessed: Dec 6, 2017.

16. Braun DL, Marzel A, Steffens D, *et al*. High rates of subsequent asymptomatic STIs and risky sexual behavior in patients initially presenting with primary HIV-1 infection. *Clin Infect Dis* Published Online First: 5 October 2017. doi:10.1093/cid/cix873

17. Cohen CE, Winston A, Asboe D*, et al.* Increasing detection of asymptomatic syphilis in HIV patients. *Sex Transm Infect* 2005;**81:**217-219.

18. Rompalo AM, Cannon RO, Quinn TC, *et al.* Association of biologic false-positive reactions for syphilis with human immunodeficiency virus infection. *J Infect Dis* 1992; **165**:1124–2

19. 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;**18**(15):2075–9.

20. Palacios R, Jimenez-Onate F, Aguilar M, *et al*. Impact of syphilis infection on HIV viral load and CD4 cell counts in HIV-infected patients. *J Acquir Immune Defic Syndr* 2007; **44**(3):356–9.

21. Sadiq ST, McSorley J, Copas AJ, *et al*. The effects of early syphilis on CD4 counts and HIV-1 RNA viral loads in blood and semen. *Sex Transm Infect* 2005;**81**:380–5.

22. Rolfs RT, Joesoef MR, Hendershot EF, *et al*. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group, *N Engl J Med* 1997;**337**:307-15.

23. Workowski KA, Berman SM. Centers for Disease C, Prevention. Sexually transmitted diseases treatment guidelines. *MMWR Recomm Rep* 2010;**59**(12):1-110.

24. Libois A, De Wit S, Poll B, *et al*. HIV and syphilis: when to perform a lumbar puncture. *Sex Transm Dis* 2007;**34**(3):141-4

25. Marra CM, Maxwell CL, Smith SL, *et al*. Cerebrospinal fluid abnormalities in patients with syphilis: association with clinical and laboratory features. *J Infect Dis* 2004;**189**:369-76.

26. Ghanem KG, Moore RD, Rompalo AM, *et al*. Neurosyphilis in a clinical cohort of HIV 1 infected patients. *AIDS* 2008;22(10):1145-51.

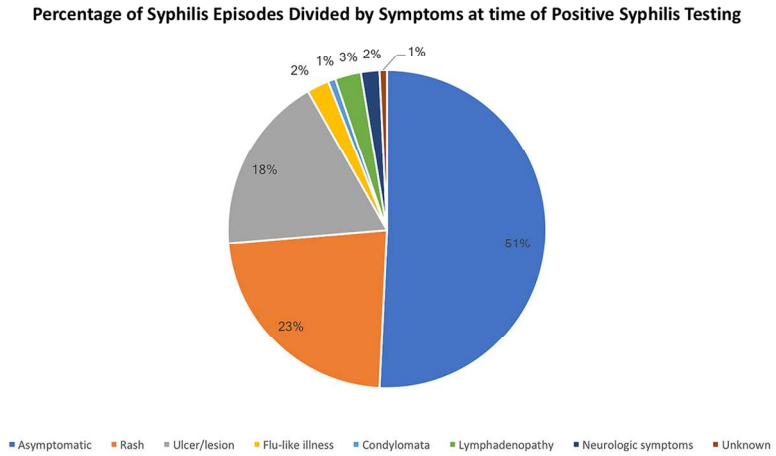

Figure 1: Percentage of episodes of syphilis diagnosed based on symptoms in a HIV-infected population.

127x71mm (300 x 300 DPI)

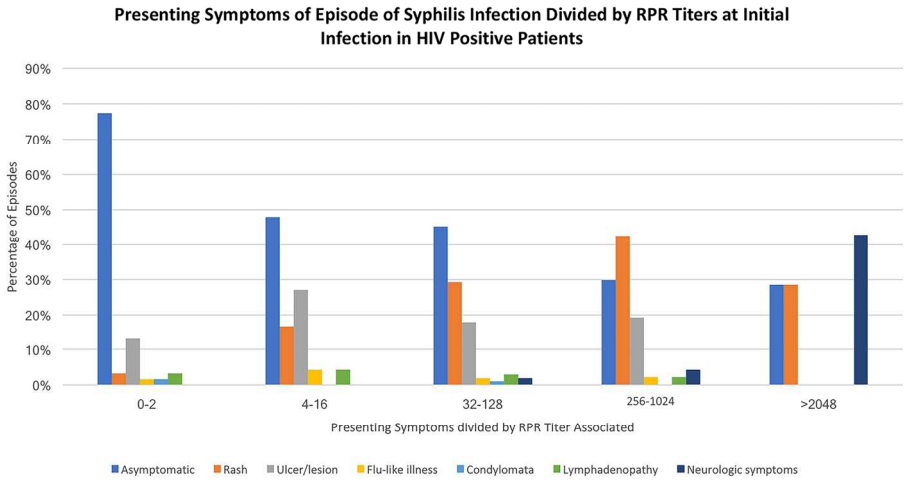

Figure 2: Percentage of syphilis episodes divided by symptom at presentation based on initial RPR titer. Individuals who had symptoms compared to those that did not were more likely to have a higher initial RPR (P=0.0339). The most common symptoms were rash and ulcer/lesion with flu-like illness, condylomata and lymphadenopathy being relatively rare. Those with neurologic symptoms had a significant elevation of their initial RPR titers compared with all other symptoms (P=<0.001) and there were no cases of neurosyphilis with RPR titers less then 1:32 dilutions.

71x40mm (600 x 600 DPI)

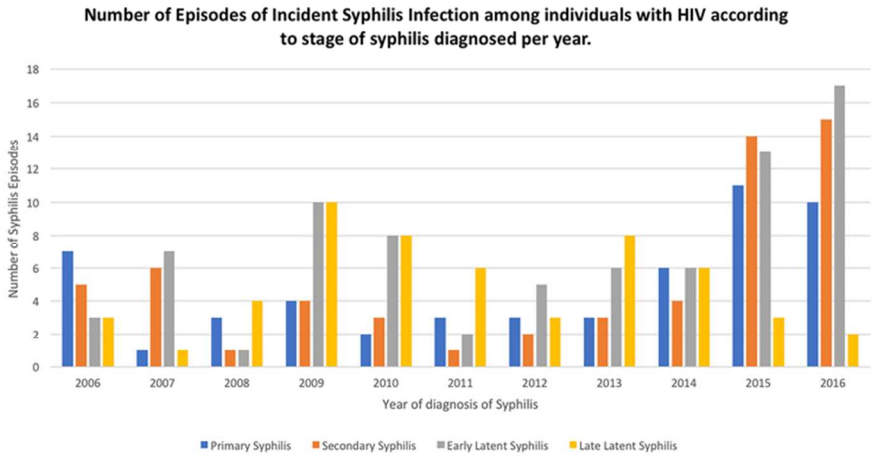

Figure 3: There is an increased number of incident syphilis infections among HIV positive individuals who are active in care programs from 2006-2016. There is an apparent trend of decreased proportion of late latent disease.

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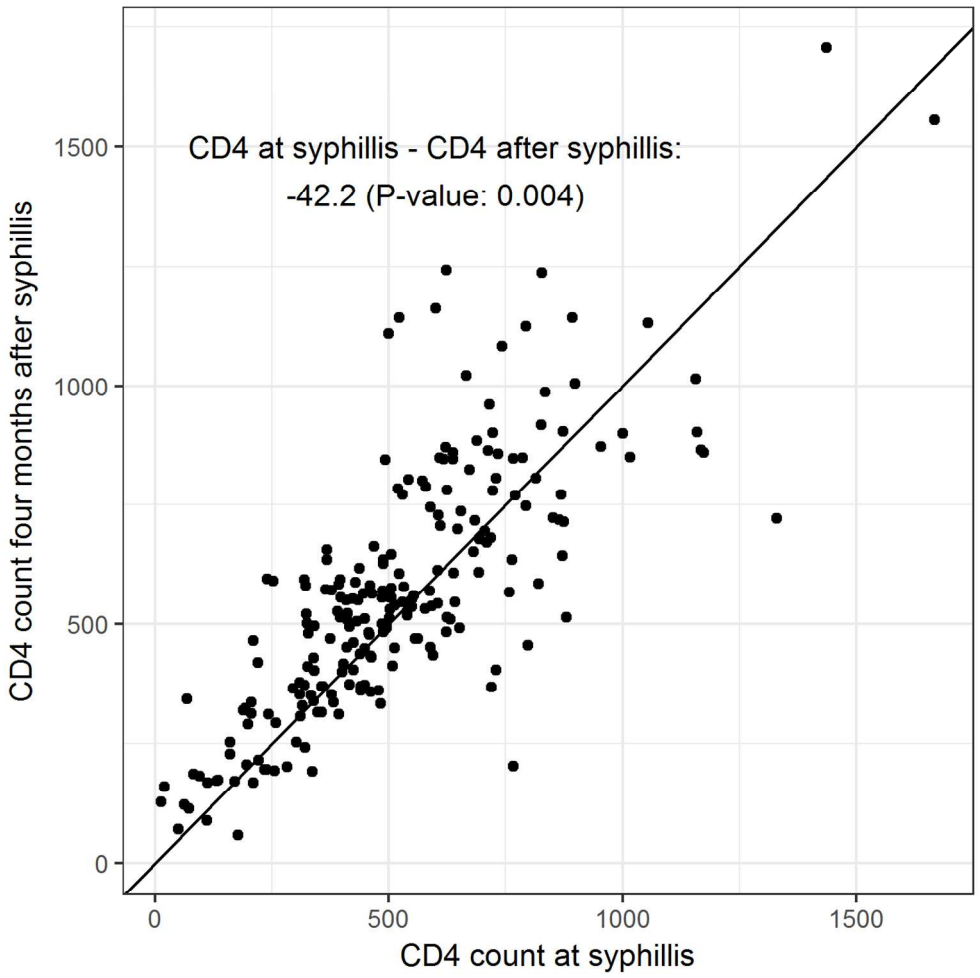

Figure 4: Scatter plot of CD4 count at syphilis diagnosis versus CD4 count at follow up appointment after treatment of syphilis. CD4 count was noted to decrease by an average of 42.2 cells/mm3 (P =0.004).

127x127mm (300 x 300 DPI)

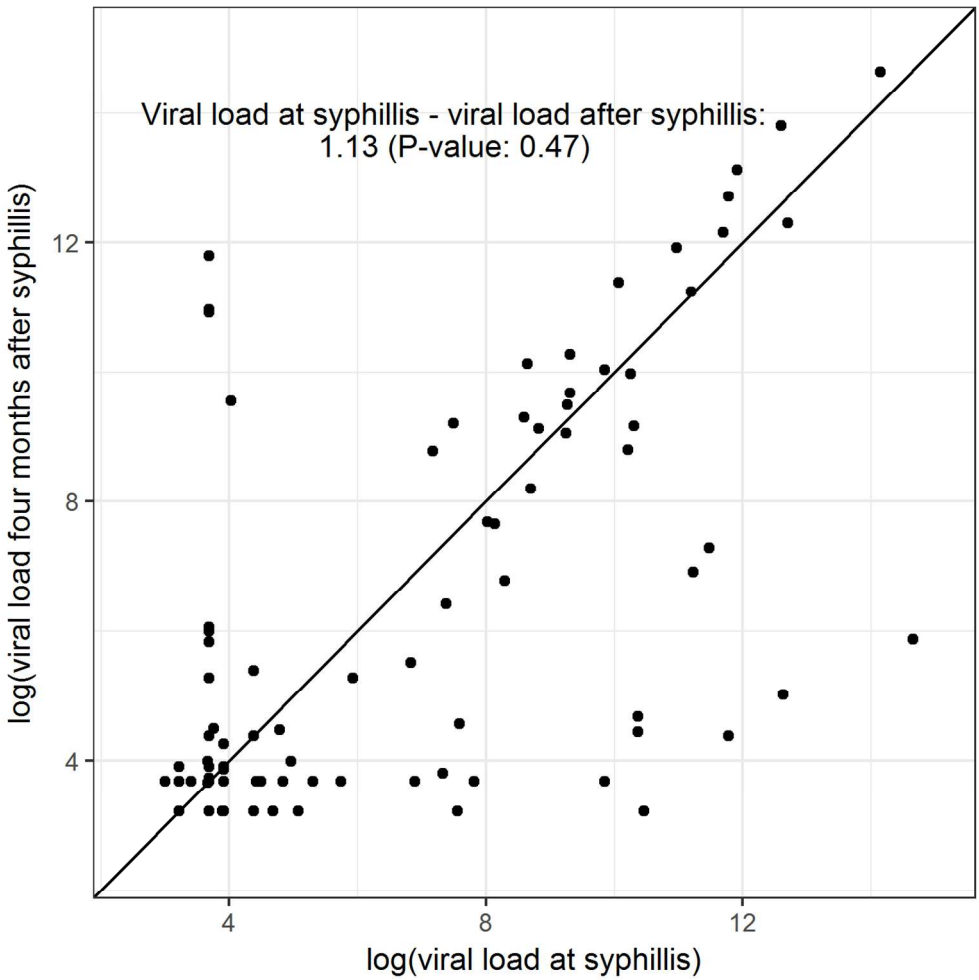

Figure 5: Scatter plot of viral load (adjusted on a logarithmic scale to account for wide variation in values) at syphilis diagnosis versus viral load at follow up appointment after treatment of syphilis. Viral load was noted to increase by an average of 3.09 copies/mL in relation to syphilis co-infection (P =0.47).

127x127mm (300 x 300 DPI)

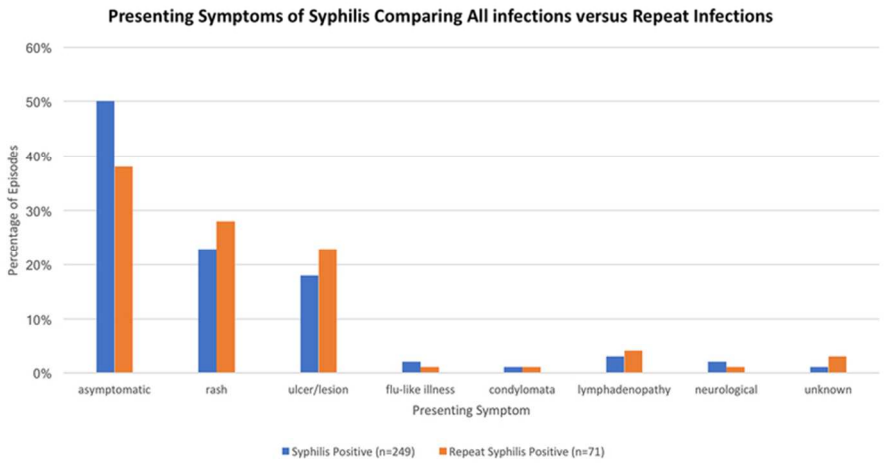

Figure 6: The percentage of syphilis episodes comparing initial symptom presentation divided by recurrent infections. There is a trend demonstrating that individuals with recurrent syphilis infections were more likely to be symptomatic on presentation, however this did not reach significance (P=0.0799).

71x40mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract Page 1,2	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale Page 4	2	Explain the scientific background and rationale for the investigation being reported
Objectives Page 5	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design Page 5	4	Present key elements of study design early in the paper
Setting Page 6	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants Page 5	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables Page 5,6, 7	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement Page 6,7	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Page 7	9	Describe any efforts to address potential sources of bias
Study size Page 8	10	Explain how the study size was arrived at
Quantitative variables Page 7	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods Page 7	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants Page 8	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data Page 8,9	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data Page 8	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results Page 10, 11, 12	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses Page 12, 13	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results Page 13, 14, 15	18	Summarise key results with reference to study objectives
Limitations Page 3	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation Page 16	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability Page 3	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding Page 18	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

A retrospective study of the clinical features of new syphilis infections in a HIV positive cohort in Alberta, Canada.

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Secondary Subject Heading:	HIV/AIDS, Sexual health, Public health
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES

TITLE: A RETROSPECTIVE STUDY OF THE CLINICAL FEATURES OF NEW SYPHILIS INFECTIONS IN A HIV POSITIVE COHORT IN ALBERTA, CANADA.

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ABSTRACT

Objectives: Syphilis is a global health concern with an estimated twelve million infections occurring annually. Due to the increasing rates of new syphilis infections being reported in HIV-infected patients, and their higher risk for atypical and severe presentations, periodic screening has been recommended as a routine component of HIV care. We aimed to characterized incident syphilis presentation, serologic features and treatment response in a well-defined, HIV-infected population over 11 years.

Methods: Since 2006, as routine practice of both the Southern Alberta Clinic (SAC) and Calgary STI Programs (CSTI) syphilis screening has accompanied HIV viral load measures every four months. All records of patients who, while in HIV care, either converted from being syphilis seronegative to a confirmed seropositive or were re-infected as evidenced by a four-fold increase in rapid plasma reagin (RPR) after past successful treatment, were reviewed.

Results: We identified 249 incident syphilis infections in 194 different HIV-infected individuals; 72% were initial infections whereas 28% were reinfections. Half (50.8%) of the infections were asymptomatic and identified only by routine screening. Symptomatic syphilis was more common when rapid plasma reagin (RPR) titers were higher (P=0.03). In patients with recurrent syphilis infection, a trend was noted favoring symptomatic presentation (62%, P=0.07). All 10 patients with CNS syphilis involvement presented with an RPR titer ≥1:32. Following syphilis infection a decline of 42 cells/mm^3 in CD4 (P=0.004) was found, but no significant changes in viral load occurred. No association was found with the stage of syphilis or symptoms at presentation and ART use, CD4 count or virologic suppression.

Conclusion: Routine screening of our HIV-infected population identified many asymptomatic syphilis infections. The interaction of HIV and syphilis infection appears to be bidirectional with effects noted on both HIV and syphilis clinical and serological markers.

ARTICLE SUMMARY

Strengths and limitations of this study

1. All HIV and STI care in our region is highly centralized and coordinated allowing for detailed analyses of our population.
2. Routine syphilis serology regardless of risk behaviors or symptomatology was obtained every four months in our HIV-infected population, allowing close monitoring of clinical characteristics, bidirectional interactions as well as inclusivity of incident syphilis infections.
3. The study population, while comprehensive and representing a Canadian perspective, is from a single regional area and may not be representative of populations elsewhere that have different rates of unprotected sexual activity and both prevalent HIV and syphilis infections. In addition, access to care varies between centers and populations and our rates and identification methods may not precisely match others.
4. This study may underestimate the clinical impact of syphilis in an HIV-infected population as patients not accessing care and individuals infected but lost to follow up or moving out of Alberta were not analyzed.

INTRODUCTION:

Syphilis continues to be a major public health concern globally, with an estimated twelve million new infections annually[1]. HIV-infected individuals are eight times more likely to become infected with syphilis than the general population[2]. In 2016, in Alberta Canada, over 25% of all new syphilis infections occurred in men who have sex with men (MSM) co-infected with HIV[3]. It has been suggested that increased use of social media including websites and mobile apps targeted towards meeting sex partners as well as serosorting (finding sex partners with the same HIV serostatus for unprotected sex), may be contributing to the rebound of high risk sexual activity in this population[2, 4]. The suppression of HIV viral replication (viral load <1,000 copies/mL) using antiretroviral therapy (ART) resulting in minimal risk for sexual transmission of HIV has received legal recognition in Canada[5]. As noted in a 2015 Swiss HIV Cohort study by Kouyos et al. there has been an accelerated rate of condomless sex since the recognition of HIV treatment as prevention. The reasons for increased risk behavior, particularly condomless sex are believed to be multifactorial, however in turn may be driving an increase in sexually transmitted infections (STIs)[6].

Syphilis in HIV-infected patients can present in atypical or severe forms, such as ulcerative skin lesions, persistent chancres, gummatous disease, ocular disease and neurosyphilis[7-11]. One study showed that HIV-infected individuals have multiple chancres and are more likely to experience Jarisch-Herxheimer reactions (22% vs 12% respectively), and another showed that concomitant genital ulcers were more common in patients with secondary syphilis and HIV[7, 8]. STIs may increase the risk of HIV acquisition via interruption of mucosal barriers and increased viral shedding[11-13]. It has also been suggested that ART may inadvertently increase

the incidence of syphilis by altering innate and acquired immune responses that may enhance susceptibility to syphilis infection[14]. Due to these increasing rates of syphilis and the higher likelihood of atypical and severe presentation, routine periodic screening (2-4 times annually) of HIV-infected persons has been recommended[4, 11, 15-17].

The aim of this retrospective cohort study was both to characterize syphilis presentation, serologic features and treatment response in a large cohort of HIV-infected individuals engaged in HIV care and receiving regular syphilis testing, as well as to examine the effect of incident syphilis on HIV disease markers.

METHODS

Study Population

The Southern Alberta Clinic (SAC) and Calgary STI Clinic (CSTI) provide exclusive care to HIV-infected individuals living in southern Alberta, Canada. In a quality assurance project (approved by University of Calgary Bioethics committee) at both programs between January 1, 2006 and December 31, 2016, routine syphilis serology regardless of risk was ordered every four months accompanying HIV viral load testing. The records of all incident syphilis infections occurring in HIV-infected patients were reviewed. Every indeterminate or positive syphilis serology for a SAC patient was discussed with or referred to CSTI at the time of testing.

All individuals with at least one visit between January 1, 2006 and December 31, 2016 were studied. Patients were followed until December 31, 2016 or until they moved, died or were lost-to-follow-up. All patients, who while in HIV care, converted from being seronegative for

syphilis to a confirmed positive status or were re-infected with syphilis were reviewed through the SAC database and a CSTI chart review.

Diagnosis

The syphilis screening algorithm and confirmatory testing was achieved using indirect serologic methods. Initially screening for syphilis was done with the non-treponemal rapid plasma reagin (RPR), however in 2008 the screening test was changed to an enzyme immunoassay (EIA), a treponemal test. The RPR continued to be used as a confirmatory test as well as for monitoring response to therapy[15, 18]. In Calgary, the secondary confirmatory test was either the fluorescent treponemal antibody absorption test (FTA-ABS) or the line immunoassay (INNO-LIA)[19].

Recurrent syphilis episodes were identified by a four-fold increase in RPR after a prior documented successful treatment course for syphilis and were evaluated and staged by an STI specialist (RR). Neurosyphilis was documented by a positive CSF-VRDL (Venereal disease research laboratory) on lumbar puncture as well as evaluated by an STI specialist (RR). HIV PrEP use was not extensively used in the community during the study period and any potential role seemed unlikely.

Data Collection

Detailed standardized information was collected by one physician (RL), through a comprehensive review of both SAC and CSTI charts and databases. Multiple data sources in

these records were accessed including nursing interviews, social work reports, self-administered questionnaires, laboratory reports, and physician notes.

From the SAC database, we identified the number of syphilis tests performed yearly at the clinic per patient as well as the interval between tests. Demographic data was collected at the time of HIV diagnosis and incorporated into the SAC database. These data included: gender (i.e. male, female, transgendered), self-reported ethnicity (i.e. Caucasian, Indigenous, African/Caribbean/Black (ACB), Other) and most likely HIV exposure risk (i.e. MSM, HET-heterosexual sex, PWID (persons who inject drugs), and other).

The stage of syphilis (i.e. primary, secondary, early latent, late latent) and symptomatology at presentation (i.e. rash, ulcer/lesion, flu-like illness, condylomata, lymphadenopathy, neurological (tinnitus/ocular), asymptomatic, other) were collected via review of CSTI charts. All episodes of syphilis were staged by an STI specialist (RR) based on both clinical and laboratory investigations. In the absence of symptoms, the staging of primary versus latent syphilis was based on the timing of rising RPR titers in relation to most recent prior titer. Prior history of comorbid infections including *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were self-reported at the time of syphilis diagnosis.

The initial RPR was documented at the time of syphilis diagnosis and recorded in CSTI charts. HIV viral load and CD4 counts were measured at the time of syphilis diagnosis and subsequently at the next routine HIV follow-up appointment. HIV viral suppression was defined as a plasma viral load <40 copies/mL. Treatment modalities (i.e. Benzathine Penicillin, Doxycycline,

Penicillin G) and response to therapy were reviewed retrospectively through a comprehensive chart review. All data was anonymized prior to analysis.

Patient and Public Involvement

Routine testing for syphilis was introduced as standard care initiative. Patients were made aware of the new testing when given their routine HIV laboratory test requisitions and advised they have the option to delete the test if they wish. After identifying the large number of incident syphilis cases with half being asymptomatic we incorporated our local findings into our routine patient safer sex counselling. Our findings have been provided to local public health and will be used in broader STI control initiatives.

Statistical Analysis

Demographic and clinical factors of patients were compared using chi-square test. Viral load and CD4 counts prior to and following episode of syphilis infection were compared using linear mixed effect model while accounting for repeated measurement and more than one episode for some patients. Subgroup analyses were performed on neurosyphilis infections and those with recurrent episodes of syphilis. Patients not accessing care and individuals infected but lost to follow up or moving out of Alberta were not analyzed. All statistical analysis was performed using R (R Development Core Team, 2005). All charts were created with Microsoft Excel and R.

RESULTS:

Demographics

Between 2006 and 2016, there were 20,203 syphilis tests done on a total of 2,448 patients who attended at least one regular SAC visit during that time. On average, there were 180 days between each syphilis test per patient. The average number of syphilis screening tests that were done per patient each year over the 11-year period was 2.1. In 2006, the average number of tests per year was 1.3, whereas in 2016 this was 2.8. For high risk patients (MSM), screening rates were more frequent with the average testing over 11 years being 2.4 tests per year.

Of the 2,448 HIV-infected individuals at SAC and CSTI programs encompassing 15,175 person years of follow up between 1/1/2006 and 12/31/2016, we identified 322 incident syphilis infections, occurring in 267 different patients. There were 73 syphilis episodes in 73 patients that were excluded. Of those excluded; 41 patients, while being tested in Alberta, had moved out of province resulting in incomplete clinical data, and in 32 patients, there was inadequate basic information available for study inclusion. We therefore analyzed 249 episodes in 194 individuals.

Of the 249 infections, 178 (72%) were first episode of a syphilis infection, whereas the remaining 71 (28%) were recurrent episodes. The annual incidence rates of syphilis in our HIV-infected population tripled from 2011, 8.08/1000 patient-years (95% confidence interval (CI): 4.14-14.75), to 27.04 per 1000 person-years (95% confidence interval (CI): 19.45-36.76) in 2016[3]. Prior history of STI's included; 32% of cases having a self-reported history of *Neisseria gonorrhoeae* and 24% having had *Chlamydia trachomatis* infection. The characteristics of the 194 individuals included in this analysis are described in table 1.

Table 1: Characteristics of HIV+ patients regularly followed at the Southern Alberta Clinic between 1/1/2006 and 12/31/2016 comparing patients who were negative for syphilis (Syphilis Neg) to patients who ever tested positive for syphilis (Syphilis Pos).

	Syphilis Neg	Syphilis Pos	P-value
N (%)	2254 (92.1)	194 (7.9)	
Age at HIV Diagnosis (years)			
Mean (range)	35 (1-79)	35 (16-69)	0.893
<30	813 (36.1)	75 (38.7)	0.801
30-39	802 (35.6)	66 (34.0)	
40-49	438 (19.4)	37 (19.1)	
≥50	201 (8.9)	16 (8.3)	
Gender			
Male	1675 (74.3)	183 (94.3)	**<.001**
Female	572 (25.4)	11 (5.6)	
Transgendered	7 (0.3)	0 (0.0)	
Self-reported Ethnicity[1]			
Caucasian	1259 (56.0)	140 (72.2)	**<.001**
Indigenous	216 (9.6)	6 (3.1)	
ACB	536 (23.8)	24 (12.4)	
Other	243 (10.8)	24 (12.4)	
Most Likely HIV Exposure Category[2]			
MSM	915 (40.6)	145 (74.4)	**<.001**
HET	512 (22.7)	14 (7.2)	
PWID	731 (32.4)	30 (15.6)	
Other	96 (4.3)	5 (2.6)	

[1]*Indigenous people includes Aboriginal, Metis and Inuit; ACB includes African, Caribbean, Black; Other includes IndoAsian, Hispanic, East Asian, and other*

[2]*MSM=self-reported men who have sex with men identification; HET=self-reported heterosexual identification; PWID=self-reported intravenous drug use identification; Other HIV Risk factor behavior includes: blood transfusions, hemophiliac, neonatal, postnatal infection, unknown or not reported.*

Symptomatology

Asymptomatic syphilis episodes

Just over half of the episodes (50.8%) of incident syphilis infections were asymptomatic and identified by routine screening (Figure 1). RPR titers were higher in patients with symptomatic versus asymptomatic syphilis (P=0.03) (Figure 2). The majority of episodes with an initial RPR

of 1:4 or less were asymptomatic (71%). Those with lower CD4 (<200 cells/mm^3) counts at syphilis diagnosis had no significant differences in symptomatology as opposed to those with CD4 counts >200 cells/mm^3 (P= 0.65). Neither virologic suppression of HIV nor ART use influenced the individual's likelihood to present with symptomatic syphilis.

Symptomatic syphilis episodes

The most common presenting symptom was rash (23%), followed by skin lesion or ulceration (18%). Uncommon presentations included lymphadenopathy, flu-like illness, condylomata lata and neurological symptoms (Figure 1). The most common presenting symptom in primary syphilis was skin ulceration/lesion (57%) and in those with secondary syphilis was a rash (76%). However, 15% of those diagnosed with secondary syphilis also complained of skin ulceration or lesion in addition to a rash. Although rare overall as presenting symptoms, lymphadenopathy (86%), flu like illness (50%) and condylomata (100%) were most seen in primary syphilis.

Stage of Syphilis

Both ART and virologic suppression of HIV had no association with the individual's stage of syphilis at diagnosis. Of those diagnosed with late latent syphilis, 98% had an initial RPR of 1:16 or less. Patients with secondary syphilis tended to present with a higher RPR, 33% having an RPR of 1:256 or higher.

Since 2008, the proportion of late latent syphilis infections diagnosed among our HIV-infected patients in care had decreased from 44% to 4.4% (Figure 3). Caucasian individuals were more likely to present with primary (24%) or secondary (28%) syphilis (P=<0.001), whereas the non-

Caucasian population were more likely to present with latent disease (74%) (P=<0.001). In males, the majority of infections were early latent (34%) and the minority being late latent (18%). However, in females 77% of infections were late latent.

Effect of Syphilis on Markers of HIV

As the interaction of HIV and syphilis infection may be bidirectional we explored CD4 and viral load response to syphilis infection. A significant decrease in CD4 count of 42.2 cells/mm^3 (P=0.004) was noted in association with syphilis coinfection (Figure 4). However, there was no change in HIV viral load noted in association with syphilis coinfection (P=0.47) (Figure 5).

Effect of HIV on Markers of Syphilis

Nearly half (49%) of all patients presented with RPR (non-treponemal) titers between 1:32-1:128. There were two episodes presenting with an initial RPR greater than 1:2048; both patients were not HIV virologically suppressed (HIV plasma viral load >1,000 copies/mL) at the time of syphilis infection (Figure 2). The individuals viral load (P=0.82) or CD4 count (P=0.48) did not appear to have any correlation with the initial RPR titer. We were unable to evaluate if the absence of ART had an impact on RPR titer due to the small number of patients not on ART (n=48).

Recurrent Episodes of Syphilis

In patients with recurrent syphilis infection, a trend (P=0.07) was noted favoring symptomatic presentation (62%). Rash and skin lesion/ulceration also remained the most common complaint (Figure 6). Recurrent episodes of syphilis were much less likely to be late latent disease (3%)

and instead more likely to be primary (28%), secondary (28%) or early latent disease (39%). Of those with a recurrent syphilis episode, 29% had RPR titers over 1:256, compared to 18% in the study population. Only 10% of the patients with prior syphilis exposure had an initial RPR less than 1:4 compared to 32% in the study population, however this did not reach significance (P=0.604).

Neurosyphilis

CNS involvement was noted in 10/249 (4%) episodes with a positive CSF-VDRL on lumbar puncture. Ocular symptoms with blurred vision or painless visual loss occurred in four patients, tinnitus in three patients and three were asymptomatic. Nine patients were male and Caucasian with eight being >40 years old. Eight were initial syphilis episodes and two were reinfections. Seven of the ten patients were on ART, five were virologically suppressed with seven having a CD4 count > 500 cells mL. The RPR titer at diagnosis was ≥1:32 in all episodes of CNS involvement with five having an RPR titer of ≥1:512 and two of these episodes diagnosed with initial RPR titers of 1:8192. These RPR titers were much higher than any other symptom presentation (P=<0.001) (Figure 2). All patients with CNS involvement were treated successfully, based on both clinical and serologic response, with intravenous penicillin G for 14 days.

Treatment

A standard three-week course of weekly intramuscular injections of Benzathine penicillin (2.4MU per dose) was used for 77% of the patients, while 10% received an oral course of doxycycline, and 10% received a combination of the two medications. Successful completion of

the full course of treatment was achieved in 94% (with 5% requiring retreatment from inadequate initial adherence and 1% never completing their full course).

DISCUSSION:

Our introduction in 2006 of syphilis screening to accompany routine HIV viral load testing allowed for the identification and analysis of incident syphilis infections in the HIV population in care in Calgary, Alberta. Our results confirm prior findings that co-infection with HIV can result in atypical or severe syphilis presentations[8-11]. Compared to non HIV-infected populations, prior studies have found higher rates of asymptomatic primary syphilis, which may result in missed diagnosis and increased episodes of secondary syphilis[11, 20]. In our study population, 50.8% (135) syphilis episodes were asymptomatic at presentation, including 21% (10) of the primary syphilis infections. Braun et al. recently published a study evaluating symptoms of syphilis in 19 HIV-infected individuals and found the rate of asymptomatic syphilis infections in HIV-infected individuals to be 40%[21]. Routine syphilis screening has been confirmed to be effective in detecting early asymptomatic syphilis in HIV-infected outpatients[20].

Our study demonstrated a decline in latent syphilis between 2008 (44%) and 2016 (4%). In 2008, the high numbers of latent syphilis may be reflective of a change to the testing algorithm for syphilis, from an initial RPR to enzyme immunoassay (EIA), resulting in an improved test sensitivity and the identification of latent syphilis[18, 19]. While latent episodes have been steadily declining since 2013, the number of primary syphilis diagnoses are increasing. Through regular syphilis screening in this HIV-infected population, earlier detection of syphilis in its

primary stage has been achieved, leading to prompt therapy, which may decrease ongoing syphilis transmission[4].

The interaction of HIV and syphilis infection appears to be bidirectional with effects noted on both HIV and syphilis serologic and clinical markers[11]. Prior studies have reported that syphilis infection may increase HIV viral load and decrease CD4 count[22-25]. We observed a statistically significant decrease in CD4 count associated with incident syphilis infections, but no change in viral load was noted. This difference in findings compared with past studies may in part, be explained by the majority of our patients being on ART, which are perhaps more potent in suppressing viral replication.

An increased prevalence of neurologic manifestations has been reported in HIV-infected individuals[2, 4]. Approximately one third of any patient with early syphilis will have treponemal invasion into their CNS regardless of their HIV status[4]. However, an increased rate of early neurosyphilis among HIV-infected individuals has been noted and may be linked to the patient's inability to control the CNS infection rather than increased invasion into the CNS[4, 25]. Our data revealed 10/249 (4%) of the syphilis episodes diagnosed in our HIV-infected cohort were neurosyphilis.

Neurosyphilis is more likely to be asymptomatic in HIV co-infected individuals and therefore a more difficult diagnosis[4]. Three of our ten neurosyphilis episodes were indeed asymptomatic. As a response to the absence of symptoms, CDC guidelines recommend HIV-infected individuals who receive a diagnosis of late latent syphilis, unknown duration of disease, have

neurologic symptoms or treatment failure should undergo CSF evaluation[4, 26]. It is controversial whether all HIV co-infected individuals require evaluation for neurosyphilis at the time of syphilis diagnosis[4].

Recent data suggests that there is an association with RPR titers ≥1:32 and laboratory defined neurosyphilis (sensitivity of 100%, specificity of 40%)[24, 25]. This is in keeping with our study findings, deducing that lumbar puncture could be restricted to the subgroup of patients with neurologic manifestation or a serum RPR of ≥1:32[27, 28]. Prior studies have found that patients with CD4 counts <350mm^3, may be at increased risk for neurosyphilis, however we identified no specific correlation[4, 28, 29]. We did note that five of the individuals with neurosyphilis were not HIV virologically suppressed, suggesting that there may be a link between increased HIV viral loads and neurosyphilis, however this requires further study.

CONCLUSIONS:

Through routine screening of an HIV-infected population engaged in care, many asymptomatic syphilis episodes were identified and treated resulting in a shift in diagnostic stage of syphilis infection from latent to primary and a theoretical decrease in ongoing transmission. Individuals with symptomatic syphilis infections were more likely to have higher RPR titers and those with highest RPR titers were at greater risk of having neurosyphilis. ART, CD4 count and virologic suppression of HIV had no association with the individual's stage of syphilis or symptoms at diagnosis. Syphilis infection was associated with a temporary decrease in CD4 count with no impact on HIV viral load. As the rates of syphilis rise among the HIV-infected population,

ongoing vigilance in screening and treatment is required in addition to further examination of co-infection interactions.

KEY MESSAGES

1. Through routine syphilis testing of an HIV-infected population many asymptomatic syphilis episodes were detected and treated.
2. Symptomatic individuals at diagnosis were more likely to have higher RPR titers.
3. Syphilis coinfection was associated with a temporary decrease in CD4 count, but no change in viral load was noted.
4. Patients with neurosyphilis were more likely to have higher RPR titers at diagnosis with no cases occurring in patients with titers <1:32.

FIGURE LEGENDS:

Figure 1: Percentage of episodes of syphilis diagnosed based on symptoms in a HIV-infected population.

Figure 2: Percentage of syphilis episodes divided by symptom at presentation based on initial RPR titer. Individuals who had symptoms compared to those that did not were more likely to have a higher initial RPR (P=0.0339). The most common symptoms were rash and ulcer/lesion with flu-like illness, condylomata and lymphadenopathy being relatively rare. Those with neurologic symptoms had a significant elevation of their initial RPR titers compared with all other symptoms (P=<0.001) and there were no cases of neurosyphilis with RPR titers less then 1:32 dilutions.

Figure 3: There is an increased number of incident syphilis infections among HIV positive individuals who are active in care programs from 2006-2016. There is an apparent trend of decreased proportion of late latent disease.

Figure 4: Scatter plot of CD4 count at syphilis diagnosis versus CD4 count at follow up appointment after treatment of syphilis. CD4 count was noted to decrease by an average of 42.2 cells/mm^3 (P =0.004).

Figure 5: Scatter plot of viral load (adjusted on a logarithmic scale to account for wide variation in values) at syphilis diagnosis versus viral load at follow up appointment after treatment of syphilis. Viral load was noted to increase by an average of 3.09 copies/mL in relation to syphilis co-infection (P =0.47).

Figure 6: The percentage of syphilis episodes comparing initial symptom presentation divided by recurrent infections. There is a trend demonstrating that individuals with recurrent syphilis infections were more likely to be symptomatic on presentation, however this did not reach significance (P=0.0799).

DECLARATIONS:

Ethics approval and consent to participate: Ethics approval was obtained through the University of Calgary Bioethics committee as a quality assurance project through A Project Ethics Community Consensus Initiative (ARECCI). Approval was granted both verbally and written on Aug 23, 2016.

Data sharing: The datasets generated and/or analyzed during the current study are not publicly available due to patient confidentiality. The sensitive nature of this information as well as the relatively small number of patients included in this dataset may lead it to be identifying and therefore does not allow this dataset to be made public.

Funding: No funding was received for this work.

Conflicts of interest: We have no relevant conflicts of interest to disclose.

This work was previously presented at ID week 2017 in San Diego, California.

Authors' contributions: RL, RR, HK and JG were involved in study design, data extraction, data analysis, drafting and final review of this work. SR, MP, and QV were involved in data extraction, data analysis and final review of this work. All authors read and approved the final manuscript.

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REFERENCES:

1. World Health Organization, Dept of Reproductive Health and Research. Global incidence and prevalence of selected curable sexually transmitted infections – 2008. 2012. Available at: http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/. Date accessed: Dec 6, 2017.

2. Karp G, Schleffer F, Jotkowitz A, *et al*. Syphilis and HIV co-infection. *Eur. J. Intern. Med* 2009;**20**:9-13.

3. 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**:125.

4. Zetola NM, Klausner JD. Syphilis and HIV Infection: An Update. *CID* 2007;**44**:1222-8.

5. R. *v.* Mabior, 2012 SCC 47, [2012] 2 S.C.R. 584. Available at: https://scc-csc.lexum.com/scc-csc/scc-csc/en/item/10008/index.do. Date accessed: Dec 1, 2017.

6. Kouyos RD, Hasse B, Calmy A, *et al.* Increases in Condomless Sex in the Swiss HIV Cohort Study. *Open Forum Infect Dis* 2015;**2**(2):ofv077.

7. Rolfs RT, Joesoef MR, Hendershot EF, *et al*. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group, *N Engl J Med* 1997;**337**:307-15.

8. Rompalo AM, Joesoef MR, O'Donnell JA, *et al*. Clinical Manifestations of Early Syphilis by HIV Status and Gender: Results of the Syphilis and HIV Study. *Sex Transm Dis* 2001;**28**(3):158-65.

9. Marra CM, Tantalo LC, Sahi SK, *et al*. Reduced Treponema pallidum-Specific Opsonic Antibody Activity in HIV-Infected Patients with Syphilis. *J Infect Dis* 2016;**213**:1348-54.

10. Collis TK, Celum CL. The Clinical Manifestations and treatment of Sexually Transmitted Diseases in Human Immunodeficiency Virus-Positive Men. *Clin Infect Dis* 2001;**32**:611-22.

11. Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. *Lancet Infect Dis* 2004;**4**:456-66.

12. McCoy SI, Eron JJ, Kuruc JD, *et al*. Sexually transmitted infections among patients with acute HIV in North Carolina. *Sex Transm Dis* 2009;**36**(6):372–4.

13. Fleming DT. Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* 1999;**75**:3–17.

14. Rekart ML, Ndifon W, Brunham RC, *et al*. A double-edged sword:does highly active antiretroviral therapy contribute to syphilis incidence by impairing immunity to *Treponema pallidum*? *Sex Transm Infect* 2017;**0**:1-5.

15. Branger J, Van Der Meer JT, Van Ketel RJ, *et al*. High Incidence of Asymptomatic Syphilis in HIV-Infected MSM Justifies Routine Screening. *Sex Transm Dis* 2009;**36**(2):84-5.

16. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines, 2015. Available at: https://www.cdc.gov/std/tg2015/tg-2015-print.pdf. Date accessed: Feb 26, 2017.

17. European AIDS Clinical Society (EACS). Guidelines Version 9.0. 2017. Available at: http://www.eacsociety.org/files/guidelines_9.0-english.pdf. Date accessed Feb 26, 2017.

18. Ratnam S. The laboratory diagnosis of syphilis. *Can J Infect Dis Med Microbiol* 2005;16(1):45-51.

19. Public Health Agency of Canada. Canadian Guidelines on Sexually Transmitted Infections: Syphilis. 2014. Available at: http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/section-5-10-eng.php. Date accessed: Dec 6, 2017.

20. Cohen CE, Winston A, Asboe D*, et al.* Increasing detection of asymptomatic syphilis in HIV patients. *Sex Transm Infect* 2005;**81:**217-219.

21. Braun DL, Marzel A, Steffens D, *et al*. High rates of subsequent asymptomatic STIs and risky sexual behavior in patients initially presenting with primary HIV-1 infection. *Clin Infect Dis* Published Online First: 5 October 2017. doi:10.1093/cid/cix873

22. 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;**18**(15):2075–9.

23. Palacios R, Jimenez-Onate F, Aguilar M, *et al*. Impact of syphilis infection on HIV viral load and CD4 cell counts in HIV-infected patients. *J Acquir Immune Defic Syndr* 2007; **44**(3):356–9.

24. Sadiq ST, McSorley J, Copas AJ, *et al*. The effects of early syphilis on CD4 counts and HIV-1 RNA viral loads in blood and semen. *Sex Transm Infect* 2005;**81**:380–5.

25. Rolfs RT, Joesoef MR, Hendershot EF, *et al*. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group, *N Engl J Med* 1997;**337**:307-15.

26. Workowski KA, Berman SM. Centers for Disease C, Prevention. Sexually transmitted diseases treatment guidelines. *MMWR Recomm Rep* 2010;**59**(12):1-110.

27. Libois A, De Wit S, Poll B, *et al*. HIV and syphilis: when to perform a lumbar puncture. *Sex Transm Dis* 2007;**34**(3):141-4

28. Marra CM, Maxwell CL, Smith SL, *et al*. Cerebrospinal fluid abnormalities in patients with syphilis: association with clinical and laboratory features. *J Infect Dis* 2004;**189**:369-76.

29. Ghanem KG, Moore RD, Rompalo AM, *et al*. Neurosyphilis in a clinical cohort of HIV 1 infected patients. *AIDS* 2008;22(10):1145-51.

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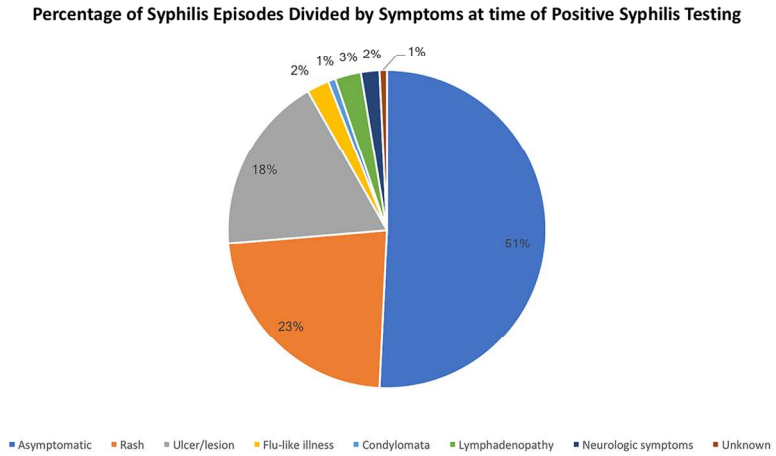

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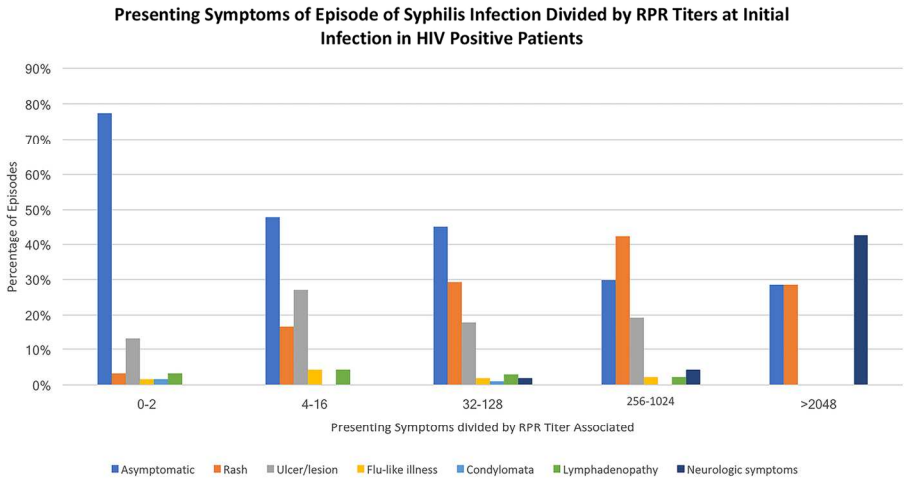

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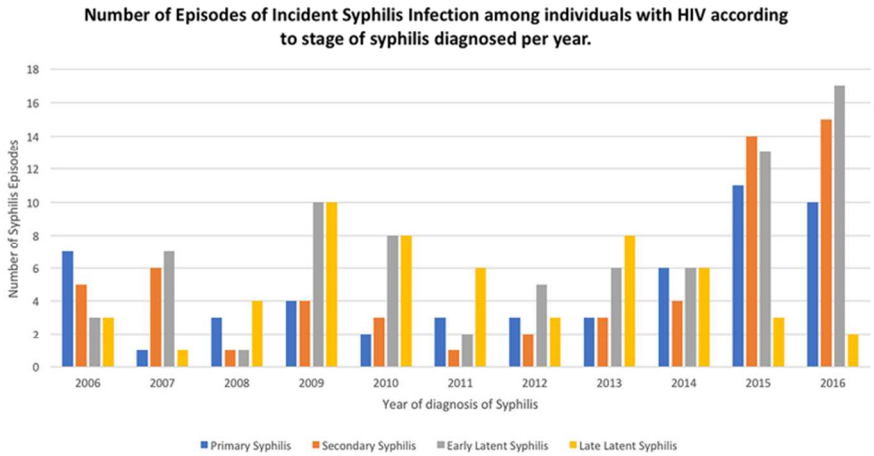

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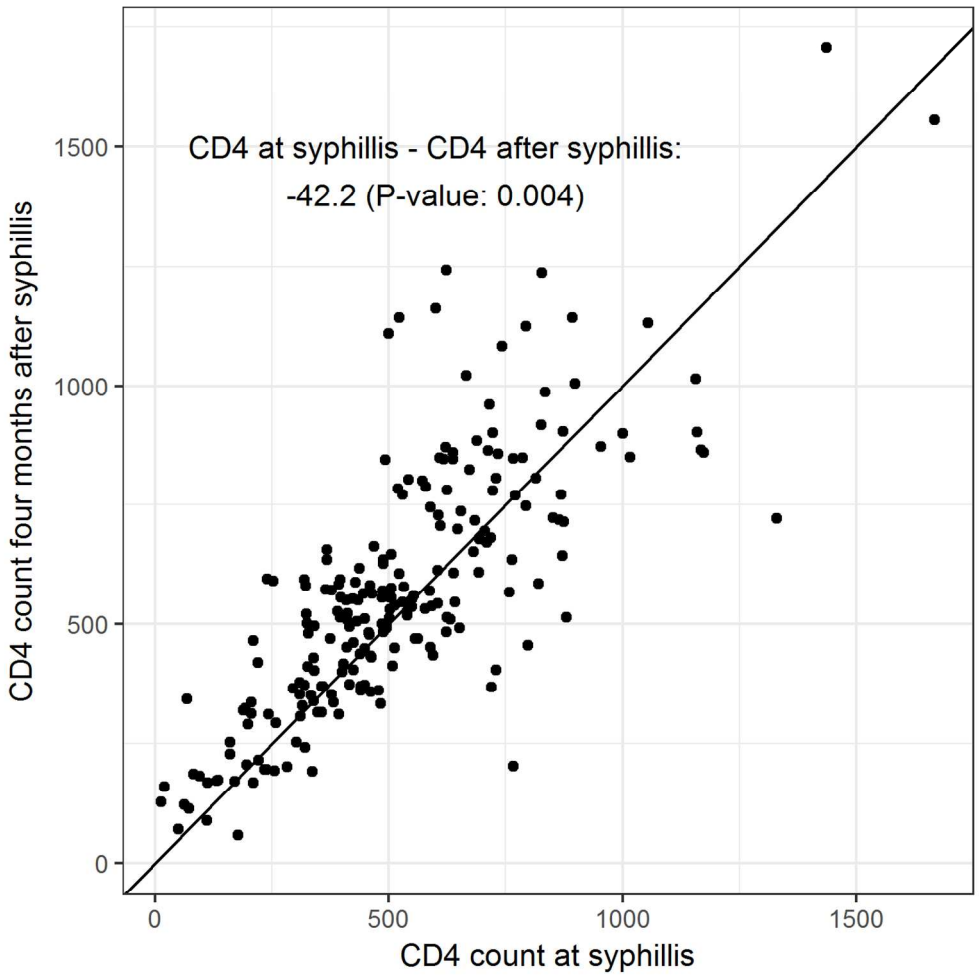

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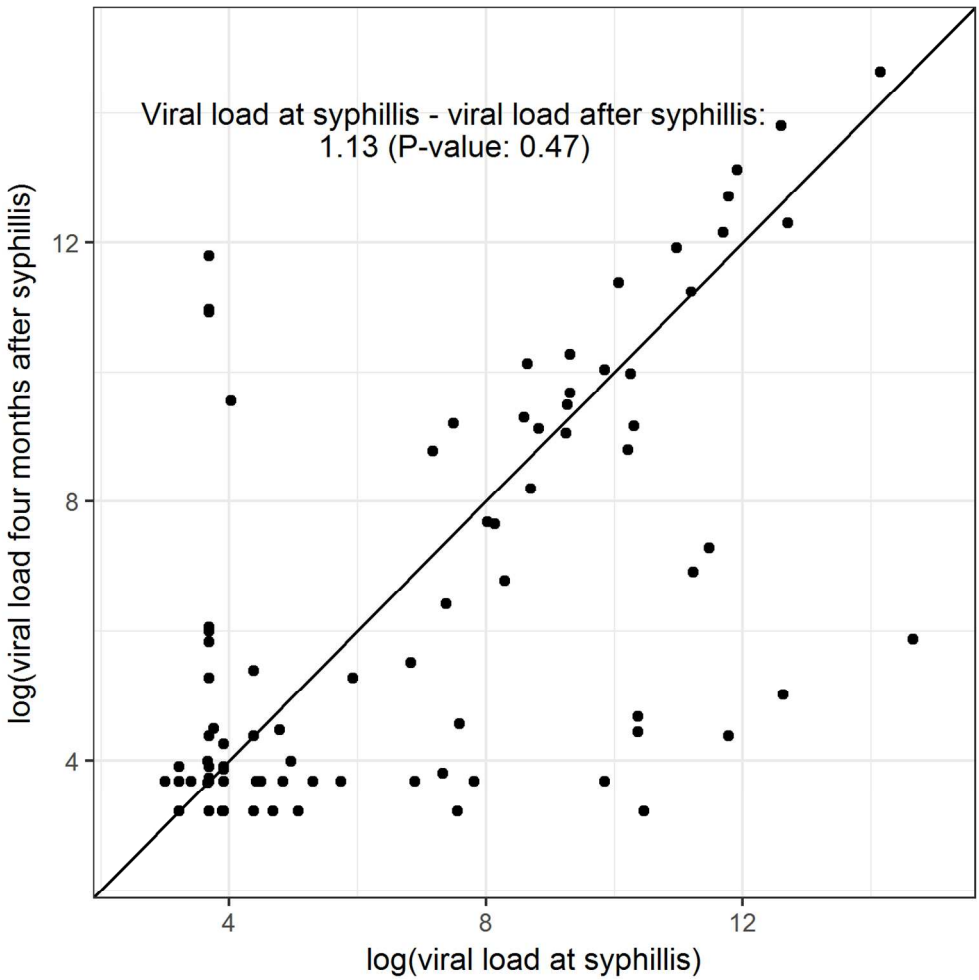

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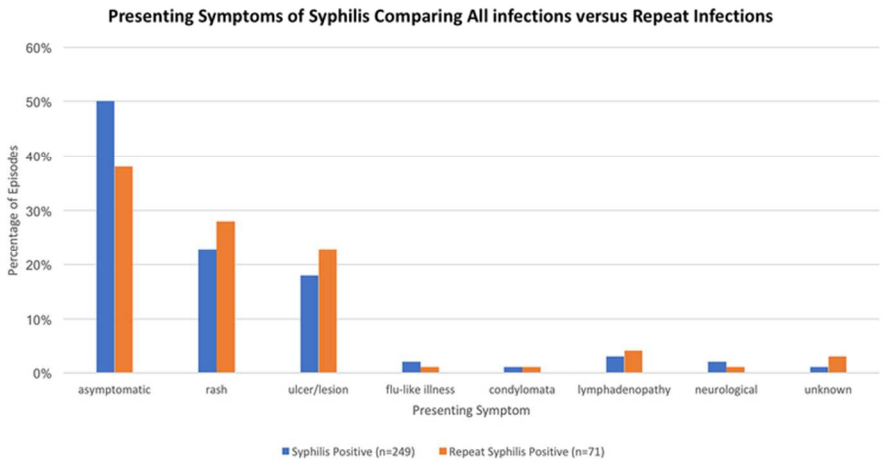

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71x40mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract Page 1,2	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale Page 4	2	Explain the scientific background and rationale for the investigation being reported
Objectives Page 5	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design Page 5	4	Present key elements of study design early in the paper
Setting Page 6	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants Page 5	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables Page 5,6, 7	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement Page 6,7	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Page 7	9	Describe any efforts to address potential sources of bias
Study size Page 8	10	Explain how the study size was arrived at
Quantitative variables Page 7	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods Page 7	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants Page 8	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data Page 8,9	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data Page 8	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results Page 10, 11, 12	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses Page 12, 13	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results Page 13, 14, 15	18	Summarise key results with reference to study objectives
Limitations Page 3	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation Page 16	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability Page 3	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding Page 18	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

A retrospective study of the clinical features of new syphilis infections in a HIV positive cohort in Alberta, Canada.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-021544.R2
Article Type:	Research
Date Submitted by the Author:	25-Apr-2018
Complete List of Authors:	Lang, Raynell; University of Calgary Cumming School of Medicine, Department of Medicine Read, Ron; University of Calgary Cumming School of Medicine, Department of Medicine Krentz, Hartmut ; Alberta Health Services, S. Alberta HIV Clinic Peng, Mingkai; University of Calgary, Department of Community Health Sciences Ramazani, Soheil; Alberta Health Services, S. Alberta HIV Clinic Vu, Quang; Alberta Health Services, S. Alberta HIV Clinic Gill, M John; University of Calgary Cumming School of Medicine, Department of Medicine; Alberta Health Services, S. Alberta HIV Clinic
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	HIV/AIDS, Sexual health, Public health
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES

TITLE: A RETROSPECTIVE STUDY OF THE CLINICAL FEATURES OF NEW SYPHILIS INFECTIONS IN A HIV POSITIVE COHORT IN ALBERTA, CANADA.

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ABSTRACT

Objectives: Syphilis is a global health concern with an estimated twelve million infections occurring annually. Due to the increasing rates of new syphilis infections being reported in HIV-infected patients, and their higher risk for atypical and severe presentations, periodic screening has been recommended as a routine component of HIV care. We aimed to characterized incident syphilis presentation, serologic features and treatment response in a well-defined, HIV-infected population over 11 years.

Methods: Since 2006, as routine practice of both the Southern Alberta Clinic (SAC) and Calgary STI Programs (CSTI) syphilis screening has accompanied HIV viral load measures every four months. All records of patients who, while in HIV care, either converted from being syphilis seronegative to a confirmed seropositive or were re-infected as evidenced by a four-fold increase in rapid plasma reagin (RPR) after past successful treatment, were reviewed.

Results: We identified 249 incident syphilis infections in 194 different HIV-infected individuals; 72% were initial infections whereas 28% were reinfections. Half (50.8%) of the infections were asymptomatic and identified only by routine screening. Symptomatic syphilis was more common when rapid plasma reagin (RPR) titers were higher (P=0.03). In patients with recurrent syphilis infection, a trend was noted favoring symptomatic presentation (62%, P=0.07). All 10 patients with CNS syphilis involvement presented with an RPR titer ≥1:32. Following syphilis infection a decline of 42 cells/mm^3 in CD4 (P=0.004) was found, but no significant changes in viral load occurred. No association was found with the stage of syphilis or symptoms at presentation and ART use, CD4 count or virologic suppression.

Conclusion: Routine screening of our HIV-infected population identified many asymptomatic syphilis infections. The interaction of HIV and syphilis infection appears to be bidirectional with effects noted on both HIV and syphilis clinical and serological markers.

ARTICLE SUMMARY

Strengths and limitations of this study

1. All HIV and STI care in our region is highly centralized and coordinated allowing for detailed analyses of our population.
2. Routine syphilis serology regardless of risk behaviors or symptomatology was obtained every four months in our HIV-infected population, allowing close monitoring of clinical characteristics, bidirectional interactions as well as inclusivity of incident syphilis infections.
3. The study population, while comprehensive and representing a Canadian perspective, is from a single regional area and may not be representative of populations elsewhere that have different rates of unprotected sexual activity and both prevalent HIV and syphilis infections. In addition, access to care varies between centers and populations and our rates and identification methods may not precisely match others.
4. This study may underestimate the clinical impact of syphilis in an HIV-infected population as patients not accessing care and individuals infected but lost to follow up or moving out of Alberta were not analyzed.

INTRODUCTION:

Syphilis continues to be a major public health concern globally, with an estimated twelve million new infections annually[1]. HIV-infected individuals are eight times more likely to become infected with syphilis than the general population[2]. In 2016, in Alberta Canada, over 25% of all new syphilis infections occurred in men who have sex with men (MSM) co-infected with HIV[3]. It has been suggested that increased use of social media including websites and mobile apps targeted towards meeting sex partners as well as serosorting (finding sex partners with the same HIV serostatus for unprotected sex), may be contributing to the rebound of high risk sexual activity in this population[2, 4]. The suppression of HIV viral replication (viral load <1,000 copies/mL) using antiretroviral therapy (ART) resulting in minimal risk for sexual transmission of HIV has received legal recognition in Canada[5]. As noted in a 2015 Swiss HIV Cohort study by Kouyos et al. there has been an accelerated rate of condomless sex since the recognition of HIV treatment as prevention. The reasons for increased risk behavior, particularly condomless sex are believed to be multifactorial, however in turn may be driving an increase in sexually transmitted infections (STIs)[6].

Syphilis in HIV-infected patients can present in atypical or severe forms, such as ulcerative skin lesions, persistent chancres, gummatous disease, ocular disease and neurosyphilis[7-11]. One study showed that HIV-infected individuals have multiple chancres and are more likely to experience Jarisch-Herxheimer reactions (22% vs 12% respectively), and another showed that concomitant genital ulcers were more common in patients with secondary syphilis and HIV[7, 8]. STIs may increase the risk of HIV acquisition via interruption of mucosal barriers and increased viral shedding[11-13]. It has also been suggested that ART may inadvertently increase

the incidence of syphilis by altering innate and acquired immune responses that may enhance susceptibility to syphilis infection[14]. Due to these increasing rates of syphilis and the higher likelihood of atypical and severe presentation, routine periodic screening (2-4 times annually) of HIV-infected persons has been recommended[4, 11, 15-17].

The aim of this retrospective cohort study was both to characterize syphilis presentation, serologic features and treatment response in a large cohort of HIV-infected individuals engaged in HIV care and receiving regular syphilis testing, as well as to examine the effect of incident syphilis on HIV disease markers.

METHODS

Study Population

The Southern Alberta Clinic (SAC) and Calgary STI Clinic (CSTI) provide exclusive care to HIV-infected individuals living in southern Alberta, Canada. In a quality assurance project (approved by University of Calgary Bioethics committee) at both programs between January 1, 2006 and December 31, 2016, routine syphilis serology regardless of risk was ordered every four months accompanying HIV viral load testing. The records of all incident syphilis infections occurring in HIV-infected patients were reviewed. Every indeterminate or positive syphilis serology for a SAC patient was discussed with or referred to CSTI at the time of testing.

All individuals with at least one visit between January 1, 2006 and December 31, 2016 were studied. Patients were followed until December 31, 2016 or until they moved, died or were lost-to-follow-up. All patients, who while in HIV care, converted from being seronegative for

syphilis to a confirmed positive status or were re-infected with syphilis were reviewed through the SAC database and a CSTI chart review.

Diagnosis

The syphilis screening algorithm and confirmatory testing was achieved using indirect serologic methods. Initially screening for syphilis was done with the non-treponemal rapid plasma reagin (RPR), however in 2008 the screening test was changed to an enzyme immunoassay (EIA), a treponemal test. The RPR continued to be used as a confirmatory test as well as for monitoring response to therapy[15, 18]. In Calgary, the secondary confirmatory test was either the fluorescent treponemal antibody absorption test (FTA-ABS) or the line immunoassay (INNO-LIA)[19].

Recurrent syphilis episodes were identified by a four-fold increase in RPR after a prior documented successful treatment course for syphilis and were evaluated and staged by an STI specialist (RR). Neurosyphilis was documented by a positive CSF-VRDL (Venereal disease research laboratory) on lumbar puncture as well as evaluated by an STI specialist (RR). HIV PrEP use was not extensively used in the community during the study period and any potential role seemed unlikely.

Data Collection

Detailed standardized information was collected by one physician (RL), through a comprehensive review of both SAC and CSTI charts and databases. Multiple data sources in

these records were accessed including nursing interviews, social work reports, self-administered questionnaires, laboratory reports, and physician notes.

From the SAC database, we identified the number of syphilis tests performed yearly at the clinic per patient as well as the interval between tests. Demographic data was collected at the time of HIV diagnosis and incorporated into the SAC database. These data included: gender (i.e. male, female, transgendered), self-reported ethnicity (i.e. Caucasian, Indigenous, African/Caribbean/Black (ACB), Other) and most likely HIV exposure risk (i.e. MSM, HET-heterosexual sex, PWID (persons who inject drugs), and other).

The stage of syphilis (i.e. primary, secondary, early latent, late latent) and symptomatology at presentation (i.e. rash, ulcer/lesion, flu-like illness, condylomata, lymphadenopathy, neurological (tinnitus/ocular), asymptomatic, other) were collected via review of CSTI charts. All episodes of syphilis were staged by an STI specialist (RR) based on both clinical and laboratory investigations. In the absence of symptoms, the staging of primary versus latent syphilis was based on the timing of rising RPR titers in relation to most recent prior titer. Prior history of comorbid infections including *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were self-reported at the time of syphilis diagnosis.

The initial RPR was documented at the time of syphilis diagnosis and recorded in CSTI charts. HIV viral load and CD4 counts were measured at the time of syphilis diagnosis and subsequently at the next routine HIV follow-up appointment. HIV viral suppression was defined as a plasma viral load <40 copies/mL. Treatment modalities (i.e. Benzathine Penicillin, Doxycycline,

Penicillin G) and response to therapy were reviewed retrospectively through a comprehensive chart review. All data was anonymized prior to analysis.

Patient and Public Involvement

No patients or public were involved in the present study. Our findings have been provided to local public health and will be used in broader STI control initiatives.

Statistical Analysis

Demographic and clinical factors of patients were compared using chi-square test. Viral load and CD4 counts prior to and following episode of syphilis infection were compared using linear mixed effect model while accounting for repeated measurement and more than one episode for some patients. Subgroup analyses were performed on neurosyphilis infections and those with recurrent episodes of syphilis. Patients not accessing care and individuals infected but lost to follow up or moving out of Alberta were not analyzed. All statistical analysis was performed using R (R Development Core Team, 2005). All charts were created with Microsoft Excel and R.

RESULTS:

Demographics

Between 2006 and 2016, there were 20,203 syphilis tests done on a total of 2,448 patients who attended at least one regular SAC visit during that time. On average, there were 180 days between each syphilis test per patient. The average number of syphilis screening tests that were done per patient each year over the 11-year period was 2.1. In 2006, the average number of tests

per year was 1.3, whereas in 2016 this was 2.8. For high risk patients (MSM), screening rates were more frequent with the average testing over 11 years being 2.4 tests per year.

Of the 2,448 HIV-infected individuals at SAC and CSTI programs encompassing 15,175 person years of follow up between 1/1/2006 and 12/31/2016, we identified 322 incident syphilis infections, occurring in 267 different patients. There were 73 syphilis episodes in 73 patients that were excluded. Of those excluded; 41 patients, while being tested in Alberta, had moved out of province resulting in incomplete clinical data, and in 32 patients, there was inadequate basic information available for study inclusion. We therefore analyzed 249 episodes in 194 individuals.

Of the 249 infections, 178 (72%) were first episode of a syphilis infection, whereas the remaining 71 (28%) were recurrent episodes. The annual incidence rates of syphilis in our HIV-infected population tripled from 2011, 8.08/1000 patient-years (95% confidence interval (CI): 4.14-14.75), to 27.04 per 1000 person-years (95% confidence interval (CI): 19.45-36.76) in 2016[3]. Prior history of STI's included; 32% of cases having a self-reported history of *Neisseria gonorrhoeae* and 24% having had *Chlamydia trachomatis* infection. The characteristics of the 194 individuals included in this analysis are described in table 1.

Table 1: Characteristics of HIV+ patients regularly followed at the Southern Alberta Clinic between 1/1/2006 and 12/31/2016 comparing patients who were negative for syphilis (Syphilis Neg) to patients who ever tested positive for syphilis (Syphilis Pos).

	Syphilis Neg	**Syphilis Pos**	**P-value**
N (%)	2254 (92.1)	194 (7.9)	
Age at HIV Diagnosis (years)			
Mean (range)	35 (1-79)	35 (16-69)	0.893

<30	813 (36.1)	75 (38.7)	0.801
30-39	802 (35.6)	66 (34.0)	
40-49	438 (19.4)	37 (19.1)	
≥50	201 (8.9)	16 (8.3)	
Gender			
Male	1675 (74.3)	183 (94.3)	**<.001**
Female	572 (25.4)	11 (5.6)	
Transgendered	7 (0.3)	0 (0.0)	
Self-reported Ethnicity[1]			
Caucasian	1259 (56.0)	140 (72.2)	**<.001**
Indigenous	216 (9.6)	6 (3.1)	
ACB	536 (23.8)	24 (12.4)	
Other	243 (10.8)	24 (12.4)	
Most Likely HIV Exposure Category[2]			
MSM	915 (40.6)	145 (74.4)	**<.001**
HET	512 (22.7)	14 (7.2)	
PWID	731 (32.4)	30 (15.6)	
Other	96 (4.3)	5 (2.6)	

[1]Indigenous people includes Aboriginal, Metis and Inuit; ACB includes African, Caribbean, Black; Other includes IndoAsian, Hispanic, East Asian, and other

[2]MSM=self-reported men who have sex with men identification; HET=self-reported heterosexual identification; PWID=self-reported intravenous drug use identification; Other HIV Risk factor behavior includes: blood transfusions, hemophiliac, neonatal, postnatal infection, unknown or not reported.

Symptomatology

Asymptomatic syphilis episodes

Just over half of the episodes (50.8%) of incident syphilis infections were asymptomatic and identified by routine screening (Figure 1). RPR titers were higher in patients with symptomatic versus asymptomatic syphilis (P=0.03) (Figure 2). The majority of episodes with an initial RPR of 1:4 or less were asymptomatic (71%). Those with lower CD4 (<200 cells/mm^3) counts at syphilis diagnosis had no significant differences in symptomatology as opposed to those with CD4 counts >200 cells/mm^3 (P= 0.65). Neither virologic suppression of HIV nor ART use influenced the individual's likelihood to present with symptomatic syphilis.

Symptomatic syphilis episodes

The most common presenting symptom was rash (23%), followed by skin lesion or ulceration (18%). Uncommon presentations included lymphadenopathy, flu-like illness, condylomata lata and neurological symptoms (Figure 1). The most common presenting symptom in primary syphilis was skin ulceration/lesion (57%) and in those with secondary syphilis was a rash (76%). However, 15% of those diagnosed with secondary syphilis also complained of skin ulceration or lesion in addition to a rash. Although rare overall as presenting symptoms, lymphadenopathy (86%), flu like illness (50%) and condylomata (100%) were most seen in primary syphilis.

Stage of Syphilis

Both ART and virologic suppression of HIV had no association with the individual's stage of syphilis at diagnosis. Of those diagnosed with late latent syphilis, 98% had an initial RPR of 1:16 or less. Patients with secondary syphilis tended to present with a higher RPR, 33% having an RPR of 1:256 or higher.

Since 2008, the proportion of late latent syphilis infections diagnosed among our HIV-infected patients in care had decreased from 44% to 4.4% (Figure 3). Caucasian individuals were more likely to present with primary (24%) or secondary (28%) syphilis (P=<0.001), whereas the non-Caucasian population were more likely to present with latent disease (74%) (P=<0.001). In males, the majority of infections were early latent (34%) and the minority being late latent (18%). However, in females 77% of infections were late latent.

Effect of Syphilis on Markers of HIV

As the interaction of HIV and syphilis infection may be bidirectional we explored CD4 and viral load response to syphilis infection. A significant decrease in CD4 count of 42.2 cells/mm^3 (P=0.004) was noted in association with syphilis coinfection (Figure 4). However, there was no change in HIV viral load noted in association with syphilis coinfection (P=0.47) (Figure 5).

Effect of HIV on Markers of Syphilis

Nearly half (49%) of all patients presented with RPR (non-treponemal) titers between 1:32-1:128. There were two episodes presenting with an initial RPR greater than 1:2048; both patients were not HIV virologically suppressed (HIV plasma viral load >1,000 copies/mL) at the time of syphilis infection (Figure 2). The individuals viral load (P=0.82) or CD4 count (P=0.48) did not appear to have any correlation with the initial RPR titer. We were unable to evaluate if the absence of ART had an impact on RPR titer due to the small number of patients not on ART (n=48).

Recurrent Episodes of Syphilis

In patients with recurrent syphilis infection, a trend (P=0.07) was noted favoring symptomatic presentation (62%). Rash and skin lesion/ulceration also remained the most common complaint (Figure 6). Recurrent episodes of syphilis were much less likely to be late latent disease (3%) and instead more likely to be primary (28%), secondary (28%) or early latent disease (39%). Of those with a recurrent syphilis episode, 29% had RPR titers over 1:256, compared to 18% in the study population. Only 10% of the patients with prior syphilis exposure had an initial RPR less than 1:4 compared to 32% in the study population, however this did not reach significance (P=0.604).

Neurosyphilis

CNS involvement was noted in 10/249 (4%) episodes with a positive CSF-VDRL on lumbar puncture. Ocular symptoms with blurred vision or painless visual loss occurred in four patients, tinnitus in three patients and three were asymptomatic. Nine patients were male and Caucasian with eight being >40 years old. Eight were initial syphilis episodes and two were reinfections. Seven of the ten patients were on ART, five were virologically suppressed with seven having a CD4 count > 500 cells mL. The RPR titer at diagnosis was ≥1:32 in all episodes of CNS involvement with five having an RPR titer of ≥1:512 and two of these episodes diagnosed with initial RPR titers of 1:8192. These RPR titers were much higher than any other symptom presentation (P=<0.001) (Figure 2). All patients with CNS involvement were treated successfully, based on both clinical and serologic response, with intravenous penicillin G for 14 days.

Treatment

A standard three-week course of weekly intramuscular injections of Benzathine penicillin (2.4MU per dose) was used for 77% of the patients, while 10% received an oral course of doxycycline, and 10% received a combination of the two medications. Successful completion of the full course of treatment was achieved in 94% (with 5% requiring retreatment from inadequate initial adherence and 1% never completing their full course).

DISCUSSION:

Our introduction in 2006 of syphilis screening to accompany routine HIV viral load testing allowed for the identification and analysis of incident syphilis infections in the HIV population in care in Calgary, Alberta. Our results confirm prior findings that co-infection with HIV can result in atypical or severe syphilis presentations[8-11]. Compared to non HIV-infected populations, prior studies have found higher rates of asymptomatic primary syphilis, which may result in missed diagnosis and increased episodes of secondary syphilis[11, 20]. In our study population, 50.8% (135) syphilis episodes were asymptomatic at presentation, including 21% (10) of the primary syphilis infections. Braun et al. recently published a study evaluating symptoms of syphilis in 19 HIV-infected individuals and found the rate of asymptomatic syphilis infections in HIV-infected individuals to be 40%[21]. Routine syphilis screening has been confirmed to be effective in detecting early asymptomatic syphilis in HIV-infected outpatients[20].

Our study demonstrated a decline in latent syphilis between 2008 (44%) and 2016 (4%). In 2008, the high numbers of latent syphilis may be reflective of a change to the testing algorithm for syphilis, from an initial RPR to enzyme immunoassay (EIA), resulting in an improved test sensitivity and the identification of latent syphilis[18, 19]. While latent episodes have been steadily declining since 2013, the number of primary syphilis diagnoses are increasing. Through regular syphilis screening in this HIV-infected population, earlier detection of syphilis in its primary stage has been achieved, leading to prompt therapy, which may decrease ongoing syphilis transmission[4].

The interaction of HIV and syphilis infection appears to be bidirectional with effects noted on both HIV and syphilis serologic and clinical markers[11]. Prior studies have reported that

syphilis infection may increase HIV viral load and decrease CD4 count[22-25]. We observed a statistically significant decrease in CD4 count associated with incident syphilis infections, but no change in viral load was noted. This difference in findings compared with past studies may in part, be explained by the majority of our patients being on ART, which are perhaps more potent in suppressing viral replication.

An increased prevalence of neurologic manifestations has been reported in HIV-infected individuals[2, 4]. Approximately one third of any patient with early syphilis will have treponemal invasion into their CNS regardless of their HIV status[4]. However, an increased rate of early neurosyphilis among HIV-infected individuals has been noted and may be linked to the patient's inability to control the CNS infection rather than increased invasion into the CNS[4, 25]. Our data revealed 10/249 (4%) of the syphilis episodes diagnosed in our HIV-infected cohort were neurosyphilis.

Neurosyphilis is more likely to be asymptomatic in HIV co-infected individuals and therefore a more difficult diagnosis[4]. Three of our ten neurosyphilis episodes were indeed asymptomatic. As a response to the absence of symptoms, CDC guidelines recommend HIV-infected individuals who receive a diagnosis of late latent syphilis, unknown duration of disease, have neurologic symptoms or treatment failure should undergo CSF evaluation[4, 26]. It is controversial whether all HIV co-infected individuals require evaluation for neurosyphilis at the time of syphilis diagnosis[4].

Recent data suggests that there is an association with RPR titers ≥1:32 and laboratory defined neurosyphilis (sensitivity of 100%, specificity of 40%)[24, 25]. This is in keeping with our study findings, deducing that lumbar puncture could be restricted to the subgroup of patients with neurologic manifestation or a serum RPR of ≥1:32[27, 28]. Prior studies have found that patients with CD4 counts <350mm^3, may be at increased risk for neurosyphilis, however we identified no specific correlation[4, 28, 29]. We did note that five of the individuals with neurosyphilis were not HIV virologically suppressed, suggesting that there may be a link between increased HIV viral loads and neurosyphilis, however this requires further study.

The key strength of our study is the detailed longitudinal analysis of clinical, serologic and treatment outcomes in our population that is made possible by the highly centralized HIV and STI care programs in our region. The study population, while comprehensive, is from a single regional area and may not be generalizable to populations elsewhere. Rates of unprotected sexual activity, prevalent HIV and syphilis infections, and access to care varies between centers and populations, therefore our rates and identification methods may not match others. Limitations of our study include a potential underestimation of the clinical impact of syphilis in this HIV-infected population as patients not accessing care and individuals infected but lost to follow up or who moved from Alberta were not analyzed.

CONCLUSIONS:

Through routine screening of an HIV-infected population engaged in care, many asymptomatic syphilis episodes were identified and treated resulting in a shift in diagnostic stage of syphilis infection from latent to primary and a theoretical decrease in ongoing transmission. Individuals

with symptomatic syphilis infections were more likely to have higher RPR titers and those with highest RPR titers were at greater risk of having neurosyphilis. ART, CD4 count and virologic suppression of HIV had no association with the individual's stage of syphilis or symptoms at diagnosis. Syphilis infection was associated with a temporary decrease in CD4 count with no impact on HIV viral load. As the rates of syphilis rise among the HIV-infected population, ongoing vigilance in screening and treatment is required in addition to further examination of co-infection interactions.

FIGURE LEGENDS:

Figure 1: Percentage of episodes of syphilis diagnosed based on symptoms in a HIV-infected population.

Figure 2: Percentage of syphilis episodes divided by symptom at presentation based on initial RPR titer. Individuals who had symptoms compared to those that did not were more likely to have a higher initial RPR (P=0.0339). The most common symptoms were rash and ulcer/lesion with flu-like illness, condylomata and lymphadenopathy being relatively rare. Those with neurologic symptoms had a significant elevation of their initial RPR titers compared with all other symptoms (P=<0.001) and there were no cases of neurosyphilis with RPR titers less then 1:32 dilutions.

Figure 3: There is an increased number of incident syphilis infections among HIV positive individuals who are active in care programs from 2006-2016. There is an apparent trend of decreased proportion of late latent disease.

Figure 4: Scatter plot of CD4 count at syphilis diagnosis versus CD4 count at follow up appointment after treatment of syphilis. CD4 count was noted to decrease by an average of 42.2 cells/mm^3 (P =0.004).

Figure 5: Scatter plot of viral load (adjusted on a logarithmic scale to account for wide variation in values) at syphilis diagnosis versus viral load at follow up appointment after treatment of syphilis. Viral load was noted to increase by an average of 3.09 copies/mL in relation to syphilis co-infection (P =0.47).

Figure 6: The percentage of syphilis episodes comparing initial symptom presentation divided by recurrent infections. There is a trend demonstrating that individuals with recurrent syphilis infections were more likely to be symptomatic on presentation, however this did not reach significance (P=0.0799).

DECLARATIONS:

Ethics approval and consent to participate: Ethics approval was obtained through the University of Calgary Bioethics committee as a quality assurance project through A Project Ethics Community Consensus Initiative (ARECCI). Approval was granted both verbally and written on Aug 23, 2016.

Data sharing: The datasets generated and/or analyzed during the current study are not publicly available due to patient confidentiality. The sensitive nature of this information as well as the relatively small number of patients included in this dataset may lead it to be identifying and therefore does not allow this dataset to be made public.

Funding: No funding was received for this work.

Conflicts of interest: We have no relevant conflicts of interest to disclose.

This work was previously presented at ID week 2017 in San Diego, California.

Authors' contributions: RL, RR, HK and JG were involved in study design, data extraction, data analysis, drafting and final review of this work. SR, MP, and QV were involved in data extraction, data analysis and final review of this work. All authors read and approved the final manuscript.

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REFERENCES:

1. World Health Organization, Dept of Reproductive Health and Research. Global incidence and prevalence of selected curable sexually transmitted infections – 2008. 2012. Available at: http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/. Date accessed: Dec 6, 2017.
2. Karp G, Schleffer F, Jotkowitz A, *et al*. Syphilis and HIV co-infection. *Eur. J. Intern. Med* 2009;**20**:9-13.
3. 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**:125.
4. Zetola NM, Klausner JD. Syphilis and HIV Infection: An Update. *CID* 2007;**44**:1222-8.

5. R. *v.* Mabior, 2012 SCC 47, [2012] 2 S.C.R. 584. Available at: https://scc-csc.lexum.com/scc-csc/scc-csc/en/item/10008/index.do. Date accessed: Dec 1, 2017.
6. Kouyos RD, Hasse B, Calmy A, *et al.* Increases in Condomless Sex in the Swiss HIV Cohort Study. *Open Forum Infect Dis* 2015;**2**(2):ofv077.
7. Rolfs RT, Joesoef MR, Hendershot EF, *et al*. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group, *N Engl J Med* 1997;**337**:307-15.
8. Rompalo AM, Joesoef MR, O'Donnell JA, *et al*. Clinical Manifestations of Early Syphilis by HIV Status and Gender: Results of the Syphilis and HIV Study. *Sex Transm Dis* 2001;**28**(3):158-65.
9. Marra CM, Tantalo LC, Sahi SK, *et al*. Reduced Treponema pallidum-Specific Opsonic Antibody Activity in HIV-Infected Patients with Syphilis. *J Infect Dis* 2016;**213**:1348-54.
10. Collis TK, Celum CL. The Clinical Manifestations and treatment of Sexually Transmitted Diseases in Human Immunodeficiency Virus-Positive Men. *Clin Infect Dis* 2001;**32**:611-22.
11. Lynn WA, Lightman S. Syphilis and HIV: a dangerous combination. *Lancet Infect Dis* 2004;**4**:456-66.
12. McCoy SI, Eron JJ, Kuruc JD, *et al*. Sexually transmitted infections among patients with acute HIV in North Carolina. *Sex Transm Dis* 2009;**36**(6):372–4.
13. Fleming DT. Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* 1999;**75**:3–17.

14. Rekart ML, Ndifon W, Brunham RC, *et al*. A double-edged sword:does highly active antiretroviral therapy contribute to syphilis incidence by impairing immunity to *Treponema pallidum*? *Sex Transm Infect* 2017;**0**:1-5.

15. Branger J, Van Der Meer JT, Van Ketel RJ, *et al*. High Incidence of Asymptomatic Syphilis in HIV-Infected MSM Justifies Routine Screening. *Sex Transm Dis* 2009;**36**(2):84-5.

16. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines, 2015. Available at: https://www.cdc.gov/std/tg2015/tg-2015-print.pdf. Date accessed: Feb 26, 2017.

17. European AIDS Clinical Society (EACS). Guidelines Version 9.0. 2017. Available at: http://www.eacsociety.org/files/guidelines_9.0-english.pdf. Date accessed Feb 26, 2017.

18. Ratnam S. The laboratory diagnosis of syphilis. *Can J Infect Dis Med Microbiol* 2005;16(1):45-51.

19. Public Health Agency of Canada. Canadian Guidelines on Sexually Transmitted Infections: Syphilis. 2014. Available at: http://www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/section-5-10-eng.php. Date accessed: Dec 6, 2017.

20. Cohen CE, Winston A, Asboe D*, et al.* Increasing detection of asymptomatic syphilis in HIV patients. *Sex Transm Infect* 2005;**81:**217-219.

21. Braun DL, Marzel A, Steffens D, *et al*. High rates of subsequent asymptomatic STIs and risky sexual behavior in patients initially presenting with primary HIV-1 infection. *Clin Infect Dis* Published Online First: 5 October 2017. doi:10.1093/cid/cix873

22. 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;**18**(15):2075–9.

23. Palacios R, Jimenez-Onate F, Aguilar M, *et al*. Impact of syphilis infection on HIV viral load and CD4 cell counts in HIV-infected patients. *J Acquir Immune Defic Syndr* 2007; **44**(3):356–9.

24. Sadiq ST, McSorley J, Copas AJ, *et al*. The effects of early syphilis on CD4 counts and HIV-1 RNA viral loads in blood and semen. *Sex Transm Infect* 2005;**81**:380–5.

25. Rolfs RT, Joesoef MR, Hendershot EF, *et al*. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group, *N Engl J Med* 1997;**337**:307-15.

26. Workowski KA, Berman SM. Centers for Disease C, Prevention. Sexually transmitted diseases treatment guidelines. *MMWR Recomm Rep* 2010;**59**(12):1-110.

27. Libois A, De Wit S, Poll B, *et al*. HIV and syphilis: when to perform a lumbar puncture. *Sex Transm Dis* 2007;**34**(3):141-4

28. Marra CM, Maxwell CL, Smith SL, *et al*. Cerebrospinal fluid abnormalities in patients with syphilis: association with clinical and laboratory features. *J Infect Dis* 2004;**189**:369-76.

29. Ghanem KG, Moore RD, Rompalo AM, *et al*. Neurosyphilis in a clinical cohort of HIV 1 infected patients. *AIDS* 2008;22(10):1145-51.

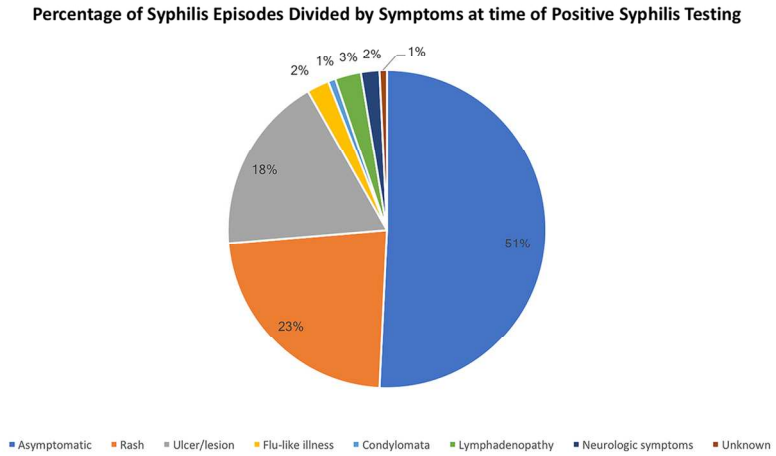

Figure 1: Percentage of episodes of syphilis diagnosed based on symptoms in a HIV-infected population.

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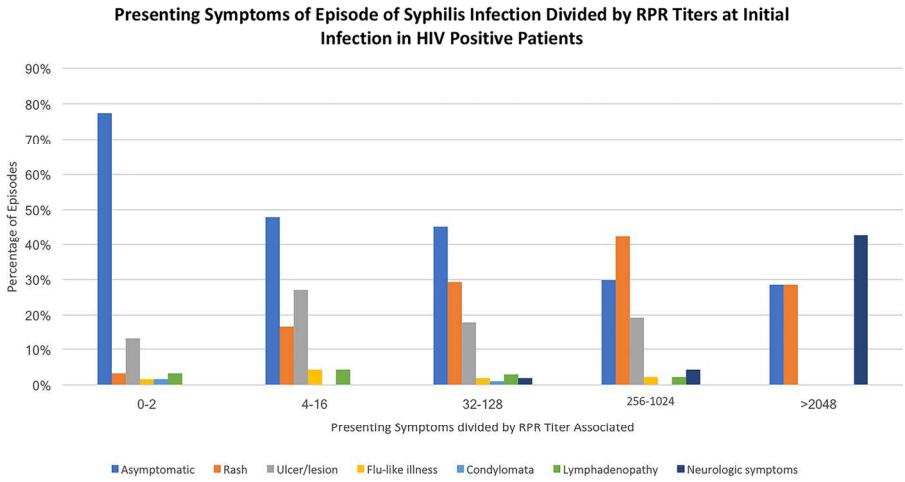

Figure 2: Percentage of syphilis episodes divided by symptom at presentation based on initial RPR titer. Individuals who had symptoms compared to those that did not were more likely to have a higher initial RPR (P=0.0339). The most common symptoms were rash and ulcer/lesion with flu-like illness, condylomata and lymphadenopathy being relatively rare. Those with neurologic symptoms had a significant elevation of their initial RPR titers compared with all other symptoms (P=<0.001) and there were no cases of neurosyphilis with RPR titers less then 1:32 dilutions.

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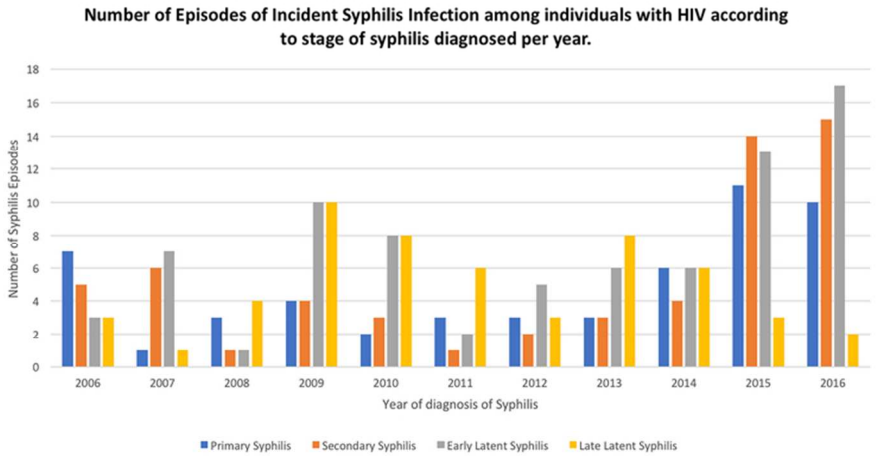

Figure 3: There is an increased number of incident syphilis infections among HIV positive individuals who are active in care programs from 2006-2016. There is an apparent trend of decreased proportion of late latent disease.

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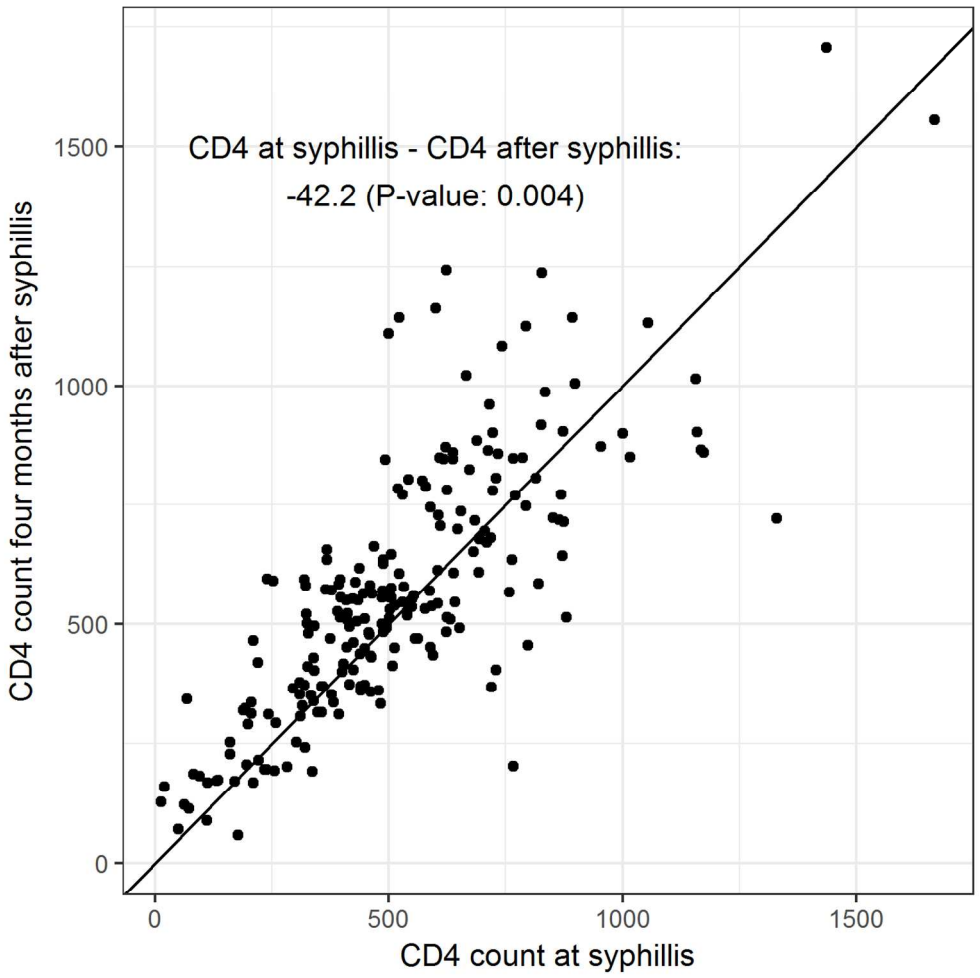

Figure 4: Scatter plot of CD4 count at syphilis diagnosis versus CD4 count at follow up appointment after treatment of syphilis. CD4 count was noted to decrease by an average of 42.2 cells/mm3 (P =0.004).

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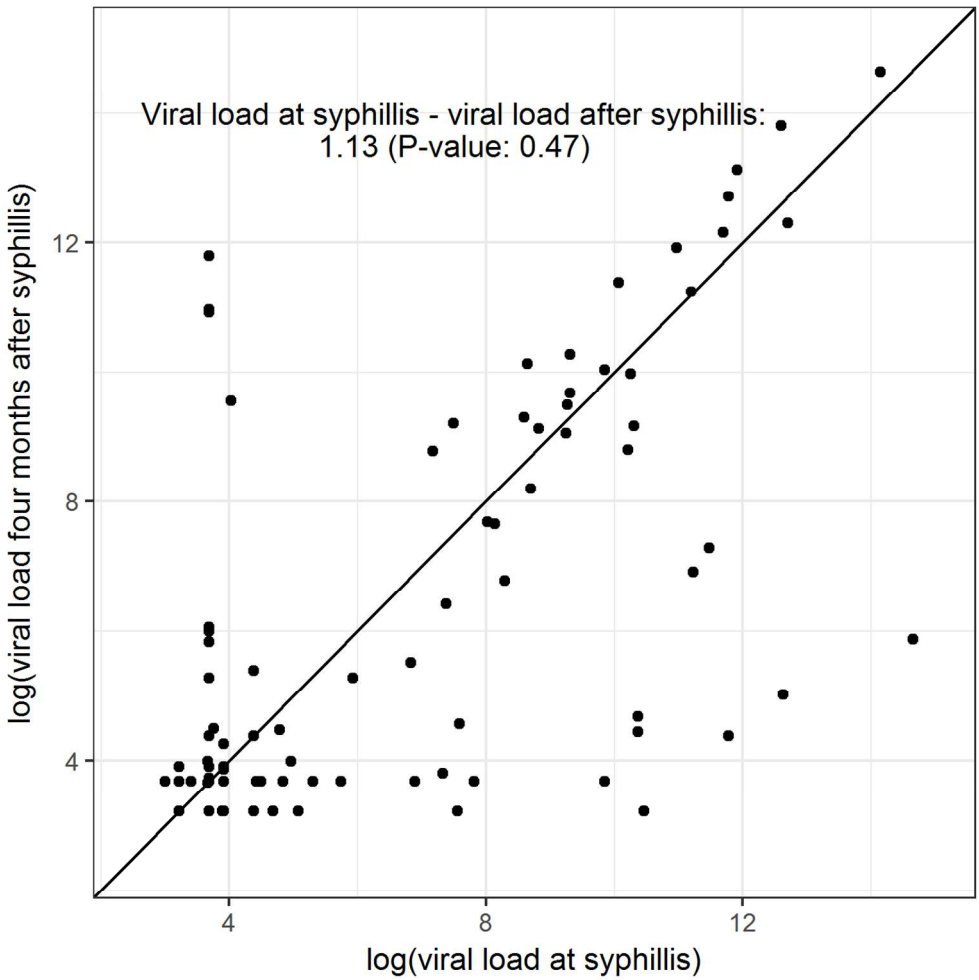

Figure 5: Scatter plot of viral load (adjusted on a logarithmic scale to account for wide variation in values) at syphilis diagnosis versus viral load at follow up appointment after treatment of syphilis. Viral load was noted to increase by an average of 3.09 copies/mL in relation to syphilis co-infection (P =0.47).

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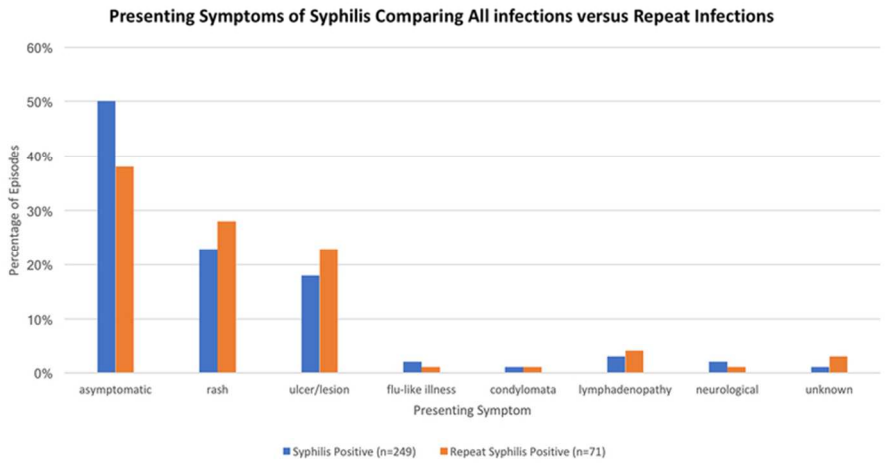

Figure 6: The percentage of syphilis episodes comparing initial symptom presentation divided by recurrent infections. There is a trend demonstrating that individuals with recurrent syphilis infections were more likely to be symptomatic on presentation, however this did not reach significance (P=0.0799).

71x40mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract Page 1,2	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale Page 4	2	Explain the scientific background and rationale for the investigation being reported
Objectives Page 5	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design Page 5	4	Present key elements of study design early in the paper
Setting Page 6	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants Page 5	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables Page 5,6, 7	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement Page 6,7	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Page 7	9	Describe any efforts to address potential sources of bias
Study size Page 8	10	Explain how the study size was arrived at
Quantitative variables Page 7	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods Page 7	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants Page 8	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data Page 8,9	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data Page 8	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results Page 10, 11, 12	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses Page 12, 13	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results Page 13, 14, 15	18	Summarise key results with reference to study objectives
Limitations Page 3	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation Page 16	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability Page 3	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding Page 18	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.