Syphilis in HIV-Infected Mothers and Infants: Results from the NICHD/ HPTN 040 Study

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ABSTRACT:

Background: Untreated syphilis during pregnancy is associated with spontaneous abortion, stillbirth, prematurity and infant mortality. Syphilis may facilitate HIV transmission, which is especially concerning in low and middle income countries where both diseases are common.

Methods: We performed an analysis of data available from NICHD/HPTN 040 (P1043), a study focused on the prevention of *intrapartum* HIV transmission to 1684 infants born to 1664 untreated HIV-infected women. The present analysis evaluates risk factors and outcomes associated with a syphilis diagnosis in this cohort of HIV-infected women and their infants.

Results: Approximately 10% (n=171) of women enrolled had serological evidence of syphilis without adequate treatment documented and 1.4% (n=24) infants were dually HIV and syphilis infected. Multivariate logistic analysis showed that compared to HIV-infected women, co-infected women were significantly more likely to self-identify as non-white (AOR 2.5, 95% CI 1.5-4.2), to consume alcohol during pregnancy (AOR 1.5, 95% CI 1.1-2.1) and to transmit HIV to their infants (AOR 2.1, 95% CI 1.3-3.4), with 88% of HIV infections being acquired in-utero. As compared to HIV infected or HIV exposed infants, co-infected infants were significantly more likely to be born to mothers with VDRL titers $\geq$1:16 (AOR 3, 95% CI 1.1-8.2) and higher viral loads (AOR 1.5 95% CI 1.1-1.9). Of 6 newborns with symptomatic syphilis, 2 expired shortly after birth, and 2 were HIV-infected.

Conclusion: Syphilis continues to be a common co-infection in HIV-infected women and can facilitate *in utero* transmission of HIV to infants. Most infants are asymptomatic at birth, but those with symptoms have high mortality rates.

Congenital syphilis is a significant public health problem, affecting an estimated one million pregnancies per year throughout the world.[1] Pregnant women diagnosed with early stages of syphilis are at the highest risk for vertically transmitting syphilis to infants: 60-90% of pregnant women with untreated primary or secondary syphilis will transmit syphilis to their fetus as compared to less than 10% of women with late latent syphilis.[2] If not fatal, the bones, liver, pancreas, intestine, and kidneys of the fetus can be severely affected, and overt infection can manifest in the fetus, the newborn, or later in childhood.[3] The diagnosis of congenital syphilis is complicated by the fact that more than two-thirds of neonates are asymptomatic at birth and treatment delay can lead to permanent damage and disability.[4] Because of this, congenital syphilis is diagnosed presumptively based on the mother's reactive non-treponemal titer, reactive treponemal (confirmatory) testing and lack of adequate treatment with a penicillin regimen more than 4 weeks prior to delivery as defined by the Center for Disease Control Sexually Transmitted Diseases (CDC STD) guidelines.[5]

Congenital syphilis often occurs in areas with high HIV prevalence.[6] Identifying potential risk factors and associated outcomes in women and infants infected with HIV and syphilis would benefit global efforts to eliminate both diseases in infants.[7,8] The NICHD /HPTN 040 (P1043) was a study designed to prevent *intrapartum* HIV transmission to infants born to untreated HIV-infected women.[9] Formula-fed live infants born to 1664 mothers diagnosed with HIV during the time of labor and delivery were randomly assigned to one of three antiviral regimens. The study results demonstrated that a multi-drug infant regimen is superior to zidovudine alone in preventing primary HIV infection at 3 months in infants uninfected at birth.[9] Prior observational studies have shown that concurrent syphilis infection leads to increased HIV mother-to-child transmission rates for reasons that are not completely understood.[10-12] However, there is a

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paucity of literature regarding perinatal HIV and syphilis dual exposure and early clinical

outcomes in this population. In this analysis, we focused on identifying risk factors associated

with maternal syphilis as well as factors associated with syphilis and HIV co-infection in infants

enrolled in NICHD /HPTN 040 (P1043) in order better clarify the relationship between HIV and

syphilis in this vulnerable population.

MATERIALS AND METHODS:

We performed a secondary analysis of data collected in the NICHD /HPTN 040 (P1043) study[9]

which enrolled HIV-infected mothers who had not received antiretroviral therapy (ART) prior to

labor. We excluded 11 mothers whose confirmatory testing was not consistent with HIV

infection (false-positives). All mothers provided written informed consent. Enrollment occurred

at 17 sites in Brazil, South Africa, Argentina and the United States. The study was approved by

local and collaborating institutional review boards.

At study enrollment, all mothers were interviewed about risk behaviors including illegal

substance use, alcohol use, tobacco use, as well as receipt of prior prenatal care. Maternal HIV

RNA levels, T lymphocyte cell subsets, and Venereal Disease Research Laboratory (VDRL)

titers were obtained at the time of labor/delivery. Confirmatory syphilis testing with Treponema

Pallidum Particle Agglutination Assay (TPPA), Treponema Pallidum Particle Hemagglutination

Assay (TPHA), or Fluorescent Treponemal Antibody-absorption (FTA-ABS) was conducted on

women with positive VDRL results as per local standard of care.   Infant data included birth

weight, gestational age, HIV antiretroviral treatment arm, whether zidovudine (ZDV) was

received during the *intrapartum* period by the mother, and if performed by the site, infant's

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VDRL titer. If infants were admitted for treatment of syphilis, this information was also recorded with a brief summary of their clinical course.

HIV Diagnosis: Infant HIV infection was diagnosed with two positive HIV DNA PCR results in blood specimens collected at birth, 10-14 days, 4-6 weeks, 3 months and 6 months. Infants with positive DNA PCR within 48 hours of birth and confirmatory results on repeat testing were classified as having *in utero* HIV infection. Infants with a negative result at birth and positive results on subsequent testing were classified as acquiring HIV infection through the *intrapartum* route. If the infant expired prior to receiving two virologic tests, the HIV status was listed as *unknown*. Ninety-seven (5%) infants discontinued the study before reaching the 3 month primary endpoint for HIV diagnosis for reasons including death (n=21), withdrawal of consent (n=37), loss to follow up (n=32), and relocation (n=7). While it is impossible to conclusively confirm or exclude HIV infection as these infants lacked confirmatory tests, of the 97 infants with unknown HIV status, 2 had one positive HIV DNA assay before 3 months of age (the study endpoint) and 95 had one negative viral test also before 3 months of age. All infants were exclusively formula-fed to avoid post-partum transmission.   These 97 infants were censored from our analysis in Table 2.   All laboratory assays were performed by quality assured laboratories supported by the NICHD and the International Maternal Pediatric Adolescent AIDS Clinical Trials group.

Syphilis Diagnosis: For this analysis, current maternal infection with syphilis (presumably untreated, i.e., without treatment documentation) was defined as any reactive VDRL result with a reactive confirmatory test, or a reactive VDRL titer without a documented confirmatory test. Those with non-reactive confirmatory tests were considered to have false positive VDRL results

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and were excluded from further analysis. Congenital syphilis was considered in infants born to mothers who were diagnosed with current syphilis at the time of delivery or after birth without any evidence of treatment more than 4 weeks prior to delivery, per CDC guidelines scenario 2.[5] Confirmed cases included infants who were diagnosed with syphilis based on abnormal physical exam, or a serum quantitative non-treponemal serologic titer that was fourfold higher than the mother's titer (CDC guidelines scenario 1).

In this secondary analysis, we employed a slightly different definition of syphilis than in the primary parent study in order to more closely follow CDC guidelines. In the initial published study, in the absence of confirmatory treponemal testing, higher maternal VDRL titers of $\geq$1:16 (n=19) were required for a case definition of congenital syphilis. However, since syphilis transmission can occur even with low maternal VDRL titers, we expanded the case definition to include all cases of untreated syphilis irrespective of the VDRL titers and added 20 extra case mothers to study cohort. Therefore, our final cohort in this analysis included 132 mothers with positive confirmatory testing for syphilis and 39 with presumed syphilis, but without confirmatory testing.

Statistical Analysis: Two-sample T-test and Pearson chi-square statistics were used to analyze continuous and categorical outcomes, respectively. Univariate and multivariate logistic regression was performed to assess predictors associated with positive syphilis results in mothers. Variables included in the multivariate model were age, ethnicity, tobacco use, illegal drug use, alcohol use, continent of origin, presence of prenatal care, CD4 T lymphocyte count and plasma $\log_{10}$ HIV RNA levels. Univariate and multivariate logistic regression was performed to assess predictors associated with co-infection with HIV and congenital syphilis in infants.

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Covariates with a P value of less than 0.10 were included in the multivariate model comprised of birth weight, maternal VDRL titers, maternal HIV RNA $\log_{10}$ viral load and whether patient was from South America or South Africa. All computations were done using STATA version 11.1. (Cary, NC).

RESULTS:

We identified 182 pregnant women with reactive VDRL results among the 1664 newly diagnosed HIV-infected women enrolled in the study (Figure 1). Among these women, 132 had a reactive confirmatory treponemal test and 39 had no recorded confirmatory test result, totaling 171 women who were presumably co-infected with syphilis and HIV. Eleven women with a reactive VDRL had nonreactive confirmatory tests, and were presumed to have false positive VDRL titers (VDRL false positive rate of 8%). Of the 171 women with presumed untreated syphilis (serological evidence of infection without treatment documentation), 123 were from Brazilian sites, 46 were South African, and 2 were from Argentina. Prevalence rates of syphilis in this cohort of newly diagnosed HIV positive women ranged from 6% in Johannesburg to more than 17% in Cape Town, South Africa and Belo Horizonte, Brazil. (Figure, Supplemental Digital Content 1, http://links.lww.com/INF/C31). A total of 144 women had documented referral for syphilis treatment in the postpartum period. The most common recorded reason for no treatment referral was past treatment (n=12), although none reported treatment during current gestation. Other common reasons for no treatment included low VDRL titer (n=6) and repeat negative results (n=5). However, we did not have documentation of negative result results and so these 5 individuals were included in our subsequent analyses.

Comparing the 171 HIV-infected women with serological evidence for syphilis to those who had

negative serological testing (n=1439), those who were co-infected with syphilis and HIV were

significantly more likely to be non-white (AOR 2.5, 95% CI 1.5-4.2), and to report drinking

alcohol during pregnancy (AOR 1.5, 95% CI 1.1-2.3) (Table 1). There was a slight yet

statistically significant difference in age between the two groups, with co-infected women having

a mean age of 28 years (range of 15-44 years) as compared to a mean age of 27 years (range of

13-47 years) in women only infected with HIV. We did not see a significant difference in the

amount of prenatal care between the two groups in our adjusted model, with a median of 4 visits

in both groups. In this subanalysis, mothers co-infected with HIV and syphilis had twice the

odds (AOR 2.1, 95% CI 1.3-2.4) of transmitting HIV infection to their babies. Of the 1684

infants born to 1664 mothers enrolled in the NICHD/HPTN 040 (P1043) trial, 24 (1.4%) were

co-infected with HIV and syphilis, and of these infants, 21 (88%) acquired HIV infection in

utero. Among the 116 infants infected with HIV whose mothers did not have serological

evidence of untreated syphilis infection, 62% of them acquired HIV in-utero. (Table 1)

Table 2 shows the results comparing infants who were co-infected with both HIV and congenital

syphilis with the remaining HIV exposed/uninfected infants and HIV infected infants. Our

multivariate logistic regression model showed that co-infected infants were more likely born to

mothers with VDRL titers ≥1:16 (AOR 3, 95% CI 1.1-8.2), higher maternal viral loads (AOR

1.5, 95% CI 1.1-19) and were also significantly more likely to have lower birth weights,

although this difference was not significant in the multivariate analysis. Exploratory analysis

was done comparing co-infected babies to infants infected only with syphilis or HIV, and

similarly revealed lower birth weight in co-infected babies, which was no longer significant in

the multivariate model (data not shown).

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Of these 173 infants born to 171 HIV-infected mothers with serologic evidence of untreated syphilis infection, 120 infants were recorded as being hospitalized to receive treatment for congenital syphilis. Six infants had physical examinations suggestive of "confirmed" congenital syphilis disease per CDC guidelines scenario. (Table, Supplemental Digital Content 2, http://links.lww.com/INF/C32) Two of the patients died prior to HIV diagnostic testing and two acquired HIV *in utero*. One infant had a rash on the extremities presumed to be secondary to syphilis although the mother's non-treponemal titer was initially non-reactive, which is suggestive of previously treated infection. However, this infant's VDRL result was positive at 1:4 and mother's subsequent testing performed within 10 days after giving birth was similarly positive. None of the infants had VDRL titers four-fold or greater than their mother's titers. All other infants who were treated for syphilis were asymptomatic.

DISCUSSION:

Inadequately treated *T. pallidum* infection can result in transplacental infection of the fetus, causing perinatal death, prematurity, low birth weight, congenital anomalies and long term sequelae including deafness and neurological impairment.[2,13-15] Since most infants are asymptomatic at birth, all infants born to untreated infected mothers should be presumptively evaluated for congenital syphilis by cerebral spinal fluid analysis for VDRL titers, cell count, and protein, a blood sample for a complete blood count with differential, and platelet count, and long-bone radiographs. Treatment with penicillin for up to 10 days should be considered.[5] In the present analysis of the NICHD /HPTN 040 (P1043) cohort which included 1664 mothers diagnosed with HIV during the time of labor and delivery, 171 (10%) mothers were noted to have serological results suggestive of untreated syphilis infection. This is consistent with a

review suggesting that 9.5% of all HIV-infected adults are co-infected with syphilis.[16] Prior studies show that inadequate prenatal care appears to be the biggest contributor to delivering an infant with congenital syphilis, but the magnitude of VDRL titers,[17-20] gestational week of syphilis treatment,[21] low maternal socioeconomic status,[22] multiple sexual partners[22] and maternal drug use[18] could all contribute to this outcome. Our results are unique in that they focus specifically on an HIV infected population, but are similar to the above studies showing that alcohol use during pregnancy and non-white ethnicity are associated with HIV infected mothers being co-infected with syphilis. In both Brazil and South Africa, non-white ethnicity may act as a marker for social inequities and poor access to medical care as opposed to a biological "predisposition" to disease.[23] In our univariate analysis, the lack of prenatal care and illegal substance use were also associated with active syphilis infection. As these women had been diagnosed late in pregnancy with HIV infection and 38% had no prenatal care, they represent a highly vulnerable sub-population at great risk for poor outcomes. Even those women who received prenatal care (with a median of 4 prenatal visits in both infected and non-infected cohorts) were not diagnosed and treated for HIV and syphilis until enrollment into this study, which is an issue of great concern as diagnosis and treatment for syphilis is widely available and should be immediately offered during pregnancy. Of note, others have shown that a higher number of prenatal care visits corresponds with lower rates of congenital syphilis, but we did not see this trend in our analysis.[18] Widespread use of rapid tests to accurately diagnose these diseases combined with improved infrastructure and training of medical staff may help ameliorate this significant deficiency in health care systems.

Infants born to mothers infected with both untreated HIV and syphilis were more likely to have acquired HIV infection *in utero*, likely secondary to placental inflammation caused by syphilis

infection.[24]   Furthermore, syphilis, like many other acute infections, can increase HIV viral load

and decrease CD4+ T lymphocyte cells, although we did not note this association in our study

population.[25]   In general, syphilis has been estimated to increase sexual HIV transmission rates

2- to 9-fold and HIV acquisition 2- to 4-fold,[26, 27] consistent with our results. For this analysis,

we used a broader definition of maternal syphilis infection as supplied by the CDC STD

guidelines and included all mothers who had positive VDRL titers even if they did not have

confirmatory testing performed, increasing the original syphilis cohort by 20 subjects.   Using

this definition, we did see a two-fold risk (AOR 2.1, 95% CI 1.3-2.4) of HIV acquisition in

infants exposed to syphilis. Moreover, as VDRL titers are used to track disease activity, with

elevated titers being associated with more severe infection, it is predictable that elevated VDRL

titers would signal increased syphilitic disease activity and inflammation, thus augmenting the

risk of HIV acquisition by infants. All other parameters evaluated in the present analysis

including maternal zidovudine administration during delivery and infant and antiretroviral

treatment arm had no effect in preventing co-infection. This is consistent with our study design

since >85% of co-infected patients had acquired HIV *in utero*, prior to any intrapartum

interventions.

 Although we do not have follow up data on all infants including serial VDRL titers and physical

exams, as this was not the main outcome of the parent study, we did notice that most infants

(>95%) were asymptomatic of disease at birth and none had 4-fold or higher titers than their

mothers. One case report suggests that HIV-exposed uninfected infants may have negative

serological test for syphilis because of immunological dysregulation secondary to exposure to

HIV antigens.[28] We also noted that one infant was diagnosed with congenital syphilis based on

physical examination (Table, Supplemental Digital Content 2, http://links.lww.com/INF/C32,

Patient 6); although mother's initial VDRL test was nonreactive. This false negative result could potentially represent a new syphilis infection where delivery occurred prior to mother developing antibodies as mother's repeat VDRL titer performed shortly after birth was 1:4. Furthermore, there have been reports of false negative serological tests in HIV-infected individuals secondary to immune dysregulation.[29-31] It is significant to note that of our six infants with symptomatic syphilitic disease, two died shortly after birth.

Our study's strength is that it comprises one of the only analyses looking at a large cohort of HIV-infected mothers and their HIV exposed/infected infants with high rates of untreated maternal syphilis because the women had not accessed prenatal care. One main study limitation is that the primary focus of the parent study was not syphilis outcomes, thus we do not have follow up syphilis evaluations for all infants unless they were hospitalized. Another potential weakness is that we may have included infants who were not infected with syphilis among those with probable congenital syphilis because the widely accepted case definition is solely based on maternal history of diagnosis, a common problem with most studies evaluating early congenital syphilis.[32] We included 39 women who had positive VDRL results and no confirmatory testing. However, we performed an exploratory analysis removing all 39 individuals with positive VDRL results who did have any confirmatory testing from our cohort. Despite this exclusion, maternal syphilis infection continued to be significantly associated with mother-to-child HIV transmission (OR 2.2, 95CI 1.3-3.71).

In conclusion, this study demonstrates that co-infected infants with HIV and symptomatic syphilis face high rates of mortality. All pregnant women, especially those diagnosed with HIV, should be immediately screened for syphilis, and if co-infected, should receive appropriate therapy to avoid adverse outcomes including *in utero* HIV infection of the infant and fetal/

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neonatal death. Infants born to HIV and syphilis co-infected mothers should receive full

evaluation for syphilis and appropriate antibiotic management to avoid complications associated

with untreated infection including skeletal deformities, developmental delay, and organ

damage.[31]

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Legend for Figure 1

Figure 1: Algorithm describing HIV infected mothers with positive syphilis serology. Boxes shaded in grey were not included in the subanalysis. (VDRL: Venereal Disease Research Laboratory, HIV: Human Immunodeficiency Virus)

Figure Supplemental Digital Content Legend

Figure Supplemental Digital Content: Prevalence of maternal syphilis with no documentation of treatment among HIV positive pregnant women enrolled into NICHD/HPTN 040 at the time of labor and delivery by city in the countries of South Africa, Brazil, and Argentina. There were no cases of syphilis in patients enrolled in the United States.

Table 1: Factors associated with confirmed or presumed syphilis in HIV infected mothers and HIV transmission to infant

	Syphilis N=171	No syphilis N=1493	Odds ratio (95% CI)	Adjusted Odds ratio (95% CI)[a]
Age (mean, SD)	28 (±6.3)	27 (±6.3)	1.03 (1-1.1)*	1.03 (1-1.06)*
Ethnicity (non-white)	153 (89%)	1166 (78%)	2.4 (1.4-3.9)*	2.5 (1.5-4.2)*
Illegal substance use (yes)	24 (14%)	123 (8%)	1.8 (1.1-2.9)*	1.5 (0.88-2.7)
Alcohol use (yes)	79 (47%)	511 (35%)	1.6 (1.2-2.3)*	1.5 (1.1-2.1)*
Tobacco use (yes)	61 (36%)	472 (32%)	1.2 (0.87-1.7)	1 (0.67-1.5)
CD4 count (cells/mm^3, SD)	506 (±300)	516 (±310)	1	1
Log$_{10}$ viral load (copies/ml, SD)	9.61 (±2)	9.5 (±1.9)	1.04 (0.96-1.1)	1.01 (0.93-1.1)
Gestational age at delivery (mean, SD)	38.5 (±1.7)	38.6 (±1.7)	0.96 (0.87-1)	1
No prenatal Care	76 (45%)	554 (37%)	1.4 (1-2)*	1.5 (0.9-2.12)
Region- (South Africa)	46 (27%)	420 (28%)	0.9 (0.63-1.3)	0.68 (0.44-1)
HIV transmitted to infant	24 (14%)	116 (8%)	2 (1.2-3.2)*	2.1 (1.3-3.4)*
HIV transmitted in utero n (% of total HIV cases)	21 (88%)	72 (62%)	4.3 (1.2-15.2)*	

[a] Adjusted for age, ethnicity, tobacco use, illegal drug use, alcohol use, continent of origin, presence of prenatal care, CD4 count and viral load
*P<0.05
SD: Standard Deviation, HIV: Human Immunodeficiency Virus

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Table 2: Risk factors associated with infant syphilis and HIV co-infection				
	Co-infected infants with HIV & syphilis (n=24)	Infants not co-infected (n=1660)	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)[a]
Birth weight (mean, SD)	2.735 kg (±430g)	2.991 kg (±522g)	0.99 (0.99-.1)*	1 (0.998-1)
Gestational wk (mean, SD)	38.3 (±2)	38.6 (±1.7)	0.88 (0.71-1.1)	
Maternal VDRL Titers[#]			3.7 (1.5-9.1)*	3 (1.1-8.2)*
1:1-1:8	12 (52%)	117 (80%)		
≥1:16	11 (48%)	29 (20%)		
Maternal $\log_{10}$ viral load (copies/ml, SD)	10.8 (±1.6)	9.5 (±2)	1.5 (1.2-1.9)*	1.5 (1.1-1.9)*
HIV Treatment arm			1 (0.44-2.4)	
Zidovudine only	8 (33%)	557 (34 %)		
Two and three drug group	16 (67%)	1103 (66%)		
Delivery type-vaginal	11 (46%)	1073 (65%)	0.45 (0.2-1)	
No use of zidovudine during delivery	12 (50%)	980 (59%)	0.69 (0.3-1.5)	
Region- Born in South Africa	3 (13%)	472 (28%)	0.36 (0.11-1.2)	0.3 (0.07-1.1)
Mothers not referred for syphilis treatment during L&D admission	2 (8%)	25 (17%)	.44 (0.1-2)	

[#]3 unknown titer levels
*P value <0.05
[a] Adjusted for birthweight, maternal VDRL titers, maternal $\log_{10}$ viral load, region
SD: Standard Deviation, HIV Human Immunodeficiency Virus, VDRL: Venereal Disease Research Laboratory, L&D: Labor and Delivery
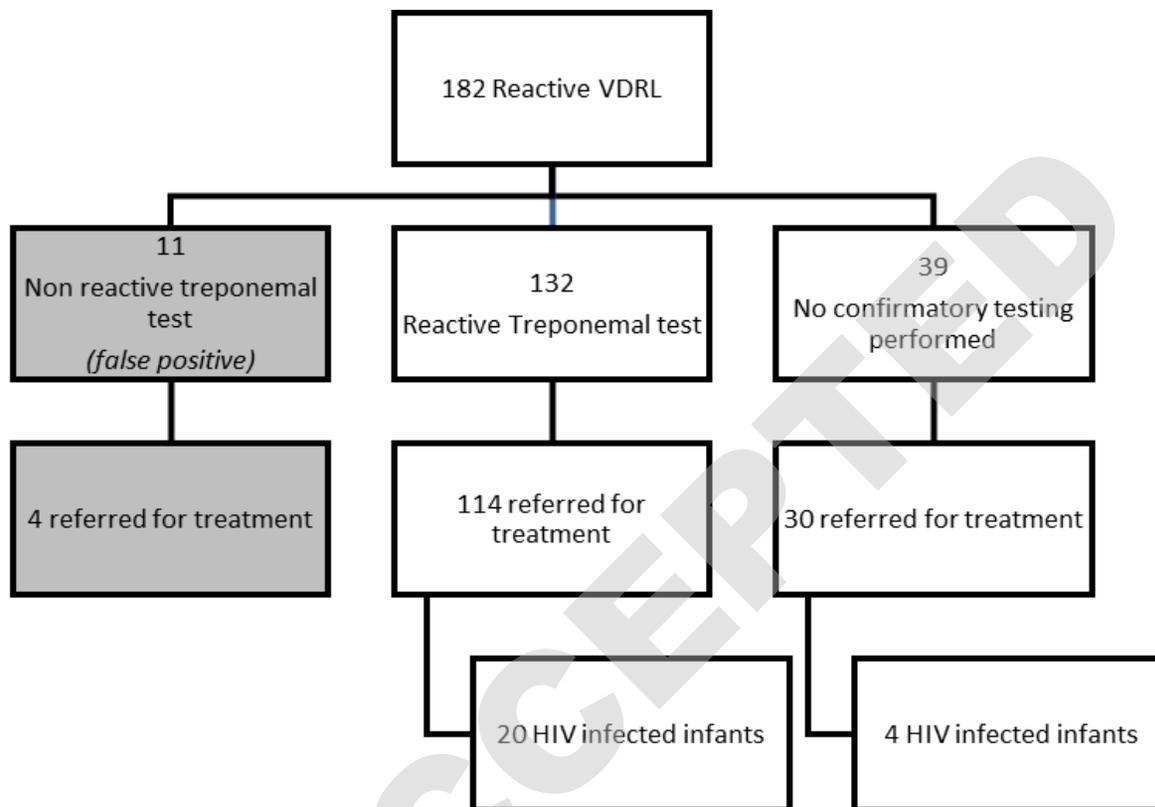
Figure 1: Algorithm describing HIV infected mothers with positive syphilis serology. Boxes shaded in grey were not included in the subanalysis. (VDRL: Venereal Disease Research Laboratory, HIV: Human Immunodeficiency Virus)
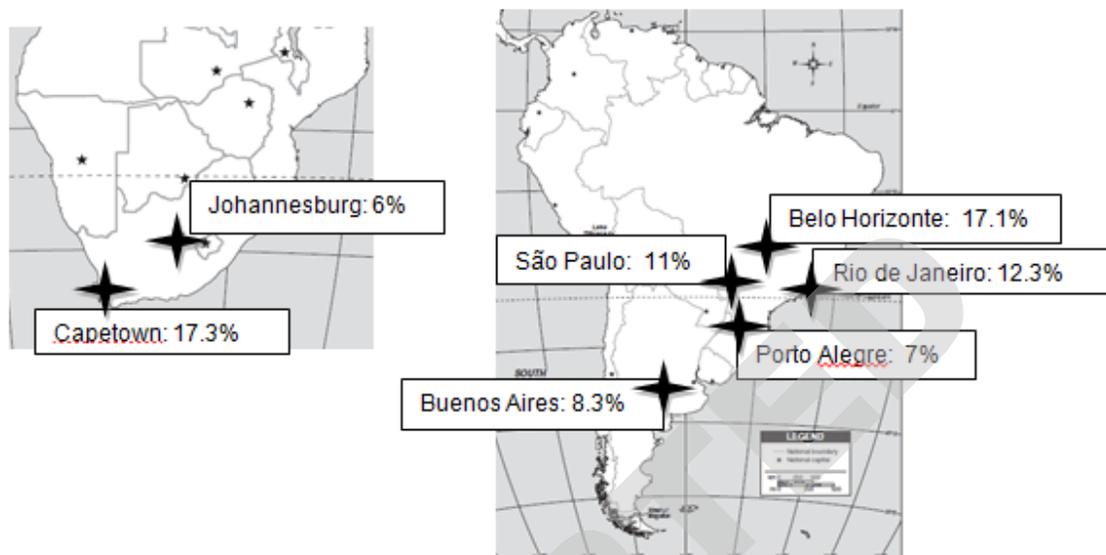
Figure (Supplemental digital content 1): Prevalence of maternal syphilis with no documentation of treatment among HIV positive pregnant women enrolled into NICHD/HPTN 040 at the time of labor and delivery by city in the countries of South Africa, Brazil, and Argentina. There were no cases of syphilis in patients enrolled in the United States.

Table in Supplemental Digital Content: Description of infants symptomatic of syphilis infection shortly after birth							
Case	Mother's VDRL	Mother's Confirmatory testing	Country	Infant's titer	Symptoms	Death	HIV Status
1	1:256	Reactive	Brazil	1:256	Premature, low birth weight, pneumonia cardiorespiratory arrest	Yes	Unknown
2	1:256	Reactive	South Africa	No titer	Hepatomegaly, sudden death	Yes	Unknown
3	1:64	Reactive	Brazil	1:16	Thrombocytopenia, hepatomegaly	No	Infected in utero
4	Reactive, no titer performed	Not performed	Brazil	No titer performed	Elevated transaminases, thrombocytopenia, jaundice, sepsis	No	Infected in utero
5	1:1024	Reactive	Brazil	1:64	Fetal distress, neonatal jaundice, anemia	No	Uninfected
6	Nonreactive	Reactive	Brazil	1:4	Rash on extremities	No	Uninfected