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Congenital CMV and HIV Perinatal Transmission

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ABSTRACT

Background: Congenital cytomegalovirus (CMV) infection (cCMV) is an important cause of hearing loss and cognitive impairment. Prior studies suggest that HIV-exposed children are at higher risk of acquiring cCMV. We assessed the presence, magnitude, and risk factors associated with cCMV among infants born to HIV-infected women, who were not receiving antiretrovirals during pregnancy.

Methods: cCMV and urinary CMV load were determined in a cohort of infants born to HIV-infected women not receiving antiretrovirals during pregnancy. Neonatal urines obtained at birth were tested for CMV DNA by qualitative and reflex quantitative real-time PCR.

Results: Urine specimens were available for 992 (58.9%) of 1684 infants; 64 (6.5%) were CMV-positive. Mean CMV load (VL) was 470,276 copies/ml (range: <200-2,000,000 copies/ml). Among 89 HIV-infected infants, 16 (18%) had cCMV versus 42 (4.9%) of 858 HIV-exposed, uninfected infants (*p* <0.0001). cCMV was present in 23.2% of infants with *in utero* and 9.1% infants with intrapartum HIV infection (*p* <0.0001). Rates of cCMV among HIV-infected infants were four-fold greater (aOR 4.4, 95% CI 2.3-8.2) and six-fold greater among HIV *in utero*-infected infants (aOR 6, 95% CI 3-12.1) compared with HIV-exposed, uninfected infants. cCMV was not associated with mode of delivery, gestational age, Apgar scores, six-month infant mortality, maternal age, race/ethnicity, HIV viral load, or CD4 count. Primary cCMV risk factors included infant HIV-infection, particularly *in utero* infection.

Conclusion*:* High rates of cCMV with high urinary CMV VL were observed in HIV-exposed infants. *In utero* HIV-infection appears to be a major risk factor for cCMV in infants whose mothers have not received combination antiretroviral therapy in pregnancy.

INTRODUCTION

Cytomegalovirus (CMV) is a significant cause of congenital infections worldwide. In the U.S. and other industrialized countries, congenital CMV affects ≤1% of all newborns and accounts for over 40,000 neonatal infections per year.[1, 2] In resource-limited countries, where maternal CMV seropositivity rates are higher and there have been limited data on congenital CMV infection (cCMV) rates, the problem of cCMV may be even more widespread.[1,3,4] cCMV's importance and potential clinical sequelae should not be underestimated. Approximately 10-15% of congenitally-infected infants have symptomatic disease that may progress to severe neurodevelopmental delays and sensorineural hearing loss.[1,5-7] Furthermore, approximately 5-17% of asymptomatic congenitally-infected infants may also develop clinical findings with later disease progression.[8-10]

Earlier studies have indicated that cCMV may be more common among HIV-exposed infants (2-7%) and among HIV-infected infants (4-26%).[11-15] These findings have particular relevance for HIV-infected infants, as they are more likely to have symptomatic cCMV, and cCMV may accelerate HIV disease progression.[11, 12, 14, 16, 17]

To investigate these issues further, we performed a study using data and specimens from the National Institute of Child Health and Human Development (NICHD) HIV Prevention Trials Network (HPTN) 040 perinatal clinical trial cohort of HIV-exposed infants. The primary objective of this sub-study was to determine the rate of CMV co-infection among HIV-exposed infants. Additional secondary objectives included comparing rates of cCMV among HIV-exposed, uninfected and HIV-infected infants (HIV acquired either *in utero* or *intrapartum*); determining predictors of cCMV; and evaluating mortality rates among infants with cCMV.

METHODS

Study Design This was a sub-study of the NICHD HPTN 040 trial, also known as the International Maternal

Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT P1043) NICHD/HPTN 040 (or P1043), a phase 3, randomized, open-label, multi-center study that evaluated the efficacy, safety, and tolerance of three different infant antiretroviral regimens for the prevention of intrapartum HIV transmission to infants born to HIV-infected pregnant women, who had not received antiretroviral drugs during pregnancy.[18] The study enrolled 1684 HIV-infected pregnant women diagnosed with HIV infection at the time of labor and delivery. All women provided written informed consent. Enrollment occurred at multiple sites in Brazil, South Africa, Argentina, and the United States. Infants <32 weeks of gestational age were excluded from the study. Maternal plasma HIV RNA levels and CD4+ T-lymphocyte subsets were obtained at the time of labor and delivery. The primary endpoint of the parent study was HIV infection status at 3 months of age. Infants were followed until 6 months of age for safety and toxicity monitoring in the parent study.

HIV Diagnosis HIV DNA polymerase chain reaction (PCR) (Roche Molecular Systems Inc., Basel, Switzerland) was performed on infant blood specimens within 48 hours of birth and at 10-14 days, 4-6 weeks, 3 months, and 6 months of age. Repeat HIV DNA PCR testing was performed to confirm a positive result. Diagnosis of infant HIV infection required two positive HIV DNA PCR test results on separate specimens. Infants with a positive HIV DNA PCR test result at birth and positive results on repeat testing were classified as having *in utero* HIV infection. Infants with a negative HIV DNA PCR result at birth and a positive HIV DNA PCR result on subsequent testing were classified as having *intrapartum* HIV infection. All HIV-exposed infants, who were enrolled in the study, were exclusively formula fed.

Specimen Collection and CMV Testing The presence of cCMV was evaluated in NICHD HPTN 040's population of HIV-exposed infants. cCMV and magnitude of urinary viral load was determined in HIV-uninfected, HIV-*in utero* infected, and HIV-*intrapartum* infected infants. Stored neonatal urine samples were collected within 48 hours of birth and frozen at – 80^{o}C and stored at study sites. Infant urines were tested by qualitative Real-Time PCR for CMV DNA (FOCUS Diagnostics CMV Analyte Specific Reagent) with quantification of positive specimens. Infants with detectable CMV in the urine in the first 48 hours of life were diagnosed with cCMV.

Statistical Analysis Chi-square or Fisher's exact tests (when more than 25% of expected cell frequencies were less than five) was used to compare differences in proportions between cCMV and CMV-non-infected infants.

Univariate and multivariable logistic regression analysis was used to examine the relationship of cCMV and HIV infection, demographic/ geographic parameters, maternal characteristics, and infant mortality. Covariates with a p-value of less than 0.15 from univariate models were included in the initial multivariable model selection. All computations were done using SAS software v9.3 (Cary, NC, USA).

Human Subjects The study was approved by the institutional review boards and national ethics committees at each of the participating study sites.

RESULTS

Rates of Congenital CMV Infection among HIV-exposed and HIV-infected Infants

Urine specimens were available for 992 (58.9%) of the 1684 infants in the original study. Of these infants, 64 (6.5%) were found to have urines with detectable CMV, with a mean virus load of 470,276 copies/ml (range: <200-2,000,000 copies/ml). Eleven (17.2%) infants with cCMV had results that were too elevated to quantify (>2,000,000 copies/ml), whereas approximately 8 (12.5%) had positive but low levels of detectable CMV (<200 copies/ml). The mean CMV urine virus load was higher for HIV-infected infants (697,698 copies/ml) than that of HIV-uninfected infants (448,897 copies/ml), but these differences were not significant.

Among the 992 infants, 89 (9%) were HIV-infected, 858 (86.6%) were HIV-uninfected, and 45 (4.5%) had unknown HIV status because of loss to follow-up or death before 3 months of age (the HIV diagnostic study endpoint). (Table 1) The rates of cCMV were significantly different when evaluated by infant HIV-status ($p < 0.0001$). The cCMV rate among HIV-infected infants was 18% (n=16 of 89 infants) compared to only 4.9% (n=42 of 858 infants) among HIV-exposed, uninfected infants and 13.3% (n=6 of 45) of infants with unknown HIV status. (Table 1) The difference was especially pronounced among HIV *in-utero*-infected infants, where 23.2% (n=13 of 56) had cCMV as opposed to 9.1% (n=3 of 33) of HIV *intrapartum*-infected infants. Compared with HIV-exposed uninfected infants, the rate of cCMV in HIV-infected infants was more than four-fold greater (OR 4.3, 95% CI 2.3-7.9) with multivariate logistic regression showing similar results aOR=4.4 (95% CI 2.3-8.2) after adjusting for study site country and maternal/race ethnicity, and was almost 6-fold greater among HIV *in-utero*-infected infants (OR 5.9, 95% CI 2.9-11.8), which was also similar in the adjusted analysis (aOR 6, 95% CI 3-12.1) after adjusting for study site country and maternal race/ethnicity. (Table 1, 2)

Deaths among infants ≤ 6 months of age occurred in 21 (2.1%) infants in the cohort with 2 deaths among 64 infants (3.1%) occurring in infants with cCMV. (Table 1) Infant mortality was not associated with cCMV. However, further evaluation revealed that HIV-exposed infants who died or were lost-to-follow-up before the 3-month HIV diagnostic endpoint also appeared to be at increased risk of cCMV (OR 2.25, 95% CI 0.98-5.20), although this was marginally significant (p=0.057).

Other Risk Factors for Congenital CMV Infection

Apart from differences noted in rates of cCMV by infant HIV status and infant HIV mode of acquisition, cCMV rates also differed by geographical location of our study sites. (Table 1) Rates of cCMV ranged from 2-16.7% from top-enrolling sites in the Americas (primarily Brazil but also the US) but were only 2.1-2.5% in South Africa. Compared to infants in South Africa, HIV-exposed infants in the U.S. (aOR 15.6, 95% CI 1.3-193) and Brazil (aOR 5.1, 95% CI 1.4-18.8) had the greatest risk of acquiring cCMV, although it should be noted that the number of US infants was small (N= 7). In contrast, cCMV was not associated with other demographic and obstetric parameters including maternal age, race/ethnicity, mode of delivery, gestational age at delivery, or infant Apgar scores. Furthermore, maternal HIV viral load and maternal CD4 count were also not significantly associated with an increased risk of cCMV. (Table 1)

DISCUSSION

This study found high rates of congenital CMV infection (cCMV) in HIV-exposed infants, whose mothers were not receiving antiretroviral drugs in pregnancy due to late detection of HIV status resulting from delayed presentation to care. The rates of cCMV were highest among HIV-infected infants, particularly among those with *in utero* acquired HIV infection.

While CMV is among the most common etiologies of congenital infection worldwide, limited studies of cCMV have been reported from low and middle-income countries, particularly from HIV-infected pregnant women living in Latin America (Brazil) and sub-Saharan Africa (South Africa).[1-4, 13, 19-23] From studies of healthy pregnant women residing in industrialized nations,[24] we know that cCMV has been estimated to affect less than 1% of all newborn infants;[1, 2]

whereas, studies from Brazil and sub-Saharan Africa have generally suggested higher cCMV rates between 1.1-2.9%[3, 4, 13] and 1.4-14%,[2, 19-23] respectively, in spite of documented high maternal CMV seroprevalence.

Our study provides further evidence demonstrating the extent to which cCMV rates may be elevated among HIV-exposed infants and HIV-infected infants. In our study, cCMV rates for HIV-exposed uninfected infants (4.9%) and HIV-infected infants (18%), particularly for infants that acquired HIV *in utero* (23%), were more than 4 to 23 times higher than cCMV rates reported among healthy infants in high-income countries (typically ≤1%).[1, 2] *In utero* HIV infection was the strongest predictor for cCMV (aOR 6, 95% CI 3-12.1).

Apart from two exceptions in smaller studies,[19, 25] the cCMV rates among HIV-exposed, uninfected infants in our sub-study were higher than those seen in the majority of other published studies ranging from 2.2% to 4.6% among studies of HIV-exposed infants in the U.S., France, South Africa, Brazil, and Thailand.[11, 13-16, 26-30] Among HIV-infected infants, cCMV rates correlated with those reported in other studies, which typically ranged from 4.3% to 21%;[11, 12, 14, 16, 29] but included some studies with rates as low as 0%[13] and others with rates as high as 26-29%.[31, 32]

Our study was designed to clearly discern between HIV *in utero* and *intrapartum* infections, since the primary objective of the parent study was to evaluate the use of postnatal antiretroviral prophylaxis in the prevention of *intrapartum* HIV transmission. Postpartum HIV or CMV transmission by breast milk was not of concern as formula feeding was an entry criterion for study participation. This distinction in the timing of HIV transmission in a large cohort of HIV-exposed infants allowed us to further explore potential associations between different transplacental infections, in this case HIV and CMV, which is a unique feature of our study.

Based on prior epidemiologic studies from Brazil and sub-Saharan Africa, it is presumed that the majority of women in our sub-study were CMV seropositive and demonstrated either CMV reinfection during pregnancy or re-activation given the risk factors of pregnancy-induced maternal immunosuppression, especially in the third trimester, and immunosuppression from undiagnosed and untreated HIV.[26] One potential explanation for the relatively high rates of cCMV in our cohort of HIV-exposed uninfected and HIV-infected infants may be that women were not diagnosed with HIV until the time of labor and delivery and were not on highly active antiretroviral treatment (HAART) during pregnancy. Some studies have suggested that the use of HAART by pregnant HIV-infected women may reduce cCMV rates among their HIV-exposed infants by improving their immune status during pregnancy.[11, 26-28, 31]

The findings of our study also provide additional evidence highlighting the complex inter-relationship "synergism" dynamics of HIV and CMV, suggesting that infection with one of these viruses may be a risk factor for infection with the other.[12, 33, 19, 29, 34] Both CMV and HIV have the ability to infect similar cells;[12, 34-36] *in vitro* studies have demonstrated that both viruses have the capability to stimulate gene expression and viral replication in the other.[12, 14, 33-35, 37-39] Other studies have also found that CMV may facilitate susceptibility to HIV infection by augmenting expression of Fc receptors, enhancing production of cytokines and other cellular products, and activating sentinel players such as T cells and monocytes.[12, 33, 39, 40] As with HIV and CMV, transplacental passage of one pathogen may facilitate passage of other concurrent pathogens, which has also been suggested for HIV and *Treponema pallidum* or even HIV and *Toxoplasma gondii*.[34, 41-45] For instance, in NICHD HPTN 040, we saw a higher rate of congenital syphilis infection overall and also among *in utero* HIV-infected infants.[45]

Although HIV-infection in infants was a risk factor for cCMV, other predictors such as lower maternal CD4 count (particularly < 200 cells/mm^3)[11, 27] and younger maternal age were not associated with an increased risk of cCMV in our study, although there have been a reported associations in other studies.[11] It is possible that younger maternal age (potentially associated with a higher likelihood of CMV primary infection) may not play a significant role in settings like ours, where CMV reactivation or reinfection are likely responsible for CMV transmission. In addition, while prior studies have suggested high rates of infant death with cCMV (nearly 27%), infant mortality was low in our study and cCMV was not associated with an increased risk for infant death.[12, 14, 16, 19]

Limitations

A major strength of our study was the relatively large sample size of HIV-exposed infants evaluated for cCMV compared to other studies, even though it was restricted to mother-infant pairs from our NICHD/HPTN 040 parent study[18] with available infant urines for specimen analysis; some sites were unable to collect urine specimens. Due to the lack of a comparison group of pregnant women without HIV, we could not evaluate cCMV rates in the general population and their risk factors for cCMV. Thus, our findings related to cCMV are only specific to infants born to HIV-infected pregnant women, who were not on HAART during pregnancy because of a late HIV diagnosis. In addition, because this was a secondary analysis, information on perinatal/postnatal CMV infection and symptomatic cCMV in infants with regards to rates of chorioretinitis, brain calcifications, microcephaly, other central nervous system anomalies, or hearing loss were not collected and is beyond the scope of this study.

CONCLUSION

HIV-exposed infants are at significant risk for acquiring cCMV during pregnancy. HIV-infected infants, particularly those who acquired HIV *in-utero*, are at greatest risk for congenital CMV. CMV screening is an important component of a comprehensive evaluation needed for HIV-exposed infants, particularly among those born to women not on antiretrovirals during pregnancy.

References

1. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. *Clinical microbiology reviews*. 2013;26(1):86-102.

2. van der Sande MA, Kaye S, Miles DJ, Waight P et al. Risk factors for and clinical outcome of congenital cytomegalovirus infection in a peri-urban West-African birth cohort. *PloS one*. 2007;2(6):e492.

3. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM et al. Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2009;49(4):522-528.

4. Yamamoto AY, Mussi-Pinhata MM, Cristina P, Pinto G, Moraes Figueiredo LT, Jorge SM. Congenital cytomegalovirus infection in preterm and full-term newborn infants from a population with a high seroprevalence rate. *The Pediatric infectious disease journal*. 2001;20(2):188-192.

5. Barbi M, Binda S, Caroppo S, Primache V. Neonatal screening for congenital cytomegalovirus infection and hearing loss. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*. 2006;35(2):206-209.

6. Boppana SB, Ross SA, Fowler KB. Congenital cytomegalovirus infection: clinical outcome. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2013;57 Suppl 4:S178-181.

7. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Reviews in medical virology*. 2007;17(5):355-363.

8. Demmler GJ. Infectious Diseases Society of America and Centers for Disease Control. Summary of a workshop on surveillance for congenital cytomegalovirus disease. *Rev Infect Dis*. 1991;13(2):315-329.

9. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. *The Pediatric infectious disease journal*. 1992;11(2):93-99.

10. Ross SA, Boppana SB. Congenital cytomegalovirus infection: outcome and diagnosis. *Seminars in pediatric infectious diseases*. 2005;16(1):44-49.

11. Guibert G, Warszawski J, Le Chenadec J, Blanche S, Benmebarek Y, Mandelbrot L, et al. Decreased risk of congenital cytomegalovirus infection in children born to HIV-1-infected mothers in the era of highly active antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2009;48(11):1516-1525.

12. Kovacs A, Schluchter M, Easley K et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. *The New England journal of medicine*. 1999;341(2):77-84.

13. Mussi-Pinhata MM, Yamamoto AY, Figueiredo LT, Cervi MC, Duarte G. Congenital and perinatal cytomegalovirus infection in infants born to mothers infected with human immunodeficiency virus. *The Journal of pediatrics*. 1998;132(2):285-290.

14. Chandwani S, Kaul A, Bebenroth D et al. Cytomegalovirus infection in human immunodeficiency virus type 1-infected children. *The Pediatric infectious disease journal*. 1996;15(4):310-314.

15. Duryea EL, Sanchez PJ, Sheffield JS et al. Maternal human immunodeficiency virus infection and congenital transmission of cytomegalovirus. *The Pediatric infectious disease journal*. 2010;29(10):915-918.

16. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. *The Pediatric infectious disease journal*. 1996;15(12):1102-1106.

17. Ellington SR, Clarke KE, Kourtis AP. Cytomegalovirus Infection in Human Immunodeficiency Virus (HIV)-Exposed and HIV-Infected Infants: A Systematic Review. *The Journal of infectious diseases*. 2016;213(6):891-900.

18. Nielsen-Saines K, Watts DH, Veloso VG et al. Three postpartum antiretroviral regimens to prevent intrapartum HIV infection. *The New England journal of medicine*. 2012;366(25):2368-2379.

19. Mwaanza N, Chilukutu L, Tembo J et al. High Rates of Congenital Cytomegalovirus Infection Linked With Maternal HIV Infection Among Neonatal Admissions at a Large Referral Center in Sub-Saharan Africa. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2013.

20. Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. *Journal of clinical pathology*. 1991;44(5):366-369.

21. Schopfer K, Lauber E, Krech U. Congenital cytomegalovirus infection in newborn infants of mothers infected before pregnancy. *Archives of disease in childhood*. 1978;53(7):536-539.

22. Olusanya BO, Slusher TM, Boppana SB. Prevalence of congenital cytomegalovirus infection in Nigeria: a pilot study. *The Pediatric infectious disease journal*. 2015;34(3):322-324.

23. Kaye S, Miles D, Antoine P et al. Virological and immunological correlates of mother-to-child transmission of cytomegalovirus in The Gambia. *The Journal of infectious diseases*. 2008;197(9):1307-1314.

24. Barbi M, Binda S, Caroppo S et al. Multicity Italian study of congenital cytomegalovirus infection. *The Pediatric infectious disease journal*. 2006;25(2):156-159.

25. Roxby AC, Atkinson C, Asbjornsdottir K et al. Maternal valacyclovir and infant cytomegalovirus acquisition: a randomized controlled trial among HIV-infected women. *PloS one*. 2014;9(2):e87855.

26. Frederick T, Homans J, Spencer L et al. The effect of prenatal highly active antiretroviral therapy on the transmission of congenital and perinatal/early postnatal cytomegalovirus among HIV-infected and HIV-exposed infants. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012;55(6):877-884.

27. Manicklal S, van Niekerk AM, Kroon SM et al. Birth prevalence of congenital cytomegalovirus among infants of HIV-infected women on prenatal antiretroviral prophylaxis in South Africa. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2014;58(10):1467-1472.

28. Gantt S, Leister E, Jacobsen DL et al. Risk of congenital cytomegalovirus infection among HIV-exposed uninfected infants is not decreased by maternal nelfinavir use during pregnancy. *Journal of medical virology*. 2016;88(6):1051-1058.

29. Khamduang W, Jourdain G, Sirirungsi W et al. The interrelated transmission of HIV-1 and cytomegalovirus during gestation and delivery in the offspring of HIV-infected mothers. *J Acquir Immune Defic Syndr*. 2011;58(2):188-192.

30. Reitter A, Buxmann H, Haberl AE et al. Incidence of CMV co-infection in HIV-positive women and their neonates in a tertiary referral centre: a cohort study. *Med Microbiol Immunol*. 2016;205(1):63-71.

31. Marin Gabriel MA, Fernandez Ibieta M, Gonzalez Tome MI et al. [Congenital cytomegalovirus infection in the infants of HIV-infected mothers]. *An Pediatr (Barc)*. 2005;62(1):38-42.

32. Slyker JA, Lohman-Payne BL, John-Stewart GC et al. Acute cytomegalovirus infection in Kenyan HIV-infected infants. *AIDS*. 2009;23(16):2173-2181.

33. McKeating JA, Griffiths PD, Weiss RA. HIV susceptibility conferred to human fibroblasts by cytomegalovirus-induced Fc receptor. *Nature*. 1990;343(6259):659-661.

34. King CC, Ellington SR, Kourtis AP. The role of co-infections in mother-to-child transmission of HIV. *Current HIV research*. 2013;11(1):10-23.

35. Davis MG, Kenney SC, Kamine J, Pagano JS, Huang ES. Immediate-early gene region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. *Proc Natl Acad Sci U S A*. 1987;84(23):8642-8646.

36. Rice GP, Schrier RD, Oldstone MB. Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. *Proc Natl Acad Sci U S A*. 1984;81(19):6134-6138.

37. Skolnik PR, Kosloff BR, Hirsch MS. Bidirectional interactions between human immunodeficiency virus type 1 and cytomegalovirus. *The Journal of infectious diseases*. 1988;157(3):508-514.

38. Ho WZ, Harouse JM, Rando RF, Gonczol E, Srinivasan A, Plotkin SA. Reciprocal enhancement of gene expression and viral replication between human cytomegalovirus and human immunodeficiency virus type 1. *The Journal of general virology*. 1990;71 (Pt 1):97-103.

39. Lathey JL, Spector DH, Spector SA. Human cytomegalovirus-mediated enhancement of human immunodeficiency virus type-1 production in monocyte-derived macrophages. *Virology*. 1994;199(1):98-104.

40. Dudding L, Haskill S, Clark BD, Auron PE, Sporn S, Huang ES. Cytomegalovirus infection stimulates expression of monocyte-associated mediator genes. *J Immunol*. 1989;143(10):3343-3352.

41. Robbins JR, Bakardjiev AI. Pathogens and the placental fortress. *Curr Opin Microbiol*. 2012;15(1):36-43.

42. Spinillo A, Iacobone AD, Calvino IG, Alberi I, Gardella B. The role of the placenta in feto-neonatal infections. *Early Hum Dev*. 2014;90 Suppl 1:S7-9.

43. Delicio AM, Milanez H, Amaral E et al. Mother-to-child transmission of human immunodeficiency virus in aten years period. *Reproductive health*. 2011;8:35.

44. Mitchell CD, Erlich SS, Mastrucci MT, Hutto SC, Parks WP, Scott GB. Congenital toxoplasmosis occurring in infants perinatally infected with human immunodeficiency virus 1. *The Pediatric infectious disease journal*. 1990;9(7):512-518.

45. Yeganeh N, Watts HD, Camarca M et al. Syphilis in HIV-infected mothers and infants: results from the NICHD/HPTN 040 study. *The Pediatric infectious disease journal*. 2015;34(3):e52-57.

Table 1. Demographic, Infant Delivery and HIV Related Characteristics by Infant CMV Status (Congenital CMV Infection)

	Overall N=992 (column %)	**Infant CMV -Infected (+cCMV)** N=64 n (column %)	**Infant CMV -Uninfected (-cCMV)** N=928 n (column %)	**Unadjusted OR (95% CI)**	**p-value**
Maternal Age, years					
Mean [std. dev.]	26.5 [6.2]	26.3 [6.8]	26.5 [6.2]		
Median [min-max]	26 [13-47]	25 [14-4]	26 [13-47]		
13-24	407 (41.0)	31 (48.4)	376 (40.5)	1.14 (0.63-2.04)	0.66
25-29	289 (29.1)	13 (20.3)	276 (29.7)	0.65 (0.32-1.33)	0.24
30 and older	296 (29.8)	20 (31.3)	276 (29.7)	1.00	
Maternal Race/Ethnicity					
Black	419 (42.2)	26 (40.6)	393 (42.4)	1.35 (0.67-2.73)	0.40
Mixed/Mulatto	316 (31.9)	26 (40.6)	290 (31.3)	1.83 (0.90-3.70)	0.09
White/Others	257 (25.9)	12 (18.8)	245 (26.4)	1.00	
Type of delivery					
Cesarean after rupture/timing unknown	130 (13.1)	8 (12.5)	122 (13.2)	0.92 (0.42-2.02)	0.83
Cesarean before rupture	276 (27.8)	17 (26.6)	259 (27.9)	0.92 (0.51-1.66)	0.78
Vaginal	586 (59.1)	39 (60.9)	547 (58.9)	1.00	
Infant Gestational age, weeks					
36 or less	92 (9.3)	9 (14.06)	83 (8.94)	1.67 (0.79-3.49)	0.18
37 or more	900 (90.7)	55 (85.94)	845 (91.06)	1.00	
Apgar score at 5 minutes					
0-3	2 (0.2)	0 (0.00)	2 (0.2)	0.00 (0.00- I)	0.99
4-6	3 (0.3)	0 (0.00)	3 (0.4)	0.00 (0.00- I)	0.99
7-10	915 (99.5)	62 (100.0)	853 (99.4)	1.00	
HIV Infant status					
Unknown	45 (4.5)	6 (9.38)	39 (4.2)	**3.07 (1.23-7.66)**	**0.02**
Positive	**89 (9.0)**	**16 (25.0)**	**73 (7.9)**	**4.26 (2.28-7.94)**	**<.0001**
Negative	858 (86.6)	42 (65.6)	816 (88.0)	1.00	
Infant HIV Mode of Acquisition					
Unknown	45 (4.5)	6 (9.4)	39 (4.2)	**3.07 (1.23-7.66)**	**0.02**

Intrapartum	33 (3.3)	3 (4.7)	30 (3.2)	1.94 (0.57-6.62)	0.29
In-utero	**56 (5.7)**	**13 (20.3)**	**43 (4.6)**	**5.87 (2.94-11.8)**	**<.0001**
Negative	858 (86.6)	42 (65.6)	816 (88.0)	1.00	
Infant Death					
No	971 (97.9)	62 (96.9)	909 (98.0)	0.65 (0.15-2.85)	0.57
Yes	21 (2.1)	2 (3.1)	19 (2.1)	1.00	
Maternal HIV Viral load, copies/mL	----	-----	----	1.29 (0.94-1.71)	0.11
Mean [std. dev.]	63,682.2 [212,837]	59,058.3 [107,208]	64,002.1 [218,294]		
Median [min-max]	14,921 [0-3,055,766]	19,181 [195- 452,741]	14,578 [0-3,055,766]		
>100,000	117 (11.8)	8 (12.5)	109 (11.8)	2.06 (0.42-10.0)	0.37
10,000-99,999	459 (46.4)	36 (56.3)	423 (45.7)	2.38 (0.56-10.2)	0.24
1,000-9,999	310 (31.3)	18 (28.1)	292 (31.6)	1.73 (0.39-7.65)	0.47
400-999	45 (4.6)	0 (0.00)	45 (4.9)	0.00 (0.00- Inf)	0.98
0-399	58 (5.9)	2 (3.1)	56 (6.1)	1.00	
Maternal CD4 counts, cells/μL	----	----	----	1.02 (0.94-1.10)	0.71
Mean [std. dev.]	515.3 [303.07]	528.9 [308.01]	514.3 [302.9]		
Median [min-max]	460 [12-2160]	486 [65-1377]	459 [12-2160]		
<350	335 (34.4)	21 (33.3)	314 (34.5)	0.93 (0.52-1.66)	0.80
350 to 499	208 (21.4)	13 (20.6)	195 (21.4)	0.92 (0.47-1.82)	0.82
500+	431 (44.3)	29 (46.0)	402 (44.1)	1.00	
Study Site					
US	6 (0.6)	1 (1.6)	5 (0.5)	8.80 (0.77- 100)	0.08
Brazil	851 (85.8)	60 (93.8)	791 (85.2)	**3.34 (1.03-10.8)**	**0.04**
South Africa	135 (13.6)	3 (4.7)	132 (14.2)	1.00	

Note: All infants of HIV-infected women who had at least one available infant urine specimen were tested for CMV. N total differences for specific evaluated risk factors for congenital CMV reflect that data may not be available for every mother-infant pair.

Table 2. Adjusted Relationship of Congenital CMV Infection (cCMV) with Potential Risk Factors

	Congenital CMV Infection (cCMV)	
	aOR (95% CI)	**p-value**
Infant HIV status		
Unknown	**5.52 (2.03-15.0)**	**0.001**
HIV positive	**4.37 (2.32-8.22)**	**<.0001**
HIV negative	1.00	
Infant HIV Mode of Acquisition		
Unknown	**5.47 (2.01-14.9)**	**0.001**
Infected intrapartum	1.97 (0.56-6.91)	0.29
Infected in Utero	**5.98 (2.96-12.1)**	**<.0001**
Negative	1.00	
Study Site Country		
US	**15.6 (1.26- 193)**	**0.03**
Brazil	**5.14 (1.40-18.8)**	**0.01**
South Africa	1.00	
Maternal Race/ethnicity		
Black	1.80 (0.86-3.76)	0.12
Mixed/mulatto	1.99 (0.96-4.09)	0.06
White/other	1.00	