

Cytomegalovirus IgG Level and Avidity in Breastfeeding Infants of HIV-Infected Mothers in Malawi

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Cytomegalovirus (CMV) infection is common among infants of HIV-infected mothers in resource-limited settings. We examined the prevalence and timing of infant CMV infection during the first year of life using IgG antibody and avidity among HIV-exposed infants in Malawi and correlated the results with the presence of detectable CMV DNA in the blood. The Breastfeeding, Antiretrovirals and Nutrition (BAN) study randomized 2,369 mothers and their infants to maternal antiretrovirals, infant nevirapine, or neither for 28 weeks of breastfeeding, followed by weaning. Stored plasma specimens were tested for CMV IgG and antibody avidity from a random subset of infants who had been previously tested with blood CMV PCR and had available specimens at birth and at 24 and 48 weeks of age. Ninety-four of 127 infants (74.0%) tested at 24 weeks of age had CMV IgG of low or intermediate avidity, signifying primary CMV infections. An additional 22 infants (17.3%) had IgG of high avidity; 19 of them had CMV DNA detected in their blood, indicating infant infections. Taken together, these results show that the estimated prevalence of CMV infection at 24 weeks was 88.9%. By 48 weeks of age, 81.3% of infants had anti-CMV IgG; most of them (70.9%) had IgG of high avidity. The CMV serology and avidity testing, combined with the PCR results, confirmed a high rate of primary CMV infection by 6 months of life among breastfeeding infants of HIV-infected mothers. The CMV PCR in blood detected most, but not all, infant CMV infections.

Cytomegalovirus (CMV) infection is the most common congenital infection, with an estimated prevalence of >1% in resource-limited settings (1, 2). Approximately 10 to 20% of congenital CMV infections result in permanent deficits, including sensorineural hearing loss, vision loss, and mental and developmental disability (3). The postnatal acquisition of CMV can occur through horizontal transmission and through breastfeeding, with a high probability of transmission in infants fed with breast milk containing infectious virus (4, 5). The infants of HIV-infected mothers may have higher rates of congenital CMV infection, particularly if the mothers are immunocompromised (6). While postnatal transmission is thought to be generally benign in healthy full-term infants, premature infants or other groups of immunocompromised infants are at risk for more extensive disease and may also experience long-term cognitive delays (7–9).

While the increased morbidity and mortality among infants coinfecting with HIV and CMV is well recognized (10–14), HIV-exposed but uninfected infants may also be at increased risk for morbidity and mortality from CMV infection (15–17). A study in Zambia found that HIV-exposed infants who were CMV seropositive had decreased length for age, reduced head size, and lower psychomotor development than those who were CMV uninfected (5). HIV is endemic in many African countries and the CMV childhood infection burden is high, which may have a substantial impact on child health in the region. It is therefore important to examine when infants acquire primary CMV infection in such settings.

In a previous analysis, we reported, using blood CMV PCR, that >70% of infants of HIV-infected mothers had acquired CMV infection by 24 weeks of age (18). Given that blood is not the optimal sample source for CMV detection, it is possible that our findings underestimated the true incidence of CMV acquisition in the infants. We thus additionally performed CMV IgG antibody

titer and avidity testing in the infant plasma specimens at different points in the infants' first year of life. The objectives of this study were to comprehensively assess the rate of CMV acquisition and to further characterize the time of CMV infection in HIV-exposed infants. Given the presence of maternal transplacentally acquired antibody in the infant and the high prevalence of CMV immunity among women in resource-limited settings, antibody avidity was used as a tool to decipher the time of primary CMV infection in the infants. We used samples collected and stored from infants enrolled in the Breastfeeding, Antiretrovirals and Nutrition (BAN) study (19), who breastfed for 28 weeks and were followed for their first 48 weeks of life in Lilongwe, Malawi.

MATERIALS AND METHODS

Study population. The BAN study was a randomized controlled clinical trial that evaluated, in a factorial design, the safety and efficacy of (i) antiretroviral prophylaxis (a maternal triple-drug antiretroviral regimen versus infant daily nevirapine administered during 28 weeks of breastfeeding versus a control arm of only 1 week of antiretroviral prophylaxis after delivery) and (ii) a maternal nutritional supplement during breastfeeding in reducing postnatal mother-to-child HIV transmission and in

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TABLE 1 Infant CMV IgG levels and IgG avidity at birth and 24 and 48 weeks of age

Measurement ^a	Results at:		
	Birth	24 wks	48 wks
CMV IgG serology			
No. of samples	28	127 ^b	107
Positive (no. [%])	27 (96.4)	116 (91.3)	87 (81.3)
Negative (no. [%])	1 (3.6)	9 (7.1)	20 (18.7)
IgG antibody level (UA/ml) ^c			
Median (IQR)	74.0 (59.0–109.0)	90.5 (69.5–116.0)	93.0 (73.0–115.0)
IgG avidity index			
No. of samples	20	116	86
Median (IQR)	0.91 (0.88–0.95)	0.73 (0.66–0.78)	0.83 (0.78–0.88)
Low (no. [%])	1 (5.0)	42 (36.2)	7 (8.1)
Intermediate (no. [%])	0	52 (44.8)	18 (20.9)
High (no. [%])	19 (95.0)	22 (19.0)	61 (70.9)

^a CMV, cytomegalovirus; IQR, interquartile range; UA, universal arbitrary units.

^b Two samples had equivocal results for IgG.

^c Antibody level calculated for those positive by IgG serology.

enhancing maternal health during breastfeeding (19). The study randomized and followed 2,369 mother-infant pairs between 2004 and 2010 in Lilongwe, Malawi; enrolled infants had to have a birth weight of at least 2,000 g and be HIV uninfected by 2 weeks of age.

We used stored infant plasma specimens from birth (28 specimens), 24 weeks of age (127 specimens), and 48 weeks of age (107 specimens), randomly selected from a subset of 492 BAN study infants who had been previously tested by CMV PCR in the plasma specimens or peripheral blood mononuclear cells (PBMC) at 24 weeks of age (18). If infants tested positive for CMV DNA at 24 weeks, they were also tested at birth; if they tested negative for CMV DNA at 24 weeks, they were also tested at 48 weeks. This study was approved by the Malawi National Health Sciences Research Committee and the institutional review boards of the University of North Carolina at Chapel Hill and of the Centers for Disease Control and Prevention.

CMV IgG and IgG avidity testing. IgG antibody against CMV was measured in plasma specimens using the Vidas instrument (bioMérieux, Lyon, France). Vidas performs an enzyme-linked fluorescent immunoassay that provides semiquantitative universal arbitrary units (UA) per milliliter. IgG avidity testing was also performed using the Vidas reagents and instrument. The avidity cutoff values were modified as described previously to provide improved sensitivity for identification of recent infection: <0.70 for low avidity (indicating recent infection), 0.70 to 0.79 for intermediate avidity (indeterminate timing of infection), and ≥ 0.80 for high avidity (indicating past infection) (20, 21). Validation of the avidity cutoffs was done on serum specimens from individuals who were well characterized regarding age, other risk for CMV infection, and IgG seroconversion. These specimens were tested for IgG avidity, and several cutoff values were applied to determine the best separation of recent and past infection (20, 21).

Statistical analysis. The IgG antibody titer and avidity index results were described using medians and interquartile ranges (IQR) at each time point. Spearman's rank correlation coefficient was used to assess the correlation between the IgG antibody level and avidity. The median IgG titer and avidity index were compared by CMV PCR test result status using Wilcoxon rank sum tests.

RESULTS

CMV IgG antibodies in the infants and their avidity at birth, 24 weeks, and 48 weeks. Of 28 samples tested by CMV serology at birth (when most of the IgG in the infant is maternal), 27 (96.4%) were positive for CMV IgG antibody, indicating a 96.4% preva-

lence of CMV immunity among the mothers. Of these IgG-positive samples, a subset of 20 with enough sample available were tested for IgG avidity; 19 (95.0%) of them had high avidity, signifying past maternal infection and transplacentally transferred maternal antibody (Table 1 and Fig. 1).

At 24 weeks of age, 116/127 (91.3%) samples tested were positive for CMV IgG. At this time point, only 19% of the positive samples had IgG of high avidity; 36.2% had IgG of low avidity, and 44.8% had IgG of intermediate avidity (Fig. 1).

At 48 weeks of age, 87/107 (81.3%) samples had anti-CMV IgG detected. The majority (70.9%) of these samples had antibody of high avidity; 8.1% had antibody of low avidity and 20.9% of intermediate avidity (Table 1 and Fig. 1).

The median IgG antibody levels for those testing positive for CMV IgG were 74.0 UA/ml at birth, 90.5 UA/ml at 24 weeks, and 93.0 UA/ml at 48 weeks (Table 1).

There was a significant correlation between the higher IgG antibody levels and increasing avidity at 24 and 48 weeks (Spearman's correlation coefficient, $r = 0.53$ at 24 weeks; $P < 0.001$; $r = 0.60$ at 48 weeks, $P < 0.001$) but not at birth ($r = 0.12$; $P = 0.63$) (Fig. 1).

Comparison of anti-CMV antibody with CMV PCR results. As previously described, eight infants had CMV DNA detected in the blood at birth (18). Of these, six had anti-CMV IgG of high avidity, indicating prior maternal infection; one infant had IgG of low avidity, indicating *in utero* infant infection with primary maternal infection, and one did not have CMV IgG, which is difficult to explain and could be a specimen labeling or laboratory error.

Of the 116 samples that were positive for CMV IgG at 24 weeks, 83 were also positive for CMV by blood PCR at the same time point (Table 2). Of the 22 samples that were positive for CMV IgG and had high-avidity IgG, 19 infants also had CMV DNA detected by PCR, signifying infant infection. For the remaining three infants, the high-avidity anti-CMV IgG could represent either persistence of maternal antibody or primary infant infection that occurred more than 3 to 4 months earlier. If we exclude these three infants, whose CMV infection status is uncertain, 113 of the 127 infants with anti-CMV IgG at 24 weeks of age can be unequivocally considered to have primary infant CMV infections. Thus,

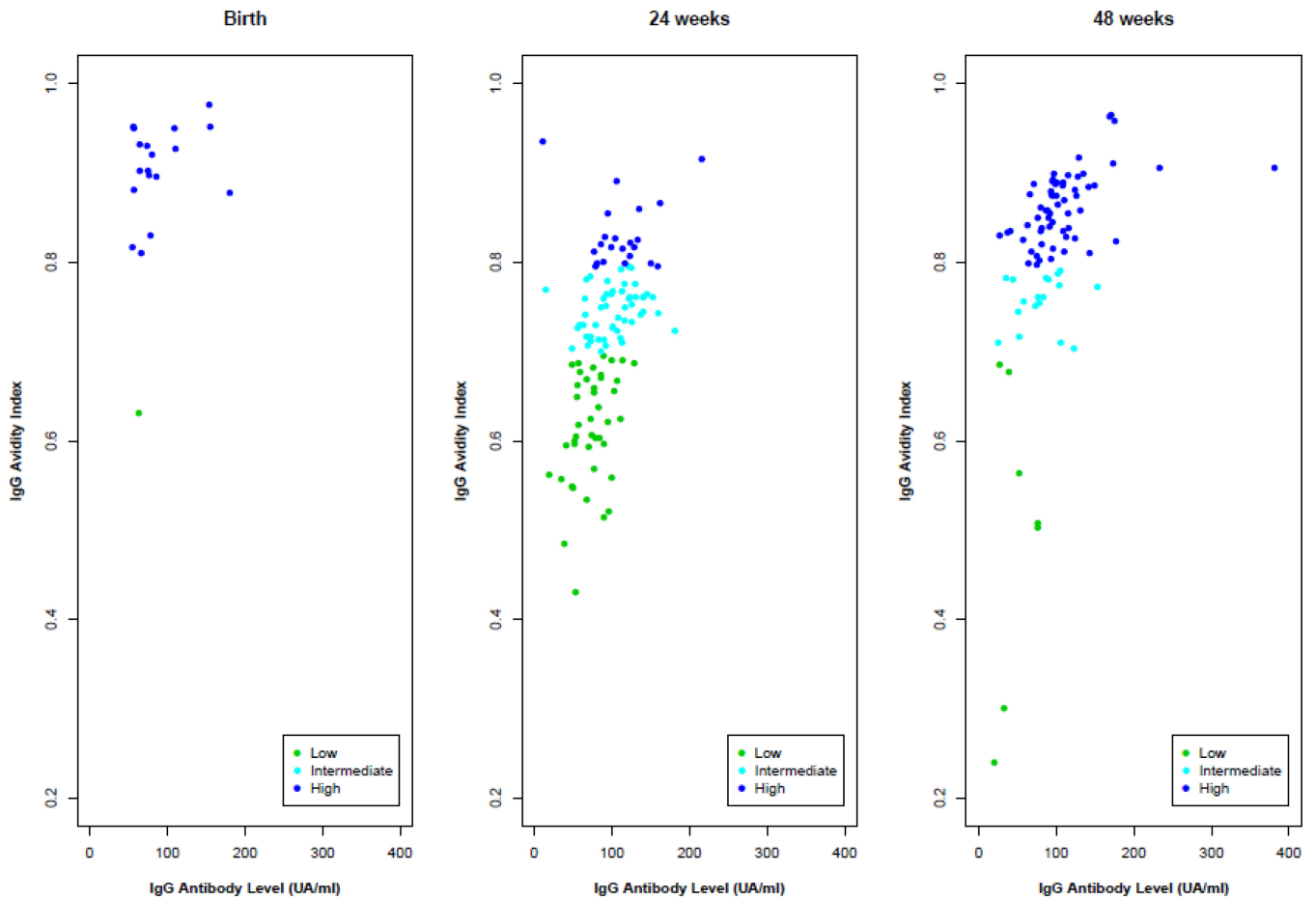


FIG 1 Association between CMV IgG antibody levels and CMV IgG avidity in infants of HIV-infected mothers in Lilongwe, Malawi, at birth and 24 and 48 weeks of age. Low (<0.70), intermediate (0.70 to 0.79) and high (>0.79) avidity index values are indicated by different colors.

taking together the results of the CMV serology and the PCR testing, we estimate the prevalence of infant CMV infection at 24 weeks to be 88.9%. Thirty cases of infant CMV infection not detected by blood PCR were detected by IgG serology at 24 weeks (representing a 23.6% increase in CMV detection by the CMV IgG testing compared with that for blood PCR alone). No samples

were negative by CMV serology and positive by CMV PCR at 24 weeks.

At 48 weeks, 56 of the 87 samples positive for CMV IgG were also positive by blood CMV PCR (Table 2). At this point, all of the high-avidity antibodies are likely to be of infant origin and thus can be assumed to be primary infant infections. Thirty-one infants

TABLE 2 CMV IgG and IgG avidity results compared with CMV PCR results in the blood of HIV-exposed, breastfeeding infants at 24 and 48 weeks of age

Measurement ^a	CMV PCR at 24 wks			CMV PCR at 48 wks		
	Positive	Negative	<i>P</i> value	Positive	Negative	<i>P</i> value
CMV IgG positive (no. of samples)	83	33		56	31	
IgG antibody level (UA/ml)						
Median (IQR)	95.0 (77.0–122.0)	73.0 (54.0–101.0)	0.005	96.5 (76.0–124.0)	90.0 (66.0–100.0)	0.09
Avidity index						
No. of samples	83	33		55	31	
Median (IQR)	0.74 (0.69–0.79)	0.68 (0.59–0.75)	0.01	0.84 (0.78–0.89)	0.83 (0.78–0.88)	0.35
Low	24 (28.9)	18 (54.5)		5 (9.1)	2 (6.5)	
Intermediate	40 (48.2)	12 (36.4)		10 (18.2)	8 (25.8)	
High	19 (22.9)	3 (9.1)		40 (72.7)	21 (67.7)	
CMV IgG negative (no. of samples)	0	9		0	20	

^a CMV, cytomegalovirus; IQR, interquartile range; UA, universal arbitrary units.

who were CMV infected as indicated by serology did not have CMV DNA detected in the blood by PCR at 48 weeks (thus, serology conferred a 35.6% increase in diagnostic sensitivity compared with that for blood PCR testing). No samples were negative by serology and positive by PCR at 48 weeks.

The median antibody levels were significantly higher among infants with CMV DNA detected in their blood than in those in whom CMV DNA was not detected at 24 weeks ($P = 0.005$) but not at 48 weeks ($P = 0.09$) (Table 2).

Maturation of anti-CMV IgG in the infants and correlation with DNA detection in the blood. Of the infants who had CMV DNA detected in the blood at 24 weeks, almost half (48.2%) had CMV IgG of intermediate avidity (median avidity index, 0.74) (Table 2). Conversely, of the samples negative by PCR at 24 weeks, the majority (54.5%) of the CMV IgG-positive samples had low-avidity antibody (median avidity index, 0.68). The median IgG avidity index was higher in CMV PCR-positive than in PCR-negative infants at 24 weeks of age ($P = 0.01$) (Table 2). At 48 weeks, of those with CMV DNA detected in the blood, the majority (72.7%) had IgG of high avidity (median avidity index, 0.84). Of those negative by CMV PCR but positive by serology at 48 weeks, the majority (67.7%) also had high-avidity IgG (median avidity index, 0.83) (Table 2). The median IgG avidity index did not differ by PCR status at 48 weeks ($P = 0.35$).

DISCUSSION

Almost all (96.4%) of the HIV-infected mothers in this setting had evidence of prior CMV infection, as indicated by the presence of anti-CMV IgG in the infants' plasma at birth. Of interest, the majority of infants with congenital infection in our study, as determined by CMV DNA detection in the blood at birth (18), had mothers with prior immunity to CMV. This is in line with other findings suggesting that many congenital infections are the result of maternal CMV reinfection or reactivation, rather than primary infection (20).

The majority of infants in this study acquired CMV rapidly during the first year of life. Using blood CMV PCR, we had previously detected CMV infection in 70% of the HIV-exposed infants in this cohort by 24 weeks and in 78.5% by 48 weeks (18). The results of the present study using CMV serology and avidity testing, when combined with the PCR results, confirm a higher rate of infection at 24 weeks (88.9%) and at 48 weeks (81%). Other studies have also shown very high rates of CMV infection in HIV-exposed infants in resource-limited settings (5, 22).

IgG avidity offered additional information on the character of the antibody detected and helped to determine whether the antibody was transplacentally acquired maternal antibody or whether it was a result of infant infection. Low CMV IgG avidity is considered a reliable indicator of primary infection within the preceding 3 to 4 months, whereas high avidity excludes primary infection within the preceding 3 months (23). The IgG avidity testing demonstrated that by 24 weeks, more than one-third of the infants had CMV IgG of low avidity and almost half of the infants had antibody of intermediate maturity. By 48 weeks, >70% of infants with detectable anti-CMV IgG had mature antibody of high avidity. At 24 weeks, <20% of infants had IgG of high avidity, which could represent either transplacentally acquired maternal antibody or already mature infant antibody from primary infection. Our results indicate that most maternal antibody has waned by 6

months, consistent with a study from China suggesting that the maternally acquired anti-CMV IgG in infants disappears before 8 months of age (24); similarly, a study from Germany concluded that maternal antibody against CMV decayed by 12 months (25).

Our findings confirm that lower IgG antibody avidity was associated with younger age and with lower antibody titers, as expected with primary infection and as has been previously observed (26). However, the median CMV IgG level and avidity were higher in infants who had CMV DNA detected in their blood than in those who did not at 24 weeks of age (although not at 48 weeks). This finding is surprising, as circulating levels of CMV and shedding in body fluids are higher during acute infection (26, 27). Nevertheless, CMV is primarily shed in other body fluids, such as urine or saliva, and the CMV levels in the blood are generally lower (27). Indeed, the CMV viral loads in the blood were quite low in this group of infants (median, 176.4 copies/ml; IQR, 88.6 to 433.3 at 24 weeks of age) (18). It is possible that studying other body fluids would lead to different results, as was found in another study that examined saliva (27).

Viral cultures or PCRs on urine (28) or saliva (29) samples are the gold standard methods for CMV detection. CMV DNA PCR is used to detect clinically significant levels of CMV DNA in plasma, such as with symptomatic CMV infection, a severe clinical course (30), or sequelae such as hearing loss (31). Our findings indicate that CMV PCR in the blood captures most, but not all, primary infant CMV infections, as confirmed by CMV IgG serology results. Increases in diagnostic sensitivity of 24 and 36%, respectively, were observed by using CMV IgG serology in addition to CMV DNA testing in the blood at 24 and 48 weeks of age.

The strength of this study is that the use of multiple assays, including CMV IgG serology and avidity testing and DNA detection in the blood, allowed precise determination of both the magnitude of infant CMV infection in the study population and the timing of such infection. Almost 90% of breastfeeding infants of HIV-infected mothers in Malawi will have already acquired CMV infection by 24 weeks of age. Given the emerging evidence on the negative repercussions of CMV infection on the health of these infants (5, 18), an antiviral approach to prevent or delay CMV transmission through reductions in maternal shedding or the development of a CMV vaccine administered very early in such settings is worth further exploration.

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