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Assignment of cytomegalovirus infection status in infants awaiting solid organ transplant: Viral detection methods as adjuncts to serology

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Abstract

Assignment of CMV infection status in infants awaiting SOT is challenging as passive maternal antibody can lead to false-positive serology. Since 2000, our protocol has recommended sending throat and urine samples for CMV viral detection, culture, or NAAT, for CMV-seropositive infants <18 months awaiting SOT. We reviewed pretransplant CMV serology for 152 infants and, for CMV seropositives, examined relationships between CMV IgG OD values, age, and CMV viral detection to explore time to clearance of maternal CMV IgG and evaluate viral detection in assignment of pretransplant CMV infection status. The proportion of CMV-seropositive infants decreased from 52% in infants 0-6 months of age to 28% in those 12-18 months. Among CMV-seropositive infants, median OD was significantly higher in the 6- to 12- and 12- to 18-month groups compared to the 0- to 6-month group. Distribution of OD by age group suggested that maternal antibody cleared before 12 months. Of 59 eligible CMV-seropositive infants, 49 (83%) had CMV viral detection studies and 18 of 49 (36.7%) had detectable CMV: 9 of 30 (30.0%) infants 0-6 months, 7 of 15 (46.7%) infants 6-12 months, and 2 of 4 (50.0%) infants 12-18 months. CMV viral detection studies are useful to confirm positive CMV infection status in CMV-seropositive infants awaiting SOT. Maternal CMV IgG likely clears before 12 months.

KEYWORDS
cytomegalovirus, pediatric, transplant recipients, virus shedding

1 | INTRODUCTION

Determination of pretransplant CMV infection status is critical in stratifying the risk of CMV disease and affects decisions regarding the use of antiviral prophylaxis and monitoring for CMV infection post-transplant.[1-3] Unfortunately, serology-based identification of CMV infection status has limitations and may be falsely positive in situations where passive antibodies exist, such as in infants who may have maternal antibody and in individuals who have received a recent transfusion of plasma-containing blood products or IVIG. The time to clearance of maternal antibodies to CMV (CMV IgG) is not well established, but current guidelines suggest that CMV serology may be unreliable for infants less than 12 or 18 months of age.[2,3]

When serology is unreliable, culture or NAATs of urine or throat swabs may be useful to identify CMV shedding, a marker of true infection, in CMV-seropositive infants, but the utility of these tests in assigning pretransplant CMV infection status has not been evaluated.[2,3] If CMV culture or NAAT are negative or are not performed,

Abbreviations: CMV, cytomegalovirus; CMV IgG, immunoglobulin G antibody to cytomegalovirus; IVIG, intravenous immunoglobulin; NAAT, nucleic acid amplification test; OD, optical density; PCR, polymerase chain reaction; SOT, solid organ transplant.

guidelines recommend assuming the highest risk donor/recipient CMV scenario for CMV-seropositive infants.[2,3] As this strategy may lead to unnecessary antiviral use and additional monitoring, which is associated with potential toxicity and additional costs, it is important to clearly identify the group of infants in whom serology may be unreliable and to evaluate the role of CMV viral detection assays as an adjunct to serology in assignment of pretransplant CMV infection status.

Our goal was to more clearly define the group of infants in whom serology may be unreliable and to evaluate our experience with the use of CMV culture and NAAT to detect viral shedding among CMV-seropositive infants awaiting SOT. We reviewed pretransplant CMV serology as well as CMV throat and urine viral culture and NAAT results for infants <18 months of age awaiting SOT at our institution looking for differences in CMV seropositivity proportions and CMV IgG OD values, a surrogate of CMV IgG titer, by age group. In CMV-seropositive infants, we also examined relationships between CMV viral shedding, age, and CMV IgG OD values to determine whether CMV IgG OD values and/or detection of viral shedding, used alone or together, may improve recipient CMV serostatus classification, assisting in differentiating CMV-infected infants from uninfected infants with passive maternal or transfusion-acquired antibody.

2 | METHODS

2.1 | Institutional protocols

CMV serology was routinely performed in all children being assessed for SOT since 1994. Since January 2000, our local protocol has also recommended submitting a throat swab and urine sample for CMV viral detection on all CMV-seropositive infants <18 months of age awaiting SOT. CMV was detected using shell-vial culture between 2000 and 2012; in January 2013, CMV culture was replaced with CMV DNA detection by NAAT.

2.2 | Detection of CMV-specific antibodies (Serology) and CMV DNA

CMV-specific IgG antibodies (serology) were detected by enzyme immunoassay (Siemens Enzygnost Anti-CMV/IgG, Siemens Healthcare Diagnostics Products GmbH, Marburg/Germany). Serology results used were as reported by the laboratory and were based on laboratory guidelines for OD cutoffs of <0.1 AU/mL for negative, >0.2 AU/mL for positive, and between 0.1 and 0.2 AU/mL for indeterminate CMV serology. Though considered semi-quantitative, the OD value is a surrogate of CMV IgG antibody titer.

Throat swabs, collected in viral transport media, and urine specimens, collected in sterile containers, were processed for CMV shell-vial culture according to routine procedures (Merifluor CMV, Meridian Bioscience Inc., Cincinnati, OH, USA). Nucleic acid amplification testing (NAAT) for CMV from throat swab, collected in universal transport media (UTM-RT, COPAN diagnostics INC, USA) and urine samples, collected in sterile containers, was performed using the RealStar® CMV PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany).

2.3 | Data collection

2.3.1 | Infants

Data on the age and CMV serology results from all infants <18 months awaiting SOT at our institution between April 1994 and September 2014 were obtained from a transplant database. CMV IgG OD values, for all infants with positive CMV serology, were extracted from the Provincial Laboratory for Public Health laboratory information system. Results of CMV culture or NAAT testing of throat swab or urine samples, for CMV-seropositive patients transplanted after January 2000, were also extracted from this system. Transfusion data were obtained from the Stollery Children's Hospital blood bank database. We considered infants to have had relevant recent transfusion if they had received transfusions of red cells, plasma, platelets, cryoprecipitate, or whole blood or if they had received low-dose (<1 mg/kg) intravenous immune globulin within the 3 months prior to the date of their CMV serology test or high-dose intravenous immune globulin (≥1 g/kg) within 6 months of CMV serology. The CMV prevalence in Canadian blood donors is 42% (personal communication, Canadian Blood Services).

2.3.2 | Normative adult OD values

To establish a reference range of OD values in CMV-seropositive adults, we reviewed CMV serology and OD values for 1016 sequential Canadian Blood Services (Edmonton) blood donors. Samples were collected in 2015 and tested in our research laboratory with the same CMV serology assay (Siemens Enzygnost Anti-CMV/IgG) as the infants in our study.

2.4 | Data analysis

For analysis, infants were classified into 3 age groups based on age at the time of sampling for serology: 0-<6 months, 6-<12 months, and 12-18 months. For the analysis of pretransplant CMV serology by age group, a single CMV serology result was used for each infant; if there were multiple pretransplant CMV serology results, the pretransplant serology result closest to the time of transplant was used. For analysis of age at sero-reversion, we documented the age when infants with a previous positive CMV serology were first found to have a negative CMV serology result.

For the analysis of CMV-seropositive infants, all infants who had a positive CMV serology at some point pretransplant were included, even if the CMV serology immediately pretransplant was no longer positive. If there were multiple positive pretransplant CMV serology results, the serology result closest to the time of CMV viral detection test (urine or throat culture or NAAT) was used. If there were multiple viral detection tests performed, the result closest to the time of transplant was used. If there was no

TABLE 1 Age and CMV serostatus by allograft type for infants awaiting transplant

	Allograft Type				
	Liver n = 89	Heart n = 57	Multivisceral n = 5	Kidney n = 1	Total n = 152
CMV seropositive[a] n (%)	43 (48.3%)	21 (36.8%)	1 (20.0%)	0	65 (42.8%)
Age (y) at CMV serology, Median (25, 75$^{\%ile}$)	0.74 (0.56, 0.99)	0.45 (0.22,0.69)	0.77 (0.45,1.05)	1.41	0.64 (0.45,0.93)

[a]If there were multiple pretransplant CMV serology results available, the serology result closest to the time of transplant was used.

viral detection test performed, the positive serology result closest to the time of transplant was chosen. Infants were classified as CMV culture positive or NAAT positive if at least one of their samples (throat or urine) was positive.

In addition to analyzing OD values in CMV-seropositive adult blood donors, comparisons were made between OD values in CMV-seropositive women of childbearing age (18-45 years) and OD values in CMV-seropositive infants in each of the 3 age groups, to examine the impact of potential passive maternal antibody.

Continuous and ordinal variables are presented as medians with 25th and 75th percentiles. To compare medians, we used the Kruskal-Wallis test and the Mann-Whitney U test for pairwise comparisons with Bonferroni correction for multiple pairwise comparisons. Categorical variables are presented as counts with percentages and were analyzed using the chi-squared test or Fisher's exact test. All tests were two-sided, and a *P*-value of <.05 was considered statistically significant. When multiple pairwise comparisons were made, *P* values were multiplied by the number of comparisons and reported as an adjusted *P* value.[4] Data analysis was performed with STATA 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX , USA: StataCorp LP). Ethics approval for this study was obtained from the University of Alberta Health Research Ethics Board.

3 | RESULTS

3.1 | Pretransplant CMV serology for infants <18 months of age

There were 152 infants, less than 18 months of age, transplanted at our institution between January 1994 and September 2014; Table 1 summarizes pretransplant CMV serostatus and age, by allograft type. The proportion of CMV-seropositive infants decreased from 52% (25/48) in the 0- to 6-month group to 43% (32/75) in the 6- to 12-month group and 28% (8/29) in the 12- to 18-month group but there were no statistically significant differences in the proportion of CMV-positive infants between the three groups (*P* = .109). CMV serology was indeterminate in 4.2% (2/48) in the 0- to 6-month group, 4.0% (3/75) in the 6- to 12-month group, and 3.4% (1/29) in the 12- to 18-month group.

3.2 | CMV IgG OD in CMV-seropositive infants

A total of 73 of the 152 infants (48.0%) had a positive CMV serology result at some point prior to transplant. At the time of transplant 65 remained CMV seropositive, 6 had sero-reverted to negative and 2 had indeterminate CMV serology. CMV IgG OD values were available for 72 of 73. The distribution of CMV IgG OD by age group is shown in Figure 1. Median OD increased from 0.78 (0.36, 1.12) in the 0- to 6-month group to 1.33 (0.81, 1.16) in the 6- to 12-month group and 1.62 (1.36, 2.41) in the 12- to 18-month group. Median OD was significantly higher in both the 6- to 12- and 12- to 18-month groups compared to the 0- to 6-month group (adjusted *P* = .007, adjusted *P* = .004), but there was no significant difference in median OD between the 6- to 12- and 12- to 18-month groups (adjusted *P* = .25). Compared to the 0- to 6-month group, there is a wider spread of CMV OD values in the 6- to 12-month group with a suggestion of clustering in a higher OD group, likely reflecting true CMV infection, and a lower OD group, likely reflecting waning maternal antibody. By 12-18 months, the lower OD cluster is no longer present and all CMV OD values are greater than 1. Complete transfusion data were available for 67 of 72 infants (93.1%): 32 of 67 (47.8%) had relevant recent transfusions so may have had transfusion-related passive CMV IgG. When the transfused infants are excluded, there is more clustering into higher and lower OD groups in the 6- to 12-month age group.

FIGURE 1 CMV IgG OD by age group in CMV-seropositive infants awaiting transplant. Median OD was similar between the 6- to 12- and 12- to 18-month age groups (*P* = .25). *Indicates significant difference when compared to 0-6 mo group (*P* < .05)

For comparison, we reviewed the CMV serology OD values for 1016 sequential Canadian Blood Services donors, age 18-76 years; 39% were CMV seropositive with a median OD value

TABLE 2 CMV viral detection in CMV-seropositive infants awaiting transplant

	Age Group			
	0-6 mo	6-12 mo	12-18 mo	Total
CMV viral detection: Any[a]	n = 30	n = 15	n = 4	n = 49
Positive n (%)	9 (30.0)	7 (46.7)	2 (50.0%)	18 (36.7)
CMV viral detection: Culture[b]	n = 29	n = 14	n = 2	n = 45
Positive n (%)	8 (27.6%)	7 (50%)	0	15 (33.3)
CMV viral detection: NAAT[b]	n = 3	n = 2	n = 2	n = 7
Positive n (%)	2 (66.7%)	0	2 (100%)	4 (57.1)

[a]Considered positive if CMV culture and/or NAAT from throat and/or urine sample was positive.
[b]3 children, 2 age 0-6 mo and 1 age 6-12 mo, had CMV culture and CMV NAAT performed. All results were concordant.

among seropositives of 1.58 (1.17, 2.14). There were 280 women of childbearing age (18-45 years) in this cohort: 41% were CMV seropositive with median OD value among seropositives of 1.49 (1.13, 1.98). The median OD of seropositive women of childbearing age was significantly higher than in seropositive infants age 0-6 months (adjusted $P < .001$), but there were no significant differences in median OD between women of childbearing age and either the 6- to 12 or 12- to 18-month groups (adjusted $P = .073$, adjusted $P = 1.0$).

Six infants sero-reverted while awaiting transplant. The age of documented sero-reversion ranged from 0.2 to 1.12 years, and all of the infants had an OD value <1 on their positive sample(s) (OD range 0.206-0.93). Four of these infants had received recent transfusions prior to their initial positive serology, and transfusion history was unavailable in one child so it is unclear whether the sero-reversion was due to disappearance of passive maternal or passive transfusion-acquired antibodies or both. The child with no history of blood transfusion had documented sero-reversion at 0.36 years.

3.3 | CMV viral detection assays in CMV-seropositive Infants

Of the 59 infants <18 months of age transplanted between January 2000 and September 2014, 83.0% (49/59) had at least one sample (throat or urine) sent for CMV viral detection and 18 of 49 (36.7%) had detectable virus/viral DNA in at least on sample. CMV viral detection results by age group, for the 49 infants with samples sent, are summarized in Table 2. There were no cases of congenital CMV identified in this population; only 7 infants had CMV viral detection studies at ≤3 weeks of age (all CMV culture), and all were negative. The median time between sample collection for CMV serology and CMV viral detection was 2 (0,6) days. Transfusion data were available for all 59 infants. Recent transfusion was common in this population and was more frequent in infants with negative compared with positive CMV viral detection studies (54.8% [17/31] vs 27.8% [5/18]), but this difference did not reach statistical significance ($P = .082$).

CMV culture was performed in 45 seropositive infants (from urine in 44 and throat in 23). There were no statistically significant differences in the proportion with a positive CMV culture between the age groups ($P = .20$). Of the 22 infants who had both a urine and throat sample sent for CMV culture, the results were concordant in 20 cases (17 negatives and 3 positives). In 2 cases, a 2.9 and a 4.8 month old, the urine CMV culture was positive and the throat culture was negative.

CMV NAAT was only performed in 7 infants and was positive in 4 (57.1%): 4 had throat samples (3 positives), 2 had urine samples (1 positive), and 1 had urine and throat samples (both negative). Only 3 children had both CMV culture and CMV NAAT performed, and results were concordant in all 3 cases (2 negatives and 1 positive).

3.4 | CMV IgG OD and CMV culture in CMV-seropositive Infants

Figure 2A illustrates the distribution of CMV IgG OD values by age in CMV-seropositive infants with positive and negative CMV cultures. Among CMV-seropositive infants with positive CMV culture, there is an increasing trend in OD with increasing age. Among CMV-seropositive infants with negative CMV cultures, there is also an overall increasing trend in OD with age. These trends remain when transfused infants are excluded (data not shown).

Median OD by CMV culture status and age group are presented in Figure 2B. There are no statistically significant differences in median OD between infants with positive and negative CMV culture overall ($P = .13$) or within age groups (0-6 months $P = .43$, 6-12 months $P = .22$). While there were no significant differences in median OD between the 3 age groups, among CMV-seropositive culture negative infants ($P = .09$), median OD value was higher in the 6- to 12-month group compared with the 0- to 6-month group which is contrary to what we would expect based on waning passive maternal CMV antibody. The 2 seropositive infants >12 months of age had high OD values but negative CMV culture, and neither had a history of recent transfusion.

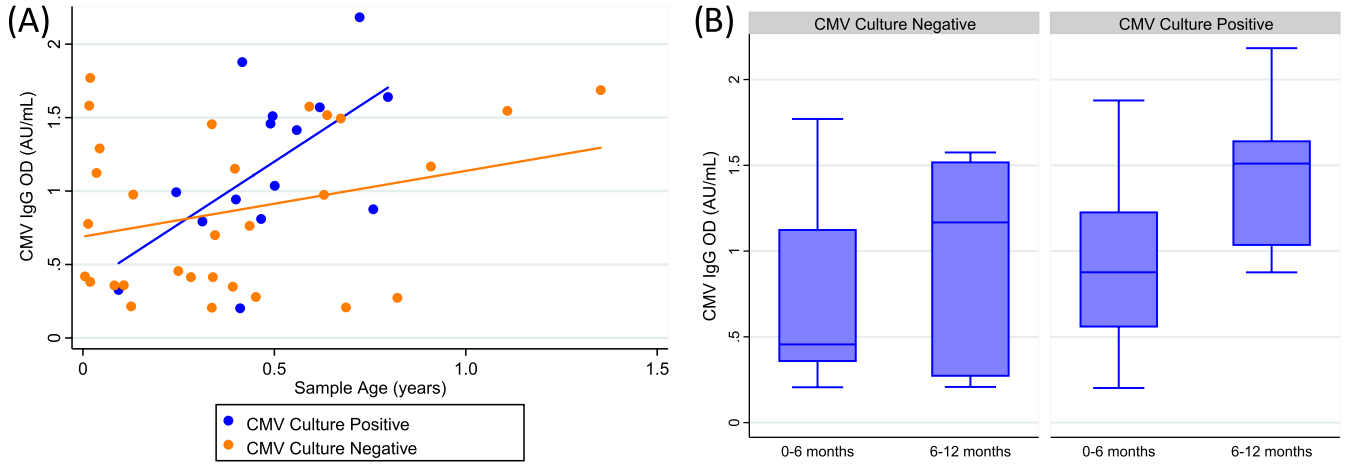

FIGURE 2 CMV IgG OD in CMV-seropositive Infants. A, By age and CMV culture. B, median CMV IgG OD by CMV culture and age group

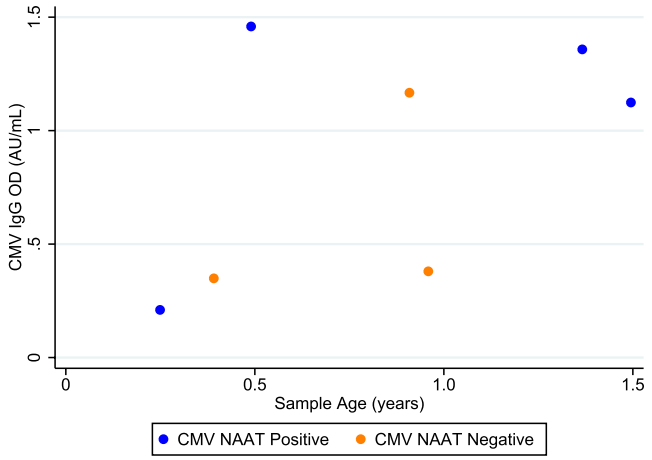

FIGURE 3 CMV IgG OD in CMV-seropositive infants with CMV NAAT testing, by age. All of the infants with negative CMV NAAT were <12 mo of age and had recent transfusion

3.5 | CMV IgG OD and CMV NAAT in CMV-seropositive infants

Figure 3 illustrates the distribution of CMV IgG OD values by age in the 7 CMV-seropositive infants with samples tested for CMV NAAT. The 3 CMV-seropositive infants with negative CMV NAAT were <12 months of age and all 3 had a history of recent transfusion; 2 of the infants, 4.7 and 11.5 months of age, had relatively low OD values (<0.5 AU/mL), while an 11-month-old infant had a high OD value (>1 AU/mL) but had received IVIG the day prior to serologic testing. Both CMV-seropositive infants >12 months of age had a high OD value (>1 AU/mL) and had positive CMV NAAT.

4 | DISCUSSION

In our cohort, the proportion of CMV-seropositive infants decreased from 52% in the 0- to 6-month group to 43% in the 6- to 12-month group and 28% the 12- to 18-month group, consistent with waning passive maternal antibody. Studies of CMV seroprevalence in infants in Turkey and China, where almost all pregnant women are seropositive, described the highest seroprevalence in infants 0-6 months of age and the lowest in infants 6-12 months, consistent with waning passive maternal antibody, then increasing seroprevalence after 12 months of age, reflecting true CMV infection.[5,6] While early studies support a sharp decline in CMV seropositivity in the first 12 months of life, current seroprevalence of CMV in infants less than 1 year of age in countries more similar to Canada, with lower prevalence of CMV, is largely unknown.[7-9] Seroprevalence studies in the United States and in Germany, where the overall prevalence of CMV is approximately 50%, report estimated prevalence of CMV IgG positivity in 1- to 2-year-old children of 12.3% and 21.5%, similar to our cohort.[10-12]

The current American Society of Transplantation guidelines suggest that passively acquired maternal CMV IgG should be considered in pediatric SOT donors and recipients <18 months of age and the International Consensus Guidelines on the management of CMV in SOT suggest a cutoff of 12 months.[2,3] The relatively high rate of CMV infection in infants from CMV-seropositive mothers, related to transmission through breastmilk and from close contact with other infected secretions, makes it difficult to accurately establish the time to clearance of passive maternal CMV IgG. The CMV OD data from our cohort suggest that passive maternal CMV IgG clears before 12 months, as the CMV-seropositive infants in the 12- to 18-month group all had higher OD values (>1 AU/mL) which would be expected with true CMV infection as opposed to with waning passive maternal antibody, and had median CMV OD that was very similar to our comparison group of seropositive adult blood donors. A study of 121 CMV seropositive very low birthweight preterm Korean infants documented a mean age of sero-reversion of 5.5 months, with 97.5% of infants becoming seronegative by 10 months of age, but these results may not be generalizable to full-term infants who would be expected to have a higher CMV IgG titer at birth.[13] A Chinese study investigating the kinetics of CMV IgG levels in serial samples in 40 infants born to

CMV-seropositive mothers found that 8 infants cleared maternal CMV IgG between 3.5 and 8 months of age, and the other 32 infants had decreasing levels of CMV IgG over the first 3.5 months and then had significantly higher levels at 8 months, consistent with true infection prior to 8 months of age.[5] Studies looking at the time to clearance of maternal IgG antibodies to other viruses that are less commonly acquired in the first year of life, including measles, mumps, rubella, and varicella, report rapid decreases in virus-specific IgG antibody levels in the first 3-6 months of life and little or no detectable antibody by 12 months.[14,15] Based on our study and the limited additional available evidence, it appears that the 18-month cutoff to consider passive maternal antibody, when establishing pretransplant CMV infection status, is likely too long and a 12-month cutoff is more appropriate.

Transfusion was common in our cohort of CMV-seropositive infants in the months before transplant. As administration of blood products complicates the interpretation of serology, every attempt should be made to collect and store a tube of serum prior to administration of blood products for any child who may require a transplant.

Current guidelines suggest that, in infants whose serology may be unreliable due to passive maternal antibody, CMV culture or NAAT of urine and throat swabs may be helpful in identifying truly infected infants, but there are limited data to support this recommendation.[2,3,16] In our study, positive CMV viral detection results confirmed true-positive CMV infection status in a large proportion of CMV-seropositive infants (37%), including 30% of young infants (<6 months). The relatively high prevalence of true CMV infection in these young pretransplant infants also highlights the importance of repeating CMV viral detection studies, in infants whose initial CMV detection studies are negative, at the time of transplant to detect primary CMV infection. Most of our data was using CMV culture, but given the well-recognized improved sensitivity of CMV NAAT over CMV culture, CMV NAAT may be a more promising adjunct in the assignment of CMV infection status in infants pretransplant, and sampling at multiples sites, urine, and throat, as well as repeated sampling may improve the sensitivity of the detection of CMV viral shedding.[17]

While positive CMV viral detection results in CMV-seropositive infants are clearly useful in assigning positive CMV infection status, the predictive value of negative CMV viral detection tests, in this population, is difficult to establish. In exploring relationships between CMV OD and CMV culture in CMV-seropositive infants, we found wide variation in CMV OD among infants with positive CMV culture but an overall increasing trend in OD with increasing age in this group, which may reflect that most infants are being infected early and that OD is low in acute infection and increases over the next 2-4 months.[18,19] Surprisingly, among CMV-seropositive infants with negative CMV culture, there was also an increasing trend in OD with age which raises concern that CMV culture may not be sensitive enough to pick up true CMV infection, especially at older ages. A recent cross-sectional US study assessed CMV shedding in saliva by NAAT, in healthy CMV-seropositive children found that 23% (3/13) of infants 0-3 months were shedding CMV, all infants aged 4-12 months (8/8) were shedding CMV, and 64% (9/14) of infants aged 13-24 months were shedding CMV.[20] This suggests that passive maternal antibody accounts for the majority of the CMV seropositivity in infants <4 months of age and that at a very young age (<12 months) truly infected infants have detectable viral shedding but the prevalence of viral shedding among truly CMV-infected CMV-seropositive children decreases with increasing age. A companion repeated-measures study of CMV shedding patterns study enrolled some of the same CMV-seropositive children and found that CMV shedding was intermittent but was highly correlated with initial shedding status: seropositive children shedding at the initial visit had CMV DNA detected in 84% of follow-up saliva specimens while those not shedding at the initial visit only had CMV DNA detected in 16% of follow-up saliva specimens.[21] Importantly, due to the way the saliva and urine samples were collected and processed to allow home collection in these studies, the limits of NAAT detection were considerably higher for the saliva (1600 copies/mL) and urine (16 000 copies/mL) than they would be for samples collected in a hospital setting where the approximated limits of detection would be closer to 500 copies/mL. A small study investigating the kinetics of CMV clearance in urine in infants with congenital CMV, who are presumed to have more prolonged CMV shedding than children with postnatally acquired CMV, found that over 20% of asymptomatic congenitally CMV-infected infants had cleared CMV DNA from the urine by 12 months of age.[22] A negative CMV viral detection result in a CMV-seropositive infant should not be definitive evidence of a true negative CMV status, especially at >12 months of age, as CMV shedding can be intermittent and it appears that older infants are less likely to have detectable shedding than younger infants.

The major limitation of our study is that, for most subjects, we had only a single serology result so we could not document CMV sero-reversion and establish a true negative CMV infection status in infants suspected to have false-positive CMV serology from passive maternal antibody; thus, we could not determine the negative predictive value of CMV culture or NAAT nor could we definitively establish the age of clearance of maternal CMV IgG. We did not have access to maternal CMV serology results so we made the assumption that young CMV-seropositive infants who had not been transfused could have had passive maternal antibody. Unfortunately, we had a very small number of infants in the 12- to 18-month age group which limited our ability to draw strong conclusions about the time to clearance of maternal CMV IgG and the sensitivity of CMV viral shedding assays in these older infants.

Despite these limitations, our study clearly highlights that CMV viral detection studies are a useful adjunct to CMV serology in CMV-seropositive infants awaiting SOT and supports that 12 months, as opposed to 18 months, may be a more appropriate cutoff for considering potential passive maternal antibody. Although our experience with CMV NAAT was limited, our preliminary results, along with the well-recognized improved sensitivity of CMV NAAT compared to CMV culture, are encouraging and more research is warranted in evaluating the role of CMV NAAT for establishing CMV infection status in infants awaiting transplant.

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AUTHORS' CONTRIBUTIONS

Catherine Burton: Involved in study design, data analysis and interpretation, writing the article and approval of the submitted version. Tatiana Dragan: Involved in the study design as well as data collection, critical review of the article, and approval of the submitted version. Curtis Mabilangan: Involved in data collection and analysis, critical review of the article, and approval of the submitted version. Sheila O'Brien, Margaret Fearon, and Vito Scalia: Involved in data collection, critical review of the article, and approval of the submitted version. Jutta Preiksaitis: Involved in study design, data analysis and interpretation, critical revision of the article, and approval of the submitted version.

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