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Postnatal cytomegalovirus infection: a pilot comparative effectiveness study of transfusion safety using leukoreduced-only transfusion strategy

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Abstract

BACKGROUND—The optimal mitigation strategy to prevent transfusion transmission of cytomegalovirus (TT-CMV) in preterm very low birthweight infants remains debated. Hospitals caring for this patient population have varied practices.

STUDY DESIGN AND METHODS—A prospective observational comparative effectiveness pilot study was conducted to determine the feasibility for a larger study. The pilot was carried out at hospitals using a leukoreduction (LR)-only transfusion strategy. Specimen and data collection for this study was performed in a similar approach to a study completed at Emory University that employed the CMV-seronegative plus LR approach. All testing was performed at one laboratory. The rates of TT-CMV using the two transfusion strategies were compared.

RESULTS—Zero incidence of TT-CMV was detected in infants (n = 20) transfused with LR-only blood (0/8; 95% confidence interval [CI], 0-25.3%) and is consistent with the previously reported zero incidence of TT-CMV finding in a cohort of infants transfused with CMV-negative plus LR blood (0/310; 95% CI, 0%–0.9%). The seroprevalence rate among enrolled mothers (n = 17) was 60%. Forty percent of those infants (8/20) received 43 transfusions; five were transfused with one or more CMV-seropositive blood components. One infant had tested positive for CMV before receiving blood transfusions; the infant's mother was CMV immunoglobulin (Ig)G positive and IgM negative.

CONCLUSIONS—Using the LR-only transfusion approach, zero cases of TT-CMV were detected in this pilot study. A larger study is needed to reliably determine the most effective strategy for prevention of TT-CMV in this population.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

Very low birthweight (VLBW, ≤1500 g) preterm infants are at risk for significant morbidity and mortality from cytomegalovirus (CMV) infection. CMV infection may be acquired from the mother, through maternal placental transfer (congenital), perinatally through the birth canal, postnatally through breast milk, through blood transfusion, or through exposure to other bodily fluids via human contact.[1–3] Although extensively studied, the appropriate mitigation strategy to halt transfusion transmission of CMV (TT-CMV) remains controversial.[4–6] Preventative measures include the use of blood products from donors that are CMV seronegative (CMV-NEG), the filtering out of white blood cells (WBCs) that carry CMV using leukoreduction (LR), as suggested by the Food and Drug Administration, or both.[7] The lack of accepted practice by neonatologists and transfusion medicine specialists has led to variation in center-specific policies for transfusion of neonatal patients throughout the United States. In a survey of 183 American centers that ranged from academic to community hospitals, blood centers and governmental or military facilities, approximately 38% use CMV-NEG only, 23% use LR only, 15% use either, 24% use LR when CMV-NEG is not available, and 22% of centers use CMV-NEG plus LR in preterm infants.[8]

Breakthrough infection in recipients has been demonstrated with both the CMV-NEG–only and LR-only transfusion, although the reasons for the residual infectivity are distinct.[6] In the CMV-NEG transfusion approach, the window period is the most likely reason for breakthrough infections.[9] Blood donor CMV viremia was found in 1.6% of seroconverting donors at the time of donation before their first evidence of CMV seroconversion.[10] Approximately 1.1% of CMV-NEG blood donors seroconvert each year; DNA may remain detectable up to 84 days later.[11] When using LR only, the concern is that breakthrough infection occurs because LR filters may not remove enough latently infected monocytes, lymphocytes, and natural killer cells (estimated as one infected WBC per 1,000-10,000).[12,13] Although 10^5 to 10^6 WBCs may remain after LR using modern filters, finding CMV DNA detection has proven difficult in LR blood products.[10,11] There are no direct studies of the CMV-NEG plus LR approach to gauge the safety of combining these methods to either one of them alone.

To compare the effectiveness of the LR-only versus CMV-NEG plus LR transfusion approaches in preventing TT-CMV in VLBW infants, we undertook a comparative effectiveness study that capitalized on the local preference at two institutions. A randomized study comparing the two approaches would be destined for enrollment failure if providers insist on CMV-NEG plus LR approach. Emory University (EU) conducted a prospective observational natural birth history cohort study in the metro-Atlanta area to assess the incidence of TT-CMV when the CMV-NEG plus LR approach was utilized.[14] Herein we describe a similar prospective observational pilot cohort study conducted in Seattle where the LR-only transfusion approach is utilized. By using a similar study design approach at each site, the difference in the rate of TT-CMV may be estimated and provide support for the superiority, or lack thereof, of one of these two common transfusion policy approaches (CMV-NEG plus LR or LR only). To align the study outcomes of these two separately conducted studies, all laboratory testing, including residual WBC counts in blood products and CMV nucleic acid testing (NAT) of maternal infant, breast milk, and blood product samples was carried out in one laboratory and all data collection and statistical analysis was performed by the same data coordinating center.

MATERIALS AND METHODS

The study protocol was approved by the human subjects review board of the University of Washington and Seattle Children's Hospital. VLBW infants admitted to either the University of Washington Medical Center or Seattle Children's Hospital, within the first 5 days of life, regardless of maternal CMV status, were identified by the mother of the child's (MOC) physician or medical record review. Infants were excluded if they have received a red blood cell (RBC) or platelet (PLT) transfusion before transfer to the study facilities or if the infant was not expected to live past the first 7 days of life (assessed by neonatologist), if the infant had a severe congenital abnormality, or if the MOC had previously participated in this study. Demographic and baseline medical information was collected about the birth of the VLBW and about the MOC, as is previously described.[14] Infants were followed during their hospital stay up to Day 40 or discharge, whichever occurred first. Follow-up laboratory results were collected on Days 21 and 40 or discharge (whichever occurred sooner). Data on blood transfusions were recorded whenever they occurred. All cellular blood components transfused were leukoreduced and irradiated. Each variable of the Seattle study was performed to mirror the EU study as much as was possible. For additional details of the EU study methods and design, please refer to previous study.[15]

CMV surveillance

Peripheral blood from enrolled MOCs was screened for past or current CMV infection using a CMV serology assay to detect both immunoglobulin (Ig)G and IgM antibodies and by CMV NAT to detect viral copies; breast milk samples were tested by NAT. All testing was performed at EU, using methods previously described.[14] Mothers who are IgM or NAT positive may be experiencing an acute infection or reactivation of existing CMV infection; these results were conveyed to the infant's neonatologist. All enrolled infants were tested using CMV NAT from discarded blood samples from previously drawn clinical samples from 0 to 5, 21 ± 7, and 40 ± 10 days of life. In the event that no serum or plasma was available for retesting, urine was collected for CMV NAT testing. Urine is an adequate substitute and the best available in the setting of a study on VLBW; viral shedding can be detected in the urine longer than in the blood. Positive or low positive results were repeated for confirmation. If a VLBW infant tested positive for CMV NAT during the study, the breast milk and blood product administered closest to the time of detection were subsequently tested outside the routine batching and testing regimen.

Before each transfusion, a sample of the blood product was reserved for CMV NAT and quantitation of resid ual WBCs (cellular products only; PLTs and RBCs). To measure the association between CMV DNA and WBC counts in transfused units due to LR failures, a sensitive method was used to detect residual WBC counts in the blood products.[14] LR failures were defined as more than 15×10^6/L as detected by flow cytometry.[14]

Comparative effectiveness

The incidence estimation of TT-CMV was compared between the Emory and Seattle sites. Additionally, overall CMV infection rates and CMV infection rates by maternal CMV-

seropositive status were compared. Confidence intervals (CIs; 95%) were calculated for incidence rates at the two sites.

RESULTS

A total of 20 infants born to 17 mothers were enrolled in the study from the University of Washington Medical Center (n = 18) and Seattle Children's Hospital (n = 2) between August 2013 and August 2014. Many of the deliveries were associated with premature rupture of membranes (47%). There were a myriad of common pregnancy, labor, and delivery complications such as incompetent cervix, pre/eclampsia, fetal distress, and bleeding, affecting 12% to 29% of study subjects. From the study subjects, one enrolled mother was affected by each of the following: chronic hypertension, cardiac disease, renal disease, diabetes, and chorioamnionitis (Table 1).

Eleven mothers were determined to be CMV seropositive out of 16 tested (69%; sample was not obtained for one mother). Ten of 11 CMV-seropositive mothers tested IgM negative (90.9%). One mother who tested IgM positive was also determined to be positive for IgG. All mothers tested CMV NAT negative at enrollment.

Forty percent of infants (8/20) received at least one transfusion: 43 transfusions were given to eight infants from 24 units during the course of the study, five infants were transfused with CMV-seropositive blood components from 7 units (17 transfusions total). Two infants died while on study (10%), one infant was discharged early (Day 19), and one infant was lost to follow-up (transferred to non–study-affiliated hospital on Day 17). The remaining 16 infants reached 40 days on study. One CMV-NEG mother was tested for seroconversion at end of study and was CMV NAT negative.

One VLBW infant had positive blood and urine samples for CMV on Days 36 and 52, respectively. Blood tested low positive (<600 IU/mL) and urine had 289,063 IU/mL (Table 2). The infant's mother was CMV seropositive (IgG) and IgM negative. This infant received two RBC aliquot transfusions before testing CMV positive (on Days 13 and 23 of life) from one CMV-NEG unit; the unit tested CMV negative by NAT. The MOC's breast milk was collected and CMV NAT performed on Days 3 (indeterminate result), 19 (positive, 9251 IU/mL), and 46 (positive, 6422 IU/mL). A sample of donor breast milk that had been given to the infant was collected and tested on Day 3 (indeterminate result). These results rule out TT-CMV as the cause of infection; this infant very likely became infected due to feeding breast milk containing CMV, derived either from the mother or from donated breast milk.

The overall TT-CMV rate when using the LR-only transfusion strategy was 0% (0/8; 95% CI, 0%–25.3%), which is comparable to the 0% TT-CMV rate when using the CMV-seronegative plus LR approach (0/310; 95% CI, 0%–0.9%, EU study). Analysis of the blood products transfused during the course of the current study found that 50% of units (12/24) were CMV-NEG, 29.2% (7/24) were CMV seropositive, and CMV status was not known for 20.1% of units (5/24). Twelve of 24 units (50%) were available for testing with CMV NAT; all results were negative. Eight of 15 (53%) cellular blood products (6/10 RBC units, 2/5 PLT units) were tested for residual WBC count. WBCs were not detected in 2 units (<0.2 ×

10^6 cells/L) and were detected in 6 units (counts ranged 0.2×10^6–3.8×10^6 cells/L). The detectable counts all were below the LR failure lower limit of 15×10^6 cells/L.

DISCUSSION

We describe a pilot study investigating the mode of CMV transmission to VLBW infants who were treated at partner institutions that use a LR-only approach for protection from TT-CMV. Moreover, we have compared these results to the results of a larger study which employed the CMV-NEG plus LR approach and have found no differences in TT-CMV infection. These results have further provided important preliminary data to better ascertain feasibility around the ability to enroll mother/infant pairs at the Seattle sites, as well as the ability to gather historical CMV data on blood donors.

Our pilot study found no cases of infant TT-CMV, with a 60% seroprevalence among enrolled mothers. However, the study was not powered to detect this endpoint given the pilot design (Table 3). The CMV seroprevalence of mothers in Seattle was lower than that reported by the study at EU (76%; 352/462).[14] In the EU study, the cumulative incidence of postnatal CMV infection at 12 weeks was 6.9% (95% CI, 4.2%–9.2%) among 539 enrolled VLBW infants; five infants with postnatal CMV infection developed symptomatic disease or died. Although 58% (310/539) of infants received 2061 transfusions, none of the CMV infections were linked to transfusions, resulting in a CMV infection incidence of 0.0% (95% CI, 0.0%–0.3%) per unit of CMV-seronegative and LR blood. Twenty-seven of 28 postnatal infections occurred among infants fed CMV-positive maternal breast milk (12-week incidence, 15.3%; 95% CI, 9.3%–20.2%). Thus, transfusion of CMV-NEG plus LR blood products effectively prevents transmission of CMV to VLBW infants, and maternal breast milk is the primary source of postnatal CMV infection. Together, across the metro-Atlanta sites in the Emory study and Seattle, none of the 559 patients enrolled at either site had evidence of TT-CMV, even though the sites used different strategies to protect the infants from CMV transmission.

Historically, the study of TT-CMV in the clinical setting has been plagued with conflicting results. Two landmark studies of CMV-NEG–transfused adult hematopoietic stem cell transplant (HSCT) patients prospectively studied CMV acquisition and came to different conclusions. Bowden and coworkers[16] employed a randomized controlled trial (RCT) design of LR versus CMV-NEG cellular blood products in 521 HSCT patients and monitored for infection with culture and CMV antigenemia assay. They restricted their primary outcome to 21 to 100 days post-HSCT to exclude possible environmental CMV infection acquired pre-HSCT. The trial found a nonsignificant difference (1.3% in CMV-NEG vs. 2.4% in LR arms) of CMV breakthrough infection. Although not the primary endpoint, analysis of Days 0 to 100 post-HSCT, Bowden and colleagues found a significantly decreased rate (0% vs. 2.4%, respectively $p = 0.03$) of CMV infection. They concluded that LR is an acceptable alternative to CMV-NEG. The second study by Nichols and colleagues[17] used a prospective cohort design of 807 HSCT patients. They compared two periods: CMV-NEG or LR by direct filtration was provided (Period 1) and CMV-NEG or LR by apheresis collection was provided (Period 2), the difference being that the second period had a change in PLT collection technology which allowed more (potentially seropositive) LR products into the

inventory. The study found that patients in the first period received more seronegative blood products and had a lower rate of CMV antigenemia compared to patients in the second period who received more seropositive LR products and had more CMV breakthrough infection (1.7% vs. 4.0%, p = 0.05, respectively). The authors cautioned that moving to 100% LR blood products without regard for donor CMV serostatus, particularly in patients without active surveillance, may be unwise. However, they noted that those patients receiving CMV-NEG products only still experienced a 2% residual rate of CMV infection. More recently, a meta-analysis of TT-CMV prevention strategies did not find a superior approach, suggesting clinical equipoise.[18]

Until the publication of the Emory-conducted study, investigation of TT-CMV in pediatric populations had not been carried out on a large scale. A small Japanese RCT study of 80 infants, which did not control for maternal CMV seropositivity and used unbalanced randomization (more infants received LR), did not find a significant difference in CMV infection (9% vs. 5%, respectively) when patients were randomized LR versus non-LR using largely CMV-seropositive blood donor base.[19] The authors point out that in their CMV-hyperendemic region, the route of CMV infection is likely from sources other than blood transfusion. A larger study of 164 neonates comparing CMV-NEG to LR found 0 and 13.5% rates of CMV infection, respectively.[20] Of the 10 CMV-infected patients, half manifested serious or lethal CMV disease. The study used LR technology that is not current with today's highly efficient LR filters for WBC removal. The EU study provided statistically strong evidence that the CMV-NEG plus LR approach is protective against TT-CMV infection in transfused neonatal patients.

Although this study used a pilot design, it is the first of its kind that we are aware of to compare the safety of blood components transfused to VLBW infants (≤1500 g) that are both CMV-NEG plus LR to the LR-only approach in this preterm infant population. In the future, to be able to gain Level 1 evidence, a study that randomly assigns patients to CMV-NEG plus LR or LR-only transfusion approaches that is appropriately powered would be necessary. However, zero incidence of TT-CMV was found in both the CMV-NEG plus LR and LR-only cohorts; thus, finding a difference between these two groups would require a very large study population. For instance, assuming TT-CMV rates of 1 and 2% in the CMV-NEG plus LR cohort and LR-only cohort, respectively, a sample size of 3000 transfused infants per group (6000 total) would achieve 87% statistical power to detect at least a 1% difference in the percentages of infants with TT-CMV between groups. A RCT of this size would require a large amount of resources. Enrollment could be problematic if there is continued concern about the LR-only approach at some centers. An observational comparative effectiveness design would feasibly allow for the comparison of otherwise similar centers without randomization. Based on these pilot data, our estimate of the rate of TT-CMV in the LR-only cohort is 0%. Thus, the same sample size justification for a comparative effectiveness study, as used in the CMV-NEG plus LR cohort at EU would be appropriate; 300 transfused infants are needed to achieve an upper bound of 1% on the one-sided 95% CI for a proportion of 0. Expressly, this sample size is needed to replicate the results of the CMV-NEG plus LR cohort in an LR-only cohort to demonstrate that the LR-only transfusion approach is comparable to CMV-NEG plus LR approach in preventing TT-CMV. Although much smaller than the RCT, the comparative effectiveness study would still

require multiple study sites and would benefit from a central testing laboratory to normalize results.

Before a definitive study of TT-CMV transfusion prevention is completed, practical approaches are required.[21] The EU study found that breast milk largely accounts for mother-to-infant postnatal CMV transmission. In this study, one infant had CMV transmission from maternal breast milk, further supporting the evidence from the EU study. Further study of breast milk CMV transmission is needed to understand this important finding. RBC transfusion trigger studies to date do not yield consensus recommendations for when to transfuse; thus, it would seem prudent to not withhold transfusion due to concerns of TT-CMV in the age of universal LR, as the risk appears to be small.

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ABBREVIATIONS

CMV-NEG	cytomegalovirus seronegative
EU	Emory University
HSCT	hematopoietic stem cell transplant
LR	leukoreduction
MOC	mother of the child
RCT	randomized controlled trial
TT-CMV	transfusion transmission of cytomegalovirus
VLBW	very low birthweight.

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TABLE 1

Infant and maternal demographic and baseline characteristics[*†]

Infant (n = 20)	
Gestational age (weeks)	28.4 ± 3.0
Birth weight (g)	1031 ± 290
Male sex	12 (60)
Hispanic ethnicity	2 (10)
Race	
White	18 (90)
American Indian/Alaska Native	1 (5)
More than one race	1 (5)
Singleton birth	14 (70)
Born outside of study hospital	3 (15)
1-min Apgar score[‡§]	4 (2-6)
5-min Apgar score[‡§]	7 (6-8)
Score for neonatal acute physiology[§]	17.5 (12.5-20)
Born to a CMV-positive mother[‖]	13 (68)
Mother (n = 17)	
Age (years)	30.6 ± 6.4
CMV-positive serology[‖]	11 (68.8)
At least one prenatal visit	16 (94.1)
Premature rupture of membranes	8 (47.1)
Rupture of membranes greater than 18 hr	1 (5.9)
Chorioamnionitis	1 (5.9)
Cesarean delivery	12 (70.6)
Receipt of antenatal steroids	14 (82.4)
Indications for premature delivery[¶]	
Premature rupture of membranes (<37 weeks)	8 (47.1)
Bleeding complications	5 (29.4)
Preeclampsia	4 (23.5)
Fetal distress	4 (23.5)

[*] Unless otherwise noted, continuous variables are reported as mean ± SD and categorical variables are reported as number (%).

[†] Groups are compared with a two-sample t test for continuous variables and a chi-square test for categorical variables.

[‡] Apgar scores unavailable for three infants born outside of study hospital.

[§] Variable reported as median (interquartile range).

[‖] CMV serostatus was not determined for one mother.

[¶] Four most common reported indications given.

TABLE 2

Infant and breast milk CMV NAT results

CMV NAT	CMV NAT performed	CMV NAT positive
In infant blood		
Day of birth	16/20 (80%)	0/16 (0%)
Day 21	9/16 (56%)	0/8 [*] (0%)
Day 40/end of study	7/16 (44%)	1 [†]/7 (14.3%)
In infant urine		
Day of birth	13/20 (65%)	0/13 (0%)
Day 40/end of study	10/16 (63%)	0/10 (0%)
Unscheduled	8/20 (40%)	1 [†]/8 (12.5%)
In breast milk		
Week 1	14/17 (82%)	4/14 [‡] (28.6%)
Week 3	10/13 (77%)	6/10 (60%)
Day 40/end of study	8/13 (62%)	4/8 (50%)

[*] One test was not performed due to insufficient quantity of sample.

[†] One infant tested low positive for CMV (viral load, <600 IU/mL) in blood on Day 36 and subsequently tested positive for CMV (viral load, 289,063 IU/mL) in urine on Day 52; mother's breast milk tested CMV NAT positive on Day 19 (viral load, 9251 IU/mL) and Day 46 (viral load, 6422 IU/mL).

[‡] Two test results were indeterminate.

TABLE 3

CMV infection rates in infants and mothers

Group	LR-only cohort (n = 20)	95% CI	CMV-NEG plus LR cohort (n = 539)	95% CI
Overall	1/20 (5%)	0.9-23.6	29/539 (5.4%)	3.8-7.6
CMV infection by mother's CMV serostatus *				
Infants born to CMV− mothers	0/6 (0%)	0-31.1	0/127 (0%)	0-2.1
Infants born to CMV+ mothers	1/13 (7.7%)	1.4-33.3	29/412 (7.0%)	4.9-9.9
CMV infection by source				
TT-CMV †	0/8 (0%)	0-25.3	0/310 (0%)	0-0.9
Breast milk CMV ‡	1/8 (12.5%)	2.2-47.1	27/221 (12.2%)	8.5-17.7
Other §	0/20 (0%)	0-11.9	2/539 (0.4%)	0-2.0
CMV DNA lactia by mother's CMV serostatus *				
CMV-NEG mothers	0/4 ‖ (0%)	0-40.3	0/81 (0%)	0-4.5
CMV seropositive mothers	6/10 ‖ (60%)	31.3-83.2	189/255 (74.1%)	69.7-80.3

* Of 17 mothers enrolled, 16 were tested for CMV serostatus at baseline. A blood sample was not obtained for testing for one mother.

† TT-CMV reported in infants who received transfusions.

‡ Breast milk CMV reported in infants who received breast milk from mothers whose milk contained CMV according to NAT.

§ Vertical transmission or unknown source.

‖ Of five CMV-NEG mothers, four had at least one test result available; of 11 seropositive mothers, 10 had at least one test result available.