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**Pre-Emptive Screening Strategies to Identify Postnatal CMV Diseases on the Neonatal Unit**

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## **Background**

CMV is the most common congenital infection.<sup>1</sup> Congenital CMV (cCMV) is diagnosed if the virus is isolated in the first three weeks of life. It is challenging to differentiate between congenital and postnatal infection (pCMV) if the virus is detected after this time point.

Retrospective diagnosis of cCMV requires identification of the virus on the Dried Blood Spot, a method which has been shown to be insensitive.<sup>2</sup> Additionally, there are no internationally accepted definitions for symptomatic pCMV.

More than 90% of seropositive mothers shed CMV into their breast milk; breast milk is therefore an important mode of transmission of CMV to newborn infants.<sup>3</sup>

The great majority of term newborns acquiring CMV infection postnatally remain asymptomatic and have no long term consequences. This contrasts with cCMV infection and may be explained by the intensity and route of exposure: CMV viral loads in urine are lower in infants with pCMV compared to infants with cCMV.<sup>4</sup> Nonetheless very premature (gestational age <32 weeks) or very low birth weight (VLBW, <1500 grams) infants are susceptible to developing symptomatic illness following acquisition of CMV in the postnatal period. A recent prospective multicenter study in Atlanta of 539 VLBW infants revealed that the incidence of CMV acquisition at 12 weeks of age was 6.9% (95% CI, 4.2% - 9.2%).<sup>5</sup> Of these, 17% developed symptomatic disease or died.

Various clinical signs and syndromes have been described in infants with pCMV infection, including severe sepsis-like syndrome (SLS), pneumonia, hepatitis, renal impairment and thrombocytopenia.<sup>6</sup> Table 1 summarizes the clinical manifestations and investigations that should be considered in managing infants with pCMV.

A key issue is the potential for pCMV infection to cause adverse long-term outcomes. To date, studies investigating clinical sequelae have been small, single center and open to confounding due to study design. Although no impact on hearing has been reported in any study, a small case controlled study showed that children at school age who had pCMV as a preterm (through breast milk acquisition) had poorer cognitive function and motor skills, although still within the normal range, compared to controls.<sup>7</sup> Another prospectively controlled study of 42 VLBW infants showed that infants with pCMV had significantly lower cognitive results using the Kaufman Assessment Battery for Children (K-ABC).<sup>8</sup> Other studies of postnatally infected preterm infants have shown that white matter changes including lenticulostriate vasculopathy are more common than in non-infected infants, although the significance of this for long-term outcomes remains unknown.<sup>4,9</sup>

These potential long-term effects, in addition to sometimes very severe acute illness, raise the question of whether antiviral therapy may have a role in pCMV infection.

There are no controlled studies which have evaluated the efficacy or safety of antiviral or immunoglobulin based treatment for symptomatic infants with pCMV, nor are there robust data to show improved short or long term outcomes. In particular, there are very limited safety and pharmacokinetic data on antiviral treatment in preterm infants; the group most likely to have severe disease.

As a consequence, the evidence on which to base treatment guidelines for the management of pCMV is sparse. Many clinicians therefore reserve treatment for babies with significant disease.<sup>10</sup>

There are therefore significant uncertainties regarding the management of infants with pCMV disease, in part due to our limited understanding of the natural history of disease.

This review will therefore focus on possible strategies to minimize CMV transmission and disease in this vulnerable group by focusing on prevention and pre-emptive therapy.

### **Prevention.**

It is possible to prevent transmission of CMV by treating breast milk through heat pasteurization or freeze-thawing. However, freeze thawing does not fully prevent transmission and heat pasteurization can negate some of the benefits of breast milk by decreasing its fat and lactose constituents.<sup>11, 12</sup> The most recent conclusion from the American Academy of Pediatrics is that the benefits of giving fresh breast milk in CMV positive mothers outweigh the risks.<sup>13</sup>

To our knowledge, only one placebo controlled trial of CMV immunoglobulin has been published.<sup>14</sup> This study was conducted before CMV negative blood transfusions were used as part of routine clinical care and showed that immunoglobulin reduced the likelihood of transmission compared to placebo (12.5% VS 3.2%). The results of a prospective cohort study conducted in Solid Organ Transplant (SOT) recipients to evaluate the neutralizing capacity of monoclonal antibodies in preventing viral transmission are awaited (NCT01753167). Promising results in this population could lead to similar trials to reduce transmission in maternal-fetal transfer.

### **Pre-emptive screening in SOT recipients**

The adoption of CMV specific antiviral therapy screening strategies has reduced the incidence of CMV disease amongst SOT recipients.<sup>15</sup> Pre-emptive screening involves administration of antiviral therapy in response to laboratory triggers, such as increasing viral load. Pre-emptive treatment is used as an alternative to antiviral prophylaxis for SOT recipients at high risk of CMV disease who are prospectively screened for CMV viremia. This strategy is based upon

knowledge of the natural history of CMV viremia in adult SOT patients and recognition that a “threshold” can exist below which virus is tolerated without associated disease.

A meta-analysis by Strippoli concluded that compared with placebo or standard care, pre-emptive treatment significantly reduced the risk of CMV disease and comparative trials of pre-emptive therapy versus prophylaxis showed no significant differences in the risks of CMV disease.<sup>16</sup> These studies are limited to adult SOT populations and there are no data which define viral threshold limits in pCMV.

### **Identifying pre-symptomatic pCMV infection**

A successful pre-emptive screening strategy would require a readily available, rapid, sensitive and easy to use surveillance test for CMV detection. Real-time PCR on plasma or whole blood has been shown to be sensitive with turnaround times of 24 hours.<sup>17</sup> Point-of-care PCR assays are now also commercially available but have not yet entered clinical trials in neonates. Saliva swabs require no skills to obtain and are therefore a painless and highly accurate method of detecting the virus.<sup>17</sup>

### **Pre-emptive screening in preterms**

Serial saliva samples could be obtained in preterm infants to test for CMV DNA using PCR to enable early identification of infection. Developing a strategy whereby saliva samples are taken at regular time intervals by health care staff on the neonatal unit in infants less than 32 weeks and sent to a regional laboratory for same day testing is conceivable. Batch testing of samples would further reduce costs. When CMV is detected treatment could be started before symptoms develop.

Such a strategy could be coupled with prospective data collection in order to monitor outcomes and to model and define the relevance of different virologic parameters, using similar methodologies to those utilized in adult SOT populations.<sup>18</sup>

There are many questions which need to be addressed before considering a strategy of pre-emptive screening for pCMV. The clinical burden of pCMV disease is largely unknown, in part due to the limited long term outcome data available. No studies have yet shown that antiviral treatment in infants with asymptomatic CMV infection is beneficial and the side effects of therapy in this preterm population still need to be clearly defined.

Linking outcomes in this well-defined patient group to existing neonatal datasets would, however, enable detailed data collection and allow comparison with non-CMV infected groups to start to address some of these questions.

### **Conclusions**

Postnatal acquisition of CMV can cause severe acute disease in preterm infants but the longer-term consequences remain uncertain. The options available for prevention, such as pasteurization of breast milk, are limited and their evidence base is inadequate. An approach which has been successfully adopted in a different setting (adult organ transplantation) is pre-emptive screening of susceptible patients to detect pre-symptomatic infection and then treatment of those with a high risk of severe disease.

Recruiting subjects to a randomized controlled trial to evaluate the efficacy and safety of oral valganciclovir in postnatally acquired cases would take several years and need multiple recruiting sites due to the inherent difficulties of conducting antiviral treatment trials for rare diseases in the neonatal population. Disease registries, however, can efficiently collect high quality safety and pharmacokinetic data in infants treated with antivirals which in turn can be

used to guide dosing and duration of treatment recommendations. Improving our understanding of the epidemiology of pCMV disease is also essential to inform evidence based management.

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Table 1: Summary of risk factors, clinical features and investigations that should be considered when managing an infant with pCMV. This table was derived from data from key publications 3,13, 19

Risk factors	Gestational age <32 weeks, birth weight <1500gm, seropositive mothers breast feeding, early detection of virolactia, high virus burden in breast milk and long duration of virus detection in breast milk
Diagnosis	CMV positive sample (saliva or urine sample to test for CMV DNA PCR) detected >21 days of life and CMV negative sample collected <21 days of life
Clinical features	CMV sepsis-like syndrome (triad of apneas, bradycardias, grey / pallor), acute hepatitis, hepatosplenomegaly and CMV pneumonia, CMV enterocolitis, jaundice, cholestasis, petechiae and lymphadenopathy
Laboratory investigations	Full Blood Count (thrombocytopenia and neutropaenia) and Liver Function Test (elevated liver enzymes)
Neuroimaging	Serial cranial ultrasound (CrUSS) from diagnosis to hospital discharge to assess for cerebral abnormalities including lenticulostriate vasculopathy. Consideration of MRI if abnormalities seen on CrUSS
Treatment	Consider treatment in severely symptomatic infants using ganciclovir/valganciclovir. Any decision to commence treatment should be made after discussion with local pediatric infectious diseases team