Acquisition of cytomegalovirus infection by premature neonates

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Summary

In order to evaluate the risk of premature infants acquiring cytomegalovirus (CMV) infection, a prospective study was made of infants of 32 weeks gestation or less admitted to a neonatal unit. Infants were assessed at birth to exclude congenital CMV infection and followed to the age of 1 month post-term.

A total of 16/131 (12.2%) infants acquired CMV infection; 6 of CMV antibody-negative and 10 of CMV antibody-positive mothers. Six of 65 (9.23%) infants of CMV antibody-negative mothers who had been transfused with blood acquired CMV infection compared with 0 of 18 infants of CMV antibody-negative mothers, and who had not been transfused with blood.

Acquired CMV infection was also more common in infants of CMV antibody-positive mothers and who had been transfused with blood, i.e. 9 of 34 (26.47%) compared with 1 of 14 (7.14%). The relative risk was 3.7 (95% confidence interval 1.53-5.87).

This study suggests that all premature infants requiring blood transfusion should be transfused with CMV antibody-negative blood regardless of the CMV immune status of their mothers.

Introduction

Transfusions of blood for treating anaemia, hypotension, or chronic loss of blood are part of modern neonatal care. Many infants of very low birthweight receive multiple transfusions with small volumes of blood in the early weeks of life.1 Several reports have highlighted the risks of CMV infection in preterm babies who have been transfused with CMV antibody-positive blood and in whom serious multi-system illness may result.2-5 Furthermore, such infections take place when the babies are relatively immune deficient and when the nervous system is at a sensitive stage of development. CMV infection complicating broncho-pulmonary dysplasia considerably worsens the prognosis,6 and CMV infected babies may have an increased risk of developmental defects of the nervous system.7

We therefore made a prospective study in order to determine the frequency of acquired CMV infection in premature infants of less than 33 weeks gestation who had been transfused with blood from donors whose blood had not been routinely tested for CMV antibody.

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Methods

The study was in two parts. In part 1, we studied babies of 32 weeks gestation or less, who had received one or more blood transfusions. In part 2, we studied all babies of 32 weeks gestation or less whether or not they had been transfused with blood.

Clinical details which included mode of delivery, illness complicating the neonatal period and the method of feeding were recorded for all babies. Babies were either fed with untreated expressed maternal breast milk (EBM) or were breast fed directly. Some were given banked breast milk or drip breast milk (DBM) which had been pasteurised at 65 °C for 30 min so as to kill CMV. Various infant formulae or a combination of the above-mentioned feeds were given also.

From each mother, a blood sample was collected as soon after the birth as possible for serological testing. In relevant cases, antenatal blood samples, if available, were tested also.

From each baby, a cord blood sample or a blood sample collected in the first 2 days of life, before any blood transfusion had been given, was tested for CMV antibody and CMV-specific IgM. Isolation of CMV was attempted from a sample of urine obtained from each baby in the first week post-term before any transfusion of blood had been given. At one month post-term, serological tests and virus cultures were repeated. In study 2, weekly samples of urine were collected for CMV culture throughout each baby's stay in the neonatal unit.

A baby was recorded as having acquired CMV infection if an initial sample of urine was negative for CMV but CMV was grown from a subsequent sample.

Serology

Samples of serum were tested for CMV antibody by means of a complement fixation test based on the method of Bradstreet and Taylor. The CMV antigen used was supplied by the Division of Microbiological Reagents, Central Public Health Laboratory, Colindale, London. Samples having a CMV antibody titre of 8 or more were regarded as positive. CMV-specific IgM was detected by means of radioimmunoassay.

Detection of CMV in urine

From each baby, a 5 ml sample of urine was collected in 5 ml sorbitol transport medium and transported directly to the laboratory. Each sample was inoculated into each of two tubes of MRC-5 cells and incubated for 21–28 days at 37 °C. Cytomegalovirus was identified by its characteristic cytopathic effect and its identity confirmed by staining with peroxidase-labelled goat anti-CMV antibody.

Transfusions of blood

The dates and volumes of all transfusions of blood and the related donors' reference numbers were recorded. Citrate-phosphate-dextrose (CPD) blood obtained from the National Blood Transfusion Service and which was less
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than 5 days old was used. The CMV antibody status of donors was not known prospectively.

In the first part of the study, samples of all units of blood transfused were tested for CMV antibody by the complement fixation test described above. In the second part, samples of the blood used to transfuse the babies were stored and tested only when babies were found to be infected with CMV.

Results

In part 1 of the study, 69 babies were initially entered. Seven babies died and nine were lost to review. The remaining 53 babies were followed for up to one month post-term. Gestational ages ranged from 25–32 weeks (mean 29 weeks) and birthweights ranged from 663–2143 g (mean 1224 g).

Of the 53 babies, 10 acquired CMV infection. Three of them had CMV antibody-negative mothers and seven CMV antibody-positive mothers.

Nine of the infected babies, from whom CMV was isolated and who had serological evidence of CMV infection, were asymptomatic. The tenth infant (Case No. 7) developed CMV hepatitis at the age of 2 months. This was confirmed by a rise in titre of CMV antibody and a positive test for CMV-specific IgM, although CMV was not grown from this baby’s urine because of bacterial contamination. When he died from severe bronchopulmonary dysplasia, CMV was found in many organs post mortem. Part 2 of the study included 75 babies of whom three died. Of all the babies in part 2, 26 had CMV antibody-positive mothers while 49 had CMV antibody-negative mothers. Each of 40 babies received one or more blood transfusions. Of these, 11 were babies of CMV antibody-positive mothers while 29 were babies of CMV antibody-negative mothers. Of 32 babies who were not transfused, 14 were of CMV antibody-positive mothers and 18 of CMV antibody-negative mothers.

Of 72 surviving babies in this part of the study, six acquired CMV infection. Three were babies of CMV antibody-positive mothers and three of CMV antibody-negative mothers. Each of these babies, except one born to a CMV antibody-positive mother, received one or more blood transfusions. Interestingly, one of the babies of a CMV antibody-negative mother, and who became infected, received only one transfusion with blood that was CMV antibody-negative on testing. His twin brother, however, who was not transfused, did not show evidence of having acquired CMV infection at the age of 1 month post-term.

Of a total of 131 babies studied, 16 (12.2%) acquired CMV infection. Details of these babies are shown in Tables 1 and 2. Of 83 babies of seronegative mothers, 6/65 (9.23%), who were transfused, acquired infection, whereas none of 18 babies who were not transfused became infected.

Of 48 babies of CMV antibody-positive mothers, 9/34 babies (26.47%) who were transfused became infected compared with 1/14 (7.14%) who were not transfused. (Relative risk 3.7. C.I. 1.53–5.87).
Table I  *Babies born to CMV antibody-negative mothers*

<table>
<thead>
<tr>
<th>Baby no.</th>
<th>Gestation (weeks)</th>
<th>Birth weight (g)</th>
<th>Type of delivery</th>
<th>Age at which infection was confirmed (days)</th>
<th>Transfusions with CMV-positive blood/total transfusions</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>1212</td>
<td>VD</td>
<td>180</td>
<td>3/5</td>
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<td>26</td>
<td>875</td>
<td>CS</td>
<td>139</td>
<td>5/6</td>
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<tr>
<td>3</td>
<td>28</td>
<td>663</td>
<td>CS</td>
<td>159</td>
<td>2/9</td>
</tr>
<tr>
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<td>1556</td>
<td>VD</td>
<td>94</td>
<td>0/1</td>
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<tr>
<td>5</td>
<td>30</td>
<td>1380</td>
<td>CS</td>
<td>131</td>
<td>3/4</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>622</td>
<td>CS</td>
<td>63</td>
<td>7/9</td>
</tr>
</tbody>
</table>

VD, Vaginal delivery; CS, caesarean section.

Table II  *Babies born to CMV antibody-positive mothers*

<table>
<thead>
<tr>
<th>Baby no.</th>
<th>Gestation (weeks)</th>
<th>Birth weight (g)</th>
<th>Type of delivery</th>
<th>Age at which infection was confirmed (days)</th>
<th>Transfusions with CMV-positive blood/total transfusions</th>
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<td>1/1</td>
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<td>VD</td>
<td>105</td>
<td>1/3</td>
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<td>848</td>
<td>CS</td>
<td>152</td>
<td>1/7</td>
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<tr>
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<td>1458</td>
<td>CS</td>
<td>35</td>
<td>?/1</td>
</tr>
<tr>
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<td>30</td>
<td>1420</td>
<td>VD</td>
<td>157</td>
<td>1/1</td>
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<tr>
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<td>28</td>
<td>1279</td>
<td>VD</td>
<td>116</td>
<td>0/3</td>
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<tr>
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<td>940</td>
<td>CS</td>
<td>138</td>
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<td>35</td>
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<td>1487</td>
<td>VD</td>
<td>135</td>
<td>0/0</td>
</tr>
</tbody>
</table>

VD, vaginal delivery; CS, caesarian section.

**Maternal infection**

Combining both these studies, 58 / 171 (34%) pregnant women were CMV antibody-positive.

**Discussion**

In the neonatal period and early infancy, infants of CMV antibody-negative mothers may develop symptomatic CMV infection from transfusion of blood from a CMV antibody-positive donor or as a result of nosocomial infection. Infants of CMV antibody-positive mothers may also acquire CMV infection in the perinatal period from their mothers' cervical secretions during vaginal delivery or from breast milk, although symptomatic disease so acquired is rare.15, 16

Infants of CMV antibody-positive mothers are thought to be protected from symptomatic CMV infection by antibodies acquired antenatally. It may be, however, that the risk of symptomatic infection is higher in those born prematurely before they have received, transplacentally, adequate amounts of
maternal anti-CMV antibody. In such babies, the concentration of antibody is likely to fall even lower postnatally.\textsuperscript{16,17} Such babies may then be at risk of symptomatic disease derived from any of the above sources of infection.

Blood from CMV antibody-positive donors is potentially a major cause of CMV infection in all preterm babies and such babies may become seriously ill. Ballard et al.\textsuperscript{6} found the babies to have respiratory deterioration, grey pallor, enlargement of the liver and spleen as well as lymphocytosis. In the study of Yeager et al.,\textsuperscript{7} half of the infected babies had serious manifestations such as pneumonia, hepatitis, haemolysis, and thrombocytopenia. Sawyer et al.\textsuperscript{6} showed a 75\% incidence of bronchopulmonary dysplasia in infants who acquired CMV infection compared with 38\% in uninfected controls. Moreover, infected infants required more respiratory support and a longer stay in hospital than uninfected infants.

The risks of transfusing the premature infant of a CMV antibody-negative mother with untested blood are therefore well established. This study confirmed that babies of CMV antibody-negative mothers and who were not transfused did not acquire CMV infection whereas 6/65 (9.23\%) of the transfused babies of CMV antibody-negative mothers acquired the virus.

It is interesting to note that one baby of a CMV antibody-negative mother, and from whose urine CMV was isolated, received only one transfusion with blood reported to be negative for CMV antibody. The urine of his twin, who was not transfused, did not yield CMV in culture. This observation suggested that nosocomial infection was unlikely. The blood may have been incorrectly classified as CMV antibody-negative.\textsuperscript{18}

In this study, babies of CMV antibody-positive mothers were also at greater risk of acquiring CMV infection if they were transfused (26.5\% compared with 7.14\%).

One baby (case no. 7) is of particular interest and details of his case have been published elsewhere.\textsuperscript{14} He developed fatal infection despite having been born to a CMV antibody-positive mother. The evidence suggested that blood-transmitted infection was very likely. No other baby developed clinical signs of CMV infection but the design of the study did not allow detailed clinical review of all babies.

All the CMV-infected babies of CMV antibody-positive mothers, except one, received one or more blood transfusions. Seven babies received one or more transfusions of blood that was recorded as positive for CMV antibody. All these babies, however, had received some of their mothers expressed, untreated breast milk and five of them were born by vaginal delivery. Babies of CMV antibody-positive mothers were, perhaps not surprisingly, more likely to become infected than those of antibody-negative mothers. Maternal sources of infection must, therefore, be implicated in these infections. Babies of CMV antibody-positive mothers can be infected from maternal sources despite their having passively acquired antibody but overt infection is rare. It was impossible to define the exact source of infection in these babies. Even so, this study showed that infants of CMV antibody-positive mothers had a relative risk of acquiring CMV infection of 3.7 (C.I. 1.53–5.87) if they were transfused in the neonatal period as compared with untransfused infants of CMV antibody-positive mothers.
These data confirm the need for all very preterm babies to be transfused with CMV antibody-negative blood, regardless of the CMV status of their mothers. This practice is now becoming routine in neonatal units in the U.K. Furthermore, infants of extremely low birthweight of antibody-positive mothers may be at some risk of acquiring CMV infection from maternal sources, such as untreated maternal breast milk, at a time when their antibody concentrations are low. More studies aimed at investigating this risk would be worthwhile.

References