

# Joint UKBTS / NIBSC Professional Advisory Committee (\*)

## Position Statement

### CMV Seronegative vs. Leucodepleted blood components for at risk recipients

14 November 2007 (confirmed November 2008)

*Prepared by:* Standing Advisory Committee on Transfusion Transmitted Infections.

*November 2008 - The contents of this document are believed to be current in the absence of any new information since November 2007. Please continue to refer to the website for in-date versions.*

## Executive summary

- The balance of evidence from clinical studies suggests that acceptable CMV safety can be achieved by pre-storage leucodepletion.
- It is likely that leucodepleted blood components are comparable to seronegative blood components with respect to the risk of transfusion-acquired CMV.
- However, there is insufficient evidence to date to firmly recommend the discontinuation of CMV serological testing in the context of leucodepletion.
- It is unlikely that this position will change unless suitable randomised, prospective, controlled trials are conducted in the future to answer this question.
- The requirement for CMV serological testing could be reviewed in the event that the use of pathogen inactivated components is generally adopted.
- Tests to detect CMV DNA in blood donors do not add an increased level of detection beyond that provided by current serological screening assays.
- In a clinical situation where a patient requires a blood component with some urgency and a suitable CMV antibody negative component is not available, it is reasonable for the doctor to prescribe a leucodepleted component that is fully compliant with the current Red Book specifications for leucodepletion.

## 1. Purpose of this paper

To provide a position statement on the efficacy of CMV testing vs leucodepletion in the light of recent publications and to determine if there is any evidence to suggest that CMV serological testing can be abandoned in the context of leucodepletion. This does not represent an exhaustive literature review or detailed analysis of different testing options.

## 2. Background of CMV

Human CMV is a highly species specific  $\beta$  herpes virus which solely infects humans. Primary infection, i.e. the infection of normal individuals previously uninfected by CMV, rarely causes severe disease. Commonly acquired in childhood, the infection is mild and usually is limited

## **Joint UKBTS / NIBSC Professional Advisory Committee (\*)**

### **Position Statement**

#### **CMV Seronegative vs. Leucodepleted blood components for at risk recipients**

**14 November 2007 (confirmed November 2008)**

to a non-specific pyrexial illness. In adults, it may cause a Paul Bunnell negative glandular fever-like syndrome with prolonged malaise and occasionally organ specific disease, e.g. hepatitis. In the elderly adult the primary infection, often acquired from young children, may cause a severe and prolonged feverish illness. In the immunocompetent child and adult, the acute illness of primary infection is self-limiting, and followed by life long viral persistence with clinical rather than viral latency. The sites of viral persistence remain undefined. They probably include CD34+ haemopoietic progenitor cells in the bone marrow and CD13+, CD14+ peripheral blood monocytes.

The prevalence of seropositivity is age dependent. CMV may be first acquired early in infancy, from either the mother who may shed the virus during the puerperium or from other children. The prevalence of infection rises again with the onset of sexual maturity and continues to rise with age. Estimates in adults vary from country to country. In a study of blood donors in the West of Scotland in the 1980s the prevalence of anti-CMV was 26% aged 18-25 years, 44% aged 26-35 years and 73% aged 46-55 years (B. Dow, personal communication). The seroconversion rate in seronegative blood donors is estimated to be approximately 1% per year.<sup>1</sup>

Other than during the acute, pre-seroconversion phase of primary infection, CMV-infected hosts can best be identified by the detection of antibody to CMV (anti-CMV). In effect, all people who have serum anti-CMV are infected with CMV. However not all will have virus replication at a level which either leads to viral shedding (infectivity) or is manifest by detectable virus in the peripheral blood. Bursts of viral replication are not uncommon, as is true for all the  $\alpha$  and  $\beta$  herpes viruses, and may be triggered by inter current infections, stress, immune activation and most importantly by any cause of immunosuppression which allows CMV to escape from immunological control. Clinical disease is the result of virus induced cytopathology in a number of organs and tissues. This most seriously arises in patients whose immune system is damaged and is associated with high levels of virus in the infected organs, in blood and in body secretions. The most severe form of clinical disease is primary CMV infection in an immunocompromised individual. This presents as an acute, pyrexial multiorgan disease and is often fatal in spite of antiviral therapy. Equally important but of more indolent onset is end organ CMV disease in seropositive patients who are immunosuppressed either through intercurrent disease (e.g. HIV infection) or through drug therapy. The manifestations are protean, and diagnosis may be difficult, requiring tissue from the affected organs. Usually there is also systemic CMV infection with high levels of virus and a high viraemia.

CMV transmission to immunocompetent hosts rarely causes clinically important consequences.

There are two main strategies for reducing the risk of transfusion-transmitted (TT) CMV to high-risk recipients of blood component transfusion. These are:

- Excluding the use of blood components from seropositive donors
- Leucodepletion of blood components

There is extensive literature which deals with the efficacy and limitations of these methods in preventing TT-CMV. Both methods may fail (around 1% failure rate) and this has led to

# Joint UKBTS / NIBSC Professional Advisory Committee (\*)

## Position Statement

### CMV Seronegative vs. Leucodepleted blood components for at risk recipients

14 November 2007 (confirmed November 2008)

controversy over the merits of serological screening versus leucodepletion. The occasional report of infection by CMV antibody negative blood components may be due to the presence of plasma viraemia prior to seroconversion or the failure correctly to identify seropositive donors. Infection attributed to leucodepleted units may be due to failure to achieve adequate removal of white cells or to persistent or recurrent plasma viraemia so that infective virus traverses the filter, allowing transmission to occur.

### 3. Risk of transfusion-transmitted CMV in the UK

#### 3.1. Donor and recipient factors

Recipients at risk are almost exclusively those with some level of immunosuppression. To acquire CMV from a blood donor, a blood transfusion recipient must be exposed to donor white cells that are infected with CMV. CMV then must be reactivated from these cells, and the cell must survive long enough in the host to release the infectious virus. Plasma viraemia may also explain some cases of TT-CMV and in a recent study plasma CMV DNA was detected in a small percentage of seroconverting blood donors, although not in donors with CMV antibody.<sup>2</sup>

Factors that influence these events include the type of blood component unit transfused and the age of that unit. Recipient factors that influence the risk of CMV disease include the number and the type of blood components received, HLA-matching between donor and recipient, the degree of immunosuppression of the recipient and the cytokine environment present in the host.<sup>3</sup> For example, patients with prolonged sepsis and burns who have increased cytokine production may be particularly vulnerable to CMV reactivation. Another important recipient factor, in bone marrow transplant patients, is the use of anti-viral treatment (ganciclovir) which can prevent both re-activation of the patient's own latent virus and potential transfusion-transmitted infection. For anti-viral treatment to be effective routine quantitative monitoring for CMV load in blood is necessary.

The pathogenesis of transfusion-acquired CMV can be summarised as complex, involving multiple donor and recipient factors. It is therefore difficult to assign specific risk per blood component transfused and to extrapolate the efficacy of CMV risk mitigation strategies from one recipient population to another.<sup>1</sup>

### 4. Detection of CMV infection

Detection of individuals likely as donors to transmit infection is best done by seeking anti-CMV. For investigation of clinical disease the presence of virus is the bench mark method.

#### 4.1. Detection of anti-CMV

##### 4.1.1. ELISA based assays (EIA)

Current serological tests are sensitive, specific, rapid and automated for high sample

## **Joint UKBTS / NIBSC Professional Advisory Committee (\*)**

### **Position Statement**

### **CMV Seronegative vs. Leucodepleted blood components for at risk recipients**

**14 November 2007 (confirmed November 2008)**

throughput. Many of these assays are biased in favour of IgG detection. Newly infected or window-period donors are normally IgM predominant and it has been questioned whether it would therefore be preferable to use an EIA which will detect efficiently both CMV-specific IgG and IgM. However, the reported prevalence of CMV-IgM in blood donors is highly variable, ranging from 13% (indirect immunofluorescence) to 0.9% (EIA) and the sensitivity and specificity of this marker for predicting the infectivity of blood components is considered to be poor<sup>1</sup>. It should also be noted that discrepant results may arise even between sensitive and specific serological assays, such that false negative (and false positive) results are not uncommon.<sup>4</sup> Assays currently available and any new assays are subject to a process of assessment (of sensitivity and specificity) and approval by an appropriate Kit Evaluation Group.

#### **4.1.2. Latex agglutination assays**

This method is quick and easy to perform but is expensive and not easily automated. It is less sensitive than EIA.

### **4.2. Detection of CMV**

#### **4.2.1. CMV nucleic acid testing (PCR)**

In the recent past this methodology has virtually replaced culture based systems. There are differing opinions over which analyte is best for routine use. Though one can use the whole range of fluids previously acquired for culture purposes, the only appropriate one for blood donors is blood.

CMV can be found in the blood of patients with CMV-disease in both the whole blood, cellular components and in the plasma. Whole blood or plasma CMV DNA quantification is used widely for monitoring bone marrow patients after transplantation. The assays used nowadays still vary considerably in sensitivity and users must define this parameter. A recent paper on this subject concluded that CMV DNA is only rarely detectable in the whole blood of seropositive donors and that the use of both commercial and in-house CMV PCR assays of moderate sensitivity but with optimal performance characteristics did not increase the detection of potentially infectious blood components beyond that provided by current serological screening assays.<sup>4</sup> It was considered that more data are required to determine the efficacy of CMV NAT for the prevention of TT-CMV. These include further studies on the incidence of CMV reactivation and primary CMV in blood donors, duration of infectivity and their contribution<sup>5</sup>.

#### **4.2.2. Tests for virus antigens in blood or plasma<sup>6</sup>**

This method is based on detection of CMV tegument phosphoprotein pp65. This is relatively insensitive at low viral loads and therefore has limited value in determining CMV status of latently infected blood donors.

## **Joint UKBTS / NIBSC Professional Advisory Committee (\*)**

### **Position Statement**

### **CMV Seronegative vs. Leucodepleted blood components for at risk recipients**

**14 November 2007 (confirmed November 2008)**

#### **5. Leucodepletion**

CMV is transmitted via leucocytes so that removal of these may be an effective way of reducing risk of CMV transmission. There is continuing debate about the relative effectiveness of leucodepleted and CMV seronegative blood components in minimising the risk of TA-CMV infection. The degree of leucocyte removal required to prevent CMV transmission is not known.<sup>3</sup> The American Association of Blood Banks has suggested that residual leucocyte levels  $<5 \times 10^6$  render blood components CMV "safe" but in theory a single latently infected donor leucocyte could be sufficient to infect a recipient, although this seems unlikely in practice, and the term "safe" may be highly recipient dependent.<sup>1</sup>

The current UK specification is that  $>99\%$  of components should achieve  $<5 \times 10^6$  residual leucocytes/unit of red cells or adult therapeutic dose of platelets with 95% confidence. Numbers tested are not less than 1% of production. Failure rates are currently around 0.2%. The Council of Europe guidelines<sup>7</sup> specification is  $<1 \times 10^6$  per unit and this lower limit is achieved by the National Blood Service (NBS) on most occasions (failure rates up to ~2%).

A study of CMV-spiked units of whole blood and platelets, in which the above level of leucodepletion is achieved, has shown that although leucodepletion by filtration reduces the viral burden it does not completely remove CMV from blood components.<sup>8</sup> Extrapolating the results of this spiking study to the likely viral burden in CMV seropositive donors suggests that leucodepleted components will contain no more than 0.01-0.1 viral copies/ $\mu$ l and it is not known whether this level is capable of infecting at risk recipients.

Pre-storage leucodepletion of blood components may reduce the risk of CMV transmission by reducing the number of latently infected cells infused and by decreasing the probability of initiating CMV reactivation events driven by cytokine release from donor leucocytes in a blood component before infusion.<sup>1</sup> In addition, the transfusion of allogeneic lymphocytes constitutes an immunological stimulus which provides a trigger for recipient CMV reactivation and therefore leucodepletion may reduce the risk of CMV by reducing the probability of an allogeneic reaction.<sup>1</sup> There is evidence that bedside filtration may be less effective.

#### **6. CMV testing vs. leucodepletion**

Numerous trials have shown the efficacy of leucodepletion in the prevention of post-transfusion CMV infection in various patient populations. However most of the trials were small case series with large 95% confidence intervals. Pamphilon et al reviewed 9 reports of patients, mainly with haematological disease, in whom a combination of leucocyte-reduced and CMV seronegative components were used to prevent TT-CMV.<sup>9</sup> They concluded that there is evidence to suggest that both approaches are equally effective. A Canadian Consensus Conference in January 2000 was not able to reach a consensus with regard to the question of whether or not CMV serological testing could be abandoned in the setting of pre-storage leucodepletion and 7 of the 10 expert panellists recommended continued provision of both white cell reduced and CMV seronegative blood components for certain at risk groups.<sup>10</sup>

## **Joint UKBTS / NIBSC Professional Advisory Committee (\*)**

### **Position Statement**

#### **CMV Seronegative vs. Leucodepleted blood components for at risk recipients**

**14 November 2007 (confirmed November 2008)**

There is only one prospective, randomised, controlled trial (in 1995) which compared the use of bedside-filtered leucodepleted blood components with CMV-seronegative components for the haematological support of 502 CMV-seronegative BMT recipients.<sup>11</sup> The incidences of CMV infection between the two groups were not significantly different. This study concluded that leucodepleted blood components are comparable to seronegative blood components with respect to the risk of CMV transmission to BMT recipients. There has been controversy over this conclusion as the incidence of CMV disease was significantly higher in the filtered arm group (2.4% vs. 0%). Five of the 6 patients who developed CMV infection whilst receiving filtered components died of CMV pneumonia. It is important to note that bedside filtration (which is less reliable than pre-storage leucodepletion) was used in this study and that the method used for the detection of sero-positive donors was the latex agglutination assay, which is less sensitive than ELISA.

Two retrospective studies have drawn differing conclusions. The impact, on the development of CMV disease, of using leucodepleted platelets in 215 allogeneic bone marrow transplant recipients, where both donor and recipient were CMV seronegative showed no difference when compared to historical controls treated with CMV seronegative blood components<sup>12</sup> This contrasts with the most recent and largest study which concluded that it would be premature to abandon CMV serological testing even if universal leucodepletion were in place.<sup>13</sup> This study reported that the use of seropositive, leucodepleted red cells was associated with the development of post-transfusion CMV infection but the results should be interpreted with caution. Although based on a large patient cohort it was retrospective in nature and poor study design included the lack of defined treatment arms and the low usage of CMV seropositive units. These weaknesses combined with incomplete statistical analysis and poorly controlled confounding factors (such as an increased use of all blood components, irrespective of CMV status in those who became CMV infected) produced data that is considered to be of questionable validity.

In summary, over the years there have been a large number of studies examining strategies to prevent transfusion-acquired CMV. Interpretation of these is difficult as many were not randomised, failed to use a control group or used historical controls or were retrospective. Other confounding factors have been changing transfusion practices over time (for example variable efficacy of leucodepletion and sensitivity of serological tests) and geographic variability in risk in defined populations.<sup>1</sup>

### **7. Conclusion**

The balance of evidence from clinical studies suggests that acceptable CMV safety can be achieved by pre-storage leucodepletion. Although it is likely that leucodepleted blood components are comparable to seronegative blood components with respect to the risk of transfusion-acquired CMV, there is insufficient evidence to date to firmly recommend the discontinuation of CMV testing in the context of leucodepletion. It is unlikely that this position will change unless suitable randomised, prospective, controlled trials are conducted in the future to answer this question. The requirement for CMV testing could be reviewed if pathogen reduction of components were implemented. Tests to detect CMV DNA in blood donors do not appear to add an increased level of detection beyond that provided by current serological screening assays.

## **Joint UKBTS / NIBSC Professional Advisory Committee (\*)**

### **Position Statement**

### **CMV Seronegative vs. Leucodepleted blood components for at risk recipients**

**14 November 2007 (confirmed November 2008)**

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## **Joint UKBTS / NIBSC Professional Advisory Committee (\*)**

### **Position Statement**

#### **CMV Seronegative vs. Leucodepleted blood components for at risk recipients**

**14 November 2007 (confirmed November 2008)**

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