Serological screening for cytomegalovirus in a leucodepleted blood supply: a systematic review.

Running title: CMV and leucodepletion

Authors

Philip J. Crispin [1,2,3]

Danielle Edwards [4]

Karen Beattie [1,5]

Kristen Brown [1,6]

Lisa Clarke [1,7,8]

Fiona King [1,9]

Amanda Ormerod [1,10]

Bryony Ross [1,11,12]

Affiliations

1. Australian and New Zealand Society of Blood Transfusion Clinical Transfusion Practice Committee, Sydney, NSW, Australia
2. Australian National University School of Medicine and Psychology, Canberra, ACT, Australia
3. Haematology Department, Canberra Hospital, Garran, ACT, Australia
4. Hunter New England Local Health District, NSW Australia
5. Western New South Wales Local Health District, Orange, NSW Australia
6. Murrumbidgee Local Health District, Wagga Wagga, NSW Australia
7. Australian Red Cross Lifeblood, Sydney, NSW Australia
8. Sydney Adventist Hospital, Sydney, NSW, Australia
9. New Zealand Blood Service, Wellington, New Zealand
10. La Trobe Regional Health, Traralgon. Vic, Australia

11. John Hunter Children's Hospital, Newcastle, NSW, Australia
12. University of Newcastle, Newcastle, NSW, Australia

Corresponding author

A/Prof Philip Crispin

Chief Pathologist and Clinical Haematologist

Canberra Hospital

PO Box 11 WODEN ACT 2606 Australia

Philip.crispin@anu.edu.au

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Abstract

Background and objectives:

Cytomegalovirus causes harm in at risk populations. Selection of seronegative donors has been used to prevent transmission. Leucodepletion reduces the potential for cytomegalovirus transmission, however the residual risk is uncertain leading to variability practice. This study systematically reviews the risk of cytomegalovirus transmission in leucodepleted blood products compared to seronegative blood products.

Materials and Methods: A systematic review identified comparative studies of cytomegalovirus infections rates following transfusion of leucodepleted blood from cytomegalovirus negative or unselected donors. Preclinical studies on blood product cytomegalovirus transmission, reported cases and studies that informed population risk were also reviewed. Meta analysis was performed on comparative studies.

Results: There was no difference in the rate of infection following transfusion of leucodepleted cellular products with or without donor cytomegalovirus seronegativity selection, with a relative risk of 1.21 (95% CI 0.42-3.49). No confirmed cases of cytomegalovirus transmission were found. Pre-clinical studies show a significant reduction in transmissible virus with leucodepletion, although no threshold could be defined. Cell free cytomegalovirus is not removed by filtration and although it may remain a potential source of infection, there was no evidence of transmission through plasma, possibly due to detectable virus not reflecting intact transmissible virus.

Conclusion: Selecting cytomegalovirus seronegative donors did not reduce the risk of transmission when transfusing leucodepleted blood products due to high efficiency of filters in removing transmissible cellular virus. This finding suggests cytomegalovirus donor negative selection does not substantially contribute to donor safety.

Keywords: Cytomegalovirus, transfusion transmitted infection, leucodepletion, donor testing

Highlights

- Cytomegalovirus is potentially transmitted through blood and can cause severe problems in susceptible recipients
- There is no clear evidence of CMV transmission with transfusion of leucodepleted blood products, irrespective of CMV serological status of the donor
- The selection of CMV negative blood products in leucodepleted blood supplies does not add to recipient safety and removing CMV negative requirements for all transfusion recipients may improve inventory management

Introduction

Cytomegalovirus (CMV) is a human herpes virus considered clinically relevant in transfusion medicine. Transmission was first reported in the 1960s, explaining a frequently observed mononucleosis syndrome after cardiac surgery.[1] Although some have questioned it,[2] molecular characterisation of virus has confirmed transmission via blood.[3] High rates of community transmission confound attribution of a post transfusion infection to blood. Release into saliva, urine and breast milk are common, may be prolonged after infection and may re-emerge during otherwise latent lifelong infection.

CMV can lead to diverse clinical manifestations. Classically described as a mononucleosis-like illness in immunocompetent people, it may also be asymptomatic. In a look-back of blood donors, viral symptoms were frequent and similar between seroconverting and control donors.[4] In immunocompromised donors retinitis, pneumonitis, hepatitis, enterocolitis and marrow suppression are common and have been common causes of death, particularly in the post-transplant setting, both from primary infection and reactivation. Congenital CMV is the most common congenital infection in humans and is acquired from the mother during pregnancy. It is more likely in maternal primary infection during or leading up to pregnancy than reactivation or secondary infection. While transmission rates appear lower during first trimester, the resulting disease is more severe, with few longer-term severe sequelae if acquired in second and third trimesters.[5] Deafness and neurodevelopmental delay can develop during childhood even in children asymptomatic at birth. CMV is the most common congenital infection in humans. Prophylactic antiviral therapy has been used in immunocompromised patients with rising CMV viral loads and has recently been recommended also for women acquiring CMV in early pregnancy.[6, 7]

Leucocytes, and in particular monocytes, are known to latently harbour CMV and are considered the major source of CMV transmission in blood products.[8, 9] Cell free CMV deoxyribonucleic acid (DNA) may be seen in plasma, but there is at most a low possibility of transmission and plasma is not supplied based on CMV serostatus. The addition of leucocyte reduction for cellular blood products is expected to reduce infection risks for leucocyte associated infections, including CMV. Furthermore, the

potential for CMV to be present in window period donations after recent CMV acquisition has highlighted that seronegative products are not absolutely risk free. Low levels of viral DNA in long term seropositive donors also suggest that seropositive units from otherwise well donors are also a relatively low risk.

CMV serologically negative cellular products were initially recommended for the prevention of transfusion transmitted CMV (TT-CMV). The addition of leukodepletion significantly reduces the risk. A prior systematic review determined that there was insufficient evidence for any one approach to CMV prevention over another.[10] The number of patients in comparative studies was small, so although there was a trend towards improved safety with CMV negative blood, there was a high level of uncertainty. Consequently there is widespread variation in clinical practice and in practice guidelines.[11-17] Expert opinion-based guidelines have increasingly limited the target populations where CMV negative blood is recommended and some centres no longer recommend CMV seronegative units in addition to leucodepletion. The previous systematic review included only comparative studies, since it sought to compare two approaches. Additional comparative studies have since been reported to add further data. Furthermore, the addition of risk assessments based on TT-CMV cases from leucodepleted blood and the preclinical studies are useful to determine the risk of TT-CMV. This study aimed to review the evidence for requiring CMV seronegative blood in addition to standard of care leucodepletion.

Methods

The study aimed to determine whether transfused patients receiving blood from CMV serology negative donors reduces the rate of cytomegalovirus infection compared with unselected donors when all transfusions undergo pre-storage leucodepletion. The study did not restrict the population, noting that the potential for transmission is largely dependent on the product, while the clinical outcome is heavily dependent on risk factors in the population.

The review undertook a single search to retrieve articles in different evidence domains. Using the terms transfusion and cytomegalovirus, searches were performed in May 2024 in Ovid Medline, PubMed, the Cochrane Library. Articles were restricted to those published in English from 1990. Prospero was reviewed for

ongoing research or completed protocols. Additional cases were sought from Serious Hazards of Transfusion (SHOT) reports and Australian haemovigilance data.

Titles and abstracts were reviewed for selection by two reviewers (PC, DE) and where assessments were discrepant, independently by a third (BR). Full text articles were further reviewed and articles selected to meet the following domains and inclusion criteria:

1. Comparative studies reporting frequency of CMV in leucodepleted blood recipients since 1990, including randomised and non-randomised, cohort and case-control studies. Systematic reviews were also retrieved.
2. Studies that informed the risk associated with leucodepleted products, including reports of donor population prevalence, pre-clinical studies of leucoreduction efficacy and studies that calculated residual risk in leucodepleted blood product recipients
3. Confirmed cases of CMV with leucodepleted blood since 1990. Due to the frequency of alternate transmission routes, transfusion transmitted cases were defined as:
    - Definite: Confirmed CMV in a transfusion recipient genotyped to match CMV from the donor
    - Probable: Primary infection in a blood transfusion recipient within three months of a transfusion and where the donor has confirmed seroconversion or symptomatic CMV
    - Where CMV infection was defined as viraemia, or persistent seroconversion or nucleic acid detection not attributable to passive transfer

The primary endpoint was the difference in rate of CMV infection in comparative studies with and without donor CMV serology testing. Secondary endpoints included the number of definite of probable infections in each group and derived risk estimates, the rates of CMV transmission with plasma products, estimated residual transmission risk associated with leucodepletion and comparisons of CMV negative unfiltered blood with filtered blood. Methodological quality of studies was undertaken for comparative studies using ROBINS-I or RoB2 for non-randomised and randomised studies, respectively.[18, 19] Where appropriate, meta-analysis was

performed for comparative studies, assuming a random effects model using MetaAnalysisOnline.[20] Differences were considered to be statistically significant when $p<0.05$. Rates of CMV were drawn from studies reporting them after leucodepleted transfusion with descriptive and pooled estimates reported. The number of transfused unit donor exposures was calculated from studies when reported. Where pooled platelets were reported, a pool was considered to be 4 units unless otherwise stated. In order not to underestimate risks, the lowest possible estimate was used, which equalled the study population if the number of transfusions was not stated.

Results

The primary literature search found 1591 unique references. Of these, 129 full text articles were selected for review based on screening of title and abstract. An additional three papers were identified from review of references. Studies that determined population or donor population frequency constituted the largest group of studies (n=37), followed by review papers (n=33). Single arm studies reporting on infection frequency after transfusion (n=11) and comparative studies (n=5) were included in the numerical analysis. Studies exploring pre-clinical measures of filter efficacy (n=4) and residual risk estimates were also included. The selection of studies is shown in Figure 1.

There were no randomised trials meeting inclusion criteria for the primary analysis. A single randomised study compared unfiltered CMV negative transfusions with leucodepleted blood. There was no difference in the rate of CMV infections between the two groups in the a priori analysis of infections between days 21-100 after seronegative donor and recipient bone marrow transplants, or in secondary analysis with infections from day 0 to day 100.[21] As all infected patients in the filtered blood arm developed CMV disease, and none in the seronegative arm, there was an unexpected difference in CMV disease in secondary analysis. Most infections in the first 21 days were considered most probably recipient-derived due to equivocal CMV serology at baseline. The study was thought to have some concerns for risk of bias due to the number and asymmetrical nature of protocol violations (Table 1).

There were three observational studies comparing CMV negative and leucodepleted blood products with leucodepleted alone.[22-24] These all studied rates of CMV viraemia by polymerase chain reaction (PCR) in seronegative recipients of seronegative bone marrow transplants who underwent weekly monitoring. This model minimises ascertainment bias and overall the reports were rated as low risk of bias (Table 1), The three studies had low clinical and statistical heterogeneity and showed no difference in the rate of CMV infection in CMV negative and leucodepleted transfusion recipients compared with leucodepletion alone (RR 1.21, 95% CI 0.42-3.49, Figure 2).

A fourth comparative observational study determined CMV infection rates after CMV negative or leucodepleted transfusions.[25] The study arose following the observation of an increase in CMV cases, in the bone marrow transplant setting. The authors hypothesised that this was related to apheresis platelet preparation, which was disproven. Secondary post-hoc analysis found an increased rate of CMV infections after transfusion with leucocyte filtered red cells (OR 1.32, 95%CI 1.08-1.61), Multiple secondary analyses reported on only one of two cohorts and unrelated to the primary hypothesis led to a classification of serious risk of bias. Leucocyte depleted platelets were not associated with an increased risk of CMV (OR 1.02, 95% CI 0.97-1.08). This study was judged to be at critical risk of bias and excluded from the primary analysis, although the reported rates of CMV were included in totalling all cases as risk of bias was not performed on all studies included in this analysis.

A sensitivity analysis including all studies that compared CMV serologically negative (with or without leucodepletion) and leucodepletion alone was also performed. There were 5 studies included with 1367 patients showing no difference between treatment arms. The risk ratio for leucodepletion was 0.67, 95% CI 0.33-1.38 (figure 3). In addition, we calculated using a binomial model [26] that a hypothetical randomised controlled trial to compare CMV seronegative and leucodepleted products with leucodepleted alone would require over 275000 patients to have 80% power of showing the difference observed in our primary analysis within 95% confidence limits.

The rates of CMV in recipients post leucodepleted transfusion were reported in 19 observational studies, including the 4 comparative studies noted above.[22-25, 27-40] The settings included stem cell transplantation to seronegative recipients, neonatal intensive care and chronically transfused patients. The were a median of 63 (range 23-235) patients receiving leucodepleted only products in 18 studies and 105.5 (range 33-310) patients in 4 studies who had both leucodepleted and CMV seronegative transfusions (Table 2). There were 29 CMV cases 1323 patients receiving leucodepleted only units (2.2%, 95% CI 1.4-3.0%) and 9 cases in 554 patients (1.6%, 95% CI 0.6-2.7%) with both leucodepletion and CMV negative donor selection (Table 3). Rates for CMV leucodepleted only were 4 in 194 (2.1% 95% CI 0.6-4.1%) in the single study identified. Following estimation of individual transfusion exposures, the rates of CMV were 0.17% per unique donor exposure (0.11-0.23%) for leucodepleted only and 0.22% (0.08-0.37%) for CMV negative and leucodepleted. These figures do not include the potential for a single individual to have acquired infection twice.

Only one study examined infectivity of known CMV DNA positive leucoreduced blood.[38] This study identified 39 seronegative recipients of 40 blood products (4 receiving plasma only) who had follow up serology for at least 38 days. No cases of CMV transmission were identified amongst the recipients, all of whom were immunocompetent.

Preclinical studies identified in this search have shown that CMV spiked into blood products is reduced but not completely eliminated by leucocyte filtration.[41, 42] Despite the presence of CMV DNA, viral cultures were negative post-filtration.[41] The infectivity of CMV has been modelled using murine CMV, which have shown that low levels of leucocyte exposure ($<1x10^4$, $<4x10^5$ /kg) prevent murine CMV transmission [43]. These studies suggest that leucocyte depletion is likely to reduce the number of transfused while cells sufficiently to prevent CMV transmission.

Pathogen reduction techniques are used to prevent viral and bacterial growth in and transmission by blood products. CMV DNA remains in platelet concentrates after amotosalen pathogen reduction but it effectively reduces viral replication to prevent transmission.[44-46]

Zieman and colleagues showed that whole blood CMV DNA positivity was more common in long term seropositive (>12 months) than seronegative donors, although most cases have very low levels of DNA and only a single, non-reproducible positive result.[47] Confirmed CMV DNA was found in similar rates and <0.1% in both long term seropositive and seronegative donors. Low rates were confirmed in a random sample of 1000 USA donors, which found only 2 were reproducibly CMV DNA positive, both serologically positive[48]. Recently positive donors had high rates and the highest levels of CMV DNA in both whole blood and plasma.[47, 49]

Plasma CMV DNA levels are high during primary infection with recently seropositive donors.[47, 49] In seroconverting donors, 3 of 12 donors in one study had positive plasma CMV DNA from the last seronegative sample within 35 days.[50]. The amount of viral DNA exposure was higher in plasma transfusions than in leucodepleted cellular products. Selecting CMV negative plasma was not recommended.[51] In preclinical studies, cell free CMV virus spiked into plasma passes through leucocyte filters.[40] While CMV in plasma could represent an avenue for breakthrough infection, no reports of transfusion-associated CMV from plasma were identified in our case search.

The study plan included reviewing the rates of CMV and CMV seroconversion in donors and populations representative of donors in order to assist in calculating the risk of transmission. Donor prevalence varied between studies, but was universally high with continuous seroconversion through life in unaffected donors.[52-54] Despite this, CMV DNA detection was rare in healthy donors.[48] It was concluded that the population frequency was unlikely to impact the potential rate of transmission, given the low rates found in leucodepleted blood. It was noted that maintaining a CMV blood supply is more difficult in regions where seroprevalence is high.[55, 56]

The risk of CMV transmission was calculated by Seed and colleagues in a leucodepleted blood supply[57]. The key assumptions included that a CMV viral exposure of $<5x10^6$ would prevent infection, based on extrapolation from mouse models,[43] and that cell free DNA was not a significant source of infection, based on murine models[43], a lack of known transmission through fresh frozen plasma[58] and evidence that plasma CMV DNA is fragmented rather than live virus[59]. Thus,

the risk of transmission was a function of the likelihood of viraemia and filter failure and gave an estimate of approximately 1 in $13.5x10^6$, with the 95% confidence limit maximum rate being approximately 1 in 1.7×10^6 transfusions.

Discussion

This review found the rates of CMV infection were similar in recipients of leucodepleted and CMV negative products and leucodepleted only products. While CMV does occur after transfusion, this is not unexpected due to the high frequency of seroconversion in the general population and at-risk groups. No confirmed cases of CMV transmission by leucodepleted blood were found in our review of the literature and haemovigilance programs. In vitro data support a low risk for CMV transmission with leucodepletion.

Preclinical data identified during this review showed that whole blood viral load detected by nucleic acid testing declined with leucocyte reduction. There is less impact on plasma viral loads with filtration, but the role of cell free DNA is uncertain and viral cultures from plasma usually negative[8]. CMV DNA in plasma has been shown to be highly fragmented, the lack of intact virus potentially explaining the lack of infectivity.[59] The presence of CMV DNA is therefore not a surrogate for infectivity. Although the lack of detection by polymerase chain reaction is regarded as a marker for an inability to transit, no safe level has been absolutely determined and it remains impossible to exclude transmission as a rare event. Murine CMV models suggest that CMV is latent within mononuclear leucocytes, as it is in humans, making it a useful model for preclinical studies on CMV transmission. Low inoculation levels in mice suggest that modern effective leucoreduction should reduce CMV in donor units below theoretical levels needed for transmission[43]. These models also suggest that pre-storage leucocyte reduction may have a role not only in preventing transmission but also viral reactivation. Lipopolysaccharide, tumour necrosis factor-alpha and interferon-gamma, the latter potentially induced by allogeneic leucocyte interactions, contribute to macrophage differentiation and CMV reactivation.[8] Leucocyte reduction may also therefore reduce CMV reactivation in carriers by removing leucocytes that stimulate mononuclear cell activation.

In support of the preclinical findings, we found no confirmed cases of CMV transmission with leucodepleted blood products, or with clinical use plasma, with or without pathogen inactivation. Similarly, the pooled estimated rates of CMV acquisition following transfusion were not significantly different with or without CMV serology for selection in a leucodepleted blood supply. An additional risk associated with using CMV untested blood could therefore not be calculated. Prior modelling has estimated a residual risk of less than 1 in 13 million transfusions, an effect that would not be determined in the population based studies and could even be missed despite years of haemovigilance monitoring.[57] It also remains uncertain whether CMV negative donors are less likely to transmit virus in blood given the high levels of CMV viraemia associated with seroconversion and rare but low detection of CMV in the blood of healthy donors.[50] With our estimate of more than 275000 patients required for a definitive randomised trial, further clinical studies comparing the rate of transmission are extremely unlikely to show a difference given the very low estimated frequency of transmission with or without selection of CMV negative donors.

The risk of CMV acquisition may relate primarily to the quantity of intact virus present in the transfused product. In this respect, the fact that most studies measuring transmission rates focused on immunosuppressed patients, particular seronegative transplant recipients, provides confidence that the rate is low in this vulnerable group. Preterm neonates are another vulnerable group where clinical implications are also high, however transmission through blood was not detected in one study, negligible to the high rate of acquisition from breast milk.[60] Maternal transmission remains a key concern as there is potentially a very high impact, especially when acquired in early pregnancy. Strategies to prevent maternal acquisition and vertical transmission need to be implemented universally as the periconceptual period carries a high risk for long term harm if vertical transmission occurs.[5] In this regard, universal leucodepletion is preferred over CMV negative blood without leucodepletion.

The recognition that CMV transmission is primarily acquired from non-transfusion related sources is critical in appropriately targeting preventative efforts. CMV testing of leucodepleted blood products is a high cost, low value intervention. While beyond the scope of this review, efforts to prevent harm from CMV in critical settings now

focus on detection of rising viral loads and pre-emptive treatment or public health measures to prevent primary infection.[6, 7]

Our study differs from previously published systematic reviews where the evidence was considered insufficient to make a recommendation for one approach to CMV prevention over another.[10, 51] The current review acknowledges that many blood banks use pre-storage leucodepletion and specifically examined whether CMV testing is useful over and above leucodepletion. Although some included studies used bedside filtration, where universal leucodepletion is not performed, CMV testing may still be considered to ensure safety. This study therefore also searched for and included preclinical studies. This was considered essential as population based studies as proving a negative – that CMV cannot be transmitted to various at risk groups - is impossible, while showing the lack of, or marked reduction in transmissible virus does not rely solely on having an affected or potentially affected population.

There are several limitations in the evidence found. The lack of a defined threshold for CMV load in humans remains problematic. While murine cytomegalovirus appears to be a reasonable model, viral infectivity can vary even with different strains of the one virus and it is unknown whether this applies to human CMV or between species. The lack of reports in haemovigilance programs supports the very low potential for transmission, however passive surveillance data may systemically under report. Prospective active monitoring is more reliable. In many included studies these rates were non-zero, although no different from CMV seronegative blood. The confounding effect of CMV acquisition within healthy populations, likely to be well-above transfusion transmission, also limits the ability to quantify and compare rates with and without donor testing. Finally, it should be noted that these findings apply only to leucodepleted products. Where leucodepletion is not routine, CMV negative products should still be preferred for high risk recipients. Products that cannot be leucodepleted, in particular stem cells and granulocytes, should also be preferentially CMV seronegative for at-risk recipients.

This study found no cases of confirmed CMV transmission from leucodepleted blood products. There was no difference in the rates of CMV in recipients of CMV unscreened or serologically negative leucodepleted blood products. Although

confounding by high rates of CMV acquisition present in everyday life prevent calculation of an absolute risk estimate with leucodepleted blood, it is very low. The addition of CMV serological testing has not been shown in this review to improve safety. While prevention of CMV remains important in transfusion, serological testing over and above leucodepletion is of low to no value.

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Table 1: Risk of bias assessments for all comparative studies

Randomised trials (ROB-2)									
Study	**Comparison**	**Randomisation**	**Deviations from intended intervention**	**Missing outcome data**	**Measurement of outcome**	**Selection of the reported results**			**Overall**
Bowden 1995	Leucodepleted blood v CMV negative	Low	Some concerns	Low	Some concerns	Low			Some concerns
Non-randomised studies (ROBINS-I V2)									
Study	**Comparison**	**Confounding**	**Classification**	**Selection**	**Deviations**	**Missing data**	**Outcome measurement**	**Selection of reported result**	**Overall**
Kekre 2013	Leucodepleted only v CMV negative and leucodepleted	Low, except for concerns about uncontrolled bias.	Low	Low	Low	Low	Low	Low	Low
Llungman 2002	Leucodepleted only v CMV negative and leucodepleted	Low, except for concerns about uncontrolled bias.	Low	Low	Low	Low	Low	Low	Low
Zantomio 2023	Leucodepleted only v CMV negative and leucodepleted	Low, except for concerns about uncontrolled bias.	Low	Low	Low	Low	Low	Low	Low
Nichols 2003	Leucodepleted v CMV negative	Low, except for concerns about uncontrolled bias.	Low	Low	Low	Low	Low	Critical	Critical

Table 2: Observational Studies: Filtered Only

Study	Study type	Population	Number of patients	Estimated Donor exposures	Infections
Delaney 2016	Prospective observational	Pre term very low birthweight infants	20	24	0
Hall 2015	Retrospective single arm single centre	Seronegative recipients from seronegative donors. Most T cell depleted in vivo with alemtuzumab or ATG.	76	1862	0
Kekre 2013	Retrospective, pre and post practice change	Seronegative SCT recipients	77	1386	1
Kim 2005	Prospective comparison between 2 NICUs	Very low birthweight infants	80	360	2
Ljungman 2002	Retrospective, pre and post practice change	Seronegative BMT recipients	49	49	6
Narvios 1998	Retrospective	Seronegative BMT recipients	45	270	1
Narvios 2005	Retrospective single arm single centre	Seronegative BMT recipients	72	3934	2
Narvios 2001	Retrospective single arm single centre	Seronegative BMT recipients	36	36	0
Nash 2012	Retrospective review of prospectively collected data	Seronegative BMT recipients	100	6133	0
Nichols 2003	Retrospective, pre and post practice change	Seronegative BMT recipients	235	235	14
Ronghe 2002	Retrospective, pre and post practice change	Seronegative BMT recipients	93	93	0
Shigemura 2019	Retrospective single arm single centre	Seronegative cord blood recipients	41	925	0
Thiele 2011	Prospective observational	Seronegative BMT recipients	23	23	0
van Prooijen 1994	Retrospective single centre	Seronegative BMT recipients	60	60	0
Voruz 2020	Retrospective single centre	Seronegative BMT recipients	165	165	0
Wu 2010	Prospective observational	Patients >13 years with expected	46	1316	3

		recurrent transfusion requirement			
Zantomio 2023	R Retrospective comparison of 2 centres	Seronegative BMT recipients	66	66	0
Zieman 2017	Retrospective on prospective samples	Blood donor CMV DNA	39	40	0
Totals N=18			**1323**	**16977**	**29**

Table 3: Observational Studies: Filtered and CMV seronegative

Study	Study type	Population	Number of patients	Estimated Donor exposures	Infections
Josephson 2014	Prospective cohort, 3 NICUs	Very low birthweight neonates	310	1038	0
Kekre 2013	Retrospective, pre and post practice change	Seronegative BMT recipients	89	2830	3
Ljungman 2002	Retrospective, pre and post practice change	Seronegative BMT recipients	33	33	3
Zantomio 2023	Retrospective comparison of 2 centres	Seronegative BMT recipients	122	122	3
Totals N=4			**554**	**4023**	**9**

Figure Legends

Figure 1: Flow chart of study selection

Figure 2: Forest plot of observational studies comparing CMV negative and CMV unselected donors all with leucocyte filtration, excluding critical bias (IV: Inverse variance)

Figure 3: Forest plot for sensitivity analysis of all comparative studies. Analysis includes four observational studies and one randomised study with selection for cytomegalovirus serology negative (with or without leucodepletion) with leucocyte filtration only, irrespective of risk of bias assessment. (IV: Inverse variance)