Prevention of Transfusion-Transmitted Cytomegalovirus Infections: Which is the Optimal Strategy?

Malte Ziemann · Holger Hennig

Institute of Transfusion Medicine, University Hospital of Schleswig-Holstein, Lübeck, Germany

Keywords

Human cytomegalovirus · CMV · Blood donor ·
Transfusion-associated infections · Transfusion

Summary

Traditionally, leukoreduction and selection of blood products from seronegative donors have been used as alternative strategies to reduce the risk of transfusion-transmitted cytomegalovirus infections (TT-CMV) in at-risk patients. After the introduction of universal leukoreduction for red blood cell and platelet concentrates in Germany, a controversy evolved as to whether the additional selection of blood products from seronegative donors would reduce or even increase the risk of TT-CMV. This review summarizes the current knowledge about CMV infections in blood donors and the implications of this information on the effect of potential transfusion strategies. Even though there are conflicting data about the incidence of TT-CMV remaining after the introduction of leukodepletion, it has been clearly shown that both prevalence and concentration of CMV DNA in peripheral blood are highest in newly seropositive donors. Therefore, avoidance of blood products from these donors is the most important goal of any transfusion strategy. This goal can be reached by: i) selection of blood products from seronegative donors, ii) provision of CMV DNA-negative blood products, or iii) provision of blood from long-term seropositive donors. In cases of suspected TT-CMV, all implicated donors should be investigated carefully to gather further knowledge on which donors confer the lowest risk for TT-CMV.

Introduction

Human cytomegalovirus (CMV) is a ubiquitous beta-herpesvirus transmitted by direct person-to-person contact and causing mostly asymptomatic or mild mononucleosis-like infections in immunocompetent subjects [1]. Antibodies against CMV are detected in about 40–60% of German blood donors [2, 3].

Transfusion-transmitted CMV infections (TT-CMV) were first described by Kääriäinen and co-workers in 1966 [4]. After development of suitable biochemical methods (restriction endonuclease analysis), the molecular evidence for TT-CMV was provided by Tolpin and colleagues in 1985 [5]. Up to the 1980s, TT-CMV were a frequent problem even for example in patients after cardiac surgery, where TT-CMV occurred in up to 67% of seronegative patients, and about a third of seronegative patients developed symptomatic CMV disease with fever and elevated liver enzymes [6]. In the 1980s and 1990s, the provision of blood products from seronegative donors and leukoreduction of blood products were introduced to reduce the incidence of TT-CMV in risk populations [7, 8]. Both strategies showed a reduction of TT-CMV by more than 90% [9].

Currently, mainly seronegative patients undergoing hematopoietic stem cell transplantation (HSCT), low-birth-weight infants, fetuses (intrauterine transfusions), CMV-seronegative pregnant women and severely immunocompromised patients are considered as being at risk for TT-CMV [10].

Incidence of Transfusion-Transmitted CMV Infections

After the universal introduction of leukodepletion for red blood cell and platelet concentrates, there is an ongoing controversy as to whether TT-CMV still exists in at-risk patients.

Thiele and colleagues [11] published a study of 23 CMV-seronegative patients undergoing HSCT from CMV-seronegative stem cell donors (CMV^{neg/neg}). The patients received 1,847 leukoreduced, but CMV-untested, blood products from 3,180 donors (median 55 units per patient, range 3–313 units per patient). None of the patients developed CMV disease or CMV-associated complications after HSCT. CMV antibodies were tested in 22 patients. All patients remained negative for IgM antibodies, but IgG antibodies were detected in 17 patients after HSCT (77%). CMV nucleic acid testing (NAT) was performed twice weekly starting at the day after HSCT, and remained negative throughout the study period (at least until day 100 after transplantation). Therefore, CMV antibodies were attributed to passive antibody transfer by blood products or intravenous immunoglobulin (IVIG).

Due to the low number of patients, the data of Thiele and co-workers correspond only to an incidence of TT-CMV of less than 14% in seronegative HSCT patients with seronegative stem cell donors. After publication of the data of Thiele and colleagues [11], Nash and co-workers [12] reported in a letter to the editor a further 100 cases of CMV^{neg/neg} patients with allogeneic HSCT who received leukoreduced but mainly CMV-untested blood products. 54 patients should have been transfused with CMV-seronegative blood products, but due to product shortages, only 12% of the transfused units were actually CMV seronegative. 46 other patients were transfused with CMV-untested blood products only. In total, 6,465 units were transfused (median 25 units per patient, range 0–877 per patient), without any case of clinical CMV disease or positive CMV NAT during weekly post-transplant NAT monitoring. 2 seroconversions were attributed to passive antibody transfer. Taken together, the data of Thiele et al. [11] and Nash et al. [12] correspond to an incidence of TT-CMV in CMV^{neg/neg} patients with allogeneic HSCT of less than 2.4%.

Contrarily to these data, Wu and colleagues [13] reported 3 cases of probable TT-CMV in 46 seronegative patients, who received 1,316 leukoreduced, but CMV-untested, cellular blood transfusions. The study included patients who were 13 years of age or older, and were projected to receive multiple transfusions. 44 patients (96%) were hematological or oncological patients, and 24 patients (52%) underwent HSCT. 3 patients (7%) developed CMV antibodies concurrent with possibly CMV-related symptoms (e.g. fever and elevated liver enzymes). 2 patients were CMV DNA-positive in several plasma samples drawn during the first weeks of seroconversion. In the third patient, no CMV DNA was detected, but the interval between the last seronegative sample and the first seropositive sample was 2 months, so that a short period of systemic viremia might have been missed. All 3 patients received blood transfusions from seropositive donors prior to seroconversion. No CMV DNA was detected in stored plasma samples of the donors, but only some donors could be tested by CMV NAT. Therefore, both free CMV in plasma as well as residual leukocytes, despite pre-storage filtration with 3rd

generation filters, might have caused these cases of TT-CMV, but even community-acquired CMV infections could not be ruled out by the authors.

In summary, only the study of Nash and colleagues [12] is sufficiently powered to argue against a clinical relevance of TT-CMV in HSCT patients, while no current study provides clear evidence for the existence of residual cases of TT-CMV. Therefore, more data about the actual incidence of TT-CMV are needed.

For other risk groups, like low-birth-weight infants, to our knowledge no current data about the incidence of TT-CMV are available. In a prospective study planned to be completed in 2015, Josephson and co-workers [14] aim to determine the incidence of TT-CMV for these patients, providing the traditional ‘belt and suspender strategy’ of supplying leukoreduced blood products from seronegative donors (ClinicalTrials.gov no. NCT00907686).

In Germany, all suspected cases of TT-CMV should be reported to the national authority, the Paul-Ehrlich Institute. Until 2010, only 2 suspected cases had been reported, which could not be confirmed by the Paul-Ehrlich Institute [15]. There may be, however, a considerable number of unrecognized and/or unreported cases.

Physiology of CMV Infections in Blood Donors

In contrast to the ongoing controversy about current rates of TT-CMV, the information gathered over the last few years has led a rather clear picture of the course of CMV infections in blood donors. The annual seroconversion rate in previously seronegative blood donors is about 1% in Germany [2, 3]. Primary CMV infections in blood donors occur in all age groups [2]. Mononucleosis-like symptoms due to primary CMV infections are rare. In a recent study, for example, none of the 13 donors with primary CMV infection developed mononucleosis-like symptoms [3]. Unspecific symptoms of viral disease were common but not significantly increased compared to a matched control group [3]. In the study of Zanghellini et al. [16], all 6 adolescents with primary CMV infection were asymptomatic.

In connection with seroconversion, CMV DNA is regularly detected in plasma samples [17]. An important factor influencing the frequency of detection of CMV DNA in peripheral blood is the sampling interval. The closer the interval between the last seronegative and the first seropositive sample, the higher is the incidence of CMV DNA (fig. 1). In donors with low interdonation intervals (e.g. apheresis donors), CMV DNA can be detected in up to 25% in the last seronegative sample and 83% in the first seropositive sample [18].

Both prevalence and concentration of CMV DNA were shown to be higher in the first seropositive than in the last seronegative sample both for plasma and whole blood samples [18, 19]. In donors who have been shown to be seropositive for the first time, CMV DNA concentrations of up to

Fig. 1. Detection of CMV DNA before and after seroconversion. The figure is taken from Ziemann et al. [18] with kind permission of John Wiley and Sons Inc. Donors were categorized according to the interval between the last seronegative and the first seropositive sample. The rate of detection of CMV DNA in plasma is given with its 95% interval of confidence.

1,600 IU/ml plasma and 4,000 IU/ml whole blood have been detected so far [18, 19]. However, as the sampling interval ranged between several weeks to several months, even higher peak concentrations during primary CMV infection might have been missed.

The detection of peak CMV DNA concentrations after seroconversion is in accordance with the results of Zhang and colleagues [20], who screened young seronegative women for primary CMV infections in a placebo-controlled clinical trial of recombinant CMV glycoprotein B (gB) vaccine. By regular screening with viral cultures, viable CMV in body fluids was detected between 0 and 12 weeks (median 2 weeks) after the first seropositive sample.

A delayed peak of CMV DNA and viable virus after development of antibodies has been explained by the hypothesis that primary CMV infections caused by direct person-to-person contact first lead to a localized lytic infection cycle, e.g. in the nasopharyngeal mucosa, initiating production of antibodies against non-structure and tegument proteins [18]. After an interval of days to several weeks, this local infection is followed by viremia during which the virus spreads to other organs, like the kidneys. At this stage, antibodies against glycoproteins of the viral envelope, e.g. gB, are induced, which probably contribute to termination of the viremic phase [18]. The duration of viremia in immunocompetent subjects seems to be short in most subjects, but even cases with prolonged viremia up to several months have been reported [3]. After detection of antibodies against gB, only a low concentration of CMV DNA was detectable in peripheral blood, and no CMV DNA was found in donors with CMV antibodies with high avidity [3, 19].

In donors with remote CMV infection (e.g. seropositive for at least 1 year), CMV DNA is rarely detectable. In 2 studies, all 1,086 plasma samples from long-term seropositive donors were CMV DNA negative [17, 21]. These data contradict the findings of Dumont and co-workers [22], who reported fre-

quent reactivations of latent CMV infections in immunocompetent blood donors in temporal association to the pine tree pollen season. This astonishing association could not be reproduced by others, and it is unclear whether the reactivation had been caused by, e.g. treatment of allergic symptoms with systemic steroids. In a recent study, CMV DNA in plasma was detected in only 1 of 7,303 long-term seropositive donors (0.01%) [19]. This donor had only weak antibodies against gB, and low concentrations of CMV DNA in plasma (<30 IU/ml). CMV DNA was detected in whole blood at low concentrations (800 IU/ml or less) in about 0.2% of long-term seropositive donors. These findings might correspond to viral DNA incorporated in latently infected white blood cells (WBCs).

Identification of At-Risk Donors

Despite early assumptions that donations from only a small subgroup of donors can cause TT-CMV [23–25], traditionally all seropositive donors have been regarded as potentially infectious for at-risk patients [26]. To identify potentially infectious donors, testing the donors' urine for CMV has been suggested by Kane and colleagues [23] because CMV viruria is frequent in primarily infected donors, and urine usually contains a higher viral load than peripheral blood. Presumably more feasible for daily practice are the suggestions of testing donors for rising antibody titers or IgM antibodies [24]. Alternatively, CMV-antibody positive, but gB-antibody-negative donors or donors with low-avidity antibodies can be regarded as potentially infectious [3, 19].

Even if there is no doubt that CMV can be transferred by non-leukoreduced blood products from seropositive blood donors, viable CMV in peripheral blood of donors has only been detected in a single study by Diosi and co-workers [27]. Numerous attempts to reproduce these findings involving

more than 1,500 donors failed [28]. These difficulties might be caused by the relatively high limit of detection of common viral culture or shell vial assays and only low concentrations of CMV in peripheral blood of immunocompetent subjects [29]. CMV DNA in plasma correlates with CMV disease [30] and is recommended as a surrogate parameter in monitoring at-risk patients for active CMV infections [31]. Therefore, the presence of CMV DNA in plasma should also correspond to systemic viremia. To our knowledge, there are no data about the infectious dose of CMV, but even low concentrations might be infectious for immunocompromised patients.

In a study of 6 adolescents with primary CMV infection, Zanghellini and colleagues [16] were able to detect viable CMV in 1 WBC sample obtained 10 weeks after the presumed date of seroconversion. Another 53 WBC samples obtained after seroconversion were negative in viral culture, even if CMV DNA was detected in 75–80% of samples drawn during the first 16 weeks. No window-phase samples were analyzed in this study.

Taken together, all data are in accordance with the hypothesis that blood donors within the first year after primary CMV infection confer the highest risk of TT-CMV for at-risk patients.

Transfusion Strategies to Reduce Transfusion-Transmitted CMV Infections

After introduction of general leukodepletion, it is mainly blood donors with primary CMV infections who are potentially infectious. Deferral of these donors prior to donation is not possible because most infections are oligo- or asymptomatic [3]. Even nonspecific markers like WBC count, neopterin or alanine aminotransferase have too-low specificity and sensitivity [17].

Several transfusion strategies have been proposed to reduce the risk of TT-CMV in addition to leukoreduction:

- i) provision of seronegative blood products [7];
- ii) provision of CMV DNA-negative blood products (NAT-negative products) [32]; and
- iii) provision of blood products from long-term seropositive donors [33].

A recent study compared these strategies on the basis of the results for CMV DNA in whole blood samples from

22,904 donations [19]. In this study, the classic strategy of seronegative blood products showed the lowest risk of transfusing cell-associated CMV. This effect was only significant, however, if even very low numbers of infected cells were taken into account. NAT-negative blood products showed the lowest risk for free CMV in plasma, but this difference reached significance only for comparison with CMV-untested donors. Blood products from long-term-seropositive donors contained potentially neutralizing antibodies against membrane proteins like gB, which might impair infection of at-risk patients by low concentrations of CMV.

Whole blood NAT testing would be an ideal option to detect both cell-associated and free CMV, but this approach is challenging, especially as a low limit of detection is required. To our knowledge, only plasma NAT, but not whole blood NAT, is used by some blood transfusion services.

To weigh the advantages and disadvantages of the alternative transfusion strategies, detailed knowledge would be necessary about the comparative infectivity of low concentrations of cell-associated versus free CMV, and the influence of neutralizing antibodies on the infectivity of low concentrations of CMV.

Until these facts are known, at least products from newly seropositive donors should be avoided for at-risk patients. This was also recommended in 2012 by the section ‘Safety of Blood Products’ of the German Society of Transfusion Medicine and Immunohematology. To reach this goal, all 3 above-mentioned strategies are suitable: provision of seronegative blood products, CMV-NAT-negative blood products, or blood products from long-term seropositive donors. In cases of suspected TT-CMV, both the serostatus of all implicated donors (seronegative, newly seropositive, long-term seropositive) and the CMV DNA concentration in stored plasma samples should be determined to gather further knowledge on which donors confer the lowest risk for TT-CMV.

Furthermore, conditions and duration of storage of blood products prior to transfusion could influence the stability of CMV. Therefore, the interval between blood donation and transfusion of the respective blood products should be investigated.

Disclosure Statement

None of the authors declares a conflict of interest.

References

- 1 Klemola E, Kaariainen L: Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. *Br Med J* 1965;6:1099–1102.
- 2 Hecker M, Qiu D, Marquardt K, et al: Continuous cytomegalovirus seroconversion in a large group of healthy blood donors. *Vox Sang* 2004;86:41–44.
- 3 Ziemann M, Unmack A, Steppat D, et al: The natural course of primary cytomegalovirus infection in blood donors. *Vox Sang* 2010;99:24–33.
- 4 Kääriäinen L, Klemola E, Paloheimo J: Rise of cytomegalovirus antibodies in an infectious-mononucleosis-like syndrome after transfusion. *Br Med J* 1966;1:1270–1272.
- 5 Tolpin MD, Stewart JA, Warren D, et al: Transfusion transmission of cytomegalovirus confirmed by restriction endonuclease analysis. *J Pediatr* 1985; 107:953–956.
- 6 Endresen K, Gjesdal K, Orstavik I, et al: Primary cytomegalovirus infection following open heart surgery. *Acta Med Scand* 1985;218:423–428.

- 7 Yeager AS, Grumet FC, Haffleigh EB, et al: Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J Pediatr* 1981;98: 281–287.
- 8 Bowden RA, Slichter SJ, Sayers M, et al: A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplant. *Blood* 1995;86: 3598–3603.
- 9 Vamvakas EC: Is white blood cell reduction equivalent to antibody screening in preventing transmission of cytomegalovirus by transfusion? A review of the literature and meta-analysis. *Transfus Med Rev* 2005;19:181–199.
- 10 Schottstedt V, Blümel J, Burger R, et al: Human cytomegalovirus (HCMV) – Revised. *Transfus Med Hemother* 2010;37:365–375.
- 11 Thiele T, Krüger W, Zimmermann K, et al: Transmission of cytomegalovirus (CMV) infection by leukoreduced blood products not tested for CMV antibodies: a single-center prospective study in high-risk patients undergoing allogeneic hematopoietic stem cell transplantation (CME). *Transfusion* 2011;51:2620–2626.
- 12 Nash T, Hoffmann S, Butch S, et al: Safety of leukoreduced, cytomegalovirus (CMV)-untested components in CMV-negative allogeneic human progenitor cell transplant recipients. *Transfusion* 2012; 52:2270–2272.
- 13 Wu Y, Zou S, Cable R, et al: Direct assessment of cytomegalovirus transfusion-transmitted risks after universal leukoreduction. *Transfusion* 2009;50:776–786.
- 14 Josephson CD, Castillejo M-I, Caliendo AM, et al: Prevention of transfusion-transmitted cytomegalovirus in low-birth weight infants (≤ 1500 g) using cytomegalovirus-seronegative and leukoreduced transfusions. *Transfus Med Rev* 2011;25:125–132.
- 15 Funk M, Günay S, Volz-Zang C: Haemovigilance report of the Paul-Ehrlich-Institut 2010: Assessment of the Reports of Serious Adverse Transfusion Reactions pursuant to Section 63 c AMG (Arzneimittelgesetz, German Medicinal Products Act), 2012. Available at: www.pei.de/SharedDocs/Downloads/vigilanz/haemovigilanz/publikationen/haemovigilance-report-2010.pdf.
- 16 Zanghellini F, Boppana SB, Emery VC, et al: Asymptomatic primary cytomegalovirus infection: Virologic and immunologic features. *J Infect Dis* 1999;180:702–707.
- 17 Ziemann M, Krueger S, Maier AB, et al: High prevalence of cytomegalovirus DNA in plasma samples of blood donors in connection with seroconversion. *Transfusion* 2007;47:1972–1983.
- 18 Ziemann M, Heuft H-G, Frank K, et al: Window period donations during primary cytomegalovirus infection and risk of transfusion-transmitted infections. *Transfusion* 2013;53:1088–1094.
- 19 Ziemann M, Juhl D, Görg S, Hennig H: The impact of donor cytomegalovirus DNA on transfusion strategies for at-risk patients. *Transfusion* 2013;53:2183–2189.
- 20 Zhang C, Buchanan H, Andrews W, et al: Detection of cytomegalovirus infection during a vaccine clinical trial in healthy young women: Seroconversion and viral shedding. *J Clin Virol* 2006;35:338–342.
- 21 Drew WL, Tegtmeier G, Alter HJ, et al: Frequency and duration of plasma CMV viremia in seroconverting blood donors and recipients. *Transfusion* 2003;43:309–313.
- 22 Dumont LJ, Luka J, VandenBroeke T, et al: The effect of leukocyte-reduction method on the amount of human cytomegalovirus in blood products: a comparison of apheresis and filtration methods. *Blood* 2001;97:3640–3647.
- 23 Kane RC, Rousseau WE, Noble GR, et al: Cytomegalovirus infection in a volunteer blood donor population. *Infect Immun* 1975:719–723.
- 24 Beneke JS, Tegtmeier GE, Alter HJ, et al: Relation of titers of antibodies to CMV in blood donors to the transmission of cytomegalovirus infection. *J Infect Dis* 1984;150:883–888.
- 25 Foster KM, Jack I: A prospective study of the role of cytomegalovirus in post-transfusion mononucleosis. *N Engl J Med* 1969;280:1311–1316.
- 26 Gajewski JL, Johnson VV, Sandler SG, et al: A review of transfusion practice before, during, and after hematopoietic progenitor cell transplantation. *Blood* 2008;112:3036–3047.
- 27 Diosi P, Moldovan E, Tomescu N: Latent cytomegalovirus infection in blood donors. *Br Med J* 1969;4:660–662.
- 28 Bayer WL, Tegtmeier GE: The blood donor: detection and magnitude of cytomegalovirus carrier states and the prevalence of cytomegalovirus antibody. *Yale J Biol Med* 1976;49:5–12.
- 29 Boeckh M, Gallez-Hawkins GM, Myerson D, et al: Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation: Comparison with polymerase chain reaction using peripheral blood leukocytes, pp65 antigenemia, and viral culture. *Transplantation* 1997;64:108–113.
- 30 Emery VC, Sabin CA, Cope AV, et al: Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. *Lancet* 2000;355:2032–2036.
- 31 Kotton CN, Kumar D, Caliendo AM, et al: International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation. *Transplantation* 2010;89:779–795.
- 32 Roback JD, Drew WL, Laycock ME, et al: CMV DNA is rarely detected in healthy blood donors using validated PCR assays. *Transfusion* 2003;43: 14–321.
- 33 Drew WL, Roback JD: Prevention of transfusion-transmitted cytomegalovirus: reactivation of the debate? *Transfusion* 2007;47:1955–1958.