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

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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<sup>neg/neg</sup>). 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<sup>neg/neg</sup> 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<sup>neg/neg</sup> 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 ‘belts and suspenders 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
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].

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 frequent 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).
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


