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Received 6 March 1990.
Accepted 12 June 1990.

0041-1337/90/5006-0964$02.00/0
TRANSPLANTATION

Vol. 50, 964–968, No. 6, December 1990
Printed in U.S.A.

PREVENTION OF PRIMARY CYTOMEGALOVIRUS INFECTION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION BY USING LEUKOCYTE-POOR RANDOM BLOOD PRODUCTS FROM CYTOMEGALOVIRUS-UNSCREENED BLOOD-BANK DONORS

T. DE WITTE,[1,2] A. SCHATTENBERG,[1] B. A. VAN DIJK,[3] J. GALAMA,[4] H. OLTHUIS,[5] J. W. W. VAN DER MEER,[6] AND V. A. J. M. KUNST[3]

Division of Hematology, Division of General Internal Medicine, Department of Internal Medicine, University Blood Transfusion Service, Department of Microbiology, University Hospital Nijmegen; and Red Cross Donor Bank, Nijmegen, The Netherlands

Cytomegalovirus infection was studied in 59 seronegative recipients of bone marrow depleted of lymphocytes by counterflow centrifugation. Eighteen patients died within 3 months after bone marrow transplantation without evidence of CMV infection, and they were excluded from analysis. Twenty-eight valuable seronegative patients received marrow from a seronegative donor, and 13 from a seropositive donor. All but 2 patients received acyclovir orally (4×400 mg/day) from days −9 to +60. CMV prophylaxis with immunoglobulin preparations was not given. All blood products were prepared from random, CMV-unscreened blood-bank donors. The red cell concentrates were depleted of leukocytes by filtration, and leukocytes were removed from the platelet concentrates by centrifugation. None of the patients

[1] Division of Hematology, University Hospital Nijmegen.
[2] Address correspondence to: T. De Witte, Division of Hematology, Department of Internal Medicine, University Hospital Nijmegen, 8 Geert Grooteplein Zuid, 6525 GA Nijmegen, The Netherlands.
[3] Department of Internal Medicine, University Blood Transfusion Service, University Hospital Nijmegen.
[4] Department of Microbiology, University Hospital Nijmegen.
[5] Red Cross Donor Bank, Nijmegen.
[6] Division of General Internal Medicine, University Hospital Nijmegen.

with seronegative donors showed any clinical sign compatible with CMV infection. Two nonfatal primary CMV infections occurred in the recipients of bone marrow from CMV-positive donors. One of the 59 patients developed interstitial pneumonia, in this case caused by *Pneumocystis carinii*.

Leukocyte depletion of blood products from random CMV-unselected blood donors appeared to prevent primary infection in CMV-seronegative BMT recipients. We conclude that prophylactic use of immunoglobulin preparations is not necessary to prevent CMV primoinfection in patients receiving leukocyte-depleted blood products and acyclovir prophylaxis during the first 2 months postgrafting.

Cytomegalovirus infection is the most common infectious cause of death after bone marrow transplantation (*1*). CMV infection after BMT has been reported to occur in 31–80% of the recipients (*1–3*). CMV infection may arise from reactivation of a latent endogenous virus, from donor bone marrow, blood products, or close physical contacts (*4*). In CMV-seropositive recipients, reactivation of latent endogenous virus is probably the most frequent source of infection, as may be concluded from the high incidence of CMV infection in recipients with positive CMV serology prior to BMT, as compared to the relatively low incidence of CMV infection in CMV-seronegative recipients (*5, 6*). The incidence of primary CMV infection in seronegative recipients ranged from 24% to 42% in five recent reports (*1, 7–10*).

Several approaches may reduce the incidence of CMV infection in seronegative recipients. Seronegative patients receiving transplants from seronegative donors were found to have the lowest risk of CMV infection (*1*), but seronegative donors are not readily available. The use of blood products from seronegative blood donors has been successfully applied in BMT with marrow from seronegative donors, whereas no protection was observed among patients receiving seropositive marrow (*8, 9*). Oral acyclovir given to prevent herpes infections may also provide protection against CMV infection (*11, 12*). Several studies have suggested that passive immunization with CMV hyperimmune globulin may reduce the severity of CMV infection and the incidence of CMV interstitial pneumonia (IP)* after BMT (*10, 13*). In seronegative recipients, conflicting data have been reported. In one study prophylactic use of hyperimmune globulin did not reduce the incidence of CMV infection (*8*), but Meyers et al. reported complete prevention in 11 seronegative recipients with seronegative-marrow donors (*10*). Since the leukocyte fraction of donor blood has been implicated as a source of virus (*14, 15*), a potential alternative is the use of leukocyte-poor blood products. We presented here the 8-year experience in a bone marrow transplant program with a blood transfusion policy of leukocyte-poor blood products obtained from volunteer blood donors not selected for CMV-antibody status.

MATERIALS AND METHODS

Patients. One hundred and twenty consecutive patients received a bone marrow graft from a sibling between May 1981 and March 1989. Fifty-nine bone marrow recipients were CMV seronegative and 61 seropositive. Eighteen of the seronegative patients died within 3 months after transplantation without evidence of CMV infection and were excluded from analysis. The remaining 41 patients with negative CMV serology formed the main group of this analysis. The median age of this group was 27 years (range: 13–44 years).

The indications for BMT were: acute myeloid leukemia in 11 patients (10 in first complete remission [CR], 1 in partial remission); acute lymphocytic or undifferentiated leukemia or lymphoblastic lymphoma in 16 patients (12 in first CR, 4 in second CR); chronic myelogenous leukemia (CML) in 9 patients (6 in first chronic phase, 3 in accelerated phase); myelodysplastic syndromes in 4 patients; and severe aplastic anemia (SAA) in 1 patient.

Conditioning. The transport conditioning consisted of cyclophosphamide 60 mg/kg body weight (days −6 and −5), and total-body irradiation in two equal fractions of 450 cGy each on 2 consecutive days (days −2 and −1) using an 18-mV photon-beam linear accelerator (Saturne, CGR, BUC, France) at a dose rate of 5.5 cGy/min in 22 patients and 22 cGy/min in 16 patients. Lung and eye shielding was used on the second day of irradiation (*16, 17*). Anthracyclines to reduce leukemic relapse after transplantation were added to the conditioning regimen in 22 patients (*18*). The only patient with a mismatched family donor (1 mismatch at A-locus) received a total TBI dose of 12 Gy (two fractions of 6 Gy). One patient with SAA received total-lymphoid irradiation (six fractions with a total dose of 12 Gy). One patient who relapsed after BMT was retransplanted with unmanipulated marrow from the same donor, and the conditioning regimen consisted of busulphan (4 mg/kg body weight) orally for 4 days, followed by cyclophosphamide (50 mg/kg body weight) intravenously for 4 days.

Donor marrow. Donor marrow was depleted of 97–98% of lymphocytes by counterflow-centrifugal elutriation as described before (19). Bone marrow was infused 24 hr after completion of the total-body irradiation.

Management of the patients. Details have been described in earlier reports (*16, 17*). Twelve patients received immunoprophylaxis consisting of CsA followed by weekly injections of methotrexate; 2 patients received methotrexate alone, and 27 patients CsA alone. All patients except the first 2 patients in this analysis received acyclovir orally (4×400 mg/day) from days −9 to +60 for prophylaxis of herpes virus infections. Acyclovir was given intravenously (3×5 mg/kg/day) when oral intake was impaired. CMV prophylaxis with hyperimmune globulin was not given. All patients were managed in single rooms with filtered air under positive pressure throughout the transplant period, and all received selective gut decontamination including co-trimoxazole for *Pneumocystis carinii* prophylaxis.

Blood products. All cell-containing blood products were irradiated (20 Gy) and prepared from random blood donors (nonpaid volunteers, about 50% of them have positive CMV serology). Leukocytes were removed from fresh red-cell concentrates (within 36 hr after collection) immediately prior to use by fiber filtration (NPBI CellSelect filter). This resulted in a white cell count of $<5\times10^7$/L in all instances, but most of the time the WCC was below the detection level of $<1\times10^7$/L (*20*). Platelet concentrates were usually prepared from 6 buffy coats (one "random" platelet concentrate = 6 units) and subsequently subjected to an additional centrifugation step for removal of the leukocytes (*21*). The number of nucleated cells per concentrate was less than 1×10^8 in 93% of the 286 tested concentrates. In case of refractoriness to platelets from random blood donors, platelet concentrates were prepared from CMV-unscreened HLA-matched blood-bank donors or relatives of the patient using cell separators. The single-donor-platelet concentrates contained less than 1×10^8 nucleated cells in 75% of the 60 tested concentrates. No granulocyte transfusions were given.

The general policy in our institution and in most referring hospitals was to give leukocyte-poor blood products to all BMT candidates and to all marrow recipients.

CMV monitoring. IgG, IgA, and IgM antibodies to CMV were tested using enzyme-linked immunosorbent assays prior to BMT in serum of both donor and recipient (*22, 23*). Reciprocal antibody levels of >10 arbitrary ELISA units (AEU) were considered positive (*22, 23*). A serum titer rise of 4 times or higher was considered significant. Serology was performed once weekly until 16 weeks after BMT and thereafter 3

* Abbreviations: AEU, arbitrary ELISA units, CML, chronic myelogenous leukemia; CMV-EA, cytomegalovirus-induced early antigen; CR, complete remission; IP, interstitial pneumonia; SAA, severe aplastic anemia.

monthly until 12 months after transplantation. The presence of CMV in cultures was determined by indirect immunofluorescence using monoclonal antibodies against CMV-induced early antigen (CMV-EA). CMV-EA was determined in the urine of the recipient prior to BMT and weekly thereafter until 16 weeks postgrafting (*24*). When CMV-IP was suspected (vide infra) a bronchoalveolar lavage was always performed, and the lavage material was cultured for the presence of CMV.

Definitions. CMV infection was defined either as primary infection or as reactivation: (1) primary infection was defined as a seroconverison with antibody levels persistently above 10 AEU and/or positive cultures in a previously seronegative recipient; and (2) reactivation was defined as a rise in antibody level of at least fourfold and/or positive cultures in a previously seropositive patient.

CMV disease was defined as a clinical syndrome characterized by fever, leukocytopenia, hepatitis, interstitial pneumonia, or encephalitis associated with CMV-positive cultures obtained from the suspected sites.

CMV interstitial pneumonia was defined as a syndrome consisting of fever, tachypnea, hypoxia, chest X-ray compatible with IP, and demonstration of virus in bronchoalveolar lavage.

RESULTS

Seronegative marrow recipients. Twelve of the 41 evaluable seronegative patients died later than 3 months after BMT. Ten patients died due to recurrence of the underlying disease and 2 patients due to infections: generalized candidiasis in one patient and interstitial pneumonia caused by *Pneumocystis carinii* in the other. These diagnoses were confirmed at autopsy in both patients. The median follow-up of the nonsurviving patients was 253 days (range: 124–1179 days). Median follow-up of the surviving patients was 760 days (range: 330–2160 days).

CMV status of bone marrow donors and CMV infection. Twenty-eight evaluable seronegative patients received marrow from seronegative donors and 13 from seropositive donors. None of the 28 CMV-seronegative recipients with a CMV seronegative donor showed any evidence of CMV infection. Two of the 13 patients with seropositive bone marrow donors developed evidence of CMV infection. One of them seroconverted 6 weeks after BMT with rapidly rising IgG, IgM, and IgA antibody titers. CMV was excreted in the urine for more than 6 months. The patient never developed signs of CMV disease, and therefore specific anti-CMV treatment was not instituted. The other patient developed IgG antibodies with concomitant positive urine cultures. He developed fever, signs of gastroenteritis, and mild myelosuppression. Treatment with ganciclovir (5 mg/kg i.v. 3 times daily for 7 days) (DHPG, Syntex Maidenhead, U.K.), and intravenous hyperimmune globulin (2 ml/kg body weight) (Cytotect, Biotest Pharma, GMBH, Frankfurt am Main, FRG) was immediately instituted and may have contributed to the rapid resolution of all symptoms. Three infusions with identical dosages of hyperimmune globulin were given during 6 additional weeks. No relapse of clinical disease was observed. Neither patient had any symptom of acute nor chronic graft-versus-host disease. Both patients are currently alive and in remission more than 3 and 2 years after BMT, respectively.

CMV infection and transfused blood products. Six patients with CML received no blood products prior to BMT. The number of transfused blood products prior to BMT was unknown for 6 patients. The remaining 29 patients received a median number of 6 red-cell concentrates (range: 0–31 units) in the 3 months prior to BMT. A median number of 30 units "random" platelets (range: 6–108 units) and 2 units from single donors (range: 2–10 units) were given to 13 and 6 patients, respectively, in the 3 months immediately prior to BMT (Table 1). In the first 3 months after BMT, a median number of 12 red-cell units was given (range: 6–30); the median number of units of "random" platelets (for 38 patients) was 36 (range: 6–222), and a median number of 4 units (range: 1–10) from single donors was given to 16 patients. Of the 2 patients with CMV infection, one had not received any transfusion prior to BMT, but he received 16 units of red-cell concentrates, and 30 units of "random" platelets after BMT. The other patient received 16 units of red cells prior to BMT and 16 units after BMT. She also received 108 units of "random" platelets and 7 units prepared from single donors in the posttransplant period.

Seropositive marrow recipients and CMV infection. IgM antibodies, indicating recent or active CMV infection, were determined in 57 of the 61 CMV-seropositive recipients prior to BMT. Four of the 57 evaluated recipients had positive IgM antibodies. Three patients had received transfusions in other institutions. The fourth patient developed CMV interstitial pneumonia at presentation with CML before any transfusion was given. This patient received a transplant uneventfully 6 months after full recovery from the pneumonia that had required artificial ventilation for 2 weeks. Nine patients died within 3 months after BMT without evidence of CMV infection. The remaining 48 patients were evaluated for CMV infection. Eleven patients received marrow from a seronegative donor. All developed CMV infection leading to fatal CMV-IP in 1 patient and fatal CMV aplasia in another patient. Thirty-seven patients received marrow from a seropositive donor or a donor with unknown CMV serology. CMV infection was detected in 31 patients leading to CMV-IP in 7 patients (fatal in 4) and a nonfatal gastroenteritis in 1 patient (Table 2). CMV was excreted in the urine of 22 of 35 patients with regular surveillance cultures of the urine.

TABLE 1. Blood products administered in the 3 months pre- and 3 months postgrafting

	Pre-BMT		Post-BMT	
	No. patients	Transfusions	No. patients	Transfusions
RCC[a]	29	6 (0–31)[b]	41	12 (6–30)
Random platelets	13	30 (6–108)	38	36 (6–222)
Single donor platelets	6	2 (2–10)	16	4 (1–10)

[a] Red-cell concentrates.
[b] Median number of donor units (range).

TABLE 2. CMV status of marrow donor and recipient in relation to CMV infection or CMV disease

	No. patients[a]	CMV infection	CMV disease[b]	CMV-IP[c]
Seroneg. recipient/seroneg. donor	28	0	0	0
Seroneg. recipient/seropos. donor	13	2	1 (0)[d]	0
Seropos. recipient/seroneg. donor	11	11	1 (1)	1 (1)
Seropos. recipient/seropos. donor	37	31	1 (0)	7 (4)

[a] Number of evaluable patients.
[b] CMV-IP excluded.
[c] CMV-IP: cytomegalovirus interstitial pneumonia.
[d] Number of fatal cases is given in parentheses.

DISCUSSION

Despite the use of blood products from CMV-unselected blood donors, only 2 of the 41 recipients with negative-CMV serology before BMT developed a nonfatal, primary infection. None of the 18 other seronegative recipients who died too early for complete follow-up showed evidence of CMV infection despite serial and careful monitoring for CMV infection. Both patients who developed a primary CMV infection received marrow from a CMV-seropositive donor. It is tempting to consider the donor marrow as the source of CMV infection in these 2 cases. This is supported by the observation that rat donor marrow can readily transfer CMV to allogeneic recipients (*25*). Other studies have also observed a relation between donor CMV serology and CMV infection after BMT (*7, 8*). The absolute absence of CMV infection in the 28 evaluable seronegative recipients who received marrow from seronegative donors supports convincingly the hypothesis that a transfusion policy of leukocyte-poor blood products is sufficient to prevent primary CMV infections. Our data are in agreement with the results of a multicenter controlled trial in CMV-negative newborn infants with seronegative mothers that showed that transfusion-acquired CMV infection can be prevented by leukocyte filtration of unscreened blood products (*26*), and a nonrandomized study of 59 seronegative patients with hematologic malignancies treated with intensive chemotherapy (*27*). In a recent study (*28*), 3 of the 36 seronegative bone marrow recipients transfused with blood products from CMV-unscreened blood donors developed CMV infection. However, only red-cell concentrates were depleted of leukocytes in this study (*28*). The absence of CMV-IgM antibodies prior to BMT in patients with leukemia—treated at our institution with intensive chemotherapy—excluded a high incidence of recent primary CMV infections. And this was also an indication of the low CMV transmission rate from random leukocyte-poor blood products. In contrast, Kelsey et al. (*29*) have found that patients receiving random blood products not depleted of leukocytes during treatment with intensive chemotherapy for leukemia showed an increase of seropositivity for CMV from 43% to 56%.

Transfusion of blood products from seronegative blood donors reduces the incidence of primary CMV infections (*8, 9*), but this approach excludes about 40–60% of potential blood donors (*30*). Bowden et al. (*8*) emphasized that it was a major undertaking to provide seronegative blood products to a population of approximately 50 marrow-transplant recipients. Platelet transfusions from seronegative donors in combination with leukocyte-poor red-cell concentrates prevented primary CMV infection (*31*). However, this was only a partial solution of the problem of the availability of seronegative blood donors, as it was stressed in a recent review on blood-component therapy of bone marrow transplant recipients (*32*).

Additional factors may have contributed to the prevention primary CMV infections. GVHD is associated with a high incidence of CMV infections and fatal CMV interstitial pneumonitis (*1, 12, 33*). The incidence of acute and chronic GVHD this study was low due to the lymphocyte depletion by counterflow centrifugation (*12, 16, 17*). All patients received acyclovir for prevention of herpes infections. Prophylactic use acyclovir has proven to reduce significantly the risk of CMV infection in seropositive patients after allogeneic bone marrow transplantation, but its protective role in seronegative recipients remains conjectural (*12*). Passive immunization with hyperimmune globulin is still a controversial issue in bone marrow transplantation (*8, 10*), but it was not used for prophylaxis in this study.

Bone marrow transplant recipients with primary CMV infection may experience overwhelming CMV infection (*34*), but both patients with primary CMV infection in this study had easily controllable or subclinical disease. This may have been due to absence of GVHD at the time of developing CMV disease (*1, 33*) or to the use of acyclovir to prevent herpes infections (*11, 12*). T cell depletion has been reported to increase the overall incidence of fatal viral infections after BMT (*35*). This phenomenon probably depends on the method and degree of T cell depletion since several other studies on T cell depletion in allogeneic BMT, including the analysis of lymphocyte depletion by counterflow elutriation in our own institute, could not confirm this observation (*36, 16*).

This study showed that the use of leukocyte-poor blood products, both red-cell and platelet concentrates, from CMV-unselected blood-bank donors (volunteers) can prevent primary CMV infection in CMV-seronegative BMT recipients with CMV-negative marrow donors. More than 98% of the leukocytes were removed from blood products either by leukocyte filtration or by an additional centrifugation step. This approach to prevent CMV infection is relatively simple, inexpensive, and effective, as shown in this study. It also prevents immunization to human leukocyte antigen alloantigens and sensitization to random donor platelets (*37*). Prophylactic hyperimmune globulin administration did not appear necessary to prevent primo-infection with CMV in this category of patients.

Acknowledgments. We thank Mrs. Yvonne Poort and the nursing staff of the bone marrow transplant unit for their contributions to this study.

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Received 27 February 1990.
Accepted 26 June 1990.