

A Comparison of Filtered Leukocyte-Reduced and Cytomegalovirus (CMV) Seronegative Blood Products for the Prevention of Transfusion-Associated CMV Infection After Marrow Transplant

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We performed a prospective, randomized trial in CMV seronegative marrow recipients to determine if filtered blood products were as effective as CMV-seronegative blood products for the prevention of transfusion-transmitted CMV infection after marrow transplant. Before transplant, 502 patients were randomized to receive either filtered or seronegative blood products. Patients were monitored for the development of CMV infection and tissue-documented CMV disease between days 21 and 100 after transplant. Infections occurring after day 21 from transplant were considered related to the transfusion of study blood products and, thus, were considered evaluable infections for the purpose of this trial. In the primary analysis of evaluable infections, there were no significant differences between the probab-

ity of CMV infection (1.3% v 2.4%, $P = 1.00$) or disease (0% v 2.4%, $P = 1.00$) between the seronegative and filtered arms, respectively, or probability of survival ($P = .6$). In a secondary analysis of all infections occurring from day 0 to 100 post-transplant, although the infection rates were similar, the probability of CMV disease in the filtered arm was greater (2.4% v 0% in the seronegative arm, $P = .03$). However, the disease rate was still within the prestudy clinically defined acceptable rate of $\leq 5\%$. We conclude that filtration is an effective alternative to the use of seronegative blood products for prevention of transfusion-associated CMV infection in marrow transplant patients.

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PRI-MARY cytomegalovirus (CMV) infection caused by transfusion is a major problem for immunocompromised CMV-seronegative patients. For seronegative marrow transplant patients who receive standard blood products, the risk of CMV infection is between 28% and 57%.¹ Although infections can be asymptomatic, symptomatic CMV disease including pneumonia and gastroenteritis occurs in $\approx 30\%$ of all CMV-infected patients with substantial mortality, despite recent improvements in treatment.²⁻⁵

Delivery of CMV-seronegative screened blood products reduces the incidence of infection to 1% to 4% in CMV-seronegative marrow⁶⁻⁸ and solid organ transplant recipients⁹ as well as in infants born to CMV-seronegative mothers.¹⁰ The use of CMV-seronegative blood products has now become the standard of care for marrow transplant patients who are seronegative and who have seronegative marrow donors. However, the demand for CMV-safe blood has increased dramatically as the number of transplants has increased and as physicians try to maintain the seronegative status of potential transplant candidates. These indications plus requests for seronegative blood products for less proven indications (eg, in the seronegative patient receiving a sero-

positive organ allograft) have often resulted in the demand for CMV-seronegative blood products exceeding the supply at many blood centers.

Manipulations to remove CMV from blood before transfusion would greatly increase the availability of CMV-safe blood. Both clinical and laboratory observations have shown the leukocyte to be the vehicle of transmission of CMV by transfusion.^{11,12} Over the past 5 years, controlled^{13,14} and uncontrolled¹⁵⁻¹⁷ studies have shown that leukocyte reduction of blood products may significantly reduce the risk of CMV transmission. However, there are no comparative studies evaluating the relative safety and efficacy of leukocyte-reduced versus CMV-seronegative blood products for the prevention of CMV infection and disease.

We performed a prospective, randomized, controlled study to compare the effectiveness of 3-log₁₀ reduction of leukocytes by filtration from both red blood cell (RBC) and platelet transfusions with that of blood product screening in preventing CMV infection and disease in both seronegative allogeneic and autologous recipients after marrow transplantation. Analysis of survival and relapse rates between patients receiving either type of blood products was also performed.

MATERIALS AND METHODS

Patients and study design. Either autologous or allogeneic marrow transplant recipients admitted to the Fred Hutchinson Cancer Research Center (FHCRC) or to the University of Minnesota (UM) were eligible for study if both they and their marrow donors were CMV seronegative before transplant. Serologic testing of patients and marrow donors was performed both by latex agglutination (CMVSCAN Card test; Becton Dickinson, Baltimore, MD) and also by enzyme-linked immunosorbent assay (ELISA) (CMV STAT; Whittaker Bioproducts, Walkersville, MD) in Seattle. When patients having both tests performed had discrepancies between the two test results (ie, one was positive, one was negative) or when one test result was equivocal (ie, the result was between 0.8 and 1.0 in the ELISA index range and, thus, could not be interpreted as either positive or negative), samples were retested by latex agglutination and two of three negative tests defined seronegative status. Throughout the course of the study at UM, and during the last year of study in Seattle, ELISA was used as a single test.

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After initial CMV serology testing 2 to 4 weeks before transplant, patients were randomized by a central randomization center (FHCRC) to receive either CMV-seronegative screened blood products (seronegative arm) or filtered blood products (filtered arm). Patients received assigned blood products until the development of CMV infection, oncologic relapse, death, or day 100 after transplant. No intravenous Ig or antiviral prophylaxis was used routinely, with the exception of acyclovir (250 mg/m² every 12 hours) for prevention of herpes simplex infection. Conditioning for transplant and post-transplant immunosuppression to prevent acute graft-versus-host disease (GVHD) have been described.^{18,19} All patients consented for study in compliance with the standards set by the Institutional Review Boards of the two study sites.

Preparation of blood products. The number of red blood cell (RBC) products and the number, type, and source of each platelet product (ie, random-donor platelet concentrates or family member or community single-donor apheresis platelets) provided to the patients were recorded as single units. Both the pooled random-donor platelet concentrate transfusions given during the study and the apheresis products contained the equivalent of approximately six platelet concentrates. Blood products were provided by the blood centers at each trial site (Puget Sound Blood Center, Seattle, WA; the American Red Cross, St. Paul, MN; and the University of Minnesota Hospital Blood Banks, Minneapolis, MN). Seronegative blood donors were identified by latex agglutination at both trial sites. Because the study was not blinded and all apheresis donors were tested for CMV, we attempted to maintain a balance of seronegative and seropositive apheresis donors. To achieve this, the serostatus of the apheresis donors used for the filtered arm was not made available to those selecting the platelet donors. Platelets and RBCs were filtered in-line at the bedside using Pall filters (Pall Biomedical Products Corporation, Glen Cove, NY). Platelet filters were either the PL100 or PL50, each was used during approximately half of the study. RBC units were filtered using the Pall RC 100 filter. All of these filters are made from compressed polyester fibers and are known to consistently remove in excess of 3 log₁₀ of total leukocytes (including granulocytes) and in excess of 4 log₁₀ of total B and T cells (including CD4 and CD8 cells), and monocytes as assessed by flow cytometry studies.²⁰ Because it is not possible to obtain a representative postfiltration sample to quantitate the degree of leukocyte reduction achieved by bedside filtration,²¹ no postfiltration leukocyte counts were performed.

Evaluation of CMV infection and disease. Based on previous studies, patients who develop CMV infections ≤ 21 days from study entry may have had a recent prior infection, but either have not had the immunologic competence or time to seroconvert or had such a low antibody titer that the antibody could not be reproducibly detected.^{1,6,8} Thus, it was decided at the outset of the study that the primary endpoint of the study would be an analysis of patients who developed their infections more than 21 days after transplant to exclude the possibility that early infections were related to prestudy viral exposure. Although CMV disease was also monitored throughout the study, it is recognized that the progression of CMV infection to disease is primarily determined by immunologic factors at the time an infection is acquired rather than by some intrinsic property of blood product transfused.¹ Thus, CMV infection rates can be attributed directly to blood product exposure, while progression of CMV infection to disease is primarily controlled by immunosuppression.¹

CMV infection was defined as the identification by culture or CMV antigen detection of CMV from any clinical specimen, and CMV disease was defined as biopsy evidence of CMV in tissue with compatible clinical symptoms. CMV pneumonia was defined either by tissue biopsy or bronchoalveolar lavage (BAL) with a new or changing infiltrate on chest radiograph. All patients were evaluated

for the development of CMV infection with cultures of urine, throat, and blood by standard tube culture techniques. All FHCRC patients had cultures obtained weekly through day 100. However, cultures were obtained only every other week for $\approx 50\%$ of UM patients after discharge from the hospital. The remainder had weekly cultures and all patients returned to the transplant center at day 100 and cultures were obtained. Culture was used as the method of virus detection because the interpretation of seroconversion would be confounded by passively acquired antibody from filtered blood products or intravenous Ig.

Patients developing signs or symptoms of CMV disease during the first 100 days after transplantation underwent a diagnostic procedure to obtain tissue samples for culture and histopathologic examination. All tissue specimens were cultured and examined for typical histologic inclusions, including autopsy samples. BAL fluid was also evaluated by direct CMV-specific fluorescent antibody staining and by shell vial and standard tube culture techniques.

Statistics. The purpose of this study was to compare the relative efficacy of the two treatment modalities for the prevention of CMV infection and/or disease. Based on a projected incidence of CMV infection of 1% to 3% in the seronegative arm, we prospectively accepted a difference of $\leq 5\%$ in infection/disease rates between the two study arms as being clinically equivalent. We projected a sample size of 250 in each arm to have an 80% power to detect a difference of 5% or more at the 0.05 level by two-sided testing.

The incidence of CMV infection, disease, and survival to day 100 after transplantation was estimated using the Kaplan-Meier product limit method and the time to an event was compared using the exact version of the log-rank test. Analysis of other discrete demographic variables was performed using either Fisher's exact or Chi-square tests.

The primary endpoint of the trial was an analysis of the evaluable infections, ie, only those infections that developed between days 21 and 100 after transplant. For completeness, a secondary analysis for all infections occurring from day 0 to 100 was also performed. Patients reached a censoring endpoint at the time of death, malignancy relapse, on the day that they received in excess of six transfusions of nonassigned blood products (defined prestudy), on the day the last viral surveillance was obtained, or on the day that patients were lost to follow-up or at day 100 after transplant, whichever occurred first. CMV endpoints included the day of first positive culture from any site (infection) or positive tissue documentation, including BAL, either by culture or typical histologic inclusions (disease).

RESULTS

Patients. Five hundred and twenty-one transplant patients were randomized to the study between July of 1989 until April, 1993. Nineteen were considered ineligible for analysis because they either (1) refused participation in the study after randomization, but before any study transfusions were given (N = 4, seronegative arm; N = 9, filtered arm), (2) died or left the center before receiving a transplant (N = 2, seronegative arm; N = 3, filtered arm), or (3) had no data available after randomization (N = 1, filtered arm). Table 1 shows the demographic characteristics of the remaining 502 transplant patients, including 306 (61%) at FHCRC and 196 patients (39%) at UM. There were no statistically significant differences in any of the demographic characteristics listed.

Blood product delivery. Approximately 50% of the blood donors at both centers were seropositive. The only significant difference between blood product use by study

Table 1. Characteristics of 502 Patients According to Treatment Group

| Characteristic | Screened Blood (N = 252) | Filtered Blood (N = 250) |
|------------------------------------|-----------------------------|-----------------------------|
| Age | 28 (1-63) | 31 (1-59) |
| Sex (M:F) | 158:94 | 159:91 |
| Underlying diagnosis | | |
| ALL | 47 | 42 |
| ANL | 55 | 47 |
| CML | 51 | 68 |
| Lymphoma | 49 | 46 |
| Other | 50 | 47 |
| Transplant type | | |
| Allogeneic related | 99 | 97 |
| Allogeneic unrelated | 58 | 49 |
| Autologous | 93 | 103 |
| Twin | 2 | 1 |
| GVHD (allogeneic only) | | |
| Grade 0-1 | 49 | 40 |
| Grade 2-4 | 108 | 106 |
| Preparatory Regimen | | |
| TBI + CYT | 143 | 141 |
| Busulfan + CYT | 65 | 60 |
| TBI + other chemotherapy | 16 | 16 |
| Chemotherapy only | 28 | 32 |
| Other | 0 | 1 |
| GVHD prophylaxis (allogeneic only) | | |
| MTx | 27 | 24 |
| MTx + CSP | 85 | 83 |
| Other | 45 | 39 |

Abbreviations: MTx, methotrexate; CSP, cyclosporine; TBI, total body irradiation; CTX, cyclophosphamide; ALL, acute lymphocytic leukemia; ANL, acute non-lymphocytic leukemia; CML, chronic myelogenous leukemia; GVHD, graft-versus-host disease.

arm was a higher mean number of family donor apheresis platelets used in the filtered arm compared with the screened arm ($P = .005$) (Table 2). In general, the designated blood products were delivered without major difficulties throughout the study period. A total of 37 patients were censored for receiving more than 6 U of blood in nonstudy transfusions; 22 in the seronegative arm and 15 in the filtered arm. Censoring occurred for the following reasons: (1) 15 patients

Table 2. Mean (and Range) of RBC and Platelet Units by Donor Source for Each Study Arm

| | Screened Blood | Filtered Blood | <i>P</i> Value |
|------------------------------|-------------------|-------------------|-------------------|
| Mean platelet units (range)* | | | |
| Random-donor concentrates | 64 (0-500) | 64 (0-630) | NS |
| Apheresis platelets* | | | |
| Community | 13 (0-135) | 14 (0-113) | NS |
| Family | 5.6 (0-55) | 6.6 (0-61) | .005 |
| Mean RBC units (range)* | 18 (0-130) | 18 (2-106) | NS |

Abbreviation: NS, not significant.

* This table reports units of product (ie, 1 U from one donor). For apheresis single-donor platelets, one apheresis procedure was considered to be equivalent to 6 U of random platelets, but is reported here as 1 U because it came from one donor.

Table 3. Incidence (and Actuarial Probability) of CMV Infection and Disease by Study Arm

| CMV Event | Seronegative Blood (N = 252) | Filtered Blood (N = 250) | <i>P</i> Value* |
|--------------------------------|------------------------------------|--------------------------------|-----------------|
| Primary analysis (day 21-100) | | | |
| All CMV infections + disease | 2 (1.3%) | 3 (2.4%) | 1.0 |
| CMV disease only | 0 (0%) | 3 (1.2%) | 0.25 |
| Secondary analysis (day 0-100) | | | |
| All CMV infections + disease | 4 (1.4%) | 6 (2.4%) | 0.5 |
| CMV disease only | 0 (0%) | 6 (2.4%) | 0.03 |
| Survival | 79% | 82% | 0.56 |

Values given are the actual number of patients, with the % actuarial probability in parentheses.

* *P* values are determined from the log-rank test.

in the seronegative arm who received more than 6 filtered transfusions because the patients developed febrile transfusion reactions; (2) 5 patients in the seronegative arm and 13 in the filtered arm erroneously received more than 6 unscreened or unfiltered transfusions, respectively; (3) 1 patient in the seronegative arm required HLA-matched platelet apheresis collections from known CMV seropositive blood donors who were the only compatible donors available; and (4) transfusion needs were so acute that neither blood product type could be obtained in time for one patient in the seronegative arm and 2 patients in the filtered arm.

Cytomegalovirus infection and disease. Greater than 90% of weekly cultures were obtained for FHCRC patients on study to day 100. For UM patients, a mean of 1.37 culture sets were taken per week during an average hospital stay of 2 to 4 weeks and 0.45 times per week from hospital discharge until day 100. Approximately 50% of patients (49.9% of filtered blood patients and 50.1% of seronegative blood patients) remained on study for the entire 100 days of study. There were no statistically significant differences between the numbers of patients who were censored for protocol violations, death, relapse, or who were lost to follow-up in either arm.

On analysis of the data for the primary endpoint of CMV infections that occurred between days 21 and 100 after transplant, there were a total of 5 CMV infections, 2 among the 249 seronegative recipients (0.8%; confidence interval [CI] = 0.1% to 2.8%) and 3 among the 247 filtered blood recipients (1.2%; CI = 0.3% to 3.5%) (not significant [NS]) (Table 3). Of the 2 infections in the seronegative arm, 1 was viremia that occurred at day 35 after transplant, and the other was viruria at day 48. Of the three infections in the filtered arm, one was viremia at day 48 that progressed to pneumonia on day 52. The second patient developed pneumonia as the first sign of infection on day 52 and the third patient developed CMV gastroenteritis on day 56 after transplant. The actuarial probability of patients developing CMV infection by day 100 after transplant was 1.3% in the seronegative arm and 2.4% in the filtered arm ($P = 1.0$). Neither of the 2 infected patients in the seronegative arm developed CMV disease (0/249 or 0%; CI = 0.0% to 2.8%) whereas the 3 patients in the filtered arm (3/249 or 1.2%; CI = 0.3% to 3.5%) devel-

Table 4. Mean (and Range) of RBC and Platelet Units by Donor Source Given to Infected Versus Noninfected Patients

| | Infected* (N = 10) | Noninfected (N = 492) | P Value |
|------------------------------|-----------------------|--------------------------|---------|
| Mean platelet units (range)† | | | |
| Random-donor | 87 (0-318) | 64 (0-630) | NS |
| Apheresis platelets | | | |
| Community | 8 (0-38) | 14 (0-135) | NS |
| Family | 4.6 (0-13) | 6.3 (0-56) | NS |
| Mean RBC units (range)† | 16 (0-35) | 18 (1-130) | NS |

Abbreviation: NS, not significant.

* Includes all infections occurring between day 0 and 100 posttransplant.

† This table reports units of product (ie, 1 U from one donor). For apheresis single-donor platelets, one apheresis procedure was considered to be equivalent to 6 U of random platelets, but is reported here as 1 U because it came from the same donor.

oped CMV disease; however, the disease rate between the arms was not significantly different ($P = .25$).

In the secondary analysis, five additional patients developed early CMV infection between the time of randomization and before day 21 after transplantation, two in the seronegative arm and three in the filtered arm. Neither of the two additional patients in the seronegative arm developed CMV disease, whereas the three patients in the filtered arm developed disease. Including all infections between days 0 and 100 in a secondary analysis, the actuarial probability of developing CMV infection in the seronegative arm (1.4%) was not significantly different than the probability in the filtered arm (2.4%) ($P = .5$). Of particular note is that four of the five patients who developed CMV infections before day 21 had either equivocal or discrepant serologic test results at the time of study entry, suggesting there was some CMV antibody present even though they were defined as seronegative by serologic testing at the time of randomization. However, the probability of developing CMV disease was greater in the filtered arm (2.4% v 0%, $P = .03$). CMV disease was diagnosed by BAL lavage ($n = 3$), by endoscopy ($n = 1$), or at autopsy as an incidental finding ($n = 2$, and both in the lung). All five patients with CMV pneumonia had fatal outcomes, whereas the patient with enteritis survived. In neither the primary nor in the secondary analysis did the CMV infection/disease rates exceed the prestudy-defined clinically significant difference between the arms of 5%.

In the secondary analysis, there were no significant differences in either the type or number of blood products received by CMV-infected patients compared with noninfected patients (Table 4). All patients developing CMV infection received a combination of random platelet concentrates and apheresis platelets. In addition, there were no appreciable differences in the number of nonstudy blood products received by infected compared with uninfected patients in either study arm. In the seronegative arm, 22 patients received a mean of 2 U (range, 1 to 6 U) of unfiltered blood products with or without filtration in error, but only one of these patients (5%), who received 1 U seropositive unfiltered product in error, became infected. In the filtered arm, 13 patients (15%) received an average of 2 U unfiltered product (range,

1 to 6 U), but only one patient (8%) who received 1 U CMV-seropositive unfiltered blood in error, became infected. Three infections occurred while the PL100 platelet filters (first half of study) were being used and three infections while the PL50 filters were being used (second half of study).

We also examined risk factors known to increase the incidence of CMV infection and disease after marrow transplantation, and found no apparent differences between patients with CMV disease receiving filtered compared with seronegative products, including GVHD or its treatment, type of transplant, or the use of fractionated total body irradiation. Three infected patients in the seronegative arm and two patients in the filtered arm were recipients of an autologous transplant; the remaining infected patients were allogeneic transplant recipients.

Finally, survival in all patients was not significantly different at day 100 between the two arms with a projected survival of 82% in the filtered arm and 79% in the seronegative arm ($P = .56$). In addition, there was no significant difference in probability of malignant relapse at or before day 100 between patients in the study arms ($P = .42$).

DISCUSSION

Both uncontrolled trials¹⁵⁻¹⁷ and a controlled marrow transplant study¹⁴ have provided strong evidence that CMV transmission by transfusion can be prevented by leukocyte reduction. We undertook the present study using recently developed filters for both RBC and platelet transfusions to test whether 3-log₁₀ reduction of leukocytes by filtration was as effective as seronegative blood products for the prevention of transfusion-associated CMV infection. We also wanted to determine if allogeneic transplant recipients, who are at higher risk than autologous recipients for the development of life-threatening CMV disease, could be equally protected, as our previous trial had been performed primarily in autologous transplant recipients.¹⁴

The CMV infection and survival rates in patients were comparable in the two arms of this study, whether the data were analyzed for the primary endpoint of evaluable infections (those infections occurring between days 21 and 100 after transplant) or for any infection occurring through day 100. Thus, the present study shows that the administration of either leukocyte reduction by filtration (3 log₁₀) of both RBC and platelet products and CMV-seronegative blood products have approximately the same efficacy in preventing transfusion-transmitted CMV infection. The overall infection rates were low in both arms with actuarial probabilities of 1.4% for seronegative blood and 2.4% for filtered blood, which were similar to results of previously screened blood studies.⁶⁻⁸ Both seronegative and filtered blood were clearly superior to the previously reported 28% to 57% incidence of transfusion-associated infection observed when unfiltered blood products were used to support CMV-seronegative patients after marrow transplantation.⁶⁻⁸ Filtration was successful despite the exposure to large numbers of blood products required by marrow transplant patients and despite the likely shift to a higher than usual percentage of seropositive blood products given to patients in the filtered arm. This probable shift may have occurred because a large proportion of the

available seronegative blood products in each community were being given to patients in the seronegative arm. Both allogeneic and autologous transplant recipients appeared equally protected from infections by filtered blood products (incidence of CMV infections of 1.4% and 0.9%, respectively).

CMV disease rates in the two study arms were also not statistically significantly different for evaluable infections ($P = .25$), but were significantly different when all infections were analyzed ($P = .03$), with less CMV disease in patients receiving seronegative blood. While including all infections may be the preferred analysis from a statistical point of view, prospectively defined rules for evaluability were based on data that early infections likely result from the presence and possible reactivation of unrecognized virus acquired before randomization. Therefore, we believe excluding early infections is more meaningful and clinically relevant.

The results of this study support this decision. Four of the five patients whose infections developed before day 21 had either equivocal or discrepant serologic test results at the time of randomization, suggesting that these patients were likely infected before entering the study.^{1,6-8} Because serologic screening tests are not 100% accurate, the entry of a small number of false-negative patients into this study was anticipated. In a study at FHCRC, where the concordance rate between two different serologic assays was compared in 409 sera, we observed equivocal results in 4.1% and either false-positive or false-negative results in 1.1% of the pairs when samples were retested by a third test.⁷ In the present study, there were 11/306 (4%) equivocal results and 36/306 (12%) discrepant results, consistent with the previous report. Furthermore, the results presented here strongly suggest that more sensitive serologic screening tests would be the best way to improve upon the results of this study.

The difference in CMV disease rates between the two arms when all infected patients were included in the secondary analysis is not easily explained based on our understanding of CMV pathobiology. The observed incidence of any CMV disease was only 2.4% in the filtered arm compared with 0% in the seronegative arm. Therefore, both techniques achieved the prestudy target goal of less than 5% difference in infection or disease rates as being clinically equivalent because we did not expect either method to be perfect in preventing either CMV infection or disease. However, CMV disease usually develops in less than 50% of patients acquiring primary culture or histologically proven CMV infections, whether in known seropositive patients,¹ seronegative patients getting seronegative blood and marrow from a seropositive marrow donor⁸ (R. Bowden, unpublished data, December 1989) or from blood^{1,6-8} or granulocyte transfusions.²² Therefore, it was surprising and remains unexplained why 100% of the infected patients who received filtered blood products developed disease. In fact, during the 6 months after completion of this study, two cases of CMV disease were observed in patients receiving seronegative blood products; one infection occurred early and one occurred after the first 21 days posttransplant. Because the probability of CMV infection was similar in both groups in the present study, we have no biologic explanation for the higher incidence of

CMV disease in the filtered group, particularly because the immunosuppressive risks were the same in both patient groups. It is not realistic to expect that whatever method used to prevent infection would necessarily prevent the development of CMV disease in the few patients who experience breakthrough infections. Although the readers must draw their own conclusions, we believe that the very low number of infections in the current study is too small for us to draw conclusions about the significance of CMV infections resulting in disease in either arm. Finally, it is possible that either filter failures or the higher percentage of single family donors used in the filtered arm (ie, some CMV-seropositive donors may have been more likely to transmit CMV continuously than single seropositive donors would), may have increased the disease risk in the filtered arm. However, if this were true, one would be more likely to expect this to result in a higher infection rate in the filtered arm, not a higher disease/infection ratio. If this explanation is correct, one would also expect a high disease/infection ratio among patients receiving unselected blood or granulocyte transfusions. This has not been observed.^{6-8,22} It is unknown what impact a higher proportion of seropositive blood donors would have on the incidence of CMV infections in the filtered arm.

In summary, the present study shows that filtration of blood products is as effective as CMV-seronegative blood products in preventing transfusion-acquired CMV infection after allogeneic or autologous marrow transplant. This study showed equivalency of the two methods for prevention of CMV infection despite exposure to blood products from a very large number of different donors. Although, we can offer no satisfactory biologic explanation as to why more CMV disease occurred in the filtered group, this observation reached marginal statistical significance only when early infections were included in the analysis. The risk of developing CMV disease has uniformly been shown to be related to factors associated with immunosuppression of the host and not to the type of exposure.

Thus, we believe that the results of this study justify abandoning the maintenance of dual inventories of seronegative and seropositive/unselected blood products. In fact, the need to perform serologic screening of blood products for CMV could be eliminated altogether. However, we do agree, based on the unresolved question as to why more CMV disease occurred in the filtered blood group or more likely on cost considerations, that some blood centers or transfusion services may choose to maintain dual inventories until the time when newer screening techniques become available to better identify the truly seronegative recipient,²³ more efficient filters are developed, or new studies show that this CMV disease risk is not reproduced. At the current time, the blood centers or transfusion services involved with this study are using the two methods interchangeably to provide CMV-safe blood products. What products are used have been selected on cost, availability, and physician ordering practices at each institution.

Other immunocompromised patient populations such as newborns and solid organ transplant patients will likely also be protected from CMV infection by filtered blood products,

however, further study in these patient populations may be warranted. Although it is likely that the risk of CMV disease in these less immunocompromised patients would be lower, there may be qualitative differences in these patient populations that require further study.

Although bedside delivery of filtered blood products successfully prevented CMV infection in this study, we believe that filtration in the blood center should further improve the amount and consistency of leukocyte reduction. In addition, random prefiltration and postfiltration quality control samples can be obtained for leukocyte counting to ensure that appropriately leukocyte reduced products are being provided. In addition, since the initiation of this study newer filters have become available that can achieve leukocyte reduction of up to $3 \log_{10}$, further improving the potential to provide reliable blood products capable of preventing transfusion-acquired CMV infection. We believe that leukocyte-reduction by filtration represents a major advance in the prevention of transfusion-transmitted CMV infection in truly seronegative recipients. Only relatively minor additional reductions in the incidence of CMV infection will likely be achieved by more reliable screening of patients to identify the truly seronegative patient, more reliable screening of donors to insure only truly seronegative blood is delivered, or improved leukocyte-reduction methods.

REFERENCES

- Meyers JD, Flournoy N, Thomas ED: Risk factors for cytomegalovirus infection after human marrow transplantation. *J Infect Dis* 153:478, 1986
- Reed EC, Bowden RA, Dandliker PS, Lilleby KE, Meyers JD: Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann Intern Med* 109:783, 1988
- Emanuel D, Cunningham I, Jules-Elysee K, Brochstein JA, Kerman NA, Laver J, Stover D, White DA, Fels A, Polsky B, Castro-Malaspina H, Peppard JR, Bartus P, Hammerling U, O'Reilly RJ: Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. *Ann Intern Med* 109:777, 1988
- Schmidt GM, Kovacs A, Zaia JA, Horak DA, Blume KG, Nademanee AP, O'Donnell MR, Snyder DS, Forman SJ: Ganciclovir/immunoglobulin combination therapy for the treatment of human cytomegalovirus-associated interstitial pneumonia in bone marrow allograft recipients. *Transplantation* 46:905, 1988
- Enright H, Haake R, Weisdorf D, Ramsay N, McGlave P, Kersey J, Thomas W, McKenzie D, Miller W: Cytomegalovirus Pneumonia After Bone Marrow Transplantation. *Transplantation* 55:1339, 1993
- Bowden RA, Sayers M, Flournoy N, Newton B, Banaji M, Thomas ED, Meyers JD: Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. *N Engl J Med* 314:1006, 1986
- Bowden RA, Sayers M, Gleaves CA, Banaji M, Newton B, Meyers JD: Cytomegalovirus-seronegative blood components for the prevention of primary cytomegalovirus infection after marrow transplantation: Considerations for blood banks. *Transfusion* 27:478, 1987
- Miller WJ, McCullough J, Balfour HH Jr, Haake RJ, Ramsay NKC, Goldman A, Bowman R, Kersey J: Prevention of cytomegalovirus infection following bone marrow transplantation: A randomized trial of blood product screening. *Bone Marrow Transplant* 7:227, 1991
- Preiksaitis JK, Rosno S, Grumet C, Merigan TC: Infections due to herpesviruses in cardiac transplant recipients: Role of the donor heart and immunosuppressive therapy. *J Infect Dis* 147:974, 1983
- Yeager AS, Grummet FC, Haffleigh EB, Arvin AM, Bradley JS, Prober CG: Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J Pediatr* 98:281, 1981
- Einhorn L, Öst Å: Cytomegalovirus infection of human blood cells. *J Infect Dis* 149:207, 1984
- Rice GPA, Schrier RD, Oldstone MBA: Cytomegalovirus infects human lymphocytes and monocytes: Virus expression is restricted to immediate-early gene products. *Proc Natl Acad Sci USA* 81:6134, 1984
- Gilbert GL, Hayes K, Hudson IL, James J, and the Neonatal Cytomegalovirus Infection Study Group: Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leucocytes. *Lancet* 1:1228, 1989
- Bowden RA, Slichter SJ, Sayers MH, Mori M, Cays MJ, Meyers JD: Use of leukocyte-depleted platelets and cytomegalovirus-seronegative red blood cells for prevention of primary cytomegalovirus infection after marrow transplant. *Blood* 78:246, 1991
- Verdonck LF, de Graan-Hentzen YCE, Dekker AW, Mudde GC, de Gast GC: Cytomegalovirus seronegative platelets and leukocyte-poor red blood cells from random donors can prevent primary cytomegalovirus infection after bone marrow transplantation. *Bone Marrow Transplant* 2:73, 1987
- DeWitte T, Schattenberg A, Van Dijk BA, Galama J, Olthuis H, Van Der Meer JWW: Prevention of primary cytomegalovirus infection after allogeneic bone marrow transplantation by using leukocyte-poor random blood products from cytomegalovirus-unscreened blood-bank donors. *Transplantation* 50:964, 1990
- de Graan-Hentzen YCE, Gratama JW, Mudde GC, Verdonck LF, Houbiers JGA, Brand A, Sebens FW, van Loon AM, The TH, Willemze R, de Gast GC: Prevention of primary cytomegalovirus infection in patients with hematologic malignancies by intensive white cell depletion of blood products. *Transfusion* 29:757, 1989
- Nash RA, Pepe MS, Storb R, Longton G, Pettinger M, Anasetti C, Appelbaum FR, Bowden R, Deeg HJ, Doney K, Martin PJ, Sullivan KM, Sanders J, Witherspoon RP: Acute graft-versus-host disease: Analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. *Blood* 80:1838, 1992
- Weisdorf D, Hakke R, Blazar B, Miller W, McGlave P, Ramsay N, Kersey J, Filipovich A: Risk factors for acute graft-versus-host disease in histocompatible donor bone marrow transplantation. *Transplantation* 51:1197, 1991
- Wenz B, Burns ER: Phenotypic characterization of white cells in white cell-reduced red cell concentrate using flow cytometry. *Transfusion* 31:829, 1991
- Williamson LM, Wimperis JZ, Williamson P, Coppleson JA, Gooi HC, Morgenstern GR, Norfolk DR for The Alloimmunisation Study Group: Bedside filtration of blood products in the prevention of HLA alloimmunization—A prospective randomized study. *Blood* 83:3028, 1994
- Hersman J, Meyers JD, Thomas ED, Buckner CD, Clift R: The effect of granulocyte transfusions on the incidence of cytomegalovirus infection after allogeneic marrow transplantation. *Ann Intern Med* 96:149, 1982
- Zhang LJ, Hanff P, Rutherford C, Churchill WH, Crumpacker CS: Detection of human cytomegalovirus DNA, RNA, and antibody in normal donor blood. *J Infect Dis* 171:1002, 1995