Leukocyte-Reduced Red Blood Cell Transfusions in Patients With Anemia and Human Immunodeficiency Virus Infection

The Viral Activation Transfusion Study: A Randomized Controlled Trial

Ann C. Collier, MD
Leslie A. Kalish, ScD
Michael P. Buseh, MD, PhD
Terry Gernsheimer, MD
Susan F. Assmann, PhD
Thomas A. Lane, MD
David M. Asmuth, MD
Michael M. Lederman, MD
Edward L. Murphy, MD, MPH
Princy Kumar, MD
Meera Kelley, MD
Timothy P. Flanigan, MD
Deborah K. McMahon, MD
Henry S. Sacks, MD, PhD
Melanie S. Kennedy, MD
Paul V. Holland, MD

for the Viral Activation Transfusion Study Group

ADVANCES IN ANTIRETROVIRAL treatment have markedly improved prognosis of persons infected with human immunodeficiency virus (HIV). However, acquired immunodeficiency syndrome (AIDS) continues to occur since antiretroviral therapy is not always tolerated, successful, or available and persons may be unaware of their infection until late clinical manifestations occur.

One complication of advanced HIV infection is anemia, which may be caused by HIV, opportunistic diseases, and medications. An estimated 21% to 58% of HIV patients are anemic, with increasing frequency in advanced HIV. Anemia is associated with shorter survival.

Management of anemia includes stopping marrow-suppressive medications, use of erythropoietin, and

Context Allogeneic blood transfusions have immunomodulatory effects and have been associated with activation of human immunodeficiency virus (HIV) and cytomegalovirus (CMV) in vitro and of HIV in small pilot studies. Retrospective studies suggest that transfusions adversely affect the clinical course of HIV. Data in selected non–HIV-infected patients requiring blood transfusion have suggested clinical benefit with leukocyte-reduced red blood cells (RBCs).

Objective To compare the effects of leukoreduced and unmodified RBC transfusions on survival, complications of acquired immunodeficiency syndrome, and relevant laboratory markers in HIV-infected patients.

Design and Setting Double-blind randomized controlled trial conducted in 11 US academic medical centers from July 1995 through June 1999, with a median follow-up of 12 months (24 months in survivors).

Patients A total of 531 persons infected with HIV and CMV, aged 14 years or older, who required transfusions for anemia; 259 received leukoreduced transfusions and 262 received unmodified transfusions (10 did not receive the planned transfusion).

Main Outcome Measures Survival and change in plasma HIV RNA level 7 days after transfusion, compared by type of transfusion.

Results At entry, the groups were similar in demographic, clinical, and relevant laboratory characteristics. A total of 3864 RBC units were transfused. Two hundred eighty-nine deaths occurred (151 with leukoreduced transfusion; 138 with unmodified transfusion); median survival was 13.0 and 20.5 months, respectively (relative hazard [RH], 1.20; 95% confidence interval [CI], 0.95-1.51; log-rank P = .12). Analyses adjusted for prognostic factors suggested possible worse survival with leukoreduction (RH, 1.35; 95% CI, 1.06-1.72). There was no difference in time to new opportunistic event/death or frequency of transfusion reactions. No changes in plasma HIV RNA level were seen in either group at days 7, 14, 21, or 28, even in patients not taking antiretroviral drugs. There were no differences in trends between groups in CMV DNA, CD4 cell counts, activated (CD38% or human leukocyte antigen-DR) CD8 cell counts, or plasma cytokine levels.

Conclusions We found no evidence of HIV, CMV, or cytokine activation following blood transfusion in patients with advanced HIV infection. Leukoreduction provided no clinical benefit in these patients. These data demonstrate the importance of conducting controlled studies of effects of leukoreduction in different patient populations, since smaller studies in other patient populations have suggested leukoreduction may be beneficial.

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that RBC transfusions containing leukocytes would result in immune and viral activation, and adversely affect the course of HIV disease. The data from this study are unique since this is one of the largest randomized studies of leukoreduction, and the only large-scale prospective study of blood transfusions in HIV-infected persons.

METHODS
Study Design and Treatment
The study was a randomized, double-blind, comparative study of leukoreduced vs unmodified RBCs. The participants, investigators, study coordinators, and persons having any contact with the patients were blinded to study treatment assignments. Blood bank technical staff who prepared the blood products were aware of the treatment assignments. All required procedures of the Food and Drug Administration and American Association of Blood Banks were followed during collection, testing, administration of blood components to the study enrollees, and leukoreduction (ie, no more than 5 × 10^9 µL of leukocytes per unit of blood). Leukoreduction involves the use of a sterile, single-use filter that is inserted in the tubing between the RBCs and a secondary collection bag. The blood is filtered using gravity, and the filter removes leukocytes by mechanical trapping and absorption. The RBC leukoreduction occurred prior to storage and within 72 hours of blood collection. At time of transfusion, RBC units were to be no more than 14 storage-days old. If enrollees required platelet transfusion, they received leukoreduced platelets. No screening of blood products for CMV antibodies was performed prior to transfusion. If enrollees developed a new medical condition that required irradiated blood products, they continued to receive their blinded, assigned blood components, and irradiation was allowed. Gamma irradiation performed under controlled procedures using regulated blood bank instruments results in damage to nucleic acids that precludes leukocytes, particularly lymphocytes, from proliferating and causing graft-vs-host disease. It has no significant effect on the integrity of other blood elements, including RBCs, platelets, and plasma proteins, nor does it have an impact on the infectivity of viruses or other pathogens.

Patients
Patients had confirmed HIV infection, symptomatic anemia requiring RBC transfusion as defined by their physician, and were to receive nonemergent RBC transfusions within 72 hours of enrollment. Enrollees were 14 years or older, had documented CMV infection (by chart review or antibody testing), a Karnofsky performance score of 40 or higher, and expected survival of more than 1 month. Patients were ineligible if they had a surgical reason for transfusion, a prior history of transfusion, renal failure requiring dialysis, thrombocytopenic purpura, used intravenous immunoglobulin within 6 weeks of entry, or had started new antiretroviral drugs or systemic immunomodulators (eg, interleukin [IL]-2, interferons, granulocyte colony-stimulating factor) within 2 weeks of entry.

Study Procedures
The study was approved by the institutional review boards at the 11 participating institutions. Patients gave written informed consent. Treatment allocation was made centrally by the study coordinating center, using stratified permuted blocks with dynamic balancing within each center. Patients were stratified by (1) CD4 cell count of less than 50 × 10^3/µL or greater and (2) presence of CMV end organ disease. Patients received transfusions as ordered by their physician and underwent monitoring during transfusions per local policies.

Patients underwent standardized evaluations. Occurrence of serious HIV-related complications, nonstudy transfusions, and type and dates of HIV-associated medications were assessed at each visit. Use of antiretroviral therapy

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was classified as (1) potent (≥3 antiretroviral drugs with at least 1 protease inhibitor or 1 nonnucleoside reverse transcriptase inhibitor), (2) other antiretroviral drugs, and (3) no antiretroviral drugs. Patients had dilated eye examinations by an ophthalmologist every 6 months. At entry and every 3 months, patients had complete BCB and CD4 cell counts and plasma HIV RNA and CMV DNA assays were performed.

Since it was hypothesized that immune or viral activation might differ between first and later transfusions, we defined 2 transfusion series for the purpose of assessing possible short-term changes in laboratory end points. The first series started at the patient’s first transfusion and included all subsequent RBC units transfused less than 7 days after a prior unit. The second was defined only for patients who had 1 or more transfusions starting at least 7 days after the end of the first series, and again included all subsequent RBC units transfused less than 7 days after a prior unit. The HIV RNA and CMV DNA levels were measured pretransfusion and at weeks 1, 2, 3, and 4 after the start of each transfusion series. Lymphocyte activation markers were measured pretransfusion and at weeks 1 and 4; cytokine levels were measured pretransfusion and at week 1. Patients were evaluable for a transfusion series if they had a pretransfusion and at least 1 posttransfusion sample available.

Laboratory testing was done centrally on batched specimens that were separated within 4 hours of collection and stored at −70°C. The CMV antibodies were assayed by enzyme immunoassay. After enrollment, blood from the baseline blood draw was tested for CMV antibodies at the Viral Activation Transfusion Study (VATS) central laboratory. Each unit of donor blood was also tested retrospectively for CMV antibodies. Lymphocyte subsets (CD3/CD4, CD3/CD8, and the proportion of CD8 cells expressing activation markers CD38% and human leukocyte antigen-DR) were measured by flow cytometry (FACScan, Becton Dickinson, San Jose, Calif). CD3/CD4 lymphocytes, often called CD4 T cells, are a major target of HIV, and their depletion is used to measure progression of HIV disease. The HIV RNA level was measured using a reverse transcriptase polymerase chain reaction assay with a lower limit of quantification of 200 copies/mL (Amplicor Monitor assay, Roche Molecular Systems, Branchburg, NJ). The CMV DNA was initially assayed using a qualitative polymerase chain reaction assay, and if positive, a quantitative polymerase chain reaction assay was subsequently applied (Roche Molecular Systems). The lower limit of the quantitative assay was 400 copies/mL. Tumor necrosis factor, tumor necrosis factor receptor, IL-6, and β₂-microglobulin plasma levels were assayed by enzyme immunoassays as detailed elsewhere. Quality control of residual leukocyte content of leukoreduced RBCs and platelets was previously described.

End Points and Statistical Methods

Primary end points were originally time to death or first serious HIV-related complication and change in plasma HIV RNA level from baseline to week 1 after first transfusion series. Planned accrual was 430, but 3 months into the study, additional resources were obtained to increase accrual to 640 and change the primary clinical end point to survival. The final design assumed 427 deaths, and had 85% power for detecting a relative hazard (RH) of 1.33 for the survival comparison, using a 2-sided test (α = .05). With improvement in the course of HIV, there were 289 deaths, resulting in 85% power to detect an RH of 1.42 for survival.

3Antiretroviral drugs with at least 1 protease inhibitor or 1 nonnucleoside reverse transcriptase inhibitor,

2Antiretroviral drugs, and

1No antiretroviral drugs.

Bacteremia†

Catheter-related

Noncatheter-related

Other serious bacterial/fungal infections (isolation from a normally sterile site)

Cervical cancer

Coccidioidomycosis (disseminated or extrapulmonary)

Cryptococcus (extrapulmonary)

Cryptosporidiosis (>1 mo)

Cytomegalovirus end organ disease

Histoplasmosis (disseminated or extrapulmonary)

Kaposi sarcoma (visceral)

Mycobacterium avium complex (disseminated or extrapulmonary)

Mycobacterium kansasii (disseminated or extrapulmonary)

Mycobacterium tuberculosis

Non-Hodgkin lymphoma (Burkitt, immunoblastic, or primary brain)

Other mycobacterial species (disseminated or extrapulmonary)

Pneumocystis carinii pneumonitis

Progressive multifocal leukoencephalopathy‡

Toxoplasmosis (brain)§

‡To be classified as a bacteremia, patients had to have systemic or focal symptoms or have 2 or more cultures positive for the same organism. To be classified as a catheter-related bacteremia, patients had no source of infection other than a catheter, and if they only had 1 positive blood culture, they had to be treated with antimicrobial therapy and have had a favorable clinical response.

§Required positive polymerase chain reaction for JC virus and compatible clinical syndrome or diagnostic brain biopsy.

Toxoplasmosis (brain)§

†Potent (≥3 antiretroviral drugs with at least 1 protease inhibitor or 1 nonnucleoside reverse transcriptase inhibitor), 2 other antiretroviral drugs, and 3 no antiretroviral drugs.

+Defined as CD4 cells <200 cells/µL in the absence of other causes.

†To be classified as a bacteremia, patients had to have systemic or focal symptoms or have 2 or more cultures positive for the same organism. To be classified as a catheter-related bacteremia, patients had no source of infection other than a catheter, and if they only had 1 positive blood culture, they had to be treated with antimicrobial therapy and have had a favorable clinical response.
Secondary objectives included time to death or first serious HIV-related complication, time to new or progressive CMV end organ disease, plasma HIV RNA and CMV DNA levels, lymphocyte subset markers, and change in cytokine and lymphocyte activation markers. The HIV-related complications were predefined and included specific AIDS-defining conditions and serious bacterial infections associated with median survival times of less than 1 year or an acute mortality of more than 5% (Box).

Definitive diagnoses were required, except for toxoplasmosis, in which patients were required to meet a standardized case definition. Progressive CMV retinitis required an ophthalmologist’s diagnosis, as well as a change in therapy. Each clinical endpoint was confirmed to meet the study criteria.

Survival and other time-to-failure probabilities were estimated by the Kaplan-Meier method. Log-rank tests and proportional hazards models (with time-varying covariates for factors determined after randomization) were used to compare failure-time curves. The HIV RNA and CMV DNA levels and CD4 cell counts were log transformed before analyses. Changes in laboratory parameters were calculated by comparison with pretransfusion values. Treatments were compared at selected time points using Wilcoxon rank-sum tests, and more globally using repeated measures models adjusted for baseline value. Long-term trends in HIV RNA level and CD4 cell count were assessed with linear random effects models. Discrete outcomes were analyzed with the Fisher exact test. All P values are 2-sided.

RESULTS

Patients and Follow-up

A total of 531 patients were enrolled from July 1995 through July 1998 (Figure 1). Treatment groups were well balanced (Table 1). All patients had advanced HIV infection and 78% had had an AIDS-defining illness. Median CD4 cell count was 15/µL. Median plasma HIV RNA level was 4.8 log_{10} copies/mL (mean, 4.5 log_{10} copies/mL). Median and mean entry hemoglobin were 7.3 g/dL. More than 85% of patients had taken antiretroviral drugs, 69% had taken them within 30 days of entry. Five percent used erythropoietin within 30 days prior to entry. Forty-three patients were found to be ineligible after enrollment (21 leukoreduced, 22 unmodified); 29 had added antiretroviral or immunomodulator drugs within the excluded 2-week time frame. Reasons for ineligibility were similar between groups. All patients are retained in the analyses (analyses were done by intention-to-treat, so no patients found to be ineligible retrospectively were dropped from the analyses). Of the 531 patients enrolled, 10 (6 leukoreduced, 4 unmodified) did not receive the planned transfusion.

Follow-up ended June 30, 1999. Mean follow-up was 12 months (24 months among patients who survived). Seven (1%) patients withdrew from study components (5 continued follow-up) and 61 (11%) terminated participation early (Figure 1). Reasons for early termination were similar. Thirty-five patients (19 leukoreduced, 16 unmodified) had no follow-up for HIV-related complications and were excluded from analyses of associated outcomes. The mean (SD) number of follow-up quarterly visits was 3.7 (4.1) and 4.3 (4.4) for the leukoreduced and unmodified groups, respectively; the median numbers were 2 and 3.

Blood Components

A total of 3864 RBC units were transfused; 79 (2%) units for 25 (5%) patients were not prepared per randomized assignment (Table 2). Median age of RBC units was 9 days. Eighty-five percent of patients received at least 1 CMV-seropositive unit, as anticipated with CMV-seroprevalence among blood donors of approximately 50% and with most patients receiving multiple RBC units. Residual leukocyte counts for leukoreduced RBC units were greater than 5 x 10^3/µL for 15 (<1%) of 1869 units tested. Four percent of platelet units had greater than 5 x 10^3/µL residual leukocyte counts. The groups were similar for all parameters related to blood components except for weight of RBC units, which was less in the leukoreduced arm (P<.001). One patient in the unmodified arm received irradiated RBCs and 1 patient in each arm received irradiated platelets.
Table 1. Baseline Clinical and Laboratory Characteristics of the Study Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Leukoreduced (n = 265)</th>
<th>Unmodified (n = 266)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (mean [SD]), y</td>
<td>37 (38.3 [8.2])</td>
<td>38 (38.4 [8.3])</td>
</tr>
<tr>
<td>Men, %</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td>Black</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Hispanic</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Human immunodeficiency virus (HIV) risk, %*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men having sex with men</td>
<td>60</td>
<td>57</td>
</tr>
<tr>
<td>Injection drug use</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Heterosexual sex</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cytomegalovirus end organ disease, %</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Prophylaxis for Pneumocystis carinii pneumonia†</td>
<td>88</td>
<td>87</td>
</tr>
<tr>
<td>Antiretroviral therapy, %†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potent combinations§</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>None</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Zidovudine use, %†</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Karnovsky score</td>
<td>Median§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70 (60-80)</td>
<td>70 (60-80)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>71.4 (13.2)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>Median§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5 (6.6-8.2)</td>
<td>7.3 (6.6-7.9)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>7.3 (1.5)</td>
</tr>
<tr>
<td>Plasma HIV RNA, log_{10} copies/mL</td>
<td>Median§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.8 (3.9-5.4)</td>
<td>4.9 (4.0-5.3)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>4.5 (1.1)</td>
</tr>
<tr>
<td>CD4 cells/µL, median§</td>
<td>16 (3-71.5)</td>
<td>12.5 (4-76)</td>
</tr>
<tr>
<td>CD8 cells/µL, median§</td>
<td>212 (92-408)</td>
<td>244.5 (110-449)</td>
</tr>
<tr>
<td>CD38% of CD8 cells, median§</td>
<td>43.8 (33.5-54.4)</td>
<td>45.9 (35.1-57.9)</td>
</tr>
<tr>
<td>Interleukin 6, pg/mL, median§</td>
<td>11 (5-30)</td>
<td>13 (6-41)</td>
</tr>
<tr>
<td>Tumor necrosis factor, pg/mL, median§</td>
<td>10 (6-17)</td>
<td>10 (6-17)</td>
</tr>
<tr>
<td>β2-Microglobulin, mg/L, median§</td>
<td>6.0 (4.2-8.7)</td>
<td>5.8 (4.3-8.1)</td>
</tr>
</tbody>
</table>

*Patients could have multiple risk factors.
†Within the last 30 days.
§Potent combination therapy was defined as 3 or more antiretroviral drugs, including at least 1 protease inhibitor or non-nucleoside reverse transcriptase inhibitor.
¶Values in parentheses are 25th and 75th percentiles.

Survival and Clinical Events

Survival. A total of 289 deaths occurred (151 leukoreduced and 138 unmodified). There was no significant difference in time to death between groups (P = .12; Figure 2). Median survival was 13 months for the leukoreduced group and 20.5 months for the unmodified group (RH of death, 1.20; 95% confidence interval [CI], 0.95-1.51).

Exploratory analyses were performed to investigate the nonsignificant trend toward worse survival in the leukoreduced group. Analysis using a Cox proportional hazards model, adjusting for baseline CD4 cell count and plasma HIV RNA level, suggested possible worse survival in the leukoreduced group (RH of death, 1.35; 95% CI, 1.06-1.72). Results were similar when use of potent antiretroviral therapy was added as a time-dependent covariate (RH, 1.39; 95% CI, 1.09-1.78). Adjustment for sex, use of prophylaxis or treatment for Mycobacterium avium complex, history of CMV disease, or cytokine levels did not affect the treatment RH of death (L.A.K. and S.F.A, unpublished data from the VATS Group, 2000). The trend toward a higher risk of death in the leukoreduced group was accentuated in the subgroup of enrollees with CD4 cell counts of less than 10/µL (RH, 1.67; 95% CI, 1.21-2.31), but this was not significantly different from patients with CD4 cell counts of 10/µL or greater (interaction P = .08). Increased exposure to RBCs did not significantly affect the results. Among persons receiving 1 to 2, 3 to 6, or 7 or more units, the treatment RHs of death were 1.02, 1.13, and 1.27, respectively (P = .77 for heterogeneity).

Clinical Events. There was no difference in clinical events between groups; 323 patients had either a new serious HIV-related complication or died (164 leukoreduced and 159 unmodified). Median time to first event was 6 months in the leukoreduced group and 7.8 in the unmodified group (P = .28; Figure 3). The RH for first clinical event (leukoreduced vs unmodified group) was 1.13 (95% CI, 0.91-1.40). Similar results were seen using Cox proportional hazards models that adjusted for baseline CD4 cell count and plasma HIV RNA level (hazard ratio, 1.17; 95% CI, 0.94-1.47), and additionally for use of potent antiretroviral therapy as a time-dependent covariate (hazard ratio, 1.19; 95% CI, 0.94-1.50).

Types of first clinical events and of all new clinical events are shown in Table 3. Similar events occurred in both groups and were typical of advanced HIV. New or progressive CMV disease occurred in a total of 33/246 (13%) leukoreduced and 30/250 (12%) unmodified patients. The groups were similar in time to first occurrence of the more common events: CMV retinitis (P = .64); catheter-related (P > .99) and noncatheter-related bacteremia (P = .26), nontuberculous mycobacterial infections (P = .07); and CMV end organ disease of any type (P = .47).

Transfusion Reactions

Signs and symptoms related to transfusion did not differ significantly between groups. No life-threatening transfusion reactions occurred. A temperature increase of 1°C or higher occurred with 16% of leukoreduced and 18% of unmodified transfusions; a drop in systolic blood pressure (≥ 30% or to < 90...
mm Hg) occurred in 5% of leukoreduced and 6% of unmodified transfusions. Two patients were removed from study components because of transfusion reactions, but neither had their assignment unblinded.

**Laboratory End Points**

**Change in Plasma HIV RNA Level.** Plasma HIV RNA levels were stable in both groups; no differences were seen between groups in the primary laboratory end point of change in HIV RNA levels at 1 week (P = .97; Figure 4A) or during the first 4 weeks (P = .65; Figure 4A) after initial transfusion. A pretransfusion and at least 1 posttransfusion specimen were available for 90% of patients in the first transfusion series. Results were similar in patients categorized by antiretroviral therapy type, including patients not taking antiretroviral drugs (P = .69 for treatment comparison at 1 week, Figure 4B). A pretransfusion and at least 1 posttransfusion specimen were available for 89% of the 239 patients who received a second transfusion series. Plasma HIV RNA levels also remained stable in both treatment groups following the second transfusion series (L.A.K. and S.F.A., unpublished data from the VATS Group, 2000). There were 520 patients in the first transfusion series and both groups received a median of 2 units—258 patients in the leukoreduced group (mean [SD] units, 2.55 [1.05]) and 262 in the unmodified group (2.56 [1.73]) (Wilcoxon P = .40). There were 239 patients in the second transfusion series and both groups received a median of 2 units—114 patients in the leukoreduced group (mean [SD] units, 2.65 [1.52]) and 125 in the unmodified group (2.69 [1.33]) (Wilcoxon P = .68).

Longer-term trends in plasma HIV RNA levels over time, presumably as a result of increasing use of potent antiretroviral drugs (−0.04 log10 copies/mL per year in the unmodified arms; P = .12), were also investigated. Both groups had decreases in mean plasma HIV RNA levels over time, presumably as a result of increasing use of potent antiretroviral drugs (−0.04 log10 copies/mL per year in the unmodified arms; P = .12).

**CMV Viremia.** Of 520 patients tested, 99% were confirmed to be CMV seropositive on retest by the central laboratory. At baseline, 21% were positive for plasma CMV DNA, with similar frequencies in both groups. Among patients with positive baseline qualitative CMV DNA, there was no evidence of CMV activation in either group, and no difference in CMV DNA quantitation between groups (P = .88 at day 7; Figure 5). Among patients with negative baseline CMV DNA, similar proportions in each treatment group became DNA-positive 1 to 4 weeks after transfusion; for example, at day 7, 10 (6%) of 169 leukoreduced vs 18 (10%) of 173 of unmodified group patients developed detectable CMV DNA (P = .17).

**Lymphocyte Subsets and Cytokines**

The CD4 cell counts remained stable following the first and second transfusion series in both groups (P = .28 for test of treatment differences and for first transfusion; Figure 6). However, long-term trends in CD4 cell counts showed increases in both groups (35/µL per year in leukoreduced and 36/µL per year in the unmodified arms, P = .84). The number of CD8 cells increased following transfusion in both groups with median

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Table 3. Frequency of Death or Leading Types of Complications by Treatment Groupa

<table>
<thead>
<tr>
<th></th>
<th>First HIV-Related Complications</th>
<th>New HIV-Related Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leukoreduced (n = 246)</td>
<td>Unmodified (n = 250)</td>
</tr>
<tr>
<td>Death</td>
<td>93 (38)</td>
<td>91 (36)</td>
</tr>
<tr>
<td>Cytomegalovirus retinitis</td>
<td>13 (5)</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncatheter-related</td>
<td>13 (5)</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Catheter-related</td>
<td>8 (3)</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Mycobacterial infection</td>
<td>14 (6)</td>
<td>5 (2)</td>
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<tr>
<td>Pneumocystis carinii pneumonia</td>
<td>6 (2)</td>
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<tr>
<td>Other cytomegalovirus disease</td>
<td>6 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4 (2)</td>
<td>6 (2)</td>
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<tr>
<td>Other complications</td>
<td>7 (3)§</td>
<td>9 (4)§</td>
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<tr>
<td>Other serious bacterial infection</td>
<td>NA</td>
<td>NA</td>
</tr>
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Values are expressed as number (percentage). NA indicates data not applicable.

†Includes Mycobacterium avium complex, Mycobacterium kansasi, and other nontuberculous mycobacteria.
§Four different types of events with less than 1.5% of patients for each event.

Figure 4. Changes in Plasma HIV RNA Level Following the First Transfusion

A All Patients

<table>
<thead>
<tr>
<th>Days</th>
<th>Change in log_{10} HIV RNA Copies/mL From Baseline</th>
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<tbody>
<tr>
<td>7</td>
<td>0.05</td>
</tr>
<tr>
<td>14</td>
<td>-0.2</td>
</tr>
<tr>
<td>21</td>
<td>0.05</td>
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<tr>
<td>28</td>
<td>0.05</td>
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B Patients Not Receiving Antiretroviral Therapy

<table>
<thead>
<tr>
<th>Days</th>
<th>Leukoreduced</th>
<th>Unmodified</th>
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<tbody>
<tr>
<td>7</td>
<td>0.05</td>
<td>-0.2</td>
</tr>
<tr>
<td>14</td>
<td>-0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>21</td>
<td>0.05</td>
<td>-0.2</td>
</tr>
<tr>
<td>28</td>
<td>0.05</td>
<td>-0.2</td>
</tr>
</tbody>
</table>

The boxes represent the median and 25th and 75th percentiles. The vertical lines connected to the boxes extend to the 10th and 90th percentiles. Outlying values are depicted as individual dots. Change is measured from pretransfusion baseline to weekly time points during the next 4 weeks. For A, 5 observations with changes tend to the 10th and 90th percentiles. Outlying values are depicted as individual dots. Change is measured from pretransfusion baseline to weekly time points during the next 4 weeks. For A, 5 observations with changes tend to the 10th and 90th percentiles. Outlying values are depicted as individual dots. Change is measured from pretransfusion baseline to weekly time points during the next 4 weeks. For A, 5 observations with changes tend to the 10th and 90th percentiles. Outlying values are depicted as individual dots. Change is measured from pretransfusion baseline to weekly time points during the next 4 weeks. For A, 5 observations with changes tend to the 10th and 90th percentiles. Outlying values are depicted as individual dots.

increases of 11/µL and 34/µL on days 7 and 28 and of 25/µL 3 months after the first transfusion series (P<.008 for each). However, there was no difference between groups. Activation markers on CD8 cells suggested modest, transient activation following the first transfusion series as measured by CD38% and human leukocyte antigen-DR, but were not different between groups (L.A.K. and S.F.A., unpublished data from the VATS Group, 2000). Plasma levels of IL-6, tumor necrosis factor, tumor necrosis factor receptor, and β2-microglobulin were generally stable after the first and second transfusion series in both groups, with no differences between groups.

**COMMENT**

This large, double-blind, randomized study failed to confirm the hypotheses that leukoreduction improves survival and clinical outcome in HIV-infected patients requiring blood transfusion and that viral activation of HIV occurs following allogeneic RBC transfusion. In contrast, trends in survival favored patients assigned to the unmodified arm in unadjusted analyses, and more so in analyses adjusted for relevant confounders, such as plasma HIV RNA level and CD4 cell counts. However, we must interpret these adjusted analyses with caution since they were not the a priori primary end point. Moreover, there was no significant increase in differences between the groups with increased exposure to RBC units. It is possible that the groups differed in risk of HIV disease progression, although they were similar in commonly used predictors of clinical outcome for HIV infection. Alternatively, outcomes may actually be better in anemic HIV-infected patients who receive unmodified RBCs. In that regard, recent studies have suggested that immunization with allogeneic leukocytes increases expression of β chemokines and other soluble factors that may limit HIV replication.

Additionally, we observed no evidence of differences in occurrence of transfusion reactions, serious HIV-related diseases, new CMV disease, or CMV reactivation between patients receiving leukoreduced and unmodified RBCs. Increases in CD8 cell counts were seen in both groups following transfusion, but no cytokine activation was seen. Leukoreduced RBC units had a lower mass, but this did not appear to have detectable clinical consequences or result in increased transfusion requirements. Clinical and laboratory outcomes in this study are internally consistent, and contrast with results from retrospective studies that suggested accelerated HIV disease and smaller prospective studies that showed increases in quantitative measures of HIV after RBC transfusion.

The lack of favorable laboratory or clinical effects of leukoreduction in this study is especially important in light of data in other patient populations that have led to universal leukoreduction of all allogeneic blood transfusions (including those in persons with HIV infection) in many European countries and Canada, and recommendations for institution of similar policies in the United States.

We cannot identify alternative explanations for the failure of leukoreduction to improve clinical outcome in this study.
study. The groups were well matched with respect to clinical and laboratory factors recognized to affect HIV disease progression. Overall, there was good adherence to the protocol: 98% of RBC units were prepared per study randomization. Although accrual was slower and clinical event rates lower than originally anticipated, the study still had adequate power to detect a clinically meaningful difference between groups.

Possible explanations for lack of benefit seen in the leukoreduced arm include storage age of the blood, efficacy of filtration, advanced stage of HIV-induced immunosuppression, and effects of potent antiretroviral therapy. As the median age of RBC units in this study was 9 days, it is possible that greater immunologic effects would have been seen with younger or older blood since functional properties of leukocytes decline, and levels of cell-free cytokines and other inflammatory mediators increase during RBC storage.33,64-70 We sought to use relatively fresh RBC units to maximize the probability of observing an effect on the parameters we studied, and we made major efforts to ensure comparability of the age of the units in the 2 arms of the study. The mechanisms and threshold number of viable leukocytes needed to cause immunological consequences after transfusion are unknown.52,32,36,71 If the number of viable leukocytes necessary to cause adverse effects was lower than achieved by our filtration methods, more effective filters could have resulted in a different outcome (differences between groups may have been more apparent). We feel this is not likely since previous studies have shown that immunomodulatory effects, such as alloimmunization, are decreased with the degree of leukoreduction achieved in this study.72-73 Our study is representative of contemporary transfusion practice in the United States with respect to efficacy of leukoreduction.

Preliminary studies that suggested HIV activation occurs after allogeneic transfusion were done in less immunosuppressed patients and prior to the era of potent antiretroviral therapy. Median CD4 cell count at baseline in our enrollees was 15/µL. The capacity of these patients to mount an immune response to allogeneic leukocytes may have been compromised.42 This was evidenced by lack of induction of activation markers typically seen in nonimmunosuppressed patients following transfusions.22,32,74 Since combination antiretroviral therapy can suppress HIV RNA level increases associated with administration of immunomodulators, such as IL-2,73 it is also possible that the stimulatory effects of leukocytes on HIV-infected cells are counterbalanced by the suppressive effects of potent antiretroviral therapy on HIV replication. Our data do not support this theory, however, since viral activation was also not seen in patients not taking antiretroviral drugs.

During planning for this study, there was substantial discussion about use of CMV-seropositive vs CMV-seronegative blood products. Leukodepletion decreases but does not eliminate risk of exposure to CMV in seropositive blood.76-80 Acquisition of multiple strains of CMV has been demonstrated in immunocompromised hosts.81-83 Since we did not include a group who received CMV-seronegative blood, we cannot assess whether the results would have been different. However, the groups were balanced with respect to the CMV serostatus of transfused units. Lack of difference in outcomes between groups demonstrates that leukoreduction did not result in improved CMV outcomes in this study.

Clinical events in this study were typical for advanced HIV infection. The study was designed to include only events associated with poor outcomes and high mortality as end points. This may underestimate total morbidity, but results are likely to be clinically relevant.

From a pragmatic standpoint, these data suggest there is no need to offer leukoreduced blood transfusions to persons infected with HIV and CMV in an attempt to prolong survival, decrease serious HIV-related complications, or reduce transfusion reactions. In an era of high-level scrutiny of blood safety issues and cost-consciousness,41 failure to demonstrate an advantage for a procedure with potential adverse effects and additional financial cost (estimated at $25-$35 per unit) is a useful, if unexpected, outcome. These data emphasize the importance of conducting rigorously controlled studies of effects of leukoreduction in different patient populations before adoption of a universal leukoreduction policy.

Author Affiliations: School of Medicine, University of Washington, Seattle (Drs Collier and Gernsheimer); New York Blood Center, New York; Fred Hutchinson Cancer Research Center, Seattle (Dr. Doms); Dana-Farber Cancer Institute, Boston (Dr. Greene); University of California, Los Angeles School of Public Health (Dr. Koester); University of California, San Francisco School of Medicine (Dr. Price); Armed Forces Institute of Pathology, Washington, DC (Dr. Warm; Reprinted) JAMA, March 28, 2001—Vol 285, No. 12 1599
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England Research Institutes, Watertown, Mass (Dr Kalish, Dr Asmuth); Blood Centers of the Pacific, San Francisco, Calif (Dr Busch); School of Medicine, University of California, San Francisco (Dr Busch and Murphy); Puget Sound Blood Center, Seattle, Wash (Dr Germsheimer); University of California, San Francisco, San Diego (Dr Kellogg); School of Medicine, University of Texas, Galveston (Dr Asmuth); School of Medicine, Case Western Reserve University, Cleveland, Ohio (Dr Lederman); School of Medicine, University of North Carolina, Chapel Hill (Dr Kellogg); School of Medicine, Brown University, Providence, RI (Dr Flanigan); School of Medicine, University of Pittsburgh, Pittsburgh, Pa (Dr McMahon); School of Medicine, University of New Mexico, Albuquerque, NM; and was involved with development of assays for CMV DNA and HLA-typed patients.

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Critical revision of the manuscript for important intellectual content: Collier, Kalish, Busch, Germsheimer, Asmuth, Lane, Asmuth, Lederman, Murphy, Kumar, Kellogg, Flanigan, McMahon, Sacks, Kennedy, Holland.

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