

## PHOTOPHERESIS FOR THE PREVENTION OF REJECTION IN CARDIAC TRANSPLANTATION

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### ABSTRACT

**Background** Photopheresis is an immunoregulatory technique in which lymphocytes are reinfused after exposure to a photoactive compound (methoxsalen) and ultraviolet A light. We performed a preliminary study to assess the safety and efficacy of photopheresis in the prevention of acute rejection of cardiac allografts.

**Methods** A total of 60 consecutive eligible recipients of primary cardiac transplants were randomly assigned to standard triple-drug immunosuppressive therapy (cyclosporine, azathioprine, and prednisone) alone or in conjunction with photopheresis. The photopheresis group received a total of 24 photopheresis treatments, each pair of treatments given on two consecutive days, during the first six months after transplantation. The regimen for maintenance immunosuppression, the definition and treatment of rejection episodes, the use of prophylactic antibiotics, and the schedule for cardiac biopsies were standardized among all 12 study centers. All the cardiac biopsy samples were graded in a blinded manner at a central pathology laboratory. Plasma from the subgroup of 34 patients (57 percent) who were enrolled at the nine U.S. centers was analyzed by polymerase-chain-reaction amplification for cytomegalovirus DNA.

**Results** After six months of follow-up, the mean ( $\pm$ SD) number of episodes of acute rejection per patient was  $1.44 \pm 1.0$  in the standard-therapy group, as compared with  $0.91 \pm 1.0$  in the photopheresis group ( $P=0.04$ ). Significantly more patients in the photopheresis group had one rejection episode or none (27 of 33) than in the standard-therapy group (14 of 27), and significantly fewer patients in the photopheresis group had two or more rejection episodes (6 of 33) than in the standard-therapy group (13 of 27,  $P=0.02$ ). There was no significant difference in the time to a first episode of rejection, the incidence of rejection associated with hemodynamic compromise, or survival at 6 and 12 months. Although there were no significant differences in the rates or types of infection, cytomegalovirus DNA was detected significantly less frequently in the photopheresis group than in the standard-therapy group ( $P=0.04$ ).

**Conclusions** In this pilot study, the addition of photopheresis to triple-drug immunosuppressive therapy significantly decreased the risk of cardiac rejection without increasing the incidence of infection. (N Engl J Med 1998;339:1744-51.)

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**M**ODERN immunosuppressive regimens, consisting of cyclosporine-based triple-drug therapy, with or without monoclonal or polyclonal antibodies, have dramatically increased survival among organ-transplant recipients. However, these regimens improve graft survival by nonspecific immunosuppression, leaving the host at increased risk for opportunistic infection and the development of malignant tumors and vulnerable to the adverse effects of these drugs. Moreover, considerable morbidity and mortality persist as a result of acute episodes of organ rejection, particularly in the first few months after transplantation, and of chronic forms of rejection, such as graft vasculopathy.<sup>1</sup> Treatments directed at suppressing donor-specific T-cell clones in the recipient have the potential to decrease graft rejection without increasing the toxicity of immunosuppressive drugs.

Photopheresis is one such technique. In photopheresis the patient's peripheral blood is removed and separated into leukocyte-depleted blood, which is returned to the patient, and leukocyte-enriched plasma, which is exposed to ultraviolet light in the presence of extracorporeally administered liquid methoxsalen. Methoxsalen, which is photoactive, covalently binds to DNA pyrimidine bases, cell-surface molecules, and cytoplasmic components in the exposed white cells, causing a lethal defect. These cells are then reinfused into the patient and die over a one-to-two-week period, but during that interval they stimulate an autologous suppressor response, in part mediated by T cells, that targets nonirradiated T cells of similar clones.<sup>2</sup>

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The first use of photopheresis in humans was in the treatment of cutaneous T-cell lymphoma, a disease characterized by malignant expansion of a single clone of T cells.<sup>3</sup> An important aspect of this first clinical application was the sparing of general immune competence in the treated patients, as demonstrated by the absence of infectious complications and the persistence of skin-test reactivity. The use of photopheresis in the treatment of other diseases that are potentially mediated by expanded populations of abnormally responsive T cells, including scleroderma, pemphigus vulgaris, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis, has also been addressed in laboratory and clinical studies.<sup>4-8</sup> Graft rejection after organ transplantation is characterized by clonal expansions of activated effector T cells and has therefore become an area of active research in photopheresis.

Perez et al.<sup>9</sup> demonstrated that BALB/c splenic lymphocytes harvested from BALB/c mice that had received skin transplants from CBA/j mice, treated with methoxsalen and ultraviolet A light, and reinfused into naive BALB/c mice, decreased proliferation in mixed-lymphocyte cultures, decreased cytotoxic activity in response to CBA/j antigens, and significantly increased the survival of CBA/j skin allografts. These treated mice retained their immune response to third-party skin allografts, demonstrating that the immunosuppression was donor-specific. Pepino et al.<sup>10</sup> showed in a model of heterotopic cardiac transplantation from cynomolgus monkeys to baboons that prophylactic photopheresis starting three days after transplantation, in addition to standard immunosuppression, increased donor-specific immunosuppression, as evidenced by inhibited responses in mixed-lymphocyte cultures, decreased formation of lymphocytotoxic antibodies to the donor, and prolonged xenograft survival, as compared with results in controls.

Rose et al.<sup>11</sup> used photopheresis to treat four patients undergoing cardiac transplantation who had elevated levels of non-donor-specific anti-HLA antibodies and who were at high risk for rejection. They found a decrease in the levels of non-donor-specific anti-HLA antibodies and a relatively low incidence of rejection. Costanzo-Nordin et al.<sup>12</sup> used a single photopheresis treatment in patients with hemodynamically stable cardiac rejection and compared the results with those in a control group that received high-dose corticosteroids for three days. Although less successful than corticosteroids in resolving rejection episodes, photopheresis alone was capable of reversing acute cellular rejection in most cases and was associated with fewer post-treatment infections.

Barr et al.<sup>13</sup> performed a prospective study in which the addition of photopheresis at monthly intervals to standard triple-drug therapy was evaluated for safety and for its ability to reduce levels of panel-

reactive antibodies. Treated patients had an early reduction in the levels of non-donor-specific, panel-reactive anti-HLA antibodies. Of greater clinical importance was the finding of a statistically significant decrease in coronary-artery intimal thickness as measured by intravascular ultrasonography at one- and two-year follow-up examinations.

In a study by Meiser et al.,<sup>14</sup> who tested a regimen in which photopheresis was performed more frequently and liquid methoxsalen was added directly to the extracorporeal buffy coat, there was a reduction in the incidence of acute cardiac rejection. This study as well as previous work by Knobler et al.<sup>15</sup> confirmed that the absorption of oral methoxsalen and its subsequent blood level are highly unpredictable; it also demonstrated that the extracorporeal addition of the liquid form of methoxsalen resulted in reliable levels in the buffy coat. We undertook a preliminary study to investigate the effect of prophylactic photopheresis on the incidence of acute cellular rejection and infection in recipients of cardiac transplants.

## METHODS

### Patients

A total of 60 consecutive eligible adult recipients of primary cardiac transplants at 12 clinical sites (9 in the United States and 3 in Europe) were randomly assigned to receive either standard triple-drug immunosuppressive therapy (cyclosporine, azathioprine, and prednisone; 27 patients) or standard triple-drug therapy plus photopheresis (33 patients). Patients were stratified and the groups balanced according to age and sex. To be eligible, patients were required to be living within a reasonable commuting distance of the transplantation center (less than two hours' travel by automobile) and to have adequate peripheral venous access; approximately 25 percent of eligible patients were not randomized, because they did not meet these criteria. The study design was approved by all relevant institutional review boards.

### Study Design

Patients randomly assigned to received photopheresis plus standard triple-drug therapy were treated with photopheresis according to the following schedule: days 1 and 2, 5 and 6, 10 and 11, 17 and 18, and 27 and 28 during month 1 after transplantation; two successive days every two weeks during months 2 and 3; and two successive days every four weeks for months 4, 5, and 6, for a total of 24 photopheresis procedures per patient. The therapy was designed to be most intense early in the postoperative period, when the incidence of rejection is highest, and to coincide with the schedule of cardiac biopsies in order to maximize convenience and compliance on the part of the patient and allow use of an internal jugular venous sheath for access if needed.

Follow-up monitoring was standardized during the six-month study period for all the patients in both groups. Routine laboratory studies and measurements of cyclosporine levels in the blood were performed according to a standardized schedule. Endomyocardial biopsies were obtained according to the following schedule: weekly during the first postoperative month; every two weeks during months 2 and 3; and every four weeks during months 4, 5, and 6, for a total of 11 biopsies.

The primary end point of the study was the number and frequency of acute rejection episodes as defined histologically in a blinded manner at the central pathology laboratory. Secondary end points included the incidence of clinically treated infections,

TABLE 1. TREATMENT OF REJECTION.\*

BIOPSY GRADE	DEGREE OF HEMODYNAMIC COMPROMISE		
	NONE	MILD TO MODERATE	SERIOUS (REQUIRING INOTROPIC SUPPORT)
0, 1A, or 1B	Prednisone, 2 mg/kg/day for 3 days (grade 1B only)	Prednisone, 2 mg/kg/day for 3 days	IV corticosteroids, 1 g/day for 3 days; treatment with muromonab-CD3 or ATG optional
2	Prednisone, 2 mg/kg/day for 3 days	IV corticosteroids, 1 g/day for 3 days	IV corticosteroids, 1 g/day for 3 days; treatment with muromonab-CD3 or ATG optional
3A, 3B	Prednisone, 2 mg/kg/day for 3 days, or IV corticosteroids, 500 mg/day for 3 days (for patients <50 kg); 1 g/day for 3 days (for patients ≥50 kg)	IV corticosteroids, 1 g/day for 3 days	IV corticosteroids, 1 g/day for 3 days, plus muromonab-CD3, 5 mg/day for 7 to 14 days, or ATG, 10 mg/kg/day for 7 days (if muromonab-CD3 previously used)
4	IV corticosteroids, 1 g/day for 3 days, plus muromonab-CD3, 5 mg/day for 7 to 14 days, or ATG, 10 mg/kg/day for 7 days (if muromonab-CD3 previously used)	IV corticosteroids, 1 g/day for 3 days, plus muromonab-CD3, 5 mg/day for 7 to 14 days, or ATG, 10 mg/kg/day for 7 days (if muromonab-CD3 previously used)	IV corticosteroids, 1 g/day for 3 days, plus muromonab-CD3, 5 mg/day for 7 to 14 days, or ATG, 10 mg/kg/day for 7 days (if muromonab-CD3 previously used)

\*Muromonab-CD3 is anti-CD3 monoclonal antibody (Ortho Biotech, Raritan, N.J.). IV denotes intravenous, and ATG polyclonal antithymocyte globulin (Upjohn, Kalamazoo, Mich.).

detection of plasma cytomegalovirus (CMV) DNA by polymerase-chain-reaction (PCR) assay performed by investigators at a central reference laboratory who were unaware of the patients' treatment assignments, and survival without the need for a second transplantation. The study end points were monitored and the patients followed for six months after transplantation; safety and survival follow-up was continued for an additional six months, during which time management at each center reverted to non-standardized institutional protocols.

#### Immunosuppression and Prophylaxis against Infection

All the patients received triple-drug immunosuppressive therapy (cyclosporine, azathioprine, and prednisone). Prophylactic use of monoclonal or polyclonal antibodies was not permitted. All the institutions targeted the trough levels of cyclosporine in whole blood to achieve the equivalent of 250 to 350 ng per milliliter by fluorescence polarization immunoassay (TDX, Abbott, Abbott Park, Ill.). Azathioprine treatment began with a single preoperative dose of 4 mg per kilogram of body weight, followed by postoperative administration of 0 to 5 mg per kilogram per day, adjusted to achieve a white-cell count of 4000 to 8000 per cubic millimeter. Corticosteroids were administered beginning with a 500-to-1000-mg dose of intravenous methylprednisolone preoperatively or intraoperatively. Tapered administration of prednisone was then initiated, beginning at 1 mg per kilogram per day, with the goal of reaching a dose of 0.1 mg per kilogram per day by the fifth month after transplantation.

All the patients received standardized prophylaxis against infection. For protection against candida infection, patients received oral mycostatin for the first three months after transplantation. For prevention of *Pneumocystis carinii* pneumonia, patients received 160 mg of trimethoprim in combination with 800 mg of sulfamethoxazole three times per week, starting in the second week after surgery and continuing throughout the study period. For prophylaxis against CMV infection, all the recipients of CMV-positive organs, whether the recipient was CMV-positive or

CMV-negative, received 10 mg of intravenous ganciclovir per kilogram per day for the first 10 to 14 days after transplantation.

#### Photopheresis

Patients randomly assigned to the photopheresis group received treatments by means of the UVAR Photopheresis System (Therakos, Exton, Pa.). Blood was removed from the patient with a 16-gauge antecubital angiocatheter (or an 8-French internal jugular venous biopptome sheath if access to a peripheral vein could not be obtained) and passed through the device's centrifuge. During the discontinuous leukapheretic processing, a total of approximately 240 ml of buffy coat and 300 ml of plasma was collected and mixed. The final buffy-coat preparation contained approximately 25 to 50 percent of the patient's total peripheral-blood mononuclear-cell compartment, with a hematocrit of approximately 5 percent. Two hundred micrograms of liquid methoxsalen (Uvadex, Therakos) was injected into the bag of buffy coat. The buffy coat then passed as a 1-mm-thick film through a cassette, where it underwent a 180-minute exposure to ultraviolet A light, yielding an average exposure of 1 to 2 J per square centimeter of leukocyte-surface area. After exposure of the cells to the ultraviolet light, the buffy coat was reinfused into the patient. Each complete procedure took approximately four hours.

#### Detection and Treatment of Rejection

Over the six-month period, 11 transvenous endomyocardial biopsies were performed according to a standardized schedule, as outlined above. All biopsy specimens were evaluated by a single pathologist in a blinded manner at the central pathology laboratory according to the standards published by the International Society for Heart and Lung Transplantation.<sup>16</sup> Specific standardized treatment regimens based on the severity of rejection (evaluated in a blinded manner within 24 hours by a pathologist at the local institution) were used (Table 1). All the patients with grade 3A, 3B, or 4 biopsy specimens were treated. Patients with a grade 2 biopsy specimen in month 1 or two successive grade 2

specimens in months 2 through 6 were treated. Patients with three successive grade 1B specimens were treated. A prolonged episode of rejection (as ascertained by two or more consecutive abnormal specimens) was counted as a single rejection episode if the biopsy grade remained higher than 0 or 1A.

**PCR Assay for CMV DNA**

For all the patients enrolled at U.S. centers (34 patients, 19 of whom received photopheresis and 15 of whom received standard therapy), serial blood samples (taken every two weeks from weeks 2 to 12 and monthly from months 4 through 6, for a total of nine samples) were analyzed by PCR amplification of plasma CMV DNA. The qualitative PCR procedure used has been described by Wolf and Spector.<sup>17</sup> Plasma samples were maintained at -20°C until processed in a blinded manner at the central laboratory. Ten microliters of plasma diluted 1:10 was passed through three freeze-thaw cycles, dissolved in PCR buffer, and incubated with proteinase K at 60°C for one hour. Samples were then heated at 95°C for 10 minutes, centrifuged at 12,000×g for 5 minutes, and directly amplified by PCR. For amplification, two sets of primer pairs were constructed for the *EcoRI* fragment D region of human CMV strain AD169. The template was combined with the appropriate primer pair (50 pmol of each), deoxyribonucleoside triphosphates (200 μM each; Pharmacia LKB, Piscataway, N.J.), and *Taq* polymerase (2.5 U; Perkin-Elmer Cetus, Norwalk, Conn.) in a total volume of 100 μl of PCR buffer and then amplified by 35 cycles of denaturation, primer annealing, and chain extension. After amplification, 10 μl of the product was denatured at 94°C and immediately hybridized at 55°C with a <sup>32</sup>P-end-labeled probe. The hybrid heteroduplexes were then resolved by electrophoresis on 6 percent polyacrylamide gels, and autoradiographs were obtained.

The sensitivity of the procedure was considered to be 6000 replicate copies of the viral DNA. All specimens were tested at least twice in separate PCR procedures, and buffer controls and control samples were run with each reaction. All the positive and negative controls were required to demonstrate the appropriate signal before any result was considered for analysis.

**Statistical Analysis**

We compared demographic factors and randomization strata (sex and age) between the treatment groups using Fisher's exact test. The frequency of rejection episodes in each of the two treatment groups was analyzed by means of a chi-square test for trend, with one degree of freedom. A collapsed version of this analysis compared the number of patients who had one rejection episode or none with the number who had two or more, and other dichotomous variables, with use of Fisher's exact test. Survival estimates in the form of survival functions were generated by the Kaplan-Meier product-limit method. The survival of the patients was analyzed with the log-rank test for comparison of the survival curves. Comparison of the infection rates in the two groups was performed with either a Fisher's exact test or a chi-square test for trend, with one degree of freedom. For the detection of CMV DNA by PCR, the percentage of time a patient had a positive test result was calculated. A Wilcoxon rank-sum test was used to compare the treatment groups. All P values were two-tailed.

**RESULTS**

**Characteristics of the Patients**

The patients' base-line characteristics are shown in Table 2. There were no significant differences between patients who received standard immunosuppression and those who received standard immunosuppression and photopheresis with respect to sex, age, race or ethnic group, cardiac disease before transplantation, duration of graft ischemia, or CMV match-

**TABLE 2. CHARACTERISTICS OF THE PATIENTS IN EACH STUDY GROUP.\***

CHARACTERISTIC	PHOTOPHERESIS PLUS STANDARD THERAPY (N=33)	STANDARD THERAPY ALONE (N=27)
Sex — no. (%)		
Male	23 (70)	21 (78)
Female	10 (30)	6 (22)
Age — yr	50.9±11.6	51.9±13.2
Race or ethnic group — no. (%)		
White	23 (70)	16 (59)
Black	6 (18)	5 (18)
Asian or Pacific Islander	0	1 (4)
Hispanic	1 (3)	0
Other	3 (9)	5 (18)
<b>Preoperative</b>		
Disease before transplantation — no. (%)		
Ischemic cardiomyopathy	13 (39)	10 (37)
Dilated cardiomyopathy	16 (48)	13 (48)
Valvular heart disease	2 (6)	2 (7)
Other	2 (6)	2 (7)
CMV status — no. (%)†		
Donor +, recipient -	9 (27)	7 (27)
Donor +, recipient +	12 (36)	8 (31)
Donor -, recipient +	7 (21)	5 (19)
Donor -, recipient -	5 (15)	6 (23)
Duration of graft ischemia — min	171.6±55.4	183.3±65.0
<b>Postoperative</b>		
Cyclosporine dose — mg/kg/day		
Months 0 to 3	4.3±1.1	4.6±1.6
Months 4 to 6	4.4±1.2	4.7±1.9
Cyclosporine level in whole blood — ng/ml	309.3±110.8	288.0±91.1
White-cell count — ×10 <sup>-3</sup> /mm <sup>3</sup>	10.2±3.0	9.8±2.7
Total cholesterol — mg/dl‡	162.2±87.2	152.3±97.5

\*Plus-minus values are means ±SD. Statistical comparison of the two groups showed no significant difference in any variable. Because of rounding, not all percentages total 100.

†For one patient in the group receiving standard therapy, the CMV status of the donor and recipient was unknown.

‡To convert values to millimoles per liter, multiply by 0.02586.

ing or mismatching. No significant differences were noted between the two groups in postoperative cyclosporine doses and levels, white-cell counts, or cholesterol levels.

**Survival**

The rate of survival at six months did not differ significantly between the group receiving standard therapy (25 of 27 patients) and the group that received photopheresis (31 of 33 patients). The mean (±SE) proportion surviving, as calculated with Kaplan-Meier estimates, was 0.93±0.05 in the standard-therapy group and 0.94±0.03 in the photopheresis group. Comparison of the two survival curves with use of a log-rank test also showed no significant difference (P=0.86). The two deaths in the photopheresis group resulted from acute rejection in one patient (who died on postoperative day 30) and from bacterial sepsis after drainage of an oral abscess in the other (on postoperative day 92). The two

deaths in the standard-therapy group resulted from mucormycosis and multiorgan failure in one patient (who died on postoperative day 122) and from renal failure and bacterial pneumonia in the other (on postoperative day 158). At the 12-month follow-up, one additional patient in the photopheresis group had died, as a result of pulmonary embolism (on postoperative day 317). The proportion surviving at 12 months was  $0.93 \pm 0.05$  in the standard-therapy group and  $0.91 \pm 0.05$  in the photopheresis group ( $P=0.80$ ).

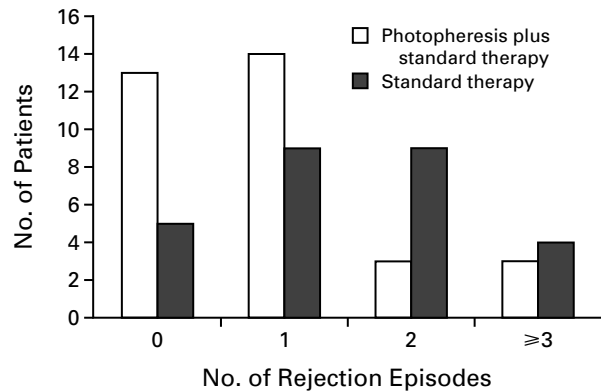
#### Complications Related to Photopheresis

One patient had bacteremia due to catheter-related infection with *Serratia marcescens* and was successfully treated with intravenous antibiotics, and one patient lost 300 ml of the plasma-buffy-coat mixture during a treatment session because of a leak in the disposable circuit but had no adverse sequelae. In six patients, venous access was difficult, necessitating repeated attempts at catheter placements or the use of a central venous line for access. Of these six patients, three missed at least four photopheresis treatments during the six-month period; one of these patients refused further treatment after three months. Despite these protocol violations and cases of noncompliance, all data on rejection and infection for the duration of the study were obtained for all patients.

#### Cardiac Rejection

The average ( $\pm$ SD) number of acute rejection episodes per patient, according to data collected at the central pathology laboratory, was  $1.44 \pm 1.0$  in the standard-therapy group and  $0.91 \pm 1.0$  in the photopheresis group ( $P=0.04$  by two-sample t-test). In the standard-therapy group, five patients (18 percent) had no rejection episodes, nine (33 percent) had one rejection episode, nine (33 percent) had two episodes, and four (15 percent) had three or more episodes (Fig. 1). In the photopheresis group, 13 patients (39 percent) had no rejection episodes, 14 (42 percent) had one rejection episode, 3 (9 percent) had two episodes, and 3 (9 percent) had three or more episodes ( $P=0.03$  by chi-square test for trend, with one degree of freedom).

The percentage of patients with two or more rejection episodes was significantly lower in the group receiving photopheresis (18 percent) than in the group receiving standard therapy (48 percent) ( $P=0.02$ ). The results of post hoc analyses for other comparisons between the two groups were not significant: one or more rejection episodes ( $P=0.10$ ) and three or more rejection episodes ( $P=0.69$ ). In terms of relative risk, the likelihood that a patient would have no rejection episodes in the photopheresis group was 2.1 times that in the standard-therapy group (95 percent confidence interval, 0.9 to



**Figure 1.** Number of Patients with and without Rejection Episodes during the Six-Month Follow-up.

Two-tailed  $P=0.03$  for the comparison between groups by the chi-square test for trend, with one degree of freedom.

5.2), and the likelihood of having two or more rejection episodes in the standard-therapy group was 2.6 times that in the photopheresis group (95 percent confidence interval, 1.2 to 6.0).

The time to a first rejection did not differ significantly between the two groups ( $38.9 \pm 42.1$  days in the photopheresis group and  $30.4 \pm 32.8$  days in the standard-therapy group). There was no statistical difference between the two groups with regard to the percentage of patients who had rejection associated with hemodynamic compromise (15 percent in the photopheresis group and 18 percent in the standard-therapy group) or in the percentage of patients who required antibody treatment for rejection (6 percent in the photopheresis group and 11 percent in the standard-therapy group). In analyses that compared the number of rejection episodes according to age, sex, and race or ethnic group, no significant differences were found to suggest that the effect of photopheresis was dependent on any of these factors. In addition, a comparison of rejection rates among different centers demonstrated no significant differences that would suggest a center-specific response to photopheresis. During the second six-month period of safety follow-up, the average number of acute rejection episodes per patient was  $0.22 \pm 0.58$  in the standard-therapy group and  $0.27 \pm 0.52$  in the photopheresis group ( $P=0.72$ ).

#### Infection

##### Clinical Infection

A comparison of the two groups with respect to the number of bacterial, viral, protozoal, and fungal infections requiring therapeutic intervention is shown in Table 3. No significant difference was demonstrated between patients receiving photopheresis plus standard therapy and those receiving standard ther-

apy alone in the overall rate of infection or in the rate of any specific type of infection. There was one episode of infection in the photopheresis group that was classified as life-threatening by the individual investigator (culture-negative sepsis), and there were four such episodes in the standard-therapy group (*Staphylococcus aureus* mediastinitis, *Pseudomonas aeruginosa* pneumonia, CMV pneumonia, and mucormycosis). During the second six-month period of safety follow-up, the average number of infections requiring treatment in each patient was  $0.33 \pm 0.73$  in the standard-therapy group and  $0.39 \pm 0.75$  in the photopheresis group ( $P=0.75$ ).

**PCR Analysis for CMV**

A frequency distribution of the percentage of tests that were positive for CMV in the two treatment groups at the centers in the United States is shown in Figure 2. Among patients receiving photopheresis, the largest proportion (47 percent) had positive results on 21 to 40 percent of the tests for CMV, whereas among patients receiving only standard therapy, the largest proportion (45 percent) had positive results on 61 to 80 percent of CMV tests. A comparison of the two treatment groups demonstrated that a significantly larger proportion of patients in the standard-therapy group had more frequent positive PCR tests for CMV ( $P=0.04$ ). A discriminant analysis with use of logistic transformation showed that neither the donor's nor the recipient's CMV status nor the treatment for rejection was a significant covariate ( $P=0.64$  and  $P=0.41$ , respectively). Treatment assignment to photopheresis or to standard therapy was the only significant factor ( $P=0.03$ ).

**DISCUSSION**

This preliminary study demonstrated a statistically significant reduction in the number of acute rejection episodes in recipients of cardiac transplants who received photopheresis therapy in addition to standard triple-drug immunosuppression. No significant differences were seen between the two groups in the time to a first rejection or in the incidence of rejection associated with hemodynamic compromise, and no significant difference was noted between the groups in survival at 6 and 12 months. Longer follow-up will be required to assess the effects of a reduction in the risk of acute rejection on long-term graft function, the long-term survival of graft recipients, and the development of graft vasculopathy.

From a safety standpoint, it is important that photopheresis treatment did not increase the rate of infectious complications and that during the second six-month period of follow-up after the discontinuation of photopheresis, there was no increase in the frequency of rejection (rebound effect). In clinical analyses, no significant difference in the incidence of

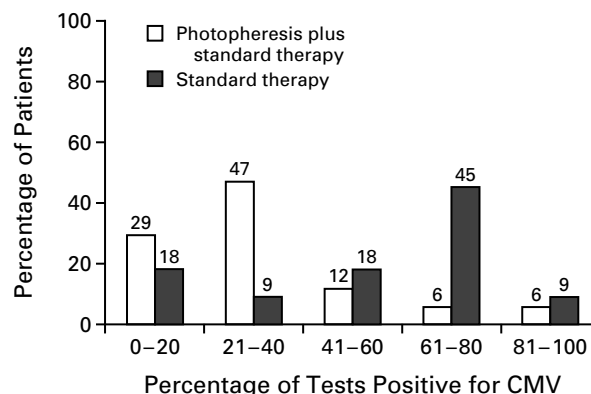
**TABLE 3.** NUMBER OF PATIENTS WHO REQUIRED TREATMENT FOR INFECTION.

TYPE AND NO. OF TREATED INFECTIONS	PHOTOPHERESIS PLUS STANDARD THERAPY (N=33)	STANDARD THERAPY ALONE (N=27)	P VALUE*
Bacterial			0.76†
0	18	17	
1	11	6	
≥2	4	4	
Viral			0.76‡
0	26	20	
1	7	7	
≥2	0	0	
Fungal			0.26†
0	30	23	
1	3	2	
≥2	0	2	
Parasitic or protozoal			0.45‡
0	33	26	
1	0	1	
≥2	0	0	

\*P values were calculated for each type of infection.

†Two-tailed probability was calculated with the chi-square test for trend, with one degree of freedom.

‡Two-tailed probability was calculated with Fisher's exact test.



**Figure 2.** Frequency Distribution of Positive CMV Tests by PCR Analysis in the 34 Patients at U.S. Centers.

$P=0.04$  by the Wilcoxon rank-sum test for the comparison between groups of the percentage of each patient's tests that were positive.

infection was noted between the photopheresis group and the standard-therapy group, but PCR analysis of plasma demonstrated a statistically significant decrease in detectable CMV DNA in patients treated with photopheresis in the United States. Multivariate analysis revealed that this decrease was independent of the donor's and recipient's CMV status and treatment of rejection, and that use of photopheresis treatment was the only variable significantly associated with the decrease.

The mechanism by which photopheresis blunts the acute rejection response is unknown, but several theories have been proposed. It is known that photoactivated psoralen cross-links the DNA of treated leukocytes and causes a lethal defect. However, since in one photopheresis treatment only 2 to 5 percent of the patient's population of mononuclear cells is affected,<sup>18</sup> this direct action cannot fully explain the immunomodulatory effect of photopheresis. It has been shown that photopheresis-treated lymphocytes secrete inflammatory mediators, such as interleukin-6 and tumor necrosis factor  $\alpha$ , that affect the entire immune-cell population.<sup>19</sup> Other studies have suggested that photopheresis-treated mononuclear cells stimulate the generation of a population of clone-specific suppressor T cells.<sup>9,20</sup> Apoptosis has been observed in photopheresis-treated peripheral-blood lymphocytes and may play an important part in immunoregulation.<sup>21</sup> Another possibility is that photopheresis alters the unique T-cell receptor associated with the expanded T-cell clone, making it more susceptible to clearance by the immune system.<sup>21</sup>

In terms of limitations of this study, the number of patients was relatively small, particularly with respect to the PCR analysis for CMV. However, analysis of demographic data on the subgroup of patients who had PCR analysis showed no significant differences from the study group as a whole. Another limitation of the study was that the investigators at the local centers were not blinded to patients' treatment-group assignments; however, the pathologist at the central laboratory analyzed the histologic features of biopsy specimens in a blinded manner, as did the investigator at the laboratory performing the PCR. There was no sham or placebo control, because such a control could involve the placement of a central venous line for access in patients, a step we considered overly invasive.

Despite the small size of the study group in this trial, the design of the study successfully minimized differences in center-specific diagnosis and treatment of rejection and infection, differences that have traditionally been a problem in multicenter studies of transplantation. Maintenance immunosuppression (including tapered administration of corticosteroids), the definition and treatment of rejection episodes, the use of prophylactic antibiotics, and the schedule for cardiac biopsies were strictly standardized among all the centers. The primary end point of the study, acute rejection, was assessed at the central pathology laboratory in a blinded manner. This feature of the design is important in transplantation research, in which less rigorous end points are frequently used or assessments are performed at local centers without blinding.

This study presents a preliminary look at the potential clinical value of photopheresis as an addition to standard immunosuppression in cardiac transplan-

tation. Photopheresis is a mildly invasive treatment without important toxic effects and appears to lower the rates of acute rejection in recipients of cardiac transplants. Although there was no difference in the frequency of clinically significant episodes of infection with the addition of photopheresis, there was a reduction in the detection of CMV in the plasma by PCR, suggesting that this therapy has a direct antiviral effect, a finding consistent with *in vitro* work.<sup>22</sup> Future trials will need to include an analysis of the cost-benefit ratio of photopheresis, and additional clinical studies and long-term follow-up will be required to assess the value of photopheresis in recipients of other solid-organ transplants and the ultimate effect of this treatment on graft and patient survival.

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