Extracorporeal Photopheresis for Steroid Resistant Graft Versus Host Disease in Pediatric Patients

A Pilot Single Institution Report

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Summary: This study was aimed at ascertaining whether extracorporeal photopheresis (ECP) is an effective treatment for pediatric patients with steroid resistant graft versus host disease (GvHD). Fifteen patients with acute GvHD (aGvHD) and 10 patients with chronic GvHD (cGvHD) were enrolled in the study. At the start of the ECP protocol, aGvHD was staged as II (n = 7), III (n = 4), and IV (n = 4). The response rate was 100% for aGvHD II, 75% for aGvHD III, and finally 0% for aGvHD IV ($P = 0.02$). In multivariate analysis, the strongest predictor for ECP response was the aGvHD severity: aGvHD II 100%, aGvHD III-IV 30% [relative risk (RR) 5.071, confidence interval (CI) 95% 2.2-5.5, $P = 0.0016$], this translates in a higher risk of transplant-related mortality for ECP nonresponders (RR 5.26, CI 95% 3.4-6.2, $P = 0.02$). cGvHD was diagnosed as limited n = 3, and extensive n = 7; the response rate was 100% and 28% for limited or extensive cGvHD, respectively ($P = 0.03$). For cGvHD the strongest predictor for ECP response was the absence of visceral organ involvement (RR 5.17, CI 95% 2-4.9, $P = 0.001$), and the highest risk of transplant-related mortality was among patients not responding to ECP (RR 12.4, CI 95%, $P = 0.02$). Our results suggest that ECP can rescue good-risk GvHD-patients, whereas for advanced, poor-risk GvHD patients, new therapies are required.

Key Words: extracorporeal photopheresis, GvHD, transplant-related mortality

(*J Pediatr Hematol Oncol* 2007;29:678–687)

Graft versus host disease (GvHD) is the main cause of morbidity and mortality after allogeneic hemopoietic stem cell transplantation (HSCT). It occurs in up to 30% to 50% of human leucocyte antigen (HLA)-identical sibling [matched sibling donor (MSD)] and up to 80% of unrelated donor (UD) HSCTs.[1–3]

At present, systemic steroid treatment represents the first line therapy for acute GvHD (aGvHD). Steroid resistant patients have a high risk of death from aGvHD. Since 1998, a multicenter Italian study showed how patients grafted with a MSD who develop aGvHD and fail to respond to methyl-prednisolone at 2 mg/kg are at a very high risk of transplant-related mortality (TRM, 46%).[4,5] Steroids are also considered the standard of care for initial treatment of chronic GvHD (cGvHD), but only a minority of patients have a lasting response to them and, so far, these patients are subject to long-term steroid-complications, and the management of steroid resistant cGvHD is not well defined.[6] Extracorporeal photopheresis (ECP) was introduced in 1987 to treat cutaneous T-cell lymphoma and autoimmune diseases, such as scleroderma. This procedure has proved effective in the treatment of acute lung, heart, and kidney allograft rejection and in the last 10 years for the treatment of aGvHD and cGvHD.[7,8] Furthermore, in adult patients grafted from MSD or UD enrolled for ECP therapy as a second line therapy for aGvHD II+ resistant to steroid treatment there was 100% complete response if aGvHD was staged II, 67% if grade III, and 12% if grade IV.[9] Finally, a recently reported series of ECP pediatric patients showed how the ECP responders had 96% of 5-year overall survival compared with 58% of non-responders.[10]

Here, we report our single center experience on ECP treatment for both aGvHD and cGvHD in pediatric patients, in which, despite the low number of reported patients, in both the aGvHD and cGvHD groups, the ECP responsiveness might predict an improved GvHD-free survival rate and this translates into a lower risk of nonrelapse mortality.

PATIENTS AND METHODS

Design and End Points of the Study

In this retrospective case-series study, we enrolled 25 pediatric patients with steroid resistant aGvHD

Received for publication June 29, 2006; accepted June 26, 2007.

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(n = 15) or cGvHD (n = 10), enrolled for ECP therapy in our Center from October 2001 to July 2005. The primary end point of this study was to assess the effectiveness of ECP on steroid resistant aGvHD and cGvHD. The secondary end points were (1) to evaluate if the long lasting GvHD-remission (GvHD-free survival) translates into a lower TRM; (2) factors affecting the probability of ECP-responsiveness in both aGvHD and cGVHD cohorts; (3) side effects of ECP treatment in low-weight children. Inclusion criteria were (1) diagnosis of both aGvHD or cGvHD based upon clinical, laboratory, and in some cases histologic documentation. Diagnosis of GvHD was made by an experienced pediatric transplant physician and by an pediatric dermatologist; (2) age < 18 years at the start of ECP; (3) previous therapy with steroids for at least 7 days, cyclosporine with therapeutic blood level ± other immunosuppressive treatments; (4) no previous treatment with either antithymocyte globulin (ATG) or monoclonal antibodies within 1 month or no other immunosuppressive treatments given within 14 days before ECP start; (5) no previous ECP treatment. Informed consent for ECP was provided according to the Helsinki Declaration. Informed consent for ECP treatment was obtained from parents if patients were younger than 8 years old. Together with the parents' consent, patients older than 8 and younger than 18 signed the appropriate form themselves. The Hospital ethical committee approved the study. For HLA matching, donor-recipient pairs were typed by high-resolution polymerase chain reaction (PCR)-sequence-based typing for loci HLA-A, B, Cw, DRβ1, and by PCR-sequence-specific priming for the loci DRβ3/4/5, DQα1, and DQβ1.[11–16]

aGvHD Patients

Fifteen patients underwent ECP for aGvHD. At the start of the ECP procedures, aGvHD was staged as II for 7 patients (47%); grade III for 4 patients (26%), and grade IV for 4 patients (26%). In this patient group, the median age at HSCT was 10.7 years (5.8 to 17.5) and the median age at ECP start was 10.8 years (5.8 to 18), 7 patients out of 15 were males. The diagnosis were acute leukemia for 6 patients (40%); other hematologic malignant diseases for 6 patients (40%), thalassemia major for 2 patients (12%); and finally McKusick syndrome with pan-cytopenia for 1 patient (6%). The stem cell sources were bone marrow (BM) for 11 patients (73%), mobilized peripheral blood for 2 patients (13%), and cord blood for 2 patients (13%). For 9 patients the preparative regimen was total body irradiation-based (60%). Five patients received HSCT from a MSD (33%) and 10 patients from a UD (66%). The clinical details and the outcome of patients who underwent ECP for aGvHD is outlined in Table 1.

cGvHD Patients

Ten patients were treated for cGvHD. At the start of the ECP procedures, 3 patients had limited cGvHD (30%) and 7 patients had extensive cGvHD (70%). Patients with limited cGvHD were also treated with ECP because of long-lasting cGvHD with previous aGvHD and steroid resistance. In this patient group, the median age at HSCT was 11.2 (6.9 to 18) and at the start of ECP 11.9 (7 to 18.5). Six patients out of 10 were males. The diagnoses were acute leukemia for 6 patients (60%); chronic myeloid leukemia for 1 patients (10%); other nonmalignant hematologic disease for 2 patients (20%); and finally relapsed Ewing sarcoma for 1 patient (10%). Seven patients had BM as stem cell source (70%), and 3 patients mobilized peripheral blood (30%). Five patients had an MSD HSCT (50%) and 5 patients had UD HSCT (50%). For 5 patients the preparative regimen was total body irradiation-based (50%). cGvHD was de novo in 3 cases (30%), quiescent in 4 cases (40%), and progressive cGvHD in 3 cases (30%). The clinical details and the outcome of patients who underwent ECP for cGvHD are outlined in Table 2.

Clinical GvHD Evaluation

The clinical stage of involved organs was staged and then combined to obtain an overall grade, according to published criteria for either aGvHD and cGvHD.[17–20] Clinical examination and hematologic monitoring were performed regularly. All patients had stable hematologic engraftment at the time of GvHD diagnosis. GvHD diagnosis was made upon clinical and laboratory evaluation and, in some cases, a histologic study was performed. Briefly, for aGvHD the severity of skin involvement was measured by skin surface involved (extent of surface skin involved) recorded on a body diagram used to calculate surface burns in children at monthly intervals. Gut involvement was based upon ruling out gastrointestinal infections by routine diagnostic tests, that is, cultures for bacterial, fungal (candida), and viral (cytomegalovirus, adenovirus, and rotavirus) agents, and by searching *Clostridium difficile* toxins and antigens for adenovirus and rotaviruses. Liver involvement was assessed by liver function tests before starting ECP and after each subsequent ECP cycle.

Patients were considered as having cGvHD if they had a long-lasting manifestation of GvHD, including lichenoid or sclerodermal skin involvement, ocular dryness that could not be relieved by artificial tears, dryness or lichenoid involvement of oral or vaginal mucosa, gastrointestinal strictures. Hyperbilirubinemia or alkaline phosphate elevations were considered to be secondary to liver cGvHD if no other causes of hepatic dysfunctions were found or in association with other symptoms of cGvHD. Diarrhea, nausea, and vomiting were considered secondary to gastrointestinal GvHD if biopsy-proven or concomitant to other symptoms and signs of cGvHD. All our patients with lung cGvHD had bronchiolitis obliterans, diagnosed following the presence of symptoms (dyspnea, cough, and wheezing) and the evidence of (1) decrease in 1-second forced expiratory value > 20% within 1 year (for patients older than 5 y); (2) evidence of air-trapping or small airway thickening or bronchiectasis at high-resolution chest computed

TABLE 1. Patients Underwent ECP for aGvHD

UPN	Age at HSCT	Diagnosis	Disease Status	Stem Cell Source	Donor Type	Conditioning Regimen	GvHD Prophylaxis	aGvHD	Skin	Gut	Liver	Previous Treatment	Body Weight	Overall ECP Response	Outcome
299	5.8	ALL	CR2	BM	MSD	TBI 1200, CTX 120, VP16 20	CyA	II	III	0	0	M-Pred	16.7	CR	A&W +63 mo
338	10.6	CML	CP1	BM	MSD	TBI 1200, CTX 120	CyA	II	II	I	I	M-Pred	46	CR	A&W +52 mo
323	15	CML	CP1	BM	MSD	TBI 1200, CTX 120	CyA	IV	III	IV	IV	M-Pred, MMF	52	NR	Died for cGvHD +10 mo
346	13.8	CML	CP1	BM	MSD	TBI 1200, CTX 120	CyA	III	0	0	III	M-Pred	42	CR	A&W +50 mo
412	11.8	AREB-T	Untreated disease	BM	MSD	BUS 16, CTX 120, L-PAM 140	CyA	III	III	I	0	M-Pred	46.6	CR	DOD +10 mo
378	8.9	T-NLH	CR2	BM	MUD	TBI 1200, CTX 100, TT 10	CyA, MTX, ATG	III	III	I	III	M-Pred	25	CR	DOD +6.2 mo
386	8.5	McKusick syndrome	NA	BM	MUD	BU 16, CTX 200	CyA, MTX, ATG	II	III	I	0	M-Pred	21	CR	A&W +40.2 mo
402	15.6	CML	CP1	BM	MUD	TBI 1200, TT 10, CTX 100	CyA, MTX, ATG	IV	IV	IV	0	M-Pred, ATG	56	NR	Died of cGvHD +10.8 mo
294	9.1	ALL	CR6	CB	MUD	L-PAM 160, FLU 200, TT 10	CyA, ATG, M-Pred	II	III	0	0	M-Pred	21	CR	A&W +66.2 mo
321	13.4	ALL	CR2	PB	MUD	TBI 1200, CTX 120, TT 10	CyA, MTX, ATG	IV	I	III	IV	M-Pred	43	NR	Died of aGvHD +4.4 mo
353	5.8	Cooley	NA	BM	MUD	BU 14, TT 6, FLUDARA 120	CyA, MTX, ATG	II	III	III	II	M-Pred	18	NR	A&W +47.8 mo
395	8.3	ALL	CR2	PB	MUD	TBI 1200, CTX 120 TT 10	CyA, MTX, ATG	II	III	0	0	M-Pred	24	CR	A&W +36.3 mo
419	17.5	ALL	CR1	BM	MUD	TBI 1200, CTX 120, TT 10	CyA, MTX, ATG	III	II	III	0	M-Pred, MMF, ATG	72	MR	Alive with cGvHD +29.4 mo
381	10.7	ALL	CR3	CB	MUD	TT 5, FLUDARA 120, LPAM 140	CyA, ATG, M-Pred	II	III	0	0	M-Pred	24	CR	A&W +40.7 mo
403	10.2	Cooley	NA	BM	MUD	BU 14, TT 6, FLUDARA 120	CyA, MTX, ATG	IV	III	IV	II	M-Pred, MMF	24	NR	Alive with cGvHD +34.7 mo

A&W indicates alive and well; AREB-T, refractory anaemia with excess of blasts in transformation; BU, busulphan; CB, cord blood; CML, chronic myeloid leukemia; CP1, first chronic phase; CR1, first complete remission; CR2, second complete remission; CR3, third complete remission; CR6, sixth complete remission; CTX, cyclophosphamide; D, de novo; DOD, dead of disease; FLUDARA, fludarabine; L-PAM, melphalan; M-pred, methyl-prednisolone; MTX, methotrexate; MUD, matched unrelated donor; NA, not applicable; P, progressive; PB, peripheral blood; Q, quiescent; TBI, total body irradiation; T-NHL, T-cell non-Hodgkin lymphoma; TT, thiotepa; UPN, unique patient number; VP16, etoposide.

TABLE 2. Overall Description of Patients who Underwent ECP for cGvHD

UPN	Body Weight	Age at HSCT	Disease	Disease Status	Stem Cell Source	Donor Type	Conditioning Regimen	GvHD Prophyl-axis	aGvHD	Landsky/ Karnofsky (%)	cGvHD Onset	cGvHD Type	Organ Involved	Platelets Count	Previous Therapy	Overall ECP Response	Outcome
193	60	8.1	SAA	NA	BM	MSD	CTX 200	CyA	II	70	Q	Limited	Skin	266,000	M-Pred	CR	A&W +106 mo
262	51	13.7	AML	CR1	BM	MSD	TBI 1200, CTX 120	CyA	III	70	Q	Extensive	Skin, joints	250,000	M-Pred CyA	CR	A&W
295	27	8	ALL	CR2	BM	MSD	TBI 1200, VP16 20, CTX 100	CyA	II	90	Q	Limited	Skin	226,000	M-Pred CyA	CR	A&W
401	52	18	AML	CR1	PB	MSD	TT 10, CTX 100	CyA MTX	0	70	D	Extensive	Skin, oral-mucosae, joints, gut	55,000	M-Pred CyA	NR	Alive with cGvHD
370	40	17.2	Ewing Sarcoma	CR2	PB	MSD	TT 10, FLUDARA 60, CTX 60	CyA MTX	0	70	D	Extensive	Skin, lung	154,000	M-Pred CyA	NR	Alive with cGvHD
269	32	18	Cooley	NA	BM	MUD	BU 14, TT 10, CTX 120	Cya MTX CTX	III	60	P	Extensive	Skin, muscles, gut	23,000	M-Pred MMF	NR	Alive with cGvHD +74.8 mo
332	24	7.2	AML	CR2	BM	MUD	BU 8, CTX 100, L-PAM 140	Cya MTX ATG	0	80	D	Extensive	Skin, oral-mucosae	44,000	M-Pred CyA	PR	DOD +14.7 mo
178	63	15.3	CML	CP1	BM	MUD	TBI 1200, TT 10, CTX 120	CyA MTX ATG	II	40	P	Extensive	Skin, Liver, gut, oral-mucosae, lung	134,000	CyA M-Pred MMF	NR	Died of cGvHD +74.3 mo
371	47	11.2	ALL	CR2	BM	MUD	TBI 1200, TT 10, CTX 120	CyA MTX ATG	II	90	P	Limited	Skin	139,000	M-Pred	CR	A&W +43.4 mo
221	23	6.9	ALL	CR2	PB	MUD	TBI 1200, TT 10, CTX 120	CyA MTX ATG	II	70	Q	Extensive	Skin, eye-mucosae, liver	400,000	CyA M-Pred MMF	NR	Alive with cGvHD +94.7 mo

A&W indicates alive and well; AML, acute myeloid leukemia; BU, busulphan; CML, chronic myeloid leukemia; CP1, first chronic phase; CR1, first complete remission; CR2, second complete remission; CTX, cyclophosphamide; D, de novo; DOD, dead of disease; FLUDARA, fludarabine; M-pred, methyl-prednisolone; MTX, methotrexate; MUD, matched unrelated donor; P, progressive; Q, quiescent; SAA, severe aplastic anemia; TBI, total body irradiation; TT, thiotepa; UPN, unique patient number; VP16, etoposide.

tomography scan; and (3) no evidence of active infections in the respiratory tract, documented by radiologic studies or microbiologic cultures.

Pre-ECP GvHD Treatment

Steroid resistance was defined as a lack of stable clinical improvement after treatment with prednisolone at 2 to 5 mg/kg/d for at least 7 days and cyclosporin-A (CSA) with therapeutic drug level.

Eleven patients of the aGvHD patient group received steroid-based therapy (74%), the remaining patients underwent ECP therapy after steroids and mofetil mycofenolate (MMF) (n = 2) or ATG (n = 1) or both treatments (n = 1). The median interval between aGvHD diagnosis and ECP was 25 days (13 to 55). The intervals between aGvHD and the start of ECP were 20 days (15 to 31) and 34 days (21 to 55) for patients receiving only steroids or combination therapy, respectively (P = NS). Moreover, the interval between the latest line of immunosuppression before ECP start was 16 days (12 to 20) for MMF and 31 days for ATG.

Patients affected by cGvHD were treated with steroids only (n = 2, 20%), steroids plus CSA (CyA) (n = 5, 50%), steroids plus MMF (n = 1, 10%), and steroids plus MMF and CyA (n = 2, 20%). The median interval between cGvHD start and ECP start was 650 days (21 to 3455): it was 455 days (12 to 903) for patients having received steroids only and 750 days (21 to 3455) for patients treated with combination therapy (P = NS). The intervals between the latest line of immunosuppression and ECP start was 33 days in the case of CyA (19 to 3455), and 782 in the case of MMF (138 to 1187).

ECP Protocol

ECP was performed on both an outpatient and an inpatient basis. All patients who underwent ECP received packed red cells the day before if hemoglobin was lower than 9 g/dL. Patients were treated with ECP on 2 consecutive days at weekly intervals for the first month, every 2 weeks during the second and third month, and then at monthly intervals for a further 3 months.[10] Briefly, patients with aGvHD were ruled out from the ECP protocol if they had completed their planned 22 procedures or, if they had aGvHD progression under ECP. Patients with cGvHD were ruled out from ECP therapy if they reached complete response (CR), partial response (PR), or minor response (MR) following the 22 planned ECP procedures or if they had aGvHD progression under ECP. Three patients continued ECP therapy behind 22 procedures as only GvHD treatment, because of severe toxicity given by other immunosuppressive drugs.

Progressive tapering and discontinuation of ECP was decided after evaluating individual response. Any concomitant immunosuppressive medication was initially maintained and then modified or discontinued according to the clinical response (except for ATG or monoclonal antibody therapies that were stopped before ECP start). If patients weighed < 40 kg lymphocytoapheresis were performed by means of a continuous flow cell separator using peripheral venous access. At least 2 blood volumes were processed: the mean volume was 140 mm with the percentage of mononuclear cell ranging from 75% to 95%. Normal saline was added to the collection bag to reach a final volume of 300 mm, and a hematocrit < 2%. The yielded buffy coat was transferred into a thin plastic bag and the 8-methoxypsoralen (8-MOP) was added to a final concentration of 200 ng/mL. Finally, the product was exposed to ultraviolet (UV) A irradiation (365 nm, 2 J/cm^2) and then reinfused in patients.

If patients were over 40 kg, the ECP procedures were performed using an UVAR Photopheresis Instrument (Therakos, Exton, PA). After collecting 240 mL of mononuclear cells, 300 mL plasma was added to 200 mL of normal saline. 8-MOP (100 μg) (Gerot, Wien, Austria) in aqueous solution was then added and finally, the buffy coat and plasma were passed in as a thin film through a disposable plastic device, exposed to UVA light (2 J/cm^2) for 90 minutes and then returned to the patients.

The average duration of the procedure was 180 to 240 minutes for both techniques. During the ECP procedures patients were monitored for blood pressure, heart rate, and body temperature. Full blood count, liver and kidney function tests and coagulation parameters were obtained before and after each procedure.

Response to ECP

Response to therapy was assessed after 3 months from the end of ECP or after 6 months if the ECP protocol was prolonged and the following response criteria were considered:

(1) criteria for defining responses to ECP for aGvHD were as previously reported.[9] Briefly, CR: overall GvHD grade 0-I after 3 months after stopping ECP-therapy; PR: > 50% of organ involvement (skin, gut, and liver); MR: tapering of immunosuppressive agents with stable GvHD; stable GvHD [stable disease (SD)]: < 50% response of organ involvement (skin, gut, and liver); progressive disease (PD): any worsening of organ involvement or new sign/symptoms of GvHD. Patients with SD or PD were considered as nonresponders;

(2) criteria for defining responses to ECP for cGvHD were CR: complete regression of skin, liver, gastrointestinal, lung, oral, joint, or eye manifestations; PR: > 50% in terms of organ involvement. In this case, due to the complexity inherent to the assessment of response in each organ, we defined PR as follows:

- skin GvHD: for lichenoid rashes a minimum reduction in the body surface area involved by 50%. For sclerodermatous involvement, any improvement in the skin score or range of motion, with an improvement of Zubrod/Eastern Cooperative Oncology Group performance status of 1
- ocular GvHD: subjective improvement and reduction in the frequency of artificial tears administration by

50%, or improvement in Schirmer test for one or both eyes
- oral GvHD: improvement by 50% in the mucosal area involved with lichenoid and/or ulcerative changes
- gastrointestinal and liver: decrease by 50% in the volume of diarrhea, bilirubin, or alkaline phosphatase
- bronchiolitis obliterans: sustained improvement in pulmonary function test (1-second forced expiratory value) and/or the ability to taper steroids by 50% with no deterioration of pulmonary function. MR: tapering of immunosuppressive agents with stable GvHD; SD: < 50% in organ involvement; PD: worsening of organ involvement or new sign/symptoms of GvHD. Patients with SD or PD were considered as nonresponders. Patients with a CR or PR in one organ and a simultaneous PD in another were considered as nonresponders.

Statistical Analysis

The end points of this study were the aGvHD and cGvHD response, the GVHD-free survival after ECP start and the TRM. The GvHD-free survival was defined as the GvHD-remission and no flare up after stop therapy until the last follow-up or neoplasia recurrence or death due to any causes. Deaths because of the relapse of underlying primary diseases were censored at the time of relapse. GvHD-free survival was assessed through Kaplan-Meier statistics[21] and the univariate comparisons were made using the log-rank test. Variables found to be significant at the P value < 0.2 level were entered into a proportional hazard regression analysis using a backward stepping procedure.[22] All analyses were performed with the Statistica base software (www.statsoft.com). The closing date was July 31, 2005.

RESULTS

Causes of Death

A total of 7 patients (28%) of patients treated with ECP died. The primary cause of death was GvHD (n = 4, 57%). The median follow-up of these patients from entering the ECP protocol was 227 days (76 to 942).

aGvHD Patients

The median number of ECP procedures was 12 (range 4 to 21). The first ECP procedures were performed at 38 days (median value) after HSCT (range 15 to 97), and the median interval between aGvHD diagnosis and ECP was 25 days (13 to 55). The intervals between aGvHD and ECP start were 20 days (15 to 31) and 34 days (21 to 55) for patients receiving only steroids or combination therapy, respectively (P = NS). Moreover, the interval between the latest line of immunosuppressive therapy before ECP start was 16 days (12 to 20) for MMF and 31 days for ATG. At the end of the scheduled ECP procedures the overall GvHD-free survival response to ECP was 62%. The aGvHD-free survival was 100%, and 30% if the aGvHD was II, or III to IV, respectively (P = 0.006) (Fig. 1). The single target organ response is outlined in Table 3. Briefly, no patients having life-threatening aGvHD IV responded to ECP, whereas all patients enrolled in the ECP protocols with early disease (aGvHD grade II) had complete response. In particular, with severe single organ involvement, responses were 80% for skin aGvHD III to IV, 50% for liver aGvHD III to IV, and 33% for gut aGvHD III to IV.

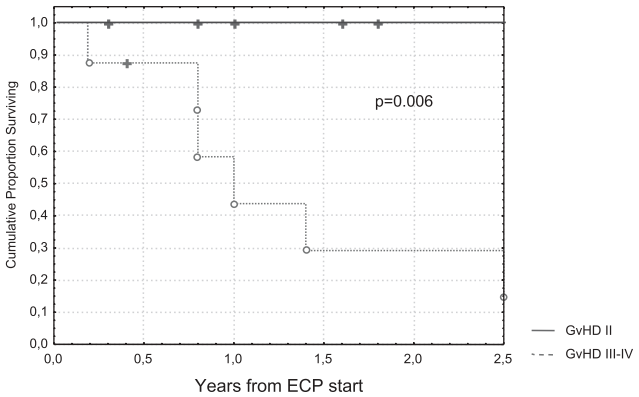

FIGURE 1. Overall survival for patients enrolled to ECP according to aGvHD severity.

Factors Affecting aGvHD-free Survival

Two patients affected by T-cell non-Hodgkin lymphoma and refractory anaemia with excess of blasts in transformation relapsed at 161 and 181 days after ECP start, and their follow-up was censored at those days. Three patients died of uncontrolled GvHD with concomitant ab ingestis pneumonitis, lung aspergillosis, and liver failure at 276, 301, and 76 days after ECP start. The median follow-up was 1.6 years (range: 0.8 to 4), 1.7 years (range: 0.8 to 4), and 0.9 years (range: 0.8 to 2.5) for ECP responders and nonresponders, respectively (P = 0.18).

TABLE 3. aGvHD Response After ECP Treatment

	CR	PR	MR	NR
Overall				
GvHD II	7/7 (100%)	—	—	—
GvHD III	2/4 (50%)	1/4 (25%)	—	1/4 (25%)
GvHD IV	—	—	—	4/4 (100%)
Target organ analysis				
Skin				
Grade IV	—	—	—	1/1 (100%)
Grade III	5/9 (55%)	3/9 (33%)	1/9 (11%)	—
Grade II	2/2 (100%)	—	—	—
Grade I	1/1 (100%)	—	—	—
Liver				
Grade IV	—	—	—	2/2 (100%)
Grade III	2/2 (100%)	—	—	—
Grade II	1/2 (50%)	1/2 (50%)	—	—
Grade I	—	1/1 (100%)	—	—
Gut				
Grade IV	—	1/3 (33%)	—	2/3 (66%)
Grade III	1/3 (33%)	—	—	2/3 (66%)
Grade II	—	—	—	—
Grade I	4/4 (100%)	—	—	—

NR indicates no response.

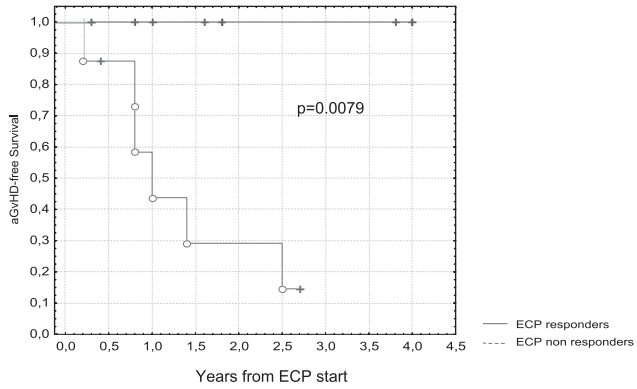

FIGURE 2. aGvHD-free survival according to ECP responsiveness.

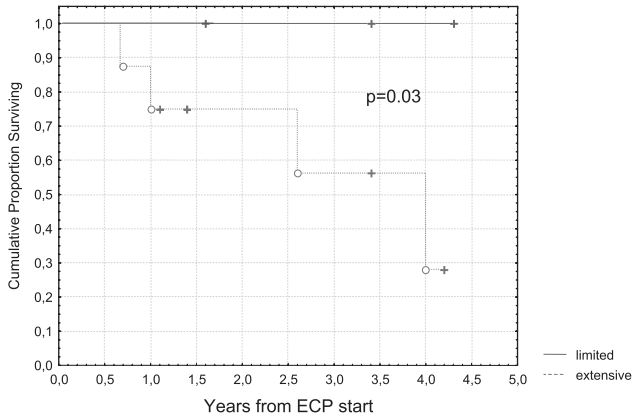

FIGURE 3. Overall survival for patients enrolled to ECP according to cGvHD extension.

In our series, we found that the only predictive factors for better aGvHD-free survival was aGvHD grading on entering the analysis (aGvHD II 100% compared with aGvHD III to IV 30%, $P = 0.006$), and the first line therapy (patients receiving only steroids had 72% of aGvHD-free survival compared with 10% for patients receiving combined therapy, $P = 0.03$). However, factors having a P value < 0.2 were run in multivariate analysis by Cox-regression model. Results of the multivariate analysis showed how the only factor statistically associated with a better aGvHD-free survival was the aGvHD grading at entering the analysis [aGvHD II vs. III to IV, relative risk (RR) 5.071, confidence interval (CI) 95% 2.2-5.5, $P = 0.016$]. Moreover, all our ECP responders had 100% of aGvHD-free survival compared with 15% for patients who did not respond to ECP ($P = 0.0079$, Fig. 2).

Factors Affecting TRM for Patients Treated With aGvHD

Because aGvHD grading was the strongest predictor of better aGvHD-free survival after ECP, we then evaluated whether this last variable translates into a lower TRM risk. Briefly, patients enrolled in ECP procedures with aGvHD II had 0% TRM compared with 42% for patients with aGvHD III to IV ($P = 0.05$). The only other factor statistically associated with a lower TRM was the ECP responsiveness, with 0% TRM for ECP responders compared with 50% for nonresponders ($P = 0.022$). Other factors had no significant impact of TRM risk in univariate analysis. However, factors with P value < 0.2 in univariate analysis were run in multivariate analysis. ECP nonresponders (RR 5.26, CI 95% 3.4-6.2, $P = 0.02$) and patients having received several lines of therapy before ECP (RR 4.96, CI 95% 2.6-5.9, $P = 0.02$) were found to be predictive factors for higher TRM risk in multivariate analysis.

cGvHD Patients

The median number of ECP procedures was 22 (range: 10 to 98) and the median follow-up from ECP start was 942 days (range: 563 to 1588). The median interval between cGvHD start and ECP start was 650 days (21 to 3455): it was 455 days (12 to 903) for patients having received steroids only and 750 days (21 to 3455) for patients treated with combination therapy (P = NS). The intervals between the latest line of immunosuppression and the ECP start was 33 days in the case of CyA (19 to 3455), and 782 in the case of MMF (138 to 1187).

At the end of the ECP procedures the overall cGvHD-free survival response was 40%. It was 100% for limited cGvHD and 28% for extensive cGvHD ($P = 0.03$) (Fig. 3). We also compared the effectiveness of ECP procedures on single organ involvement. Briefly, if cGvHD was skin-limited the overall response was 90%, whereas if the cGvHD reaction involved visceral organs such as liver, gut, or lung, the response rate was lower: 50% for liver, and 0% for gut or lung, respectively (Table 4).

Factors Affecting cGvHD-free Survival

One patient affected by acute myeloid leukemia M0 relapsed on day 443 from UD HSCT and at 269 days from ECP start and finally the follow-up was censored at the day of relapse. One patient died of uncontrolled cGvHD at 2229 from transplantation (lung cGvHD).

Briefly, the only factors associated with improved cGvHD-free survival were the absence of severe (grade II+)

TABLE 4. cGvHD Response After ECP Treatment

	CR	PR	MR	NR
cGvHD				
Limited cGvHD	3/3 (100%)	—	—	—
Extensive cGvHD	1/7 (14%)	1/7 (14%)	—	5/7 (71%)
Target organ analysis				
Skin	5/10 (50%)	4/10 (40%)	—	1/10 (10%)
Oral mucosae	—	2/3 (33%)	—	1/3 (33%)
Eye mucosae	—	—	—	1/1 (100%)
Joints	1/2 (50%)	1/2 (50%)	—	—
Liver	—	1/2 (50%)	—	1/2 (50%)
Gut	—	—	—	3/3 (100%)
Lung	—	—	—	2/2 (100%)

NR indicates no response.

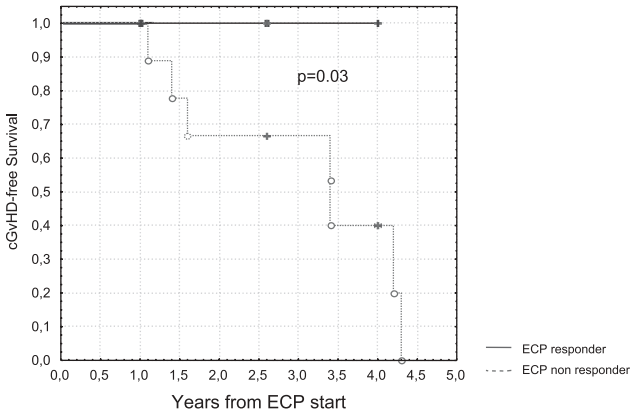

FIGURE 4. cGvHD-free survival according to ECP responsiveness.

gut, liver, or lung involvement, 100% compared with 0% ($P = 0.017$), the GvHD prophylaxis with only CSA 100% compared with other regimens 20% ($P = 0.05$) and finally the lower $CD3^+$ lymphocyte content of the stem cell grafts (60% compared with 0%, $P = 0.048$). However, factors with P value < 0.2 were run in a multivariate analysis by Cox-regression model. The results of the multivariate analysis show how only cGvHD with severe liver, gut, or lung involvement had a significant poor effect on the cGvHD-free survival (RR 5.17, CI 95% 2-4.9, and $P = 0.001$). ECP responders had 100% of cGvHD-free survival compared with 0% for ECP nonresponders ($P = 0.03$, Fig. 4).

Factors Affecting TRM for Patients With cGvHD

Factors affecting the probability of cGvHD-free survival were also run to evaluate their predictive role for TRM. Factors statistically associated with a lower probability of death from TRM were ECP responsiveness with 0% TRM for ECP responder compared with 50% for nonresponders ($P = 0.022$), HLA-matching at high resolution for 10/10 loci (0% compared with 40%, $P = 0.011$), type of cGvHD onset (being 0% for de novo or quiescent type compared with 68% for the progressive form, $P = 0.023$), and finally the lower $CD3^+$ lymphocytes given with the stem cell inoculum (0% vs. 100%, $P = 0.05$). All factors with P value < 0.2 were run in multivariate analysis by Cox regression model and the only factors found significantly associated with higher TRM were the ECP nonresponsiveness (RR 12.4, CI 95% 8.4-16.9, $P = 0.02$), the cGvHD with visceral involvement (RR 11.49, CI 95% 6-13.5, $P = 0.041$) and the higher $CD3^+$ content (RR 10.9, CI 95% 5.9-16.8, $P = 0.04$).

Side Effects and Fate of Patients Failing the ECP Therapy

The side effects were mild and more frequent in advanced disease GvHD patients. The most frequent symptom was abdominal pain during the procedures (5/25, 20%), although no severe hypotension requiring the stop procedures occurred, also for low weight children. A transient reduction in platelet and white blood cell counts was observed after ECP procedures in 20/25 patients (80%). No severe infections occurred during ECP procedures, and no patients required sedation or general anesthesia during the procedures. Two out of 25 patients required a de novo catheter placement. No patients had catheter-related infections, and finally no grade III to IV organ toxicity according to NCI CTC version 2.0 was observed.

All patients having no response to ECP therapy (10 patients, 40%) underwent several lines of GvHD-salvage therapies. Of them 4 patients (40%) had no benefit and finally died of GvHD and its complications (Table 5).

TABLE 5. Fate of Patients Who Died of GvHD

UPN	Response to ECP	Post-ECP Therapy	Complications	Cause of Death
323	NR	ATG, steroids, tacrolimus	Seizures due to cerebral vasculitis, GvHD-related cachexia, CMV reactivation, respiratory distress	Ab ingestis pneumonia +10 mo
402	NR	ATG (2 courses), steroids, daclizumab	Respiratory distress, CMV reactivation, seizures due to cerebral vasculitis	ARDS lung aspergillosis +10.8 mo
321	NR	ATG, steroids, tacrolimus	CMV reactivation	Liver failure +4.4 mo
178	NR	ATG, steroids, MMF	Seizures due to cerebral vasculitis, bilateral retinal detachment, CMV reactivation	ARDS +74.3 mo

CMV indicates cytomegalovirus; Daclizumab, monoclonal antibody directed to the IL-2Rá receptor of T cells; NR, no response; UPN, unique patient number.

DISCUSSION

GvHD is one of the major limitations to successful HSCT with a substantial impact on survival and quality of life in otherwise cancer-free patients. Several studies have demonstrated that T lymphocytes can be functionally inactivated in vitro with 8-MOP and UVA treatment.[23,24] This was followed by showing that photoinactivation of donor T-lymphocytes with 8-MOP and psoralen + UVA before infusion prevents the development of GvHD in a murine experimental model.[25] In light of this and following previous reports on the activity of ECP for GvHD in adult patients,[26–31] a relatively low number of ECP procedures was reported for the treatment of aGvHD and cGvHD in pediatric patients,[10] in particular a recently reported series by the Associazione Italiana di Ematologia e Oncologia Pediatrica-Bone Marrow Transplantation group, patients enrolled for ECP for steroid-resistant aGvHD had a very high success rate of 75% (complete + partial response) for GvHD I to IV, whereas for cGvHD the response rate was 73% (complete + partial response). Our first priority was to evaluate the GvHD-free survival (both acute and chronic) and the second one was to evaluate whether those patients refractory to ECP procedures had a greater risk of death for TRM. Data

from our analysis, as reported above, clearly show how the severity of the GvHD (both acute and chronic) did correlate significantly with ECP responsiveness.

Specifically, the aGvHD-free survival was 100% for patients with grade II and 30% for grade III to IV; whereas the second factor was the previous therapy. Patients given only steroids experienced a higher aGvHD-free survival rate, 72% compared with 10% ($P = 0.03$). This might be explained by the fact that early treatment in the early phase with ECP rescued patients with aGvHD progression and finally at a higher risk of no longer being responsive to ECP or other therapies. However, these data need to be correlated to the low patient number reported and the lack of controls. Despite these limitations our data agree with the results proposed by Greinix et al[32] in which patients with aGvHD ECP responders had 59% of survival compared with 11% for patients who were ECP nonresponders.

Regarding the TRM risk for the patients treated with ECP for steroid resistant aGvHD, we found the only predictive factor was the aGvHD grading [TRM 0% for aGvHD II and 42% for aGvHD III to IV ($P = 0.05$)]. This data confirms previously reported data indicating that uncontrolled GvHD is the main reason for nonrelapse mortality.[4,5,33]

As far as ECP effectiveness on cGvHD is concerned, it has to be pointed out that we had 70% of extensive cGvHD patients. The poorer cGvHD-free survival was among patients with severe liver, gut, or lung involvement [(II+), (0%)], and CSA plus other drugs as GvHD prophylaxis alone (20%). The multivariate analysis shows how the only significantly associated factor with a poorer cGvHD-free survival was the severe liver, gut, or lung involvement. The TRM risk for patients who underwent ECP for steroid resistant cGvHD was associated with HLA-mismatched pairs (40%), and the cGvHD type of onset (68%). The multivariate analysis shows, together with the ECP resistance, the cGvHD involving liver, gut, or lungs (RR 11.49, CI 95% 6-13.5, $P = 0.041$) and the higher $CD3^+$ lymphocyte content of the stem cell graft (RR 10.9, CI 95% 5.9-16.8), were significantly associated with a higher risk of TRM. Our results agree with adults' data in which the ECP responders had lower TRM compared with nonresponders (16% compared with 80%).[34]

In conclusion, in this study we have shown that ECP is feasible in low-weight children with no severe side effects.

Remarkably the absence of long term side effect after ECP treatment is very important in comparison to the long term side effects of steroid treatment (such as bone necrosis, cataracts, growth retard) and this data should also be considered when the GvHD diagnosis is made.

Moreover, despite the low number of enrolled pediatric patients, data from our analysis suggest that ECP is effective in the early phase of GvHD and this is followed by a significant reduction of nonrelapse mortality. In particular, it would be appropriate to enroll patients in an early phase of disease if steroid-refractoriness is documented.

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