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Treatment of Acute Graft-versus-Host Disease in Childhood with Extracorporeal Photochemotherapy/Photopheresis: The Padova Experience

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A B S T R A C T

Acute graft-versus-host disease (aGVHD) is the major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation. Systemic steroid treatment represents the first-line therapy for aGVHD and is associated with a response rate of 30% to 60%. Steroid-resistant patients have a poor prognosis with high transplantation-related mortality (TRM). Several second-line therapies have been proposed for the management of unresponsive aGVHD, without proven beneficial effects on patients' outcome or overall long-term survival. For these reasons, extracorporeal photochemotherapy/photopheresis (ECP), a cell-based approach to control GVHD that spares generalized immunosuppression, seems to be promising. In this study, we report the outcome of 72 consecutive pediatric patients treated with ECP between 1997 and 2013 for aGVHD. Among them, 21 patients had steroid-resistant aGVHD, 42 had steroid-dependent aGVHD, and 9 did not receive steroid as first-line therapy because of clinical contraindications. A complete response was obtained in 72% of patients, a partial response was observed in 11%, and there was no response in 17% of patients. At day +180, TRM was 4% in the whole cohort; TRM was 3% and 20% among responders and nonresponders to ECP, respectively ($P < .0001$). The 5-year overall survival was 71%, showing a difference between responders and nonresponders of 78% and 30%, respectively ($P = .0004$). The 5-year time to progression of primary disease was 81%, without any significant difference between the 2 groups. Moreover, the 5-year progression-free survival of primary disease was 72%, with a significant difference ($P = .0007$) between responders (79%) and nonresponders (30%) to ECP. In conclusion, this study demonstrates that ECP is highly effective in aGVHD without a negative impact on primary disease.

INTRODUCTION

Allogeneic (allo) hematopoietic stem cell transplantation (HSCT) is increasingly used as a therapeutic approach for hematological and nonhematological diseases. In the last decade, improvements in infection monitoring and prophylaxis, immunosuppressive (IS) strategies, high-resolution HLA typing, and supportive care measures led to better outcomes after this procedure. Despite these advancements, acute graft-versus-host disease (aGVHD) remains the major cause of morbidity and mortality after allo-HSCT [1,2].

To standardize diagnosis and management of aGVHD, a British guideline was published by a joint working group of the British Committee for Standards in Haematology and the British Society for Bone Marrow Transplantation. This document included recommendations for diagnosis and management of aGVHD as well as primary treatment options for patients with steroid-refractory (SR) disease [3].

Standard management of aGVHD included steroids at different doses depending on aGVHD grade. If no improvement of aGVHD after 7 days was noted or progression within 72 hours were observed, then the addition of second-line agents should be considered. Second-line options are

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mycophenolate mofetil (MMF), anti-TNF antibodies, mammalian target of rapamycin inhibitors, IL-2 receptor antibodies, and extracorporeal photochemotherapy/photopheresis (ECP) [3].

Unfortunately, despite multiple clinical trials, no single agent improving overall survival (OS) for patients who failed steroid treatment has been identified [4,5].

Moreover, the current survival at years in patients who respond to steroids is about 36% versus 17% in nonresponders (NR) [6] and it has been shown that transplantation-related mortality (TRM) is higher in steroid-resistant patients [7,8].

In our study, we focused on ECP, one of the most promising treatments for aGVHD. Briefly, ECP consists of 3 procedures: collection of peripheral leukocytes cells, irradiation of cells by ultraviolet A light in presence of 8-methoxypsoralen (8-MOP), and reinfusion of treated cells in the patient. The underlying mechanism of action of ECP in GVHD is not completely understood [9,10]. Within 24 hours, this process induces apoptosis of all treated cells (including T cells) and subsequent phagocytosis by antigen-presenting cells; as a result, this might regulate immune homoeostasis, modulate the cytokine production, and induce tolerance of antigen-presenting cells [10-13].

ECP has been demonstrated to be effective in treating both steroid-resistant and steroid-dependent patients with aGVHD [14-17]. In the pediatric setting in particular, the reported response rate ranges from 50% to 100%, according to the organs involved. In aGVHD steroid-resistant patients, 5-year OS is significantly increased in complete responders to ECP, 69% compared with 12% for NR [18].

In 2013, the Italian Society of Haemapheresis and Cell Manipulation and the Italian Group of Bone Marrow Transplantation elaborated best practice recommendations for ECP, which reflected the common clinical practice in most Italian transplantation centers where ECP is performed with a total of 4500 procedures per year [19]. Despite this large use of ECP, most of the published reports deal with retrospective data that are difficult to compare, as patients' selection criteria, treatment schedules, patients' monitoring, and patients' assessment protocols differ among institutions. Moreover, no randomized studies have been conducted in patients with aGVHD.

Here, we report our single-center experience on ECP treatment in 72 pediatric patients with aGVHD. The response to ECP, TRM, OS, progression-free survival (PFS) of primary disease, and time to progression (TTP) of primary disease of patients treated with ECP were analyzed.

MATERIAL AND METHODS

Patients

From January 1997 to June 2013, 72 consecutive pediatric patients (44 males, 28 females) affected by aGVHD were treated with ECP at the HSCT Unit of University Hospital of Padova. Fifteen of these 72 patients have been previously reported [20]. The clinical characteristics of patients are shown in Table 1. The median age at ECP was 8.3 years (range, .9 to 20.3 years) and the median body weight was 25 kg (range, 7 to 98 kg). Fifteen children weighted less than 15 kg. The last follow-up was fixed on June 2014. In detail, 21 patients were treated with ECP for *aGVHD refractory to steroids*, which was defined as a progression or no improvement in aGVHD after at least 3 days or 7 days on methylprednisolone (MP) $\geq$ 2 mg/kg body weight, respectively (SR group); 21 patients for *steroid-dependant aGVHD*, defined as a flare-up of aGVHD during the tapering of MP (SD group); and 30 children with aGVHD who required a reduction of pharmacological IS or contraindications to IS therapy for viral reactivations, systemic mycoses, or intolerable side effects (group with infectious complications [IC]). In particular, 9 of 30 patients in the IC group (IC-A group) underwent ECP without steroids as a first-line therapy because of contraindications: 1 for TCR $\alpha\beta$ and CD19–depleted haploidentical transplantation with probable invasive pulmonary aspergillosis (IPA) and adenovirus (ADV), 1 for proven IPA, 1 for concomitant proved IPA and cytomegalovirus (CMV) reactivation, 1 for probable IPA and CMV and BK virus (BKV) reactivations, 1 for proven IPA and multiple viral reactivations, including ADV, CMV, and Epstein-Barr virus (EBV), 2 for CMV reactivation, 1 for CMV and EBV reactivations, and 1 for CMV, EBV, ADV, and BKV reactivations. The other 21 patients (IC-B group) had SD aGVHD and cyclosporin A (CsA)–related renal insufficiency in only 1 patient; SD aGVHD and concomitant infections in the remaining 20 patients: CMV reactivations, 2; CMV and EBV reactivations, 4; CMV and BKV reactivations, 2; CMV, ADV, and BKV reactivations, 1; EBV reactivations, 4; EBV and ADV reactivations, 1; EBV reactivations and probable aspergillosis, 1; hepatitis B virus, 1; hepatitis B virus and EBV reactivations, 1; CMV, EBV, and BKV reactivations, 1; ADV, human herpesvirus-6, BKV, and coronavirus, 1; hepatitis C virus, CMV, and proven IPA, n = 1.

In our practice, surveillance for viral and fungal infections in blood is routinely performed during the first 100 days after HSCT in all patients and includes EBV-DNA, CMV-DNA, ADV-DNA, human herpesvirus-6 DNA, BKV-DNA, JC-DNA, and galactomannan antigen search. This schedule is performed once each week in allo-HSCT recipients from HLA-identical sibling and twice each week in allo-HSCT recipients from unrelated or haploidentical donors. Monitoring viral infections in urine comprises CMV-DNA, BKV-DNA, JCV-DNA in a weekly search. Blood, urine, and stool cultures; nasal and throat swabs; and nasopharyngeal aspirates were weekly performed. Search for other viruses or microbiological agents was performed upon clinical symptoms. Viral reactivations were detected by PCR positivity for EBV-DNA (cut-off: 1000 copies/mL), CMV-DNA (cut-off: 1000 copies/mL), and ADV-DNA in qualitative test. Clinical systemic fungal infections were defined proved or probable according to European Organization for Research and Treatment of Cancer criteria [21]. The cut-off for the galactomannan index was set at .5 (Enzyme Immuno Assay (E.I.A.) method).

The algorithm for aGVHD treatment used in our center is shown in Figure 1.

GVHD Prophylaxis

CsA was administered for 6 and 12 months in children who received HSCT from an HLA-identical sibling or unrelated donor, respectively. In unrelated HSCT, short-term methotrexate and rabbit antithymocyte globulin (ATG) were given. In unrelated cord blood HSCT, prophylaxis included CsA and ATG. In haploidentical setting, ex vivo elimination of $\alpha\beta$ T cells and CD19 B cells was done and ATG was administered before the cells were infused; no other IS therapy was given after HSCT.

Informed consent was obtained from patients' parents, as well as from the patients themselves when possible, and the use of ECP was approved by the ethical committee of the Hospital of Padova.

aGVHD Evaluation

The clinical organ involvement was graded and then combined to obtain an overall grade, according to Glucksberg criteria for aGVHD [22]. Histological confirmation was obtained whenever clinically indicated to confirm GVHD diagnosis.

Eligibility Criteria for ECP

Eligibility criteria for ECP treatment were as follows: children with SR aGVHD (n = 21); children with SD aGVHD (n = 21); patients with aGVHD in whom IS therapy was contraindicated or who required a rapid decrease of IS therapy for increasing EBV viral load, CMV reactivation in 2 subsequent samples, systemic fungal infections, intolerable side effects (n = 30). All children must present in complete hematological remission and full donor chimerism; white blood cell (WBC) count $> 1 \times 10^9$/L, and no concomitant treatment with either ATG or monoclonal antibodies.

ECP Procedure and Technical Elements

ECP was performed using 2 different techniques: "in-line" treatment (UVAR Photopheresis Instrument, Therakos, Exton, PA) was used in 19 of 72 patients and the "off-line" technique (Cobe Spectra, BCT Terumo, Lakewood, CO) was used in 53 of 72 children. Technical descriptions have already been published [19]. The "off-line" technique was introduced in 2003 to treat low-weight children.

For patients weighing less than 15 kg, priming of the leukapheresis circuit with irradiated and leuko-reduced red blood cells (regardless of baseline hemoglobin level) was performed, as recommended in the Italian Society of Haemapheresis and Cell Manipulation-Italian Group of Bone Marrow Transplantation indications [19,23]. Pre-ECP hemoglobin levels were maintained between 10 g/dL and 12 g/dL. The cell product was treated with 8-MOP and diluted to a final concentration of 20 μg/100 mL to 34 μg/100 mL, according to specific procedures (in-line technique, 34 μg/100 mL; off-line technique, 20 μg/100 mL).

Table 1
Clinical Characteristics of Patients Treated with ECP

Characteristic	No. Patients	Group by Reason for ECP			
		SR	SD	IC-A	IC-B
	72	21	21	9	21
Sex (M/F)	44/28	12/9	14/7	7/2	11/10
Disease					
ALL	37	8	13	6	10
AML	16	9	3	0	4
MDS/AML secondary	6	1	2	1	2
CML	4	1	2	0	1
NHL	4	2	1	0	1
Others	5	0	0	2	3
Disease status at HSCT					
CR1/CR2/CR3/other	26/30/3/13	12/6/1/2	6/11/0/4	2/2/2/3	6/11/0/4
Source of HSC					
URD	54	17	11	7	19
BM; PBSC; CB	40; 8; 6	13; 1; 3	8; 2; 1	6; 1; 0	13; 4; 2
HLA identical sibling	15	3	9	1	2
BM; CB	14; 1	3; 0	8; 1	1; 0	2; 0
HLA-identical familiar donor	1	1	0	0	0
Haplo, TCRαβCD19 depleted	2	0	1	1	0
Donor					
Age, median (range), yr	28 (1-54)	28 (16-49)	27 (2-54)	27 (1-40)	27 (10-44)
Match/mismatched*	41/31	12/9	13/8	4/5	12/9
Sex mismatched	25/72	6/21	7/21	3/9	9/21
Female donor/male recipient	10/72	2/21	3/21	2/9	3/21
Conditioning regimen					
Myeloablative: yes/no	70/2	21/0	21/0	8/1	20/1
TBI: yes/no	44/28	13/8	14/7	5/4	13/8
aGVHD: overall clinical grade at start of ECP					
Grade I; II; III; IV	8; 29; 17; 18	0; 4; 6; 11	2; 9; 6; 4	3; 5; 1; 0	3; 11; 4; 3
aGVHD: organ involvement and grade at start of ECP					
Skin	64	19	20	6	19
Grade I; II; III; IV	10; 21; 20; 13	2; 3; 8; 6	3; 8; 5; 4	2; 4; 0; 0	3; 6; 7; 3
Gut	55	18	17	6	14
Grade I; II; III; IV	27; 18; 2; 8	4; 5; 1; 8	9; 8; 0; 0	5; 0; 1; 0	9; 5; 0; 0
Liver	12	7	2	1	2
Grade I; II; III; IV	5; 4; 3; 0	1; 3; 3; 0	2; 0; 0; 0	1; 0; 0; 0	1; 1; 0; 0
Therapies before ECP					
CsA (no steroid)	9	0	0	9	0
Steroids (+ others)	63	21	21	0	21
Age at ECP, median (range), yr	8.3 (.9-20.3)	7.9 (1.5-17.9)	8.3 (.9-20.3)	8.3 (1.8-17.1)	7.9 (1.6-18.3)
Body weight at ECP, median (range), kg	25 (7-98)	25 (9.6-85)	25 (7-98)	24 (13-38)	25 (10-52)
Interval HSCT to aGVHD, median (range), d	16 (6-64)	15 (6-32)	16 (8; 41)	17 (14-64)	16 (12-50)
Interval aGVHD to ECP, median (range), d	22 (4-81)	24 (4-63)	22 (14-81)	18 (5-29)	22 (5-56)

M indicates male; F, female; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; NHL, non-Hodgkin lymphoma; HSC, hematopoietic stem cell; URD, unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; Haplo, haploidentical parental donor; TBI, total body irradiation.
IC-A group is those with infectious complications and no steroid before ECP; the IC-B group is those with infectious complications and steroid before ECP.
* HLA match considered 6/6.

Vascular access

In all patients, a 7 to 9 French Hickman double-lumen central catheter was systematically used for the procedure. To provide adequate flow rates, ie, 1 to 2 mL/kg/minute, anticoagulation with urokinase 10,000 U for 2 hours as lock-therapy was performed on the day of the procedure.

Product's characteristics ("off-line" technique)

The leukapheresis product contained a median of WBC of 19.4×10^3/uL (range, 10.7 to 70.1 $\times 10^3$), a median of mononuclear cells (MNC) of 80.5% (range, 50% to 90%). The median number of WBC reinfused to the patients was 2970×10^6 (range, 1150 to $10{,}420 \times 10^6$), whereas the median number of MNC reinfused to the patients was 2794×10^6 (range, 782.3 to 9805.4×10^6).

Treatment protocol

Patients were treated with ECP twice each week for the first month, every 2 weeks during the second and third months, and then monthly for at least 3 more months, for a total of 22 procedures. Progressive tapering and discontinuation of ECP were decided upon evaluation of individual response. Any concomitant IS therapy was initially maintained, then modified or discontinued according to the clinical response.

Response Criteria to ECP

Criteria for defining response to ECP were previously reported [20]. All patients enrolled for ECP before day +100 were included in this group and response to ECP was evaluated at day +28, day +56, and at the end of ECP treatment.

Complete response (CR) was defined as the resolution of all signs of aGVHD and *partial response* (PR) as at least a 50% improvement in the clinical signs. In the latter case, given the complexity of assessing response, we defined PR for each organ as follows: for the skin, at least a 50% reduction in the body surface area affected; for the GI tract and liver a 50% reduction in the volume of diarrhea or value of bilirubin.

Any worsening of organ involvement, as well as the appearance of new signs or symptoms of GVHD, was defined as *progressive disease* (PD). Patients with stable or PD were considered *NR*.

aGVHD

Seventy-two consecutive patients with aGVHD received ECP at a median time of 46 days (range, 13 to 91) after HSCT and 22 days (range, 4 to 81) from the diagnosis of aGVHD. Sixty-four patients had skin involvement (grade IV, n = 13; grade III, n = 20; grade II, n = 21; grade I, n = 10). Fifty-five patients had gastrointestinal (GI) aGVHD (grade IV, n = 8; grade III, n = 2; grade II, n = 18; grade I, n = 27). Twelve patients had liver involvement (grade III, n = 3; grade II, n = 4; grade I, n = 5). Regarding the number of organs involved: in 17 patients skin was affected and 7 presented GI involvement, whereas 36 patients had combined skin and GI aGVHD, 1 patient had combined GI and liver aGVHD, and 11 patients had combined skin, GI, and liver aGVHD.

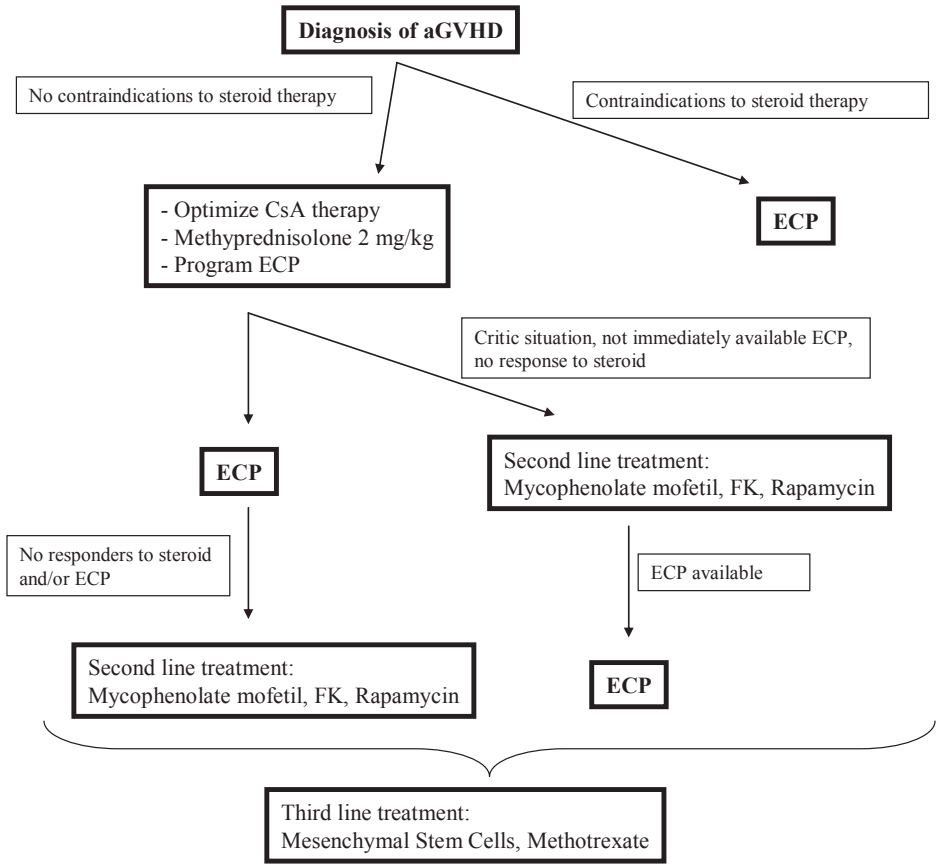

Figure 1. Algorithm for aGVHD treatment used in our center.

The overall clinical grading of aGVHD was as follows: grade I, n = 8; grade II, n = 29; grade III, n = 17; and grade IV, n = 18; details of different grades in the patients' subgroups can be found in Table 1. The median duration of treatment was 4 months (range, 1.1 to 10.2) for a median number of 18 procedures (range, 8 to 90). Forty-one out of 72 patients stopped ECP early because of CR to ECP (20 patients), NR (8 patients), relapse of primary disease (6 patients), clinical contraindications (such as sepsis in 6 patients), and anaphylaxis (1 patient).

Clinical evaluation of the patients was conducted at every ECP procedure. Sixty-three of 72 patients with aGVHD grades I to IV received 2 mg/kg/day of MP as first-line therapy. The median dose of steroid at the beginning of ECP was 2 mg/kg/day. In detail, the IS therapies before ECP were CsA, n = 9; CsA plus steroid (2 mg/kg), n = 42; tacrolimus plus steroid (2 mg/kg), n = 12; and CsA or tacrolimus plus MMF plus steroid, n = 9. ECP was used as first-line therapy in 8 of 72 patients, as second line therapy in 43 of 72 patients (among them, 1 haploidentical HSCT was treated only with CsA), as third-line in 15 of 72 patients, and in 6 of 72 patients as fourth-line therapy.

Statistical Analysis

Patients' characteristics were compared using the chi-squared or Fisher's exact test (as appropriate) in case of discrete variables, or the Mann-Whitney test in case of continuous variables. TRM was calculated from the date of HSCT to day +180 and to the last follow-up, considering as event any nonrelapse cause of death. OS was calculated from the date of HSCT to the date of death from any cause, or to the last follow-up. PFS was calculated from the date of HSCT to the date of relapse of underlying primary disease or death for any cause or to the last follow-up. TTP was calculated from the date of HSCT to the date of relapse of primary disease or to the last follow up. Cumulative incidences (CI) of relapse of underlying disease were estimated in the competing risk model, considering death from any cause or cGVHD as the competing events. Survival analysis was performed using Kaplan-Meier method with 95% confidence interval. Standard error (SE) for each survival and incidence rate is given. Differences between groups were compared using the log-rank test and the Gray's test. All reported *P* values were 2-sided, and statistical significance was set at $\alpha = .05$ (SAS Institute, Cary, NC; release 8.2) [24].

RESULTS

Clinical Response to ECP

Response to ECP treatment, evaluated according to the overall grading of aGVHD and to the organ involvement at day +28, day +56, and at the end of ECP, is summarized in Table 2.

At the end of treatment with ECP, 52 of 72 (72%) patients had a CR, 8 of 72 (11%) had a PR, and 12 of 72 (17%) were NR. Among the 52 patients showing a CR, 7 patients had aGVHD grade I, 22 patients had grade II, 12 had grade III, and 11 had grade IV. In particular, the CR rate for patients with aGVHD grades I and II and grades III and IV were 78% and 66%, respectively ($P = .70$), whereas the PR rate for patients with aGVHD grades I and II and grades II to IV were 5% and 17%, respectively ($P = .80$). No significant statistical difference in CR rate was observed according to the subgroups analyzed (SR, 67%; SD, 81%; IC groups, 70%) ($P = .91$).

At ECP discontinuation, CR of aGVHD manifestations of skin, gut, and liver was observed in 78%, 76%, and 84% of patients, respectively. Maximal response to ECP was observed after 8 weeks of treatment (16 procedures).

As a result of ECP, at the end of treatment, it was possible to discontinue IS therapy in 12 patients (17%) and reduce it in 44 patients (61%), of them 32 who received allo-HSCT from an unrelated donor. Regarding the steroid tapering, in 63 patients treated with 2 mg/kg/day before ECP, the steroid dose was reduced by 80% after 1 month of ECP treatment, 84% after 2 months, and 88% after 3 months of ECP treatment. The median Lansky/Karnofsky performance score improved from 70% before ECP to 100% after completing the treatment.

Table 2
Outcomes of Patients Treated with ECP according to Overall Grading of aGVHD and Organ Involvement

	No. of Patients	At Day +28			At Day +56			Stop ECP		
		CR	PR	NR	CR	PR	NR	CR	PR	NR
Overall grade										
Grade I	8	6	0	2	7	0	1	7	0	1
Grade II	29	6	8	15	19	5	5	22	2	5
Grade III	17	5	8	4	9	4	4	12	2	3
Grade IV	18	4	12	2	11	5	2	11	4	3
Total (%)	72	21 (29%)	28 (39%)	23 (32%)	46 (64%)	14 (19%)	12 (17%)	52 (72%)	8 (11%)	12 (17%)
Organ involvement										
Skin										
Grade I	10	5	0	5	7	0	3	9	0	1
Grade II	21	8	8	5	16	4	1	17	2	2
Grade III	20	10	10	0	14	6	0	16	4	0
Grade IV	13	3	9	1	8	3	2	8	2	3
Total (%)	64 (100%)	26 (41%)	21 (42%)	11 (17%)	45 (70%)	13 (20%)	6 (10%)	50 (78%)	8 (13%)	6 (9%)
Gut										
Grade I	27	15	0	12	22	0	5	23	0	4
Grade II	18	10	4	4	12	2	4	14	1	3
Grade III	2	1	0	1	1	0	1	1	0	1
Grade IV	8	3	4	1	4	3	1	4	4	0
Total (%)	55 (100%)	29 (53%)	8 (14%)	18 (33%)	39 (71%)	5 (9%)	11 (20%)	42 (76%)	5 (9%)	8 (15%)
Liver										
Grade I	5	4	0	1	5	0	0	5	0	0
Grade II	4	2	1	1	3	0	1	3	0	1
Grade III	3	0	3	0	2	1	0	2	1	0
Grade IV	0	0	0	0	0	0	0	0	0	0
Total (%)	12	6 (50%)	4 (33%)	2 (17%)	10 (84%)	1 (8%)	1 (8%)	10 (84%)	1 (8%)	1 (8%)

No association was found between responders and NR to ECP and the major clinical risk factors affecting aGVHD (Table 3).

cGVHD

Twenty-three of 72 patients (32%) presented clinic manifestations of cGVHD (Table 4). In detail, 19 patients (26%) had progressive cGVHD (11 NR and 8 PR to ECP) and 4 patients (5%) had quiescent cGVHD onset after a median of 6 months (range, 5 days to 16 months) from the end of ECP. Overall grading of cGVHD, based on the National Institutes of Health Consensus [25], was mild for 6 patients, moderate for 10 patients, and severe for 7 patients.

Among the patients with progressive cGVHD, ECP was used with other IS therapies in 4 of 19 patients, obtaining CR in only 1 of them. Overall, 10 of 19 patients were alive at the last follow-up: 9 of 10 had no cGVHD and discontinued IS therapy, whereas only 1 patient presenting with cGVHD was still in treatment.

All the patients with quiescent cGVHD were alive at the last follow-up: 2 patients were free from cGVHD and without IS therapy and the other 2 patients had cGVHD and were still on treatment with IS therapy plus ECP.

No association was found between responders and NR to ECP and the onset of quiescent cGVHD.

Table 3
Major Risk Factors of aGVHD and Response to ECP

Risk Factors	*P* Value
Malignant disease (yes/no)	1.00
Median age of patient at HSCT (8.3 years)	1.00
Myeloablative conditioning regimen (yes/no)	.48
TBI (yes/no)	.67
Stem cell source: BM versus PBSC versus CB	.84
Type of HSCT: URD versus HLA-identical Sibling	1.00
Donor gender: sex mismatch versus matched	.56
Donor gender: F donor/M recipient versus others	.45
Median age of donor: 28 yr	.56
HLA: match versus mismatched	.86
Hematopoietic stem cells infused*	1.00
Neutrophils engraftment (d +15)	1.00
CMV reactivation (yes/no)	.95
ECP technique (in-line/off-line)	.73
Median interval of ECP's beginning from onset of aGVHD (day +22)	.50
Median number of WBC infused (2970×10^6)	.72
Median number of MNC infused (2794×10^6)	.55

* BM: $> 3 \times 10^8$ TNC/kg; PBSC: 5 to 10×10^6 CD34+/kg; CB $>3 \times 10^7$ TNC/kg.

Survival and Immunosuppression

At day +180, the overall TRM was 4% (SE, 1%). TRM was 3% (SE, 2%) and 20% (SE, 13%) for responders and NR to ECP, respectively ($P < .0001$). At last follow-up, the overall TRM was 11% (SE, 4%), whereas TRM stratified between responders and NR was 3% (SE, 2%) and 58% (SE, 20%), respectively ($P < .0001$) (Figure 2A,B).

The 5-year OS was 71% (SE, 5%) with a statistically significant difference between responders and NR (78%; SE, 5% versus 30%; SE, 14%, respectively; $P = .0004$) (Figure 3A,B). The 5-year PFS of primary disease for all the group was 72% (SE, 5%), with a significant difference ($P = .0007$) between responders (79%; SE, 5%) and NR (30%; SE, 14%) (Figure 4A,B). Overall, the 5-year TTP of primary disease was 81% (SE, 5%), without any significant difference between the 2 groups (responders: 82%; SE 5% versus NR: 78%; SE, 14%; $P = .65$) (Figure 5A,B).

We compared patients' survival rates on ECP treatment used as first, second, or third/fourth-line therapy. No difference was observed at 5-years between responders and NR in term of OS ($P = .56$), PFS ($P = .55$), and TTP ($P = .62$).

The overall 5-year CI of relapse of the underlying disease was 20% (SE, 5%); in particular, it was 21% (SE, 6%) and 20% (SE, 9%) for responders and NR to ECP, respectively (Figure 6A,B).

Overall, at the last follow-up (median time from HSCT of 5 years; range, .18 to 17.6 years), 51 patients were alive (71%);

Table 4
Follow-Up of Patients with cGVHD

Patient	Group	aGVHD Grade	No. of ECP	ECP Line	Response to ECP	Status at Last FU	Last FU, yr*	Cause of Death	cGVHD Grade	IS for cGVHD	cGVHD at Last FU	IS at Last FU
Progressive cGVHD												
1	SR	4	12	2	PR	Alive	11.2		Moderate	FK + steroid + ECP	No	No
2	SR	4	10	2	PR	Alive	11.2		Moderate	FK	No	No
3	SR	4	26	3	NR	Alive	27.3		Severe	High-dose steroid + MMF + FK steroid + imatinid FK + steroid	Yes	Yes
4	SR	3	18	2	PR	Dead	1.3	Relapse	Moderate	CSA + steroid	Yes	No
5	SR	4	16	2	PR	Alive	14.6		Moderate	CSA + MMF + steroid	No	No
6	SR	4	90	3	NR	Dead	2.6	GVHD (MOF)	Severe	FK + MMF + steroid FK + steroid + ECP FK + MMF + rituximab + ECP FK + steroid + imatinib	Yes	Yes
7	SD	3	20	2	NR	Dead	.03	Infection	Moderate	MMF + steroid	Yes	Yes
8	SD	2	28	2	NR	Alive	7.8		Mild	FK + MMF + steroid	No	No
9	SD	4	12	3	PR	Alive	7.6		Moderate	FK + steroid	No	No
10	SD	2	14	4	NR	Dead	.3	Infection	Severe	FK + MMF + steroid CSA + steroid + ECP	Yes	Yes
11	IC(A)	3	18	1	NR	Alive	3.2		Severe	FK + steroid, Rapamycin + steroid	No	No
12	IC(B)	2	16	3	NR	Dead	.5	Relapse	Mild	FK	Yes	No
13	IC(B)	2	20	3	PR	Dead	.2	Relapse	Mild	FK + steroid	Yes	No
14	IC(B)	2	28	2	PR	Alive	9.1		Moderate	FK + MMF	No	No
15	IC(B)	3	14	2	PR	Alive	8.4		Moderate	FK + steroid	No	No
16	IC(B)	2	6	2	NR	Dead	.3	Encephalopathy	Moderate	MMF	No	No
17	IC(B)	2	8	2	NR	Dead	.5	Relapse	Moderate	FK + steroid	No	No
18	IC(B)	1	15	2	NR	Alive	2.5		Severe	FK + MMF + steroid Mesenchymal stem cells Imatinib + FK + seroid FK + steroid	No	No
19	IC(B)	4	22	2	NR	Dead	.7	GVHD (MOF)	Severe	FK + steroid + imatinib, FK + steroid + Imatinib + PUVA FK + steroid + CPM + MTX + imatinib + ECP	Yes	Yes
Quiescent cGVHD												
20	SR	3	30	4	CR	Alive	3.7		Severe	FK + MMF + steroid + ECP Rapamycin PUVA + Rituximab FK + MMF + steroid + PUVA FK + imatinib + PUVA	Yes	Yes
21	IC(A)	2	22	1	CR	Alive	9.2		Mild	FK + steroid MMF	No	No
22	IC(B)	2	22	2	CR	Alive	7.2		Mild	CSA	No	No
23	SD	4	11	2	CR	Alive	.4		Mild	CSA + steroid + ECP	Yes	Yes

FU indicates follow-up; FK, tacrolimus; MOF, multi-organ failure; PUVA, psoralen combined with ultraviolet A; CPM, cyclophosphamide; MTX, methotrexate. IC(A): infection complications, no steroid before ECP; IC(B): infection complications, steroid-dependent.

* Median time from end of ECP to last follow-up.

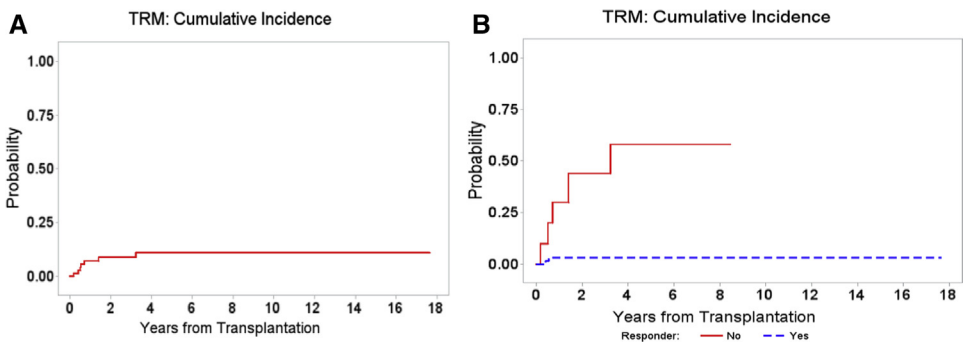

Figure 2. (A) TRM for all patients. (B) TRM for responders and NR to ECP.

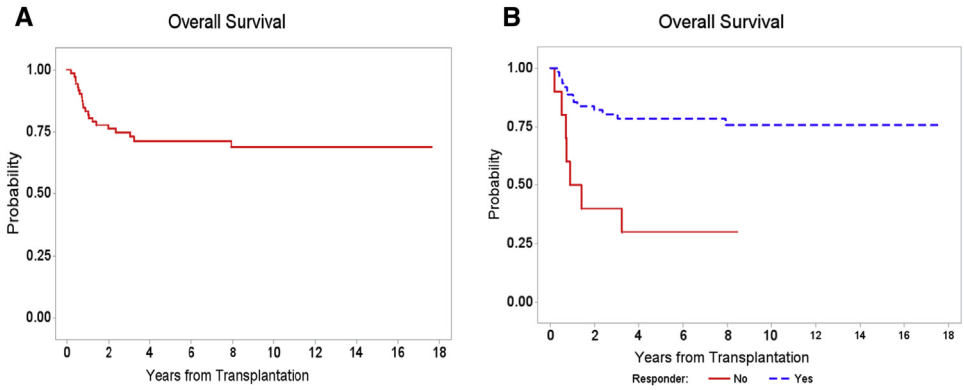

Figure 3. (A) Five-year OS for all patients treated with ECP. (B) Five-year OS for responders and NR to ECP.

48 of them (94%) were without GVHD and without any IS therapy. Twenty-one patients (29%) died: 14 from relapse of primary disease and 7 from NRM. Among this last group, 1 patient with aGVHD died at day +65 from HSCT because of sepsis; 5 patients with cGVHD died from CMV pneumonia (1 case), acute hepatitis from HCV infection (1 case), encephalopathy (1 case), and multiorgan failure (2 cases); and 1 patient died from CMV pneumonia at day +135 from HSCT, without evidence of cGVHD.

Complications

Side effects observed during ECP were generally mild and more frequent in low-weight children. ECP caused mild hypotension in 10 patients associated with abdominal pain in all cases (16 episodes out of 1382 apheresis sessions). These adverse effects did warrant suspending the procedure. A transient reduction in hemoglobin, platelet, and/or WBC count during the ECP treatment, likely independent from the post-transplantation course and putatively ECP-related, was observed in 26, 20, and 25 patients, respectively. One patient with grade IV aGVHD on high-dose steroid therapy (5 mg/kg/day) experienced acute GI bleeding after the second course of ECP: GI endoscopy showed multiple ulcers in the stomach. A girl with pre-existing cardiac impairment showed acute heart failure for fluid overload after the procedure that quickly responded to adequate therapy. One girl, after 10 ECP procedures, had anaphylaxis (cough, vomiting, abdominal pain, hypotension, and palpebral edema) a few minutes after the end of 8-MOP irradiated bag infusion. She responded to antihistamine and steroid therapy, but ECP treatment was then stopped.

DISCUSSION

The aim of this retrospective study was to analyze the role of ECP for the treatment of aGVHD. The efficacy of ECP is well established for treatment of cGVHD [26,27], whereas in aGVHD, no prospective randomized studies have been published. However, the use of ECP is suggested as second-line therapy, together with mammalian target of rapamycin inhibitors, MMF, IL-2 receptor antibodies, and anti-TNF antibodies [3]. The largest phase 2 prospective study exploring feasibility and efficacy of ECP in treatment of aGVHD in adults, involving 59 patients, was performed by Greinix et al. [14] and reported a CR rates of 82% for skin and 61% for liver and GI aGVHD. So far, data on 207 pediatric patients treated with ECP for aGVHD have been reported, showing an overall CR rate ranging from 32% to 73% and a survival rate ranging from 44% to 85% [18,20,28-34].

To date, our is the largest pediatric case series treated in a single center. In our sample size, we found a higher overall response rate to ECP compared with a multicenter retrospective study of the Italian Association for Pediatric Hematology/Oncology (AIEOP) (72% versus 54%) [18]. We attempted to determine the factors that may have influenced our observed higher response. In the last 15 years, many changes have been introduced in HSCT, such as high-resolution HLA typing, new agents in the conditioning regimen, more use of ATG, monoclonal antibodies, and new

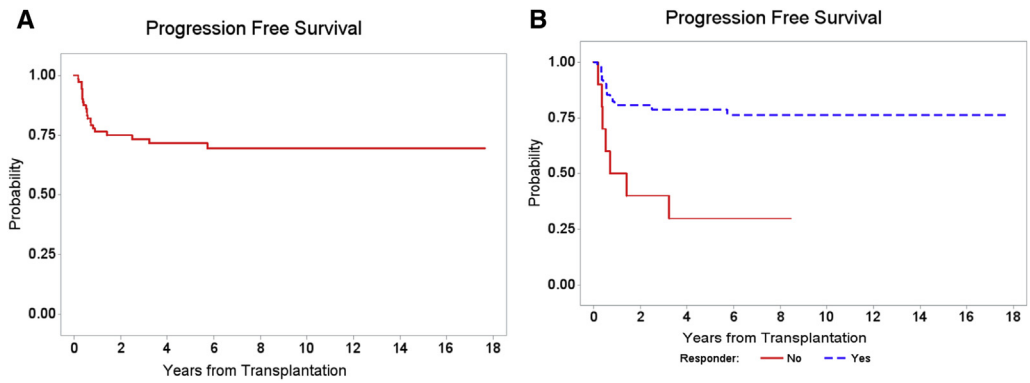

Figure 4. (A) Overall 5-PFS of primary disease. (B) Five-year PFS of primary disease according to response to ECP.

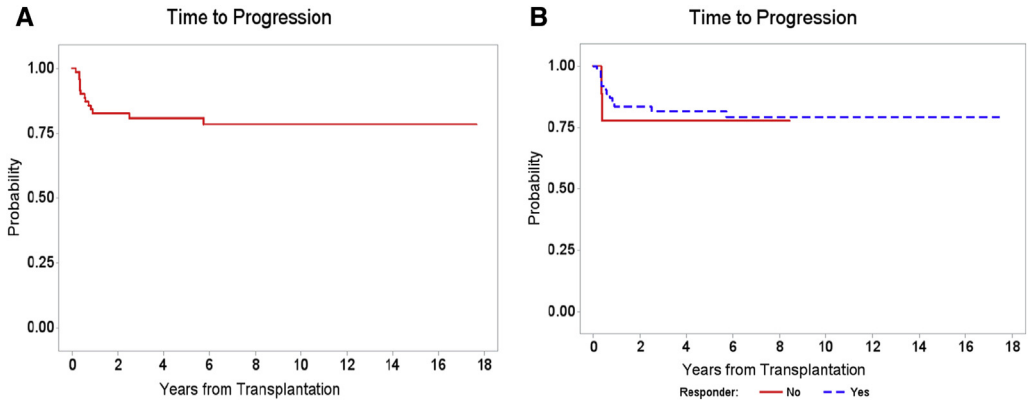

Figure 5. (A) Overall 5-year TTP of primary disease. (B) Five-year TTP of primary disease according to response to ECP.

antifungal drugs. It is difficult to determine which modification may have influenced the outcome. We could also hypothesize that specific expertise in pediatric HSCT and earlier treatment with ECP (22 days in our series versus 30 days in AEIOP study) may have improved the overall outcome. Further studies are needed to address this topic.

No association was found between responders and NR setting to ECP and major risk factors for aGVHD. In addition, in our series, no difference was found according to the grade of GVHD (grade I and II, 78%; versus grade III and IV, 66%; $P = .70$) and to the subgroups of patients analyzed (SR, 67%; SD, 81%; IC, 70%; $P = .91$). Our results showed better response rate than those reported in literature for advanced stages of disease, where higher grades and poorer response to IS therapy correlate with a worst outcome [6]. Nevertheless, higher CR rates were observed in grade II GVHD, suggesting that an early start of ECP sessions may be beneficial, even if in our study the timing to start ECP (<22 versus >22 days) did not influence the response. In our group, ECP seemed to be effective in all the involved organs. As previously reported, our results support that ECP is a steroid-sparing treatment; in fact, the steroid dose was reduced by 80%, 84%, and 88% after 1, 2, and 3 months, respectively from the onset of ECP. We performed ECP as front-line therapy in 8 patients with fungal infection and viral reactivation and aGVHD, with complete response in 7 of them. To our knowledge, this is the first report of ECP as first-line treatment. IS therapy was either discontinued or reduced in 78% of responding patients. It is well known from the literature that IS therapy increases the risk of infectious complications and relapse of underlying disease after allo-HSCT [1,2,18,20,28,35].

In children, who may be particularly vulnerable to the consequence of GVHD itself and prolonged treatment with IS agents, the use of ECP is particularly appealing. The efficacy of ECP in controlling GVHD did not affect the preservation of graft-versus-leukemia effect; in fact, the low incidence of relapse of underlying disease was recorded by us and others [17,18,20,28].

Many concerns has been raised related to the technical aspects of apheresis in the pediatric setting. In children with low body weight, the caregivers should carefully monitor patients for signs and symptoms of hypovolemia. In our experience, ECP was well tolerated, with few and mild adverse effects, the most frequent of which were mild hypotension, abdominal pain, and headache. Curiously, these symptoms were recorded more often during ECP compared with other apheretic procedures [19,34]. The majority of side effects were observed in the earliest period in which ECP was performed in our center. All these observations support the idea that there has been a learning process for the management of technical elements and side effects. In our experience, ECP was feasible even in 15 very young children with low body weight (<15 kg). Technically, we performed priming of the circuit with irradiated and leukodepleted red blood cells (regardless of baseline hemoglobin level). Some authors recently reported that saline infusion or albumin boluses may be an alternative priming approach in patients with body weight ranging from 19 to 39 kg [36]. However, it

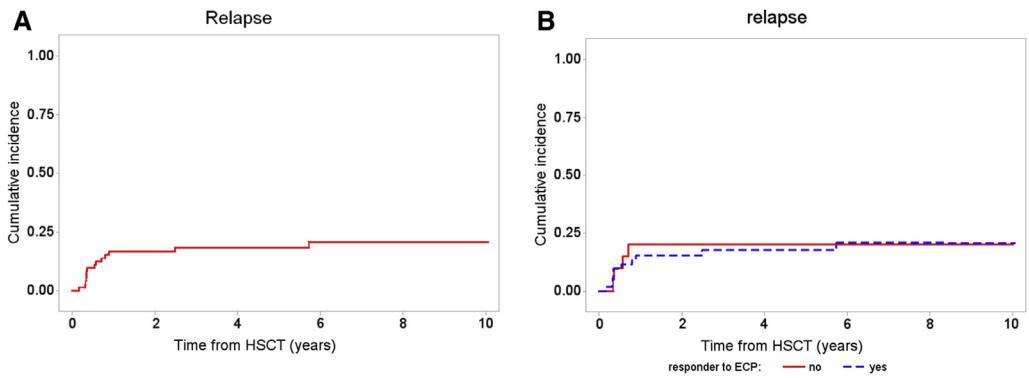

Figure 6. (A) Overall cumulative incidence of relapse of primary disease. (B) Cumulative incidence of relapse of primary disease for responders and NR to ECP.

should be proven that this approach could be safely transferred to population weighting less than 15 kg. Currently used ECP techniques include the "off-line" and the "in-line" devices [19]. In our center, both techniques were used in different time periods with no difference in response rate observed. The number of WBC collected and MNC reinfused did not affect clinical outcome. Notably, all patients underwent the procedure with the bilumen central venous line already in place (Hickman-Broviac Bard Access Systems, Salt Lake City, UT, USA), which is different from the majority of reports, in which a larger central venous line (for instance, quinton) is placed. It would be interesting to extend our experience to determine if urokinase anticoagulation allows the proper flow rate of pre-existing central venous line. Further, because of the experience of the staff in completing the procedure, no patient required sedation.

The CI of cGVHD in pediatric population ranged from 6% to 65% according to the source of stem cells (HLA-identical sibling cord blood versus matched unrelated donor peripheral blood) [37,38], whereas in the AIEOP experience, the CI of cGVHD was reported to be 27% [39]. In our small series, the incidence of cGVHD was 32%. The majority of our children presented progressive cGVHD (26%) and few had quiescent cGVHD (5%). For this reason, it is hard to determine if patients previously treated with ECP for aGVHD could benefit from retreatment.

Our data are consistent with literature and the results encourage us in exploiting this promising approach for aGVHD. In conclusion, a standardized approach to ECP treatment is needed for pediatric patients. From this perspective, sharing single-center experience is of great value in building experience; however, it is time to propose randomized prospective trials.

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