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Source / Izvornik: **Journal of Clinical Apheresis, 2019, 34, 468 - 473**

Journal article, Accepted version

Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

<https://doi.org/10.1002/jca.21700>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:105:587362>

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Download date / Datum preuzimanja: **2024-07-24**



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An Automated Mini Buffy Coat Preparation Method for Use in Mini Extracorporeal Photopheresis Treatment of Graft-versus-Host-Disease in a Low Body Weight Pediatric Patient

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Conflict of interest disclosure: The authors declare no competing financial interests. There is no conflict of interest.

Key words: GVHD, mini-ECP, pediatrics

Running head: Mini-ECP in a low weight child

ABSTRACT

A mini extracorporeal photopheresis (mini-ECP) “off line” technique has been developed for use in the treatment of small children and patients with apheresis contraindications. Until now various methods have been used for buffy coat separation from whole blood. In this report we describe a protocol for mini buffy coat preparation using the automated Sepax laboratory separator for “off line” ECP treatment in a low body weight child with graft-versus-host-disease. According to our results this alternative method has been proven feasible and tolerable.

INTRODUCTION:

Conventional extracorporeal photopheresis (ECP) performed using apheresis devices has proven efficient for the treatment of acute and chronic graft-versus-host-disease (GVHD).^{1,2} The experimental modifications of the conventional ECP procedure have been developed, involving ECP-treated allogeneic cells, ECP treated enriched or depleted cell populations, mini-ECP, and cryopreservation of ECP-treated cells.³ The performance of conventional ECP can be particularly challenging in young children because of factors such as their low body weight with limited extracorporeal volume, adequate vascular access, maintenance of intravascular fluid balance, patient's tolerance of the lengthy procedure, and psychological implications.⁴ Mini-ECP is an "off line" technique that has been developed for use in the treatment of small children and patients with apheresis contraindications. In mini-ECP the whole blood is collected from the patients and various methods have been used for mononuclear cell (MNC) separation and buffy coat preparation.⁵⁻⁷ In this report we describe an alternative method for mini buffy coat preparation using the automated Sepax cell laboratory separator (Biosafe, Switzerland) for the "off line" technique ECP treatment of a low body weight child with graft-versus-host-disease. The "UCB protocol" designed for isolation of the buffy-coat fraction from umbilical cord blood was used. We evaluated the efficiency of the separation procedure, as well as its feasibility and safety.

MATERIAL AND METHODS

A toddler with juvenile myelomonocytic leukemia (JMML) developed acute GVHD, after a matched unrelated peripheral blood stem cell transplantation (SCT) performed at the age of 12 months at the University Hospital Center Zagreb. Acute GVHD of the skin (grade 2, stage I) occurred three weeks after SCT and completely resolved after high dose steroids (4 mg/kg methylprednisolone) with cyclosporine (blood trough concentration 150-200 ng/ml), but recurred 25 weeks after SCT together with suspected GVHD of the liver (acute GVHD of the skin grade 3,

acute GVHD of the liver grade 2, stage II). Biopsy of the skin confirmed acute GVHD, but the biopsy of the liver was not done, because the parents did not consent to this invasive procedure. Because of the resistant disease, after three weeks of therapy with cyclosporine (blood trough concentration 150-200 ng/ml) and steroids (4mg/kg methylprednisolone), ECP treatment had been considered. Since the patient was 18 months old and his weight was only 8.0 kg, we decided to perform the mini-ECP.

Blood was collected from a tunneled central venous catheter (CVC) and the collected volume was simultaneously replaced with saline infusion. In order to prevent hypovolemia, the collected blood volume shouldn't exceed 15% of the patient's total blood volume, therefore 10 mL of blood per kilogram of the patient's body weight was collected. During the whole collection procedure, the patient's vital signs were monitored. The cord blood collection bag (MSC1201DU, Macopharma, Mouvaux, France), containing 21 mL citrate phosphate dextrose (CPD) anticoagulant solution, was used for the whole blood collection, and 8 mL CPD from the attached rinsing pouch was used for rinsing the blood remaining in the collection line into the bag. In order to enable connection of cord blood collection set with CVC, the needle of the collection set was replaced by an infusion system line that ended with luer lock using TSCD II sterile tubing welder (Terumo BCT, Lakewood, CO). Whole blood was processed using the Sepax cell laboratory separator on "UCB protocol" (Figure 1). This protocol allows processing of the initial blood volume from 35 mL to 290 mL, including the anticoagulant, and does not require the addition of a sedimentation agent. The final volume of buffy coat was set at 25 mL. The extracted buffy coat was transferred into the UV-A Illumination Eva Bag (Macopharma, France) and diluted with saline solution up to 200 mL. 8-methoxypsoralen (8-MOP) (Gerot, Austria) was injected directly into the UV-A illumination bag. Since the sterile barrier could be compromised when adding 8-MOP, it must be added in a clean room in a laminar flow cabinet of GMP grade A with background environment at least equivalent to GMP grade D as required by directive 2006/86/EC. The cells were afterwards irradiated by the UV-A illumination

device Macogenic 2 (Macopharma, France) with 2,5 J/m². TSCD II sterile tubing welder was used for connecting the whole blood collection bag with the processing kit, and the buffy coat bag with irradiation bag. UV-A irradiated cells were pooled with the autologous red blood cells remaining after Sepax separation, and were infused back to the patient together. The plasma obtained after the buffy coat separation was not infused back, and was discarded because of its expected high cytokine content, as they are associated with the development of acute GVHD.⁸

The peripheral blood samples before mini-ECP, and the product samples from the whole blood, buffy coat and diluted irradiated product bags were analyzed for complete blood counts using an automated cell counter (ADVIA 120; Bayer, Leverkusen, Germany). The data are presented as arithmetic mean \pm standard deviation (SD). Due to the possible microbial contamination during the off line procedure, microbial testing (aerobic, anaerobic, and fungal cultures) was done on the final product.

The Sepax separator and the Macogenic 2 illumination device were located in our Cell Bank which is 5 minutes walk away from the pediatric ward where the blood was collected, and where the irradiated cells were reinfused. The overall time delay between the whole blood collection and reinfusion of treated mini-buffy coat to the patient was 120 minutes.

RESULTS

ECP procedures were performed 3 times a week for 6 weeks, followed by 2 times a week for the next 5 weeks. In a three-month period 28 mini-ECP procedures were performed.

Peripheral blood white blood cell (WBC) count before mini-ECP was $12,4 \pm 3,2 \times 10^9/L$, hemoglobin $98,2 \pm 11,1$ g/L, hematocrit $30,5 \pm 3,3$, and platelets $72,1 \pm 15,8 \times 10^9/L$. The volume of collected whole blood was 90.3 ± 9.9 mL, and the volume of buffy coat 26.3 ± 9.1 . The recovery of WBC and MNC after separation on the Sepax were $88.3 \pm 9.4\%$, and $90.8 \pm 11.4\%$, respectively.⁵ The hematocrit in the buffy coat product was 45.8 ± 5.7 %, and in the final diluted irradiated product

it was $4\pm 0.7\%$. Regarding the characteristics of the UVA illumination, the UVA dose of 2 J/cm^2 recommended for diluted apheresis product with the low hematocrit ($<2\%$), was increased to $2,5\text{ J/cm}^2$ due to higher hematocrit (4%) in diluted buffy coat separated on the Sepax. The results of microbial testing were sterile for all products. The patient was reinfused with $10.0\pm 3.3 \times 10^7$ WBC /kg body weight, $6.13\pm 2.02 \times 10^7$ MNC /kg body weight, and 5.6 ± 1.9 lymphocyte $\times 10^7$ /kg body weight.

The total volume of pooled irradiated cells and autologous red blood cells was $233,9\pm 12\text{ mL}$, and it was infused slowly over 60 min to prevent fluid overload. The patient tolerated well both the blood collection, as well as infusion of irradiated cells, and no adverse events were observed.

Mini-ECP treatment was continued for three months without technical difficulties. After one month of ECP treatment with the same dosing of steroids and cyclosporine, GVHD of the skin resolved completely. (Figure 2.) The steroids were gradually tapered with no recurrence of the skin GVHD. Suspected GVHD of the liver showed no improvement, and even slowly progressed to grade 3 after four weeks of ECP treatment, so other therapies had to be introduced. Six weeks after the beginning of ECP treatment cyclosporine was shifted to tacrolimus (blood trough concentration 10-15 ng/ml), and mycophenolate mofetil (blood trough concentration 2-3 $\mu\text{g/ml}$) and methotrexate (10 mg/m² once weekly) were introduced, but with tapering of steroids. GVHD of the liver improved to grade 1 after three weeks with this therapy, and resolved after two more months, after which the immunosuppressive therapy was tapered. (Figure 3)

DISCUSSION:

First-line therapy for extensive GVHD, both acute and chronic, is generalized immunosuppression with calcineurin inhibitors and corticosteroids being the most widely used agents. Because of its limited effectiveness (40-50% in children) and toxicity issues, second-line therapy is often necessary.⁹ Numerous treatment modalities for steroid-refractory aGVHD have been investigated, but no clear recommendations for second-line therapy have been established to date.¹⁰ ECP treatment is being widely used, especially for the cutaneous involvement.¹

There are two conventional techniques of ECP, open „off-line“, and closed „in-line“ technique. In „off-line“ technique the target cells are collected by a conventional apheresis device and afterwards irradiated using an external UV-A light irradiation device in the presence of the photosensitizer 8-MOP. The „in-line“ technique uses a single-unit system in which the whole treatment is performed as a closed, one-step procedure. A mini-ECP is an alternative “off line” technique that is used for the treatment of small children and patients with apheresis contraindications. Various methods have been used for buffy coat separation from whole blood (Table 1.).

It is important to emphasize that the dose of the collected target cells is lower in mini-ECP than in conventional apheresis ECP. But there is still no recommendation of the minimum number of cells to be processed per ECP session or amount of blood volume to be processed for cell collection.³ The data from an animal model showed that as few as 0.2% of the body's blood volume irradiated are sufficient to achieve an immune response after photopheresis.¹⁴ Based on these data Schreiner et al. calculated that ECP with mononuclear cells from 50 ml peripheral blood of a patient with cutaneous T-cell lymphoma should yield a clinical effect.⁶ They developed small scale photopheresis procedure, and concentrate mononuclear cells (MNC) by density gradient centrifugation with Lymphoprep (Nycomed, Oslo, Norway).

Hackstein et al. prepared the buffy coat for pediatric patients using fully automated separator device Compomat G4 (Fresenius, Bad Homburg, Germany).^{5,1} Recently, Gramber et al. developed an alternative mini buffy coat preparation method for adult patients using the Spectra Optia Apheresis System (Terumo BCT, Lakewood, CO).⁷ Their technique resulted in similar WBC yields and higher lymphocyte yields than the Compomat device using method, but since it requires minimum processing blood volume of 300 mL its use is limited for the adult patients. The recovery of WBC and MNC after separation on the Sepax were $88.3 \pm 9.4\%$, and $90.8 \pm 11.4\%$, respectively, which were comparable to the Compomat technique.⁷

Various authors have assumed an impact of collected and treated cell numbers on patient's outcomes. Compared with the apheresis ECP methods, the lowest cell numbers are collected and treated in mini-ECP procedures. Hackstein et al. have showed in their follow-up study of 16 patients, including 13 with aGVHD, that mini-ECP treatments were effective in pediatric patients.¹¹ Their patients received a mean dose of 10.4×10^6 WBCs/kg body weight per procedure, which is substantially lower compared with conventional ECP. Patients with aGVHD who responded to mini-ECP received significantly higher doses of UV-A-irradiated WBCs than patients who exhibited no response to therapy (12.4×10^6 vs. 2.9×10^6 WBCs/kg body weight/treatment). Worel et al. in their recent report observed, in multivariate analyses, no significant differences in treated WBCs, MNC and lymphocytes between patients with or without a response to conventional "in line" ECP.¹² In their study, ECP-responding patients received 35.2×10^6 WBCs/kg whereas non responders received 33.8×10^6 WBCs/kg. These doses were considerably higher than those reported by Hackstein and et al.^{5,11} Worel et al. assumed that a minimum number of cells for an effective ECP treatment might be necessary to obtain a response to therapy, and that all of their patients, including the non responders, received cell doses above that threshold cell number. Our patient received 5.6 ± 1.9 lymphocyte $\times 10^7$ /kg, and $6.13 \pm 2.02 \times 10^7$ MNC /kg, that was more than the cutoffs of 8.4×10^6 /kg lymphocytes, and 13.9×10^6 /kg MNC

treated per single procedure which could predict an overall response to ECP at 1 month with 75% sensitivity.¹²

The red blood cell status should be assessed prior to mini-ECP, and in case of anaemia, it should be corrected with a transfusion. The withdrawal of whole blood and its replacement with saline infusion led to normovolemic hemodilution, but since the autologous red blood cells remaining after separation were reinfused back, in our patient there was no need for additional red blood cell transfusion after the mini-ECP procedure.

Whole blood collection is associated with fewer adverse reactions than apheresis procedure, but nevertheless it should be performed under the supervision of a pediatrician. The child must be carefully observed, and vital signs monitored before and throughout blood collection as well as during reinfusion. According to our experience, very important is an adequate surrounding in which a small child as well as parents feel comfortable, and can better tolerate this procedure.

One of the advantages of the mini-ECP is that it reduces overall buffy coat collection time from 240 min. in conventional „off-line“ apheresis ECP, to 30 min. in whole blood collection, and so it interferes less with other therapy that needs to be administered.

An “off line” ECP procedure always carries the risk of the misidentification of cellular products during collection and processing. In order to prevent it, all our products were labelled with an ISBT 128 code consisting of a unique donor identification number, and a product description code. The labels were generated using the Tissue, Cord Blood, Stem Cells (TCS) software system (MAK System, Paris, France) which enables automatic labelling control.

It is well known that the treatment of GVHD complications results in the high expenses, and one of the obstacles to ECP treatment is the substantial cost associated with this long-term therapy.^{15,16}

The cost of blood processing on the Sepax separator is equal to that of a conventional “off line” ECP apheresis procedure.

According to our results, buffy coat separation using automated Sepax system separator has been proven feasible and tolerable. In our presented case, a positive effect of mini ECP was achieved in the skin GVHD, but further clinical studies are required to confirm that this technique is an effective alternative method for buffy coat preparation in ECP treatment in low body weight children or patients with contraindications for apheresis, who are in need of second- or third-line therapy for GVHD.¹

Figure 1. Sepax cell laboratory separator (Biosafe, Switzerland) used for mini buffy coat preparation

Figure 2. Photodocumentation of the clinical response of the skin GVHD: (A) Blood collection from central venous catheter during the first week of mini-ECP treatment. (B) Two months after the start of mini-ECP treatment.

Figure 3. Timeline of the events and treatments of acute GVHD.

References

1. Kanold J, Merlin E, Halle P, Paillard C, Marabelle A, Rapatel C, Evrard B, Berger C, Stephan JL, Galambrun C, Piguet C, D'Incan M, Bordigoni P, Deméocq F. Photopheresis in pediatric graft-versus-host disease after allogeneic marrow transplantation: clinical practice guidelines based on field experience and review of the literature. *Transfusion*. 2007;47:2276-89.
2. Schneiderman J, Jacobsohn DA, Collins J, Thormann K, Kletzel M. The use of fluid boluses to safely perform extracorporeal photopheresis (ECP) in low-weight children: a novel procedure. *J Clin Apher*. 2010;25(2):63-9.
3. Raval JS, Ratcliffe NR. Extracorporeal photopheresis and personalized medicine in the 21st century: The future's so bright! *J Clin Apher*. 2018 May 7. doi: 10.1002/jca.21633. [Epub ahead of print]
4. DeSimone RA, Schwartz J, Schneiderman J. Extracorporeal photopheresis in pediatric patients: Practical and technical considerations. *J Clin Apher*. 2017;32(6):543-552.
5. Hackstein H, Misterek J, Nockher A, Reiter A, Bein G, Woessmann W. Mini buffy coat photopheresis for children and critically ill patients with extracorporeal photopheresis contraindications. *Transfusion*. 2009;49:2366-73.
6. Schreiner T, Gaczkowski A, Scharffetter-Kochanek K, Borberg H. Small-scale extracorporeal photopheresis for the treatment of cutaneous T-cell lymphoma: a report of 3 cases. *Transfus Apher Sci*. 2005;32:197-203.
7. Grabmer C, Schlager S, Mayer G, Streif D, Lener T, Schallmoser K, Rohde E. An alternative mini buffy coat preparation method for adult patients with extracorporeal photopheresis contraindications. *J Clin Apher*. 2017;32:12-15.

8. Toubai T, Tanaka J, Paczesny S, Shono Y, Reddy P, Imamura M. Role of cytokines in the pathophysiology of acute graft-versus-host disease (GVHD): are serum/plasma cytokines potential biomarkers for diagnosis of acute GVHD following allogeneic hematopoietic cell transplantation (Allo-HCT)? *Curr Stem Cell Res Ther.* 2012;7(3):229-39.
9. Messina C, Locatelli F, Lanino E, Uderzo C, Zacchello G, Cesaro S, Pillon M, Perotti C, Del Fante C, Faraci M, Rivabella L, Calore E, De Stefano P, Zecca M, Giorgiani G, Brugiolo A, Balduzzi A, Dini G, Zanesco L, Dall'Amico R. Extracorporeal photochemotherapy for paediatric patients with graft-versus-host disease after haematopoietic stem cell transplantation. *Br J Haematol.* 2003;122:118-27.
10. Martin PJ, Rizzo JD, Wingard JR, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant.* 2012;18:1150-63.
11. Hackstein H, Amoros JJ, Bein G, Woessmann W. Successful use of miniphotopheresis for the treatment of graft-versus-host disease. *Transfusion.* 2014;54:2022-7.
12. Worel N, Lehner E, Führer H, Kalhs P, Rabitsch W, Mitterbauer M, Hopfinger G, Greinix HT. Extracorporeal photopheresis as second-line therapy for patients with acute graft-versus-host disease: does the number of cells treated matter? *Transfusion.* 2018;58(4):1045-1053.
13. Perseghin P, Galimberti S, Balduzzi A, Bonanomi S, Baldini V, Rovelli A, Dassi M, Rambaldi A, Castagna L, Corti P, Pogliani EM, Uderzo C. Extracorporeal photochemotherapy for the treatment of chronic graft-versus-host disease: trend for a possible cell dose-related effect? *Ther Apher Dial.* 2007 Apr;11(2):85-93.
14. van Iperen HP, Beijersbergen van Henegouwen GM. Clinical and mechanistic aspects of photopheresis. *J Photochem Photobiol B* 1997;39:99–109.

15. Im A, Pavletic SZ. Deciphering the Mystery: Extracorporeal Photopheresis in Graft-versus-Host Disease. *Biol Blood Marrow Transplant*. 2015;21(11):1861-2.
16. de Waure C, Capri S, Veneziano MA, Specchia ML, Cadeddu C, Di Nardo F, Ferriero AM, Gennari F, Hamilton C, Mancuso A, Quaranta G, Raponi M, Valerio L, Gensini G, Ricciardi W. Extracorporeal Photopheresis for Second-Line Treatment of Chronic Graft-versus-Host Diseases: Results from a Health Technology Assessment in Italy. *Value Health*. 2015;18(4):457-66.