Impact of extracorporeal photopheresis on blood and coagulation parameters

Thilo Gambichler | Maria Chatzipantazi | Rene Stranzenbach | Alicia Feldkamp | Laura Susok

Department of Dermatology, Ruhr-University Bochum, Bochum, Germany

Correspondence
Prof. Dr. Thilo Gambichler, Department of Dermatology, Ruhr-University Bochum, Gudrunstrasse 56, 44791 Bochum, Germany.
Email: t.gambichler@klinikum-bochum.de

Abstract
Extracorporeal photopheresis (ECP) is considered a safe treatment modality. We aimed to assess blood parameters including coagulation during ECP over time. We performed a long-term retrospective single-center chart review (laboratory parameters) of adult patients \( n = 172 \) who had received ECP for any indication. We observed a significant decrease \( (p < 0.05) \) in erythrocytes, hemoglobin, and leukocytes compared to baseline levels after only one ECP procedure. This decrease persisted after 3-, 6-, 9-, and 12-months of ECP. A significant pathological decline of hemoglobin was observed in a higher proportion (26.4% and 25.2%, respectively) of patients after 6 \( (p = 0.0007) \) and 12 \( (p = 0.012) \) months of ECP. Mean corpuscular volume as well as hematocrit was significantly decreased at 3-, 6-, 9-, and 12-months of evaluation compared to baseline \( (p < 0.05) \). After 9 and 12 months of ECP we observed a further decline in lymphocyte counts \( (p < 0.05) \). Various coagulation parameters did not change significantly during ECP treatment. Even though not all alterations observed in peripheral blood of ECP patients in the present study were of clinical significance, risk for developing persistent anemia must be considered in patients undergoing ECP.

KEYWORDS
blood parameters, coagulation, extracorporeal photopheresis

1 | INTRODUCTION
Extracorporeal photopheresis (ECP) procedure involves three stages: (a) leukapheresis, (b) treatment of the collected buffy coat with the photosensitizer 8-methoxypsoralen followed by UV-A irradiation, (c) retransfusion of the treated buffy coat. In detail, the ECP method includes using small amounts of blood, which are treated with heparin as an anticoagulant, because the leukocytes (should be used consistently in the text...if it’s better in American style with leucocytes pl use this consistently) re subsequently collected in 3–6 cycles. At the end of each leukocyte collection cycle, an accumulation of blood (extracorporeal volume: 120–150 ml), with a reduced number of leukocytes, is reinfused from the ECP device into the peripheral circulation before preparing for the next cycle. When all cycles are completed, leukocytes are pretreated with 8-methoxypsoralen and then exposed to UV-A irradiation (corresponding to about 5% of all circulating cells). Thereafter, leukocytes are reinfused into the patient. Edelson et al. introduced ECP in the early 80s. Although ECP was initially developed for patients with cutaneous T-cell lymphoma (CTCL), it has also shown promising efficacy in a number of other severe and difficult-to-treat diseases, including graft-versus-host disease (GvHD), systemic sclerosis (SSc), and atopic...
dermatitis (AD). Even though ECP is generally well tolerated, there is a lack of studies systematically investigating laboratory-based adverse events (AEs) under ECP. Based on these considerations, we chose a reasonable sample size of ECP patients to evaluate acute and chronic changes in peripheral blood parameters including coagulation.

2 | MATERIAL AND METHODS

2.1 | Patients

For this retrospective analysis, we included patients who were treated with ECP over the past 20 years at the Department of Dermatology, Ruhr-University of Bochum Germany. Only patients who had a history of at least 3 months of ECP treatment were included. Concomitant therapies were also evaluated. The study was conducted according to the declaration of Helsinki and followed a protocol approved by our institutional ethics review board (#4222–12).

2.2 | Extracorporeal photopheresis

ECP treatment was performed using UVAR XTS (Therakos Inc., Raritan, NJ, USA) or CELLEX (Mallinckrodt, Dublin, Ireland) instruments as recommended by updated European dermatology forum guidelines. Most patients started ECP via peripheral venous access. During the course of treatment, a port catheter (TITAN-PORT D, Pakumed GmbH, Germany) was implanted in some patients. In most cases, ECP was performed on two consecutive days every 4 weeks, except for patients with CTCL and AD receiving two consecutive ECP procedures every 2 weeks over a 6–12-week period. Heparin was used as an anticoagulant.

2.3 | Blood parameters

The following laboratory parameters were collected prior to ECP procedures at baseline (ECP naïve), 1 day after the first ECP, after 3-, 6-, 9-, 12-months of ECP; and after 2-, 3-, 4-, 5-years of ECP: blood erythrocytes, hemoglobin, mean corpuscular volume, hematocrit, blood leucocytes, total lymphocyte count, thrombocytes, international normalized ratio (INR), and partial prothrombin time (PTT). All blood collections were performed by venipuncture in the morning.

2.4 | Statistics

Data analysis was performed using the statistical package MedCalc Software version 19.6.1 (MedCalc. Ostend, Belgium). Distribution of data was assessed by the D’Agostino-Pearson test. For non-normally distributed data, the median and range were calculated. Data were analyzed using the Friedman-ANOVA, Spearman correlation procedure, and Chi²-test. p-values less than 0.05 were considered significant.

3 | RESULTS

All in all, 172 patients were included (103 males, 69 females; median [range] age: 53 years [18–84]). Among these were patients with AD (n = 69), CTCL (n = 40), various connective tissue diseases, predominantly SSc (n = 39), GvHD (n = 19), and miscellaneous (n = 5) who were treated with ECP over the last 20 years in our department. The median number of ECP cycles was 19 (3–158) with a median duration of 18.5 (3–188) months (Table 1). With respect to the acute impact of

| TABLE 1 | Patients (n = 172) with different indications who were treated with extracorporeal photopheresis (ECP) |
|---------------------------------|-----------------|-----------------|
| Gender | Male/female | 103/69 | (59.9%/40.1%) |
| Indications | Cutaneous T cell lymphoma (CTCL) | n = 40 (23.3%) |
| | Connective tissue diseases (CTD) | n = 39 (22.7%) |
| | Atopic dermatitis (AD) | n = 69 (40.1%) |
| | Graft-versus-host disease (GvHD) | n = 19 (11%) |
| | Miscellaneous | n = 5 (2.9%) |
| | Nephrogenic fibrosing dermopathy, linear IgA dermatosis, pemphigus foliaceus | |
| Median (range) disease duration before ECP | 57 months (1–720) |
| Median (range) ECP cycles in total | 19 (3–158) |
| Median (range) on ECP | 18.5 months (3–188) |
| Concomitant systemic therapy | None | n = 62 (36%) |
| | Immunosuppressants including corticosteroids | n = 67 (39%) |
| | Combined treatments and miscellaneous | n = 43 (25%) |
| | Cutaneous T cell lymphoma (systemic corticosteroids, methotrexate) | |
| | Connective tissue diseases (systemic corticosteroids, methotrexate, azathioprine) | |
| | Atopic dermatitis (systemic corticosteroids, cyclosporine, azathioprine, methotrexate) | |
| | Graft-versus-host disease (cyclosporine, tacrolimus, mofetil mycophenolate) | |
| | Miscellaneous (systemic corticosteroids, azathioprine, methotrexate) | |
| Anticoagulation | No/yes | 11 /21 | (87.7%/12.3%) |
| Time Medians (range) | Baseline before first ECP (A) | Day after first ECP (B) | 3 months (C) | 6 months (D) | 9 months (E) | 12 months (F) | Friedman-ANOVA/Chi²-test p-values (multiple comparisons) |
|----------------------|-------------------------------|-------------------------|--------------|--------------|--------------|--------------|----------------------------------------------------------|
| Erythrocytes (4.2–5.4 Mio/μl) | 4.6 (3.49–5.56) | 4.46 (3.50–5.7) | 4.44 (1.41–5.45) | 4.49 (3.46–5.57) | 4.55 (2.88–13.7) | 4.54 (3.44–13.6) | = 0.00013 (A vs. B–F) |
| Hemoglobin (Hb) (12–16 g/dl) | 13.85 (9.9–17.2) | 13.70 (9.90–16.9) | 13.20 (8.2–16.5) | 12.85 (8.5–16.9) | 13.05 (7.9–16.9) | 12.70 (9.10–17.4) | <0.00001 (A vs. B–F) |
| Decreased Hb No/yes | 149/23 (86.6%/13.4%) | - | - | 106/38 (73.6%/26.4%) | - | 83/28 (74.8%/25.2%) | = 0.0007 (A vs. D) = 0.012 (A vs. F) |
| Mean corpuscular volume (85–95 fl) | 90.2 (75.9–101) | 90.80 (76.9–100.3) | 89.1 (75.2–107.4) | 88.5 (65–105.1) | 87.4 (65.1–107.3) | 86.1 (63.8–106.4) | <0.00001 (A vs. C–F) |
| Hematocrit (36%–46%) | 41.65 (30.6–51.3) | 40.85 (30–49.6) | 39.65 (25.5–48.3) | 39.15 (27.9–48.8) | 39 (25.5–48) | 38.45 (31.4–48) | <0.00001 (A vs. C–F) |
| Leukocytes (4600–9500/μl) | 7810 (3810–111,120) | 7020 (3690–89,990) | 7670 (3030–41,990) | 7580 (2460–37,720) | 7640 (1300–27,210) | 6560 (1070–35,400) | <0.00001 (A vs. C–F) |
| Lymphocytes (1000–4050/μl) | 1530 (350–50,940) | 1530 (740–50,000) | 1470 (510–37,080) | 1460 (390–29,660) | 1330 (330–22,760) | 1240 (290–1750) | = 0.00086 (A vs. B–F) |
| Thrombocytes (150,000–400,000/μl) | 261,000 (92000–572,000) | 230,000 (15,000–479,000) | 270,000 (122,000–2,120,000) | 254,000 (22,600–750,000) | 261,000 (101,000–818,000) | 264,000 (134,000–619,000) | <0.00001 (B vs. A, C–F) |
| International normalized ratio (0.8–1.1) | 0.99 (0.8–96) | 1 (0.8–2.2) | 1 (0.9–3.4) | 1 (0.8–2.27) | 1 (0.89–2.7) | 1 (0.89–2.53) | = 0.56 |
| Partial prothrombin time (26–40 s) | 31.4 (22–66.5) | 30.6 (22–56.4) | 30.2 (21.5–58.5) | 30.9 (23.6–59.1) | 30.6 (20–61.3) | 31 (21.5–54.6) | = 0.82 |
ECP on blood parameters we observed a significant ($p < 0.05$) decrease in erythrocyte counts, hemoglobin, and leukocyte counts on the day after the first ECP cycle. However, all other laboratory parameters analyzed did not show significant changes after the first ECP cycle ($p > 0.05$). Compared to baseline levels, erythrocyte counts, hemoglobin, and leukocyte counts remained significantly decreased after 3-, 6-, 9-, and 12-month ECP. As shown in Table 2, a significant pathological decrease in hemoglobin was observed in a higher proportion (26.4% and 25.2%, respectively) of patients after 6 ($p = 0.0007$) and 12 ($p = 0.012$) months of ECP treatment compared to other time points. Mean corpuscular volume as well as hematocrit were significantly decreased at 3-, 6-, 9-, and 12-month evaluation compared to baseline ($p < 0.05$). Correlation studies at 6 and 12 months showed a significant positive relationship between hemoglobin levels, MCV, and hematocrit with $r$-values ranging from 0.35 to 0.96 and $p$-values from 0.0002 to <0.0001. After 9 and 12 months of ECP treatment, we observed a significant ($p < 0.05$) decrease in absolute lymphocyte counts when compared to baseline. On day 2 after the first ECP cycle, thrombocyte counts showed a significant decrease ($p < 0.00001$), but did not significantly change over a longer period of time. Other coagulation parameters such as INR and PTT did not significantly change during ECP treatment. Moreover, long-term evaluation (1–5 years) of available blood and coagulation parameters listed in Table 2 did not reveal significant changes ($p > 0.05$).

4 | DISCUSSION

In general, ECP is considered a safe photochemotherapy regimen with minimal to no AEs. Acute AEs observed in ECP-treated patients are generally transient and mild, and may include hypotension, headache, chills, and hematoma/localized infection at the site of venipuncture. A decrease in platelet count may occur as a consequence of heparin-induced thrombocytopenia. Anemia has been observed in long-term ECP patients, after incomplete reinfusion, or, rarely, due to hemolysis. Kuhn et al. studied 123 ECP patients with GvHD, CTCL, solid organ transplant rejection, or other indications. Of note, about 50% of the patients in this study were already anemic prior to the first ECP cycle, without evidence of iron deficiency. Notably, at 5 years of follow-up, a cumulative incidence of iron deficiency anemia (IDA) of 68.3% was described in this study. Such a relatively high incidence of IDA may be explained by the large number of patients with GvHD in this study, which are at high risk for anemia for multiple reasons. Sanford et al. studied 36 patients with CTCL receiving 4–327 cycles of ECP treatment. Here, the incidence of IDA was 49%. By contrast, Quaglino et al. observed an incidence of only 6% ECP-treated CTLC patients who developed anemia ($n = 51$). Recently, Moosavi et al. studied 34 patients of which most patients had GvHD ($n = 19$). Interestingly, they determined that the mean drop of hemoglobin under ECP treatment significantly differed between the CELLEX and the UVAR XTS techniques (3.3 vs. 2 g/dl, respectively). In 56% of patients studied, IDA could be detected over time. In the present study, the number of patients with a pathological decrease in hemoglobin levels roughly doubled compared to baseline levels with a total incidence of 25% over time. This relatively low incidence compared to the studies discussed above may be explained by differences in the patient population. Specifically, our patient collective included a high percentage of patients with AD who are generally not prone to develop anemia. As expected, a decrease in hemoglobin levels, MCV, and hematocrit was also accompanied by a decrease of erythrocyte count. Erythrocyte loss may be explained by blood clotting that may occur during the leukapheresis procedure and technical issues during reinfusion. A transient decrease in hemoglobin after 24 h after the ECP procedure may be explained by mechanical cell destruction. By contrast, the decrease in leukocyte count detected after the first cycle of ECP persisted over time. Franklin et al. showed that neutrophils, the main proportion of leukocytes, account for the majority of cells treated during ECP. In line with our observation, they also demonstrated that patients with GvHD treated by ECP showed enhanced apoptosis and diminished half-life of neutrophils.

Furthermore, we observed a decrease in lymphocyte count after 9 and 12 months of ECP treatment. Indeed, a decrease in lymphocytes under ECP treatment has been frequently reported in the literature. Importantly, ECP represents a potent immunomodulatory treatment option for several immune-mediated conditions and hematological malignancies and has been shown to induce apoptosis in various cell types, including normal and malignant lymphocytes. However, concomitant immunosuppressive therapies administered to patients in this study are confounding factors in assessing the effects of ECP on lymphocyte and leukocyte counts. Consequently, we cannot exclude that observed decreases in lymphocyte and leukocyte counts were due to concomitant immunosuppressive therapies rather than caused by ECP.

Anticoagulation through heparins or citrate is essential for maintaining the extracorporeal blood flow on the apheresis circuit. Heparin, which was used for ECP method in the present study, can cause prolongation of PT and the PTT by acting on the common pathway factors. By contrast, the INR as another frequently used coagulation parameter is not affected by heparin directly, but can be falsely elevated by heparin due to thromboplastin test reagents. While effective, heparin anticoagulation may be associated with an increased frequency of bleeding complications. The amount of heparin returned at the end of the first cycle of ECP can vary between patients, but is probably in the range of 1000–2000 USP units in a 2-min period. To our best knowledge, evaluation of systemic coagulation parameters in ECP patients have not been reported in the literature. Based on these considerations, we assessed various coagulation parameters in patients who underwent ECP, but did not observe any significant changes over time.

The main scope of this study was to evaluate ECP effects with regard to robust laboratory-based changes or in general. Taken together, we observed significant changes in some blood parameters under ECP. Even though statistically significant, however, the magnitude of changes was pretty small and may not be of significant biological and clinical relevance. Clinical data, such as ECP outcome (clinical scores, etc.), were incomplete and therefore not included into this
Nevertheless, the correlation of decreases in hemoglobin, MCV, and hematocrit indicate that iron deficiency was likely the cause of anemia in most cases.\textsuperscript{8–10} In addition, particularly in lymphocyte and leucocyte counts could also be explained, at least in part, by concomitant immunosuppressive therapies (e.g., immunosuppressants) and/or exacerbated by the underlying disease (e.g., GvHD) without any influence by ECP.\textsuperscript{8–10} Nevertheless, the acute changes observed 24 h after the first cycle of ECP indicate that the procedure may have a direct impact on blood parameters. Further, dilution effects can be excluded, since blood collections were always performed by additional venipuncture and not performed on venous catheters or ports used for ECP.

In conclusion, ECP is an established treatment regimen with few side effects.\textsuperscript{20,21} We observed, however, in a reasonable number of patients, that ECP has acute and/or chronic impact on several blood parameters (erythrocytes, hemoglobin, MCV, hematocrit, leukocytes, lymphocytes, thrombocytes). Even though not all of these blood alterations observed in the present study are of clinical significance and/or definitively attributable to ECP treatment, in particular, a risk of anemia over time must be considered in ECP-treated patients. Hence, monitoring of blood parameters (e.g., hemoglobin, MCV, hematocrit, serum iron) is strongly recommended in patients undergoing ECP.

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CONFLICT OF INTEREST

Thilo Gambichler has received speakers and/or advisory board honoraria from BMS, Sanofi-Genzyme, MSD, Novartis Pharma, Roche, Abbvie, Almirall, Janssen, Lilly, Pfizer, Pierre Fabre, Merck-Serono, outside the submitted work. Laura Susok has received speakers and/or advisory board honoraria from BMS, Sun-Pharma, MSD, and Novartis. All other authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Thilo Gambichler and Laura Susok contributed to the study conception and design. Material preparation, data collection, analysis, and interpretation were predominantly performed by Thilo Gambichler, Maria Chatzipantazi, Rene Stranzenbach, Alicia Feldkamp, and Laura Susok. The first draft of the manuscript was written by Thilo Gambichler. All authors read the manuscript, revised it critically, and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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