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First Human Application of a Novel Adsorptive-Type Cytapheresis Module in Patients With Active Ulcerative Colitis: A Pilot Study

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Abstract: The aim of this study was to evaluate the safety, tolerability, technical performance and clinical efficacy of a novel adsorptive-type cytapheresis module in patients with active ulcerative colitis. Ten patients with ulcerative colitis (clinical activity index 6–10) were recruited. The new adsorber (Nikkiso, Tokyo, Japan) was specifically designed to remove platelets, granulocytes and monocytes from peripheral blood using an extracorporeal circulation. Cytapheresis treatments were performed weekly for five consecutive weeks (each with a 60-min duration). Safety and tolerability were evaluated by investigating vital parameters, routine laboratory tests, adverse event reporting and a questionnaire. Disease activity was evaluated by assessing the clinical activity index as well as the endoscopic index, according to Rachmilewitz. Technical performance and biocompatibility were investigated by repeated measurements of cellular blood count, complement factor C3a and cell surface markers before, during and after the apheresis treatments. The cytapheresis treatments were "well" to "very well" tolerated by the patients. All measured safety parameters remained essentially unchanged. Performance data showed that platelets, monocytes and neutrophil granulocytes were effectively reduced during the cytapheresis treatments. Apheresis treatment was associated with high remission rates (80% at week 10). Clinical remission was accompanied by the reduction of the endoscopic index in four out of the nine eligible patients. Levels of C3a did not significantly increase during cytapheresis treatments. The novel device has been shown to be safe, well tolerated and clinically efficient. It offered a very good biocompatibility and platelet elimination capacity. **Key Words:** Granulocyte and monocyte adsorptive apheresis, Inflammatory bowel disease, Ulcerative colitis.

The etiology of inflammatory bowel diseases (IBD) is still not completely understood. The main treatment goal for the active disease is to achieve and maintain clinical remission by using treatment options with less potential for inducing side-effects.

To date, 5-aminosalicylic acid (5-ASA) and corticosteroids are the first-line treatment options for inducing remission in patients with active ulcerative colitis (UC) (1). Cumulative high doses or long-term steroid administration cause severe side-effects, and steroid-refractory cases have been reported (2). In a substantial number of patients, colectomy as a last-resort treatment alternative cannot be avoided (3). Adsorptive cytapheresis has been proposed as a steroid-sparing, safe treatment strategy to achieve remission not only for IBDs but also for other immunological diseases, such as rheumatoid arthritis and systemic lupus erythematosus (4–6). When comparing the efficacy and safety of intensive granulocyte/monocyte adsorption apheresis (GMCAP) with intensive intravenous prednisolone in patients with severe UC, GMCAP was comparable to or better than prednisolone. Moreover, the response to GMCAP was slower but more sustainable (7).

Several randomized controlled trials from Japan have demonstrated the clinical benefit of apheresis treatment in addition to a standard treatment over standard treatment alone (7–9). In contrast to Japan, adsorptive cytapheresis systems are not generally

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covered by healthcare insurance in Europe, North America and most parts of the world. This is mainly because standard treatment and the selection of outcome parameters used in earlier Japanese trials (8,10) were different from European and North American standards. Moreover, two recent randomized, double-blind, sham-controlled clinical trials conducted in the US and European countries to prove clinical efficacy of GMCAP in UC (11) and Crohn's disease (12) demonstrated that the efficacy of GMCAP was not superior to sham apheresis. Thus, despite Japanese practice, current guidelines of US as well as European gastroenterological societies do not recommend GMCAP or leukocytapheresis (LCAP) for clinical practice. But, subgroup analyses within the study of Sands et al. (11) showed a clearly better outcome for the GMCAP group in patients with histologically more active UC proving the importance of a more critical patient selection in apheresis trials.

The commercially available cytapheresis systems preferentially adsorb granulocytes (46%), monocytes/macrophages (54%) and only a small number of lymphocytes (18.5%) and platelets (19%) (GMCAP) (13) or lymphocytes (30–60%), platelets (35%) in addition to granulocytes and monocytes (almost 100% of cells in the blood line of the device) (leukocytapheresis, LCAP) (14). Both methods have been reported to be effective in the induction and maintenance of clinical remission in steroid-refractory or steroid-naive UC patients and only rarely cause adverse events (9,15). Moreover, there is no significant difference in clinical response and clinical course between LCAP and GMCAP (16). Several potential mechanisms investigating the mode of action of GMCAP and LCAP have been analyzed. Both systems have similar effects, for example, a reduction of activated leukocytes, downregulation of proinflammatory cytokines and modification of adhesion molecule expression (10,17,18), which induce an anti-inflammatory status. However, the precise mode of action of these techniques, which mediate the therapeutic effects, might be more complex. There are several pieces of evidence to indicate that additional effects on the innate immune system and other regulatory processes are caused by cytapheresis (18–20).

Predictive factors for the efficacy of adsorptive cytapheresis have rarely been reported. However, several reports have indicated that a decrease of platelets may be an early marker for a beneficial response in patients with severe UC (21,22). Moreover, platelets have been recognized to play an important role in the pathophysiology of IBD. Platelet number increases during flare-ups of IBD and correlates with disease severity (23). Platelets are able to activate various cells, for example, through contact with CD40L, secretion of soluble CD40L and other chemokines mediating leukocyte adhesion and transmigration (24). Therefore, the development of new adsorber materials preferentially adsorbing platelets in addition to granulocytes and monocytes is an interesting approach for the treatment of patients with IBD.

In this study, a novel adsorptive-type cytapheresis module filled with amorphous polyarylate resin beads, which particularly removes platelets, granulocytes and monocytes from the peripheral blood, has been tested. A prospective open-label pilot study has been conducted to evaluate the safety, tolerability, technical performance and clinical efficacy of this device in patients with active UC.

PATIENTS AND METHODS

Patients

This prospective study was conducted in accordance with the principle of ISO 14155 good clinical practice and the Declaration of Helsinki. Informed consent was received by all patients after the ethics committee of Rostock University had approved the study protocol. A total of 10 patients (six males, four females, mean age: 47.1 years, minimum age: 25 years, maximum age: 73 years) with moderately to severely active UC defined by the Clinical Activity Index (CAI 6–10 according to Rachmilewitz [25]), who had failed to achieve long-term remission with steroids and/or immunosuppessive agents or who had contraindications or were intolerant to high doses of steroids and/or immunosuppressive agents, were recruited. Patients on prednisolone more than 30 mg/day were excluded from this study.

The mean disease duration was 14 years. Sixty percent of the patients suffering from active UC exhibited a left-sided colitis (distal colitis), and 40% a pancolitis. In general, the patients' medications comprised 5-aminosalicylate (5-ASA), azathioprine (Aza), tacrolimus and steroids (Table 1). Accordingly, 80% of the patients received steroids at a mean concentration of 8 mg/day. Mucosal damage was investigated by colonoscopy immediately prior to the start of the apheresis therapy. Endoscopic evaluations were performed by an endoscopist who did not have any relation to this study using the endoscopic index (EI), according to Rachmilewitz (25). The mean EI prior to initiation of the apheresis therapy was 7.4 ± 5.2 (range 6–10).

Apheresis protocol

Patients received five treatment sessions with the novel apheresis device at one-week intervals.

TABLE 1. *Patient data*

Patient no.	Sex	Age	Diagnosis[†]	Treatment	CAI before	CAI W10	EI before	EI W10
1	M	54	E2	Steroids	10	2	6	2
2	M	32	E2	Steroids 5-ASA	10	1	6	2
3[‡]	M	44	E2	Steroids Tacrolimus 5-ASA	10	8	7	7
4[‡]	M	55	E2	Steroids 5-ASA	8	(5)[§]	8	10
5	F	55	E3	Steroids 5-ASA	10	0	7	6
6	M	57	E3	5-ASA Aza	7	0	–[¶]	–[¶]
7	M	72	E2	Steroids	7	4	10	8
8	F	42	E2	5-ASA Aza	6	1	7	2
9	F	24	E3	Steroids 5-ASA Tacrolimus	9	4	10	8
10	F	28	E3	Steroids 5-ASA Aza	8	0	6	2

[†]Montreal classification (26): E2: left sided UC (distal UC), E3: extensive UC (pancolitis). [‡]Non-responder. [§]Patient received 50 mg/day steroids between W6 and W10 after treatment with the novel device. [¶]Patient did not consent to endoscopy.

Each session lasted 60 min and used a blood flow of 30 mL/min.

Immunopure device and blood sample collection

The Immunopure (Nikkiso, Tokyo, Japan) device has been designed to be used in a simple hemoperfusion setting by selectively adsorbing granulocytes, monocytes/macrophages and platelets. The device is a gamma ray-sterilized, single-use (disposable) module filled with amorphous polyarylate resin beads of 1.0 mm diameter. This material has a long history of clinical application as a part of hollow fiber dialyzers. The total adsorber volume was 350 mL. The void volume of the device was 139 mL. The apheresis system comprises the device, circuit lines (ABT-002PX, Nikkiso) and the blood monitor (LPM-01, Nikkiso Europe GmbH, Hanover, Germany).

Cytapheresis was conducted at a flow rate of 30 mL/min for 60 min. Unfractionated heparin (Ratiopharm, Ulm, Germany) was administered as a bolus (75–150 IU/kg body weight) at the beginning of the treatment, followed by the continuous infusion of 50–100 IU/kg per treatment. Blood was withdrawn from the cubital or forearm veins, circulated through the column and returned to the cubital or forearm veins of the contralateral arm. Blood samples were taken at screening (week 0, W0), intermediate evaluation (week 6, W6), final evaluation (week 10, W10) as well as before, during and at the end of each apheresis treatment (week 1–5, W1–5).

Safety, tolerability and biocompatibility

Vital signs were assessed by registering blood pressure, pulse rate and body temperature during all the visits. Blood pressure and pulse rate were measured before, at 30 min, at the end (after retransfusion) and 15 min after the apheresis session. Body temperature was measured before and 15 min after the apheresis session. Any adverse event was registered. Safety evaluations also included laboratory analyses of peripheral blood cell counts, electrolytes (Na^+, K^+, Ca^{2+}), serum glucose, fibrinogen, partial thromboplastin time (PTT), Quick value (prothrombin time ratio), international normalized ratio (INR), total protein, creatine kinase (CK), albumin, alpha 1-globulin, alpha 2-globulin, beta-globulin and gamma-globulin, IgG, M, A, E, complement factor C3a (parameter comparison between the beginning and end of one treatment session, intra-treatment analyses). Additionally, creatine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (γGT), bilirubin, C-reactive protein (CRP), and lactate dehydrogenase (LDH) were analyzed only at the beginning of all treatment sessions (parameter comparison between the treatment sessions, inter-treatment analyses). Analyses were performed by a certified external laboratory.

Tolerability was assessed for each single apheresis session (before and after) and at W6 and W10 using a questionnaire that allowed patients and physicians

to judge the treatment according to a pre-defined scheme (6 levels that ranged from "very well" to "very bad").

Clinical efficacy

Disease activity was evaluated by assessing the CAI (before the start of the therapy and at W6 and W10) as well as the endoscopic index (before the start of the therapy and W10) both according to Rachmilewitz (25). Clinical remission in UC is defined as a CAI score of 4 or less. Clinical response is defined as a CAI drop ≥ 3 or CAI ≤ 4. Endoscopic remission is defined as an EI < 4 (25).

Flow cytometry

Antibodies against the following human surface antigens were acquired from BD Biosciences (San Jose, CA, USA): CD3, CD4, CD8, γ/δ T cell receptor, HLADR, CD10, CD14, CD62L, CD11b, CD42b, CD63. Isotype-matched irrelevant antibodies against mouse IgG1 and IgG2a (BD Bioscience) were used as negative controls. In brief, the appropriate amount of an antibody was added to 50 µL of total blood. The samples were incubated for 20 min at 4°C. Then, 1 mL of 1 × BD FACS Lysing Solution (BD Bioscience) was added followed by a 10-min incubation period at room temperature in the dark. The cells were washed once with BD CellWash solution (BD Bioscience) and analyzed immediately on a FACSCalibur (BD Bioscience) flow cytometer. For the estimation of peripheral blood counts, absolute numbers of the different cell populations were calculated as follows: percentage × leukocyte number/100. The counts were hematocrit-corrected. For platelet staining, the peripheral blood was immediately fixed in 1% formaldehyde/PBS.

T_{regs} were identified using an intracellular staining kit (eBioscience, San Diego, CA, USA). Prior to staining, peripheral blood lymphocytes were isolated by Percoll gradient centrifugation. Prepared cells (1×10^6) were stained with a CD4 FITC/CD25 APC cocktail (10 µL each, eBioscience) or mouse IgG1FITC/APC isotype controls for 30 min. After washing (CellWash), the cells were incubated with 1 mL of freshly prepared Fixation/Permeabilization Buffer (kit component) for 30 min at 4°C in the dark. The samples were washed twice with Permeabilization Buffer (kit component) and incubated for 30 min with the anti-human FoxP3 (PCH101) PE antibody or rat IgG2a isotype control, respectively. The cells were washed once with Permeabilization Buffer, resuspended in 200 µL of CellWash and analyzed immediately on the cytometer. A total of 200 000 events were acquired. Data were analyzed using the CellQuest (BD Bioscience) software. The cells were differentiated into $CD4^+CD25^{high+}FoxP3^+$ and $CD4^+CD25^{intermediate+}FoxP3^+$ T_{regs}.

Statistical analysis

The data are expressed as the mean ± SD. Statistical analysis was performed by the parameter-free Wilcoxon test. *P*-values of <0.05 were considered statistically significant. Calculations were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Tolerability

The questioning, according to the ascertained steps of well-being, resulted in the overview displayed by Table 2, which led to the conclusion that the apheresis treatments displayed "well" to "very well" tolerability. With the exception of occasional light headaches and puncture problems, no further side-effects or adverse events were observed.

Safety laboratory parameters and vital signs

All 10 patients finished the study according to the suggested protocol. Safety laboratory parameters between the beginning and end of each apheresis

TABLE 2. *Results of the questioning for treatment tolerability*

Patient no.	1st therapy	2nd therapy	3rd therapy	4th therapy	5th therapy	Visit 7	Visit 8
1	Well	Well	Well	Well	Well	Well	Well
2	Well	Well	Well	Very well	Well	Well	Very well
3†	Well	Well	Well	Well	Very well	Fair	Well
4†	Well	Well	Well	Well	Well	Well	Well
5	Well	Well	Well	Well	Well	Very well	Very well
6	Well	Well	Very well	Very well	Very well	Very well	Very well
7	Well	Very well	Very well	Well	Very well	Very well	Very well
8	Well	Very well	Very well	Very well	Very well	Very well	Very well
9	Well	Well	Well	Well	Well	Well	Well
10	Well	Well	Very well	Very well	Well	Very well	Very well

†Non-responder.

TABLE 3. *Safety laboratory parameters. Intra-treatment impacts*

Parameter	Treatment start values	Treatment end values	Normal range
Fibrinogen (g/L)	4.2 ± 0.6	4.0 ± 0.7*	1.4–4.8
aPTT (sec)	31.5 ± 6.6	212.1 ± 30.2*	25–36
Albumin (g/L)	40.6 ± 3.0	37.1 ± 2.4*	35–50
Total protein (g/L)	72.3 ± 3.5	66.8 ± 5.1*	64–83
Albumin (%)	61.7 ± 3.7	60.8 ± 4.2	59.8–72.4
Alpha 1-globulin (%)	3.3 ± 0.6	4.4 ± 3.2	1.0–3.2
Alpha 2-globulin (%)	11.0 ± 2.1	11.0 ± 1.6	7.4–12.6
Beta-globulin (%)	9.9 ± 0.6	9.4 ± 0.8*	7.5–12.9
Gamma-globulin (%)	14.1 ± 2.5	14.4 ± 2.7	8.0–15.8
IgG (g/L)	10.8 ± 1.9	9.9 ± 1.9*	7.0–16.0
IgM (g/L)	1.4 ± 0.7	1.3 ± 0.6*	0.4–2.3
IgA (g/L)	1.9 ± 0.9	1.71 ± 0.7*	0.7–4.0
IgE (IU/mL)	47.5 ± 58.0	41 ± 49.4*	<220
Glucose (mmol/L)	4.9 ± 1.4	5.2 ± 1.2	<7.8
K^+ (mmol/L)	4.3 ± 0.3	4.1 ± 0.4	3.6–5.4
Ca^{2+} (mmol/L)	2.4 ± 0.1	2.3 ± 0.1*	2.1–2.55
Na^+ (mmol/L)	138.9 ± 1.4	138.0 ± 3.1	134–145
CK (µmol/sL)	1.5 ± 1.1	1.3 ± 0.9*	<3.2

10 patients, albumin %, alpha 1-globulin, alpha 2-globulin, beta-globulin, gamma-globulin, IgE: means of 10 treatment sessions (W1); fibrinogen, immunoglobulin (Ig) IgG, IgM, IgA, total protein, CK: means of 20 treatment sessions (W1, W3); glucose, K^+, Ca^{2+}, Na^+: means of 30 treatment sessions (W1, W2, W3); aPTT, albumin g/L: means of 50 treatment sessions (W1–W5), Mean ± SD, $*P < 0.05$ vs. 0-min value; Wilcoxon test.

session (intra-treatment) and all assessed parameters remained within the normal range, except for a significant increase of aPPT values due to anticoagulation (Table 3). In general, the patients had slightly increased alpha 1-globulin values, which was possibly due to the inflammatory status (CAI 6–10). There were no long-term impacts (inter-treatment) of the intermediary apheresis treatments on the safety laboratory parameters except from expected fluctuations in coagulation parameters such as Quick value, INR and aPTT values.

Body temperature did not show any distinctive changes during the treatment. Heart rate and blood pressure were slightly reduced (heart rate: from 79.7/min to 72.6/min after 30 min of treatment, 72.4/min at the end; mean arterial pressure: from 103.7 mm Hg to 97.7 mm Hg after 30 min of treatment, 100.2 mm Hg at the end), which can be ascribed to the resting position of the patients after starting the treatment. Blood pressure recovered toward the end of the treatments.

Biocompatibility

C3a values significantly decreased after 15 min of treatment in the outflow line of the device compared with the 0-min values. After 60 min, the levels of complement factor C3a were not significantly increased (Fig. 1).

Technical performance

The technical performance of the device was assessed by calculating the absolute reduction rates of peripheral blood cell populations collected from the inflow and outflow line at different time points of a single apheresis session. The hematocrit was slightly reduced after starting the treatment (–2%) until the end of the treatment because of dilutional effects (cell counts were corrected by hematocrit changes). The red blood cell count very slightly increased during the treatments (inflow to 100.4%, outflow to 100.7% after 15 min of treatment; 100.5% at the end). The total leukocyte count was effectively reduced with a maximum between 30 and 60 min and recovered to 99.6% after the treatment. Monocytes (inflow to 87.3%, outflow to 37.3% after 30 min), neutrophil granulocytes (inflow to 93.8%, outflow to 53.2% after 30 min), eosinophils (inflow to 88.4%, outflow to 51.5% after 15 min) and platelets (inflow to 98.9%, outflow to 20.3% after 15 min) were strongly reduced during the treatment. However, after the treatment, the recovery values were 95%, 99%, 107% and 92%, respectively. There was only a small impact on the lymphocytes (inflow to 99.4%, outflow to 93.8% after 30 min of treatment, 103.9% at the end; Fig. 2). There were no long-term impacts from the apheresis treatments on the peripheral blood cell counts with the exception of slight (within a normal range) hemoglobin, hematocrit and erythrocyte count decreases between W3 and W6, which completely recovered toward W10.

Modification of cell surface markers

When the results were compared before and after each apheresis session, $CD3^+CD4^+$ T helper

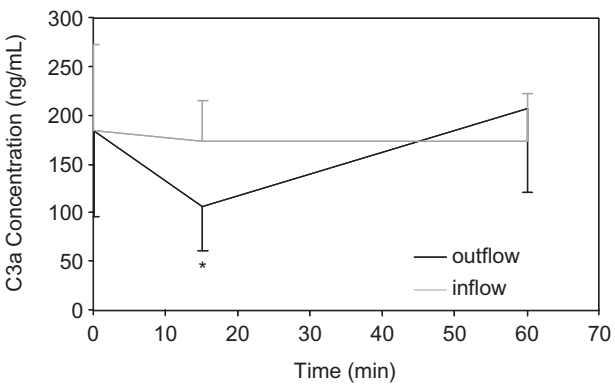

FIG. 1. Time course of the complement factor C3a during the apheresis treatments. Comparison of the start (0 min) vs. 15- and 60-min values for one treatment session (W1, W3 and W5; 10 patients, 30 treatments, Mean ± SD, $*P < 0.05$ vs. screening value; Wilcoxon test).

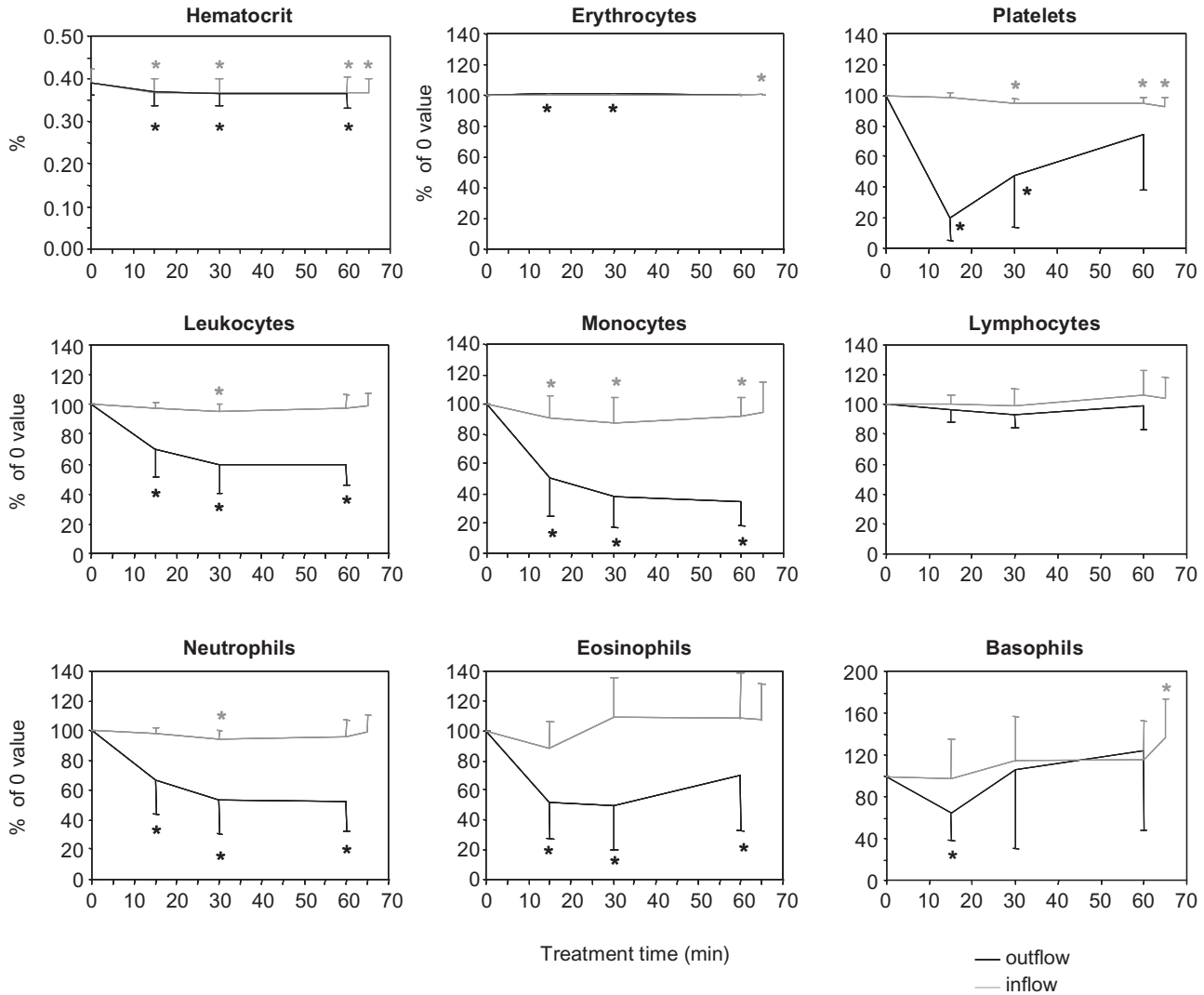

FIG. 2. Blood count graphs—intra-treatment impacts: Comparison of the start (0 min) vs. 15-, 30- and 60-min values for one treatment session (10 patients, 50 treatments, Mean ± SD, absolute counts are expressed as a % of the 0-min value [100%]).

cells slightly increased (to 114% after 60 min), while $CD3^+CD8^+$ cytotoxic T-cells remained unchanged. $CD3^+HLADR^+$ cells were moderately reduced to 71% after 60 min in the outflow, while $CD4^+CD25^+$ cells increased to 118%. T-cells bearing the γ/δ T-cell receptor were moderately reduced after 60 min in the inflow (to 91%) and in the outflow (to 78%). $CD4^+CD25^{+high}$ cells, $CD4^+CD25^{+high}FoxP3^+$ and $CD4^+CD25^{+interm}FoxP3^+$ cells significantly decreased after 60 min in the outflow (Tables 4,5).

There was a strong reduction of $CD14^+$ monocytes and $CD10^+$ granulocytes during the treatments in the outflow ($CD14^+$: to 45% after 60 min, $CD10^+$: to 54% after 60 min) but not in the inflow. With regard to $CD62L^+$ and $CD11b^+$ cells, the reduction rates were similar to those of the granulocytes ($CD62L^+$: to 57% after 60 min in the outflow; $CD11b^+$: to 53% after 60 min in the outflow). Similarly, there were no significant changes in these cells after 60 min in the inflow line.

The mean channel fluorescence of the $CD62L^+$ cells, which reflects the surface expression of this parameter on the cells, slightly decreased in the inflow after 15 min (to 92%) and in the outflow after 60 min of treatment time (to 86%). The mean channel fluorescence of $CD11b^+$ cells was moderately elevated in the column outflow only after 60 min of treatment time (to 202%). $CD42b^+CD63^+$-activated platelets increased during the treatment, especially in the outflow line (Tables 4,5). There were no long-term impacts from the apheresis treatments on the different cell populations.

TABLE 4. *Modification of cell surface markers, intra-treatment impacts (W1, W3 and W5)*

Parameter	0 min	15-min inflow	15-min outflow	60-min inflow	60-min outflow
$CD3^+CD4^+$	100.0 ± 0.0	108.7 ± 15.4*	107.4 ± 17.6*	117.8 ± 17.9*	113.6 ± 18.1*
$CD3^+CD8^+$	100.0 ± 0.0	100.6 ± 16.8	96.3 ± 18.3	100.8 ± 20.4	100.6 ± 22.2
$CD10^+$	100.0 ± 0.0	102.1 ± 12.3	66.2 ± 24.7*	99.6 ± 28.8	54.1 ± 20.0*
$CD14^+$	100.0 ± 0.0	93.0 ± 19.0*	48.0 ± 29.9*	97.2 ± 37.2	45.3 ± 36.0*
$CD62L^+$	100.0 ± 0.0	98.5 ± 6.2	67.5 ± 22.2*	98.7 ± 16.7	57.0 ± 17.6*
$CD62L^+$ Mean	100.0 ± 0.0	92.0 ± 14.9*	94.8 ± 15.7	108.8 ± 21.7*	85.6 ± 21.7*
$CD11b^+$	100.0 ± 0.0	96.7 ± 5.9*	64.4 ± 24.4*	95.4 ± 15.3	52.6 ± 20.5*
$CD11b^+$ Mean	100.0 ± 0.0	108.0 ± 43.8	103.8 ± 41.9	119.7 ± 51.4	202.2 ± 84.2*
$CD3^+HLADR^+$	100.0 ± 0.0	97.6 ± 33.4	91.9 ± 41.4	121.3 ± 82.0	71.1 ± 32.0*
$CD42b^+CD63^+$	100.0 ± 0.0	117.3 ± 107.0	389.2 ± 529.1*	194.7 ± 182.7*	409.8 ± 343.3*
$CD3^+TCR\gamma\delta^+$	100.0 ± 0.0	113.7 ± 105.1	79.9 ± 56.6*	90.5 ± 59.5*	78.4 ± 31.1*

10 patients, 30 treatments, Mean ± SD, absolute counts were hematocrit corrected (except $CD42b^+CD63^+$) and expressed as a % of the 0-min value (100%), *$P < 0.05$ vs. screening value; Wilcoxon test.

Clinical efficacy

Based on the definitions of clinical remission and clinical response (remission: CAI ≤ 4, response: CAI drop ≥ 3 or CAI ≤ 4), the remission and response rates are reflected by Table 1. The average CAI was reduced from 8.5 ± 1.5 (screening value) to 4.5 ± 4.1 at W6 and 2.2 ± 2.7 at W10 ($P < 0.05$). Remission of the disease was achieved in 8 of the 10 patients (80%) at W10.

Clinical remission was accompanied by a reduction of the endoscopic index (EI <4) in four of the nine patients (44%) who consented to endoscopic examination. The average EI of the nine eligible patients decreased from 7.4 ± 1.6 (screening value) to 5.2 ± 3.2 at W10 ($P < 0.05$). Although four patients (44%) had a clinical remission according to improvements in CAI score, five (56%) did not show remission based on their endoscopic scores (EI >4).

DISCUSSION

Ulcerative colitis patients are frequently administered high doses of corticosteroids. Cytapheresis has the potential to avoid steroid-mediated side-effects and to treat steroid-refractory patients. However, the treatment is expensive, and its therapeutic success varies. Therefore, newly developed devices should provide an improved efficacy and cost-benefit ratio.

This study is the first pilot trial of a novel adsorptive-type cytapheresis module filled with polyarylate resin beads in patients with active UC and has demonstrated that the tolerability of this device is well to very well without showing adverse side-effects. The apheresis treatments with the novel adsorber assured a high degree of safety. All measured safety parameters remained essentially unchanged both during intra-treatment and inter-treatment periods. Vital parameters, such as blood pressure, heart rate and body temperature, were essentially stable during the apheresis sessions.

The clinical response experienced in this study was promising as eight out of 10 patients were in remission at W10. In four out of nine patients who consented to endoscopic evaluation, CAI remission was accompanied by an improvement of the EI. The response rates were in full concordance with those reported in the literature for other adsorptive cytapheresis devices for patients suffering from active ulcerative colitis (4,7,27).

During blood contact with artificial surfaces, the complement system is activated via the alternative pathway to a varying degree. A low degree of complement activation is an expression of a low contact

TABLE 5. *Flow cytometry analyses of T_{regs}, intra-treatment impacts (W1, W3 and W5)*

Parameter	0 min	15-min inflow	15-min outflow	60-min inflow	60-min outflow
$CD4^+CD25^+$	100.0 ± 0.0	105.7 ± 17.0	121.1 ± 29.7*	117.6 ± 28.2*	118.2 ± 44.9*
$CD4^+CD25^{+interm}$	100.0 ± 0.0	106.5 ± 18.0	122.3 ± 31.5*	118.4 ± 30.0*	124.0 ± 44.0*
$CD4^+CD25^{+high}$	100.0 ± 0.0	104.4 ± 29.0	100.9 ± 29.9	97.6 ± 34.1	85.5 ± 34.9*
$CD4^+CD25^+FoxP3^+$	100.0 ± 0.0	104.4 ± 27.0	114.6 ± 36.7	92.8 ± 35.8	83.1 ± 45.5*
$CD4^+CD25^{+interm}FoxP3^+$	100.0 ± 0.0	102.9 ± 33.4	111.7 ± 44.3	93.6 ± 53.1	80.7 ± 59.4*
$CD4^+CD25^{+high}FoxP3^+$	100.0 ± 0.0	104.3 ± 26.3	115.8 ± 36.5*	93.7 ± 39.0	76.5 ± 38.4*

10 patients, 30 treatments, Mean ± SD, % of 0-min value (100%), *$P < 0.05$ vs. screening value; Wilcoxon test.

activation potential and high biocompatibility. Activation of the complement system by cellulose acetate beads has been shown by the increased levels of plasma C3a and C5a in the column outflow, without affecting the clinical tolerability (13). In this study, the device showed an excellent biocompatibility, as plasma C3a concentrations were not elevated after the treatment. C3a values significantly decreased after 15 min of treatment in the outflow line of the device compared with the 0-min values, which may be due to an adsorption of complement fragments to the adsorber or to cells attached to the column. However, after 60 min, differences were not significant.

Performance data from the new device showed that platelets (80%), monocytes (66%) and neutrophil granulocytes (to 47%) were effectively reduced in the outflow line during the cytapheresis treatments. At the end of the treatment, the blood samples directly collected from the patient showed that granulocyte numbers recovered, whereas monocytes and platelets still showed a reduction of 5% and 8%, respectively, compared with the basal values. Probably, this might have an influence on the overall activation status of these cells in the body even if they return to baseline after a longer observation period, which should be clarified in further studies. Lymphocytes and red blood cells remained relatively constant. Flow cytometry analyses confirmed these results and displayed a strong reduction of $CD14^+$ monocytes and $CD10^+$ granulocytes. Thus, concerning the removal characteristics, the novel device resembles the available cellulose acetate based selective GMCAP system with an additional higher platelet elimination capacity.

The primary aim of the GMCAP treatment was to remove activated granulocytes and monocytes from the circulation (28). However, even the removal of primary non-immune cells such as platelets might be important. Activated platelets participate in the pathogenesis of IBD by secreting a variety of biologically active molecules. Moreover, they are able to interact with the mucosal endothelium and T-cells and create a proinflammatory platform perpetuating the inflammatory process (24). The importance of this effect is supported by the result that the decrease in activated platelets immediately after the first LCAP may be an early marker for predicting the clinical response toward the treatment (21,22). In this study, we observed that the number of $CD42b^+CD63^+$-activated platelets increased during the treatment, especially in the outflow line. However, the platelet number at the column outlet was very low. Thus, this activation may be of decreased importance.

Generally, adsorptive cytapheresis has been ascribed an immunomodulatory effect, as shown by its beneficial long-lasting clinical effects in patients with autoimmune diseases. Alterations in cell population profiles, modulation of the cytokine response, signaling, homing and transmigration pathways have previously been discussed (29). The repeated reduction of dendritic cells, which play a substantial role in the induction of immune tolerance, has been described (19). Recent findings emphasize the role of regulatory T-cells in explaining the long-term effects of cytapheresis treatments (20,30). The flow cytometry data from this study revealed a slight increase in both $CD3^+CD4^+$ T helper cells and $CD4^+CD25^+$ cells, which suggested minor T-cell activation or redistribution of these cells because $CD4^+CD25^{+high}FoxP3^+$ T_{regs} were elevated only after 15 min of treatment but then significantly decreased. This result is in contrast to studies using other adsorbers (20,31) and may be due to the characteristics of the device. Nevertheless, our results show that $CD3^+HLADR^+$ cells (late stage-activated T-cells) and T-cells bearing the γ/δ T cell receptor were reduced and support the hypothesis that the therapy induced an anti-inflammatory effect. The analysis of the leukocyte trafficking receptors CD62L and CD11b showed downregulation of CD62L expression and upregulation of CD11b expression. This result has also been reported by other cytapheresis studies and reveals a suppression of leukocyte extravasation (31).

CONCLUSION

During this pilot trial, the novel semi-selective device has been shown to be safe, well tolerated and clinically efficient in patients with active ulcerative colitis. This study led to approval status of the device (CE marking). Our data suggest a difference between the study device and other adsorbers on the market concerning a stronger removal of platelets, an induction of a slight T-cell activation and a very low complement activation. Response rates (80% remission) in this preliminary trial with small patient numbers were comparable or even better compared to already established cytapheresis techniques. This positive impact on efficacy parameters merits further evaluation in controlled trials and dose-finding studies with larger patient numbers.

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GS: data collection, sample preparation, data analysis; HP: patient treatment; PB: data analysis; JE: study design, patient treatment; all authors: reviewing, editing and approving the final manuscript.

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