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Rheopheresis in Patients with Critical Limb Ischemia—Results of an Open Label Prospective Pilot Trial

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Abstract: Rheopheresis is a specifically designed application of double filtration plasmapheresis, for extracorporeal treatment of microcirculatory disorders. Safety and efficacy of Rheopheresis for wound healing and skin oxygenation were investigated in patients with critical limb ischemia. Twelve patients of Fontaine stage III-IV were treated with a series of 10 Rheopheresis sessions over 17 weeks. Transcutaneous oxygen pressure ($tcpO_2$) and ankle-brachial index (ABI) were repeatedly determined to monitor the effects of the Rheopheresis treatment series on microcirculation and skin blood flow. Laboratory parameters of blood rheology were measured in addition to safety parameters and course of the pain syndrome was documented. In four patients (baseline Fontaine stage III) Rheopheresis was associated with an improvement of Fontaine stage, a pronounced increase in $tcpO_2$ and complete regression of the rest pain. As an adjunct therapeutic option, Rheopheresis may preserve a functional lower extremity, delay amputation or reduce the extent of amputation.
Key Words: Critical limb ischemia, Fibrinogen, Microcirculation, Peripheral artery disease, Rheopheresis.

Lower-extremity ischemia results from occlusion of the circulation to the limb either proximally or distally in the circulatory trees. The resulting chronic disorder occurring in the artery segments of the circulatory system is called peripheral arterial disease (PAD). PAD is defined as atherosclerotic disease, causing a mismatch between oxygen supply and demand (1).

Besides already established major risk factors for PAD like cigarette smoking, diabetes mellitus, middle to old age (older than 40 years), hypertension, hyperlipidemia, and hyperhomocystinemia (2), several potential risk factors for PAD have been identified, including elevated levels of C-reactive protein (3), fibrinogen, apolipoprotein B, lipoprotein(a), and plasma viscosity (4). Elevated plasma viscosity and fibrinogen are both correlated with abnormalities in the ankle-brachial index (ABI) among patients with PAD, and elevated fibrinogen has been associated with the development, presence, and complications of PAD (5). Therefore, plasma viscosity, fibrinogen and erythrocyte rigidity (6) offer potential therapeutic targets for hemorheological effective approaches in PAD (7).

The most advanced stages of PAD can lead to critical limb ischemia (CLI). The term CLI should be used for all patients with ischemic rest pain, ulcers or gangrene, predominantly due to objectively proven arterial occlusive disease (8). In general, patients with PAD are classified either according to the Fontaine classification or the Rutherford classification. Patients with CLI have either Fontaine stage III or IV, and those patients with rest pain or tissue loss are categorized as Category 4 or 6 on the Rutherford classification. Compared to Fontaine stage III, the presence of trophic lesions in stage IV increases the risk of amputation (9).

It is estimated that approximately 15% to 20% of patients with lower extremity PAD will progress from intermittent claudication to CLI over the course of their disease (10). Recent estimates of the

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incidence rate of CLI have been reported with approximately 500–1000 individuals per million per year (11). According to current German guidelines, standard therapy for patients with CLI comprises the administration of prostaglandins, such as prostaglandin E1 (PEG1) (12). However, despite the use of PEG1 and major advances in both percutaneous and surgical techniques, the disease frequently follows an inexorable down-hill course (13). Previous revascularization procedures had been associated with a lower risk of mortality, but with a greater risk of amputation, possibly meaning that the benefit is not permanent (14). Of those patients with CLI, 30% will undergo amputation and 20% will die within 6 months (8). Consequently, the need for alternative strategies for the treatment of patients with critical limb ischemia is compelling.

New techniques including capillaroscopy, fluorescence videomicroscopy, laser Doppler flowmetry, and transcutaneous oxygen pressure ($tcpO_2$) measurement have improved the understanding of skin microcirculation, reflecting severity of disease. In contrast to the early stages of PAD, in which compromised skeletal muscle blood flow causes intermittent claudication, rest pain and trophic changes associated with CLI are predominantly related to a critical reduction in skin microcirculation (8).

Taking into consideration that fibrinogen has been clearly identified as a risk factor for atherosclerosis (15) and that many open trials reported promising results with the use of defibrinogenating agents in 50–80% of treated patients suffering PAD (10), the present open clinical trial investigates the efficacy and safety of Rheopheresis in patients with CLI. Rheopheresis is an extracorporeal treatment specifically designed for the treatment of microcirculatory disorders (16). A defined spectrum of high molecular weight proteins (such as LDL-cholesterol, fibrinogen, α_2-macroglobulin, immunoglobulin M [IgM], von Willebrand factor [vWF], and fibronectin) is simultaneously eliminated from human plasma, resulting in a pulse of lowered blood plasma viscosity, as well as reduced erythrocyte and thrombocyte aggregation. Repeated pulsed reductions in blood and plasma viscosity by the Rheopheresis treatment series can result in sustained improvement in microcirculation at a functional level (16). Rheopheresis has been successfully investigated in controlled, randomized clinical trials in ophthalmology: it was found to be an effective treatment for selected patients with age-related macular degeneration, a microcirculatory disorder of the retina (16,17). Targeting the microcirculatory dysfunction in the ischemic diabetic foot syndrome here affecting smallest capillary vessels in the lower extremities, the efficacy of Rheopheresis has already been demonstrated, with improvement of Wagner stage, associated with a pronounced increase in $tcpO_2$ (18).

The primary objective of the present open-label, prospective pilot trial was to investigate the efficacy of Rheopheresis in another microcirculatory disorder affecting the lower extremities. However, in patients with CLI not only the small capillaries but also larger arteries are affected by microcirculatory dysfunction. Patients selected were classified as Fontaine stage III and IV, were likely to face surgery (amputation) and were lacking any other promising therapeutic option. Secondary objectives were analysis of changes in $tcpO_2$ in the affected leg, measuring ankle pressures, evaluating the ABI, and analysis of laboratory parameters.

METHODS

Patients

Patients were aged 35-80 years and had PAD of Fontaine stage III-IV in one lower extremity. Arterial ankle pressures were <70 mm Hg, and arterial toe pressures <50 mm Hg. $TcpO_2$ measurements at the dorsum of the foot in recumbent position were <20 mm Hg. Further, patients included in the study suffered rest pain with regularly taken analgesics for at least 14 days, revascularization was technically not possible, and there was no indication for acute amputation. The following conditions represented PAD-specific exclusion criteria: (i) diagnosed PAD with the indication of immediate surgery; (ii) need of any kind of urgent amputation; (iii) the patient had revascularization, dilatation, lysis, prostaglandin or anticoagulants within the last 14 days; (iv) progressive deterioration of PAD within the last 7 days; and (v) ischemic diabetic foot, osteomyelitis, acute infection, or sepsis.

In total 12 patients, 11 men and 1 woman, were included in the pilot trial. Except three men with Fontaine stage IV, all patients showed Fontaine stage III. Mean age was 68.6 years (range 57–79 years). Main patient baseline characteristics, and laboratory and safety parameters, are listed in Tables 1 and 2, respectively. All patients signed an informed consent form to participate in the study. The study protocol was approved by the local ethics committee.

Study protocol

Each patient received a series of 10 Rheopheresis treatments over 17 weeks. The target for a single Rheopheresis treatment was 100% of the patient's plasma volume, determined using a plasma volume

TABLE 1. *Characteristics of patients with critical limb ischemia*

Patient number	1	2	3	4	5	6	7	8	9	10	11	12	Mean value
Sex	male	male	male	male	male	male	male	male	male	female	male	male	
Age (years)	79	77	65	59	72	57	61	61	78	78	71	66	68.7
Weight (kg)	90	75	76	62	78	59	107	95	75	70	96	84	80.6
Fontaine stage at baseline	III	III	IV	III	III	III	IV	IV	III	III	III	III	
Fontaine stage after last Rheopheresis	IIA	III	IV	III	III	III	IV	IV	IIA	IIA	IIA	III	
Number of treatments	10	2	11	10	10	9	2	10	12	10	10	7	8.6
$tcpO_2$ (mm Hg) at baseline	40	–	10	0	15	10	2	20	45	40	10	32	20.4
$tcpO_2$ (mm Hg) after last Rheopheresis	70	–	10	10	0	40	–	45	75	61	32	–	38.1
ABI at baseline	0.4	0.4	0.3	0.2	0.4	0.3	0.8	0.4	0.4	0.4	–	0	0.4
Walking distance (m) at baseline	116	30	10	150	100	0	–	60	50	50	70	70	64.2

–, not measured.

nomogram adjusted for patient gender, height, weight and hematocrit. The first two Rheopheresis treatments were performed in week 1, followed by one treatment every 2 weeks. Subjective assessment of pain and walking distance documentation, measurement of $tcpO_2$, and systolic ankle pressure were performed weekly. After 17 weeks the clinical status of the patients was compared with baseline data.

Performance of Rheopheresis

A specialized blood and plasma therapy system was used to automatically monitor blood and plasma flow, and pressure for Rheopheresis (OctoNova, Diamed Medizintechnik, Cologne, Germany). A Rheopheresis system is composed of an OP-05 polyethylene plasma separator (Asahi Kasei Medical, Tokyo, Japan) and the specifically designed AR-2000 Rheofilter (effective surface area 1.7 m^2) (Asahi Kasei Medical, Tokyo, Japan). Vascular access for the extracorporeal circuit was established by two peripheral veins. Blood was pumped at a continuous rate of 60–90 mL/min through the OP-05 plasma filter. A special plasma pump implemented in the OctoNova machine drew plasma from the outer space of the plasma separator at 10–30 mL/min (typically corresponding to one third of the blood flow). Flow balance was achieved by high-precision, peristaltic pumps implemented in the OctoNova machine. After plasma separation, separated plasma was pumped into the Rheofilter. Soluble high molecular weight plasma components larger than 25 nm or approximately 500 kDa were eliminated by the Rheofilter. Targeted high molecular weight plasma components were retained in the hollow fibers. For anticoagulation, an initial bolus of 10 000 IU unfractionated heparin was given throughout the whole study, with the option of an extra bolus of 5000 IU heparin if required. The system was primed with 3 L normal saline containing unfractionated heparin (5000 IU/L) before treatment. A schematic drawing of the extracorporeal circuit for Rheopheresis is shown in Figure 1.

Transcutaneous oximetry

$TcpO_2$ is a non-invasive method for measuring oxygen diffusing to the surface of the skin from der-

TABLE 2. *Baseline laboratory parameters*

Parameters	Patients 1	2	3	4	5	6	7	8	9	10	11	12	Mean value
Hemoglobin (g/dL)	12.4	13.7	15	15	14.5	13.8	14.3	15.2	12.6	11.9	14.2	14.6	13.93
Hematocrit (%)	37	43	45	45	44	40	43	46	40	37	40	40	42.16
Cholesterol (mg/dL)	197	162	193	208	196	269	120	229	118	154	141	175	180.2
LDL cholesterol (mg/dL)	115	99	112	134	112	–	51	149	6	76	79	77	96.81
HDL cholesterol (mg/dL)	67	29	34	54	49	16	34	38	24	50	37	45	39.75
Triglycerides (mg/dL)	134	168	237	74	174	1504	176	208	165	140	126	267	281.08
Fibrinogen (mg/dL)	366	390	317	630	478	462	700	406	567	424	714	491	495.41
α-2-macroglobulin (mg/dL)	119	140	145	135	136	458	102	–	146	217	246	138	180.18

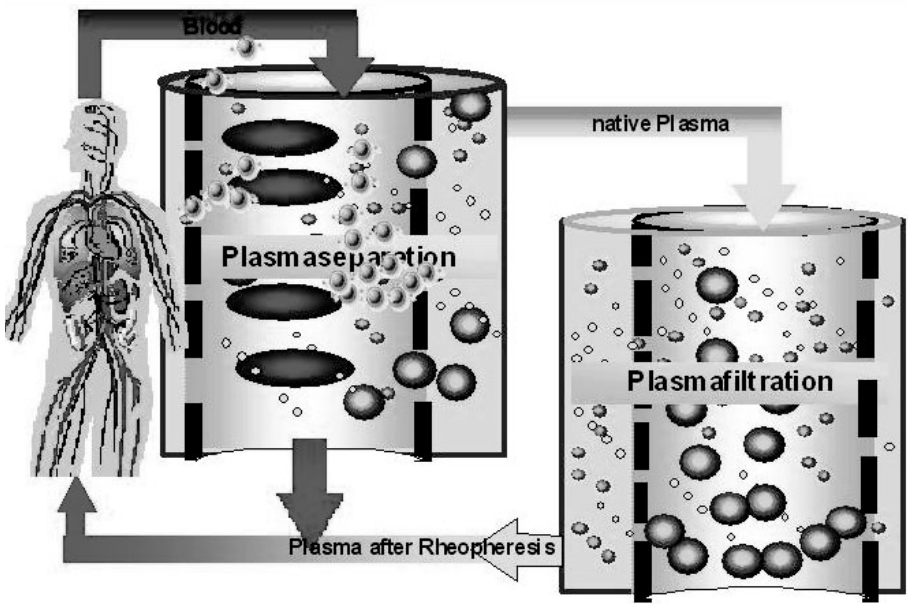

FIG. 1. Schematic drawing of the extracorporeal circuit established for Rheopheresis.

mal capillaries. The values obtained represent a complex function of cutaneous blood flow, metabolic activity, oxyhemoglobin dissociation and oxygen perfusion through tissue (8). Values of $tcpO_2$ were determined to assess the severity and clinical progression, and to evaluate cutaneous ischemia, in CLI. $TcpO_2$ has a high positive predictive value (77–87%) for classifying patients as having severe ischemia (19).

In general, an oxygen tension of 30 mm Hg suggests ischemia and non-healing but a range of ±10 mm Hg must be considered (20). Thus, it might be predicted that healing will not occur with a $tcpO_2$ under 20 mm Hg and will occur with a $tcpO_2$ over 40 mm Hg (8).

In the investigation presented here, measurements of lower extremity $tcpO_2$ were performed repeatedly at the dorsum of the foot at 44°C under standard conditions. At 44°C, the O_2 partial pressure of the skin surface is independent of the blood pressure, being solely dependent on the arterial O_2 partial pressure of the blood. After calibration of the sensor, periwound $tcpO_2$ values were also measured immediately close to the greatest wound lesion. The oxygen sensor, a modified Clark electrode, was affixed to the skin surface with a double-sided adhesive ring containing a NaCl 0.9% solution. The electrodes quantify oxygen content by measuring the rate of oxygen reduction at the cathode. The cathode and anode were suspended in an electrolyte solution behind an oxygen-permeable Teflon membrane. A constant voltage was applied between the electrodes and the current voltage was measured.

Ankle-Brachial Index (ABI)

The ankle-brachial index (ABI) is a non-invasive, quantitative measurement of the patency of the lower extremity arterial system, which involves measuring the systolic blood pressure in the ankles and arms generally using a hand-held 5–10 MHz Doppler and then calculating a ratio of systolic blood pressure in ankle and arm. This method has been validated against angiographically confirmed disease and found to be 95% sensitive and almost 100% specific (21). Nonetheless, the usefulness of the ABI as a predictor of outcome is still debated (9). The ABI can be interpreted as follows: (i) ABI < 0.40 = severe obstruction; (ii) ABI 0.40–0.69 = moderate obstruction; (iii) ABI 0.70–0.90 = mild obstruction; (iv) ABI 0.91–1.30 = normal; and (v) ABI > 1.30 = poorly compressible (5).

For each decrease in ABI of 0.1, the relative risk of developing rest pain is increased 2.2-fold and the risk of tissue loss is increased 1.9-fold (22). Claudication can be seen in patients with ABIs of less than 0.92. Values less than 0.5 are seen in patients with severe disease and pain at rest; tissue loss is typically noted with values less than 0.3 (23).

Measurement of the systolic ankle pressure was performed with the 8 Mhz Ultrasonic Doppler Flow Detection Model 811-B (Parks Medical Electronic Inc., Aloha, OR, USA), and a standard blood pressure cuff. Measurement was done on a recumbent patient, after a resting period of at least 15 min. The value was used to calculate the ABI for each patient at the initial examination.

TABLE 3. *Summary of side-effects observed during a total of 103 documented rheopheresis treatments*

	Number	Percent	Number	Percent
Side-effects			Cases with intervention/interruption/termination	
Hypotension	2	1.94%	1	0.97%
Dizziness	1	0.97%	0	0.00%
Chills	1	0.97%	1	0.97%
Total	4	3.88%	2	1.94%
Venous access problems (puncture/blood flow)			Cases with intervention/interruption/termination	
	10	9.71%	1	0.97%
Therapy terminations; mean treated plasma volume: 60.47% of target volume (due to side-effects, venous or technical problems)				
	2	1.94%		

Blood sampling and laboratory parameters

At the initial and final examination, blood samples were collected. Laboratory parameters, such as blood count, creatinine, Na, K, urea, Quick, aPTT, total protein, albumin, α_2-macroglobulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides, fibrinogen, IgM, and IgG were analyzed. Before the first Rheopheresis the infection parameters anti HIV-Ab, HBS-Ag, anti HBS-Ab and anti HCV-Ab were controlled.

For calculation of reduction rates, fibrinogen, total cholesterol, LDL-cholesterol, total protein, albumin, α_2-macroglobulin, and IgM were analyzed before and after each Rheopheresis treatment.

Descriptive statistical analysis

All values of normally distributed variables are presented as means with standard deviations (± SD). The program Statistical Package for Social Science (SPSS 11, SPSS, Chicago, IL, USA) was used for data analysis. Due to the small sample size, non-parametric tests were used for statistical analysis of the data. For the analysis of two related samples the Wilcoxon signed-ranks test was used. The Mann–Whitney *U*-test was used as non-parametric test for two independent samples. All tests were performed 2-sided and *P*-values <0.05 were considered significant.

RESULTS

Safety of Rheopheresis treatments

During 21 months of this pilot study, 103 Rheopheresis treatments were performed on 12 patients. According to the protocol, the first two Rheopheresis treatments were performed within 1 week with the following Rheopheresis treatments in intervals of 2 weeks. Mean treated plasma volume was 3020 ± 316 mL corresponding to 94% of the target plasma volume. A treated plasma volume of 70% was defined as necessary for successful Rheopheresis treatment (Table 3). Average duration of a single Rheopheresis treatment was 183 ± 33 min, and eight of 12 patients received the complete Rheopheresis series. Six of these 8 patients received the planned 10 Rheopheresis treatments, one patient received 11 (due to repeat of one treatment that had to be terminated because of technical problems) and one patient received 12 treatments (due to patient's withdrawal after two Rheopheresis treatments, and subsequent restart of the trial 8 months after withdrawal). In the other four patients the Rheopheresis series could not be completed due to patient-specific problems: (i) one patient with 9 treatments, deterioration and cardiac failure; (ii) one patient with 7 treatments, hospitalization due to ulcer surgery; and (iii) and (iv) two patients with 2 treatments: leg amputation. There were no occurrences of clinically relevant adverse events that required temporary discontinuation or termination of Rheopheresis treatments. Rheopheresis did not have any severe side-effects on hemodynamic parameters such as blood pressure and heart rate. Transient hypotension occurred in two out of 103 treatments (1.94%) and could be easily controlled. No episodes of severe arterial hypotension requiring the application of vaso-active drugs were observed. Substitution of fresh frozen plasma, albumin or other plasma products was not necessary at any time. Table 3 summarizes the side-effects observed in the present study.

Clinical course as assessed by Fontaine stages

Before the first Rheopheresis treatment, seven of 12 patients were classified as Fontaine stage III and four patients were classified as Fontaine stage IV (Fig. 2). After completion of the present pilot trial, four patients with Fontaine stage III before Rheopheresis treatments showed a clinical improvement

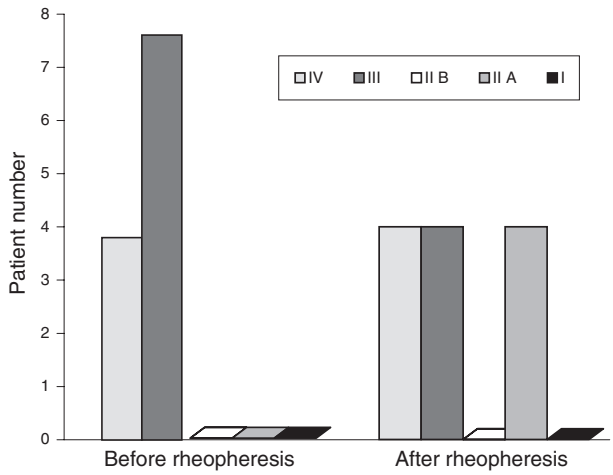

FIG. 2. Fontaine stage classification (IV, III, IIB, IIA, I) before and after Rheopheresis series for all patients.

after 10, and in case of patient 9, after 12 treatments. After the treatments these four patients could be classified as Fontaine stage IIA. In the remaining eight patients no improvement was observed (Fig. 2). However, two of these 8 patients had to stop the protocol after the second treatment due to lower leg surgery, but were further considered as intention-to-treat patients.

Thus, in total four of the 12 enrolled patients (33.3%) showed clinical improvement according to Fontaine stages. In the following discussion they will be referred to as 'responders' compared to 'non-responders' who showed no clinical improvement.

Having a great impact on the classification outcome, the complete regression of the rest pain in those four patients was, along with an increased walking distance, one of the main reasons responsible for the improvement of the Fontaine classification in the responders. Of the non-responders, six of eight patients experienced neither improvement nor deterioration in pain. In two patients, a slight improvement, and a pronounced improvement of the rest pain could be observed, respectively.

$TcpO_2$ measurements

In seven of the 12 patients an improvement in $tcpO_2$ of ≥10 mm Hg was observed during the Rheopheresis series. For the total patient group, a comparison of $tcpO_2$ values before and after Rheopheresis treatments revealed a significant increase in $tcpO_2$ values ($P = 0.024$, $N = 9$). All four responders showed a distinct but not significant increase of the $tcpO_2$ values during the Rheopheresis series ($P = 0.066$) (Fig. 3). Mean $tcpO_2$ values were 33.8 ± 16.0 mm Hg before and 59.5 ± 19.2 mm Hg after the Rheopheresis series, corresponding to a $tcpO_2$ increase of 76%. An increase of the mean $tcpO_2$ values in the non-responders was also observed, with 12.7 ± 10.1 mm Hg before and 21.0 ± 20.1 mm Hg after the treatment series, corresponding to a $tcpO_2$ increase of 65.3%.

A comparison of responder and non-responder baseline values and endpoint values, respectively, revealed no significant difference in the mean $tcpO_2$ before the start of the Rheopheresis series between responder and non-responder ($P = 0.109$, $N = 11$), but revealed a significant higher mean $tcpO_2$ value after completion of the Rheopheresis series for the responder group ($P = 0.019$, $N = 10$).

Changes in ABI

Baseline values were not available for two of the 12 patients, and for six of the 12 patients no endpoint values were available. Due to this loss of ABI values the analysis of the present ABI data is very limited. However, for the responders a trend towards an improved ABI could be detected. For the responders, the ABI increased from 0.39 ± 0.0 before Rheopheresis to 0.61 ± 0.16 after Rheopheresis. For the non-responder group with a mean ABI of 0.26 ± 0.10 before treatments and a mean ABI of 0.50 ± 0.10 after the Rheopheresis series, the ABI increase was less pronounced.

Hemorheological laboratory parameters

Rheopheresis resulted in a decrease in the concentration of hemorheologically relevant plasma proteins, such as LDL-cholesterol, fibrinogen, IgM, and α_2-macroglobulin. Mean changes in laboratory parameters before and after Rheopheresis are given in Table 4.

There is a linear relationship between plasma viscosity and levels of certain rheologically active macromolecules, especially large proteins such as LDL-

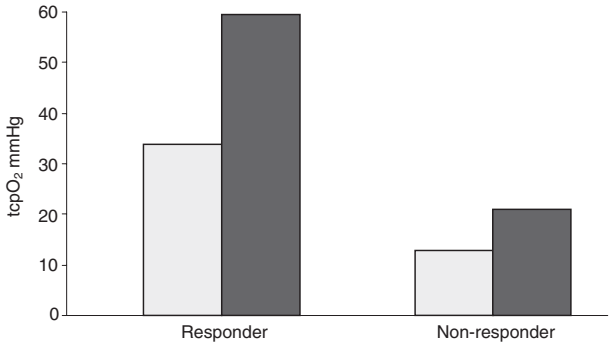

FIG. 3. Changes in $tcpO_2$ values before (left bar) and after (right bar) Rheopheresis treatments for responders ($N = 4$) and non-responders ($N = 5$). For responders, P-values (Wilcoxon signed ranks test) between $tcpO_2$ values were $P = 0.066$, and for non-responders, $P = 0.273$.

TABLE 4. *Influence of Rheopheresis on low density lipoprotein (LDL)-cholesterol, fibrinogen, immunoglobulin M (IgM), α_2-macroglobulin, and total protein (mean pre- and post-treatment levels and mean percentage reduction)*

Parameter	Pre-treatment	Post-treatment	Mean percentage reduction
LDL-cholesterol (mg/dL)	97.02 ± 24.03 $n_1 = 11$; $n_2 = 45$	46.14 ± 15.64 $n_1 = 10$; $n_2 = 32$	56.74 ± 17.00
Fibrinogen (mg/dL)	450.65 ± 109.78 $n_1 = 12$; $n_2 = 101$	224.90 ± 68.03 $n_1 = 10$; $n_2 = 87$	48.31 ± 9.98
IgM (mg/dL)	69.32 ± 41.01 $n_1 = 10$; $n_2 = 28$	29.80 ± 14.27 $n_1 = 9$; $n_2 = 19$	53.02 ± 41.98
α_2-macroglobulin (mg/dL)	147.09 ± 60.32 $n_1 = 12$; $n_2 = 31$	75.84 ± 23.16 $n_1 = 7$; $n_2 = 10$	52.41 ± 8.33
Total Protein (g/dL)	7.69 ± 4.11 $n_1 = 12$; $n_2 = 39$	5.31 ± 0.43 $n_1 = 4$; $n_2 = 7$	19.80 ± 4.68

Values are given as mean ± SD; n_1: number of patients; n_2: number of available values. Plasma concentrations were measured before (pretreatment) and after (post-treatment) Rheopheresis.

cholesterol, fibrinogen, IgM and α_2-macroglobulin (24). In this pilot investigation, LDL-cholesterol was diminished by 56.74 ± 17.00%, fibrinogen levels decreased by 48.31 ± 9.98%, and concentrations of IgM and α_2-macroglobulin fell by 53.02 ± 41.98% and 54.96 ± 32.32%, respectively, immediately after the treatment session. In addition, total protein levels were reduced by 19.8 ± 4.68% (Table 4).

DISCUSSION

In this pilot trial the efficacy of a series of Rheopheresis treatments in 12 patients with CLI referring to Fontaine stage III and IV was investigated. Before enrollment patients experienced a deterioration of their PAD despite the standard approaches in both conservative and interventional therapies. They suffered from chronic pain and showed beginning tissue lesions in the affected leg. The aim of the present pilot trial was to perform Rheopheresis to gain functional restitution of sufficient microcirculation, of muscular capacity, maximum resilience and thus of the quality of life in general.

Rheopheresis is known to be a safe and effective method of therapeutic apheresis for the treatment of microcirculatory disorders (16,18). The effect of Rheopheresis on clinical improvement was analyzed using the Fontaine classification system. Tissue repair was evaluated by measuring values of $tcpO_2$. Hemorheological properties were characterized by laboratory parameters.

Results showed that by performing a series of up to 12 Rheopheresis treatments, 33.3% of the enrolled patients experienced a clinical benefit such as complete regression of the rest pain by re-shifting from Fontaine stage III to stage IIA. Therefore, after completion of the Rheopheresis series, four patients with CLI at trial entry no longer fit the criteria for CLI. Fontaine stage IIA now again allowed additional therapy regimes that were not available at trial entry, including intensive walking training with the perspective of improving endothelial function, muscular metabolism, reduction of inflammatory processes and increase of endothelial growth factors, thus improving the probability of induction of angiogenesis (25). Additionally, the responders also experienced complete disappearance of pain and an improved mean pain-free walking distance from 71.5 ± 31.1 m before treatments to 349.0 ± 178.7 m after completion of the Rheopheresis treatments.

Next to the changes in Fontaine stages, the effect of Rheopheresis on tissue repair was investigated by analysis of the changes in $tcpO_2$ values. In PAD and CLI, respectively, $tcpO_2$ reflects local hyperemic skin blood supply. Even though it has been recently shown that $tcpO_2$ measurement does not necessarily lead to either better patient outcome, or reduction in the number of diagnostic or therapeutic interventions, $tcpO_2$ measurements offer additional information to confirm clinical diagnosis and to function as an objective criterion in clinical trials (26). This was also the case in the present pilot trial. The comparison of $tcpO_2$ for the total patient group before and after the Rheopheresis treatments with significantly higher $tcpO_2$ after the treatment series unequivocally verified the increased oxygen supply of the tissue induced by Rheopheresis. However, the improved tissue repair did not lead to clinical improvement according to Fontaine stages in all patients. Nevertheless, these results confirm the efficacy of Rheopheresis in improving microcirculatory dysfunction as is the case in patients with CLI. It is noteworthy that all patients who experienced improvement in their Fontaine stage, the responders, had a mean baseline $tcpO_2 \geq 33$ mm Hg. These results are in accord with the general opinion that a $tcpO_2$ below

20 mm Hg is associated with irreversible tissue damage, whereas a $tcpO_2$ over 40 mm Hg will lead to healing (8). During the Rheopheresis treatment series the responders approached $tcpO_2$ values comparable to those of healthy subjects. This finding underlines a strong relation between tissue oxygen supply (sufficient microcirculation), and clinical outcome (Fontaine stage classification), and can be used for the indication of Rheopheresis in patients with CLI.

The effect of Rheopheresis on $tcpO_2$, leading to an improved tissue oxygen supply and resulting in an improved clinical outcome as could be demonstrated in the present pilot trial paralleled analogous outcomes of Rheopheresis performed in patients with ischemic diabetic foot syndrome (18).

Unfortunately, due to loss of several patient values, the analysis of ABI could not be based satisfactorily on the present data. However, considering that values less than 0.5 are known for patients with severe disease and pain at rest, and tissue loss is typically expected with values of less than 0.3 (23), the available baseline values of 0.29–0.45 were at the lower limit of expected tissue loss, and consequently suggested only a moderate probability of improvement in ABI. In patients with severe disease with ABI below 0.5, the efficacy of Rheopheresis appears to be limited, as emphasized by the fact that patients with Fontaine stage IV did not benefit from Rheopheresis treatments.

By eliminating LDL-cholesterol, fibrinogen and α_2-macroglobulin Rheopheresis offers a safe and effective therapy for treating microcirculatory disorders (16,18). Increased levels of fibrinogen, α_2-macroglobulin, LDL-cholesterol and other plasma proteins lead to increased plasma viscosity, negatively affecting whole blood viscosity and finally resulting in microcirculatory dysfunction as found in patients with CLI. This result is accompanied by complex changes in the blood vessel system that are not understood in detail. Recently, it was shown that reduced blood flow leads to adaptive changes in the vascular and muscle extracellular matrix with raised circulating levels of matrix metalloproteinases and their inhibitors, reflecting an increase in proteolytic activity (27). This outcome has complex consequences of a dysregulated microcirculation, including a direct impact on disease severity in patients with CLI.

Over the last years evidence has accumulated underlining that in particular the plasma fibrinogen level is associated with the clinical progression of CLI. In the Edinburgh Artery Study a significant association of fibrinogen and vWF with the course of CLI was detected (15). Recently, it was also be demonstrated that in patients with PAD, platelet activation is increased. Particularly in patients with CLI, fibrinogen binding by stimulated platelets is significantly diminished (28), which leads to increased fibrinogen level in plasma and increased plasma viscosity. Dysregulated microcirculation in patients with CLI then worsens, impairing quality of life. Recently, a key role of fibrinogen for the course of CLI was again confirmed because there is a significant inverse linear association between physical activity and fibrinogen level (3). Since by performing Rheopheresis a fibrinogen reduction rate of approximately 50% can be achieved, the direct impact of Rheopheresis on the course of CLI becomes evident.

Considering CLI is an atherosclerotic disease and there is an established causal relation between plasma cholesterol and atherosclerosis, the effect of diminishing LDL-cholesterol and its positive impact on endothelial function by performing a series of Rheopheresis treatments becomes apparent. A reduction of LDL-cholesterol of up to 60% can be achieved by performing Rheopheresis. A recent study confirmed that the elevated plasma viscosity found in patients with CLI primarily depends on elevated plasma cholesterol levels (4).

There is an association between red blood cell (RBC) rigidity and CLI (29). RBC rigidity was found to be significantly increased in patients with CLI, resulting in impaired peripheral perfusion. By eliminating a selective spectrum of plasma proteins, Rheopheresis leads to decreased RBC rigidity. As the RBC becomes less rigid, the surface area increases and this again results in an increased oxygen delivery capacity (29).

CONCLUSION

The present pilot trial confirmed the assumption that a series of Rheopheresis treatments may have a benefit for patients with CLI. According to the presented results, patients most likely to improve clinically due to Rheopheresis are those of Fontaine stage III, with a $tcpO_2 \geq 30$ mm Hg and ABI ≥ 0.40. In contrast, in CLI patients with already irreversible changes (patients with Fontaine IV and tissue lesions), Rheopheresis appears to be of limited use. In those patients Rheopheresis showed no effect on clinical outcome, $tcpO_2$ and ABI.

An investigation of the potential long-term effect of Rheopheresis in patients with CLI, as already observed in patients with age-related macular degeneration (AMD), is required (17). In summary, as an

adjunct therapeutic option in the treatment of patient with CLI of Fontaine stage III, Rheopheresis may preserve a functional lower extremity, delay amputation or reduce the extent of amputation. A clinical trial with adequate patient numbers and follow-up to assess the relationship between Rheopheresis and frequency of amputation and mortality, is required to assess whether Rheopheresis for CLI may be a therapeutic option for clinical practice.

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