RHEOPHERESIS IN THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION

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The authors of the article declare that no conflict of interests exists in the compilation, theme and subsequent publication of this professional communication, and that it is not supported by any pharmaceuticals company. The study has not been submitted to any other journal or printed elsewhere.

Received: 16 August 2022
Accepted: 12 November 2022
Available on-line: 20 February 2023

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SUMMARY

Purpose: Evaluation of the long-term effect of rheopheresis treatment of dry form of age-related macular degeneration (AMD).
Materials and Methods: The treatment group consisted of 65 patients and 55 patients in the control group, with a minimum follow-up period of 60 months. The basic treatment consisted of 8 rheopheresis procedures, and the additional treatment (booster therapy) of 2 rheopheresis procedures 1.5–2 years after the basic treatment. We evaluated changes in best corrected visual acuity, anatomical effect, electrical activity of the retina, haematological, biochemical and immunological parameters.
Results: Rheopheresis treatment contributed significantly: 1) to stabilisation of best corrected visual acuity of the treated patients, which initially showed an insignificant increased during the 2-years follow-up period, and then slightly decreased. By contrast, visual acuity decreased in the control group, to an insignificant degree up to 4 years, then statistically significantly. 2) to an improvement of the morphological findings in 62.4% of treated patients compared to 7.5% in the control group, while disease progression to stage 3 (neovascular form of the disease or geographic atrophy) with a significant decrease of visual acuity occurred in only 7.1% of treated patients, versus 37.0% in the control group. 3) to regression, even to the attachment of drusenoid pigment epithelial detachment (DPED). To a reduction of the area of DPED in 80.4% of treated patients, in contrast with an increase in the area of DPED in 47.1% of patients in the control group, and the development of new DPED in only 2 eyes of treated patients compared with 16 eyes of patients in the control group. 4) to a preservation of the integrity of the ellipsoid layer in the fovea in 68.2% of the treated patients, while by contrast we found a damaged ellipsoid layer in the fovea in 66.6% of the control patients. 5) to a stabilisation of the activity of ganglion cells, the pineal system and the activity of the central area of the retina, with eccentricity between 1.8° and 30° in the treated patients, compared to alteration in the control group manifested mainly after 3.5 years of the follow-up period. 6) to a statistically significant improvement in rheological parameters, thereby increasing flow in microcirculation and positively influencing the metabolism in the retina. Also to a positive effect on the classical, alternative and lectin pathway of complement activation, a reduction in the level of proprotein convertase subtilisin kexin 9 (PCSK9), and thus also the level of LDL-cholesterol, and 7) Additional treatment with 2 RHF procedures (so-called "booster therapy") seems to be a safe and suitable method of prolonging the stabilisation phase, or even improving visual acuity, anatomical and functional findings.
Conclusion: We demonstrated positive changes in anatomical, functional and humoral parameters upon rheopheresis treatment of AMD. Their correlation provides a real possibility to identify patients at risk and to manage an individualised regime of rheopheresis therapy. This method of treatment is effective and safe, with a low percentage of non-serious adverse effects.
Key words: dry form of AMD, DPED, ellipsoid layer of photoreceptors, rheopheresis, ERG

Čes. a slov. Oftal., 79, 2023, No. 1, p. 8–24

INTRODUCTION

Age-related macular degeneration (AMD) is a degenerative disease of the central region of the retina and choroid. It is the most common cause of practical blindness in the population over the age of 65 years in developed countries [1]. The pathology occurs in two variants. In 80–90% of cases it concerns progressing "dry" atrophic form, and in approximately 10–20% of cases a rapidly developing "wet" exudative form develops, with a neovascular choroidal membrane [2]. A sudden transition of AMD into wet form over the course of 5 years is described in up to 26% of cases [3].

Primary changes in AMD have been described in the Bruch's membrane, in the retinal pigment epithelium (RPE) and in the choriocapillaris. A disorder of perfusion occurs in the choroid and retina, dysfunction of the RPE develops, and there is a loss of active transport of metabolites from the retina. Metabolites then accumulate in the form of drusen, as we have described ourselves [4]. Soft drusen are pale yellow coloured, with an unclear boundary, individual drusen are no larger than 165 µm in diameter. Upon the progression of the disease, they merge into confluent drusen, which have a larger diameter and a tendency to advance to a more serious stage: drusenoid pigment epithelial detachment (DPED) [5,6]. If DPED also affects the central foveola (CF), in this stage a deterioration of best corrected visual acuity (BCVA) occurs, because changes of the RPE are accompanied with a defect of the ellipsoid layer of the internal segments of the photoreceptors. This results in damage to and loss of photoreceptors [7]. With advancing age, a terrain of present DPED provides fertile conditions for transition to the wet form of AMD, with the onset of choroidal neovascularisation (CNV). This is located in the choroid and is a reaction to local hypoxia. It is part of the 3rd stage of AMD, which if untreated leads to practical blindness, reducing visual acuity to 3/50 or less. Moreover, persistent DPED may also cause a further manifestation of irreversible damage to the macula, and the progression of geographic atrophy of the RPE. Upon affliction with this form, we usually again sooner or later encounter the onset of practical blindness.

Evidence has been discovered attesting to a genetic burden of patients with AMD, and a correlation of a number of genetic abnormalities with AMD has been found [8,9]. A revolutionary transformation of opinions on the pathogenesis of the disease is represented by the "inflammatory" theory. The correlation of the progression of AMD with inflammatory and immune processes is supported by the presence of immunocomplexes (IgM, IgG) and a component of the complement system (C3, C5, CD46) in drusen. It is known that complement proteins, macrophages and cytokines accelerate the angiogenesis, formation and proliferation of CNV [10]. The onset and progression of AMD is evidently also influenced by a mutation of gene H [8] and/or oxidative damage, leading to apoptosis of the photoreceptors [11]. Naturally, endeavours to suppress the activation of complement have been commenced. Inhibitors of complement components have been developed [12], as well as monoclonal antibodies against factors B and D, or C5 inhibitors with the possibility of use in AMD, with greater effectiveness and less frequent application [8]. The trials are currently in phase 1 or 2, and will evidently continue for several years to come before they will be available in clinical practice. These observations have led us to conduct research into activation pathways of complement and H component in rheopheresis treatment as potentially significant biomarkers of the progression of AMD. It is known that complement, in particular the alternative and lectin pathways, plays an important role in microcirculation disorders in AMD [8,9]. Treatment targeted at the complement system will probably represent another new approach to the treatment or prevention of microcirculation disorders such as AMD and others. According to our hypothesis, changes in the complement components could also be a marker of the success of treatment, and a correlation could therefore exist between the extent of the decrease of activity of complement components, and the activity of the pathology and the clinical picture. It would be highly valuable if this concerned an early marker. To date the success rate of treatment has been assessed by means of clinical indicators (development of morphological changes on the retina, deterioration of BCVA etc.), which are long-term. They therefore do not enable individualised management of the frequency and intensity of therapy already during the basic series of rheopheresis treatments. Also important is the correlation of the complement changes not only with the activity of the pathology and the clinical finding, but also with other bio-indicators (endothelial activity markers, coagulation, fibrinolysis, lipoprotein metabolism) and with special indicators (apoptosis activation factors, selected interleukins, adhesion molecules).

Hypotheses concerning the pathogenesis of AMD also mention the probable contribution of the lipoprotein metabolism in the onset and progression of the disease. The retina, with its capacities for processing light, is a unique photooxidative environment among other types of nerve tissues. It therefore evidently has a capacity for uptake of lipids and their metabolism, including oxidative processes. The intake of lipids from the blood into all the cell layers of the retina has been demonstrated [13,14]. The retina is also capable of synthesising lipids and maintaining a "steady--state" in terms of lipid composition [15]. The performance of these tasks takes place thanks to the same molecules (confirmed in the retina) as in the pathophysiological process of uptake and lipid metabolism elsewhere within the internal environment [15]. An important enzyme influencing the cholesterol level is the proprotein convertase subtilisin/kexin 9 (PCSK9). This is a secretion regulator of the LDL receptor on the surface of the cells, whose function is to increase the level of LDL cholesterol [16]. More than 30% of plasmatic PCSK9 is bonded to LDL-cholesterol, and as a result we assume that rheopheresis performed for AMD could also bring about a reduction of the level of PCSK9.

Treatment of dry form of AMD currently relies upon preventive pharmacotherapy with antioxidants of vitamins E and C, beta carotene, copper, zinc and unesterified lutein (Age-related Eye Disease Study 1, Age-related Eye Disease Study Research Group 2001, Lutein Antioxidant Supplementation Trial) [17].

Rheopheresis (RHF) is considered to be an effective method of treating dry form of AMD. A number of studies, including our own, have demonstrated the effectiveness of rheopheresis [6,18-22]. According to Schwartz et al., 2013 (Writing Committee of the American Society for Apheresis), a number of case reports have been published, as well as two controlled trials and five randomised trials which demonstrate the effectiveness of rheopheresis in treating dry form of AMD [20]. On the basis of these studies, the ASFA (American Society for Apheresis) included rheopheresis in the last but one version of the

regularly updated guidelines in 2013 as the first line treatment for dry form of AMD [23]. In 2005 we were the first in the Czech Republic to commence the treatment of patients with AMD using rheopheresis. The treatment was paid for by resources from grants. The cost for 1 RHF procedure is approximately CZK 17 000.00, thus basic treatment with 8 procedures costs CZK 136 000.00.

The expected positive effects of rheopheresis on AMD are a reduction of the diffusion barrier in the Bruch's membrane, improvement of nutrition of the RPE and neuroretina, a reduction of ischaemia and the formation of vascular endothelial growth factor (VEGF) and a reduction in the activity of the inflammatory process, which are demonstrated pathophysiological mechanisms of the onset and progression of the pathology [24]. An adjustment of the parameters takes place on a molecular level, as well as activation of the functional reserves of the retina. All of these factors can contribute to the stabilisation of the morphology and function of the macula, which is the most important area for sight, as well as to a halting or at least slowing of the progression of AMD [6]. Our aim is to prevent transition to the 3rd stage of AMD, in particular to avert the onset of choroidal neovascular and geographic retinal atrophy in the macula, and therefore to protect the central foveola from affliction by pathological changes.

Rheopheresis and our own modification of the procedures

According to Klingel et al. [24], rheopheresis is a modification of membrane differential plasma filtration, in which a special secondary plasmatic filter with relatively small pores known as a rheofilter is used, which eliminates a precisely defined spectrum of proteins with a high molecular weight (above 150 000 Daltons) from the plasma passing through the filter. This especially concerns fibrinogen, α_2-macroglobulin, immunoglobulin M, LDL cholesterol, von Willebrand factor, thrombomodulin and fibronectin [25]. The elimination of these proteins leads to a reduction of blood and plasma viscosity, and also to an improvement of the aggregability and flexibility of erythrocytes [26,27]. The result of reduced blood and plasma viscosity is an improvement of blood flow through microcirculation and a therapeutically beneficial influence on certain disorders in microcirculation [10]. It brings about propitious shifts in the levels of cytokines and adhesive molecules, as well as increased production of nitrous oxide by the endothelium, and an improvement of the deformability of erythrocytes [26].

In our own modification of rheopheresis (also known as rheohemapheresis), a Cobe Spectra or Spectra Optia (Terumo BCT, Lakewood, Colorado, USA) continual separator was used for the primary separation of plasma. In the second step, the plasma passed through an Evaflux 4A (Kawasumi, Tokyo, Japan) filter. The filtration membranes have a total surface area of 2 m^2, they are produced from ethylene copolymer and vinyl alcohol and are arranged into the form of capillaries with an internal diameter of 175 µm and a pore diameter of 0.03 µm. The capillaries are enclosed in casing made of polycarbonate resin. The filter is placed and controlled by a CF 100 (Infomed, Geneva, Switzerland) device. Upon an increase in pressure in the capillary filters above 250 mm Hg, the CF-100 device automatically backwashes the capillaries with a physiological solution, which is drained off into a waste sack together with the filtered particles. All the sets of connecting tubes, the filter and the waste sack are designated for single use.

After passing through the filter, the treated plasma together with the blood cells is returned back into the patient's circulation via a second venous entry. The procedure is continuous, the patient's blood is taken and returned simultaneously. The quantity of blood and plasma currently in extracorporeal circulation is determined by the used separator (in the case of the Spectra Optia separator this is 185 ml, with the Cobe Spectra 240 ml). The procedure requires continual anticoagulation, in rheophereses we used a combination of ACD-A (Baxter, Munich, Germany) and an initial single intravenous administration of unfractionated heparin in a dose of 4000 j. The ACD-A solution was used in a ratio of 1:22 to blood. The absolute majority of the procedures were performed in outpatient care, from two peripheral venous entries (veins in the cubital fossa are most suitable).

The working procedures used in rheopheresis differ according to individual centres, each of which has its own modification of extracorporeal elimination. The key parts of our modification have been patented (patents no. 301836/2013, no. 301836/2010 and no. 3050387/2015). During the course of treatment, the necessary adjustments of the method were performed, with improvements according to past experiences (e.g. improvement

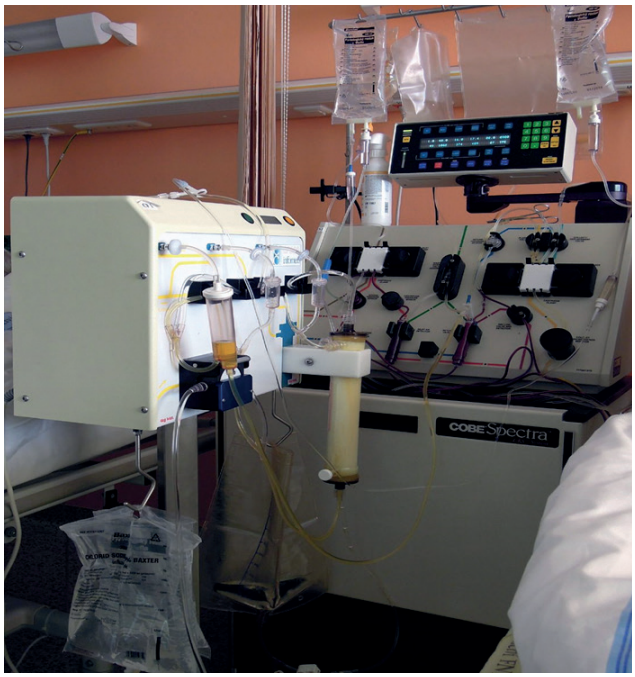


Figure 1. Rheopheresis equipment at the patient's bedside

of the method of cannulation of the peripheral veins and central catheterisation using a safer cannula with a more appropriate diameter, adjustment of the method of anticoagulation, refinement of the set of basic safeguarding haematological and biochemical markers). Patient care was also improved (experience of team, constant monitoring of 02 saturation, bedside alarm devices, heated beds, ECG, pulse and respiratory rate monitoring).

The bedside device is presented in Fig. 1, the scheme of the procedure is described in Fig. 2: the plasma is separated from the blood corpuscles with the aid of a Cobe-Spectra or Optia (Likewood, Oklahoma, USA) separator, and then pumped into the lines of a CF 100 (Infomed, Geneva, Switzerland) device, which controls the flow of plasma through an Evaflux filter (Kawasumi, Tokyo, Japan). After passing through the filter, the plasma together with the blood elements is fed back into the patient. Further details and experiences with our modification of the method are described elsewhere [10,29,30-32].

The procedures take 3–4 hours, depending on the quantity of washed blood and the through-flow of blood, which is determined primarily by the condition of the peripheral vein used for extraction. During the procedures, patients have their arms trussed for the insertion of the extraction needles into both cubital fossae. It is therefore necessary to ensure patients have small needs catered to, such as adjustment of their position in case of complaints caused by stiffness, alleviation of any itching, provision of fluids, feeding for diabetics, urination etc. Due to their age, patients with AMD usually suffer also from other complaints and diseases, which must be taken into account and respected. Monitoring of patients' clinical condition during the procedures, including essential vital functions (pulse, blood pressure, respiratory rate, or ECG monitoring), was obligatory and performed according to the Standard Operating Procedure of the Separator Centre at the University Hospital in Hradec Králové, which is regularly validated and checked by internal and external audits. In addition, a detailed protocol was prepared for the monitoring and registration of adverse reactions, either immediate or long-term – 26 items based on experience from the literature, our own previous results and observations [30], and the regulations of the WAA (World Apheresis Association) register [33]. All adverse effects in connection with apheresis were subsequently evaluated according to the uniform schema of the WAA register, and entered and sent electronically via a registration code to the WAA register [34].

Before the procedure, patients were enabled to observe other patients in natura during the course of treatment – the aim was as far as possible to allay any unnecessary fears before the first procedure, which is when a larger percentage of adverse reactions occur. Before the commencement of rheopheresis, the patient's condition was again assessed, and the medical history supplemented. Attention was focused on pharmacological history, since if patients use ACE-inhibitors there is a risk of adverse bradykinin reactions. An objective internal examination was performed, including an assessment of the condition of the peripheral veins, measurement of pulse and pressure by auscultation. The patients signed an informed consent form. During

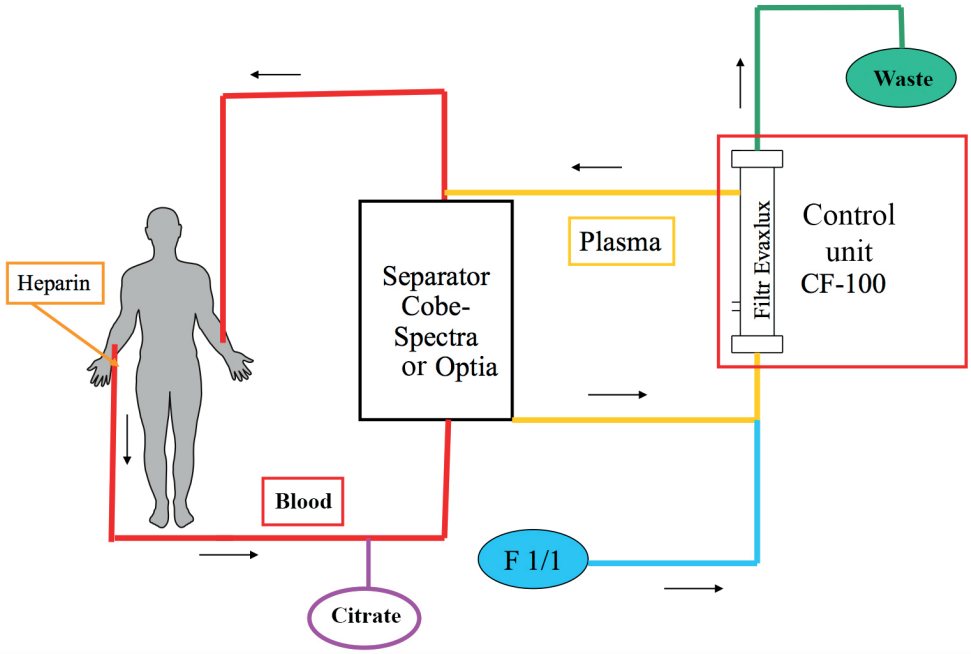


Figure 2. Schematic of the rheopheretic procedure
Legend: the plasma is separated from the blood cells using a Cobe-Spectra or Optia separator and then pumped into the working lines of the CF 100 device, which controls the plasma flow through the Evaflux filter. The plasma is led together with the blood elements back to the patient after passing through the filter

apheresis, the patients were continuously monitored by the staff of the Separator Centre. In the case of serious adverse occurrences of a severe degree, the assistance of the intensive care unit team was secured (called by the clinic alarm system, due to the close proximity it was possible to provide specialist aid within dozens of seconds).

MATERIAL AND METHODS

Cohort of patients

To date we have treated a total of 84 patients using rheopheresis, with an additional 72 patients randomised into a control group. We included 65 patients with an average age of 69.3 years (range 52–85 years; 35 women; 27 men) in the evaluation, and 55 patients with an average age of 73.5 years (range 56–82 years; 42 women and 10 men) in the control group, with a minimum observation period of 60 months (average observation period 106 months, maximum observation period 141 months). The patients in the control group were monitored at the same time intervals as the patients treated with rheopheresis.

Based on our long-term experiences we stipulated the indication criteria and contraindications of treatment by rheopheresis. Indication for treatment was the presence of AMD with soft drusen in stage 1–3 according to the EURYEYE study (Augood et al. 2004), or drusenoid ablation of the RPE (DPED), with best corrected visual acuity within the range of 20/20 to 20/125 (monocular 20/160) of ETDRS optotypes, age within the range of 50–80 years, body weight above 50 kg and good quality of peripheral veins for repeated therapeutic procedures. Ocular contraindications are the presence of wet form of AMD, geographic atrophies in the macula or other irreversible changes, pathologies of the retina and choroid other than AMD (dystrophy, inflammatory diseases), acute haemorrhage on the ocular fundus, pathology of the optic nerve including glaucoma and opacity of optic media limiting examination of the ocular fundus. General contraindications include severe general pathologies, e.g. malignancy, ST segment elevation myocardial infarction and central stroke in the last 3 months, and uncorrected arterial hypertension of ≥ 160/100.

All patients underwent a **basic treatment** of 8 rheopheresis procedures, performed twice in one week, followed by a two-week interval, and the pulse was repeated 4x. At the same time, we performed 2 additional rheopheresis "booster therapy" procedures on 20 patients who had undergone a basic series of rheopheresis procedures 1.5 to 2 years previously.

Ophthalmoscopic finding: In the group of patients who received treatment, soft drusen and confluent soft drusen were present in 60 eyes, and drusenoid ablation of the RPE was present in 56 eyes. Wet form of AMD was diagnosed in 14 eyes, and these were not included in the evaluation. In the control group, soft drusen and confluent soft drusen were present in 54 eyes, and drusenoid ablation in 38 eyes. We determined wet form in 18 eyes, which were not evaluated. However, we included only those patients on whom spectral domain optical coherence tomography (SD-OCT) had been performed from the beginning of the observation period in the evaluation of the morphological finding.

Examinations

Ophthalmological examinations

- Best corrected visual acuity (BCVA) on ETDRS optotype tables.
- Monitoring of development of soft drusen and drusenoid pigment epithelial detachment (DPED) during the course of treatment in [mm2] using the VISUPAC program on a digital fundus camera (FF 450 + IR, Zeiss).
- State of retinal circulation by fluorescence angiography (FAG) on a digital fundus camera (FF 450 + IR, Zeiss), from 2019 angio-OCT (Spectralis, Heidelberg).
- Optical coherence tomography (OCT; Cirrus, Zeiss from 2009; 2005-2009: Stratus, Zeiss) for verification of drusen deposits, determination of retinal thickness and anatomical changes, especially in the region of the ellipsoid photoreceptors.
- Electroretinography examination (ERG) for objective determination of the functional condition of the retina:

a) Flash ERG for testing activity of the rod and cone system.
b) Multifocal electroretinography (mfERG) specifically detecting localised changes of the photoreceptors and their bipolar cells in the central region of the retina.
c) Pattern-reversal ERG (pERG) recording activity of the ganglion cells and central region of the retina.

Observation of patients

Time schedule of ophthalmological examinations: before commencement of treatment, then 3, 6 and 12 months after treatment the following examinations were performed: BCVA, fundus photography, electrophysiological examinations and OCT. FAG/angio-OCT before commencement of treatment, and also in case of suspicion of progression to wet form of AMD. If additional rheopheresis was indicated after 1.5 to 2 years, the cycle of all examinations was repeated, otherwise examinations were performed at six-monthly intervals.

Biochemical, haematological and immunological parameters

We examined the complete basic biochemical and haematological profile [29,31], also parameters with vasoactive, coagulation or other pathogenetic activity, or which were defining for the effectiveness and safety of the procedures:

- lipoproteins: total cholesterol, LDL-cholesterol, HD
- cholesterol, Lp(a), apolipoprotein A and B;
- with regard to the active contribution of selectins (adhesion molecules) or other adhesion molecules and macrophages in the activity of atheromatosis: examination of sP-selectin, MCP-1 (monocyte chemotactic peptide) and endoglin;
- dynamics of special markers which could attest to inflammatory activity (IL-10), markers of the status of cell immunity (soluble antigen CD40), endothelial activity, or apoptosis markers (sAPOfas);
- monitoring of parameters of pathophysiology and function of blood platelets: number, MPV (mean platelet volume of thrombocyte), PDW (platelet distribution width

according to volume) and PCT (platelet haematocrit);
- basic indicators of endothelial activity (thrombomodulin, vW-factor) and fibrinolysis (fibrinogen, tPA = tissue plasminogen activator and its inhibitor PAI-1);
- rheological parameters (blood and plasma viscosity, fibrinogen, α2-macroglobulin, immunoglobulins);
- complement: all three basic pathways of complement activation (classic, alternative and lectin), and component of H complement;
- PCSK9: proprotein convertase subtilisin/kexin 9), secretion regulator of LDL receptor on cell surface.

RESULTS

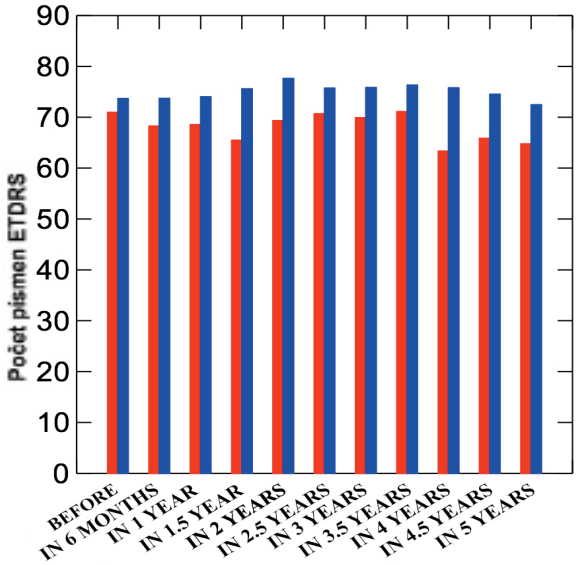
Best corrected visual acuity

Treated patients

In the observed group of patients who underwent treatment by rheopheresis, we determined only an insignificant fluctuation of BCVA over the course of a five-year observation period. Up to 2 years of observation BCVA increased to an insignificant degree, after which there is nonetheless a perceptible trend towards deterioration (Graph 1).

Control group

In the control group of patients, during the course of a four-year observation period we determined a statistically insignificant deterioration of BCVA. After five years of observation the deterioration of BCVA was now statistically significant ($p = 0.031$), see Fig. 3. The differences in BCVA between the groups of patients at the beginning and up to 4 years of observation were only insignificant. After 4 years of observation, BCVA in the control group was significantly lower in comparison with the treated patients ($p < 0.05$ and $p < 0.01$).



Graph 1. Development of BCVA over time. On the x-axis: LETTERS – the number of letters read before the procedure, in 6 months, in 1 year, in 2, 3 and 5 years. On the y-axis is the number of letters read according to ETDRS

BCVA – best corrected visual acuity; ETDRS – early treatment diabetic retinopathy study; RHF – rheopheresis

Morphological changes

We considered a reduction of the surface area of soft drusen by more than 25% of the original surface without a progression to DPED, also a reduction (by more than 25% of the original surface) or complete disappearance of DPED without the onset of subsequent RPE atrophy, without progression to the 3rd stage of AMD, to constitute an improvement of the finding. An evaluation of without change means stabilisation of the finding within the range of ±25% of the original surface of the pathological changes (surface area of drusen, DPED). We consider the progression of the surface of soft drusen and an increase in the surface of DPED by > 25% of the original surface, the onset of isolated RPE atrophy with affliction of the cent-

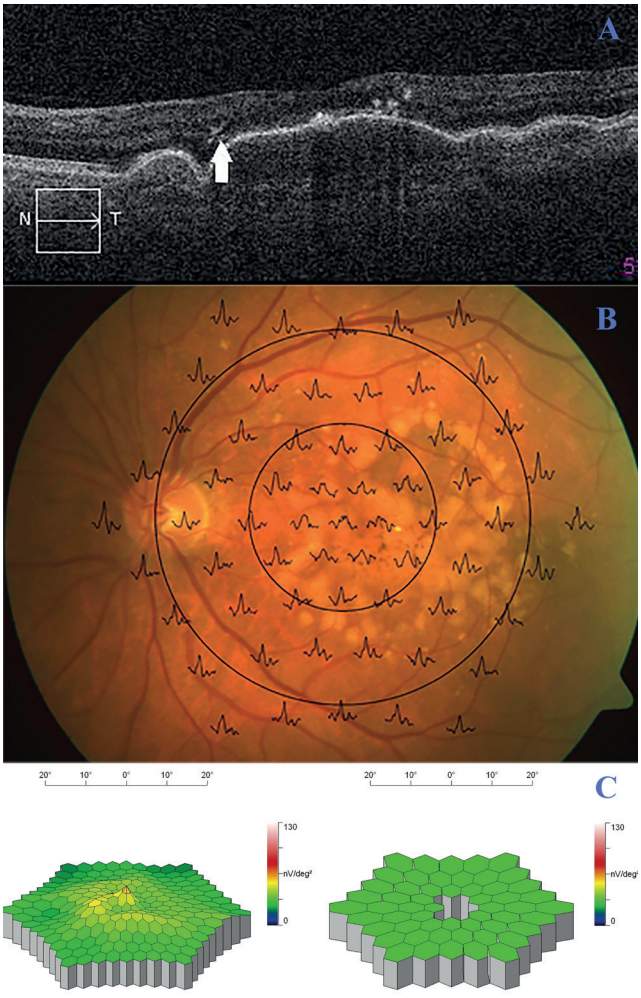


Figure 3. Ophthalmological findings of the patient before rheopheresis. (**A**) Optical coherence tomography of the patient's left eye before rheopheresis. A thin layer of photoreceptor ellipsoids begins to detach from the extensive drusen ablation of the retinal pigment epithelium (arrow). (**B**) Superposition of the mfERG response on the fundus of the patient's left eye. (**C**) Three-dimensional mfERG image. On the left, a three-dimensional image of the electrical activity of the retina of the patient's left eye; on the right, comparison with the age norm (decrease in the activity of foveolar and parafoveolar responses)

mfERG – multifocal electroretinography

ral fovea (CF), as well as the occurrence of pigmentations in this localisation, to represent a slight deterioration. By contrast, other slight increase in pigmentation may be of benefit and protect against the onset of choroidal neovascularisation. We consider the onset of geographic atrophy and a progression to wet form of AMD on the basis of the development of new CNV to represent a pronounced worsening of AMD. The development of morphological changes in the groups of patients is presented in Table 1.

In conclusion it is possible to summarise that the results demonstrate a pronounced improvement in the first years following rheopheresis, but over the course of time a process of deterioration of the morphological finding may occur. This raises the question of the suitability of repeating rheopheresis after a period of 1, 2 or 3 years for the purpose of preventing a reversal of the adjustment of the physiological finding, resulting in deterioration. Upon a comparison of both groups, a significant capacity for improvement of the morphological finding in the macula after rheopheresis dominates in comparison with the natural finding, influenced by metabolic changes in old age, in the control group.

Drusenoid ablation of the pigment epithelium

Treated patients

Before treatment we determined DPED in 56 eyes (28x RE and 28x LE). From the commencement of treatment up to 5 years of observation we recorded a reduction of the surface of DPED in 45 eyes (23x RE and 22x LE). Of these, we recorded complete disappearance of DPED in 6 eyes (3x RE and 3x LE), 4 of which were without manifestations of RPE atrophy. In 2 eyes of one patient, the disappearance of DPED was followed by the development of pronounced RPE atrophy, evidently due to the originally large surface of DPED which had persisted for several years, accompanied with a deterioration of vision. In 1 eye of this patient the pathology progressed, with the development of CNV 7 years after treatment. Geographic atrophy developed upon reduction of the surface of DPED in only 3 eyes. DPED remained without significant changes in 4 eyes (1x RE and 3x LE). We determined an increase in the surface of DPED in 6 eyes, in 2 eyes of one patient with the development of CNV within 1 year of treatment. We determined newly occurring DPED in 2 eyes (1x RE and 2x LE), of which CNV occurred in one eye 3 months after treatment, see Table 2.

Control group

Before treatment we determined DPED in 17 eyes (10x RE and 7x LE). From the commencement of treatment up to 5 years of observation we recorded a reduction of the surface of DPED in 10 eyes (7x RE and 3x LE). We recorded complete disappearance of DPED in 2 eyes (1x RE and 1x LE). The disappearance of DPED in 2 eyes and the reduction of the surface of DPED in 2 eyes was accompanied by the development of CNV. The reduction of the surface of DPED was replaced by the development of geographic atrophy in 2 eyes (1x RE and 1x LE), one eye with a reduction of DPED ended with improvement. DPED remained without significant changes in 2 eyes (2x RE). We determined an increase in the surface of DPED in 8 eyes (2x RE, 6x LE), in one patient with the development of CNV within 1 year of treatment. The differences between the patients and the control group presented in

Table 1. Development of morphological changes in patient groups

Morphology	Treated patients (85 eyes)	Control group (54 eyes)
Improvement	62.4% (53/85)	7.4% (4/54)
Unchanged	18.8% (16/85)	3.7% (2/54)
Slight worsening	11.8% (10/85)	51.9% (28/54)
Significant impairment	7.1% (6/85)	37.0% (20/54)

Table 2. Changes in DPED over time in patient groups

DPED	Patients treated (56 eyes)	Control group (20 eyes)
Area reduction	80.4% (45/56)	50% (10/20)
Unchanged	7.1% (4/56)	10% (2/20)
Area enlargement	10.7% (6/56)	40% (8/20)
The newly created DPED	2 oči	16 očí

DPED – Drusenoid Pigment Epithelial Detachment

Table 3. DPED area at the start and end of follow-up

DPED (mm²)	Start	End of follow-up	Statistics
RHF	6.78 ±3.79	4.13 ±3.84	$p < 0.001$
Controls	4.09 ±3.48	6.69 ±4.2	$p < 0.001$
Statistics	$p = 0.012$	$p = 0.015$	

DPED – Drusenoid Pigment Epithelial Detachment; RHF – rheoferéza

Table 2 by a reduction or increase of DPED are significant in favour of the treated patients, on a level of $p < 0.001$.

DPED surface area

The mean value of DPED at the beginning of observation in patients treated with rheopheresis was at a size of 6.78 ± 3.79 mm^2, and by the end of observation had been reduced to 4.13 ± 3.84 mm^2 ($p < 0.001$). In patients in the control group the mean size of DPED at the beginning of observation was 4.09 ± 3.48 mm^2, at the end of observation it had increased to 6.69 ± 4.2 mm^2 ($p = 0.001$). During the course of observation we determined the onset of CNV in two eyes in the group treated with rheopheresis, and in six eyes in the control group. A comparison of the groups is illustrated by Table 3.

Condition of ellipsoid layer of photoreceptors

We evaluated the condition of the ellipsoid layer in a subgroup of 24 patients with DPED; 12 patients (22 eyes) were treated with 8 RHF procedures, and 12 patients (18 eyes) were from the control group.

Treated patients

At the beginning of observation, in the group of treated patients the ellipsoid layer of photoreceptors was intact in 6/22 (27.3%) eyes, and remained without defect in all of these eyes 2.5 years after the procedure. In the remaining 16/22 eyes (72.7%) DPED was accompanied with detachment of the ellipsoid layer, without any defect thereof in 15/22 eyes. We determined a defect of the ellipsoid layer in only 1 eye (Fig. 3 A, B and C), and this defect persisted and spread to the foveolar region after 2.5 years of observation (Fig. 4 A, B and C).

At the end of observation we determined a reattachment of the ellipsoid layer in 8 eyes (50% of the original 16 eyes with detachment). We determined residues of detachment of ellipsoids in 7 eyes, enlarged in 1 eye and minimal in 6 eyes. In 15 eyes (68.2%) we did not find any defect of the ellipsoid layer (6 eyes with an originally continuous layer of ellipsoids and 9 eyes with original detachment of the ellipsoid layer). We observed a defect of ellipsoids in 7/22 eyes (31.8%). Fortunately this defect reached the central foveal region in only 4 eyes (8.8%), with a negative impact on the function of the photoreceptors and visual acuity (VA) (Fig. 5).

Control group

At the beginning of observation, in the control group the ellipsoid layer of photoreceptors was attached to DPED in 6/18 eyes (33.3%). In the remaining 12/18 eyes (66.6%) DPED was accompanied with detachment of the ellipsoid layer, without any defect thereof in 9 eyes, and with a defect in 3 eyes. The original detachment of the ellipsoid layer was enlarged in all 12 eyes.

At the end of the observation period, the integrity of the ellipsoid layer was preserved in only 3/6 eyes (16.7%) with an originally attached ellipsoid layer and in 3/6 a defect of the layer developed, which did not reach the foveola. In 9/12 eyes we determined the development of defects of ellipsoids, and in 3/12 eyes the original defects were enlarged. In total a defect of ellipsoids was diagnosed in 15/18 eyes (83.3%), which in 12 cases reached into the foveolar region with a negative impact on vision (Fig. 6 A, B and C). Furthermore, in 6 eyes with original detachment of ellipsoids, choroidal neovascularisation (CNV) developed. The development of CNV is often preceded by a rupture of the detached ellipsoid layer of the photoreceptors, later followed by the development of swelling of the internal layers of the retina (Fig. 7).

In conclusion we can state that at the end of observation, 15 eyes (68.2%) of the treated patients remained without damage to the ellipsoid layer. We determined defects of the

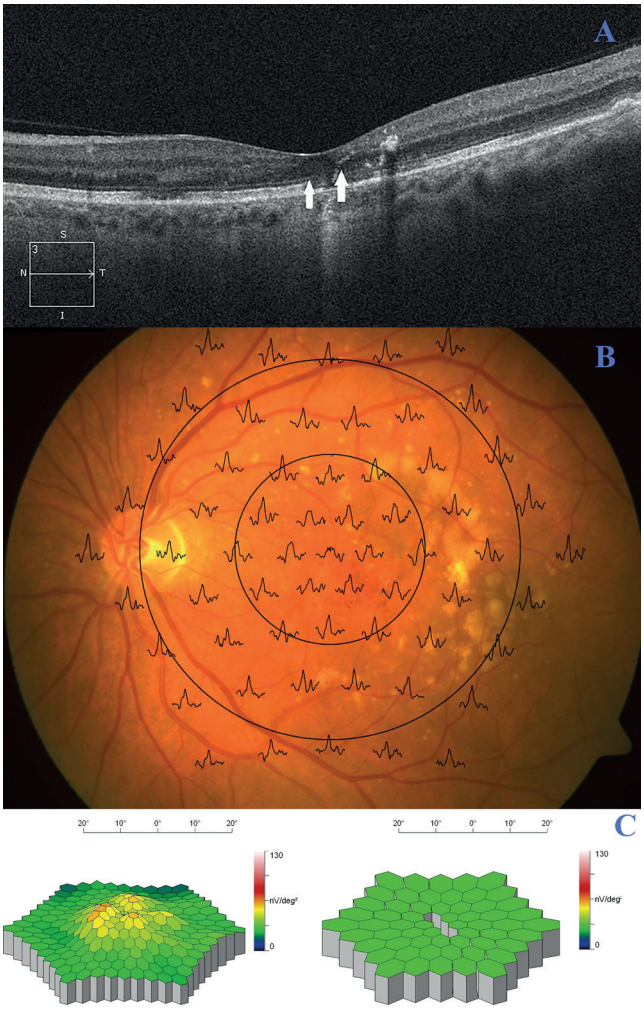


Figure 4. Ophthalmological findings of the patient from Figure 4 after rheopheresis. (**A**) Optical coherence tomography of the patient's left eye. After rheopheresis, DPED and ablation of photoreceptor ellipsoids, a risk factor for reversion to the wet form of AMD, took place. We consider the 2 small deposits of defects of the ellipsoidal layer to be the result of previous ablation of this layer (arrows). (**B**) Superposition of mfERG responses on the fundus of the patient's left eye. (**C**) Three-dimensional image of electrical activity of the retina of the left eye, rise after treatment; on the right, comparison with the age norm (increase in parafoveolar activity to the normal range)

DPED – Drusenoid Pigment Epithelial Detachment; AMD – Age-related macular degeneration; mfERG – multifocal electroretinography

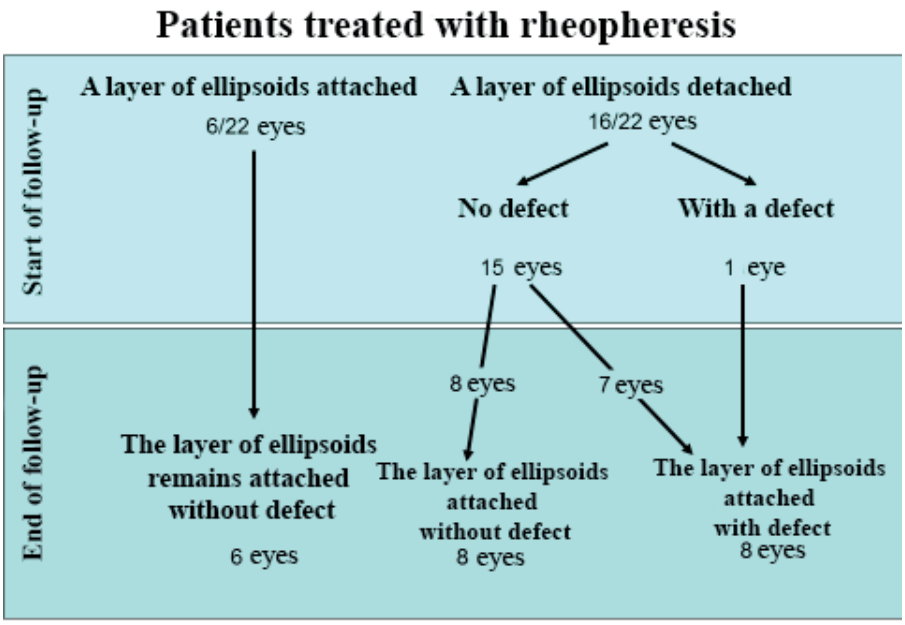
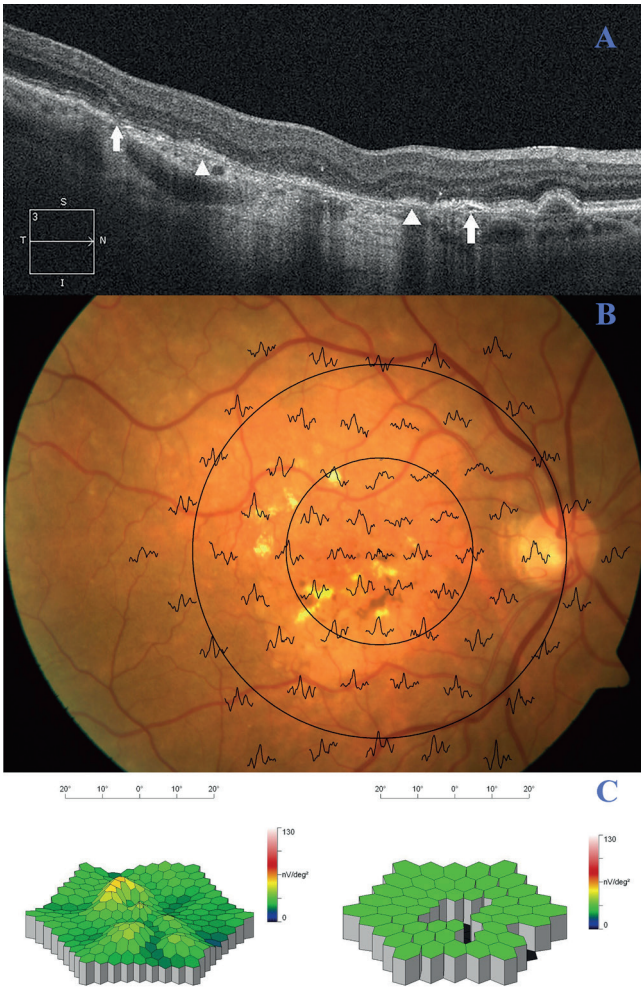


Figure 5. Status of the ellipsoid layer over time in treated patients



layer in 7 eyes, in only 4 of which the defect reached the foveola, with a negative impact on vision. In the control group we determined defects of the ellipsoid layer in 15 eyes (83%), in 12 cases of which the defect reached the foveola, causing a deterioration of VA. Multifocal electroretinography demonstrated significantly higher amplitudes of parafoveolar responses in the region between 1.8° and 7° eccentricity in the treated patients ($p = 0.04$) at the end of the observation period. The improvement of rheological parameters contributed to maintaining the integrity of the ellipsoid layer in the fovea, which is a fundamental factor for preserving visual acuity.

Electroretinography

Pattern-reversal electroretinography (pERG): Activity of central retinal region (wave P50) and ganglion cells (wave N95)

We determined an insignificant fluctuation of amplitudes of wave P50 during the entire observation period in both groups of patients. The amplitudes of wave N95 changed

Figure 6. Ophthalmological findings of a patient from the control group. (**A**) Optical coherence tomography of the patient's right eye. An asymmetrically ellipsoid layer of photoreceptors (▲) splits off from the DPED, the edges of its defect are marked with arrows. (**B**) Superposition of mfERG responses on the fundus of the patient's right eye. Residual foveolar and parafoveolar activity. (**C**) Three-dimensional mfERG image. Left: retinal electrical activity image of the right eye with absent foveolar response; on the right, comparison with the age norm (decrease in foveolar, parafoveolar and part of paramacular responses)

DPED - Drusenoid Pigment Epithelial Detachment; mfERG – multifocal electroretinography

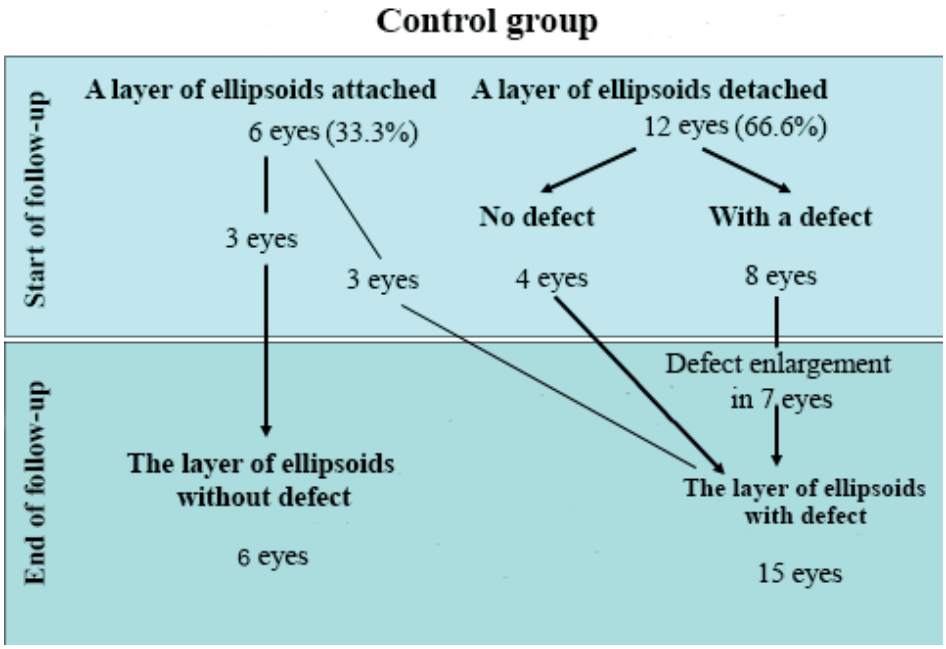
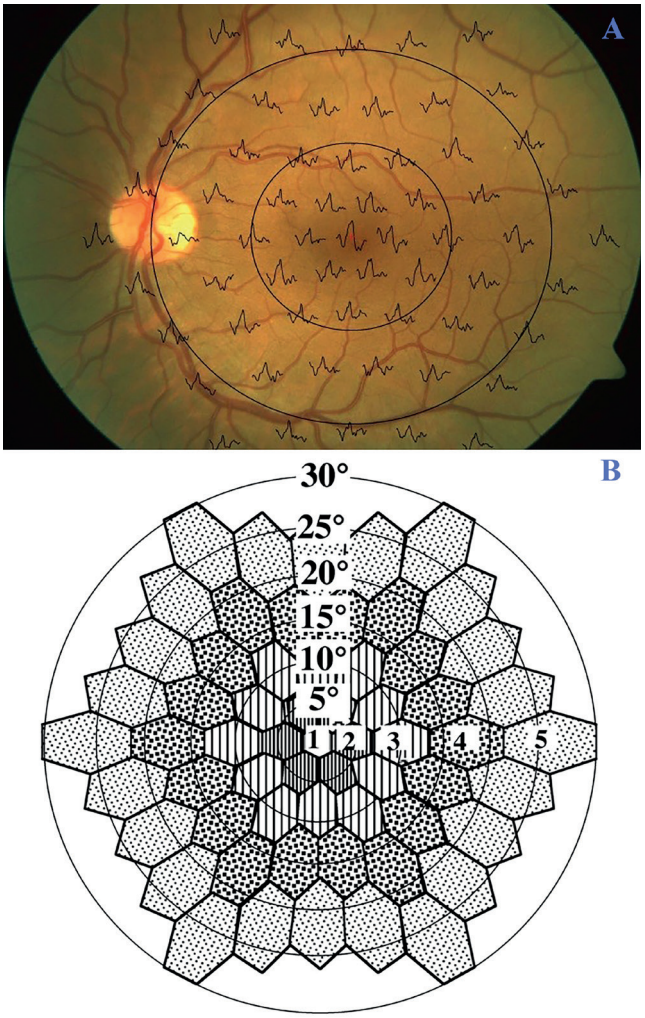


Figure 7. State of the ellipsoid layer over time in the control group



to an insignificant degree in the treated patients up to 3 years, but then showed a statistically significant reduction ($p < 0.05$ and $p < 0.01$). In the control group the waves showed a statistically significant reduction already after 1 year of observation ($p < 0.01$) and remained significantly lower in comparison with the original values ($p < 0.05$ to $p < 0.01$).

The latencies of both waves (P50 and N95) remained practically on the original level in the treated patients up to 3 years of observation, and then manifested a statistically significant prolonging ($p < 0.05$ and $p < 0.01$). By contrast, in the control group a tendency of progressive increase was manifested during the whole course of the observation period, which is predominantly statistically significant ($p < 0.05$ to $p < 0.01$).

Flash scotopic electroretinography (ERG): activity of rod system

Scotopic: activity of rod system

We predominantly determined only an insignificant fluctuation of the amplitudes of rod responses and oscillation potentials during the course of the observation of both groups of patients. The a- and b-wave amplitudes of maxi-

Figure 8. Multifocal ERG in physiological ocular findings. (**A**) Superposition of local mfERG responses on the left fundus. Sixty-one local multifocal ERG responses superimposed on a funduscopic image of the left eye with maximal response in the foveola and decreasing activity to the periphery. (**B**) Clustering of local mfERG responses into groups according to eccentricity. For routine evaluation, local mfERG responses are grouped into central: foveolar response (1) and 4 concentric circles (2–5) of increasing eccentricity, for which the mean positive peak response is calculated

mfERG ¬– multifocal electroretinography

mum response progressively decreased in both groups of patients, in the treated patients predominantly insignificantly and in the control group significantly ($p < 0.05$ to $p < 0.01$).

The latencies of scotopic responses remained practically on the original level up to 3 years in the treated patients, and afterwards they were statistically significantly prolonged ($p < 0.05$ and $p < 0.01$). By contrast, in the control group a tendency of progressive increase was manifested during the whole course of the observation period, which is predominantly statistically significant ($p < 0.05$ to $p < 0.01$).

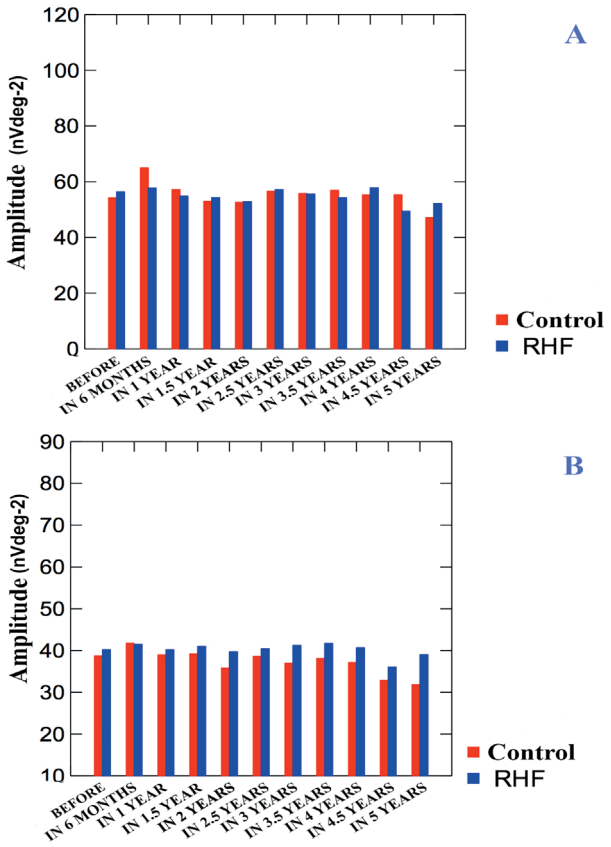
Photopic: activity of cone system

We determined relatively stable amplitudes of photopic responses in both groups of patients up to 3.5 years of observation, after which we recorded a decrease of activity, in the group of treated patients statistically insignificantly, and in the control group to a statistically significant extent ($p < 0.05$ and $p < 0.01$). The differences in amplitudes between the groups of patients were predominantly only insignificant. The latencies of the majority of responses were prolonged during the course of the observation period insignificantly in both groups of patients, and in the predominant majority were longer in the control group in comparison with the treated patients ($p < 0.05$).

Multifocal electroretinography (mfERG): activity of the central retinal region

A regression analysis confirmed only an insignificant fluctuation of amplitudes of foveolar activity in both group of patients, and furthermore determined a large dispersion of the values of amplitudes. In the parafoveolar, paramacular and peripheral regions with eccentricity between 1.8° and 30° we determined relatively stable responses in the treated patients up to 4 years of observation, after which there was a decrease of activity, predominantly only insignificant, with the exception of a significant decrease of A2 to A4 after 4.5 years. By contrast, in the control group the response amplitudes decreased significantly after 4 years of observation, and during this period they were also significantly lower in comparison with the treated patients, whereas previously they had differed only insignificantly. The latencies of the majority of responses were prolonged in both groups of patients during the course of the observation period, predominantly statistically insignificantly, the differences between the groups of patients were predominantly only insignificant, Fig. 8 A, B and Graph 2 A, B.

Central retinal activity (especially in the parafoveolar region between 1.8° and 7° eccentricity or even paramacular activity between 5° and 13°) increased in the treated patients, with an early reduction or reattachment of DPED. By contrast, in patients with long-term or persistent DPED, retinal activity and BCVA in certain cases actually deteriorated.

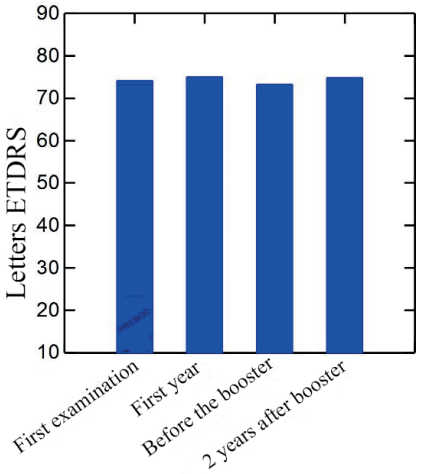


Graph 2. Multifocal ERG: mean response amplitudes of mfERG positive peaks. (**A**) Parafoveolar area in the 2nd ring of the mfERG. A2 – the amplitude of the positive wave of the average response in the 2nd circle around the foveola in both groups of patients during follow-up before the procedure, in 6 months, 1 year, 1.5 years up to 5 years. On the y-axis, the amplitude is in [nVdeg^{-2}]. (**B**) Paramacular area in the 3rd circle of mfERG. A3 – the amplitude of the positive wave of the average response in the 3rd circle around the foveola in both groups of patients during follow-up before the procedure, in 6 months, 1 year, 1.5 years up to 5 years. On the y-axis, the amplitude is in [nVdeg^{-2}]

mfERG – multifocal electroretinography

"Booster therapy"

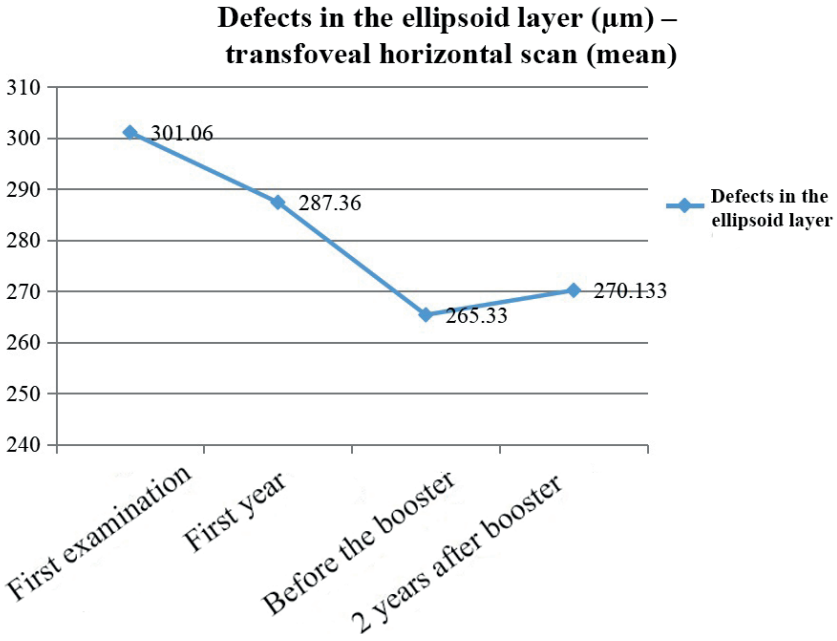
In the cohort of 65 patients with advanced dry form of AMD we therefore demonstrated a positive effect of basic



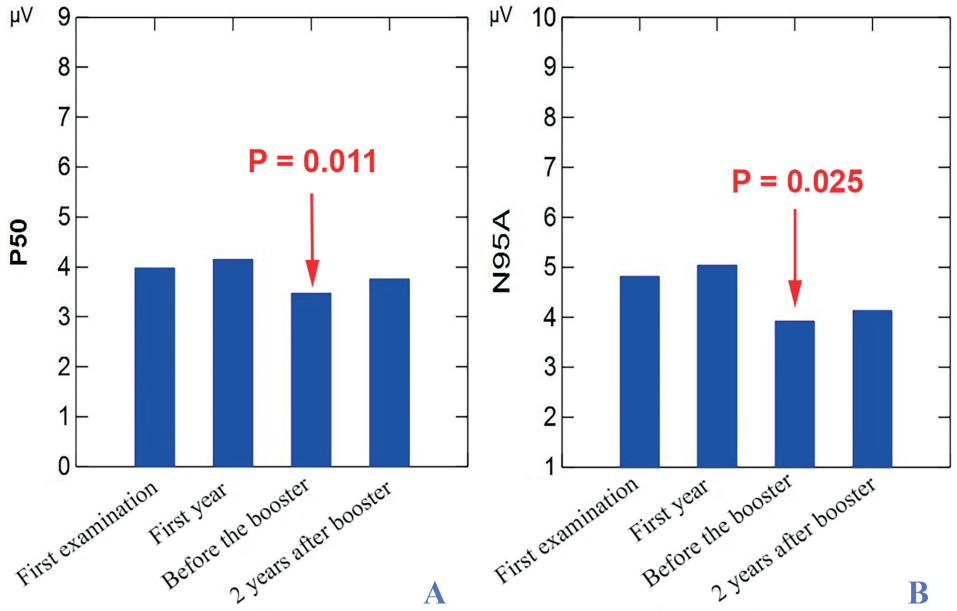
Graph 3. Development of BCVA over time: bar graph. On the x-axis: LETTERS - number of letters read. Examination dates are on the y-axis

BCVA – best corrected visual acuity

treatment with rheopheresis on the morphological and functional state of the eye, which lasts for several months, but may diminish in time. We described a significant difference in VA in favour of the treated patients in comparison with those in the control group up to 2 years, after which the difference was only insignificant. The initial positive functional effect on electroretinography was also only insignificant after 36 to 42 months. As a result, we decided to perform additional treatment 2 years after the standard RHF treatment. We performed this booster therapy on 20 patients (11 men and 9 women) who had undergone a basic series of rheopheresis procedures 1.5 to 2 years previously. We evaluated



Graph 4. Evolution of the defect size of the ellipsoid layer. On x-axis: cumulative length of defects in the layer of ellipsoids (µm). Examination dates are on the y-axis



Graph 5. ERG to a reversal stimulus: amplitudes of mean responses. (**A**) Positive P50 wave: macular function. On the y-axis is the amplitude of the positive P50 wave in [µV]. Examination dates are on the x-axis. (**B**) N95 negative wave: ganglion cell function. On the y-axis is the amplitude of the N95 negative wave in [µV]. Examination dates are on the x-axis
ERG – electroretinography

the development of 35 eyes (5 eyes already had wet form of AMD at the beginning of the observation period) up to 4 years of observation, thus 2 years after the booster therapy.

Best corrected VA changed only statistically insignificantly during the course of the examination (Graph 3).

We determined an improvement of **the morphological finding** (reduction of the surface of soft drusen and DPED) in 85.7% of cases (30/35 eyes). The ocular finding was stabilised in 8.6% (3/35 eyes). We determined a slight deterioration with the development of a small area of RPE-atrophy in the centre in 2/35 eyes (5.7%). Progression to the 3rd stage of the pathology (wet form, geographic atrophy) was not determined any of the patients. The development of the size of the defects of the ellipsoid layer is presented in Graph 4.

Electroretinography

pERG: The function of the central retinal region (P50) and the ganglion cells (N95) increased to an insignificant degree following standard RHF treatment, and subsequently significantly decreased before RHF booster therapy, after which it again increased after 2 years Graph 5 A, B).

The function of the rod and cone system, and the central retinal region on mfERG fluctuated only insignificantly during the course of the entire observation period, with a tendency of a slight increase in activity following basic RHF treatment, a reduction before booster therapy and a further increase following booster therapy.

Conclusion: improvement of visual acuity, as well as the anatomical and functional finding following standard rheopheresis treatment persists over the long term, additional booster therapy consisting of 2 RHF procedures appears to be a safe and appropriate method of prolonging this phase. Rheopheresis positively influences the natural, adverse course of dry form of AMD through a change of the activity of negatively acting factors.

Laboratory indicators in rheopheresis treatment

An evaluation of the fundamental biochemical parameters (glycaemia, minerals, kidney and liver functions) demonstrated only clinically insignificant fluctuations, even if they were statistically significant. As regards decreases in values, in which dilution changes may contribute (following the procedures there is a small blood dilution, which constitutes approximately 5 to 10%).

The fundamental haematological indicators, namely the blood count (haemoglobin, haematocrit, leukocytes) including the platelet parameters, are not significantly influenced by the procedures, even if statistical significance may be determined. A certain increase in the number of leukocytes, which is nevertheless entirely within normal values, is generally observed following rheopheresis procedures performed also in other clinical indications. The procedures are therefore safe in terms of their influence on the blood count.

Effectiveness indicators of rheopheresis treatments

We assessed the rheological success rate of the individual procedures (as a fundamental precondition for the successful treatment of AMD) according to the decrease of rheologically significant factors (α2-macroglobulin, IgM, fibrinogen, lipoproteins), see Table 4. This decrease led to a resulting effect on blood and plasma viscosity.

The effect of therapy is a reduction of a defined spectrum of high molecular weight substances. The results are illustrated below in Table 4 – it is evident that the procedures are highly effective and lead to a reduction of α2-macroglobulin by 51%, fibrinogen by 54%, IgM by 60%, LDL-cholesterol by 62%, and apolipoprotein B by 59%; a further risk factor – lipoprotein (a) – is also reduced by 54%.

The reduction of the above factors ultimately influences the rheological parameters: whole blood viscosity decreases by 9.3% and plasma viscosity by 11.1%. The result is an improvement of flow in microcirculation.

Indicators for monitoring the result of research procedures

- Complement: there is a reduction not only of the activity of the alternative pathway (by 1/3), but also of the classical pathway to 88%, the lectin pathway to 62% and H factor to 80% [10].

Table 4. Rheopheresis efficiency indicators

Parametr	Before the procedure		After the procedure		N	P	% decrease
	Mean	SD	Mean	SD			
Total cholesterol	4.13	0.79	2.18	0.35	63	< 0.0001	47.2
LDL cholesterol	1.99	0.74	0.91	0.25	63	< 0.0001	62.2
Lp(a)	12.20	24.83	4.61	9.44	63	< 0.0001	54.3
Apolipoprotein B	0.75	0.21	0.31	0.13	63	< 0.0001	58.7
α2-Macroglobulin	142.81	44.39	70.36	25.20	64	< 0.0001	50.7
Immunoglobulin M	0.48	0.24	0.19	0.08	64	< 0.0001	60.4
Fibrinogen	2.86	0.59	1.30	0.29	63	< 0.0001	54.5
Plasma viscosity	1.98	0.22	1.76	0.95	63	< 0.0001	11.1
Blood viscosity	6.11	1.02	5.54	0.82	63	< 0.0001	9.3

LDL – low-density lipoprotein; Lp(a) – Lipoprotein a

- The level of **PCSK9** in patients with AMD is significantly higher than in the control group. After the procedures it decreases significantly. It correlates with the level of total cholesterol, it does not correlate with LDL, HDL cholesterol, fibrinogen, blood and plasma viscosity, apolipoprotein B, IgM or α2-macroglobulin. The improvement or stabilisation of BCVA and the morphological finding in patients was accompanied by a reduction of LDL-CH and PCSK9, and an improvement of endothelial dysfunction markers.
- We also monitored the dynamics of special markers which could attest to inflammatory activity (IL-10), markers of the state of cell immunity (MCP-1, soluble antigen CD40), activity of the endothelium, adhesion molecule (e.g. selectin) or apoptosis markers (sAPO-fas).
- The level of **sP-selectin** decreased following the procedures, as did the level of **endoglin**. We examined the level of sE-selectin at the beginning of the study, but did not attain positive results; because the level did not change it was not monitored further.
- **IL 10** manifested a pronounced decrease after treatment upon examination of the first patients.
- The level of **sCD40L** initially decreased insignificantly, now following examination of further patients the decrease is statistically significant.
- The level of **hsCRP** decreased significantly.
- **Annexin V**, apoptosis indicator decreased numerically following the RHF procedures, but only insignificantly.
- **Soluble APO-Fas** decreased significantly following the procedures.

Adverse effects of rheopheresis

Based on our long-term experience, we can state that rheopheresis is a safe method of treatment. When performed in the above-stated indications, with respect to contraindications, mild adverse effects appear in only 5% of cases [34]. The most common reactions in our experience, and also according to the literature, are vasovagal episodes with a relatively diverse range of corresponding symptoms, as a rule nausea, weakness, small and short-term decrease of blood pressure [36,39]. A short-term interruption of the procedure, or alternatively a horizontal or Trendelenburg position was usually sufficient for the symptoms to regress.

As concerns citrate complaints: only very manifest reactions are stated. Mild reactions occur almost regularly, so in our modification of the method as a rule an input was included in the system containing 4 phials of calcium gluconicum in 100–250 ml of physiological solution. This infusion may be gradually drip fed if required. The average consumption over the entire four-hour procedure is 100–150 ml of solution. As a centre registered in the WAA – Word Apheresis Association registry, we reported this method to the centre, upon which it was accepted and recommended for application. Positive results with this prevention were published jointly in 2009 and 2010 [37,38].

A specific complication which it is necessary for us to draw attention to within the framework of objectivity, and which may be encountered in procedures with secondary processing (filtration) of plasma, is bradykinin reaction. Nevertheless, it is possible to avert this, which is necessary, since the reaction is unpleasant. In our patients our careful prevention was successful, and we did not observe any such reaction. It occurs in patients who use ACE-inhibitors. It is manifested in the form of reddening, hypotension, bradycardia and breathlessness (bradykinin also causes bronchial constriction). An activation of the kinin system takes place on the negatively charged surfaces of the filters or columns, with the subsequent formation of bradykinin. Under normal circumstances bradykinin is quickly inactivated by kinases I (ACE = angiotensin-converting-enzyme) and II (two types of peptidases), but in patients using ACE-inhibitors these enzymes are blocked. In the case of ACE blockage an accumulation of bradykinin occurs, with the onset of the symptoms described above. For this reason, in patients undergoing secondary plasma filtration the ACE inhibitor should be discontinued at least 24 hours before the procedure [39-41].

With regard to the technical aspect of performing rheopheresis (technical difficulties): the procedures rank among the most complex of hemapheretic therapeutic procedures. Difficulties depend on the theoretical preparedness and technical dexterity of the staff, who are well trained only after they have performed approximately 100 procedures. To date minor technical defects have appeared. In the case of a more serious technical failure, it may be necessary to terminate the procedure. However, over the course of the entire four years this has not yet occurred.

Upon an **evaluation of safety and tolerability**, our own modification of rheopheresis treatment was demonstrated to be safe in the hands of experienced staff. Any secondary events were easily clinically managed. Tolerance of the procedures is also acceptable. A duration of the procedure up to 4 hours appears to be an acceptable limit of tolerability, in which it is possible to attain the desired clinical result, while still remaining very tolerable for patients. However, the procedure usually takes 3 hours, and may take an hour longer only in the case of a poor condition of the peripheral veins and slow blood flow.

DISCUSSION

We indicated patients with dry form of AMD with soft drusen for rheopheresis, for the purpose of maintaining the best possible BCVA and reducing the risk of progression to the terminal stage of this disease in the form of geographic atrophy or even wet form of AMD, which are associated with a marked deterioration of VA. In long-term observation we demonstrated an improvement of BCVA in patients with advanced dry form of AMD, similar to the German authors of the MAC-I trial from the University of Cologne, the MAC-II trial from the University of Frankfurt and the MAC-III trial from the University of Hamburg [25]. In comparison with the results of the multicentric, randomised, double-blind MIRA-1 trial we demonstrated an improvement of BCVA in the treated patients, and a statistically significant deterioration of the patients in the control group in long-term observation. However, the

MIRA-1 trial was burdened in the complex evaluation by an error upon adherence to the entrance criteria of the treated patients, and a subsequent analysis following the exclusion of patients who did not meet the entrance criteria confirmed the effect of rheopheresis in one-year observation [42,43]. In contrast with the MIRA-1 trial, our trial was not double-blind, because our ethics commission did not permit extracorporeal circulation within the framework of a double-blind trial since rheopheresis in general can have serious adverse effects (despite the fact that we did not record any such effects in our study). Our long-term results demonstrated a tendency towards improvement of VA in the treated patients over the long term, although this was not statistically significant. We explain the deterioration of BCVA in the treated group by means of the delayed manifestation of the improvement of microcirculation in the retina, which demonstrably occurs following rheopheresis. The gradual deterioration of BCVA in the control group is a manifestation of the natural progression of the disease, which was expressed also by means of a higher representation of newly occurring neovascular form of AMD in the control group.

Our findings of an increase in amplitudes of pERG responses, which were significant for wave P50 and insignificant for wave N95, attest to the positive influence of rheopheresis treatment on the function of the central retinal region and the ganglion cells. We have not found any evaluation of pERG in patients with AMD treated with rheohemapheresis in the literature. In this study we determined stable photopic activity of the treated patients, in contrast with a significant decrease in the control group. Only relatively few authors have conducted an evaluation of the electrical activity of the retina in patients with AMD. They have described either insignificant changes of photopic retinal activity [6] or an increase in amplitudes of cone responses after 2.5 years [44]. Rencová et al. determined a significant increase of activity of the central retinal region in the paramacular region, with eccentricity between 5° and 13° following rheopheresis [6]. Bláha et al. describe an increase in responses in the region between 11° and 22o eccentricity in treated patients [44]. In this study, the activity of the central retinal region and BCVA increased in the treated patients, with an early decrease or disappearance of DPED. By contrast, in patients with long-term persistent DPED, paradoxically BCVA and retinal activity may decrease as a result of degenerative changes of the higher nuclear layer and the ellipsoid layer on OCT [45].

One of the aims of rheopheresis is to achieve the resorption of soft drusen. The first results are stated in the previous study [6], in which we determined the influence of rheopheresis also on the reduction to elimination of the surface area of DPED. With regard to the confluence of soft drusen into DPED, in the ordinary development of AMD this pathological change is gradually enlarged, with no tendency towards resorption. If resorption takes place after a number of years, it is generally replaced by atrophy of the retinal pigment epithelium (RPE), most commonly in the form of geographic atrophy. This terminal stage of dry form of AMD is naturally associated with a marked deterioration of VA [5]. Our results show that following rheopheresis the surface area of DPED is reduced, and in long-term observation the development of extensive geographic atrophy of the RPE therefore does not occur. Khanifar et al. refer to the danger of the progression of drusen merging into DPED and individual high drusen into wet form of AMD [46]. Sikorski et al. have used spectral OCT with high resolution capacity, and note the possibility of an accumulation of fluid beneath the ellipsoid layer of the photoreceptors in the depressions between the adjacent, partially already confluent drusen [45]. This fluid increases the distance between the surface of the RPE and the ellipsoid layer of the photoreceptors. The presence of fluid here beneath the retinal neuroepithelium surprisingly does not yet mean the presence of choroidal neovascularisation and therefore wet form of AMD [45]. We thereby explain the statistically insignificant deterioration of VA and the insignificant decrease of the values of multifocal ERG in the case that reattachment of DPED takes place after treatment, without the onset of RPE atrophy. Atrophy of the RPE after reattachment of DPED may occur following rheopheresis, but with reference to its small size, in the treated patients it was not associated with a marked deterioration of BCVA. According to Sikorski et al., at the apex of especially prominent drusen or DPED, a defect may occur in the thin ellipsoid layer adjacent to the RPE, into which fluid enters, which is now a sign of progression to wet form of AMD [45]. This occurred in only two eyes of our treated patients a number of years following the performance of rheopheresis. In comparison with the control group, the frequency of transition to neovascular AMD is lower, although due to the small size of the cohort this is not statistically significant. By contrast with the control group, in which we recorded a transition into neovascular AMD in one eye only 6 months after the beginning of the observation period, a deferral of the progression of the pathology is achieved thanks to the influence of rheopheresis. This finding leads us to the hypothesis that in order to achieve the maximum effect, it shall be necessary to repeat rheopheresis 1.5 to 2 years after the end of the last cycles. This view is reinforced also by the results of mfERG, which do not change between 30 and 42 months, despite having improved slightly prior to this.

An evaluation of the biochemical and haematological indicators before and immediately after rheopheresis showed values which, though statistically significant, were of no clinical significance. As regards the decreases of values – a role may be played here by dilution changes (following the procedures a small dilution of blood occurs, which constitutes 5–10%). A certain increase in the number of leukocytes, even if entirely within normal values, is generally observed following these procedures performed in other clinical indications. As is the case after other haemapheretic procedures, the quantity of haemoglobin and blood platelets decreases somewhat, but upon the use of the aforementioned modern separators of blood corpuscles this decrease is smaller than previously, upon the use of older types of separators. In terms of the influence on the blood count, the procedures are therefore safe. The decrease of 2-macroglobulin, IgM, fibrinogen and lipoproteins influenced the rheological parameters: whole blood viscosity decreased by 15% and plasma viscosity by 12%. The result is certainly an improvement of flow in microcirculation, which is a funda-

mental prerequisite for increasing the flow also in the choroid and improving the metabolism of the retina.

We demonstrated that certain of the above-evaluated indicators attest to a decrease in the activity of the vascular endothelium or inflammatory changes in our patients following rheopheresis treatment. The improvement of the blood perfusion of tissues following rheopheresis may also have a connection with the decrease in the level of soluble forms of the selectin adhesive modules E-selectin and P-selectin in the blood periphery [47]. These molecules, regulating the initial phase of adhesion of leukocytes and thrombocytes, are stored in intracellular granules, which following activation are very rapidly transported to the surface. E-selectin is located exclusively in Weibel-Palade bodies of the endothelial cells, P-selectin is stored both in alpha-granules of thrombocytes and in Weibel-Palade bodies of the endothelial cells. However, it has been demonstrated that soluble P-selectin (sP-selectin) in peripheral blood is practically exclusively released from the alpha granules of the thrombocytes [48]. By measuring sP-selectin and soluble E-selectin (sE-selectin) it is evidently possible to assess the influence of the procedures on the endothelial lining of the blood vessels. In our cohort, the level of sP-selectin decreased after the procedures, as did endoglin. We examined the level of sE-selectin at the beginning of the study, but did not attain positive results; because the level did not change it was not monitored further.

In patients with AMD we found a raised level of PCSK9 in comparison with control patients, but a correlation was found only between the level of PCSK9 and total cholesterol, and not between the level of PCSK9 and the level of LDL-cholesterol. However, our cohort is relatively small, and this may represent an error of small numbers. Nonetheless, we have found similar results to those of our study [49] in terms of a marked decrease of PCSK9. A reduction of the level of PCSK9 by one half, similarly as in our case, was observed by Julius et al. [49]. We did not have the opportunity to measure the level of PCSK9 after 24 hours as was performed by the above authors, who determined that the return to the original values was rapid. However, it is probable that in the case of AMD the return to the original values will be faster than in the case of the cholesterol level, which does not reach its original value until after approximately 8 days [49]. We nevertheless confirmed that the level of PCSK9 half way through the treatment (before the fourth rheopheresis procedure) and at the end of the entire series of treatments (before the eighth procedure) does not differ significantly as against the baseline values.

We also examined MCP-1 with regard to the fundamental significance of microphages in microcirculation, and we determined a significant decrease in patients with familial hypercholesterolaemia [47,50]. During this trial it was determined that it decreases in the case of AMD. As stated in the literature, this may represent a significant factor which attests to the effectiveness of influencing the activity of the inflammatory process or atherosclerosis [25,47]. The mechanism of influencing the activity of the inflammatory process by rheopheresis in the pathogenesis of the development of AMD could also be documented by the significant decrease of the inflammatory markers IL 10, Ig M, and in the case of the atherosclerotic mechanism by the decrease of the levels of CD30 and sCD40L.

CONCLUSION

On the basis of the results of long-term treatment and observation of patients, we can conclude that rheopheresis provides a possibility for improving the pessimistic outlook of patients with dry form of AMD, who are in danger of progression. Also important is the economic aspect, since medical costs increase approximately threefold upon the progression of dry form of AMD to wet form, and thus by halting the progression of dry form of AMD it would be possible to reduce the already high and ever-increasing costs for the treatment of AMD, creating a comprehensive system of optimal long-term care for patients with dry form of AMD. This is another reason why this therapeutic method is anchored in the internationally recognised guidelines of ASFA (American Society for Apheresis) as the method of first choice, which was evidently contributed to also by our results, since our study was cited as one of the most recent randomised trials in these guidelines.

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