

# Rheopheresis in vascular diseases

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**Abstract.** Rheopheresis is an extracorporeal selective double-filtration procedure. In the first part of the treatment the blood is passes through the plasma filter, which separates blood cells from the plasma. Then the plasma flow to a second filter called MONET (Membranefiltration Optimised Novel Extracorporeal Treatment). The MONET filter retains high molecular weight proteins such LDL, Lp(a), fibrinogen,  $\alpha$ 2 macroglobulin, vWF and IgM. Hereby the whole blood and plasma viscosity decrease, improves microcirculation, and has a positive effect on lipid profile as well.

According to ASFA recommendation rheopheresis is a first line treatment in age-related dry macular degeneration and in sudden sensorineural hearing loss. There are other clinical situations in which rheopheresis has been used effectively. But only few data are available and large clinical trials have not been done in these diseases. In this paper we describe a case history and laboratory findings of a patient who suffers from age related dry macular degeneration and was successfully treated by rheopheresis.

**Keywords:** Rheopheresis, microcirculation, age-related dry macular degeneration, anti-inflammatory effect, haemorheological parameters

## 1. Introduction

During the past few years several novel therapeutic apheresis methods were introduced, significantly widening the indication range of apheresis therapies. One of these new therapeutic procedures is called rheopheresis, which is classified into the group of selective extracorporeal haemotherapies. Rheopheresis is a 2-step cascade filtration procedure [20]. As the first step of the treatment cellular elements are separated from the plasma, then the plasma flows through a special filter which is able to eliminate certain plasma components based on molecular size, or at least it is able to reduce their level significantly [3].

With the help of the MONET filter (Membrane filtration Optimised Novel Extracorporeal Treatment) developed by Fresenius SE (Bad Homburg, Germany) the opportunity to perform a rheopheresis is provided. With this method proteins with molecular weights higher than 250–300 kDa can be eliminated from the plasma. After that the purified plasma is administered to the patient through a peripheral vein, together with the cellular elements. LDL, Lp(a), fibrinogen,  $\alpha$ 2 macroglobulin, vWF and IgM are some of those proteins of high molecular weight which are eliminated this way. Averagely, 1 treatment reduces the LDL level by approximately 70%, the total cholesterol level by 50%, and it decreases the triglyceride level generally by 35–40%. The fibrinogen level is also reduced to its half in general, after one treatment. On the other hand it needs to be highlighted that HDL level does not change significantly, neither do plasma albumin nor total protein levels [19].

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Besides the above mentioned facts they also observed that rheopheresis is able to decrease Lp(a), vWF and IgM levels as well. The favourable effect of the rheopheresis therapy is resulting from the beneficial changes in levels of these macromolecules. As a result of this, treatment viscosity of the plasma and the whole blood is reduced, the erythrocyte aggregation is moderated and deformability of the erythrocytes is improved [3, 22].

As a consequence of these, blood flow is improved, and dysfunctions of the microcirculation are also alleviated. It has a beneficial effect on lipid profile as well, it decreases the level of atherogenic lipid fractions in blood, but at the same time it does not significantly alter the levels of protective HDL.

Indication range of rheopheresis has been widened during the past few years. They conducted trials with rheopheresis in different disease groups, however, randomized, prospective clinical trials were only conducted in case of age-related macular degeneration and sensorineural hearing loss [4, 17]. Rheopheresis was applied in case of patients with peripheral arterial vascular diseases, who were not suitable for revascularization or who suffered from diabetic leg syndrome [9]. Furthermore beneficial effects were detected on the blood flow in the carotid artery and on the vasodilatory capacity of the coronary arteries [6].

Based on double blind, placebo-controlled trials, and 2013 guidelines of the American Society for Apheresis (ASFA), rheopheresis is the recommended first choice of therapy in case of dry form of age-related macular degeneration.

Age-related macular degeneration (AMD) is a special angiological disease, which has two different types, dry and exudative form. About 80% of all AMD cases are dry forms. It shows the significance of AMD that based on a WHO survey it is responsible for 50% of ophthalmological diseases leading to blindness in Western countries, and the ratio is similar in Hungary [15]. The disease shows direct correlation with cholesterol, fibrinogen and alpha-macroglobulin levels. Its pathomechanism is multi-factorial, in which several different mechanisms play role, such as inflammatory involvement of chorio-capillaries, lipid accumulation, haemorrhological disorders and dysfunctions of microcirculation. Among the subjective symptoms we have to outline that the patient initially sees a blurred area, which is always in the way of sight (central visual impairment). Currently there are no available causal treatments, but progression can be slowed down with conservative therapy and moderation of the risk factors. In case of dry form of AMD we can administer antioxidant vitamins, zinc, lutein, or zeaxanthin containing dietary supplements, as well as nutrients rich in unsaturated fatty acids, and in case of the exudative form of AMD, intra-vitreous pharmacotherapy (VEGF inhibitors) is recommended [1–2, 14].

## 2. Case report

In the medical anamnesis of our 68-year-old male patient, medical care due to hypertension and asthma, as well as TIA is listed. Considering his risk factors, he has been a heavy smoker for 30 years, but he quit smoking in 2009. No ophthalmological diseases are present in familial medical anamnesis.

His eye-related complaints started to emerge during summer of 2013 he experienced blurred vision and central visual field loss. Based on the dilated fundus examination, optical coherence tomography (OCT), and fluorescent angiography we diagnosed dry type age-related macular degeneration in the background of his complaints. In line with the most intensive conservative therapy (zinc and lutein containing dietary supplements) his visual loss progressed continuously. By the spring of 2014, his visual impairment made him unable to read. Because of the rapidly progressing condition—despite the conservative therapy – we decided to perform a rheopheresis treatment together with the Department of Ophthalmology. Before we initiated the therapy we obtained a licence from the local Ethics Committee and the written informed consent form signed by the patient.

80 The first rheopheresis treatment was performed in July 2014 at the ICU of our clinic. Between July  
81 2014 and July 2016 the patient received 6 cycles of rheopheresis treatment, altogether for 11 times.  
82 The rheopheresis treatment was performed with the use of a MONET filter. No complications or side  
83 effects were observed during the course of the therapy.

84 For efficacy assessment of the rheopheresis treatment we performed laboratory tests and angiological  
85 examinations—besides the ophthalmological tests—preceding the treatment cycles, as well as at the  
86 end of each therapeutic cycle. According to our assumption, rheopheresis treatment not only works  
87 via improvement of viscosity to moderate microcirculatory dysfunction, but it may have other anti-  
88 inflammatory effects, and it might decrease the level of endothelial and thrombocyte activating markers.  
89 All these beneficial effects may result in improving the endothelial functions.

### 90 3. Rheopheresis

91 The rheopheresis treatment was performed by using the MONET (Membrane filtration Optimised  
92 Novel Extracorporeal Treatment) filter developed by Fresenius Medical Care Deutschland GmbH. The  
93 MONET filters were attached to Art Universal devices. For the extra-corporeal treatment, cannula-  
94 tion of two peripheral veins is necessary. As first step of the therapy we separate the plasma from  
95 the cellular components of blood with the help of a plasma filter, then the plasma is made to flow  
96 through the MONET filter. After that the purified plasma is administered into the patient's circulatory  
97 system through a peripheral vein, together with the cellular elements. Citrate was administered as an  
98 anticoagulant.

### 99 4. Haemorheological measurements

100 Determination of the viscosity of the whole blood and plasma (WBV, PV [mPa.s]) was performed  
101 with a Hevimet-40 capillary viscometer (Hemorex Ltd., Hungary). Viscosity values at  $90\text{ s}^{-1}$  shear rate  
102 were used for comparison. According to the Mátrai-formula, the whole blood viscosity data were also  
103 corrected to 40% hematocrit value [8, 12]. Deformability of erythrocytes was measured by a rotational  
104 ektacytometer (LoRRca MaxSis Osmoscan, Mechatronics BV, The Netherlands). Elongation index (EI)  
105 was plotted in the function of shear stress (SS [Pa]). RBC aggregation was determined concomitantly  
106 with two different methods. First we used a Myrenne MA-1 erythrocyte aggregometer (Myrenne  
107 GmbH, Germany), the operation of which is based on light transmission. Aggregation indices were  
108 determined under stasis (M mode) and at  $3\text{ s}^{-1}$  shear rate (M1 mode), at the 5th and the 10th second of  
109 the aggregation (M 5 s, M 10 s, M1 5 s, M1 10 s). The LoRRca system characterizes the aggregation  
110 process based on light reflection (syllectometry). For comparison the following parameters were used:  
111 aggregation index % (AI), amplitude (Amp),  $t_{1/2}$  [s].

#### 112 4.1. Laboratory tests

113 *Phagocyte activity* was measured by chemiluminescence method. Heparinized human whole blood  
114 was used. Samples were diluted by PBS (phosphate buffered saline), in the ratio of 1 : 3. In the control  
115 tubes, 0.5 ml of diluted blood was diluted to 1 ml by adding 0.5 ml of PBS. For the stimulation of  
116 phagocytes, 0.5 ml of zymosan suspension (1 mg/ml zymosan, Sigma, MO, USA) was added. As  
117 positive control, phagocytes were stimulated by 0.5 ml PMA (0.04  $\mu\text{g/ml}$ , phorbol-myristate-acetate,  
118 Sigma). The amplification of free radical production (chemi-luminescence, CL) took place by the  
119 addition of 50  $\mu\text{l}$  luminol (20  $\mu\text{g/ml}$ , 5 amino-2,3 dihydro-1,4 phtalazinedione, Sigma). The CL was  
120 measured by an AutoLumat LB 953 multi-tube luminometer (Berthold Technologies, Germany). Both

121 the non-stimulated controls (basal CL), the zymosan-stimulated and the PMA-stimulated whole blood  
122 samples were tested in duplicate. Their CL was detected in every 3 minutes for 45 minutes. Results  
123 are presented as the area under the relative-light unit (RLU) curves and the maximum values of  
124 RLUs.

125 *Activated monocytes (CD14+CD16+)* were measured by flow cytometry from heparinized whole  
126 blood. Cells in 100  $\mu$ l blood were stained with 10-10  $\mu$ l of monoclonal antibodies (anti-CD16-FITC,  
127 anti-CD14-PE, Becton Dickinson, USA). Mouse IgG1-FITC, IgG1-PE (Becton Dickinson, USA) was  
128 used as an isotype control. After 25 min incubation the red blood cells were haemolysed and the  
129 leukocytes were washed with PBS (completed with BSA and sodium-azide). Finally the cells were  
130 suspended in 800  $\mu$ l of 1% para-formaldehyde. Samples were measured by a Beckman Coulter FC500  
131 flow cytometer and analysis was carried out by CXP software (Beckman Coulter, USA). Monocytes  
132 were gated on the basis of their side-scatter parameter and CD14 positivity. Results were expressed as  
133 the percentage of CD16+ cells in the gated CD14+ monocyte region.

134 *Determination of lipid profile* (cholesterol, triglyceride, LDL, HDL, Lp(a) levels) was performed  
135 based on routine laboratory diagnostic tests at DE KK LMI (University of Debrecen, Clinical Centre,  
136 Institute of Laboratory Medicine). Analyses were performed from 4 ml blood samples, free from  
137 anticoagulants. Determination of fibrinogen was done from blood sample treated with Na-citrate  
138 anticoagulant, according to the Clauss method. The reference range was: 1.5–4 g/L.

139 *Measurement of vWF:Ag level* was performed from blood sample treated with Na-citrate anticoag-  
140 ulant. The whole blood was first centrifuged (1500 g, 20 min, 22 °C), then from the plasma obtained  
141 this way, level of vWF:Ag was determined by immuno-turbidimetry. The degree of agglutination is  
142 directly proportional to the level of vWF found in the sample. The reference range was between 50  
143 and 160%.

144 *P-selectin expression* characterising thrombocyte activation was determined from whole blood  
145 treated with citrate anticoagulant, with the help of anti-CD-62 monoclonal antibodies. Measurements  
146 were done by flow cytometer. Reference range of thrombocyte P-selectin was: 0–2%.

147 *Flow-mediated vasodilatation (FMD)* was performed by ultrasound technic. Examinations were  
148 performed under standardized conditions on the patient, after 8 hours of fasting and 18 hours of smok-  
149 ing prohibition, on the right brachial artery. Measurements were made with Phillips (HD11XE) high  
150 resolution ultrasound device, with a 5–10 MHz linear transducer. ECG monitoring was performed  
151 during the whole examination. We took longitudinal cross-sectional images of the brachial artery  
152 proximally from the elbow, and we determined the diameter of the artery between at least 5 identi-  
153 cal points. Measurements were made synchronized to the R-wave. After that we triggered reactive  
154 hyperaemia, first by bloating the sphygmomanometer cuff placed onto the forearm for 4.5 minutes,  
155 maintaining a supra-systolic value 50 mmHg above the systolic pressure, then suddenly deflating the  
156 cuff. We detected the change in the aortic diameter within the 60th second following the cuff defla-  
157 tion, accordingly to the method detailed above. The flow-mediated dilation was given as a percentage  
158 value, which expresses the diameter change triggered by reactive hyperaemia, compared to the resting  
159 diameter.

160 *Arterial stiffness determination* (augmentation index, pulse wave velocity) was done with the help of  
161 a Tensio Clinic arteriograph (TensioMed Kft., Debrecen). The measurement is based on the principle  
162 that as a result of contraction of the heart, the first pulse wave generated in the aorta is reflected at the  
163 level of bifurcation, thus an easily detectable second wave (late systolic peak) appears during systole.  
164 Morphology of the second wave depends on stiffness parameter of the common carotid artery, on  
165 the reflection time measured on the brachial artery at 35 mmHg supra-systolic pressure (RT S35), as  
166 well as on peripheral resistance defined by the amplitude of the wave. The pulse wave velocity is  
167 the distance between the jugular fossa and the symphysis, and ratio of the RT S 35 value. Unit of the  
168 quotient obtained this way is m/s.

169 *Ophthalmological examination* determined the best corrected visual acuity of the patient on a loga-  
170 rithmic scale, we took colour photos of the eye fundus (TRC-NW7SF, Topcon, Tokyo, Japan) and we  
171 performed an OCT (Zeiss Stratus, Carl Zeiss Meditec, Inc, California USA) examination.

## 172 **5. Results**

### 173 *5.1. Ophthalmological results*

174 Even the first cycle of therapy resulted in significant improvement in visual acuity. Reading ability  
175 of the patient was restored, he was able to read with a magnifier.

176 The objective best corrected visual acuity showed fluctuation, but no progression could be detected.  
177 Based on the examination of the eye fundus, the wasting of the right eye was not aggravated as a result  
178 of the therapy, and sub-retinal bleeding of the left eye absorbed completely by the end of the third  
179 cycle of therapy (Fig. 1). The OCT examination performed prior to the treatments showed extended  
180 retinal pigment epithelium (RPE) atrophy on the right side, pigment aggregation, thinned neuroretina  
181 and presence of sub-retinal fibro-vascular membrane in the left eye. In addition, pigment epithelial  
182 abruption distorting the fovea, and nasally from the centre, a small amount of sub-retinal fluid could  
183 be detected. By the end of the therapeutic cycles the contour of the fovea on the right became even,  
184 no progression was observed, neither intra-retinal nor sub-retinal fluid was visible on the left eye, and  
185 the pigment epithelium abruption also ceased (Fig. 2).

### 186 *5.2. Changes of haemorheological and lipid parameters as a result of rheopheresis*

187 Viscosity of whole blood and the plasma was increased prior to the first treatment, and increased  
188 erythrocyte aggregation was also measured, in line with borderline fibrinogen level. It can be observed  
189 that by the end of the first therapeutic cycle viscosity of the plasma returned to the normal range, viscos-  
190 ity of the whole blood also decreased significantly, it almost reached the reference range. Erythrocyte  
191 aggregation normalized and fibrinogen level also decreased. During the additional therapeutic cycles  
192 we observed obtained similar results (Table 1). In case of our patient lipid parameters were already  
193 within the normal range before initiation of the rheopheresis, due to the long-term statin therapy that  
194 he received because of secondary prevention of the formerly developing TIA. But still, our results  
195 prove that rheopheresis therapy was able to effectively decrease the level of atherogenic lipid fractions  
196 during the treatment cycles.

### 197 *5.3. Anti-inflammatory, thrombocyte and endothelial activation moderation effects* 198 *of rheopheresis*

199 Prior to the first therapeutic cycle significantly increased phagocyte activity and increased  
200 CD14+/CD16+ cell population ratio were detected. Our results support the hypothetical inflammatory  
201 involvement of chorio-capillaries in pathogenesis of AMD. After the first treatment session, as a result  
202 of that phagocyte activity and CD14+/CD16+ cell population ratio normalized. Based on all these we  
203 assume that rheopheresis therapy has – besides the decrease in known macromolecular levels – other  
204 effects as well, for the detection of which further tests should be performed.

205 Rheopheresis treatment also moderated thrombocyte aggregation, which was evidenced by decrease  
206 and normalization of P-selectin expression.

Table 1  
Changes of laboratory parameters after rheopheresis treatments

	Treatment period 1		Treatment period 2		Treatment period 3		Treatment period 4		Treatment period 5		Treatment period 6	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Whole Blood-Plasma viscosity (mPa sec)	6.28	4.34	4.25	4.22	5.1	4.8	5	4.19	4.44	4.1	4.57	4
Ert aggregability	1.55	1.17	1.25	1.19	1.38	1.18	1.47	1.3	1.45	1.35	1.67	1.67
Fibrinogen (g/l)	Increased	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Tg (mmol/l)	3.29	2.43	3.05	2.49	4.36	2.09	4.24	2.19	3.71	3.5	3.99	3.57
Cholesterine (mmol/l)	0.8	0.6	0.5	0.4	0.7	0.9	0.7	0.7	0.7	0.6	0.8	0.7
LDL-C (mmol/l)	3.6	2.1	4.6	2.8	4.2	1.8	4.5	2.3	3.3	1.6	4.3	2.4
Lp(a) (mg/l)	2.5	0.8	3	1.2	2.7	0.7	3	1.3	2.9	1.1	2.9	1.1
CD14+/CD16+ activated monocyte ratio (%)	141	69	135	69	60	32	80	58	62	42	62	42
Fagocyte activity (RLU/30 min)	70.9	34.3	14.6	13.2	13.5	11.1	8.8	8.2	12	10	13	10
P-selectin expression (%)	12938	3452	525	422	2852	1895	122	115	43.8	108	104.9	111.8
vWF:Ag (%)	3.15	1.1	1.57	1.12	1.27	2.6	1.32	2.33	1.3	0.9	1.94	1.94
	114	90	107	89	116	86	121	81	124	110	131	80

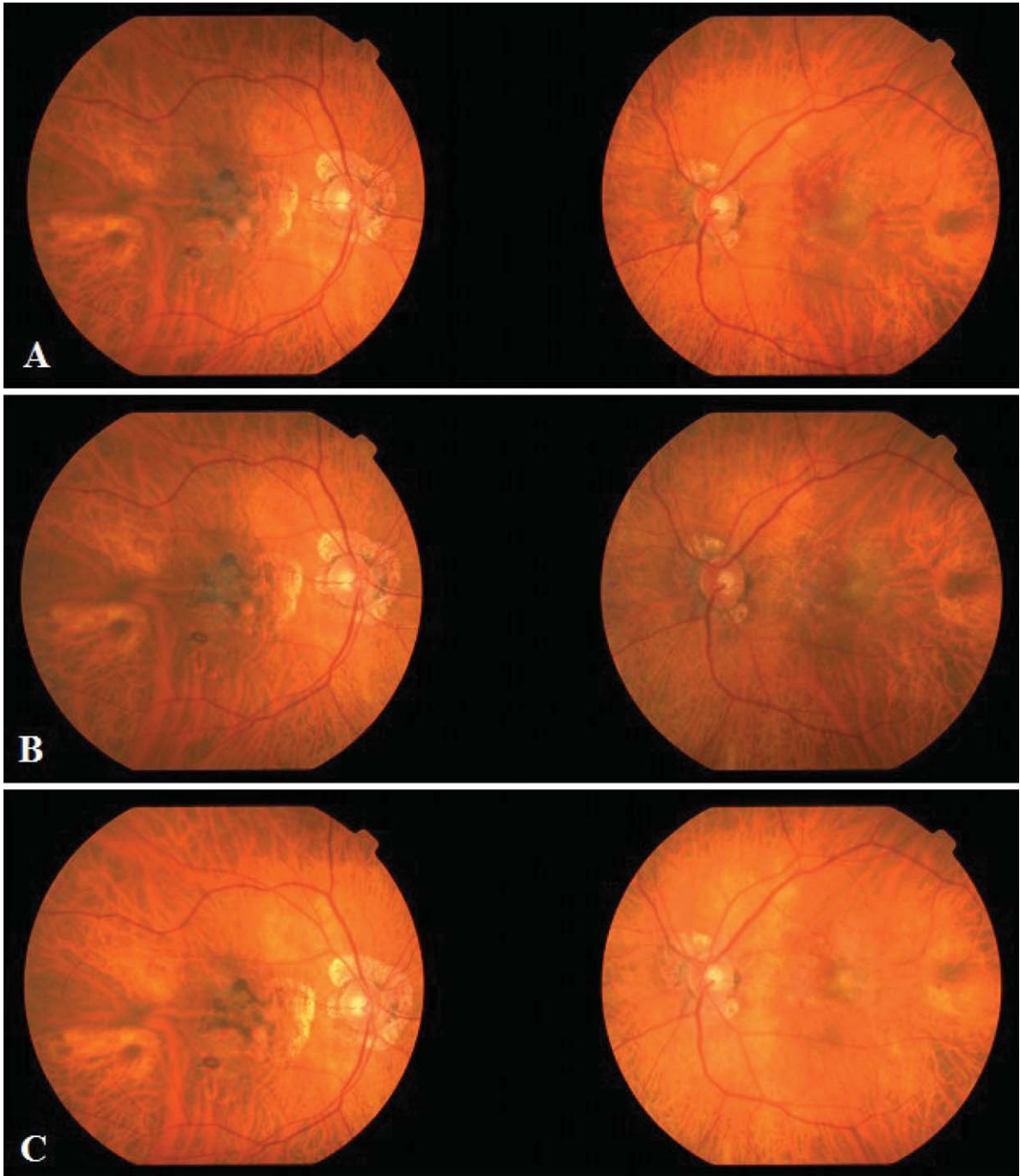


Fig. 1. Picture of the patient's coloured fundus: before the 1st treatment (A), after the 2nd (B), and after the 3rd treatment (C).

207 *5.4. Effect of rheopheresis on endothelial function and on stiffness parameters*

208 Beneficial effects emerging as a result of rheopheresis therapy may not only be exhibited via enhanc-  
209 ing blood flow and improvement of microcirculation. According to our assumption these effects can

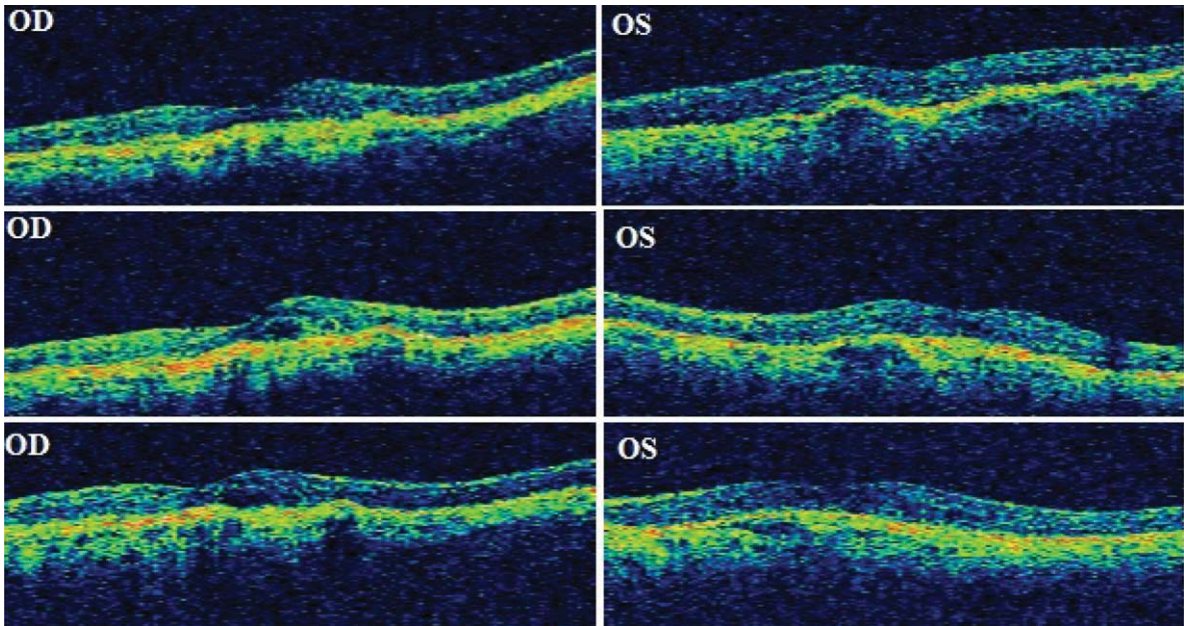


Fig. 2. OCT image: before the 1st rheopheresis treatment (on the top), after the 2nd treatment (in the middle) and after the 3rd treatment (below).

210 improve macrocirculation as well. Within the confines of this endothelial dysfunction may be improved,  
 211 as well as stiffness parameters (Fig. 3a,b,c). Our results support the observation that resulting from the  
 212 rheopheresis therapy endothelial dysfunction eased, and it also had a positive effect on vascular wall  
 213 stiffness. Based on all these rheopheresis may have potential application in the treatment of vascular  
 214 disease developing as a result of atherosclerosis.

## 215 6. Discussion

216 The age-related macular degeneration is a leading cause of severe visual impairment and blindness  
 217 of the population above the age of 60 in developed countries. Vast majority, about 80% of all AMD  
 218 cases are classified into the group of dry type.

219 The risk of developing the disease increases exponentially with ageing.

220 Smoking unambiguously affects the development and course of the disease, but alcohol consumption,  
 221 obesity, pathological BMI can also be influencing factors.

222 Dry type AMD does not have a causal therapy currently. The following new strategies are available  
 223 regarding the treatment of dry type AMD: a.) decreasing the development of drusen, b.) alleviating  
 224 the inflammation, c.) decreasing the retinal oxidative stress and accumulation of toxic metabolites, d.  
 225 increasing the chorioidea perfusion e.) enhancing regeneration of RPE and photoreceptor cells, f. gene  
 226 therapy [5].

227 Besides the above mentioned, rheopheresis also became available, at the University of Debrecen,  
 228 Hungary was the first where it was introduced.

229 The few larger trials, such as the Trial-Art and RheoNet study reported positive rheological, angi-  
 230 ological and ophthalmological effects, which we were also able to observe [10–11]. Wolf et al. have  
 231 found correlation between retinal microcirculation, plasma viscosity and visual function in patients  
 232 with macroglobulinemia [23]. Mechanism of action of the treatment at the level of the retina is



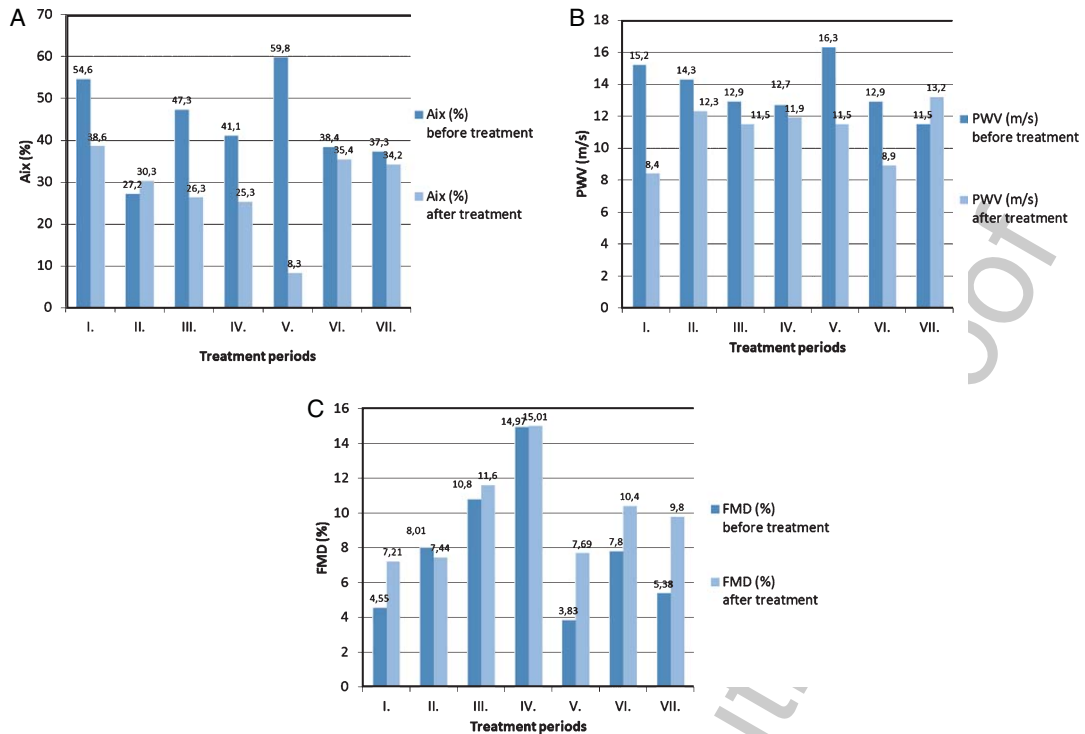


Fig. 3. (a) The effect of rheopheresis treatment on augmentation index, (b) The effect of rheopheresis on pulse wave velocity, (c) The effect of rheopheresis treatment on flow mediated vasodilatation.

probably exhibited via enhancing tight attachment of photoreceptor external/internal segments to the foveal area [18]. It can be especially efficient in such dry AMD forms, like in our case, where pigment epithelium detachment is present with sub-retinal fluid. The latter was completely absorbed by the end of the treatment, and the contour of the fovea was confirmed to be even by OCT. On contrary to this, the almost 10-year-old MIRA study was not able to provide the initially promising achievements on long term, but the selection criteria were not unified [16]. Recently, according to the guidelines of the American Society for Apheresis (ASFA), they recommend rheopheresis as a first choice of treatment in case of dry form of AMD, based on strong evidence specified in a comprehensive evidence-based meta-analysis [21].

Our examinations supported the hypothesis that besides the above mentioned effects of rheopheresis, it may have other vascular and angiological impacts. In line with normalization of viscosity parameters and decreasing the levels of atherogenic lipid fractions, it was proven that rheopheresis has anti-inflammatory effect as well, and it also moderates thrombocyte activation, which has additional beneficial effects too. In addition to all the above mentioned facts, we also found evidence that rheopheresis therapy may improve endothelial functions and stiffness parameters. These effects might have the outmost importance regarding the treatment of vascular diseases developing as a result of atherosclerosis. However, further examinations are needed to discover vascular target points of rheopheresis in details.

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