The Impact of Rheohaemapheresis on the Proprotein Convertase Subtilisin Kexin 9 (PCSK9) in Age-Related Macular Degeneration

Vladimír Blaha
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove https://orcid.org/0000-0001-8088-9919

Hana Langrová
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Milan Blaha
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Jan Studnička
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Alexander Stěpanov
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Ctirad Andrýs
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Veronika Loefflerová
Regional Hospital Liberec: Krajska Nemocnice Liberec as

Miriam Lánská
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Eva Vejražková
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

Petr Dulíček (petr.dulicek@fnhk.cz)
University Hospital Hradec Kralove: Fakultni Nemocnice Hradec Kralove

---

Research

Keywords: Proprotein convertase subtilisin/kexin 9, age-related macular degeneration, Rheohaemapheresis, LDL-cholesterol

Posted Date: November 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-101210/v1

Abstract

Background. Age-related macular degeneration (AMD) is a progressive chronic disease with resulting visual impairment or even blindness with limited therapeutic options. Because hyperlipidemia is a significant risk factor for AMD development we investigated long-term effects of rheohaemapheresis in the dry form of age-related macular degeneration on the lipid related parameters including PCSK9.

Methods. This study evaluates 31 patients with age-related macular degeneration (AMD), treated with rheohaemapheresis. The followed-up period was 7 years. Average age was 69.1 ± 4.9 years. Each treated patient received a series of 8 sessions of rheohaemapheresis. 2 additional procedures within 1-week procedures were performed to boost the effect after the 2-year period. We measured the drusenoid pigment epithelium detachment (DPED), best-corrected visual acuity (BCVA), electroretinography (ERG), lipid, inflammatory, endothelial dysfunction and rheologically important parameters.

Results. Rheohaemapheresis treatment in AMD patients was associated with a significant decrease of total cholesterol, LDL-C, HDL-C, apoprotein B, and lipoprotein (a) levels, biomarkers of inflammation, endothelial dysfunction (CD40L, MCP-1) and rheologically important parameters, and serum PCSK9 ($P<0.001$). The patients were further divided into 2 groups based on the ophtalmological examination. Successfully treated patients (n=10, with at least a 5-year follow-up) had significantly lower baseline LDL-C and ApoB ($P<0.05$) and their serum PCSK9 significantly decreased after rheohaemapheresis ($P<0.001$) in comparison to the patients where treatment failured (n=4).

Conclusion. Over the long term, rheohaemapheresis reduced the DPED, improved the function of photoreceptors, and prevented the decline of BCVA. BCVA improvement was accompanied by lowering of LDL-C and PCSK9 and improvement of endothelial dysfunction. We suggest that rheohaemapheresis and other novel anti-PCSK9 therapies may be used synergistically to reduce severity, slow down or even induce regression of AMD.

Introduction

Age-related macular degeneration (AMD) is a progressive chronic disease, and despite the best care, it remains a leading cause of visual impairment in the elderly. Moreover, the therapeutic options are limited. A hypothesis implicating hyperlipidemia/hypercholesterolemia as a risk factor in AMD has arisen from the observation that AMD and cardiovascular disease share a number of risk factors and pathophysiological pathways [1]. Hyperlipidemia is a significant risk factor for the development of a number of vascular diseases in the eye, such as retina atherosclerosis and AMD [2]. The retina has been shown to uptake circulating low density lipoprotein (LDL) [3], providing blood-borne lipids to all the cellular layers of the retina. The retina can also synthesize cholesterol to maintain its dynamic steady-state lipid composition [4]. To perform these tasks, the retina expresses the same molecules used in blood-borne lipoprotein uptake and in intraretinal lipoprotein-based lipid transport process [5] including the proprotein convertase subtilisin/kexin type-9 cascade [6].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a pivotal regulator of low-density lipoprotein (LDL) receptor, and thus of LDL-cholesterol levels (LDL-C) [7]. When cell cholesterol depletion or inhibition of intracellular synthesis occurs, PCSK9 promoter activity is raised, leading to an increased transcription [8]. A second transcription factor involved in regulation of PCSK9 is the hepatocyte nuclear factor 1 alpha (HNF1 alpha) [9]. Once secreted, PCSK9 binds the epidermal growth factor-like repeat homology domain A (EGFA-like) of the LDL receptor (LDLR) through its catalytic domain. This phenomenon fosters the degradation of LDLR in lysosomes, instead of allowing it to recycle on the cell surface. This degrading activity reduces the number of LDLR on hepatocytes, and thus the uptake of circulating LDL particles by the liver. For this reason, greater PCSK9 activity associates with higher circulating levels of LDL cholesterol, and its pharmacological inhibition has been considered as a new line of intervention for preventing cardiovascular diseases [10].

In spite of the important role of PCSK9 in LDL-C metabolism in coronary heart disease, its impact on other diseases that stem from LDL-C overload and subsequent oxidation, such as age-related macular degeneration (AMD), has thus far not been studied. The progression of AMD involves the transition from an early or intermediate stage, when extracellular deposits called drusen accumulate on the inner surface of Bruch's membrane, to an advanced stage featuring photoreceptor and retina pigment epithelium (RPE) atrophy and/or choroidal neovascularization (CNV), leading to central vision loss [11]. While the mechanisms that drive this progression are unknown, they have been linked to lipid transport and lipid metabolism in the retina, as variants in genes involved in these processes have been found to confer increased risk of AMD progression in several genome-wide association studies [12–14]. In addition, histological studies have demonstrated the accumulation of phospholipids and cholesterol in Bruch's membrane-retinal pigment epithelium complex, which increases with aging and the AMD stage [15, 16]. In the highly oxidative environment of the outer retina, these lipids have been noted to undergo conversion to oxidized species, which exert deleterious changes resembling those found in advanced AMD [17, 18].

Rheohaemapheresis is an extracorporeal membrane filtration method for the elimination of high molecular weight proteins (i.e., fibrinogen, 2-macroglobulin, immunoglobulin M, thrombomodulin), which also significantly decreases low-density lipoprotein cholesterol [19]. This method can normalize rheologically important parameters (the viscosity of blood and plasma as well as erythrocyte aggregability), can improve erythrocyte flexibility, and could lead to substantial improvement of visual functions in people suffering from AMD [20]. Consequently, this could improve blood flow in the choroid, which is reduced in the dry form of AMD [21]. Therapy using rheohaemapheresis in AMD patients may have the influence also on other proteins like PCSK9.

Primary objective of this study was to investigate for the first time whether the AMD associates with increased plasma PCSK9 which is linked to the regulation to the metabolism of LDL-C. Secondary objective was to evaluate whether the treatment of AMD by rheohaemapheresis reduces plasma PCSK9 levels as it removes LDL, and whether this will have the positive and persistent effect on the course of AMD.

Materials And Methods

Methods/Patients

The project was a prospective, open-label, controlled study involving patients with AMD. The protocol was carried out according to the Declaration of Helsinki. All examined individuals were Caucasians, and all signed informed consent forms, which, together with the protocol of the study, were approved by the institute's ethics committee (approval number 201607 S03P).

We treated 31 patients with AMD, using rheohaemapheresis between March 2012 and September 2019. The group consisted of 20 females and 11 males. Average age was 69.1 ± 4.9 years. The patients were treated for coronary heart disease, n = 2 (6%); cerebrovascular disease, n = 2 (6%); hypertension, n = 14 (45%); hyperlipoproteinemia, n = 14 (45%). None of the patients was treated for peripheral artery disease or diabetes mellitus. Statins were used in 11 patients (35%) and ezetimibe in 1 patient (3%) for the treatment of hyperlipoproteinemia.

The patients were followed up for a long period of time. Inclusion criteria were: Patients with AMD in the soft drusen stage, grade 1–3 according to the EURYEYE study [22], body weight over 50 kg and with other feasible indications for apheresis therapy (peripheral veins allowing vascular access to establish the extracorporeal circuit). Exclusion criteria were: study eye with exudative AMD, study eye with concomitant retinal or choroidal disorder other than AMD, study eye with significant central lens opacities and/or conditions that limit the view of the fundus, uncontrolled diabetes, uncontrolled arterial hypertension, insufficient antecubital venous access, haemato-oncological malignancies, patients who were unwilling to adhere to the examination visit schedule or who were in poor general condition (serious diseases – infections, cardiovascular or cerebral insufficiency, severe CAD).

For the studies examining PCSK9 levels, a group of 53 healthy blood donors (age 55.0 ± 6.7 years, 26 females, 27 males) was included.

Ophthalmological examination

An ophthalmological examination was carried out, using best-corrected visual acuity (BCVA) and ophthalmoscopy. Also, the development of soft drusen, DPED and the area of RPE atrophy was monitored using fundus photography in 7 areas, performed with the digital fundus camera (Zeiss FF 450 + IR; Zeiss, Jena, Germany). For quantification of size changes (DPED, RPE atrophy) during the course of treatment [mm^2], the VISUPAC program (Zeiss, Jena, Germany) was used. For electrophysiological examination of the retina, we used electroretinography: flash ERG for rod and cone systems, pattern ERG for ganglion cells and multifocal ERG for central retinal activity (RETI-port plus mfERG System; Roland Consult, Germany). Progression of the disease to its wet form was evaluated by fluorescein angiography (FAG; Zeiss FF 450 + IR, Zeiss, Jena, Germany). Spectral domain optical coherence tomography (Cirrus, Zeiss, Jena, Germany) was used to verify the position of drusen and to determine anatomical changes in the layer of photoreceptors, focusing on the integrity of the outer and inner segment junctions: OS / IS. An

advanced 5-line SD-OCT raster scan was used to assess disorders of photoreceptor junctions, which are important for vision.

The patients were further divided into 2 groups based on the ophtalmological examination. The first group consisted of patients who were clinically successfully treated, and the second group included patients with therapeutic failure. Patients whose disease became stable or improved (visual acuity, morphological ocular findings) were considered as being successfully treated. (Note: the group of successfully treated patients is numerically larger, as it includes patients who were found to have improved, as well as those with a stable disease. Generally, long-term stabilization is considered as successful therapy in the case of AMD). Out of 31 patients in this study, 14 patients who completed rheohaemapheresis treatment using the predetermined scheme, with at least a 5-year follow-up, were included for evaluation. Among them, 10 patients were evaluated as successfully treated, 4 patients as treatment failure.

Clinical evaluation of the patients (visual acuity, morphological ocular findings):

The most important criterion, especially from the patient's point of view, is visual acuity: this was evaluated as stabilization if the visual acuity was within ± 10 letters of the original value on a specified date (i.e. change of maximum of 2 lines of the ETDRS optotypes). Improvement of visual acuity by 11 or more letters was evaluated as an improvement; deterioration by 11 or more letters was evaluated as a worsening. Stabilization of the morphological finding was evaluated as a change in the original area of pathological changes (drusenoid pigment epithelium detachment, area of soft drusen, area of RPE-atrophy) by ± 25%. A decrease of more than 25% in the pathological changes was considered as an improvement; analogical increase by more than 25% from the baseline was considered as a worsening. The effect of the therapy was evaluated by an experienced ophthalmologist.

Rheohaemapheresis

The method of rheohaemapheresis in the treatment of the AMD has been used for more than 10 years in our hospital. In the present study, the data from 31 patients with AMD, treated using rheohaemapheresis between March 2012 and September 2019 are evaluated, as mentioned above. All patients completed the full protocol according to our study. Rheohaemapheresis therapy was used according to Borberg et al. with our own modification [23, 20]. To obtain plasma, we used continuous separators (Cobe Spectra or Spectra Optia, Terumo BCT, Lakewood, Co, USA) and Evaflux 4A filters (Kawasumi, Tokyo, Japan) to wash the obtained plasma were used. The flow through the filter was controlled, using the CF100 automatic machine (Infomed, Geneva, Switzerland). Anticoagulation was performed using a combination of heparin and ACD-A (Baxter, Munich, Germany). 1-1.5 l of blood was washed. The procedures were performed from the peripheral vein in the elbow pit or in the forearm. We presented some more detailed data separately [24–26]. The treatment scheme was performed for patients with AMD according to the MIRA-1 study [27]; 8 procedures in 10 weeks, i.e. 2 aphereses weekly at intervals of 2–4 days, followed by a break of 14 days, followed by another series of aphereses. The patients were then followed up every

6 months. The effects of a successful treatment last from 2 to 2.5 years usually, according to our experience. It is recommended (based on the literature data and our experience [19, 26] that 1–2 additional procedures are performed to boost the effect ("booster therapy") after this 2-year period. For our group of patients, we decided on 2 procedures within 1 week.

Plasma samples and blood analysis

Blood samples were collected immediately before and after rheohaemapheresis in EDTA-containing tubes and centrifuged within 30 minutes at 1500G for 15 min at room temperature. Plasma samples were aliquoted and stored at -80 °C before the proteomic analysis. The analysis was performed periodically with a consistent methodology. Sampling was done at the baseline before therapy, in the middle of the protocol (after 4th rheohaemapheresis) and at the end of the protocol (after 8th rheohaemapheresis).

TC, LDL-C, HDL-C, apoprotein B, and triglycerides were determined using a commercial kit with a Modular Roche analyzer. Haematological parameters were assessed using routine laboratory techniques (fibrinogen, viscosity of the blood and plasma).

Analysis of PCSK9

Serum concentrations of PCSK9 were analyzed using Quantikine Human Proprotein Convertase 9/PCSK9 produced by company R&D Systems (USA). Instructions from the producer were always respected. Serum samples were diluted 20x and the range of measurement was 0.3 to 40 ng/ml.

Biomarkers of inflammation and endothelial dysfunction

The levels of hsCRP were assessed by immunonephelometry with analyzer IMMAGE 800 (Beckman, USA), and results were expressed in milligrams per liter (mg/L) of serum with a detection limit of 1.0 mg/L.

The serum concentrations of the human soluble form of P-selectin were determined by sandwich enzyme-linked immunosorbent assay technique (ELISA) with Human Quantikine P-Selectin/CD62P ELISA commercial kit (R&D Systems, MN, USA) according to the manufacturer's instructions. The limit of detection of sP-Selectin was 0.05 ng/mL. Samples were diluted 1:20. Absorbance values were measured at 450 nm/620 nm by Multiskan RC ELISA reader (Thermo Fisher Scientific, MA, USA).

The level of monocyte chemoattractant protein-1 (MCP-1) was evaluated by ELISA using Quantikine Human CCL2/MCP-1 ELISA kit (R&D Systems, MN, USA) according to the manufacturer's instructions. The concentration was expressed in picograms per milliliter (pg/mL) of serum, with a detection limit of 1.7 pg/mL. Samples were diluted twice (1:1) with specific diluent. The absorbance values were measured at 450 nm by a Multiskan RC ELISA reader (Thermo Fisher Scientific, MA, USA).

The levels of CD40 ligand (CD40L) were detected by ELISA kit Quantikine Human CD40 Ligand/TNFSF5. The kit was manufactured by R&D Systems, MN, USA. The assay was run according to the instructions

for use provided by the manufacturer. Absorbance was measured at 450 nm with the microplate reader Multiskan RC ELISA reader (Thermo Fisher Scientific, MA, USA). Serum samples were 1:5 diluted, and the concentration of CD40L was expressed in pg/mL of serum, with a detection limit of 4.2 pg/mL.

Statistical analyses

Apart from absolute and relative patient frequencies, data are presented as mean (standard deviation). T-tests and (mean values with SDs) and Wilcoxon matched-paired signed rank tests were used for intergroup comparisons. A value of $P < 0.05$ was the minimum requirement for a statistically significant difference. GraphPad Prism 8.0 software (La Jolla, CA, USA), JMP (2012 SAS Institute, Inc.) and SigmaPlot 12.5 (2013 Systat Software, Inc.) statistical software were used for the statistical analyses.

Results

Rheohaemapheresis reduces total cholesterol, LDL-C, HDL-C, apoprotein B, and lipoprotein (a) levels

Rheohaemapheresis treatment was associated with a significant decrease of total cholesterol, LDL-C, HDL-C, apoprotein B, and lipoprotein (a) levels in AMD patients after all 8 rheohaemaphereses (100% cases) (**Table 1**) and also after every single rheohaemapheresis (P <0,0001, results not shown) during the monitored interval of 7 years.

Simultaneously, treatment by rheohaemapheresis resulted in different results based on the baseline lipid values. Thus, the baseline LDL-C and ApoB were significantly higher in AMD patients with therapeutic failure (**Table 2**). Hyperlipidemia treated with statins was more frequent in the group of successfully treated patients (n=5; 50%) than in the patients with therapeutic failure (n=1; 25%).

The effects of rheohaemapheresis in AMD patients on biomarkers of inflammation, endothelial dysfunction and rheologically important parameters

Rheohaemapheresis treatment was associated with a significant decrease of biomarkers of inflammation and endothelial dysfunction (CD40L, MCP-1) during the monitored interval of 7 years. The levels of hsCRP and sP-selectin did not change significantly. The concentration of the rheologically important parameters (fibrinogen, viscosity of the blood and plasma) decreased significantly after rheohaemapheresis (**Table 3**).

The effects of rheohaemapheresis in AMD patients on the levels of PCSK9

The levels of serum PCSK9 were significantly higher in AMD patients than in the healthy control group ($P<0.001$). Rheohaemapheresis treatment was associated with a significant decrease of serum concentration of PCSK9 during the monitored interval of 7 years. The levels of serum PCSK9 decreased significantly after each rheohaemapheresis (**Figure 1**).

Simultaneously, treatment by rheohaemapheresis resulted in different results based on decrease of PCSK9 values post-rheohaemapheresis. Thus, the decrease of serum PCSK9 was significant post-

rheohaemapheresis in AMD patients with successful treatment, but not in patients with therapeutic failure (**Table 4**). The concentration of PCSK9 before rheohaemapheresis was not statistically different between AMD patients with successful treatment and patients with therapeutic failure.

Discussion

In this paper, we aimed to analyze the data collected in last 7 years from patients with AMD treated with rheohaemapheresis in order to elucidate the benefit of this procedure with respect to plasma lipids, selected biomarkers of inflammation, endothelial dysfunction, and a novel regulatory protein of lipid metabolism - PCSK9.

There are three main findings in this study. (i) Treatment with rheohaemapheresis was associated with the long-lasting, significant and desirable improvement of visual acuity concomitantly with the decrease in the original area of pathological changes (drusenoid pigment epithelium detachment, area of soft drusen, area of RPE-atrophy) in 10 from the total of 31 patients (32.2%), while therapeutic failure was present in 4 from 31 patients (12.9%). 17 patients (54.8%) did not have at least a 5-year follow-up, therefore they were not included for this evaluation. (ii) Treatment with rheohaemapheresis was associated with the significant reduction of lipids (TC, LDL-C, HDL-C, apoB, Lp(a)) and PCSK9; biomarkers of endothelial dysfunction (CD40L, MCP-1); and rheologically important parameters (fibrinogen, blood and plasma viscosity) after each procedure. (iii) The improvement of the best-corrected visual acuity and ophthalmoscopic findings in successfully treated AMD patients was associated with significantly lower lipid parameters (LDL-C, apoB and PCSK9).

We have shown in the present study, that the treatment of the in patients with advanced dry AMD by rheohaemapheresis resulted in the BCVA improvement during long-term follow up. These results confirmed our previous findings, where we demonstrated improved BCVA in the group of treated patients and statistically significant deterioration in the control group of patients [26]. Similar results published the authors of the MAC-I study from the University of Cologne, MAC-II study from the University of Frankfurt, and MAC-III study from the University of Hamburg [19].

While the use of rheohaemapheresis was associated with successful treatment of AMD, the precise mechanisms have to be established. There are several plausible explanations resulting from this study with regard to lipid metabolism. The retina has multiple physiological demands for cholesterol utilization. There are physiological changes in cholesterol metabolism that occur with aging, and these affect the RPE. Drusen and basal linear deposit are quite specific for AMD, and cholesterol is present in drusen, which are pathognomonic disease markers both on clinical examination and in histopathological study [28]. Eye pathology studies demonstrate a high cholesterol concentration in classical AMD lesions, such as drusen, aging BM, and newly discovered subretinal lesions [1]. Numerous cholesterol and lipoprotein-related proteins and genes are expressed in human RPE and retina [29].

The beneficial effects of rheohaemapheresis treatment was associated with a significant decrease of total cholesterol, LDL-C, HDL-C, apoprotein B, and lipoprotein (a) levels in AMD patients after all

rheohaemaphereses during the monitored interval of 7 years. Moreover, the baseline LDL-C and ApoB were significantly higher in AMD patients with therapeutic failure than in successfully treated patients in our study. One explanation could be that treatment with statins was more frequent in the group of successfully treated patients (n = 5; 50%) than in the patients with therapeutic failure (n = 1; 25%). There are a number of mechanisms by which statins may exert protective effects in AMD. These include, but are not limited to, serum lipid-lowering that may alter BM lipid deposition [30]; preservation of the outer retinal and choroidal vascular supply by an anti-atherogenic effect [31]; anti-inflammatory properties [31]; antioxidant effects that may counter increased plasma levels of oxidized LDL [32]; and inhibition of metalloproteinases that may contribute to fissuring and rupture of plaques that lead to neovascularization [33]. HMG-CoA reductase inhibition may also have direct effects on cholesterol processing by outer retinal cells. The RPE is a native secretor of lipoproteins, and statins may affect lipidation of lipoproteins directly [34]. However, a number of studies have examined the relationship between AMD and statin effectiveness compared to other treatment options, absence of treatment, or placebo, but the results remain mixed [35].

We have shown for the first time that another key regulator of lipid metabolism which is serum PCSK9, has been significantly higher in AMD patients than in the healthy control group. This is unique finding with further implication into the possible pharmacological treatment of AMD by novel class of hypolipidemic drugs - inhibitors of PCSK9. So far, there are not any data in the literature studying the involvement of PCSK9 in the development of AMD. One experimental study used minipigs overexpressing a gain-of-function mutant (D374Y) of the human gene PCSK9 with a consequent experimental hypercholesterolemia and found advanced atheromatosis of retinal arterioles caused by lipid overload [36]. The finding of increased plasma PCSK9 in the AMD patients predispose them to the long-lasting hypercholesterolemia and related atherogenic changes in the retinal arteries. On the other hand, efficient removal of LDL-C during the treatment by rheohaemapheresis (LDL-C is rapidly reduced on average by 40% per one session) will deplete cholesterol cell content and, via upregulation of sterol regulatory element binding protein (SREBP) [37] will down-regulate *PCSK9* gene expression and thus lead to the decreased formation of PCSK9. In fact, rheohaemapheresis treatment in AMD patients was associated with a significant decrease of serum concentration of PCSK9 during the monitored interval of 7 years. We assume that the PCSK9 particles had been removed by the rheohaemapheresis filters, although we did not measure PCSK9 concentration directly in the adsorbers. However, we measured the difference of the PCSK9 concentration in the plasma at the inflow of the adsorbers and in the plasma outflow just after the adsorber in random 6 patients. We found that the immediate retention of PCSK9 in the adsorbers is in the range of 17%. Regardless the mechanism the resulting decrease of PCSK9 should lead to the decrease of LDL-C, similarly as does the therapy with PCSK9 inhibitors [38]. Moreover, treatment by rheohaemapheresis also resulted in different results based on decrease of PCSK9 values post-rheohaemapheresis. Thus, the decrease of serum PCSK9 was significant post-rheohaemapheresis in AMD patients with successful treatment, but not in patients with therapeutic failure. These findings further imply that not only lower plasma LDL-C, but also lower plasma PCSK9 should be beneficial in order to successfully treat AMD. We assume that the significant decrease of PCSK9 after individual

rheohaemapheresis is not long-lasting, similarly as the decrease of LDL-C. The rebound of LDL-C post LDL-apheresis occurs in 13 days post-treatment [39], and similar kinetics should be expected post-rheohaemapheresis. Body stores would be quickly depleted if not replaced by absorption of dietary cholesterol or newly synthesized cholesterol. The data has been shown indicating a threshold effect: reduction of LDL cholesterol to levels below 1.0 mmol/l induces an upregulation of the cholesterol biosynthesis in normocholesterolemic subjects [40]. In our study LDL cholesterol was lowered to 0.92 mmol/l immediately after rheohaemapheresis. Despite prolonged, aggressive lipid lowering with rheohaemapheresis which should stimulate cholesterol biosynthesis and thus increase LDL-C, this was not noted in our present study. Most probable explanation is the frequency of aphereses (2 aphereses weekly at intervals of 2–4 days), which prevented the increase of LDL-C.

The beneficial impact of lowering LDL-C and PCSK9 by means of rheohaemapheresis in AMD patients was accompanied also with significant decrease of biomarkers of inflammation and endothelial dysfunction (CD40L, MCP-1) during the monitored interval of 7 years. We previously evaluated MCP-1 during our earlier research of rheohaemapheresis use due to the significant importance of macrophages in the microcirculation and we found a significant decrease in patients with AMD [26]. This study demonstrated its decrease in patients with AMD. As stated in the literature, this may be a significant factor, indicating efficacy of the impact on activity of the inflammatory process or atherosclerosis [19]. The mechanism of the inflammatory process modulation by rheohaemapheresis in the pathogenesis of AMD could also be documented by a significant decrease in inflammatory marker sCD40L in case of atherosclerotic mechanism, respectively. Evaluation of rheologically important parameters before and immediately after rheohaemapheresis showed significant decrease after rheohaemapheresis (fibrinogen decreased by 48%, viscosity of the blood by 9% and viscosity of plasma by 12%). The result is undoubtedly an improved microcirculation flow, which is also a basic prerequisite for increased flow in the choroid and improved retinal metabolism.

Study limitations

Our study had a small sample size, includes only the treatment group, and we did not incorporate a control treatment arm in this study. The patients were compared based on results of ophtalmological examination as the subjects who were clinically successfully treated and/or the individuals with therapeutic failure which shortened even more the number of evaluated patients. The small number of participants in the present study may affect the accuracy of our results. Furthermore, since the lipoprotein apheresis treatment technique is carried out only in our medical centers in the Czech Republic because of the technical and economic reasons, many AMD patients are unable to receive apheresis treatment, resulting in a particular bias in patient selection. Moreover, it is necessary to diagnose AMD disease in the early stage of the dry form, which is not frequently possible. Our study's strength is the collection of a large set of data comprising the long-term monitored interval of 7 years. We have experience with the rheohaemapheresis therapy in AMD patients in our center for more than 10 years, the total number of treated patients is 74, and the PCSK9 concentration was evaluated from the year 2012 on in 66 patients. To assess the outcome in AMD it is necessary to respect its slow progression rate, therefore we set up the

interval of 5 years of follow-up and evaluate finally 31 patients who completed it. Finally, our study group is unique also because of the evaluation of the relationship of PCSK9 concentration to the AMD prognosis, markers of endothelial dysfunction, inflammation and rheologically important parameters. The results obtained in this study are valuable and will be evolved in our further research.

Conclusion

In our study we demonstrated the beneficial long-term effects of rheohaemapheresis on the dry form of AMD. BCVA improvement was accompanied by lowering of LDL-C and PCSK9 and improvement of markers of endothelial dysfunction. Anti-PCSK9 therapies, such as antibodies, are approved for cholesterol control in patients with hypercholesterolemia and atherosclerotic diseases [38]. The anti-PCSK9 therapies may reduce also the burden of AMD in patients treated by rheohaemapheresis therapy. Rheohaemapheresis and other anti-PCSK9 therapies may be even used synergistically to reduce severity, slow down or even induce regression of AMD. Further research, motivated by our findings of the effects of rheohaemapheresis on lipid-related and atherosclerotic markers, could contribute to the improved knowledge of AMD etiopathogenesis and treatment.

Abbreviations

PCSK9
Proprotein convertase subtilisin kexin 9; AMD:Age-related macular degeneration; DPED:Drusenoid pigment epithelium detachment; BCVA:Best-corrected visual acuity (BCVA); ERG:electroretinography; TC:total cholesterol; LDL:low density lipoprotein; LDL-C:low density lipoprotein cholesterol; HDL-C:high density lipoprotein cholesterol; apoB:apoprotein B; Lp (a):lipoprotein (a).

Declarations

Acknowledgements

Not applicable.

Author contributions

VB designed study experiments, wrote the manuscript. HL designed study experiments, wrote the manuscript. MB provided rheohaemapheresis, revised the text of the manuscript. JS performed ophtalmological examination, results analysis, revised the text of the manuscript. AS performed ophtalmological examination, results analysis, revised the text of the manuscript. CA performed ELISA analysis of samples. VL provided results analysis, revised the text of the manuscript. ML provided rheohaemapheresis, revised the text of the manuscript. EV calculated statistical analysis, revised the text of the manuscript. PD performed results analysis, statistical analysis, revised the text of the manuscript. All authors read and approved the final manuscript.

Funding

This study has been funded with an unrestricted grant by Ministry of Health of the Czech Republic, grant no. 17-29241A. All rights reserved. The funding body had no role in the design of the study and collection, analysis, and interpretation of data nor in writing the manuscript.

Data availability statements

The datasets generated and analyzed during the current study are not publicly available because it could compromise the anonymity and confidentiality of the patient data but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the local Ethical Committee of University Hospital Hradec Kralove. All subjects participating in the study gave written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]University Hospital Hradec Králové and Charles University, Faculty of Medicine in Hradec Králové, 3rd Department of Internal Medicine - Metabolism and Gerontology, Hradec Králové, Czech Republic

[2]University Hospital Hradec Králové and Charles University, Faculty of Medicine in Hradec Králové, Department of Ophtalmology, Hradec Králové, Czech Republic

[3]University Hospital Hradec Králové and Charles University, Faculty of Medicine in Hradec Králové, 4th Department of Medicine - Hematology, Hradec Králové, Czech Republic

[4]University Hospital Hradec Králové and Charles University, Faculty of Medicine in Hradec Králové, Department of Immunology and Allergology, Hradec Králové, Czech Republic

[5]Regional hospital Liberec, Department of Ophtalmology, Czech Republic

Literature

1. Roizenblatt M, Naranjit N, Maia M, Gehlbach PL. The Question of a Role for Statins in Age-Related Macular Degeneration. Int J Mol Sci. 2018;19(11). doi:10.3390/ijms19113688.

2. Wong TY, Larsen EK, Klein R, Mitchell P, Couper DJ, Klein BE et al. Cardiovascular risk factors for retinal vein occlusion and arteriolar emboli: the Atherosclerosis Risk in Communities & Cardiovascular Health studies. Ophthalmology. 2005;112(4):540-7. doi:10.1016/j.ophtha.2004.10.039.
3. Tserentsoodol N, Sztein J, Campos M, Gordiyenko NV, Fariss RN, Lee JW et al. Uptake of cholesterol by the retina occurs primarily via a low density lipoprotein receptor-mediated process. Mol Vis. 2006;12:1306-18.
4. Fliesler SJ, Florman R, Rapp LM, Pittler SJ, Keller RK. In vivo biosynthesis of cholesterol in the rat retina. FEBS Lett. 1993;335(2):234-8. doi:10.1016/0014-5793(93)80736-e.
5. Tserentsoodol N, Gordiyenko NV, Pascual I, Lee JW, Fliesler SJ, Rodriguez IR. Intraretinal lipid transport is dependent on high density lipoprotein-like particles and class B scavenger receptors. Mol Vis. 2006;12:1319-33.
6. https://www.proteinatlas.org/ENSG00000169174-PCSK9/tissue/retina.
7. Ferri N, Ruscica M. Proprotein convertase subtilisin/kexin type 9 (PCSK9) and metabolic syndrome: insights on insulin resistance, inflammation, and atherogenic dyslipidemia. Endocrine. 2016;54(3):588-601. doi:10.1007/s12020-016-0939-0.
8. Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2004;24(8):1454-9. doi:10.1161/01.Atv.0000134621.14315.43.
9. Li H, Dong B, Park SW, Lee HS, Chen W, Liu J. Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. J Biol Chem. 2009;284(42):28885-95. doi:10.1074/jbc.M109.052407.
10. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet. 2003;34(2):154-6. doi:10.1038/ng1161.
11. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. N Engl J Med. 2008;358(24):2606-17. doi:10.1056/NEJMra0801537.
12. Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, Thorleifsson G et al. Seven new loci associated with age-related macular degeneration. Nat Genet. 2013;45(4):433-9, 9e1-2. doi:10.1038/ng.2578.
13. Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J et al. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci U S A. 2010;107(16):7401-6. doi:10.1073/pnas.0912702107.
14. Neale BM, Fagerness J, Reynolds R, Sobrin L, Parker M, Raychaudhuri S et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci U S A. 2010;107(16):7395-400. doi:10.1073/pnas.0912019107.

15. Curcio CA, Johnson M, Huang JD, Rudolf M. Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. J Lipid Res. 2010;51(3):451-67. doi:10.1194/jlr.R002238.
16. Rodriguez IR, Clark ME, Lee JW, Curcio CA. 7-ketocholesterol accumulates in ocular tissues as a consequence of aging and is present in high levels in drusen. Exp Eye Res. 2014;128:151-5. doi:10.1016/j.exer.2014.09.009.
17. Baba T, Bhutto IA, Merges C, Grebe R, Emmert D, McLeod DS et al. A rat model for choroidal neovascularization using subretinal lipid hydroperoxide injection. Am J Pathol. 2010;176(6):3085-97. doi:10.2353/ajpath.2010.090989.
18. Tamai K, Spaide RF, Ellis EA, Iwabuchi S, Ogura Y, Armstrong D. Lipid hydroperoxide stimulates subretinal choroidal neovascularization in the rabbit. Exp Eye Res. 2002;74(2):301-8. doi:10.1006/exer.2001.1121.
19. Klingel R, Fassbender C, Fassbender T, Göhlen B. Clinical studies to implement Rheopheresis for age-related macular degeneration guided by evidence-based-medicine. Transfus Apher Sci. 2003;29(1):71-84. doi:10.1016/s1473-0502(03)00101-0.
20. Borberg H, Tauchert M. Rheohaemapheresis of ophthalmological diseases and diseases of the microcirculation. Transfus Apher Sci. 2006;34(1):41-9. doi:10.1016/j.transci.2005.09.001.
21. Zion IB, Harris A, Siesky B, Shulman S, McCranor L, Garzozi HJ. Pulsatile ocular blood flow: relationship with flow velocities in vessels supplying the retina and choroid. Br J Ophthalmol. 2007;91(7):882-4. doi:10.1136/bjo.2006.108340.
22. Augood CA, Vingerling JR, de Jong PTVM, Chakravarthy U, Seland J, Soubrane G et al. Prevalence of Age-Related Maculopathy in Older Europeans: The European Eye Study (EUREYE). Archives of Ophthalmology. 2006;124(4):529-35. doi:10.1001/archopht.124.4.529.
23. Blaha V, Blaha M, Solichová D, Krčmová LK, Lánská M, Havel E et al. Antioxidant defense system in familial hypercholesterolemia and the effects of lipoprotein apheresis. Atheroscler Suppl. 2017;30:159-65. doi:10.1016/j.atherosclerosissup.2017.05.002.
24. Rencová E, Bláha M, Studnička J, Bláha V, Brožík J, Pazderová M et al. Reduction in the drusenoid retinal pigment epithelium detachment area in the dry form of age-related macular degeneration 2.5 years after rheohemapheresis. Acta Ophthalmol. 2013;91(5):e406-8. doi:10.1111/j.1755-3768.2012.02503.x.
25. Rencová E, Bláha M, Studnička J, Blažek M, Bláha V, Dusová J et al. Haemorheopheresis could block the progression of the dry form of age-related macular degeneration with soft drusen to the neovascular form. Acta Ophthalmol. 2011;89(5):463-71. doi:10.1111/j.1755-3768.2009.01710.x.
26. Studnička J, Rencová E, Bláha M, Rozsíval P, Lánská M, Bláha V et al. Long-term outcomes of rheohaemapheresis in the treatment of dry form of age-related macular degeneration. J Ophthalmol. 2013;2013:135798. doi:10.1155/2013/135798.
27. Pulido JS. Multicenter prospective, randomized, double-masked, placebo-controlled study of Rheopheresis to treat nonexudative age-related macular degeneration: interim analysis. Trans Am Ophthalmol Soc. 2002;100:85-106; discussion -7.

28. Pikuleva IA, Curcio CA. Cholesterol in the retina: the best is yet to come. Prog Retin Eye Res. 2014;41:64-89. doi:10.1016/j.preteyeres.2014.03.002.
29. Zheng W, Reem RE, Omarova S, Huang S, DiPatre PL, Charvet CD et al. Spatial distribution of the pathways of cholesterol homeostasis in human retina. PLoS One. 2012;7(5):e37926. doi:10.1371/journal.pone.0037926.
30. Rader DJ, Maugeais C. Genes influencing HDL metabolism: new perspectives and implications for atherosclerosis prevention. Mol Med Today. 2000;6(4):170-5. doi:10.1016/s1357-4310(00)01673-7.
31. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. Prog Retin Eye Res. 2001;20(3):385-414. doi:10.1016/s1350-9462(00)00025-2.
32. Ikeda T, Obayashi H, Hasegawa G, Nakamura N, Yoshikawa T, Imamura Y et al. Paraoxonase gene polymorphisms and plasma oxidized low-density lipoprotein level as possible risk factors for exudative age-related macular degeneration. Am J Ophthalmol. 2001;132(2):191-5. doi:10.1016/s0002-9394(01)00975-8.
33. Guymer RH, Chiu AW, Lim L, Baird PN. HMG CoA reductase inhibitors (statins): do they have a role in age-related macular degeneration? Surv Ophthalmol. 2005;50(2):194-206. doi:10.1016/j.survophthal.2004.12.002.
34. Funatsu T, Suzuki K, Goto M, Arai Y, Kakuta H, Tanaka H et al. Prolonged inhibition of cholesterol synthesis by atorvastatin inhibits apo B-100 and triglyceride secretion from HepG2 cells. Atherosclerosis. 2001;157(1):107-15. doi:10.1016/s0021-9150(00)00714-0.
35. Gehlbach P, Li T, Hatef E. Statins for age-related macular degeneration. Cochrane Database Syst Rev. 2016(8):Cd006927. doi:10.1002/14651858.CD006927.pub5.
36. Bek T, Al-Mashhadi RH, Misfeldt M, Riis-Vestergaard MJ, Bentzon JF, Pedersen SMM. Relaxation of porcine retinal arterioles exposed to hypercholesterolemia in vivo is modified by hepatic LDL-receptor deficiency and diabetes mellitus. Experimental Eye Research. 2013;115:79-86. doi:https://doi.org/10.1016/j.exer.2013.06.013.
37. Costet P, Cariou B, Lambert G, Lalanne F, Lardeux B, Jarnoux AL et al. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. J Biol Chem. 2006;281(10):6211-8. doi:10.1074/jbc.M508582200.
38. Latimer J, Batty JA, Neely RD, Kunadian V. PCSK9 inhibitors in the prevention of cardiovascular disease. J Thromb Thrombolysis. 2016;42(3):405-19. doi:10.1007/s11239-016-1364-1.
39. Kroon AA, van't Hof MA, Demacker PNM, Stalenhoef AFH. The rebound of lipoproteins after LDL-apheresis. Kinetics and estimation of mean lipoprotein levels. Atherosclerosis. 2000;152(2):519-26. doi:https://doi.org/10.1016/S0021-9150(00)00371-3.
40. Feillet C, Cristol JP, Michel F, Kanouni T, Navarro R, Navarro M et al. Cholesterol biosynthesis in normocholesterolemic patients after cholesterol removal by plasmapheresis. J Clin Apher. 1997;12(3):110-5. doi:10.1002/(sici)1098-1101(1997)12:3<110::aid-jca2>3.0.co;2-d.

Tables

Table 1. The effects of rheohaemapheresis in AMD patients on total cholesterol, LDL-C, HDL-C, apoprotein B, and lipoprotein (a) levels. TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; apoB, apoprotein B; Lp (a), lipoprotein (a).

		Before rheohaemapheresis			After rheohaemapheresis			P
		N	Mean	SD	N	Mean	SD	
TC	mmol/l	94	4.22	0.94	94	2.15	0.43	< 0.0001
LDL-C	mmol/l	94	2.30	0.80	94	0.93	0.35	< 0.0001
HDL-C	mmol/l	94	1.60	0.37	94	1.09	0.24	< 0.0001
ApoB	mmol/l	94	1.20	2.99	94	0.39	0.95	0.0011
Lp (a)	nmol/l	94	20.85	31.52	94	8.06	11.88	< 0.0001

Table 2. The lipid values in successfully treated AMD patients and in patients with therapeutic failure. TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; apoB, apoprotein B; Lp (a), lipoprotein (a).

		Successfully treated			Therapeutic failure			P
		N	Mean	SD	N	Mean	SD	
TC	mmol/l	10	4.03	0.87	4	4.34	0.87	NS
LDL-C	mmol/l	10	2.03	0.60	4	2.46	0.71	0.0499
HDL-C	mmol/l	10	1.72	0.42	4	1.49	0.21	NS
ApoB	mmol/l	10	0.65	0.17	4	0.85	0.15	0.0019
Lp (a)	nmol/l	10	13.53	22.53	4	19.65	36.28	NS

Table 3. The effects of rheohaemapheresis in AMD patients on biomarkers of inflammation, endothelial dysfunction and rheologically important parameters. CD40L, CD40 ligand; hsCRP, high sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; sP-selectin, soluble plasma selectin; B viscosity, viscosity of the blood; P viscosity, viscosity of the plasma.

		Before rheohaemapheresis			After rheohaemapheresis			P
		N	Mean	SD	N	Mean	SD	
CD40L	pg/mL	90	4540.68	2130.63	90	3776.32	2196.83	0.0093
hsCRP	mg/L	90	2.83	2.15	90	2.43	1.82	NS
MCP-1	pg/mL	90	306.33	128.46	90	259.55	103.76	0.0140
sP-selectin	ng/mL	90	99.83	47.37	90	94.47	47.41	NS
Fibrinogen	g/L	94	2.97	0.61	94	1.43	1.39	< 0.0001
B viscosity	mPa.s	93	6.14	1.04	93	5.58	0.69	< 0.0001
P viscosity	mPa.s	94	2.05	0.29	94	1.82	0.26	< 0.0001

Table 4. The concentration of serum PCSK9 in successfully treated AMD patients and in AMD patients with therapeutic failure. PCK9, proprotein convertase subtilisin kexin 9. Sampling for analysis of PCSK9 was done in the AMD patients before and after each rheohaemapheresis. Results are shown as mean ± SD in successfully treated patients (n=10) and in patients with therapeutic failure (n=4).

		Successfully treated			Therapeutic failure		
		Before	After	P	Before	After	P
PCSK9	ng/mL	282 ± 115	175 ± 127	< 0.001	234 ± 86	184 ± 166	NS

Figures

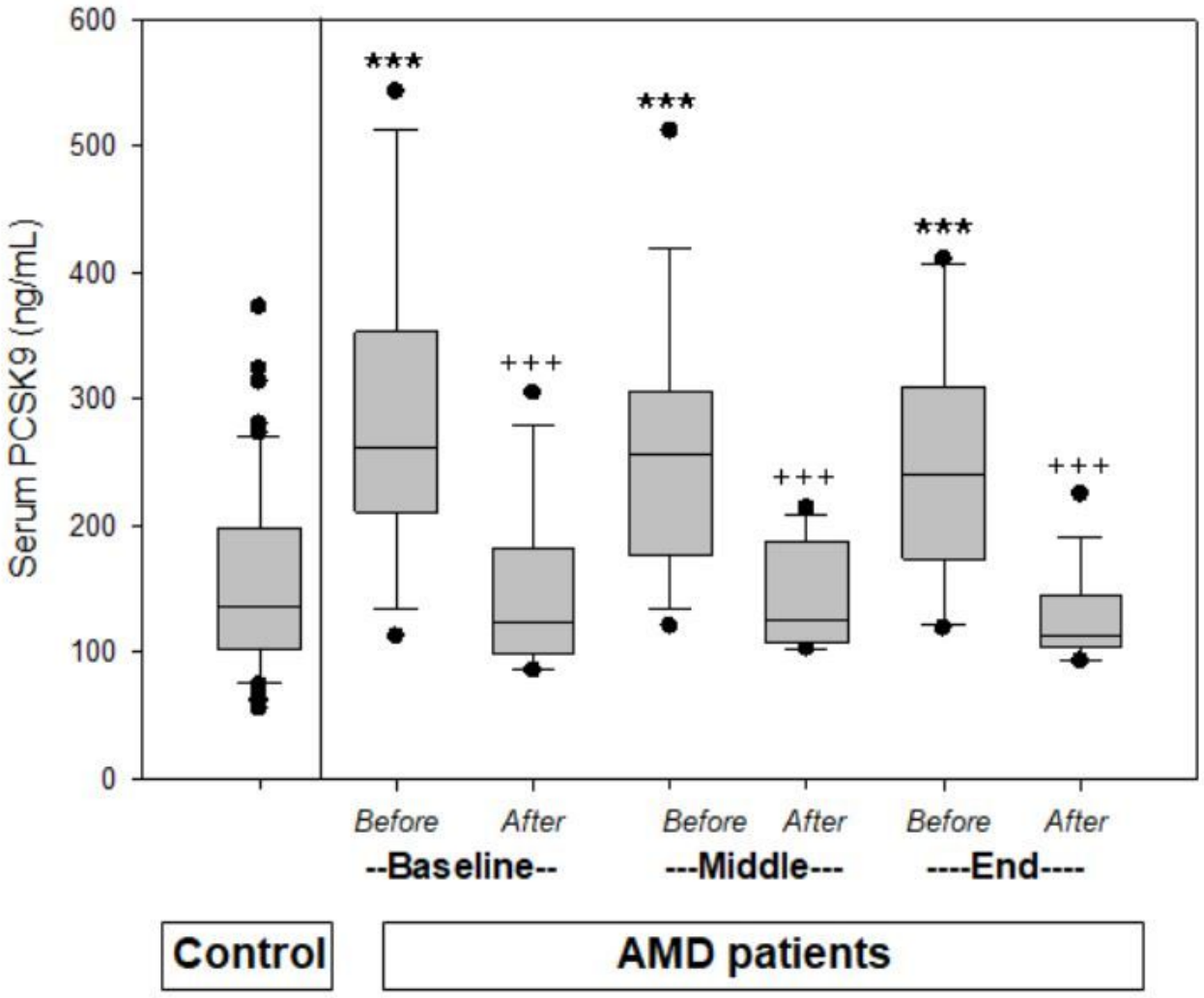

Figure 1

The effects of rheohaemapheresis in AMD patients on serum concentrations of PCSK9 Sampling for analysis of PCSK9 was done in the AMD patients at the baseline before therapy by rheohaemapheresis; Baseline), in the middle of the protocol (after 4th rheohaemapheresis; Middle) and at the end of the protocol (after 8th rheohaemapheresis; End). The boxes represent analysis before (Before) and after (After) rheohaemapheresis. Control group are healthy blood donors. Significant differences are marked by *** ($P<0.001$) for AMD patients versus control group; +++ ($P<0.001$) for AMD patients before versus after rheohaemapheresis.