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# Rheopheresis for age-related macular degeneration: clinical results and putative mechanism of action

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## ABSTRACT • RÉSUMÉ

**Background:** Rheopheresis is being evaluated in a clinical trial. The rationale and available results are presented.

**Methods:** We reviewed the literature about the pathophysiology of age-related macular degeneration (AMD) that might support the use of rheopheresis. In addition, we reviewed the previously published results of the use of rheopheresis for AMD.

**Results:** There appears to be a diffusion barrier caused by accumulation of cross-linked proteins known as advanced macular oxidation products (AMOPS) in AMD. Rheopheresis allows removal of uncross-linked proteins and facilitates antioxidant entry into Bruch's membrane, preventing further accumulation of AMOPS. The Multicenter Investigation of Rheopheresis for AMD (MIRA-1), an ongoing double-masked randomized trial, should determine the efficacy of rheopheresis in preventing the progression of AMD. The interim results, from an analysis of visual acuity data for 43 patients, are encouraging, confirming the potential of rheopheresis as a therapeutic option for dry AMD. The benefit was evident immediately after treatment and remained essentially stable throughout the 12-month period of evaluation. Eyes with late-stage, high-risk, dry AMD appeared to be at significant risk for substantial vision loss over the 12 months if not treated. Subgroup analysis demonstrated that the timing of rheopheresis in the course of a patient's disease may have a pronounced effect on outcome.

**Interpretation:** There appears to be a rationale for the use of rheopheresis in AMD. Further results of the clinical trial are awaited.

**Objet :** La rhéophérèse a été évaluée au cours d'un essai clinique. En voici le bien-fondé et les résultats disponibles.

**Méthodes :** Nous avons examiné la documentation sur la pathophysiologie de la dégénérescence maculaire liée à l'âge (DMLA) qui pourrait justifier le recours à la rhéophérèse. Nous avons aussi examiné les résultats antérieurs du traitement de la DMLA par rhéophérèse.

**Résultats :** Il semble y avoir une barrière de diffusion causée par l'accumulation de protéines réticulées, appelées produits avancés d'oxydation de la macule (PAOM), dans la DMLA. La rhéophérèse permet d'extraire les protéines non réticulées, ce qui favorise l'entrée des antioxydants dans la membrane de Bruch, prévenant

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d'autres accumulations de PAOM. L'étude MIRA-1 (*Multicenter Investigation of Rheopheresis for AMD*), une étude à double insu randomisée en cours, devrait établir l'efficacité de la rhéophérèse pour prévenir la progression de la DMLA. Les résultats provisoires, provenant d'une analyse des données sur l'acuité visuelle de 43 patients, sont encourageants en ce qu'ils confirment le potentiel de la rhéophérèse comme option thérapeutique pour la DMLA sèche. Les bienfaits sont apparus immédiatement après le traitement et demeurés essentiellement stables durant les 12 mois qu'a duré l'évaluation. Les yeux qui ont une DMLA sèche avancée et à risque élevé semblent être grandement exposés à une perte substantielle de la vue durant la période de 12 mois s'ils ne sont pas traités. L'analyse des sous-groupes a démontré que le recours à la rhéophérèse au moment opportun de la maladie peut avoir un effet marqué sur le résultat.

**Interprétation : Il semble y avoir un bien-fondé au recours à la rhéophérèse pour la DMLA. On attend les autres résultats des essais cliniques.**

Therapeutic apheresis has not previously been used in ophthalmology and therefore requires explanation of not only methodology and clinical results but also mechanism of action. Rheopheresis is a safe and effective application of double-filtration plasmapheresis for extracorporeal hemorheotherapy. The elimination of an exactly defined spectrum of high-molecular-weight proteins — including fibrinogen,  $\alpha_2$ -macroglobulin, low-density-lipoprotein (LDL) cholesterol, fibronectin, von Willebrand factor and, probably, multimeric vitronectin — reduces blood and plasma viscosity as well as erythrocyte and thrombocyte aggregation and improves erythrocyte flexibility. The serial pulses of plasma protein elimination with associated reduction of plasma viscosity can result in sustained improvements in the microcirculation at the functional levels required to sustain the perfused tissues.

At the cellular and molecular levels, age-related macular degeneration (AMD) is, at least in part, a microcirculatory disorder of the retina. A functional reserve exists in the retina affected by AMD that is determined by a pattern of reversible and irreversible morphologic and physiological changes. AMD spontaneously has a chronic and progressive course. The capacity of the individual functional reserve cannot currently be assessed by any diagnostic procedure. The goal of rheopheresis is to restore and activate or stabilize the functional reserve. This, of course, is a hypothesis, but it is confirmed by available clinical data for AMD, ischemic diabetic foot syndrome and fibrinogen/LDL apheresis for sudden deafness, and it can be linked to current concepts of the pathogenesis of AMD.<sup>1-3</sup>

### PHYSIOLOGICAL BASIS OF RHEOPHERESIS IN AMD

Rheopheresis may affect the microcirculation and subsequent function of the retina by altering the diffusion characteristics of Bruch's membrane or the rheology of the choriocapillaris or both.

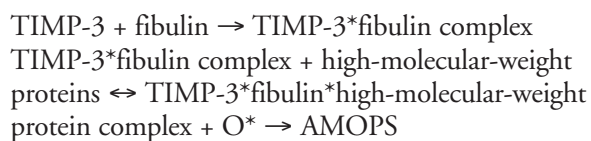
#### Diffusion model

Sorsby's dystrophy is an autosomal dominant form of macular degeneration that is characterized by night blindness and choroidal neovascularization.<sup>4</sup> It is due to an abnormality in the tissue inhibitor of metalloproteinases-3 (TIMP-3).<sup>5</sup> Another genetic disease that affects Bruch's membrane is malattia leventinese. It too is an autosomal dominant disorder and is characterized by drusen-like deposits that appear to radiate from the fundus outwards to the periphery. The genetic abnormality is in a basement-membrane-associated protein, fibulin-3 (EFEMP-1 [epithelial growth factor-containing fibulin-like extracellular matrix protein 1]).<sup>6</sup>

In Sorsby's dystrophy, it was initially thought that Bruch's membrane was a physical barrier that was disrupted by metalloproteinases that could not be inhibited from being activated by the mutant form of TIMP-3. The breakdown of Bruch's membrane would then allow for choroidal neovascularization. Recent studies, however, have shown that the abnormal TIMP-3 that is present in Sorsby's dystrophy can still inactivate metalloproteinases,<sup>7</sup> casting doubt on the importance of the physical-barrier disruption of Bruch's membrane in Sorsby's dystrophy and therefore in other forms of macular degeneration. Instead, an important study by Klenotic and colleagues<sup>6</sup> has shown that the abnormal TIMP-3 binds tightly to

EFEMP-1, creating a formidable barrier to the diffusion of substances into the overlying retinal pigment epithelium (RPE). In addition, this complex may serve as a nidus for the aggregation of other proteins, since others have an affinity to bind EFEMP-1 at sites separate from the binding site of TIMP-3. Immunohistochemical and polymerase chain reaction analysis has shown that vitronectin is a major constituent of human drusen and is expressed by local RPE cells.<sup>8</sup> Drusen-associated vitronectin is the result of selective accumulation. Vitronectin is functionally related to processes of thrombosis, fibrinolysis, inflammation and cellular adhesion. Self-association of vitronectin results in the formation of multimeric species of the protein.<sup>9,10</sup>

Recent studies have shown that other fibulins may be abnormal in at least some cases of AMD.<sup>11</sup> Because of the aggregatory effect of abnormal fibulin with other proteins, it appears that the diffusion model of AMD is accumulating evidence that it is at least an important aspect of the pathogenesis of AMD. Furthermore, Gu and associates<sup>12</sup> have shown that within the drusen in AMD there are proteins that have been modified by cross-linkage to other proteins by free radicals. We call these cross-linked proteins advanced macular oxidation products (AMOPS). It may be that the abnormal barrier formed by the fibulin and TIMP-3 proteins aggregates other proteins of high molecular weight, which are then further modified, creating an even more formidable diffusion barrier (Fig. 1). A steady-state model could be developed in the following way:



In this model, rheopheresis would work in several ways. In the short term it would decrease the amount of high-molecular-weight protein in the serum that could aggregate with the TIMP-3\*fibulin complex and would allow for removal of the nonirreversibly bound high-molecular-weight proteins from the TIMP-3\*fibulin\*high-molecular-weight protein complex in Bruch's membrane, thus allowing the barely functioning RPE cells to function better, diminishing the release of vascular endothelial growth factor (VEGF) and reducing the amount of complex available for cross-linkage. In both the short and the long term, because it changed the diffusion characteristics of

Bruch's membrane, rheopheresis would allow for entry of the antioxidant vitamins that have been shown to decrease the progression of AMD and prevent further cross-linkage.<sup>13</sup> The cross-linked proteins (AMOPS) may inhibit diffusion, cause macrophage accumulation and possibly immunologic damage to Bruch's membrane, and thus accelerate the development of AMD, akin to the situation with cross-linked advanced glycation end-products in diabetic retinopathy.<sup>14,15</sup>

If there is a problem with the microcirculation in the choroid or with the diffusion characteristics of Bruch's membrane, the overlying RPE cells and outer layer of the retina are very vulnerable to ischemia. In addition, the underlying accumulated lipofuscin that cannot be cleared because of the diffusion barrier is toxic to the RPE, causing apoptosis.<sup>16</sup> The affected cells would release cytokines, which would be neuroprotective as well as angiogenic, to help with the cells' survival in this hostile environment. Interestingly, recent studies on VEGF have shown that it is not only angiogenic but also a very potent neuroprotective agent.<sup>17</sup> The release of VEGF by ischemic retinal tissue may thus have the dual effects of angiogenesis and neuroprotection. Rheopheresis, by allowing more diffusion and thereby decreasing the ischemia, should decrease VEGF release by the RPE cells.

### Vascular model

In Friedman's vascular model it was hypothesized that lipid deposition in sclera and Bruch's membrane leads to scleral stiffening and impaired choroidal perfusion, which in turn could adversely affect metabolic transport in the RPE.<sup>18</sup> In patients with dry AMD and large, soft drusen the choroidal blood flow and volume were one-third lower than in age-matched control subjects, and in patients with bilateral AMD the pulsatile ocular blood flow was lower in eyes with drusen than in the fellow eyes with neovascular lesions.<sup>19</sup> Delayed choroidal perfusion and abnormalities in psychophysical retinal function may result from the diffusion barrier created by a thickened, lipid-laden Bruch's membrane. Age-related declines in choriocapillaris density and lumen diameter might decrease the clearance of debris from Bruch's membrane, which would contribute to thickening with age.<sup>20</sup>

Cellular functions of the RPE depend on the oxygen concentration. Rheopheresis has been demonstrated to increase oxygen concentration in ischemic tissues.<sup>21</sup> Phagocytosis is a major function of RPE

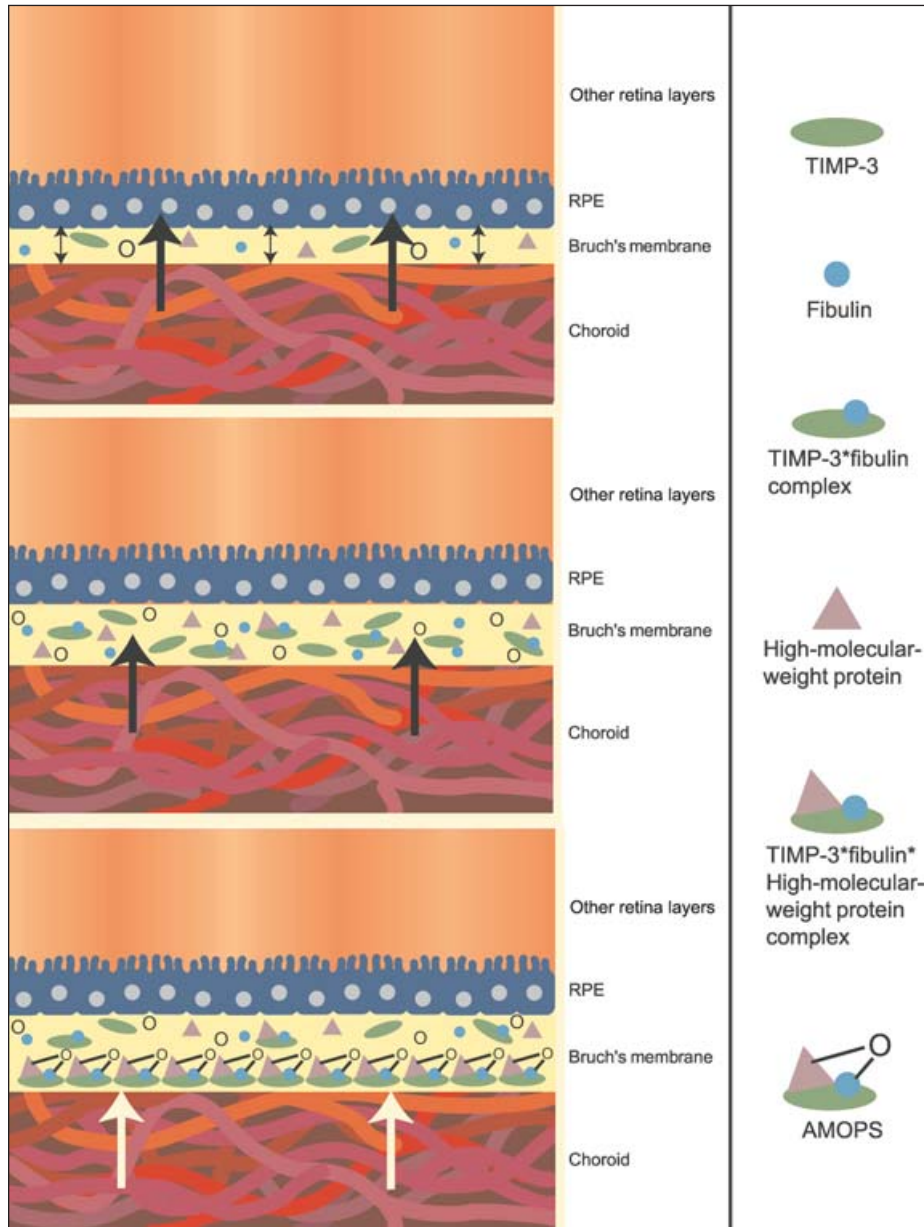


Fig. 1—Top: Normal diffusion through Bruch's membrane. Middle: Accumulation of tissue inhibitor of metalloproteinases-3 (TIMP-3), which binds fibulin. Subsequently this complex reversibly binds to other proteins. Bottom: Irreversible cross-linking of the protein complex via oxygen radicals. The membrane is now strongly diffusion-resistant. RPE = retinal pigment epithelium; AMOPS = advanced macular oxidation products.

cells and is essential for homeostasis of the microenvironment in the eye. Blood flow can have a direct functional relationship with tissue cells via shear stress. With aging, the capacity of Bruch's membrane to facilitate macromolecular exchange between the choroidal and the RPE compartments becomes reduced, representing an important aspect of retinal

microcirculation.<sup>22-24</sup> The macular region, including the fovea, is an avascular zone, much thinner than the rest of the retina, and receives nourishment by diffusion. The capacities for diffusional transport across the Bruch's membrane-choroid complex decline with aging.<sup>25</sup> Angiogenic and antiangiogenic factors in the retina coordinate vascular flow and regeneration with

the corresponding metabolic requirements of the retina. Most important are VEGF and pigment-epithelium-derived factor (PEDF), both of which are regulated by tissue oxygenation.<sup>26,27</sup> Expression of VEGF is induced by hypoxia, thus promoting neovascularization. VEGF, which was previously called vascular permeability factor (VPF), is known to be a powerful factor in the permeability and rheology of small vessels. PEDF, which is induced by an increase in oxygen concentration, inhibits neovascularization.

### **Inflammatory model**

Another model, the inflammatory model, needs to be considered. Data published online by *Science* Mar. 10, 2005, show that about 40% of patients with AMD may have a mutation in complement factor H.<sup>28</sup> There is also a mutation in this factor in membranoproliferative glomerulonephritis type II (MPGN II),<sup>29</sup> a disease that often necessitates renal dialysis. Drusen have been noted in some patients with this disease.<sup>30</sup> Previous work had shown that the serum levels of highly sensitive C-reactive protein (CRP) are elevated in AMD patients.<sup>31</sup> Complement factor 3 binds to CRP and to heparin and inhibits complement activation. In patients with MPGN II, indiscriminate complement activation causes inflammation that destroys the glomeruli through endothelial cell damage. A similar process could be occurring in the choroid, which would make the vascular model reasonable. Also, the chronic inflammation would cause fibrosis, similar to the membranoproliferative changes seen in the kidney, worsening diffusion. In addition, the accumulation of AMOPS may cause macrophages to infiltrate the area and worsen the underlying inflammation. These recent data thus put together the diffusion model, the vascular model and the inflammation model.

Rheopheresis would decrease the inflammatory components in the blood, causing a marked change in the proinflammatory milieu and allowing Bruch's membrane to reestablish a normal diffusion gradient and partial function of the overlying RPE.

## **CLINICAL TRIALS OF RHEOPHERESIS FOR AMD**

### **MAC-1, University of Cologne**

A number of German trials have studied the efficacy of rheopheresis in AMD.<sup>32-35</sup> Success in several uncontrolled case series led to the first prospective randomized controlled clinical trial in Germany

(MAC-1). Brunner and coworkers<sup>2</sup> enrolled 40 AMD patients, who were randomly assigned to receive either 5 rheopheresis treatments over a 21-week period or no treatment. Analysis of the 40 "primary eyes" immediately after treatment demonstrated a mean difference in logMAR (logarithm of the minimum angle of resolution) best-corrected visual acuity (BCVA) of 1.57 lines between the 2 groups of patients and 2.33 lines between the 2 subsets of eyes with soft drusen (11 in each group); both differences were significant at  $p < 0.01$ . Of the eyes with soft drusen, 92.5% had a baseline visual acuity (determined by Early Treatment Diabetic Retinopathy Study [ETDRS] evaluation) of less than 20/40. Electroretinography demonstrated significant improvement in the photopic a-wave amplitude ( $p = 0.009$ ) and flicker response ( $p = 0.03$ ), indicating functional improvement of the central photoreceptor complex. Improvement of the pulsatile ocular blood flow and shortening of the arteriovenous passage time in patients with AMD after rheopheresis treatments had been demonstrated earlier.<sup>32-35</sup>

### **University of Utah pilot study**

A phase II/III trial in 30 patients (10 treated with rheopheresis [10 treatments over about 16 weeks], 10 treated with a sham procedure and 10 not treated) showed a significant improvement in the rheopheresis-treated group compared with the other 2 groups with regard to ETDRS-determined visual acuity, Pepper Visual Skills Reading Test (PVSRT) scores, and results of the 14-item Visual Function questionnaire (VF-14) and a 10-item second questionnaire, used to corroborate the findings of the VF-14 questionnaire (Table 1).<sup>36</sup>

### **MIRA-1**

The Multicenter Investigation of Rheopheresis for AMD (MIRA-1), a randomized, prospective, double-masked, placebo-controlled, pivotal phase III trial, approved by the US Food and Drug Administration, was designed to compare rheopheresis and placebo treatment in 150 patients with late-stage, high-risk, dry AMD and elevated plasma levels of hemorheologic macromolecules, selected to achieve optimal homogeneity of the study groups.<sup>37</sup> Consenting patients are randomly assigned to either the rheopheresis or the placebo group in a 2:1 ratio. If both of a patient's eyes qualify, 1 eye is assigned to be the

**Table 1—Efficacy results of the University of Utah pilot study of rheopheresis for age-related macular degeneration (AMD)<sup>36</sup>**

Treatment group	n	Change from baseline*					Mean no. of improved items on 10-item second questionnaire
		ETDRS BCVA (no. of lines)	PVSRT score		Score on VF-14		
			Mean %	Median %	Mean %	Median %	
Rheopheresis	10	1.8	31.50	34.2	7.23	12.5	3.3
Sham procedure	10	0.5	6.76	-13.6	-8.49	-10.3	0.0
<i>p</i> value <sup>†</sup>		0.017	0.04		0.039		< 0.01

Note: ETDRS BCVA = best-corrected visual acuity, as determined by Early Treatment Diabetic Retinopathy Study evaluation; PVSRT = Pepper Visual Skills Reading Test; VF-14 = 14-item Visual Function questionnaire.  
\*Positive value indicates improvement.  
<sup>†</sup>The significance of the difference between the treatment groups was determined by the Wilcoxon rank-sum test.

**Table 2—Interim efficacy results\* of the Multicenter Investigation of Rheopheresis for AMD<sup>37</sup>**

Treatment group of eyes	n	Mean line difference at 12 mo	<i>F</i> (and <i>p</i> value) <sup>†</sup>			
			Group effect		Time effect	
All primary	43	1.6	12.22	0.0011	1.41	0.2560
All qualifying	54	1.5	8.50	0.0053	1.39	0.2570
All	85	1.7	9.49	0.0028	0.97	0.4093
Eyes with baseline BCVA worse than 20/40	56					
All primary	28	3.0	12.70	0.0014	1.31	0.2928
All qualifying	35	2.8	7.08	0.0122	1.36	0.2747
All	56	3.2	8.55	0.0050	1.10	0.3565

\*Based on the ETDRS BCVA data collected 3, 6, 9 and 12 months after baseline for the first 43 patients who completed treatment.  
<sup>†</sup>Determined by repeated-measures analysis (with unstructured covariance).

primary (study) eye. Both eyes have qualified in approximately 25% of enrolled patients. Each patient receives 8 rheopheresis or 8 sham procedures over 10 weeks. For the sham procedure the patient is shrouded from the neck down, the arms being covered with drapes, and receives masked needle sticks in both arms but no extracorporeal circulation of the blood. The ophthalmologic investigators are masked, since treatments are performed as nurse-directed procedures at separate locations.

Efficacy and safety parameters are evaluated at 3, 6, 9 and 12 months after baseline. The trial's primary end-point is comparison of the mean line change in logMAR BCVA (determined by ETDRS evaluation) in the primary eye. Secondary end-points include

comparisons of the BCVA in the fellow eye and scores on the PVSRT and the 25-item version of the Visual Function Questionnaire of the National Eye Institute, US National Institutes of Health. In addition, serial macular examinations, fundus photography, fluorescein angiography and other tests are performed.

Table 2 and Figs. 2 and 3 summarize the results of the interim data analysis,<sup>37</sup> which is based on the ETDRS BCVA data collected in the 12-month study period for the first 43 patients who have completed treatment. Repeated-measures analysis was selected as a comprehensive statistical tool to compare the parallel performance of the 2 treatment groups over multiple data-acquisition points at discrete intervals and

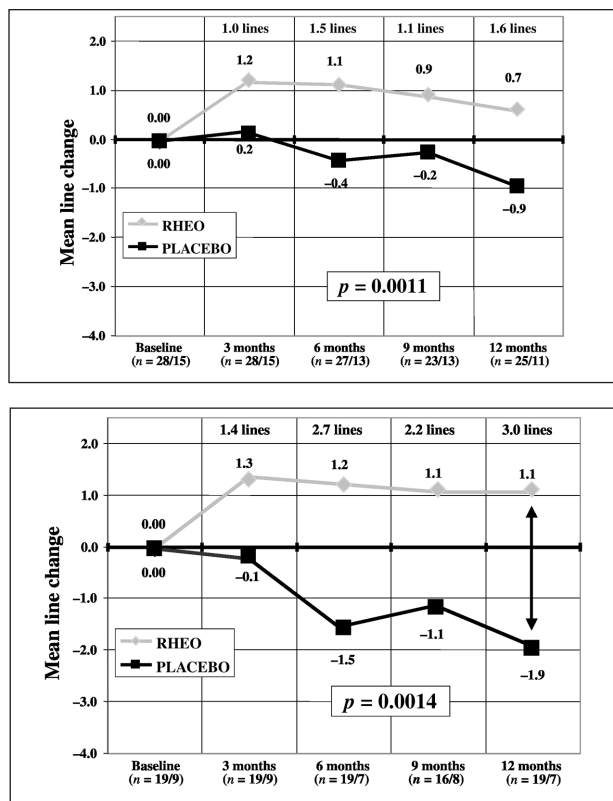


Fig. 2—Top: Changes in best-corrected visual acuity (BCVA) with time after rheopheresis or placebo treatment among all primary eyes in 43 patients. Differences between treatment groups were significant at  $p = 0.0011$  by repeated-measures analysis. Bottom: Changes in BCVA with time after treatment among the primary eyes with a baseline BCVA worse than 20/40. Differences between treatment groups were significant at  $p = 0.0014$ .

provide measures of mixed models (with unstructured covariance) looking at 2 fixed effects: group and time. The group effect provides a measure of treatment efficacy by comparing the changes in BCVA in the rheopheresis group of eyes with those in the placebo group, compiled over the 4 study intervals. The time effect detects increases or decreases in therapeutic effect over time and thus compares the variability of the group effect over the study period. For subgroup analysis, eyes having a baseline ETDRS BCVA of 20/40 or better were compared with eyes having a worse BCVA. This demarcation reflects the legal issue of ability to retain a driver's licence, which defines many people's lives as independent.

The group effect component of the model demonstrated a significant treatment effect throughout the study period for all cohorts ( $p = 0.0011$  to  $0.0122$ ), and the time effect component of the model demon-

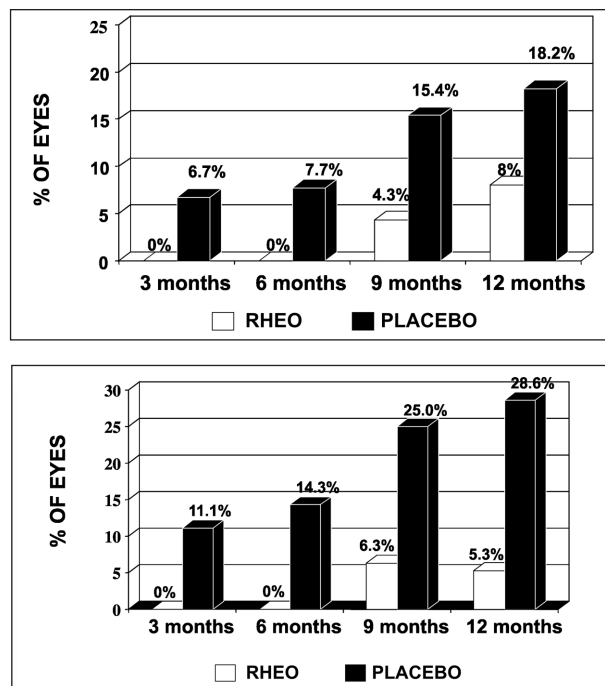


Fig. 3—Top: Proportions of eyes with at least a 2-line loss in BCVA with time after treatment among all primary eyes. Bottom: Proportions of eyes with at least a 2-line loss in BCVA with time after treatment among the primary eyes with a baseline BCVA worse than 20/40.

strated no significant change in therapeutic benefit over the 12-month study period despite the fact that treatments were completed in the first 10 weeks of the study. The subgroup of primary eyes with a baseline BCVA worse than 20/40 showed more benefit from rheopheresis than did the entire cohort of primary eyes.

The primary objective of rheopheresis is to preserve and potentially restore functional vision. Its clinical efficacy can be measured in terms of improvement in visual performance that restores some degree of autonomy to patients' lives. Some of the interim findings for the primary eyes showing trends of major clinical relevance are as follows. Rheopheresis was compared with placebo treatment with respect to 3 clinically meaningful measures of visual performance at 12 months: vision restoration, ability to drive and progression of vision loss to a BCVA worse than 20/200.

At 12 months, 28% of the rheopheresis-treated eyes vs. 18% of the placebo-treated eyes showed an increase of at least 2 lines of BCVA, and 12% vs. 0%, respectively, showed an increase of at least 3 lines. Of the subgroups of eyes with a baseline BCVA worse than 20/40, 58% of the rheopheresis-treated eyes vs.

14% of the placebo-treated eyes obtained sufficient improvement in their BCVA to qualify for a driver's licence at 12 months. None of the rheopheresis-treated eyes with a baseline BCVA better than 20/200 vs. 18% of the placebo-treated eyes progressed to worse than 20/200 at 12 months. Of all the eyes, 8% vs. 18% in the 2 respective groups showed decreases in BCVA of at least 2 lines at 12 months, and for the subgroups with a baseline BCVA worse than 20/40 the proportions showing such a decrease were 5% and 29%. More of the eyes in the placebo group than of those in the rheopheresis group exhibited visual loss at each data-collection point.

## CONCLUSION

The results of the MIRA-1 interim analysis are encouraging, having confirmed the potential of rheopheresis as a therapeutic option for dry AMD. The benefit was evident immediately after treatment and remained essentially stable throughout the 12-month period of the trial. Eyes with late-stage, high-risk, dry AMD appeared to be at significant risk for substantial vision loss over the 12 months if not treated. The subgroup analysis demonstrated that the timing of rheopheresis in the course of a patient's disease progression appears to be important and may have a pronounced effect on BCVA outcome.

As demonstrated by the data from MIRA-1 as well as from the international RheoNet registry (which has collected data from more than 3800 rheopheresis treatments), rheopheresis appears to be safe and well tolerated by elderly AMD patients (R.K.: unpublished observations). The efficacy results in the United States accord well with results from Germany and Canada, where rheopheresis is entering clinical practice, especially for high-risk patients with dry AMD and no therapeutic alternative.

There is a plausible link between current concepts of AMD pathogenesis and performance characteristics of rheopheresis. Repetitive pulses of plasma protein elimination seem to be capable of changing the activity of promoters of the natural course of AMD development and progression. This may be through diminishing AMOPS accumulation in Bruch's membrane, which causes a formidable diffusion barrier and may also cause an immunologic reaction. The microcirculation is therefore affected by the secondary release of factors from the overlying ischemic retina. Rheopheresis directly targets risk factors and pathophysiologically relevant factors for

AMD; however, these plasma parameters should be regarded as epidemiologic risk factors and do not predict the individual therapeutic response.

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