Monocyte Chemoattractant Protein 1, Intercellular Adhesion Molecule 1, and Vascular Cell Adhesion Molecule 1 in Exudative Age-Related Macular Degeneration

Jost B. Jonas, MD; Yong Tao, MD; Michael Neumaier, MD; Peter Findeisen, MD

Objective: To examine intraocular concentrations of monocyte chemoattractant protein 1 (MCP-1), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), and vascular endothelial growth factor (VEGF) in eyes with exudative age-related macular degeneration (AMD).

Methods: The investigation included a study group of 28 patients (28 eyes) with exudative AMD and a control group of 25 patients (25 eyes) with cataract. The concentrations of MCP-1, sICAM-1, sVCAM-1, and VEGF in aqueous humor samples obtained during surgery were measured using a solid-phase chemiluminescence immunoassay.

Results: The study group as compared with the control group had higher aqueous concentrations of sICAM-1 (mean [SD], 844 [2073] vs 246 [206] pg/mL, respectively; *P* < .001), sVCAM-1 (mean [SD], 7978 [7120] vs 2999 [1426] pg/mL, respectively; *P* < .001), and MCP-1 (mean [SD], 587 [338] vs 435 [221] pg/mL, respectively; *P* = .07). The concentration of VEGF did not vary significantly between the groups (*P* = .76). The MCP-1 concentration was significantly associated with macular thickness (*r* = 0.40; *P* = .004). It decreased significantly with the type of subfoveal neovascular membrane (classic membrane type, occult membrane, retinal pigment epithelium detachment) (*P* = .009). The concentrations of sICAM-1, sVCAM-1, and VEGF were not significantly associated with membrane type and macular thickness (*P* ≥ .18).

Conclusions: Concentrations of MCP-1, sICAM-1, and sVCAM-1 are significantly associated with exudative AMD, even in the presence of normal VEGF concentrations. Intraocular MCP-1 concentrations are correlated with the subfoveal neovascular membrane type and the amount of macular edema. One may infer that MCP-1, sICAM-1, and sVCAM-1 could potentially be additional target molecules in therapy for exudative AMD.


A GE-RELATED MACULAR DEGENERATION (AMD) is one of the leading causes of severe, often irreversible visual impairment in the elderly population in Western countries, while East Asian societies have a lower prevalence of the disease. The neovascular exudative form of AMD as compared with the nonexudative or dry form is responsible for most severe visual loss resulting from the disease. After the landmark studies by Aiello et al on the association of retinal neovascularization and vascular endothelial growth factor (VEGF), evidence has been accumulating that VEGF is associated also with the development of subfoveal choroidal neovascular membranes in eyes with exudative AMD. In particular, the intravitreous application of anti-VEGF drugs such as bevacizumab and ranibizumab resulted in a highly significant improvement in visual outcome compared with nontreated control groups. Studies have also shown, however, that although the intraocular concentrations of VEGF were markedly higher in eyes with diabetic retinopathy than in eyes with exudative AMD, the latter eyes did not always show significantly higher VEGF concentrations as compared with age-matched control eyes. In a similar manner, the intraocular concentrations of other cytokines such as erythropoietin and basic fibroblast growth factor were significantly higher in eyes with diabetic retinopathy than in eyes with exudative AMD, in which the levels were not significantly different from the concentrations in normal eyes.

In view of these findings that eyes with exudative AMD and age-matched control eyes did not differ markedly in the intraocular concentrations of most of the examined cytokines including VEGF, it was hypothesized that other cytokines and adhesive molecules besides VEGF may be involved in the pathogenic process of exu-
The clinical interventional comparative study included a study group consisting of patients with exudative AMD who were treated by an intraocular injection of bevacizumab and/or triamcinolone acetonide and a control group of patients with age-related cataract who underwent routine cataract surgery. All patients were treated at the same institution. An inclusion criterion was the absence of any retinal or optic nerve disease except exudative AMD in the study group and except hard drusen of the macular retinal pigment epithelium in the control group. Additionally, the volume of collected aqueous humor had to be at least 100 µL. Previous photodynamic therapy or laser photocoagulation therapy for the subfoveal neovascular membrane was an exclusion criterion, while previous intravitreal drug applications (bevacizumab or triamcinolone) were allowed. Intraocular pressure had to be within the reference range of 10 to 21 mm Hg. The Medical Ethics Committee II of the Medical Faculty Mannheim of the Ruprecht-Karls University, Heidelberg, Germany, approved the study protocol.

All patients underwent an ophthalmologic examination including refraction, corneal topometry, and slitlamp-assisted biomicroscopy of the anterior and posterior segments of the eye. The diagnosis of exudative AMD was substantiated by ophthalmoscopy, fluorescein angiography, and optical coherence tomography as described previously.1-7,11-13 Maximal thickness of the macula was measured by optical coherence tomography (OCT III Stratus; Carl Zeiss Meditec, Jena, Germany). Maximal thickness of the macula was defined as the highest elevation of the retina in the scanned macular region.

### RESULTS

The study group consisted of 28 patients (28 eyes) with exudative AMD, and the control group included 25 patients (25 eyes) undergoing routine cataract surgery. The study group and the control group did not vary significantly in age (P = .23), sex (P = .86), or intraocular pressure (P = .32). Owing to cataract-induced myopization, the mean refractive error was significantly more myopic in the study group than in the control group (P = .01) (Table). In the study group, 14 eyes (50%) were pseudophakic. Within the study group, 7 eyes (25%) showed a predominantly or purely classic type of subfoveal neovascular membrane, 18 eyes (64%) had a purely occult type or a minimally classic type of subfoveal neovascular membrane, and 3 eyes (11%) showed a detachment of the retinal pigment epithelium. On optical coherence tomography, the mean (SD) maximum retinal thickness of the macula was 396 (179) µm (median, 350; range, 245-1155 µm). The study group was further subdivided into eyes without any previous intravitreal treatment (n = 17) and eyes that had previously received intravitreal bevacizumab (n = 11). In the eyes with a previous intravitreal bevacizumab application, the mean (SD) interval between the last injection and inclusion in this study was 3.5 (2.8) months (range, 1.0-8.0 months).

### METHODS

The study group was further subdivided into eyes with a maximum macular thickness of 300 µm or less (n = 13) and eyes with a thickness above 300 µm (n = 15). Statistical analysis was performed using a commercially available statistical software package (SPSS for Windows, version 17.0; SPSS Inc, Chicago, Illinois). The refractive data were converted into the spherical equivalent for statistical analysis. Where appropriate, Kendall bivariate correlation test, independent non-parametric test (Mann-Whitney U test), and 1-way analysis of variance were used. Two-tailed probabilities of less than .05 were considered to indicate statistical significance.

### Table. Demographic Data of the Study Group of Patients With Exudative Age-Related Macular Degeneration and the Control Group of Patients Without Retinal Disorders and Undergoing Routine Cataract Surgery

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study Group (n=28)</th>
<th>Control Group (n=25)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>76.0 (8.0)</td>
<td>72.6 (11.4)</td>
<td>.23</td>
</tr>
<tr>
<td>Women, No. (%)</td>
<td>15 (54)</td>
<td>14 (56)</td>
<td>.86</td>
</tr>
<tr>
<td>Intraocular pressure, mean (SD), mm Hg</td>
<td>15.1 (2.6)</td>
<td>16.0 (3.5)</td>
<td>.32</td>
</tr>
<tr>
<td>Spherical equivalent power, mean (SD), dipters</td>
<td>0.2 (1.8)</td>
<td>−3.7 (5.7)</td>
<td>.01</td>
</tr>
</tbody>
</table>

All scans were visually inspected to make sure that the computerized thickness analysis was appropriate.

In the study group, the aqueous humor was collected after disinfection of the perilitoral skin and conjunctiva, sterile draping of the patient, and insertion of an eyelid speculum. A paracentesis was performed in the temporal limbal region, and aqueous humor was sampled using a blunt cannula and a tuberculin syringe before the intravitreal transconjunctival injection was performed in the temporal inferior quadrant. In the control group, the paracentesis and the release of aqueous humor were performed in the temporal inferior quadrant. In the control group, the paracentesis was routinely performed to create temporal access to the anterior chamber for the bimanual maneuvering of the lens nucleus and cortex during surgery. The technique has been described previously.13 The concentrations of VEGF, MCP-1, sICAM-1, and sVCAM-1 were measured using the suspension bead array technology on a Luminex 100 IS system (Luminex Corp, Austin, Texas).27
Comparing the groups with each other revealed that the exudative AMD study group as compared with the control group had higher concentrations of aqueous sICAM-1 (mean [SD], 844 [2073] vs 246 [206] pg/mL, respectively; \( P < .001 \)) and sVCAM-1 (mean [SD], 7978 [7120] vs 2999 [1426] pg/mL, respectively; \( P < .001 \)) (Figure 1A and B). The difference in the concentration of MCP-1 between the AMD group and the control group was marginally significant (mean [SD], 587 [338] vs 435 [221] pg/mL, respectively; \( P = .07 \)) (Figure 1C). The concentration of VEGF did not vary between the AMD group and the control group (mean [SD], 46 [33] vs 44 [30] pg/mL, respectively; \( P = .76 \)) (Figure 1D).

If the eyes with previous intravitreous bevacizumab therapy were excluded, similar results were obtained: the AMD group as compared with the control group had significantly higher concentrations of aqueous sICAM-1 (mean [SD], 1089 [2653] vs 246 [206] pg/mL, respectively; \( P < .001 \)) and sVCAM-1 (mean [SD], 8653 [8302] vs 3000 [1426] pg/mL, respectively; \( P = .003 \)). Again, the AMD group as compared with the control group had higher, but not significantly higher, concentrations of VEGF (mean [SD], 55.8 [33.9] vs 43.6 [30.5] pg/mL, respectively; \( P = .14 \)).

Within the study group, the concentrations of MCP-1 were significantly correlated with the macular thickness (\( P = .004; r = 0.40 \)) (Figure 2). The other adhesive molecules were not significantly associated with macular thickness (VEGF, \( P = .70 \); sICAM-1, \( P = .21 \); and sVCAM-1, \( P = .33 \)).

Comparing the different types of subfoveal neovascular membranes showed that the aqueous concentration of MCP-1 was significantly associated with the type of subfoveal neovascular membrane (\( r = 0.49; P = .009 \)) (Figure 3). The MCP-1 concentration was the highest in the subgroup with a classic or predominantly classic type of membrane (mean [SD], 851 [439] pg/mL), followed by the subgroup with an occult or minimally classic membrane type (mean [SD], 527 [260] pg/mL) and finally the subgroup with a detachment of the retinal pigment epithelium (mean [SD], 331 [129] pg/mL). Considered separately, the difference in the MCP-1 concentration was significant for the comparison of the retinal pigment epithelium detachment group vs the classic subfoveal membrane type group (\( P = .03 \)); for the comparison between the classic subfoveal membrane type group vs the minimally classic or occult subfoveal membrane type group, the difference was marginally significant.

Figure 1. Aqueous concentrations of soluble intercellular adhesion molecule 1 (sICAM-1) (A), soluble vascular cell adhesion molecule 1 (sVCAM-1) (B), monocyte chemoattractant protein 1 (MCP-1) (C), and vascular endothelial growth factor (VEGF) (D) in aqueous humor samples of a study group of patients with exudative age-related macular degeneration (AMD) and a control group of subjects undergoing routine cataract surgery. The horizontal line in the middle of each box indicates the median, and the top and bottom borders of the box indicate the 75th and 25th percentiles, respectively. The points beyond the whiskers indicate outliers beyond the 90th and 10th percentiles.
The finding that the intraocular concentrations of MCP-1 were significantly associated with the degree of macular edema and with the clinical types of subfoveal neovascular membranes is corroborated by results of previous investigations. These studies revealed that inflammation is critically involved in the formation of subfoveal choroidal neovascular membranes and may thus contribute to the pathogenesis of AMD. The recruitment of monocytes is an early step in the initiation of the inflammatory and angiogenic processes, and MCP-1 plays an important role in regulation of the migration and infiltration of monocytes and macrophages. It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules. Recently, it was put forward by Austin et al that the enhanced release of MCP-1 could attract resident ocular macrophages (microglia) and could contribute to the digestion of the retinal pigment epithelium and Bruch membrane as its basal membrane. Correspondingly, histologic examinations of surgically removed subfoveal neovascular membranes from patients with exudative AMD showed macrophages located in regions with atrophy of the retinal pigment epithelium, Bruch membrane breakdown, and choroidal neovascularization. These data suggest that MCP-1 essentially contributes to the formation of subfoveal choroidal neovascular membranes and may thus contribute to the pathogenesis of AMD. MCP-1 has been known to facilitate angiogenesis. Yamada et al demonstrated that VEGF increases MCP-1 messenger RNA levels in cultured endothelial cells and that MCP-1 participates in VEGF-induced angiogenesis and vascular leakage, which may be helpful in explaining our result that the aqueous concentration of MCP-1 is positively cor-

Figure 2. Scatterplot showing the correlation between the concentration of monocyte chemoattractant protein 1 (MCP-1) in aqueous humor and the maximal retinal thickness in the macula (measured by optical coherence tomography) in patients with exudative age-related macular degeneration (Kendall bivariate correlation test, r = -0.46, P = 0.04).

Figure 3. Aqueous concentrations of monocyte chemoattractant protein 1 (MCP-1) in patients with exudative age-related macular degeneration, differentiated into patients with predominantly or purely classic choroidal neovascular membrane (n = 7), with occult or minimally classic choroidal neovascular membrane (n = 18), and with a detachment of the retinal pigment epithelium (n = 3). The horizontal line in the middle of each box indicates the median, and the top and bottom borders of the box indicate the 75th and 25th percentiles, respectively. The whiskers above and below the box indicate the 90th and 10th percentiles, respectively. The points beyond the whiskers indicate outliers beyond the 90th and 10th percentiles.

(P = 0.06). If the occult or minimally classic membrane type group was combined with the retinal pigment epithelium detachment group, the difference from the classic membrane type group was statistically significant (P = 0.03). The concentration measurements of the other adhesive molecules and VEGF did not vary significantly (by 1-way analysis of variance) between the membrane types (VEGF, P = 0.75; sICAM-1, P = 0.18; and sVCAM-1, P = 0.56).

Comparing the eyes with a previous intravitreous bevacizumab injection with the eyes without previous intravitreous treatment revealed that the aqueous concentrations of VEGF were significantly lower in the pretreated subgroup than in the nontreated subgroup (mean [SD], 30.8 [24.9] vs 55.8 [33.9] pg/mL, respectively; P = 0.02). The pretreated and nontreated subgroups did not vary significantly in the concentrations of the 3 adhesive molecules (MCP-1: mean [SD], 550 [149] vs 621 [421] pg/mL, respectively; P = 0.89; sICAM-1: mean [SD], 466 [293] vs 1089 [2653] pg/mL, respectively; P = 0.68; and sVCAM-1: mean [SD], 6934 [4965] vs 8653 [8303] pg/mL, respectively; P = 0.52).

If eyes after an intravitreous injection of bevacizumab were excluded, the difference in the concentrations of sVCAM-1 (P < 0.001) and sICAM-1 (P = 0.003) between the study group and the control group remained statistically significant, while the 2 groups did not vary significantly in the intraocular concentrations of VEGF (P = 0.14) and MCP-1 (P = 0.27). If pseudophakic eyes were excluded from the statistical analysis, the difference in the concentrations of sVCAM-1 (P = 0.003) and sICAM-1 (P = 0.02) between the study group and the control group remained statistically significant, while the 2 groups did not vary significantly in the intraocular concentrations of VEGF and MCP-1.
related with the maximal retinal thickness of the macula in patients with exudative AMD.

Immunoglobulin superfamily (IgSF) molecules, including ICAM-1 and VCAM-1, are key indicators of vascular endothelial cell activation. Adhesion molecule expression, including ICAM-1, has been found in association with inflammatory cells in extracted subretinal neovascular membrane lesions. Moreover, ICAM-1 labeling of the choriocapillaris was typically more intense in the macula than in the peripheral choroid in human donor eyes. It implies that the macula may be subject to increased leukocyte trafficking. In agreement with these previous findings, our results showed that the intraocular concentrations of sICAM-1 and sVCAM-1 were significantly higher in the patients with exudative AMD than in the patients of the control group. In addition, sICAM-1 and sVCAM-1 as compared with VEGF appeared to be more closely related with neovascular AMD because the aqueous concentrations of VEGF did not vary markedly between the study group and the control group.

Our finding that the aqueous concentration of VEGF did not vary significantly between the patients with exudative AMD and the patients with cataract is in agreement with the study by Chan et al in which the aqueous VEGF concentration did not differ significantly between a study group with untreated choroidal neovascular neovascularization (mean [SD], 66.8 [35.1] pg/mL), a study group with recurrent choroidal neovascular neovascularization (mean [SD], 55.7 [63.0] pg/mL), and a control group consisting of patients with cataract (mean [SD], 55.7 [63.0] pg/mL), and a control group consisting of patients with cataract (mean [SD], 72 [95] pg/mL vs 72 [95] pg/mL, respectively; \( P = .10 \)).

Interestingly, within the group of patients with exudative AMD, the subgroup after an intravitreal injection of bevacizumab as compared with the subgroup without previous intravitreal bevacizumab applications showed significantly lower concentrations of VEGF but not of MCP-1, sICAM-1, or sVCAM-1. It suggests first that the preceding intravitreal injection of bevacizumab led to a decrease in the intraocular VEGF concentration and second that the inflammatory factors including MCP-1, sICAM-1, and sVCAM-1 were not remarkably inhibited by the preceding anti-VEGF therapy. One may infer that an additional anti-inflammatory treatment of exudative AMD combined with a specific anti-VEGF therapy may be useful as some laboratory and clinical studies have suggested.

Limitations of our study should be mentioned. First, the number of patients enrolled in the study is relatively low. Despite this, however, the results were statistically significant, so the relatively small number of patients may only serve to strengthen the results and conclusions of the study. One may even argue that with a larger number of study participants, the differences in the aqueous concentrations of MCP-1 between the groups may have become statistically more significant. Second, the concentrations of VEGF and the adhesive molecules were determined from aqueous samples and not from vitreous samples, which usually show higher concentrations and may reflect the situation in the retina and the sub-retinal space better. Obtaining vitreous samples in the patients is, however, not possible because it would unnecessarily expand the surgical intervention.

In conclusion, the aqueous level of MCP-1 was significantly correlated with macular thickness in patients with exudative AMD, and correspondingly the aqueous MCP-1 concentration varied between the different types of choroidal neovascular membranes. The aqueous levels of sICAM-1 and sVCAM-1 were significantly higher in patients with neovascular exudative AMD than in patients of a cataract control group. Patients who had previous intravitreal anti-VEGF therapy showed significantly lower intraocular levels of VEGF than patients without a previous intravitreal bevacizumab application, while these subgroups did not vary significantly in the intraocular concentrations of MCP-1, sICAM-1, and sVCAM-1. Parallel to previous investigations, the findings suggest that inflammatory factors including MCP-1, ICAM-1, and VCAM-1 play an essential role in the development of exudative AMD and that, in particular, MCP-1 may markedly contribute to the development of macular edema. As such, MCP-1, ICAM-1, and VCAM-1 may be additional target molecules in therapy for exudative AMD.

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REFERENCES

10. Kwant A, Ngereve PV, Berglin L, Seregard S. Subfoveal fibrovascular mem-

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branes in age-related macular degeneration express vascular endothelial growth factor.


28. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflamma-


47. Jonas JB. Intravitreal triamcinolone acetonide for treatment of intraocular oedema

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