

The Intraocular Cytokine Profile and Therapeutic Response in Persistent Neovascular Age-Related Macular Degeneration

Sandra Rezar-Dreindl,¹ Stefan Sacu,¹ Katharina Eibenberger,¹ Andreas Pollreisz,¹ Wolf Bühl,¹ Michael Georgopoulos,¹ Christoph Krall,² Günther Weigert,¹ and Ursula Schmidt-Erfurth¹

¹Department of Ophthalmology, Medical University of Vienna, Vienna, Austria

²Department of Medical Statistics, Medical University of Vienna, Vienna, Austria

Correspondence: Stefan Sacu, Department of Ophthalmology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria; stefan.sacu@meduniwien.ac.at.

Submitted: April 20, 2016

Accepted: June 13, 2016

Citation: Rezar-Dreindl S, Sacu S, Eibenberger K, et al. The intraocular cytokine profile and therapeutic response in persistent neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2016;57:4144-4150. DOI:10.1167/iovs.16-19772

PURPOSE. To investigate the course of inflammatory and angiogenic cytokines in the aqueous humor of patients with persistent/recurrent neovascular age-related macular degeneration (nAMD) under ranibizumab monotherapy (IVM) or ranibizumab plus dexamethasone combination treatment.

METHODS. In this 12-month prospective study, 40 eyes with nAMD were treated with either IVM or combined treatment with ranibizumab plus intravitreal dexamethasone implant (IVC). Patients in the IVM group were treated following an “as needed” treatment regimen; patients in the IVC group received ranibizumab and a dexamethasone implant at baseline and were re-treated with ranibizumab. At baseline and at each time of retreatment aqueous humor samples were taken.

RESULTS. Before treatment, levels of macrophage chemoattractant protein (MCP)-1, monokine induced by γ interferon (MIG), and lipocalin-2/ neutrophil gelatinase-associated lipocalin (NGAL) were elevated in nAMD patients compared to healthy controls ($P = 0.024$; $P = 0.04$; $P = 0.01$). In contrast, tumor necrosis factor α , IL-12p70, and secreted protein acidic and rich in cysteine (SPARC) concentrations were lower ($P = 0.001$; $P = 0.008$; $P = 0.03$), while vascular endothelial growth factor (VEGF) was not altered ($45 \pm 6/51 \pm 12$ pg/mL nAMD/control group; $P = 0.6$). During IVC, levels of VEGF, MIG, platelet-derived growth factor (PDGF)-AA, and transforming growth factor $\beta 1$ ($P = 0.005$; $P = 0.011$; $P = 0.008$; $P = 0.013$) were reduced. Ranibizumab monotherapy did not influence the course of any inflammatory/angiogenic cytokine. Interleukin 6 and PDGF-AA levels correlated with central retinal thickness changes ($P = 0.007$; $P = 0.022$). Over the 12-month period visual function was maintained with no significant differences during or between both treatment groups.

CONCLUSIONS. Inflammatory proteins are involved in the pathogenesis of chronic macular edema due to AMD and are associated with disease activity. During combined treatment, levels of inflammatory and angiogenic cytokines decreased over a 12-month period with no superiority in functional outcome.

Keywords: neovascular age-related macular degeneration, cytokines, dexamethasone intravitreal implant, anti-vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), as a regulator of angiogenesis, is a major contributor involved in the development of macular edema (ME) secondary to AMD. Together with VEGF, different growth factors and inflammatory cytokines have been reported to play an important role in the pathogenesis of choroidal neovascularization (CNV).¹⁻⁵ Macrophage infiltration is shown in the early phases of subretinal neovascularization, and chronic inflammatory cells are present at the level of the Bruch's membrane in experimental models.⁶ Furthermore, increased expression of macrophage chemoattractant protein (MCP)-1 and vascular cell adhesion molecule is observed in the aqueous humor in treatment-naïve eyes when compared to healthy controls.⁴ Other cytokines including angiogenin, interferon γ -induced protein 10, macrophage inflammatory protein-1 β , and monokine induced by γ interferon (MIG) are highly elevated in the

aqueous humor of nAMD eyes, highlighting involvement of inflammatory pathways.⁵

With the implementation of antiangiogenic drugs for the treatment of nAMD, improvements in visual acuity (VA) and long-term effectiveness in terms of disease stabilization could be achieved.⁷⁻¹⁰ During monotherapy a substantial proportion of patients have recurrence or persistence of disease activity, and continuous retreatment is essential to maintain visual function.^{11,12} Recent research has focused on alternative treatment options to reduce the burden of repeated treatments and improve effectiveness during long-term management of those patients. As inflammation is involved in the complex pathogenesis of nAMD, the role of intravitreal steroids, either as monotherapy or combined treatment, has been investigated to treat CNV lesions.^{13,14} A multimodal treatment approach targeting angiogenesis and inflammation has recently been



TABLE. Cytokine Concentrations of Persistent or Recurrent nAMD Patients at Baseline and Month 12 and Cytokine Concentrations of the Control Patients

Variables	Control Group	nAMD Baseline		nAMD Month 12	
	All, <i>n</i> = 15	IVM, <i>n</i> = 20	IVC, <i>n</i> = 20	IVM, <i>n</i> = 15	IVC, <i>n</i> = 9
TNF- α	1.4 \pm 0.7	0.7 \pm 0.5	0.8 \pm 0.4	0.8 \pm 0.6	0.8 \pm 0.7
IL-6	1.4 \pm 1.0	3.5 \pm 8.9	1.8 \pm 5.4	6.8 \pm 32.9	0.7 \pm 0.7
MMP-9	50.5 \pm 24.6	76.7 \pm 76.7	81.4 \pm 66.7	55.3 \pm 32.9	233.2 \pm 333.0
IL-8	4.9 \pm 3.5	4.1 \pm 3.5	6.1 \pm 4.7	7.0 \pm 13.0	7.0 \pm 6.2
IL-10	0.9 \pm 0.5	0.8 \pm 0.6	0.9 \pm 0.3	0.7 \pm 0.4	0.9 \pm 0.3
MCP-1	434.1 \pm 120.5	554.3 \pm 298.7	635.5 \pm 186.7	550.8 \pm 292.2	586.9 \pm 274.0
VEGF	51.4 \pm 43.3	41.4 \pm 46.1	48.1 \pm 28.5	26.1 \pm 34.2	29.4 \pm 40.2
IL-1 α	20.9 \pm 8.0	20.3 \pm 10.4	22.4 \pm 8.6	19.4 \pm 8.8	16.5 \pm 4.1
IL-4	8.6 \pm 2.3	7.4 \pm 4.2	8.0 \pm 2.1	6.9 \pm 3.5	7.8 \pm 2.0
IL-2	2.2 \pm 0.7	2.9 \pm 1.8	2.9 \pm 0.9	2.7 \pm 1.5	2.7 \pm 0.7
MIG	61.0 \pm 57.1	75.5 \pm 64.8	108.6 \pm 42.2	92.7 \pm 51.0	67.5 \pm 51.0
IL-5	0.3 \pm 0.3	0.3 \pm 0.2	0.3 \pm 0.3	0.2 \pm 0.2	0.2 \pm 0.2
IL-12p70	56.1 \pm 26.8	29.5 \pm 29.3	39.0 \pm 20.4	35.4 \pm 21.7	45.1 \pm 16.0
ICAM-1	3887.5 \pm 1854.5	3586.6 \pm 2401.3	3374.2 \pm 1712.6	3131.6 \pm 1809.1	3917.2 \pm 2463.8
HGF	197.6 \pm 250.4	139.7 \pm 83.5	156.3 \pm 80.8	118.1 \pm 35.8	151.4 \pm 91.0
IL-3	3.0 \pm 2.6	2.6 \pm 2.3	1.6 \pm 1.4	3.4 \pm 2.2	3.3 \pm 2.0
PDGF-BB	2.1 \pm 1.1	1.2 \pm 1.3	1.9 \pm 1.0	0.7 \pm 0.7	1.5 \pm 0.9
EGF	0.3 \pm 0.2	0.4 \pm 0.3	0.4 \pm 0.2	0.4 \pm 0.2	0.4 \pm 0.2
SPARC	40,341.1 \pm 13,124.8	28,730.3 \pm 13,294.4	34,078.0 \pm 12,599.1	24,656.2 \pm 7272.2	29,370.9 \pm 11,196.3
PAI-1	354.3 \pm 291.3	797.8 \pm 1421.1	628.3 \pm 1250.7	383.7 \pm 397.2	382.7 \pm 251.5
Thrombospondin-2	581.2 \pm 399.1	572.5 \pm 322.7	670.1 \pm 221.9	516.1 \pm 202.9	618.3 \pm 227.0
Lipocalin-2	5395.3 \pm 1582.4	8301.5 \pm 5604.3	9280.3 \pm 4047.2	10,813.8 \pm 6498.7	12,564.2 \pm 6218.8
PDGF-AA	39.3 \pm 7.9	32.6 \pm 16.8	36.3 \pm 8.8	30.3 \pm 17.7	23.8 \pm 11.2
PGF	3.8 \pm 1.1	3.2 \pm 2.4	3.6 \pm 1.6	2.5 \pm 1.3	3.6 \pm 0.9
ANG-1	29.6 \pm 11.6	25.7 \pm 16.5	31.9 \pm 12.7	25.5 \pm 15.2	27.4 \pm 12.4
TGF- β 3	41.8 \pm 40.7	33.1 \pm 43.5	54.0 \pm 49.6	41.3 \pm 48.7	20.0 \pm 44.3
TGF- β 2	341.4 \pm 341.4	823.2 \pm 505.1	1079.5 \pm 387.5	665.0 \pm 369.4	894.6 \pm 256.8
TGF- β 1	67.5 \pm 64.5	109.2 \pm 105.0	119.6 \pm 86.0	70.6 \pm 78.0	39.4 \pm 67.0

All data are presented as mean and standard deviation; cytokine concentrations are presented as pg/mL.

shown to reduce the amount of retreatments.^{13,15} However, limited clinical evidence exists about the use of combined treatments in the management of nAMD patients.

The cytokine profile of patients with recurrent or persistent CNV activity treated with a multimodal treatment approach, including anti-VEGF and corticosteroids, is unknown. The knowledge of intraocular cytokine levels and their response to treatment is necessary to better understand the pathophysiology of disease and identify targets. Therefore, this study aimed to describe the profile and course of inflammatory and angiogenic cytokines during anti-VEGF monotherapy and anti-VEGF and dexamethasone combined treatment in persistent or recurrent CNV.

METHODS

Patient Selection and Setting

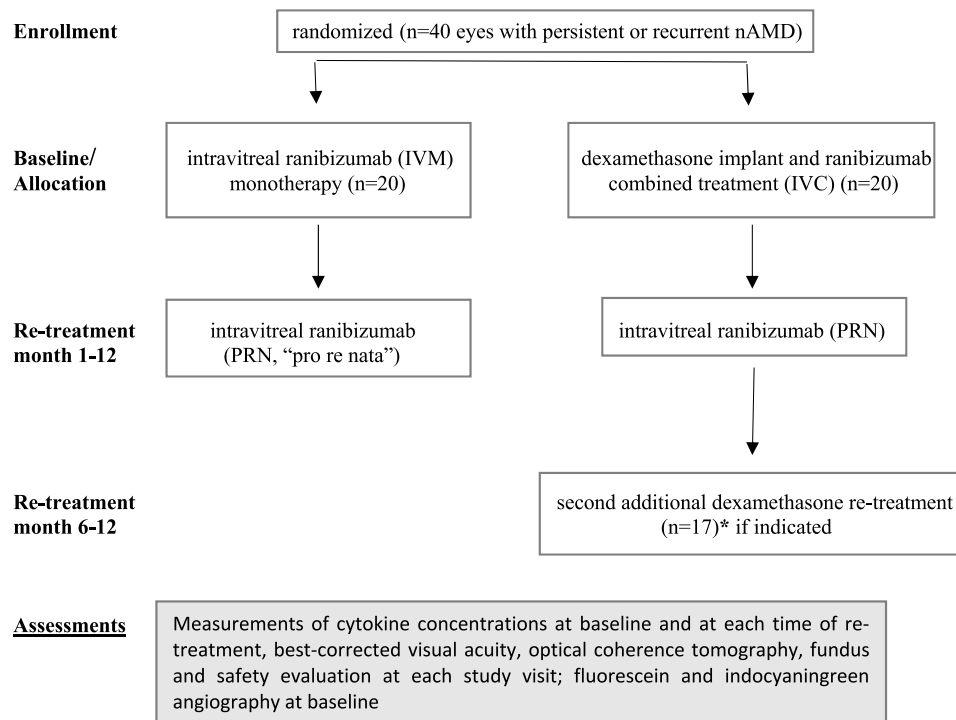
In this prospective study, 40 eyes of 40 consecutive patients with persistent/recurrent ME due to nAMD were included and the course of intraocular angiogenic and inflammatory cytokines was measured over a 12-month period with different treatment regimens. Patients were recruited at the Department of Ophthalmology and Optometry at the Medical University of Vienna. Informed consent was obtained before inclusion and the study was approved by the ethics committee of the Medical University of Vienna. The study design adhered to the tenets of the Declaration of Helsinki and followed the guidelines of "Good Clinical Practice." The study was registered at www.clinicaltrials.gov (NCT01162746; provided in the public

domain by the National Library of Medicine [NLM], National Institutes of Health [NIH], Bethesda, MD, USA).

Inclusion/Exclusion Criteria

Only patients with recurrent or persistent nAMD were included for analysis. Recurrent/persistent disease was defined as evidence of subretinal or intraretinal fluid on optical coherence tomography (OCT) after obtaining a minimum of three monthly consecutive ranibizumab injections. No other prior initial intravitreal treatment other than ranibizumab was allowed for inclusion. Visual acuity of the patients was between 24 (20/400 Snellen equivalent) and 82 ETDRS letters (20/25 Snellen equivalent). Patients with a history of photodynamic therapy, treatment with corticosteroids, uncontrolled glaucoma, active inflammation, retinal detachment, vitreous hemorrhage, or diabetic retinopathy were not included in this study. Active CNV due to nAMD was determined at baseline by clinical findings, fluorescein and indocyanine green angiography, and by the presence of subretinal or intraretinal edema involving the fovea as assessed by OCT.

The patients were randomly assigned (1:1) to either ranibizumab monotherapy (IVM, 0.5 mg/0.05 mL Lucentis; Genentech, San Francisco, CA, USA) or a combination of ranibizumab (0.5 mg/0.05 mL Lucentis) plus dexamethasone intravitreal implant (IVC, 0.7 mg Ozurdex drug delivery system; Allergan, Irvine, CA, USA) and were evaluated on a monthly basis until month 12. Patients randomized to IVM received ranibizumab at baseline, whereas patients in the IVC group received a dexamethasone implant following an intravitreal injection of ranibizumab at the same study visit.



* additional dexamethasone treatment was given in at month 6, month 7 and month 8 (n=11, n=5 and n=1) due to missing disease stability.

FIGURE 1. Diagram of the study and retreatment protocol.

At all following study visits, retreatment with ranibizumab was performed after an “as needed” (pro re nata) treatment regimen in both groups when signs of active CNV were present. Retreatment criteria included evidence of subretinal fluid or cystoid ME. After a minimum of 6 months and lack of disease stability, a second dexamethasone implant was allowed for retreatment in the IVC group. At baseline and at each time of retreatment, aqueous humor samples were taken for cytokine measurements. Figure 1 provides a diagram of the study and retreatment protocol.

Study Procedures

Intravitreal injections of ranibizumab (Lucentis) and sustained-release dexamethasone (Ozurdex; Allergan) were performed by following a standardized procedure. After thorough cleansing of the lid, lashes, and the periorbital area with an antiseptic, local anesthesia, and antimicrobial drops, the eye was covered with a sterile scarf. A sterile lid speculum was inserted and 0.1 mL aqueous humor collected by anterior chamber paracentesis with a 27-gauge needle attached to an insulin syringe. In the IVM/IVC group, 0.05 mL ranibizumab was administered 3.5 to 4.0 mm posterior to the limbus. In the IVC group following ranibizumab injection, an Ozurdex intravitreal implant was delivered by using Allergan’s proprietary Novadur solid polymer delivery system. The implant was inserted 3.5 to 4.0 mm posterior to the limbus. Immediately after intraocular injection, antimicrobial drops were administered in both groups. The patient was instructed to self-administer topical antimicrobial drops (Gentamicin) for 4 days after treatment with dexamethasone.

Aqueous humor samples were collected at baseline and whenever retreatment was necessary during the 12-month

period. The samples were immediately transferred to sterile plastic tubes, frozen, and stored at -80° . Analysis was done with bead assays (xMAP; Luminex Corp., Austin, TX, USA). Capture bead kits (Beadlyte; Upstate Biotechnology, Lake Placid, NY, USA) were used for the detection of interleukin (IL)-1 α , -2, -3, -4, -5, -6, -8, -10, -12p70; tumor necrosis factor α (TNF- α); matrix metalloproteinase (MMP)-9; monocyte chemoattractant protein (MCP)-1; vascular endothelial growth factor (VEGF); monokine induced by γ interferon (MIG/CXCL9); intercellular adhesion molecule (ICAM)-1; hepatocyte growth factor (HGF); platelet-derived growth factor AA and BB (PDGF-AA, PDGF-BB); epidermal growth factor (EGF); secreted protein acidic and rich in cysteine (SPARC); plasminogen activator inhibitor (PAI)-1; thrombospondin 2; lipocalin-1/neutrophil gelatinase-associated lipocalin (NGAL); placental growth factor (PGF); angiopoietin (ANG)-1; and transforming growth factor (TGF) β 1, β 2, β 3 (TGF- β 1, TGF- β 2, TGF- β 3).

A control group with 15 healthy age-matched patients undergoing cataract surgery was included. Aqueous humor samples of patients without retinal disease or any previous intraocular surgery were analyzed. A total of 0.1 mL aqueous humor was taken before cataract surgery by 27-gauge limbal paracentesis as described above. The samples were immediately transferred to sterile plastic tubes, frozen, and stored at -80° .

Aqueous humor samples were used undiluted and incubated overnight. Kits were used according to the manufacturers’ instructions. Curves for each cytokine were generated by using the reference cytokine concentrations supplied in the kit.

Statistical Analyses and Outcome Measures

Statistical analyses were performed by using descriptive statistics for all variables and data frequency. Main outcome

of these analyses was to describe the alterations of different angiogenic and inflammatory cytokine levels under treatment with IVM or a combination of ranibizumab and dexamethasone. Generalized linear mixed models were analyzed to investigate the effects of cytokine baseline levels on the probability of retreatment and the probability of response. Cytokines baseline values were compared by ANOVAs and post hoc Helmert tests between treatment groups and healthy control group. To investigate the correlation between number of retreatment and cytokine levels depending on the group, Spearman correlations were performed. Correlations of cytokine levels with functional and anatomic parameters over time were described by linear mixed effect models. The level of significance was set to 0.05. All analyses were performed at the Department of Medical Statistics at the Medical University of Vienna, using R 3.0.2 under RStudio (Boston, MA, USA). Figures were done by using SPSS for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Forty eyes of 40 consecutive patients with persistent or recurrent nAMD and 15 healthy age-matched controls undergoing cataract surgery were included in this analysis. Twenty patients were treated with IVM and 20 were treated with IVC. Total follow-up of the study was 12 months. Mean age was $77 \pm 7.1/75 \pm 7.5$ years [IVM/IVC] ($P=0.6$), and we included 29 (72.5%) women and 11 (27.5%) men with persistent or recurrent nAMD (15:5/14:6 W:M [IVM/IVC group]; $P=0.7$). Patients in IVM and IVC received an overall amount of 5.6 ± 3.4 (range: 3-17) and 6.7 ± 4.4 (range: 3-16) intravitreal ranibizumab injections and were included after a mean time of 11.7 ± 6.3 and 11.9 ± 7.2 months after the initial ranibizumab treatment ($P=0.4$; $P=0.8$). The time between the last ranibizumab injection before the baseline treatment in the study was a mean of 3.0/3.2 (IVM/IVC) months. No correlation was found between the baseline cytokine concentrations and the number of prior intravitreal injections before inclusion in the study or with length of disease. Visual acuity was 62 ± 15 ETDRS letters in the IVM and 68 ± 12 in the IVC group ($P=0.2$). Central retinal thickness was $485 \pm 122 \mu\text{m}$ in the IVM and $439 \pm 90 \mu\text{m}$ in the IVC group ($P=0.2$). In the monotherapy group, 70% presented with type I, 5% type II, and 25% type III lesions. In the combination therapy group, 75% were type I and 25% were type III lesions.

Baseline Cytokine Concentrations of Persistent nAMD Patients

The Table summarizes the baseline mean values of angiogenic and inflammatory cytokines for persistent or recurrent nAMD patients and control patients.

At baseline, MCP-1 (595 ± 252 pg/mL [mean \pm standard deviation]), MIG (92.5 ± 56.8 pg/mL), and lipocalin-2/NGAL (8791 ± 4913 pg/mL) concentrations were elevated in persistent nAMD patients when compared to controls (MCP-1: 434 ± 121 pg/mL; MIG: 61.0 ± 57.1 pg/mL; lipocalin-2/NGAL: 5395 ± 1582 pg/mL) ($P=0.024$; $P=0.04$; $P=0.01$). In contrast, TNF- α , IL-12p70, and SPARC concentrations were lower than in controls. Mean TNF- α concentrations were 0.8 ± 0.5 pg/mL in comparison to 1.4 ± 0.7 pg/mL in healthy controls ($P=0.001$). Interleukin 12p70 concentrations were 34.2 ± 25.7 pg/mL compared to 56.1 ± 26.8 pg/mL ($P=0.008$), and SPARC concentrations were $31,404.1 \pm 13,224.5$ pg/mL compared to $40,341.1 \pm 13,124.8$ pg/mL ($P=0.03$). All other cytokines did not differ in comparison to the control group at baseline. Vascular endothelial growth factor levels

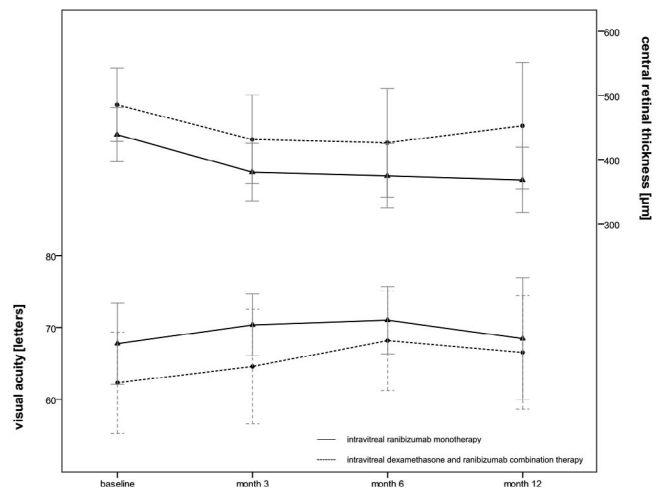


Figure 2. Course of functional and anatomic variables over the 12-month period.

were 44.8 ± 38.5 pg/mL in comparison to 51.4 ± 43.3 pg/mL in the control group ($P=0.6$).

Correlation of cytokine levels with different morphologic features seen on OCT did not show differences at baseline. No significant effect of cytokine baseline levels was detected on the probability of retreatment or the probability of response.

Functional Outcome

In all patients, functional variables could be maintained under either treatment with no significant differences between the groups over the 12-month follow-up period. Visual acuity changed from 62 ETDRS letters at baseline to 67 letters at month 12 in IVM and remained stable at 68 letters in IVC ($P=0.68$). Central retinal thickness (CRT) significantly decreased over time with no difference between the groups (IVM: $486 \mu\text{m}$ at baseline to $453 \mu\text{m}$ at month 12; IVC: 439 to $368 \mu\text{m}$; $P=0.38$). Figure 2 shows the course of VA and CRT during the study period.

Change of Cytokine Levels and Relation to Central Thickness or VA During Treatment

In the IVM group, no inflammatory or angiogenic cytokines were found to be altered under treatment in a mixed model over time. In the IVC group, generalized linear mixed models identified VEGF, MIG, PDGF-AA, and TGF- β 1 to be reduced by treatment over time ($P=0.005$, $P=0.011$, $P=0.008$, $P=0.013$).

Interleukin 6 and PDGF-AA showed positive correlation with CRT changes ($P=0.007$; $P=0.022$). In addition, IL-10 and lipocalin-2/NGAL levels were positively correlated with VA changes ($P=0.005$; $P=0.002$). Figures 3a and 3b show OCT images at time of treatment together with concentrations of cytokine levels of representative patient cases.

DISCUSSION

Persistent or recurrent manifestation is well known in the course of nAMD and has led to the search for alternative treatment strategies.^{16,17} Intravitreal triamcinolone (IVTA) has been investigated as an alternative option for the treatment of nAMD, either in monotherapy or combined with anti-VEGF drugs, but is associated with a number of side effects.^{14,15} In addition, in an animal model, high doses of IVTA have been

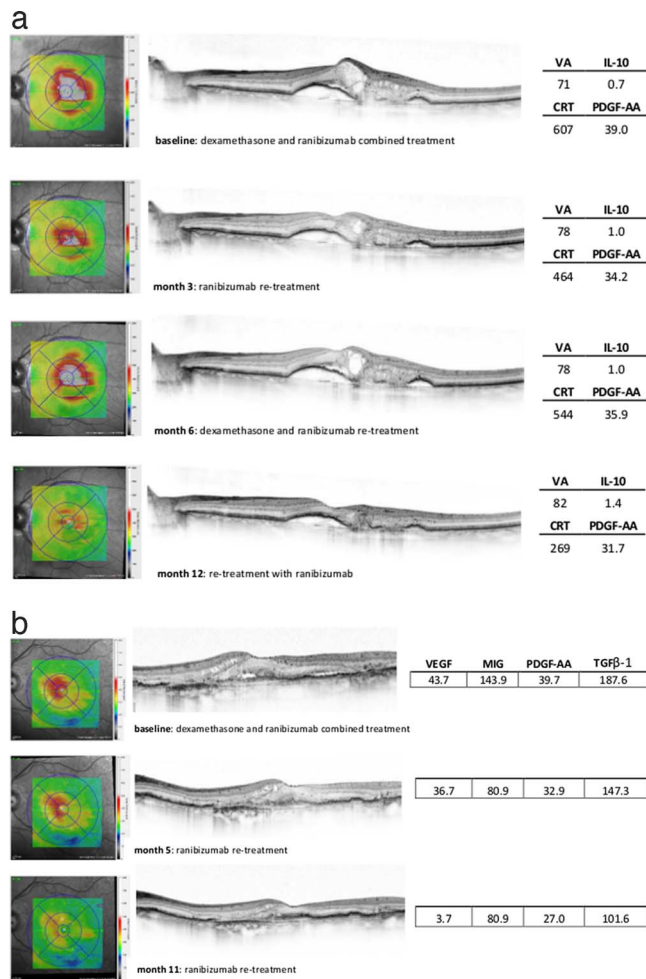


Figure 3. (a, b) Optical coherence tomography images at time of treatment together with cytokine levels of representative patient cases.

found to have outer retinal toxic effects.¹⁸ Drug concentrations are difficult to achieve owing to the short half-life of corticosteroids in the vitreous.¹⁹ Thus, dexamethasone is several times more potent than IVTA and can be applied as a sustained-release intravitreal implant providing sustained intraocular drug concentrations. Successful treatment of ME resulting from retinal vein occlusion, diabetic retinopathy, or uveitis has already been demonstrated.^{20–22} Targeting angiogenesis and inflammation during disease progression seems to be reasonable, as inflammation has been shown to be involved in the pathogenesis of ME, and the possible benefit of a multimodal treatment approach including steroids has been recently investigated.^{13,15} To date, limited evidence exists about the use of additional corticosteroid treatment in patients with nAMD. Hence, we evaluated the influence of anti-VEGF monotherapy or anti-VEGF and corticosteroid combined treatment on intraocular cytokine concentrations in persistent or recurrent nAMD patients. We aimed to determine cytokine changes in the course of repeated treatment over a 12-month period. In contrast to anti-VEGF monotherapy, we observed alterations of inflammatory and angiogenic cytokine (VEGF, MIG, PDGF-AA, and TGF-β1) concentrations during combined anti-VEGF/dexamethasone treatment. No superiority in functional outcome could be detected during the study period.

Platelet-derived growth factor AA is a potent chemoattractant and activator of neutrophils, monocytes, and fibroblasts and is expressed during angiogenesis and endothe-

lial cell activation in nAMD.^{23,24} VEGF is likely to participate together with PDGF in this process of endothelial cell regulation.²⁵ Corticosteroids act to prevent or decrease inflammation by reducing the release of various mediators of inflammation and act on the vascular endothelium, decreasing vascular permeability and edema.²⁶ In addition, an alternative anti-inflammatory effect of dexamethasone and other corticosteroids is the inhibition of PDGF-induced transcription of VEGF and VEGF release; via this mechanism, vascular permeability can be reduced.²⁷

In vivo, TGF-β is expressed by RPE cells during CNV formation and is known to modulate its process.^{1,28} This indirect angiogenic cytokine induces VEGF expression in multiple cell types including RPE cells.²⁹ In animal models, inhibition of TGF-β reduces the progression of early CNV with downregulation of VEGF. Owing to the activation of other cytokines, TGF-β inhibition may not only decrease the angiogenic process, but also affect other pathways related with disease progression.³⁰ In animal models, dexamethasone is able to modulate the TGF-β signaling pathway by downregulation of TGF-β expression.³¹ In contrast, MIG is a chemoattractant and is thought to be angiostatic. In the aqueous humor of nAMD patients, significant higher expression of MIG has been demonstrated.^{32,33} Nevertheless, the exact role of these cytokines and mechanisms of the proposed effects, as well as their role in the eye, needs to be further elucidated.

In our analysis, we found a reduction in cytokine concentrations during treatment in the combined treatment group. However, regarding visual function no superiority for either the monotherapy group or the combined treatment group existed over the 12-month period. Thus, our results indicate that in persistent/recurrent lesions the morphologic and functional assessments differ in determining the treatment response. In clinical decision making, careful evaluation of both the morphologic and functional variables should be considered. The IVM group gained more letters after 12 months than the IVC group because cataract opacity progressed in patients receiving dexamethasone, thus limiting VA outcomes in the long run. Thirty-three percent of phakic patients treated with the combined therapy were referred to cataract surgery owing to considerable lens opacification impairing VA after repeated dexamethasone treatment. After cataract surgery, VA improved in all patients. In addition, a reason for the limited improvement in visual function in both groups could be the structural or degenerative changes following tissue remodeling in advanced disease. As this analysis focused on treatment-resistant or recurrent CNV lesions, advanced structural changes may limit visual improvements. It is possible that introducing combined treatment modalities earlier in the course of the disease may result in improvements of visual function and stronger effects on intraocular cytokine concentrations. Future studies are necessary to address this question.

Vascular endothelial growth factor is the main cytokine involved in ME development and is the target for anti-VEGF therapy. Previous reports have found aqueous VEGF concentrations to be more significant in treatment-naïve nAMD eyes than controls.^{5,6,34} One investigation concentrated on the differences in cytokine profiles between naïve and recurrent or regressed CNV types with previous bevacizumab treatment, reporting differences in their cytokine profile. In treatment-naïve eyes, VEGF concentrations were significantly higher (66.8 pg/mL) than in recurrent or regressed lesions (55.7 and 9.8 pg/mL).³⁵ In addition, MCP-1 levels were highest in persistent nAMD patients, while TNF-α levels decreased in comparison to treatment-naïve patients. These results are in accordance with our findings showing intraocular VEGF levels not to be elevated (45 and 51 pg/mL nAMD/control patients)

when compared to controls, while TNF- α levels were reduced. In our investigation, MIG and lipocalin-2/NGAL also were elevated in persistent/recurrent nAMD patients. These results suggest an altered cytokine profile with an inflammatory component in patients with recurrent or persistent ME. Higher levels of inflammatory cytokines beside VEGF may have an impact on the response to anti-VEGF therapy alone. Cytokine measurements will further challenge the individual patient profile and may help to classify treatment responses and to detect patients who are most likely to respond to anti-VEGF therapy. Further investigation is essential in order to define the differences between acute and chronic disease, as well as treatment-naïve and persistent cases, to explore the optimal time point when decision for combined treatments or switching to another agent should be considered.

Furthermore, we found inflammatory cytokines to be correlated with disease activity in nAMD patients, reflecting again the crucial inflammatory involvement in the pathophysiology of ME. Inflammatory cytokines (IL-6 and PDGF-AA) showed positive correlation with the amount of ME in a statistical model over time and may be linked to its severity and disease activity.

In conclusion, we showed a distinct inflammatory involvement in persistent and recurrent nAMD patients, which could be linked to disease activity. Differences in the cytokine profile of treatment-naïve eyes and lesions not adequately responding to monotherapy should be considered. Understanding of the pathomechanism of angiogenesis and the cytokines involved is necessary for the identification of new therapeutic targets in neovascular disease. Owing to the multifactorial nature of AMD, inhibition of angiogenesis alone may not be sufficient, and new approaches may target mediators involved at different levels of its pathogenesis. Combined treatment with anti-VEGF and corticosteroids reduced angiogenic as well as inflammatory cytokines under repeated treatment at an advanced disease stage; however, no superiority in functional outcome could be achieved. Thus, treatment decisions for chronic nAMD patients should be based on morphologic as well as functional measurements. However, more studies are required to evaluate combined approaches and the influence on the specific cytokine profile. Identification of the individual cytokine pattern and response may help to guide treatment decisions and identify additional treatment modalities.

Acknowledgments

The authors thank the Vienna Study Center for their contribution and technical assistance.

Disclosure: **S. Rezar-Dreindl**, None; **S. Sacu**, None; **K. Eibenberger**, None; **A. Pollreis**, None; **W. Bühl**, None; **M. Georgopoulos**, None; **C. Krall**, None; **G. Weigert**, None; **U. Schmidt-Erfurth**, None

References

1. Campa C, Costagliola C, Incorvaia C, et al. Inflammatory mediators and angiogenic factors in choroidal neovascularization: pathogenetic interactions and therapeutic implications. *Mediators Inflamm*. 2010;2010:546826.
2. Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, Hartnett ME. Vascular endothelial growth factor in eye disease. *Prog Retin Eye Res*. 2008;27:331-371.
3. Campochiaro PA, Soloway P, Ryan SJ, Miller JW. The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration. *Mol Vis*. 1999;3:34.
4. Fauser S, Viebahn U, Muether PS. Intraocular and systemic inflammation-related cytokines during one year of ranibizumab treatment for neovascular age-related macular degeneration. *Acta Ophthalmol*. 2015;93:734-738.

5. Agawa T, Usui Y, Wakabayashi Y, et al. Profile of intraocular immune mediators in patients with age-related macular degeneration and the effect of intravitreal bevacizumab injection. *Retina*. 2014;34:1811-1818.
6. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. *Prog Retin Eye Res*. 2001;20:385-414.
7. Funk M, Karl D, Georgopoulos M, et al. Neovascular age-related macular degeneration: intraocular cytokines and growth factors and the influence of therapy with ranibizumab. *Ophthalmology*. 2009;116:2393-2399.
8. Rosenfeld PJ, Rich RM, Lalwani GA. Ranibizumab: phase III clinical trial results. *Ophthalmol Clin North Am*. 2006;19:361-372.
9. Singer MA, Awh CC, Sadda S, et al. HORIZON: an open-label extension trial of ranibizumab for choroidal neovascularization secondary to age-related macular degeneration. *Ophthalmology*. 2012;119:1175-1183.
10. Michels S, Schmidt-Erfurth U, Rosenfeld PJ. Promising new treatments for neovascular age-related macular degeneration. *Expert Opin Investig Drugs*. 2006;15:779-793.
11. Rofagha S, Bhisitkul RB, Boyer DS, et al; SEVEN-UP Study Group. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology*. 2013;120:2292-2299.
12. Broadhead GK, Hong T, Chang AA. Treating the untreatable patient: current options for the management of treatment-resistant neovascular age-related macular degeneration. *Acta Ophthalmol*. 2014;92:713-723.
13. Kuppermann BD, Goldstein M, Maturi RK, et al. Dexamethasone intravitreal implant as adjunctive therapy to ranibizumab in neovascular age-related macular degeneration: a multicenter randomized controlled trial. *Ophthalmologica*. 2015;234:40-54.
14. Jonas JB, Akkoyun I, Budde WM, Kreissig I, Degenring RF. Intravitreal reinjection of triamcinolone for exudative age-related macular degeneration. *Arch Ophthalmol*. 2004;122:218-222.
15. Lim JJ, Niec M, Wong V. One year results of a phase 1 study of the safety and tolerability of combination therapy using sustained release intravitreal triamcinolone acetonide and ranibizumab for subfoveal neovascular AMD. *Br J Ophthalmol*. 2015;99:618-623.
16. Fung AE, Lalwani GA, Rosenfeld PJ, et al. An optical coherence tomography-guided, variable dosing regimen with intravitreal ranibizumab (Lucentis) for neovascular age-related macular degeneration. *Am J Ophthalmol*. 2007;143:566-583.
17. Rayess N, Houston SK, Gupta OP, Ho AC, Regillo CD. Treatment outcomes after 3 years in neovascular age-related macular degeneration using a treat-and-extend regimen. *Am J Ophthalmol*. 2015;159:3-8.
18. Yu SY, Damico FM, Viola F, D'Amico DJ, Young LH. Retinal toxicity of intravitreal triamcinolone acetonide: a morphological study. *Retina*. 2006;26:531-536.
19. Kwak HW, D'Amico DJ. Evaluation of the retinal toxicity and pharmacokinetics of dexamethasone after intravitreal injection. *Arch Ophthalmol*. 1992;110:259-266.
20. Haller JA, Bandello F, Belfort R Jr, et al; Ozurdex GENEVA Study Group. Dexamethasone intravitreal implant in patients with macular edema related to branch or central retinal vein occlusion twelve-month study results. *Ophthalmology*. 2011;118:2453-2460.
21. Cao JH, Mulvahill M, Zhang L, Joondeph BC, Dacey MS. Dexamethasone intravitreal implant in the treatment of persistent uveitic macular edema in the absence of active inflammation. *Ophthalmology*. 2014;121:1871-1876.

22. Scaramuzzi M, Querques G, Spina C, Lattanzio R, Bandello F. Repeated intravitreal dexamethasone implant (Ozurdex) for diabetic macular edema. *Retina*. 2015;35:1216-1222.
23. Shure D, Senior RM, Griffin GL, Deuel TF. PDGF AA homodimers are potent chemoattractants for fibroblasts and neutrophils, and for monocytes activated by lymphocytes or cytokines. *Biochem Biophys Res Commun*. 1992;14:1510-1514.
24. Kliffen M, Sharmab HS, Mooy CM, Kerkvliet S, de Jong PT. Increased expression of angiogenic growth factors in age-related maculopathy. *Br J Ophthalmol*. 1997;81:154-162.
25. Shikada Y, Yonemitsu Y, Koga T, et al. Platelet-derived growth factor-AA is an essential and autocrine regulator of vascular endothelial growth factor expression in non-small cell lung carcinomas. *Cancer Res*. 2005;15:7241-7248.
26. Naucka M, Karakiulakis G, Perruchoudc AP, Papakonstantinou E, Rothc M. Corticosteroids inhibit the expression of the vascular endothelial growth factor gene in human vascular smooth muscle cells. *Eur J Pharmacol*. 1998;341:309-315.
27. Keck PJ, Hauser SD, Krivi G, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*. 1989;246:1309-1315.
28. Ogata N, Yamamoto C, Miyashiro M, Yamada H, Matsushima M, Uyama M. Expression of transforming growth factor-beta mRNA in experimental choroidal neovascularization. *Curr Eye Res*. 1997;16:9-18.
29. Bian ZM, Elnor SG, Elnor VM. Regulation of VEGF mRNA expression and protein secretion by TGF- β 2 in human retinal pigment epithelial cells. *Exp Eye Res*. 2007;84:812-822.
30. Zarranz-Ventura J, Fernández-Robredo P, Recalde S, et al. Transforming growth factor-beta inhibition reduces progression of early choroidal neovascularization lesions in rats: P17 and P144 peptides. *PLoS One*. 2013;31:8.
31. Potchinsky M, Nugent P, Lafferty C, Greene RM. Effects of dexamethasone on the expression of transforming growth factor-beta in mouse embryonic palatal mesenchymal cells. *J Cell Physiol*. 1996;166:380-386.
32. Jonas JB, Tao Y, Neumaier M, Findeisen P. Monocyte chemoattractant protein 1, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 in exudative age-related macular degeneration. *Arch Ophthalmol*. 2010;128:1281-1286.
33. Farber JM. Mig and IP-10: CXC chemokines that target lymphocytes. *J Leukoc Biol*. 1997;61:246-257.
34. Tong JP, Chan WM, Liu DT, et al. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. *Am J Ophthalmol*. 2006;141:456-462.
35. Roh MI, Kim HS, Song JH, Lim JB, Koh HJ, Kwon OW. Concentration of cytokines in the aqueous humor of patients with naive, recurrent and regressed CNV associated with amd after bevacizumab treatment. *Retina*. 2009;29:523-529.