JAMA Ophthalmology | Original Investigation

Aqueous Humor Cytokine Levels and Anatomic Response to Intravitreal Ranibizumab in Diabetic Macular Edema

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IMPORTANCE Variability in response to anti–vascular endothelial growth factor (VEGF) treatment in diabetic macular edema (DME) remains a significant clinical challenge. Biomarkers could help anticipate responses to anti-VEGF therapy.

OBJECTIVES To investigate aqueous humor cytokine level changes in response to intravitreal ranibizumab therapy for the management of DME, and to determine the association between baseline aqueous levels and anatomic response.

DESIGN, SETTING, AND PARTICIPANTS In this prospective multicenter cohort study, 49 participants with diabetes mellitus complicated by center-involving DME, with a central subfield thickness of 310 µm or greater on spectral-domain optical coherence tomography (SD-OCT), were recruited from December 22, 2011, to June 13, 2013 and statistical analysis were performed from March 1, 2017, to June 1, 2017. A total of 48 participants proceeded to follow-up.

INTERVENTIONS Participants received monthly injections of ranibizumab, 0.5 mg, for 3 months. Aqueous fluid for cytokine analysis was obtained at baseline and repeated at the 2-month visit. Multiplex immunoassay was carried out in duplicate for VEGF, placental growth factor, transforming growth factor beta 2, intercellular adhesion molecule 1 (ICAM-1), interleukin 6 (IL-6), IL-8, IL-10, vascular intercellular adhesion molecule, and monocyte chemoattractant protein 1.

MAIN OUTCOMES AND MEASURES Baseline and 2-month change in aqueous cytokine levels, 3-month change in SD-OCT central subfield thickness and macular volume (MV), and the statistical association between baseline aqueous cytokine levels and these measures of anatomic response to ranibizumab in center-involving DME.

RESULTS Among the 48 participants, the mean (SD) age was 61.9 (7.1) years and 36 participants (75.0%) were men. The following cytokines were lower at month 2 vs baseline: ICAM-1 (median change, −190.88; interquartile range [IQR], −634.20 to −26.54; $P < .001$), VEGF (median change, −639.45; IQR, −1040.61 to −502.61; $P < .001$), placental growth factor (median change, −1.31; IQR, −5.99 to −0.01; $P < .001$), IL-6 (median change, −38.61; IQR, −166.72 to −2.80; $P < .001$), and monocyte chemoattractant protein 1 (median change, −90.13; IQR, −382.74 to 109.47; $P = .01$). When controlling for age, foveal avascular zone size, and severity of retinopathy, multiple linear regression determined that increasing baseline aqueous ICAM-1 was associated with a favorable anatomic response, in terms of reduced SD-OCT MV at 3 months (every additional 100 pg/mL of baseline ICAM-1 was associated with a reduction of 0.0379 mm^3; $P = .01$). Conversely, increasing baseline aqueous VEGF was associated with a less favorable SD-OCT MV response at 3 months (every additional 100 pg/mL of baseline VEGF was associated with an increase of 0.0731 mm^3; $P = .02$) and was associated with lower odds of being a central subfield thickness responder (odds ratio, 0.868; 95% CI, 0.755-0.998).

CONCLUSIONS AND RELEVANCE Elevated aqueous ICAM-1 and reduced VEGF levels at baseline are associated with a favorable anatomic response to ranibizumab in DME, although there is not always direct correlation between anatomic and visual acuity response.

JAMA Ophthalmol. doi:10.1001/jamaophthalmol.2018.0179
Published online March 8, 2018.

Supplemental content

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Contemporary management of diabetic macular edema (DME) uses intravitreal pharmacotherapy to modulate the intraocular cytokine milieu. Agents in current use focus on suppression of vascular endothelial growth factor (VEGF) isoforms.[1] Ranibizumab, a monoclonal antibody fragment, and bevacizumab, its full-length parent antibody, bind to and block VEGF-A. Aflibercept, a recombinant fusion protein, binds to and inhibits VEGF-A, VEGF-B, and placental growth factor (PlGF). Although aflibercept is purported to have a stronger binding affinity to VEGF-A than ranibizumab or bevacizumab, the efficacy of the 3 agents in DME is similar in patients with relatively good vision.[2-4] Aflibercept may be superior to ranibizumab and bevacizumab in patients with poor vision after 1 year of treatment.[3,4] While the efficacy of these anti-VEGF agents in treating DME has been demonstrated in the scientific literature and subsequently validated by extensive clinical use, the response to anti-VEGF treatment is variable, with some patients having rapid resolution of edema with significant visual gains, whereas others may not benefit despite numerous injections.[5-9]

The reasons for this variability are incompletely understood, but likely rest with the fact that the pathogenesis of DME is related to a complex interplay of a number of different cytokine pathways rather than being solely VEGF driven. Several cytokines besides VEGF and PlGF have been implicated, including intercellular adhesion molecule 1 (ICAM-1) and interleukin 6 (IL-6). We recently demonstrated that aqueous humor levels of ICAM-1 correlated with disease severity at baseline in DME.[10] Interestingly, aqueous VEGF levels were not correlated with DME severity despite the proven efficacy of intravitreal agents targeting primarily VEGF. It is thus reasonable to suppose that perhaps suppression of VEGF by these agents results in upstream or downstream modulation of cytokines other than those in the VEGF family. Furthermore, it raises the question whether baseline aqueous levels of VEGF or these other cytokines, particularly ICAM-1, might be associated with response to treatment with anti-VEGF agents.

Thus, the objectives of this study were to (1) investigate aqueous humor cytokine level changes in response to intravitreal ranibizumab therapy for the management of center-involving DME, (2) determine the association between baseline aqueous levels of VEGF and ICAM-1 and anatomic response, and (3) explore whether other cytokines were associated with anatomic response.

Key Points

Question Are particular aqueous cytokines associated with anatomic response to ranibizumab in patients with diabetic macular edema?

Findings A prospective cohort study of 48 study participants found that elevated intercellular adhesion molecule 1 and reduced vascular endothelial growth factor levels at baseline were associated with favorable anatomic response following 3 monthly injections of ranibizumab for diabetic macular edema.

Meaning These findings suggest aqueous biomarkers of anatomic response have the potential to guide treatment algorithms for anti–vascular endothelial growth factor therapy for diabetic macular edema.

Methods

Participants

Participants were adults with diabetes (type 1 or 2) complicated by center-involving DME, with central subfield thickness (CST) 310 µm or greater in the study eye on spectral-domain optical coherence tomography (SD-OCT). Participants had already fulfilled eligibility criteria and participated in an initial baseline disease correlation phase to this study. The exclusion criteria have been detailed in our previous article.[10]

This prospective, multicenter cohort study was approved by the research ethics boards of St. Michael's Hospital and Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, and adhered to the tenets of the Declaration of Helsinki. All participants provided written informed consent to participation in the study.

Study Design

Each participant received a monthly intravitreal injection of ranbizumab, 0.5 mg, for 3 months (baseline, month 1, and month 2). Aqueous fluid (0.2 mL) for cytokine analysis was obtained at baseline and repeated at the 2-month visit, immediately before the third intravitreal ranibizumab injection. Participants were seen at month 3, to assess their anatomic response 1 month after their third and final study injection. Details of the sampling technique have been described elsewhere.[10] Multiplex immunoassay (Ciraplex; Aushon Biosystems) of all aqueous samples was carried out in duplicate for candidate cytokines that could, based on the existing scientific literature, play a potential role in the pathogenesis of DME. These were VEGF, PlGF, transforming growth factor beta 2, ICAM-1, IL-2, IL-3, IL-6, IL-8, IL-10, IL-17, vascular cell adhesion molecule 1, monocyte chemoattractant protein 1, and epidermal growth factor.

Baseline clinical observations and investigations were carried out in each patient to assess the severity of DME. These were Snellen best-corrected visual acuity (subsequent conversion to logMAR for statistical analysis), dilated ophthalmoscopy, color fundus photography, diabetic retinopathy grading,[11] SD-OCT (Cirrus™ high-definition OCT; Carl Zeiss Meditec), and fundus fluorescein angiography (standard 7 field). Details of our SD-OCT scanning protocol and image quality verification process are available elsewhere.[10] These observations and investigations were repeated according to a predefined schedule, to allow anatomic response to be monitored and quantified.

Statistical Analysis

Four cytokines were excluded from analysis (IL-2, IL-3, IL-17, and epidermal growth factor), as the data for these clustered at the lower sensitivity limit for the immunoassay used and thus there was uncertainty regarding the reliability of these measurements. To investigate aqueous humor cytokine level changes in response to intravitreal ranibizumab therapy, we

compared aqueous cytokine levels at baseline vs month 2 using the Wilcoxon signed rank test to lessen the effect of outliers. To determine the association between baseline aqueous levels of VEGF and ICAM-1 and anatomic response, we defined a response as the difference between month 3 and baseline in terms of logMAR, SD-OCT macular volume (MV), and SD-OCT CST. The association between VEGF, ICAM-1, and anatomic response was estimated by linear regression models, adjusting for age (in decades), baseline foveal avascular zone (FAZ), and either retinopathy severity or lens status. Only 1 of retinopathy severity or lens status was included in the models, as they were highly correlated with each other.

After determining that baseline aqueous VEGF and ICAM-1 were associated with anatomic response in the linear regression models, we determined whether these cytokines were also associated with SD-OCT MV and SD-OCT CST, once categorized into responders and nonresponders. An MV responder was defined as a patient with 10% or greater reduction in MV from baseline to month 3, whereas a CST responder was defined as a patient with 50% or greater reduction in excess CST (> 310 µm) from baseline to month 3. Logistic regression models estimated the odds ratios (ORs) and 95% confidence intervals for the association between VEGF, ICAM-1, and anatomic response. To explore whether other cytokines were associated with anatomic response, we tested the association of other baseline cytokines, added 1 at a time, to the multivariable models. Data analysis was performed using SAS statistical software, version 9.4 (SAS Institute).

Results

Forty-nine participants were recruited to the original baseline study from December 22, 2011, to June 13, 2013, and 48 participants proceeded to follow-up. The mean age was 61.9 years (SD, 7.1 years; range, 38-76 years), and 36 participants (75.0%) were men. At baseline, the mean best-corrected visual acuity was 20/80 (mean logMAR, 0.6; SD, 0.3; range, 20/32-20/800), the mean SD-OCT CST was 495.0 µm (SD, 134.6 µm; range, 311.0-842.0 µm), the mean SD-OCT MV was 13.0 mm^3 (SD, 2.4 mm^3; range, 9.6-19.0 mm^3), and the mean fluorescein angiographic diameter of FAZ was 847.3 µm (SD, 195.2 µm; range, 540.0-1530.0 µm) (**Table 1**). Details of the clinical response to ranibizumab therapy in our patient group are summarized in **Table 2**. No systemic or ocular complications occurred.

The following cytokines were lower at month 2 vs baseline: ICAM-1 (median change −190.88 pg/mL; $P < .001$), VEGF (median change −639.45 pg/mL; $P < .001$), PlGF (median change −1.31 pg/mL; $P < .001$), IL-6 (median change −38.61 pg/mL; $P < .001$), and MCP-1 (median change −90.13 pg/mL; $P = .01$) (**Table 3**). The median percentage change in aqueous levels of VEGF, IL-6, PlGF, ICAM-1, and MCP-1 were 97%, 63%, 51%, 15%, and 6% lower, respectively, at month 2 vs baseline.

There was a correlation between baseline ICAM-1 and change in MV baseline to month 3 ($r_s = −0.465$; $P = .002$) (eFigure 1 in the Supplement). The correlation between baseline VEGF and MV change was not statistically significant ($r_s = −0.064$; $P = .66$) (eFigure 2 in the Supplement). After adjusting for age, FAZ size, and lens status, increasing baseline aqueous ICAM-1 was found to be associated with a favorable anatomic response to ranibizumab treatment at month 3, in terms of reduced SD-OCT MV. Every additional 100 pg/mL of baseline ICAM-1 was associated with a reduction in MV of 0.0379 mm^3 ($P = .01$). Conversely, increasing baseline aqueous VEGF was found to be associated with a less favorable SD-OCT MV response. Every additional 100 pg/mL of baseline VEGF was associated with an increase in MV of 0.0731 mm^3 ($P = .02$) (**Table 4**). No other baseline cytokine or any other variable was associated with SD-OCT MV anatomic response.

Increasing baseline aqueous VEGF was the only cytokine that was associated with a less favorable anatomic response in terms of SD-OCT CST ($P = .03$) (Table 4). This association was only present after adjusting for FAZ size. When FAZ size was removed from the model, baseline VEGF was no longer associated with CST response. There was no association between

Table 1. Baseline Characteristics Features

Characteristics	Value
Demographic (n = 48)	
Age, mean (SD) [range], y	61.9 (7.1) [38-76]
Sex, No. (%)	
Male	36 (75.0)
Female	12 (25.0)
Laterality, No. (%)	
Right	26 (54.2)
Left	22 (45.8)
Lens status, No. (%)	
Phakic	36 (75.0)
Pseudophakic	12 (25.0)
Best-corrected visual acuity (n = 48)	
Snellen, mean (range)	20/80 (20/32-20/800)
LogMAR, mean (SD) [range]	0.6 (0.3) [0.2-1.6]
Angiographic features	
Diameter of focal avascular zone, mean (SD) [range], µm (n = 48)	847.3 (195.2) [540.0-1530.0]
Peripheral ischemia, mean (SD) [range], disc diameters (n = 47)	5.6 (4.7) [0.0-20.0]
OCT features	
Central macular thickness, mean (SD) [range], µm (n = 48)	495.0 (134.6) [311.0-842.0]
Baseline macular volume, mean (SD) [range], mm^3 (n = 48)	13.0 (2.4) [9.6-19.0]
Severity of nonproliferative retinopathy, No. (%)	
Mild	8 (17.0)
Moderate	22 (46.8)
Severe	17 (36.2)
HbA_{1c}, mean (SD) [range], % (n = 34)	8.2 (1.7) [6.1-11.9]
Duration of diabetes, No. (%), y	
<5	4 (9.0)
5 to <10	11 (25.0)
10 to <15	11 (25.0)
15 to <20	5 (11.4)
>20	13 (29.6)

Abbreviations: HbA_{1c}, hemoglobin A_{1c}; OCT, optical coherence tomography.

Table 2. Changes in Clinical Features Between Baseline and Month 3

Study Characteristic	Mean Change Between Baseline and Month 3, (SD) [Range]	*P* Value[a]
Best-corrected visual acuity (n = 47)		
LogMAR	−0.20 (0.23) [−1.0 to 0.12]	<.001
OCT features		
Central macular thickness, μm (n = 48)	−149 (125.62) [−571.0 to 8.0]	<.001
Macular volume, mm^3 (n = 48)	−1.57 (1.36) [−6.0 to 0.1]	<.001

Abbreviation: OCT, optical coherence tomography.

[a] Statistically significant at $P < .05$.

increasing ICAM-1 and favorable SD-OCT CST response at month 3 ($P = .07$). Increased FAZ size at baseline was also associated with more favorable CST response at month 3 ($P = .007$). None of the aqueous cytokines were associated with treatment response in terms of logMAR best-corrected visual acuity (Table 4).

Of the 48 participants, there were 30 CST responders (62.5%; 95% CI, 48.8%-76.2%) and 23 MV responders (47.9%; 95% CI, 33.8%-62.1%). Using logistic regression models, it was determined that increasing ICAM-1 was associated with higher odds of being an MV responder (OR, 1.270; 95% CI, 1.064-1.515), and increasing VEGF was associated with lower odds of being an MV responder (OR, 0.793; 95% CI, 0.658-0.955) after adjusting for age, FAZ size, and severity of retinopathy. It was also determined that increasing VEGF was associated with lower odds of being a CST responder (OR, 0.868; 95% CI, 0.755-0.998) when controlling for age, FAZ size, and severity of retinopathy. For every 100-pg/mL increase in ICAM-1, odds of MV response increased by 27%. Conversely, for every 100-pg/mL increase in VEGF, odds of MV response decreased by 20.7%. For every 100-pg/mL increase in VEGF at baseline, the odds of CST response decreased by 13.2%. No other aqueous cytokines besides VEGF and ICAM-1 were found to be associated with any measure of ranibizumab anatomic response, in any of the models considered.

Discussion

Our finding that aqueous VEGF levels were lower at month 2 vs baseline in response to intravitreal ranibizumab therapy is not surprising, as it is consistent with the intended mechanism of action of this specifically engineered recombinant antibody fragment. However, we found several aqueous cytokines to be reduced in response to ranibizumab therapy. Specifically, the median percentage change in aqueous levels of VEGF, IL-6, PlGF, ICAM-1, and monocyte chemoattractant protein-1 were 97%, 63%, 51%, 15%, and 6% lower, respectively, at month 2 vs baseline. Given that ranibizumab has no known affinity other than for VEGF-A, this emphasizes the complex regulatory interplay that exists between VEGF and these other cytokines.

Aqueous ICAM-1 has been reported to be the sole cytokine associated with SD-OCT MV at baseline in a recent article.[10] This study also proposes SD-OCT MV to be the ideal parameter for the assessment and monitoring of DME severity, both for research purposes and in clinical practice.[10,12] We chose MV to be our primary dependent outcome measure to determine whether aqueous ICAM-1 and VEGF levels are associated with treatment response following intravitreal ranibizumab in DME. Change in MV provides the most objective measure of change in edema in the macula following an injection of ranibizumab, and largely avoids the error and variability associated with measuring retinal thickness within the central Early Treatment Diabetic Retinopathy Study subfield (1 mm in diameter). Macular volume also avoids the many potential confounders and variables that may affect visual acuity measurements. To our knowledge, CST remains the most commonly used OCT measurement of DME severity in clinical practice at this time. Changes in MV may not reflect changes in CST in a given case.

The key finding of this study is that not only are aqueous ICAM-1 levels reduced in response to intravitreal ranibizumab therapy, but increasing aqueous ICAM-1 at baseline is associated with a subsequent favorable anatomic response to ranibizumab therapy at month 3, in terms of SD-OCT MV, in patients with DME. Conversely, increasing aqueous VEGF at baseline is associated with a less favorable anatomic response to ranibizumab at month 3. We also found that after adjusting for FAZ size, age, and lens status, increasing baseline aqueous VEGF was also associated with less favorable CST response. No other baseline aqueous cytokine was associated with anatomic response.

In addition to the associations described earlier from the multivariable linear regression models, we categorized patients in the study into MV responders and determined the odds of a favorable response to treatment with ranibizumab in DME. We determined that higher ICAM-1 levels are associated with a higher odds of MV response. For every 100-pg/mL increase in ICAM-1, odds of MV response increased by 27%. Conversely, for every 100-pg/mL increase in VEGF, odds of MV response decreased by 20.7%. When categorizing patients into CST responders, for every 100-pg/mL increase in VEGF at baseline, the odds of CST response decreased by 13.2%.

We hypothesize that baseline levels of ICAM-1 in the eye and the extent of its downstream suppression by ranibizumab may be particularly important in explaining the variable response to treatment that clinicians observe. In practice, patients who fail to have complete resolution of DME after a certain amount of treatment with ranibizumab are often switched to other medications. The variable responses to different agents among patients with DME might be due to varying degrees of ICAM-1 suppression, among other possible mechanisms. Diabetic Retinopathy Clinical Research Network Protocol T suggests that patients with visual acuity of 20/50 or worse may respond better to aflibercept than ranibizumab after 1 year of treatment.[3,4] It could be that this possible difference in efficacy is related to differential suppression of cytokines outside the VEGF family. Although aflibercept is reported to suppress PIGF in addition to VEGF, this study demonstrates a large reduction in aqueous PIGF following ranibizumab therapy. Therefore, specific targeting of PIGF by aflibercept may not be as relevant in explaining the clinical dif-

Table 3. Aqueous Cytokine Concentrations at Month 2 vs Baseline[a]

Cytokine[b]	Cytokine Levels, Median (IQR), pg/mL		Median Change Between Baseline and Month 2	*P* Value	% Change Between Baseline and Month 2, Median (IQR)
	Baseline	Month 2			
VEGF	752.79 (567.05 to 1309.07)	14.65 (3.98 to 137.37)	−639.45 (−1040.61 to −502.61)	.001[c]	−97.43 (−99.40 to −84.92)
PlGF	3.67 (1.59 to 8.48)	1.56 (0.59 to 3.24)	−1.31 (−5.99 to −0.01)	.001[c]	−51.06 to (−76.67 −0.23)
MCP1	1395.96 (1001.79 to 1820.66)	1210.18 (961.94 to 1652.70)	−90.13 (−382.74 to 109.47)	.01[c]	−6.06 (−24.51 to 8.57)
TGF-β2	10 284.67 (8830.35 to 12 869.56)	9302.07 (6797.03 to 12 289.40)	−345.93 to −2641.78 to 1635.07)	.20	−3.92 (−31.23 to 16.17)
IL-6	80.84 (32.97 to 363.95)	34.14 (20.94 to 95.06)	−38.61 (−166.72 to −2.80)	.002[c]	−63.15 (−82.05 to −8.25)
IL-8	12.32 (7.96 to 18.79)	12.73 (8.68 to 18.87)	0.68 (−5.80 to 4.79)	.88	5.91 (−35.15 to 59.40)
IL-10	0.24 (0.09 to 0.45)	0.12 (0.08 to 0.38)	−0.03 (−0.26 to 0.06)	.13	−15.59 (−71.88 to 62.50)
ICAM-1	1489.54 (740.56 to 1841.91)	1021.57 (660.14 to 1606.34)	−190.88 (−634.20 to −26.54)	.001[c]	−15.11 (−31.42 to −4.28)
VCAM-1	47 547.46 (26 442.41 to 65 169.37)	43 571.03 (24 957.95 to 64 530.33)	−1537.19 (−10 351.77 to 4624.50)	.38	−4.09 (−26.86 to 12.29)

Abbreviations: ICAM-1, intercellular adhesion molecule-1; IL, interleukin; IQR, interquartile range; MCP1, monocyte chemoattractant protein 1; PlGF, placental growth factor; TGF-β2, transforming growth factor-beta 2; VCAM-1, vascular intercellular adhesion molecule-1; VEGF, vascular endothelial growth factor.

[a] For certain cytokines, the concentration values clustered at the lowest test sensitivity threshold; therefore exact concentration values are not presented. The excluded cytokines and lowest test sensitivity thresholds were IL-2 (1.03 pg/mL), IL-3 (0.07 pg/mL), IL-17 (0.15 pg/mL), and endothelial growth factor (0.01 pg/mL).

[b] Sample size was 48 for all cytokines, except for ICAM-1 and VCAM-1 at baseline (n = 43), month 2 (n = 47), and change from baseline to month 2 (n = 43).

[c] Statistically significant at $P < .05$.

Table 4. Multivariate Linear Regression Models for Change in Disease Severity Measures

	Change in Best-Corrected Visual Acuity (n = 42)		Change in Central Macular Thickness (n = 43)		Change in Macular Volume (n = 43)	
Baseline Cytokines[a]	β (SE)[b]	*P* Value	β (SE)[b]	*P* Value	β (SE)[b]	*P* Value
ICAM-1, × 10^{-2}	0.0003 (0.0022)	.90	−2.3877 (1.2969)	.07	−0.0379 (0.0140)	.01[c]
VEGF, × 10^{-2}	−0.0005 (0.0048)	.92	6.2240 (2.8193)	.03[c]	0.0731 (0.0303)	.02[c]
R^2	.32		.26		.29	

Abbreviations: ICAM-1, intercellular adhesion molecule-1; VEGF, vascular endothelial growth factor.

[a] Cytokine values were divided by 100 for better interpretation of β coefficients.

[b] Coefficients are adjusted for age, lens status, and size of foveal avascular zone.

[c] Statistically significant at $P < .05$.

ference between these drugs. Baseline aqueous PIGF was not associated with either baseline disease severity or anatomic response in DME.

Intercellular adhesion molecule 1 is a member of the immunoglobulin superfamily. It is an inducible cell surface glycoprotein that resides primarily on leukocytes and endothelial cells. Intercellular adhesion molecule 1 is a ligand for the lymphocyte function-associated antigen 1 receptor, and together they govern leukocyte adhesion and migration across vascular endothelium. As a consequence, ICAM-1 upregulation promotes leukostasis, hypoxia, endothelial cell damage, and breakdown of the blood-retinal barrier—key events in DME pathogenesis.[13-15] Elevated levels of ICAM-1 (as well as other cytokines) have been identified in the aqueous and vitreous of eyes with diffuse DME, when compared with control participants.[16,17] In addition to previous findings, aqueous and vitreous ICAM-1 levels have also been correlated with SD-OCT indicators of disease severity in DME by other authors.[16-18]

It is well established that expression of VEGF and ICAM-1 in the retinal vascular endothelium are closely linked, upregulating synergistically to promote leukostasis in the diabetic eye.[14,15,19,20] At a cellular level, nitric oxide is thought to play a key role in this synergetic relationship. Endogenous VEGF has been shown to stimulate the expression of retinal endothelial nitric oxide synthase.[15] In brain microvascular endothelial cells, VEGF has been found to activate a pathway leading to induction of endothelial nitric oxide synthase, nitric oxide production, and ICAM-1 upregulation.[9] However, there is evidence to suggest that non-VEGF-mediated pathways may be of prime importance in DME pathogenesis.[21] In a diabetic rat model, etanercept (a tumor necrosis factor inhibitor) was shown to reduce retinal ICAM-1 expression and, accordingly, leukocyte adhesion and blood-retinal barrier breakdown. However, the drug does not alter retinal VEGF levels.[22] Furthermore, in the diabetic rat model, blockade of the ICAM-1 and lymphocyte function-associated antigen 1 axis prevented retinal leukostasis and promoted blood-retinal barrier integrity.[23,24]

Our analysis reveals that aqueous VEGF levels at baseline are associated with SD-OCT MV and CST anatomic response. This association remained when assessed using continuous or categorical data. Interestingly, the relationship between VEGF and anatomic response was not linear and was highly dependent on controlling for certain covariates, specifically FAZ size. There is likely a complex interplay between baseline VEGF, FAZ size, and treatment response.

The association of anatomic response with baseline VEGF may be expected; however, the direction of the relationship may be counterintuitive. Patients with increasing baseline VEGF exhibited less favorable treatment response. Patients with higher baseline aqueous VEGF levels are known to have worse diabetic retinopathy and thus may be more resistant to treatment at the 3-month point than patients with lower baseline VEGF.[25] It is conceivable that with continued anti-VEGF therapy, some of these more resistant cases would eventually respond to treatment. However, a recent subgroup analysis of randomized clinical trial data demonstrated that patients who are poor responders after 3 intravitreal ranibizumab injections are likely to remain poor responders with continued treatment.[26] Further studies will be required to explore the complex relationship between baseline aqueous VEGF levels and subsequent response to intravitreal ranibizumab in DME.

Limitations

The study has limitations. A large number of cytokines were examined leading to the possibility of statistical errors relating to multiple testing. We are reassured that the results of this study are consistent with our baseline study demonstrating the importance of ICAM-1. Although peripheral nonperfusion was not found to be significantly associated with treatment response to ranibizumab, wide-field imaging (not performed in this study) may have better quantified this. Another limitation of this study is that the assays used have likely not been tested specifically for reliability and validity in aqueous fluid and following treatment with intravitreal ranibizumab by the manufacturer.

Aqueous fluid has been used to measure cytokines that may to be associated with treatment response for a disease of the posterior segment. Although analysis of vitreous fluid would be ideal from a scientific method perspective as performed in another study,[27] it is our opinion that it is not practical at this time to take vitreous samples for research purposes from patients while under treatment with intravitreal pharmacotherapy. Therefore, we feel that aqueous fluid is presently the most appropriate and practical surrogate to use for studies of biomarkers of disease severity and treatment response in patients with DME and other retinal diseases. This may change as safer and less invasive methods of sampling the vitreous are developed. Finally, it is well known that in patients with DME, there is not always direct correlation between anatomic and visual acuity response.

Conclusions

Our data strongly support the assertion that in addition to VEGF, ICAM-1 is a crucial cytokine mediator in DME. That aqueous ICAM-1 and VEGF at baseline are strongly associated with anatomic response to ranibizumab introduces the novel paradigm of cytokine-guided treatment algorithms whereby clinicians might tailor therapy by choosing among a variety of therapeutic agents based on a patient's individual intraocular cytokine profile. Furthermore, our findings suggest ICAM-1 may be not only a biomarker of disease severity and anatomic response but also a potential therapeutic target in DME, recognizing that in patients with DME, there is not always a direct correlation between anatomic and visual acuity response.

ARTICLE INFORMATION

Accepted for Publication: December 31, 2017.

Published Online: March 8, 2018. doi:10.1001/jamaophthalmol.2018.0179

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Acquisition, analysis, or interpretation of data: Hillier, Ojaimi, Wong, Mak, Berger, Kohly, Kertes, Boyd, Eng, Giavedoni, Nisenbaum, Muni.
Drafting of the manuscript: Hillier, Mak, Kohly, Nisenbaum, Muni.
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Statistical analysis: Mak, Berger, Nisenbaum, Muni.
Obtained funding: Ojaimi, Muni.
Administrative, technical, or material support: Hillier, Ojaimi, Wong, Berger, Kohly, Kertes, Boyd, Muni.
Study supervision: Wong, Berger, Kertes, Forooghian, Muni.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Hillier reports personal fees from Novartis Inc and Alimera Sciences Ltd outside the submitted work. Dr Wong reports grants from Novartis and The Retina Foundation of Canada during the conduct of the study, grants and personal fees from Novartis, personal fees from Bayer, Valeant, Allergan, Alcon, Labtician, and DORC, and grants from Genetech outside the submitted work. Dr Berger reports honorariums from Novartis and Bayer outside the submitted work. Dr Muni reports personal fees from Bayer and Novartis and grants from Novartis outside the submitted work. No other disclosures were reported.

Funding/Support: An unrestricted research grant and study drug was provided by Novartis Pharmaceuticals Canada Inc (Dr Muni). A research grant was received from the Retina Foundation of Canada (Dr Ojaimi).

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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