Association of Inflammatory Factors With Macular Edema in Branch Retinal Vein Occlusion

Hidetaka Noma, MD; Tatsuya Mimura, MD; Shuichiro Eguchi, MD

Objective: To evaluate the association between vitreous fluid levels of inflammatory factors and macular edema in patients with branch retinal vein occlusion (BRVO).

Methods: In 39 patients with BRVO and macular edema and 21 individuals with idiopathic macular hole (MH) serving as controls, vitreous fluid samples were obtained during vitrectomy surgery, and the levels of vascular endothelial growth factor (VEGF), soluble VEGF receptor 2 (sVEGFR-2), soluble intercellular adhesion molecule 1 (sICAM-1), interleukin 6 (IL-6), monocyte chemotactic protein 1 (MCP-1), and pigment epithelium-derived factor (PEDF) were measured by enzyme-linked immunosorbent assay. Macular edema was examined using optical coherence tomography.

Results: Vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, and PTX3 were significantly higher in the patients with BRVO than in those with MH; however, the PEDF level was significantly lower in the BRVO group. Vitreous fluid levels of all 7 factors were significantly correlated with the retinal thickness at the central fovea. There were also significant correlations of sVEGFR-2 with sICAM-1, IL-6, MCP-1, and PTX3 but no correlation with VEGF. However, there were significant correlations of VEGF with sICAM-1, IL-6, MCP-1, and PEDF in the BRVO group.

Conclusions: Vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, PTX3, and PEDF are strongly correlated with retinal vascular permeability and the severity of macular edema in patients with BRVO. These findings may be useful for understanding macular edema and developing new treatments for BRVO.


B RANCH RETINAL VEIN OCCLUSION (BRVO) often results in macular edema, which is the chief cause of visual impairment in patients with BRVO. Although the pathogenesis of macular edema in these patients is unclear, retinal changes due to BRVO (including hemorrhage) are known to cause local inflammation. After retinal vein occlusion, there is increased rolling and adhesion of leukocytes to the retinal vein walls that lead to stagnation of blood flow, so inflammation may play a key role in the pathogenesis of BRVO. The role of inflammation is supported by reports that intravitreal injection of triamcinolone acetonide lessens macular edema in patients with BRVO and that the aqueous flare value is significantly higher in patients with retinal vein occlusion than in healthy individuals.

Various molecules that are secreted into the vitreous fluid may be associated with ocular abnormalities, although the vitreous levels of soluble inflammatory factors might not necessarily reflect their tissue levels, especially the amounts in the retinal microenvironment. However, the concentrations of soluble factors secreted into the vitreous fluid have been reported to influence visual prognosis. There is evidence that upregulation of inflammatory factors, including vascular endothelial growth factor (VEGF), VEGF receptor 2 (VEGFR-2), intercellular adhesion molecule 1 (ICAM-1), interleukin 6 (IL-6), and monocyte chemotactic protein 1 (MCP-1), or downregulation of anti-inflammatory factors, such as pigment epithelium-derived factor (PEDF), and a subsequent increase in leukocyte-endothelial interactions contribute to breakdown of the blood-retinal barrier (BRB). The levels of VEGF, IL-6, soluble ICAM-1 (sICAM-1), soluble VEGFR-2 (sVEGFR-2), and PEDF in the vitreous fluid are independently related to vascular permeability in patients with BRVO and macular edema. Blocking the actions of inflammatory factors has been shown to prevent leukostasis and an increase in retinal vascular permeability in rats, and development of macular edema in patients with BRVO has been reported to be accompanied by elevation of cytokines that regulate the inflammatory response. Thus, various inflammatory cytokines and other factors influence vascular permeability in the eye and are associated with macular edema in patients with BRVO.

Recently, long pentraxin 3 (PTX3) was reported to be an early indicator of myocardial infarction and a predictor of...
3-month mortality after acute myocardial events. Long pentraxin 3 is an acute-phase protein that is involved in innate immunity and inflammation. Pentraxins are a family of acute response proteins comprising 3 members—C-reactive protein, serum amyloid P, and PTX3—and these proteins are classic acute-phase reactants that closely reflect the level of inflammatory activity. Long pentraxin 3 is induced by cytokines and is produced mainly by vascular endothelial cells, fibroblasts, and cells in some other extrahepatic tissues, unlike the other 2 family members that are synthesized primarily in the liver. In an animal model, there was a rapid increase in PTX3 expression after reperfusion of the ischemic superior mesenteric artery territory. More important, overexpression of PTX3 was accompanied by an increase in death and tissue damage after intestinal ischemia and reperfusion. PTX3 increases vascular permeability. These findings suggest that PTX3 may play an important role in the pathogenesis of macular edema associated with BRVO. However, the level of PTX3 expression in patients with BRVO and its relationship to the pathogenesis of macular edema are unclear, just as the relative contribution of each of the molecules evaluated herein to the development of macular edema remains uncertain. Accordingly, we measured the vitreous fluid levels of 6 inflammatory factors (including PTX3) and 1 anti-inflammatory factor in patients with BRVO and macular edema, focusing on molecules that have been linked to the onset or exacerbation of this condition. The association between each of these molecules and the severity of macular edema was then assessed.

## METHODS

### PARTICIPANTS

Undiluted vitreous fluid samples were harvested at the start of pars plana vitrectomy (PPV) after written informed consent was obtained from each participant following an explanation of the purpose and potential adverse effects of the procedure. This study was performed in accordance with the Helsinki Declaration of 1975 (1983 revision). The institutional review boards of Tokyo Women’s Medical University and Eguchi Eye Hospital approved the protocol for collection and testing of vitreous fluid and blood samples. This was a retrospective case-control study of 60 Japanese patients who underwent PPV in 1 eye (39 with BRVO and 21 with idiopathic macular edema [MH]) to treat macular edema. Seventy-two consecutive patients with BRVO who sought care at the hospitals associated with Tokyo Women’s Medical University or Eguchi Eye Hospital between August 11, 2009, and November 15, 2011, were screened, using the criteria listed in the next sentence, and vitreous fluid samples were obtained from the 39 patients enrolled. The indications for PPV were (1) clinically detectable diffuse macular edema or cystoid macular edema persisting for more than 3 months and (2) best-corrected visual acuity worse than 20/40.

The Branch Vein Occlusion Study demonstrated the effectiveness of argon laser photocoagulation for BRVO, but it was recommended that this should not be performed within 3 months of occurrence, during which time spontaneous improvement may occur. The absence of posterior vitreous detachment can contribute to persistent macular edema in patients with retinal vascular occlusion. Sakai et al reported on the effectiveness of PPV combined with surgical posterior vitreous detachment for macular edema in patients with BRVO.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BRVO Group</th>
<th>MH Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>39</td>
<td>21</td>
<td>.66</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>69.2 (9.6)</td>
<td>68.8 (8.4)</td>
<td>.88</td>
</tr>
<tr>
<td>Blood pressure, mean (SD), mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>134 (14)</td>
<td>121 (11)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78 (8)</td>
<td>74 (8)</td>
<td>.07</td>
</tr>
<tr>
<td>Hypertension, No.</td>
<td>22</td>
<td>3</td>
<td>.002</td>
</tr>
<tr>
<td>Hyperlipidemia, No.</td>
<td>12</td>
<td>4</td>
<td>.33</td>
</tr>
<tr>
<td>Duration of BRVO, mean (SD), mo</td>
<td>5.1 (2.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BRVO, branch retinal vein occlusion; MH, macular hole.

It has also been reported that PPV contributes to an increase in oxygen tension in the inner retina. If retinal oxygen tension increases after PPV, macular edema would be lessened for several reasons. First, an increase in oxygen tension would reduce VEGF production and thus decrease vascular permeability. Second, an increase in oxygen tension would alleviate autoregulatory arteriolar vasoconstriction and thus reduce the hydrostatic pressure in the retinal capillaries and venules. This would decrease water flux from the vascular compartment to the tissue compartment and reduce edema according to the Starling law. Finally, PPV reduces the intraocular levels of various other inflammatory factors in addition to VEGF, and this may be another mechanism by which it alleviates macular edema in patients with BRVO. In fact, it has been reported that PPV improves both functional and tomographic outcomes in patients with BRVO and macular edema. Accordingly, we performed PPV in patients with clinically detectable diffuse macular edema or cystoid macular edema more than 3 months after the onset of BRVO.

Thirty-three of the 72 patients were excluded because of previous ocular surgery or intravitreous injection of anti-VEGF agents or triamcinolone acetonide in 23 patients, diabetic retinopathy in 2 patients, previous retinal photocoagulation in 7 patients, and a history of ocular inflammation or vitreoretinal disease in 1 patient. Patients with intravitreous injection of anti-VEGF agents or triamcinolone acetonide were excluded because such treatment could influence vitreous fluid levels of inflammatory factors. Vitreous fluid samples were also obtained from 21 patients with nonischemic ocular diseases as a control group (MH group). None of the patients in the MH group had proliferative vitreoretinopathy. The mean (SD) age of the BRVO group (19 men and 20 women) was 69.2 (9.6) years, and the control group (9 men and 12 women) was aged 68.8 (8.4) years. The mean duration of BRVO was 5.1 (2.4) months (range, 3–11 months). Clinical and laboratory characteristics of the BRVO and MH groups are shown in Table 1.

### FUNDUS FINDINGS

Both preoperative and operative fundus findings were recorded for each participant. A masked grader (H.N.) independently assessed ischemic retinal vascular occlusion by examining fluorescein angiograms. The ischemic region of the retina was measured with the public domain Scion Image program (Scion Corp), as reported previously. On digital fundus pho...
Table 2. Vitreous Fluid Levels of Factors in the Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>BRVO Group</th>
<th>MH Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVEGFR-2, pg/mL</td>
<td>1500 (1083-2035)</td>
<td>1020 (721-1343)</td>
<td>.002</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>229 (33.9-1353)</td>
<td>15.6 (15.6-31.2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>8.20 (5.33-15.6)</td>
<td>4.50 (3.60-5.65)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>10.7 (5.53-29.0)</td>
<td>1.00 (0.50-1.18)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MCP-1, pg/L</td>
<td>1190 (747-1993)</td>
<td>458 (375-636)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PTX3, ng/mL</td>
<td>0.86 (0.50-1.16)</td>
<td>0.50 (0.50-0.81)</td>
<td>.01</td>
</tr>
<tr>
<td>PEDF, ng/mL</td>
<td>25.6 (8.14-40.7)</td>
<td>59.9 (25.0-101)</td>
<td>.005</td>
</tr>
</tbody>
</table>

Abbreviations: BRVO, branch retinal vein occlusion; IL-6, interleukin 6; MCP-1, monocyte chemotactic protein 1; MH, macular hole; PEDF, pigment epithelium-derived factor; PTX3, pentraxin 3; sICAM-1, soluble intercellular adhesion molecule 1; sVEGFR-2, soluble vascular endothelial growth factor (VEGF) receptor 2.

Table 3. Correlation of Vitreous Factors With the Nonperfused Area and Retinal Thickness

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonperfused Area</th>
<th>Retinal Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVEGFR-2</td>
<td>0.19</td>
<td>0.36</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.77</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>0.36</td>
<td>.02</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.46</td>
<td>.004</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.52</td>
<td>.001</td>
</tr>
<tr>
<td>PTX3</td>
<td>0.37</td>
<td>.02</td>
</tr>
<tr>
<td>PEDF</td>
<td>−0.39</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: See Table 2; r, correlation coefficient.

RESULTS

The vitreous fluid concentration of sVEGFR-2 (median [interquartile range]) was significantly higher in the BRVO group (1500 pg/mL [1083-2035]) than in the MH group (1020 pg/mL [721-1343]; P = .002) (Table 2). The vitreous fluid concentration of VEGF was significantly higher in the BRVO group (229 pg/mL [33.9-1353]) compared with the MH group (15.6 pg/mL [15.6-31.2]; P < .001) (Table 2). Likewise, vitreous sICAM-1 levels were significantly higher in the BRVO group (8.20 ng/mL [5.53-15.6]) than in the MH group (4.50 ng/mL [3.60-5.65]; P < .001) (Table 2). Furthermore, the vitreous level of IL-6 was significantly higher in the BRVO group (10.7 pg/mL [5.53-29.0]) than in the MH group (1.00 pg/mL [0.50-1.18]; P < .001), as was the vitreous level of MCP-1 (1190 pg/mL [747-1993] vs 458 pg/mL [375-636]; P < .001) and the vitreous level of PTX3 (0.86 ng/mL [0.50-1.16] vs 0.50 ng/mL [0.50-0.81]; P = .01) (Table 2). In contrast, the vitreous fluid level of PEDF was significantly lower in the BRVO group (25.6 ng/mL [8.14-40.7]) than in the MH group (59.9 ng/mL [25.0-101]; P = .005) (Table 2).

Vitreous fluid levels of VEGF, sICAM-1, IL-6, MCP-1, and PTX3 were significantly correlated with the nonperfused area of the retina in the BRVO group (r = 0.77, P < .001; r = 0.36, P = .02; r = 0.46, P = .004; r = 0.52, P < .001; and r = 0.37, P = .02, respectively) (Table 3). Conversely, the vitreous fluid level of PEDF showed a significant negative correlation with the nonperfused area in the BRVO group (r = −0.39, P = .02) (Table 3). However, the vitreous fluid level of sVEGFR-2 was not significantly correlated with the nonperfused area in this group (r = 0.19, P = .25) (Table 3).

STATISTICAL ANALYSIS

Analyses were performed with commercial software (SAS, version 9.1; SAS Institute Inc). A t test was used to compare normally distributed unpaired continuous variables between the 2 groups, and the Mann-Whitney test was used for variables with a skewed distribution. The χ² test or Fisher exact test was used to compare discrete variables. Differences between the median plasma and vitreous levels were assessed with the Wilcoxon single rank test. To examine relationships among the variables, Spearman rank order correlation coefficients or Pearson correlation coefficients were calculated. Statistical significance was set at P < .05, with 2-tailed values.
Vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, PTX3, and PEDF were significantly correlated with the retinal thickness at the central fovea according to simple linear regression analysis ($r = 0.36$, $P = 0.02$; $r = 0.47$, $P = 0.003$; $r = 0.56$, $P < 0.001$; $r = 0.41$, $P = 0.01$; $r = 0.63$, $P < 0.001$; $r = 0.39$, $P = 0.02$; and $r = -0.36$, $P = 0.02$, respectively) (Table 3).

In the BRVO group, there were significant correlations between the vitreous fluid level of sVEGFR-2 and the levels of sICAM-1, IL-6, MCP-1, and PTX3 ($r = 0.76$, $P < 0.001$; $r = 0.63$, $P < 0.001$; $r = 0.69$, $P < 0.001$; and $r = 0.66$, $P < 0.001$, respectively) (Table 4). There were also significant correlations between the vitreous fluid level of VEGF and the levels of sICAM-1, IL-6, MCP-1, and PEDF in the BRVO group ($r = 0.34$, $P = 0.03$; $r = 0.41$, $P = 0.01$; $r = 0.46$, $P = 0.004$; and $r = -0.33$, $P = 0.04$, respectively) (Table 4). Furthermore, there was a significant correlation between the vitreous fluid level of sICAM-1 and the levels of IL-6, MCP-1, and PTX3 ($r = 0.63$, $P < 0.001$; $r = 0.66$, $P < 0.001$; and $r = 0.64$, $P < 0.001$, respectively) (Table 4). Moreover, there was a significant correlation between the vitreous fluid level of IL-6 and the levels of MCP-1 and PTX3 ($r = 0.70$, $P < 0.001$; and $r = 0.65$, $P < 0.001$, respectively) (Table 4) as well as a significant correlation between MCP-1 and PTX3 or PEDF ($r = 0.53$, $P < 0.001$; and $r = -0.39$, $P = 0.02$, respectively) (Table 4). In contrast, there was no significant correlation between the vitreous levels of sVEGFR-2 and VEGF ($r = 0.14$, $P = 0.38$) or between the vitreous levels of VEGF and PTX3 in the BRVO group ($r = 0.23$, $P = 0.19$) (Table 4). There were also no significant correlations between the vitreous fluid level of PEDF and the levels of sVEGFR-2, sICAM-1, IL-6, and PTX3 in the BRVO group ($r = -0.12$, $P = 0.44$; $r = 0.03$, $P = 0.87$; $r = -0.10$, $P = 0.52$; and $r = 0.04$, $P = 0.82$, respectively) (Table 4).

In the BRVO group, the vitreous fluid levels of VEGF, IL-6, and MCP-1 were significantly higher (all $P < 0.001$) than the plasma levels of these molecules (18.1 pg/mL [15.6-44.1], 0.59 pg/mL [0.35-0.98], and 142 pg/mL [117-167], respectively), whereas the vitreous levels of sVEGFR-2, sICAM-1, and PTX3 were significantly lower (all $P < 0.001$) than their plasma levels (6750 pg/mL [5895-8245], 423 ng/mL [332-508], and 3.66 ng/mL [2.66-5.11], respectively).

**Table 4. Correlation Matrix for Vitreous Factors**

<table>
<thead>
<tr>
<th>Variable</th>
<th>sVEGFR-2</th>
<th>VEGF</th>
<th>sICAM-1</th>
<th>IL-6</th>
<th>MCP-1</th>
<th>PTX3</th>
<th>PEDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVEGFR-2</td>
<td>$r = 0.14$</td>
<td>$r = 0.38$</td>
<td>$r = 0.76$</td>
<td>$r &lt; 0.001$</td>
<td>$r = 0.63$</td>
<td>$r &lt; 0.001$</td>
<td>$r = 0.69$</td>
</tr>
<tr>
<td>VEGF</td>
<td>$r = 0.34$</td>
<td>$r = 0.30$</td>
<td>$r = 0.41$</td>
<td>$r = 0.01$</td>
<td>$r = 0.46$</td>
<td>$r = 0.004$</td>
<td>$r = 0.70$</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>$r = 0.63$</td>
<td>$r &lt; 0.001$</td>
<td>$r = 0.66$</td>
<td>$r &lt; 0.001$</td>
<td>$r = 0.64$</td>
<td>$r &lt; 0.001$</td>
<td>$r = 0.03$</td>
</tr>
<tr>
<td>IL-6</td>
<td>$r = 0.12$</td>
<td>$r = 0.44$</td>
<td>$r = 0.03$</td>
<td>$r = 0.87$</td>
<td>$r = -0.10$</td>
<td>$r = 0.52$</td>
<td>$r = 0.04$</td>
</tr>
<tr>
<td>MCP-1</td>
<td>$r = 0.53$</td>
<td>$r &lt; 0.001$</td>
<td>$r = -0.33$</td>
<td>$r = 0.04$</td>
<td>$r = 0.82$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTX3</td>
<td>$r = 0.53$</td>
<td>$r &lt; 0.001$</td>
<td>$r = -0.33$</td>
<td>$r = 0.04$</td>
<td>$r = 0.82$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEDF</td>
<td>$r = 0.53$</td>
<td>$r &lt; 0.001$</td>
<td>$r = -0.33$</td>
<td>$r = 0.04$</td>
<td>$r = 0.82$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: See Table 2; $r$, correlation coefficient.

There were 3 main findings in this study. First, vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, and PTX3 were significantly higher in patients with BRVO and macular edema than in controls with MH. Second, vitreous fluid levels of sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, PTX3, and PEDF were also correlated with the retinal thickness at the central fovea. Finally, there were significant correlations among the vitreous fluid levels of sICAM-1, IL-6, MCP-1, PTX3, and sVEGFR-2 in the BRVO group, as well as among the vitreous levels of sICAM-1, IL-6, MCP-1, PEDF, and VEGF.

These findings suggest that not only VEGF but also sVEGFR-2, ICAM-1, IL-6, MCP-1, and PTX3 may play important roles in the occurrence of macular edema associated with BRVO. Vascular endothelial growth factor has a potent influence on vascular permeability, and its production is upregulated by retinal hypoxia in patients with BRVO and macular edema.5 Breakdown of the BRB and retinal vascular hyperpermeability are important pathophysiologic features of macular edema associated with BRVO, and there is evidence that inflammation is a key mediator of both endothelial cell damage and BRB breakdown.6,7 Upregulation of inflammatory factors, including VEGF, VEGFR-2, ICAM-1, IL-6, and MCP-1, as well as increased rolling and adhesion of leukocytes, is observed before and during the increase in retinal permeability.5-7 Leukocyte recruitment is modulated by PTX3 in inflammation,31 so its upregulation could also lead to an increase in vascular permeability.32 This possibility is supported by the report24 that the response of vascular permeability is less marked in PTX3-deficient mice. Thus, interactions among the network of inflammatory factors evaluated here may enhance vascular permeability. Activation of ICAM-1 and the subsequent increase in leukocyte-endothelial adhesion may be essential for VEGF to induce vascular hyperpermeability because blocking ICAM-1 activity almost completely prevents VEGF-induced leukostasis and BRB breakdown.35 However, blocking VEGF activity in the diabetic retina markedly reduces the upregulation of ICAM-1 as well as the increase in leukocyte adhesion and BRB breakdown.36 These findings suggest that VEGF is the key factor mediating the response to hypoxia in the retina.

Interestingly, we found a significant correlation between the vitreous fluid level of sVEGFR-2 and the levels of various inflammatory factors (sICAM-1, IL-6, MCP-1, and PTX3) in patients with BRVO and macular edema, but there was no significant correlation between the vitreous fluid levels of sVEGFR-2 and VEGF. Binding of VEGF to VEGFR-2 triggers a signaling cascade that results in tyro-
sine phosphorylation of phospholipase Cγ,37-39 which in turn increases the intracellular levels of inositol 1,4,5-triphosphate and diacylglycerol. Inositol 1,4,5-triphosphate increases the intracellular calcium level by promoting efflux of calcium from the endoplasmic reticulum. This increase in intracellular calcium stimulates sphingo-
sine kinase to produce sphingosine 1-phosphate,36 which then activates protein kinase C (PKC). Activated phospho-
cylglycerol, and activated PKC is a strong activator of nuclear

...the expression of inflammatory proteins by vascular en-
thelial cells through binding to VEGFR-2. This is sup-
porting the expression of inflammatory factors (in-
cluding ICAM-1, IL-6, and MCP-1).42-47 Nuclear fac-
tors–kB is found in almost all cell types and is involved in
 cellular responses to stimuli such as stress, proinflamma-
tory gene expression (including cytokines, adhesion mol-
ecules, and chemokines), free radicals, UV irradiation, and
bacterial or viral antigens in addition to its central role in
the immune response.66-68 It has also been reported51-53 that
VEGF, via the VEGFR-2–PKC axis, induces the produc-
tion of proinflammatory cytokines (including IL-6 and
MCP-1) in endothelial cells. Thus, VEGF promotes the ex-
pression of inflammatory factor messenger RNAs (including
ICAM-1, IL-6, and MCP-1), mainly through the activa-
tion of PKC and NF-kB, indicating that VEGF induces the
expression of inflammatory proteins by vascular endo-
thelial cells through binding to VEGFR-2. This is sup-
ported by reports55,56 that a specific VEGF-2 antago-
nist blocks VEGF-induced expression of inflammatory
factors (including ICAM-1, IL-6, and MCP-1) and also
blocks activation of NF-kB by VEGF. Expression of the
PTX3 gene also requires the activation of NF-kB.57 In
addition, Souza et al39 reported that NF-kB activation was sig-
nificantly suppressed in PTX3-deficient mice. Taken to-
gether with our results, these reports suggest that the
vitreous level of sVEGFR-2 influences various inflamma-
tory factors (including ICAM-1, IL-6, and MCP-1, and PTX3)
in patients with BRVO and macular edema. On the other
hand, the vitreous level of sVEGFR-2 may be regulated inde-
dependently of VEGF, although the VEGF–VEGFR-2 sig-
naling pathway is considered essential for controlling vas-
cular permeability.30,39 The VEGF is upregulated by hypoxia
through hypoxia-inducible factor 1α,50 which is another
transcription factor that regulates genes responding to hy-
poxia.61 Vascular endothelial growth factor may act via an
independent pathway to promote the retinal changes that
occur in BRVO; therefore, additional studies are required
to identify the mechanism. Differences in the activation of
various transcription factors may determine the severity of
ocular ischemic and inflammatory changes.

Considering our results, as well as the balance be-
tween VEGF and inflammatory cytokines, we should sel-
ector treatment with anti-VEGF agents (to reduce the level of
free VEGF) or triamcinolone acetoneide (with a broad
spectrum of action, as appropriate). Because the aque-
ous level of VEGF is significantly correlated with the vit-
reous level of VEGF,53 measuring the concentrations of
various molecules in aqueous humor by enzyme-linked
immunosorbent assay or multiplex bead analysis could
help with the selection of treatment between anti-VEGF
agents, triamcinolone acetoneide, or combined therapy.
In addition, upregulation of inflammatory factors may
be dependent on VEGFR-2 because there were signifi-
cant correlations between the vitreous fluid level of
sVEGFR-2 and the vitreous levels of 4 inflammatory fac-
tors (sICAM-1, IL-6, MCP-1, and PTX3) in our patients
with BRVO and macular edema. Accordingly, multiple
inflammatory factors could be inhibited by an antibody
targeting VEGFR-2, so it may be worth also considering
anti–VEGFR-2 therapy to treat macular edema in this
population. However, a prospective clinical trial would be
required to investigate the efficacy of such therapy.

This study also had some other limitations. For ex-
ample, it is unclear from our data whether elevated vit-
reous levels of cytokines and chemokines were related
to increased retinal vascular permeability or local pro-
duction in the retina, but the mechanism involved may
be revealed by animal studies.

In the present study, the vitreous fluid levels of
sVEGFR-2, VEGF, sICAM-1, IL-6, MCP-1, and PTX3,
and PEDF were strongly correlated with retinal vascular
permeability and the severity of macular edema. The
dsVEGFR-2 level was significantly correlated with the lev-
els of sICAM-1, IL-6, MCP-1, and PTX3 but not with the
level of VEGF. These findings suggest the importance of
investigating relationships among VEGF and the cyto-
kine network and may contribute to understanding the
mechanism of macular edema in patients with BRVO and
developing new treatments.

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