Serum and Plasma Vascular Endothelial Growth Factor Concentrations Before and After Intravitreal Injection of Aflibercept or Ranibizumab for Age-Related Macular Degeneration

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- PURPOSE: To evaluate serum and plasma vascular endothelial growth factor (VEGF) concentrations in neovascular age-related macular degeneration patients treated bimonthly with an intravitreal injection of aflibercept or ranibizumab.
- DESIGN: Prospective, interventional case series.
- METHODS: This study includes 17 eyes of 17 patients treated with 2 mg aflibercept (the aflibercept group), 15 eyes of 15 patients treated with 0.5 mg ranibizumab (the ranibizumab group), and 12 patients with cataract (the control group). Serum and plasma VEGF concentrations were quantified using the enzyme-linked immunosorbent assay.
- RESULTS: At baseline, mean serum VEGF concentration (in picograms per milliliter) did not differ significantly among the 3 groups ($P = .99$). In the aflibercept group, it was 28.3 pg/mL at baseline, decreased to below the detectable limit at 1 week ($P < .0001$), increased to 11.7 pg/mL at 1 month, which was still significantly less than the baseline level ($P < .001$), and returned to 23.9 pg/mL ($P = .35$) at 2 months. In the ranibizumab group, there were no significant differences. At baseline, mean plasma VEGF concentration did not differ significantly among the 3 groups ($P = .64$). In the aflibercept group, it was 16.2 at baseline, decreased to less than the detectable limit at 1 week ($P < .01$) and at 1 month ($P < .05$), and returned to 13.6 pg/mL at 2 months ($P = .73$). In the ranibizumab group, there were no significant differences.
- CONCLUSIONS: Aflibercept significantly decreased serum and plasma VEGF concentrations 1 month after injection; however, ranibizumab had no significant effect on either serum or plasma VEGF level. (Am J Ophthalmol 2014;158:738–744. © 2014 by Elsevier Inc. All rights reserved.)
aimed to evaluate the change in serum and plasma VEGF concentrations over a 2-month period after 1 intravitreal injection of aflibercept or ranibizumab in patients with AMD.

METHODS

THIS PROSPECTIVE STUDY WAS REGISTERED AT http://www.umin.ac.jp (No. UMIN000010312). Its protocol was approved by the Institutional Review Board of Shiga University of Medical Science Hospital. All patients provided written informed consent before the study started.

• PATIENTS: This prospective, interventional case series investigated the levels of VEGF in serum and plasma from patients with active choroidal neovascularization secondary to AMD. Forty-four participants were enrolled, including 32 patients with choroidal neovascularization resulting from AMD (18 with neovascular AMD, 12 with polypoidal choroidal vasculopathy, and 2 with retinal angiomaticat protation), and 12 age-matched patients scheduled for cataract surgeries served as a control group. Patients with any other ocular disease that had systemic pathologic features were excluded from the study.

• TREATMENT: Treatment-naive patients with AMD underwent bimonthly intravitreal injection of aflibercept or ranibizumab without a loading phase of monthly injection. Seventeen eyes of 17 patients received 1 intravitreal aflibercept injection (the aflibercept group, 2.0 mg/0.05 mL; Eylea; Regeneron Pharmaceuticals, Inc, Tarrytown, New York, USA; and Bayer HealthCare Pharmaceuticals, Berlin, Germany). Fifteen eyes of another 15 AMD patients received 1 intravitreal ranibizumab injection (the ranibizumab group, 0.5 mg/0.05 mL; Lucentis; Novartis, Basel, Switzerland).

The bimonthly intravitreal aflibercept or ranibizumab injection were administered as described in a previous report. In brief, eyes were anesthetized with topical 4% lidocaine using a sterile lid speculum. After the ocular surface was sterilized using a povidone–iodine solution, aflibercept or ranibizumab was injected. A topical ophthalmic antibiotic was applied 4 times daily starting 3 days before the day of injection and continuing for 3 days after the injection.

• SAMPLE COLLECTING: Venous blood samples were collected from patients just before the first injection, and then 1 week, 1 month, and 2 months after the injection. For serum preparation, blood samples from 17 patients in the aflibercept group, 15 patients in the ranibizumab group, and 12 patients in the control group were obtained (allowed to clot at room temperature for 30 minutes) and then centrifuged as mentioned in a previous article. For plasma preparation, blood samples from 7 patients in the ranibizumab group, 7 patients in the aflibercept group, and 10 patients in the control group were collected in tubes with sodium citrate, theophylline, adenosine, dipyridamole (catalog no. 367599; Becton Dickinson, Rutherford, New Jersey, USA) and centrifuged for 10 minutes at 1700 g within 30 minutes after sampling. Harvested serum and plasma samples were frozen immediately and stored at −80°C until assay.

• MEASUREMENT OF VASCULAR ENDOTHELIAL GROWTH FACTOR CONCENTRATIONS: Serum and plasma VEGF concentrations were measured by enzyme-linked immunosorbent assay of human VEGF (SVE00; R&D Systems, Quantikine, Minneapolis, Minnesota, USA) according to the instructions given by the manufacturer and mentioned previously. The lower limit of VEGF detection was 9.0 pg/mL. All samples were analyzed together in duplicate.

• STATISTICAL ANALYSIS: SigmaStat statistical software version 3.1 (Systat Software, Inc, Richmond, California, USA) was used for data analysis. Baseline characteristic of the 3 groups were evaluated with a 1-way analysis of variance. The Friedman repeated-measures analysis of variance on ranks was used to compare baseline and follow-up data in each group. A P value of less than .05 indicated statistical significance. For analytic purposes, a VEGF concentration less than the lower limit of detection was considered to be 0.

RESULTS

THE BASELINE CHARACTERISTICS OF THE PARTICIPANTS are presented in the Table. There was no difference among the 3 groups. No eye or systemic adverse events were observed during the 2-month observation period.

At baseline, the mean ± standard deviation serum VEGF concentration was 28.3 ± 9.8 pg/mL (range, 12.1 to 40.2 pg/mL) in the control group, 28.1 ± 10.6 pg/mL (range, 14.0 to 50.2 pg/mL) in the aflibercept group, and 27.5 ± 13.7 pg/mL (range, 9.3 to 53.7 pg/mL) in the ranibizumab group. There were no significant differences among the 3 groups (P = .99; Figure 1).

In the aflibercept group, the mean serum VEGF concentration was significantly less than the detectable limit at 1 week after the injection in all the patients (P < .0001) and increased to 11.7 ± 12.6 pg/mL (range, 0 to 49.7 pg/mL) at 1 month after the injection. The VEGF level in 6 (35.3%) of 17 patients was still less than the lower limit of detection, meaning that it was still significantly less than the baseline level (P < .001) and returned to near-baseline levels (to 23.9 ± 18.7 pg/mL; range, 9.7 to 75.3 pg/mL) at 2 months after the injection (P = .35; Figure 2).

In the ranibizumab group, the mean serum VEGF concentration was 36.2 ± 20.8 pg/mL (range, 0 to 82.1 pg/mL)
at 1 week, 31.2 ± 19.4 pg/mL (range, 0 to 74.2 pg/mL) at 1 month, and 32.8 ± 31.6 pg/mL (range, 0 to 139.9 pg/mL) at 2 months (Figure 3). There was no significant difference among the 3 groups (P = .99).

At baseline, the plasma VEGF concentrations were 29.5 ± 44.3 pg/mL (range, 0 to 153.8 pg/mL) in the control group, 16.2 ± 9.4 pg/mL (range, 10.1 to 29.6 pg/mL) in the aflibercept group, and 19.7 ± 10.8 pg/mL (range, 0 to 30.8 pg/mL) in the ranibizumab group. No significant difference was found among the 3 groups (P = .63).

In the aflibercept group, plasma VEGF concentration was less than the limit of detection in all cases at 1 week (P < .01). It remained less than the limit of detection and significantly less than the baseline level: in 5 (71.4%) of 7 patients, the VEGF concentrations were still less than the lower limit of detection at 1 month (P < .05). Plasma VEGF concentration was at near-baseline levels (13.6 ± 5.1 pg/mL; range, 0 to 32.8 pg/mL) at 2 months (P = .73; Figure 5).

In the ranibizumab group, plasma VEGF concentrations were 18.6 ± 5.1 pg/mL (range, 11.4 to 24.9 pg/mL) at 1 week, 17.2 ± 15.2 pg/mL (range, 0 to 41.3 pg/mL) at 1 month, and 19.4 ± 11.9 pg/mL (range, 0 to 38.1 pg/mL) at 2 months. There was no statistically significant difference between baseline and after-treatment levels (P = .93; Figure 6).

FIGURE 2. Scatterplot showing serum vascular endothelial growth factor (VEGF) concentration before and after intravitreal aflibercept injection in the aflibercept group. One week after injection, serum VEGF level in all patients decreased to less than the detectable limit (****P < .0001), and although it began to increase 1 month after injection, it remained significantly less than the baseline level (****P < .001). At 2 months after injection, serum VEGF concentration returned to the baseline level (P = .35).

FIGURE 3. Scatterplot showing serum vascular endothelial growth factor (VEGF) concentration before and after intravitreal ranibizumab injection in the ranibizumab group. There was no significant difference among time points (P = .36).
DISCUSSION

THE VEGF CONCENTRATIONS IN BOTH SERUM AND PLASMA have been estimated in patients with various diseases. To our best knowledge, this is the first time that serum and plasma VEGF concentrations have been estimated in patients with AMD. Plasma in CTAD (buffered sodium citrate theophylline adenosine dipyridamole) tubes were used to measure circulating extracellular VEGF because ethylene diamine tetraacetic acid influences platelet shape by changing Ca\textsuperscript{2+} concentration, which may affect evaluation of VEGF.\textsuperscript{25}

In the current study, as in previous reports,\textsuperscript{26,27} both plasma and serum VEGF concentrations in the aflibercept, ranibizumab, and control groups were similar at baseline. Serum and plasma VEGF concentrations of all patients were less than the limit of detection at 1 week after intravitreal aflibercept injection. Although the VEGF level tended to increase subsequently, it remained at less than detection limits in 35.3% of serum samples and in 71.4% of plasma samples and remained significantly less than the baseline level at 1 month. Thus, aflibercept seems to escape into the systemic blood circulation. This hypothesis is reasonable for several reasons. First, Christoforidis and associates previously reported that after 1 intravitreal aflibercept injection into rabbit eyes,\textsuperscript{124} I-labelled aflibercept diffused into the bloodstream, became decoupled from the agent substrate, and was sequestered by the thyroid.\textsuperscript{28} Second, our 1-month results in the aflibercept group were similar to those of Zehetner and associates, who found that suppression of the plasma level of VEGF at 1 week after intravitreal injection of bevacizumab lasted up to 1 month in patients with AMD.\textsuperscript{23} Moreover, Miyake and associates reported much lower bevacizumab levels in serum than in injected eyes of macaques after intravitreal injection of bevacizumab.\textsuperscript{29} Both human and animal studies have indicated that intravitreally injected bevacizumab can enter the systemic circulation. In addition, bevacizumab (Avastin; Genentech, Inc, San Francisco, California, USA) is a 149-kDa, fully humanized immunoglobulin G monoclonal antibody against all isoforms of VEGF-A, and its Fc (which slows clearance and prolongs systemic half-life of the entire molecule) is the same as that attached to aflibercept.\textsuperscript{30,31} The half-lives of aflibercept and bevacizumab in the rabbit eye are similar (4.58 vs 4.3 days, respectively).\textsuperscript{29,32} Although there are no published reports of the half-life of intravitreal aflibercept injection in human eyes, some investigators have estimated a half-life for aflibercept of 7.13 days, which is comparable with that of bevacizumab (8.25 days).\textsuperscript{33} Therefore, based on the findings described above, it can be...

![FIGURE 4](image1.png)

FIGURE 4. Scatterplot showing plasma vascular endothelial growth factor (VEGF) concentration at baseline in the control group, aflibercept group, and ranibizumab group. The VEGF concentrations were not significantly different among the 3 groups ($P = .64$).

![FIGURE 5](image2.png)

FIGURE 5. Scatterplot showing plasma vascular endothelial growth factor (VEGF) concentration before and after intravitreal aflibercept injection in the aflibercept group. Plasma VEGF concentrations in all patients decreased to less than detectable limits at 1 week (**$P < .01$), remained less than the detectable limit and significantly lower than baseline level at 1 month (**$P < .05$), and returned to baseline level at 2 months ($P = .73$).

![FIGURE 6](image3.png)

FIGURE 6. Scatterplot showing plasma vascular endothelial growth factor (VEGF) concentration before and after intravitreal ranibizumab injection in the ranibizumab group. There was no significant difference in plasma VEGF concentration between baseline and after intravitreal ranibizumab injection ($P = .93$).
speculated that the movement of aflibercept and bevacizumab into the systemic circulation follows a similar pathway.

Further, our present results showed that serum and plasma VEGF concentrations in the aflibercept group returned to baseline levels 2 months after the injection, indicating that 2 months may be the time needed for systemic VEGF recovery to normal levels and for restoration of normal circumstances in vivo after intravitreal aflibercept injection. Hence, when compared with the standard strategy of 3 monthly doses after bimonthly injections (which could oversuppress the VEGF system during the loading phase), our regimen of bimonthly intravitreal injection of aflibercept may have less effect on the systemic VEGF level.

Our results are consistent with previous reports showing that the plasma level of VEGF does not change significantly after intravitreal ranibizumab injection in patients with AMD. In addition, we also found that serum VEGF concentrations followed the same trend. However, the trend toward increased serum VEGF concentration at 1 week may indicate that ranibizumab could reach the peripheral circulation and is consistent with previous findings in animals.35,36 Ranibizumab, an antibody Fab fragment, is cleared rapidly from the systemic circulation and reaches maximum serum concentration approximately 0.5 days after intravitreal ranibizumab injection. Ranibizumab concentrations in humans are predicted to be approximately 90,000-fold lower in serum than in vitreous.37 Therefore, using ranibizumab may have a smaller influence on systemic VEGF level.

Both serum and plasma samples are used frequently in clinical studies. However, which one is more representative of the peripheral VEGF level remains controversial. Although in the present study, VEGF concentration was higher in serum than in plasma, the trend of change in serum and plasma VEGF levels was similar in both the aflibercept and ranibizumab groups. Serum VEGF level usually is higher than plasma VEGF level because VEGF inside the platelets comes out after clotting. Therefore, both serum and plasma are important for measurement of VEGF level in peripheral blood.

In our study, the change in serum and plasma VEGF concentrations after the injection differed between the aflibercept and ranibizumab groups, but in the VIEW 1 and VIEW 2 studies, the incidence of arterial thromboembolic events as defined by the Antiplatelet Trialists’ Collaboration criteria was similar among 4 groups (0.5-mg intravitreal ranibizumab injection every 4 weeks, 2-mg intravitreal aflibercept injection every 4 weeks, 0.5-mg intravitreal aflibercept injection every 4 weeks, and 2-mg intravitreal aflibercept injection every 8 weeks after 3 monthly injections). However, most patients with AMD who need anti-VEGF injections are older people, and they may have many other medical diseases such as diabetes and hypertension that usually need long-term and frequent treatment. Patients with both AMD and diabetes have a high risk of thromboembolic events, in which VEGF play a part. Despite the limitations of the present prospective study (short-term follow-up and small population sample size), our data showed that intravitreal injection of aflibercept or ranibizumab can affect systemic VEGF level. In future treatment using intravitreal anti-VEGF agents, consideration should be given to the potential for adverse events resulting from the suppressed or enhanced expression of systemic VEGF.

In conclusion, aflibercept significantly decreased serum and plasma VEGF concentrations 1 week and even 1 month after injection. By contrast, ranibizumab had no significant effect on either serum or plasma VEGF level. Clinicians should be aware that changes in systemic VEGF levels occur after intravitreal injection of aflibercept.

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**REFERENCES**


Xiying Wang is currently an 3rd-year MD student of the Department of Ophthalmology, Harbin Medical University, Heilongjiang Province, China. During her MD period, she was doing research work on age-related macular degeneration in the Department of Ophthalmology in Shiga University of Medical Science, Shiga, Japan.