Supernormal Flicker ERGs in Eyes With Central Retinal Vein Occlusion: Clinical Characteristics, Prognosis, and Effects of Anti-VEGF Agent

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Purpose. To determine the clinical characteristics, prognosis, and effect of anti-vascular endothelial growth factor (VEGF) agents on eyes with a central retinal vein occlusion (CRVO) with and without supernormal flicker ERG amplitudes.

Methods. Forty-eight eyes of 48 patients with a CRVO were studied. Flicker ERGs were recorded from fully dilated eyes with the RET*eval* system. The amplitudes and implicit times of the fundamental component were analyzed. "Supernormal flicker ERGs" were defined as those whose amplitudes were ≥117% of the unaffected fellow eyes.

Results. Ten of the 48 eyes (20.8%) with a CRVO showed supernormal flicker ERGs before the treatment. The difference in the implicit times of these 10 CRVO eyes and those of normal fellow eyes was <4 millisecond. There was a significant correlation between the implicit time delay and the relative amplitude in the 48 CRVO eyes. All 10 CRVO eyes with supernormal flicker ERGs had the nonischemic type of CRVO and tended to have better visual acuities than did the 28 nonischemic CRVO eyes without supernormal flicker ERGs at 12 months after the treatment ($P = 0.058$). The CRVO eyes with supernormal flicker ERGs had a significant amplitude reduction after a single injection of an anti-VEGF agent.

Conclusions. These results indicated that the supernormal flicker ERGs can be a sign of a mild degree of ischemia, and these eyes have a better prognosis. The results also suggest that the supernormal flicker ERG may be caused by changes in the electrical activities of retinal cells following a mild increase in the VEGF levels in eyes with CRVO.

Keywords: electroretinogram (ERG), flicker ERG, RETeval, central retinal vein occlusion (CRVO), supernormal, VEGF, amplitude, implicit time

A central retinal vein occlusion (CRVO) is a common retinal vascular disorder. Although the exact etiology of a CRVO has not been determined, it is believed that a reduction of venous outflow caused by a narrowing of the vein or partial thrombosis induces an ischemic and hypoxic state in the retina. This leads to different pathological alterations, including macular edema and neovascularization of the anterior segment and retina.[1–3]

It is important to evaluate the ischemic state of retinas with a CRVO, not only for the prognosis but also for the treatment strategy. Fluorescein angiography (FA) has been traditionally used to classify eyes with a CRVO into ischemic and nonischemic types based on the extent of the capillary nonperfused areas.[4,5] On the other hand, other clinical tests—including the best-corrected visual acuity (BCVA), visual field, relative afferent pupillary defect, and ophthalmoscopic appearance of the fundus—have been used to assess the ischemic status of CRVO eyes.[6]

It is known that the implicit time of the 30-Hz flicker ERGs is a useful way to evaluate the degree of retinal ischemia in eyes with CRVO.[7–12] The results of earlier studies have shown that a prolongation of the implicit times of the 30-Hz flicker ERG was correlated with the degree of retinal ischemia in eyes with CRVO, and this prolongation has been a useful value in predicting the development of neovascularization of the anterior chamber angle and iris.[7–12]

We have routinely recorded flicker ERGs from all CRVO patients to evaluate the degree of retinal ischemia. The results showed that the amplitudes of the flicker ERGs in some eyes with a CRVO were often significantly larger than that of the normal fellow eyes. The amplitudes of the flicker ERGs in these eyes were stated to be supernormal.

The purpose of this study was to determine the clinical characteristics and prognosis of eyes with a CRVO that had supernormal flicker ERGs. We also studied the effect of anti-vascular endothelial growth factor (VEGF) agents on the supernormal flicker ERG amplitudes.

Methods

Study Design and Approvals

This was a retrospective study of the medical records of eyes with a CRVO examined at the Mie University Hospital and Nagoya University Hospital. Pprocedures used were approved by the Medical Ethics Committee of Mie University Hospital (No. 3243) and Nagoya University Hospital (No. 2018-0079).

The procedures used conformed to the tenets of the Declaration of Helsinki of the World Medical Association. A written informed consent was not obtained from the subjects because of the retrospective nature of this study. Instead, a home page was created with information on the purpose of this study for the subjects to read. We emphasized that there was a statement in the text that any subjects could opt out of the study at any time by telephone, fax, or e-mail. The study was also registered on the International Clinical Trial Registry Platform (UMIN Clinical Trials Registry, R000037330, http://www.umin.ac.jp/ctr/index-j.htm).

Subjects

We reviewed the medical records of patients with a CRVO who had been examined at the Mie University Hospital or Nagoya University Hospital from January 2014 to December 2017. All the patients were referred with visual symptoms caused by the CRVO and were scheduled to receive an intravitreal injection of ranibizumab or aflibercept. These CRVO patients were all $\geq$20 years, and the interval between the symptom onset to the initial visit to the hospital was $\leq$12 months. Patients who had diabetic retinopathy or other retinal diseases were excluded. Because we wanted to compare the flicker ERG parameters between CRVO eyes and normal fellow eyes, patients who had any retinal diseases in fellow eyes were also excluded. Subjects who had received any previous treatments—including vitrectomy, laser treatments, or drug injections in either eye—were also excluded. The short-term results of 15 of these eyes before and after a ranibizumab injection have been reported elsewhere.[13]

Clinical Examinations

All patients had undergone a complete eye examination that included measurements of the best-corrected visual acuity (BCVA) with a standard Japanese visual acuity chart at 5 m, slit-lamp biomicroscopy, and color fundus photography. Spectral-domain optical coherence tomography (SD-OCT) was performed with either the Spectralis OCT (HRA+OCT; Heidelberg Engineering, Inc., Franklin, MA, USA) or the Cirrus HD-OCT (version 5.1; Carl Zeiss Meditec, Jena, Germany). Fluorescein angiography was performed at the initial visit and when needed using either a digital fundus camera (TRC-50Dx; Topcon Corp., Tokyo, Japan) or the Optos ultra-widefield imaging system (Optos Panoramic 200MA; Optos PLC, Dunfermline, Scotland).

All CRVO eyes were classified as the ischemic type or nonischemic type based on the findings of the FA performed at the initial visit. The classical definition of the CVO Study was used: the CRVO was classified as the ischemic type if the eye had at least a 10-disc area of retinal capillary nonperfusion within the area of a standard photographic field.[5]

Electroretinography

Full-field flicker ERGs were routinely recorded at every visit to the Mie University Hospital or the Nagoya University Hospital to monitor the ischemic status of the retina in all CRVO patients. Full-field flicker ERGs were also recorded with the RET*eval* system (LKC Technologies, Gaithersburg, MD, USA). We have reported that the RET*eval* flicker ERGs were significantly affected by the pupillary area even after the compensation for the pupillary area.[14,15] Therefore, we recorded the full-field flicker ERGs with the ISCEV standard pupil dilation mode of the RET*eval* system from all of the CRVO eyes.

The components of the RET*eval* system have been described in detail.[13–15] Briefly, full-field stimuli were presented with a 60-mm diameter dome, and the white stimuli were created by a combination of three colored light-emitting diodes. A small red fixation spot was present at the center of the dome. We used a flash stimulus of 3.0 cd-s/m^2 with a duration of $<$1 millisecond. The frequency of the flicker stimulus was 28.306 Hz. A constant background illumination of 30 cd/m^2 was used during the recordings.

After full mydriasis with topical 0.5% tropicamide and 0.5% phenylephrine HCl, (Mydrin-P; Santen Pharmaceutical Co., Ltd., Osaka, Japan) and 10 minutes of light-adaptation to 30 cd/m^2 with the RET*eval* background illumination, the flicker ERGs were recorded. The ERGs were recorded from the CRVO eyes at all visits. Flicker ERGs were recorded from the normal fellow eyes only at the initial visit. A special skin electrode array (Sensor Strip; LKC Technologies, Inc., Gaithersburg, MD, USA) was placed 2 mm from the margin of the lower eyelid. This electrode array contained an active, a reference, and a ground electrode in a single adhesive tape. The electrical potentials were DC-amplified and digitized with a sampling rate of 2 kHz. The data resolution was 24 bits for $\pm$0.6 V, which is equal to approximately 0.07 μV.

The amplitudes and implicit times of the fundamental component were automatically measured and displayed by the RET*eval* system using a special algorithm with discrete Fourier transformation (DFT) and cross-correlation analysis.[16] We also measured the conventional peak implicit times and peak-to-peak amplitudes of the "raw" flicker ERGs, such as, the reconstructed flicker ERG waveforms using the first eight harmonic components.

Statistical Analyses

After confirming that the data were approximately normally distributed by the calculation of the skewness and kurtosis, paired *t*-tests were used to determine if the amplitudes or implicit times of flicker ERG were significantly different between the affected eyes and normal fellow eyes. The Pearson product-moment correlation coefficient was used to determine whether there was a significant correlation between the relative amplitudes and implicit time delays of the flicker ERGs. To determine whether clinical factors were significantly different among the three groups, a 1-way layout analysis of variance (ANOVA) with Tukey-type multiple comparison was used for quantitative variables, and a chi-square test with Bonferroni-type multiple comparison was used for qualitative variables. Paired *t*-tests were also used to determine if the amplitudes or implicit times of the flicker ERGs were significantly different before and after the intravitreal injection of anti-VEGF agent. The results were considered statistically significant when $P < 0.05$.

Results

Clinical Characteristics of Patients

The clinical characteristics of the 48 eyes of 48 patients (33 men and 15 women) with a CRVO are summarized in Table 1. The mean age of the patients was 68.4 years (range, 29–88 years). Thirty-one patients (64.6%) had systemic hypertension, and 10 patients (20.8%) had diabetes mellitus without diabetic retinopathy. The mean interval between the onset of the symptoms to the initial visit to the hospital was 5.4 weeks (range, 0–32 weeks). Based on the pretreatment FA findings, 10 eyes (20.8%) were the ischemic type, and the other 38 eyes (79.2%) were the nonischemic type.

Representative Flicker ERG Findings

The flicker ERGs recorded from a representative nonischemic type, and an ischemic type of CRVO eyes are shown in Figure

Table 1. Clinical Characteristics of 48 Eyes of 48 Patients With CRVO at the initial Visit to the Hospital

Parameter	Value
Number of eyes/subjects	48/48
Age, mean ± SD (range), years	68.4 ± 12.3 (29–88)
Sex	
Men	33
Women	15
Hypertension (%)	31 (64.6)
Diabetes mellitus (%)	10 (20.8)
Period from symptom onset to initial visit to hospital, mean ± SD (range), weeks	5.4 ± 5.5 (0–32)
Best-corrected visual acuity, mean ± SD (range), logMAR units	0.69 ± 0.52 (0–2.0)
Central macular thickness, mean ± SD (range), μm	683 ± 262 (313–1900)
Ischemic-type/nonischemic-type	10/38
Amplitude of fundamental component of flicker ERG in the CRVO eye, mean ± SD (range), μV	18.5 ± 8.8 (2.6–36.8)
Implicit time of fundamental component of flicker ERG in the CRVO eye, mean ± SD (range), milliseconds	31.7 ± 2.9 (26.0–38.5)

logMAR, logarithm of the minimum angle of resolution.

1. Patient #33 was 73-year-old man who noticed a sudden blurring of the vision of his right eye 4 weeks before the initial visit to the hospital. At the initial examination, his decimal BCVA was 0.3 in the affected eye. Based on the fundus photographs and FA (Figs. 1A, 1B), he was diagnosed with nonischemic CRVO associated with macular edema (Fig. 1C). The implicit time of the flicker ERG was longer than that of the normal left eye by 3.2 milliseconds (Fig. 1D). The amplitude of the ERGs in his CRVO eye was 147% larger than that of the normal fellow eye.

Patient #42 was 80-year-old woman who reported that she noticed a reduction in the vision of her left eye 8 weeks before her initial visit to the hospital. At her initial examination, her decimal BCVA of the left eye was 0.01. Based on the findings of fundus photographs and FA (Figs. 1E, 1F), she was diagnosed with the ischemic type of CRVO associated with severe macular edema (Fig. 1G). The implicit time of the flicker ERGs was markedly delayed by 9.1 milliseconds, and the amplitude was significantly smaller in the affected right eye than in the normal right eye (Fig. 1H). The amplitude of the CRVO eye was only 24% of that in the normal fellow eye.

Amplitudes and Implicit Times of Flicker ERGs in 48 CRVO Eyes

Initially, we compared the amplitudes and implicit times of the fundamental component of the flicker ERGs recorded from the eyes with a CRVO to those recorded from normal fellow eyes of the 48 patients (Fig. 2). The results of the implicit times were

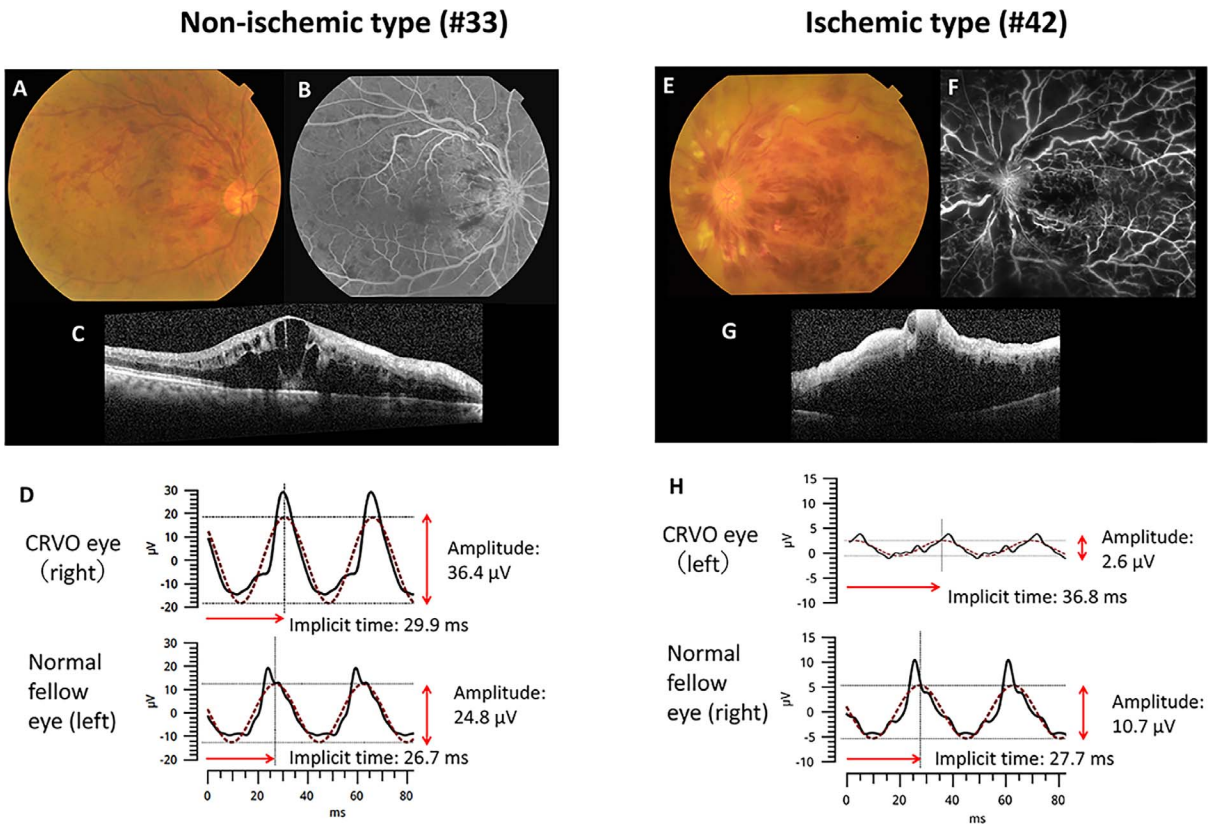

Figure 1. Fundus photographs (**A**, **E**), fluorescein angiograms (**B**, **F**), optical coherence tomographic images (OCTs; **C**, **G**), and full-field flicker ERGs (**D**, **H**) recorded from two representative cases of CRVO. *Left panel* shows the findings in a case of nonischemic CRVO. The fundamental component (*dotted red line*) is superimposed on the reconstructed flicker ERG waveforms using the first eight harmonics (*solid black line*). The implicit times of the flicker ERGs of the affected right eye was slightly delayed by 3.2 milliseconds, and the amplitude was significantly larger than that of normal left eye. The *right panel* shows the findings of a case with ischemic CRVO, and the implicit time of the flicker ERGs of the affected left eye is delayed by 9.1 milliseconds, and the amplitude was smaller than that of the normal right eye.

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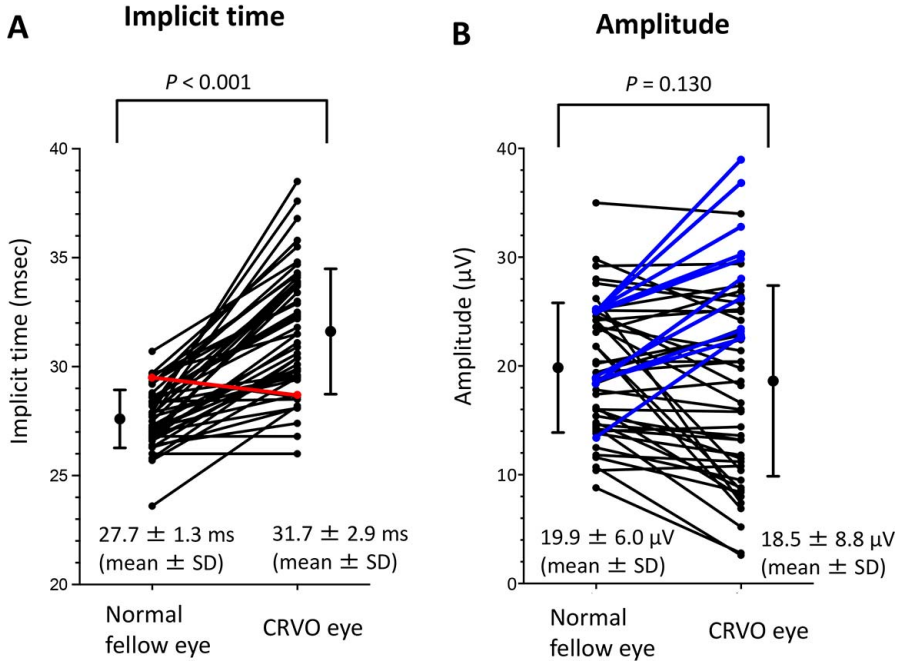

Figure 2. Comparisons of the implicit times and amplitudes of the fundamental component of flicker ERGs between the CRVO eyes and normal fellow eyes in 48 patients with a CRVO. (**A**) Plot of the implicit times of the fundamental component. Almost all CRVO eyes, except one eye (*red line*), had longer implicit times than that of the normal fellow eyes. The mean implicit times of the CRVO eyes were significantly longer than that of normal fellow eyes ($P < 0.001$). (**B**) Plot of the amplitudes of the fundamental component. There was no significant difference in the amplitude of fundamental component between the CRVO eyes and normal fellow eyes ($P = 0.130$). When the supernormal flicker ERGs were defined as those whose amplitudes were ≥117% than that of the unaffected eyes, 10 of the 48 CRVO eyes (20.8%; *blue lines*) met this criterion.

quite similar for all CRVO eyes: The implicit times of almost all CRVO eyes were delayed compared to the normal fellow eyes (Fig. 2A). Only one CRVO eye had a slightly shorter implicit time than the normal fellow eye (Fig. 2A, red line), but this difference was only 0.9 milliseconds. The average implicit times in the CRVO eyes was significant longer than that of the fellow eyes (paired *t*-tests; $P < 0.001$, Fig. 2A).

In contrast, the results for the amplitudes were more mixed: the amplitudes of CRVO eyes were smaller than those of normal fellow eyes in 28 eyes, equal to normal fellow eye in 1 eye, and larger than that of the normal fellow eyes in 19 eyes (Fig. 2B). On the average, there was no significant difference in the amplitudes between the affected and the normal fellow eyes ($P = 0.130$, paired *t*-test; Fig. 2B).

In our study, "supernormal flicker ERGs" were defined as those whose amplitudes were ≥117% of that of the unaffected normal fellow eye. This definition was based on our data that the normal range (95% reference interval) of the intraocular difference of the flicker ERG amplitudes of the fundamental component of dilated normal eyes recorded with the RET*eval* system was less than 17% ($n = 38$; age, 22–83 years). Based on this criterion, we found that 10 of the 48 CRVO eyes (20.8%) had supernormal flicker ERG amplitudes in our CRVO cohort (Fig. 2B, blue lines).

Plot of Relative Amplitudes versus Implicit Time Delays in CRVO Eyes

To determine the relationships between the implicit times and amplitudes of the CRVO eyes, the relative amplitudes—meaning the amplitude of CRVO eye/amplitude of normal fellow eye ×100—were plotted against the implicit time delays for the CRVO eyes to the normal fellow eyes (Fig. 3). The ten blue dots surrounded by a blue square show the eyes whose amplitudes were larger than 117% of those in the normal fellow eye, that is, supernormal flicker ERGs.

There was a weak but significant correlation between the implicit time delay and the relative amplitude in the 48 CRVO eyes ($r = 0.358$, $P < 0.001$). All eyes with supernormal flicker ERG amplitudes had implicit time delays of <4 milliseconds. We also noted that there was no ischemic type CRVO (Fig. 4, red dots) in the supernormal flicker ERG amplitude group.

Clinical Characteristics of CRVO Patients with Supernormal Flicker ERGs

To determine the clinical characteristics of eyes with supernormal flicker ERG amplitudes, we separated the 48 CRVO eyes into three groups: nonischemic CRVO with supernormal flicker ERGs (Group A, $n = 10$), nonischemic CRVO without supernormal flicker ERG (Group B, $n = 28$), and ischemic CRVO (Group C, $n = 10$). Then, we compared the different clinical factors among the three groups (Table 2).

The results showed that the nonischemic CRVO groups (Groups A and B) had significantly better BCVA both before the treatment and 12 months after the treatment. Both groups had thinner central macular thickness before the treatment and had significantly fewer panretinal laser photocoagulation treatments during the 12 months than did the ischemic CRVO group (Group C). These results are not too unexpected because it is known that the prognosis of nonischemic CRVO is better than that of ischemic CRVO.

The differences in the clinical characteristics between the nonischemic CRVO with supernormal flicker ERGs (Group A) and without supernormal flicker ERGs (Group B) were not significant. However, we noted that the BCVA at 12 months after treatment was better in Group A than in Group B ($P = 0.058$; multiple comparison). This suggested the possibility that the prognosis of the nonischemic CRVO with supernormal

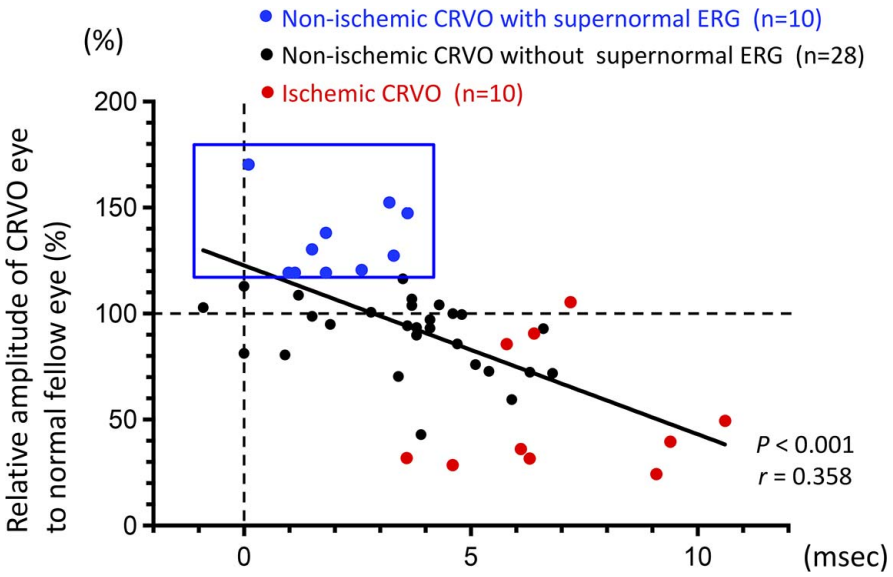

FIGURE 3. Plot of the relative amplitudes (amplitude of CRVO eye/amplitude of normal fellow eye ×100) against the implicit time delay of CRVO eyes to normal fellow eyes in milliseconds. Ten *blue dots* surrounded by a *blue square* show the eyes whose amplitudes were 117% or larger than those of the normal fellow eye (i.e., supernormal flicker ERG amplitude). There was a weak but significant correlation between the implicit time delay and relative amplitudes in the 48 CRVO eyes ($P < 0.001$, $r = 0.358$). *Red dots* show the eyes with ischemic type CRVO.

ERGs is better than that of the nonischemic CRVO without supernormal ERGs.

Changes of Flicker ERGs After Anti-VEGF Therapy

Finally, to study how the anti-VEGF treatments influenced the supernormal flicker ERG amplitudes in eyes with CRVO, we examined the changes of the flicker ERG amplitudes before and 1 month after the initial anti-VEGF drug injections for the three groups (Fig. 4A). In Group A, 9 of 10 eyes (90%) had a reduction of the flicker ERG amplitudes after a single injection of anti-VEGF drug (Fig. 4A, left column). The mean (±SD) amplitude of the flicker ERG was 27.6 ± 4.9 μV before the treatment, and it decreased to 21.6 ± 3.4 μV after a single injection of the anti-VEGF agent in Group A (Fig. 4A, left column). This amplitude decrease was statistically significant ($P = 0.002$). In contrast, the mean amplitude of Groups B and C did not change significantly after a single injection of anti-VEGF agent. (Fig. 4A, middle and right columns).

We also measured the changes in the mean implicit time of the flicker ERGs before and after intravitreal injection of anti-VEGF agent for the three groups. The mean implicit time did not change significantly before and after the anti-VEGF treatments for all three groups (Fig. 4B).

DISCUSSION

The results showed that 20.8% of the eyes with a CRVO had supernormal flicker ERG amplitudes. All of the CRVO eyes with supernormal flicker ERG amplitudes were the nonischemic type based on the FA findings, and the degree of implicit time delay of the flicker ERGs was slight (<4 milliseconds; Fig. 3). We also found that the visual acuity at 12 months after the treatment tended to be better in the nonischemic CRVO eyes with supernormal ERG than in the nonischemic CRVO eyes without supernormal ERG ($P = 0.058$; Table 2). These results suggest that the supernormal flicker ERG amplitudes can be a sign of relatively mild ischemia, and they have a better prognosis.

There are several reports of the presence of supernormal ERG amplitudes in eyes with retinal vascular disorders including eyes with diabetic retinopathy[17,18] and CRVO.[19–22] In 1992, Gouras and Mackay[21] reported that 4 of 12 eyes with a CRVO had supernormal single-flash cone ERGs when conventional white flash stimuli were used. This incidence was similar to the 20.8% results in our study. They also showed that the supernormal cone ERG amplitudes were seen more frequently when red flashes were used, suggesting that the long wavelength-sensitive cones (L-cones) contributed to this phenomenon.

Roy et al.[23] also studied the prognosis of CRVO eyes with supernormal cone ERG amplitudes elicited by red flashes. They reported that only 1 of the 15 patients (7%) who had supernormal cone ERGs developed ocular neovascularization, whereas all 6 patients with subnormal cone ERGs developed ocular neovascularization. Their findings are similar to our results; the prognosis of CRVO eyes with supernormal cone-mediated ERG amplitude is reliable.

It is difficult to speculate which retinal cells contribute to the supernormal ERG amplitude in CRVO eyes from this study because we evaluated only the results of flicker ERGs. In 1996, Matsui et al.[22] demonstrated that the amplitudes of both the a- and b-waves to bright-flash stimuli after dark-adaptation were supernormal in some patients with CRVO, suggesting that at least the electrical activities of the photoreceptors themselves might be enhanced in these retinas. Gouras and Mackay[21] reported that not only the b-waves but also the a-waves of single-flash cone ERGs were supernormal in some CRVO eyes. These findings suggest that the activities of the photoreceptors may be enhanced under mild ischemic conditions in CRVO eyes.

The most interesting finding in this study was the fact that the supernormal flicker ERG amplitudes were markedly reduced after anti-VEGF treatment. The mean amplitude of the flicker ERGs was significantly decreased from 27.6 μV to 21.1 μV just 1 month after a single intravitreal injection of anti-VEGF agent in the nonischemic CRVO eyes with supernormal flicker ERG group (Fig. 4A). The mean amplitude 1 month after the injection in Group A (21.1 μV) was close to that of normal

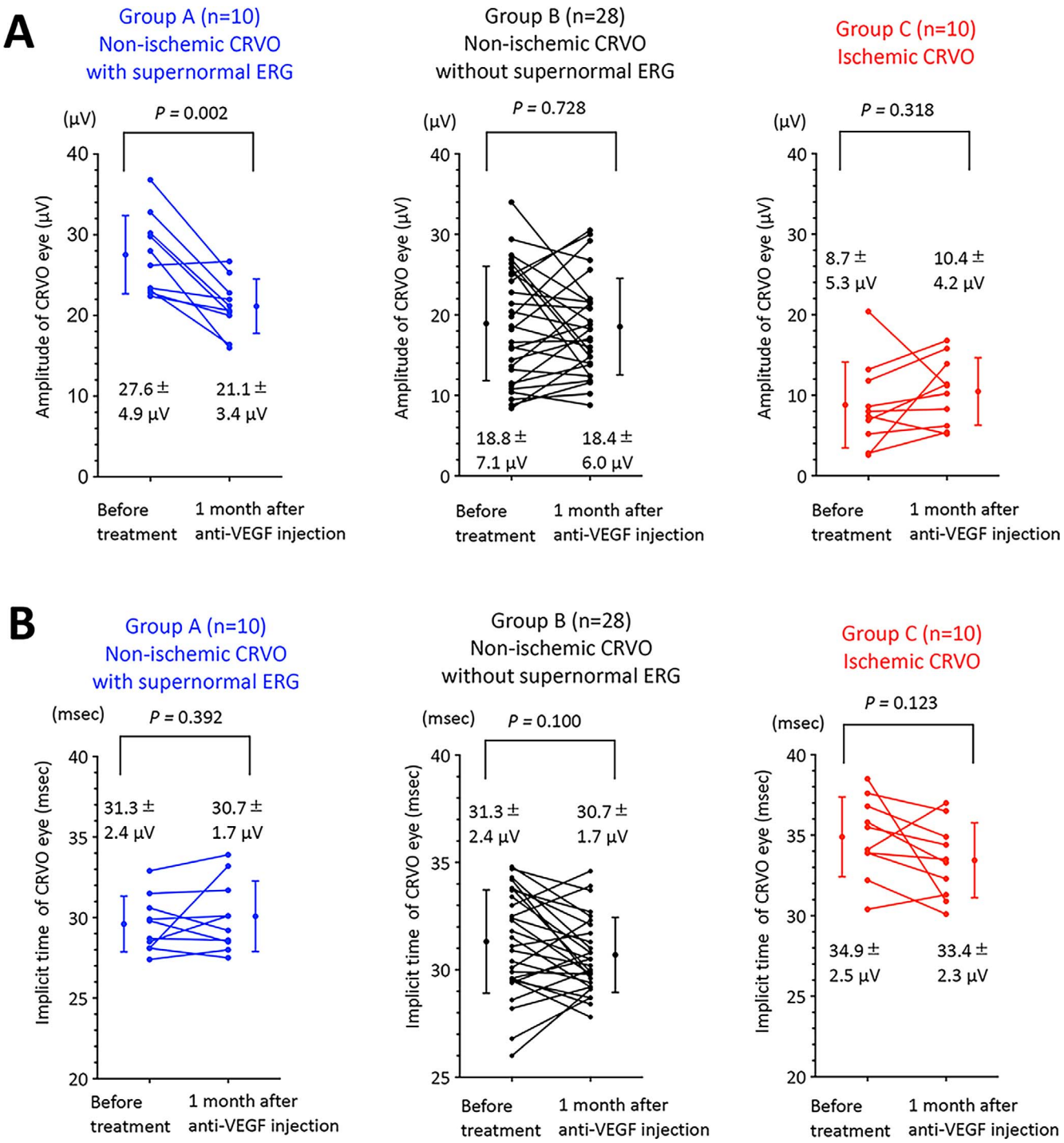

Figure 4. (**A**) Plot of the amplitudes of CRVO eyes before and 1 month after the intravitreal injection of anti-VEGF agent for three groups: nonischemic CRVO eyes with supernormal flicker ERG amplitude (Group A, $n = 10$), nonischemic CRVO eyes without supernormal flicker ERG amplitude (Group B, $n = 28$), and ischemic CRVO eyes (Group C, $n = 10$). There is a significant reduction in the amplitudes in Group A after the intravitreal injection of anti-VEGF drugs ($P = 0.002$), but there are no significant changes in Groups B and C. (**B**) Plots of the implicit time of the CRVO eyes before and 1 month after the intravitreal injection of anti-VEGF drugs for groups A, B, C. There are no significant changes in the implicit times before and after the intravitreal injection of anti-VEGF drugs for all three groups.

fellow eyes recorded at the baseline (19.9 μV; Fig. 2B). These results suggest the possibility that the supernormal flicker ERG in CRVO may be caused by the changes in the electrical activities of retinal cells through an increase in the level of VEGF in the retina. It is known that the VEGF receptors are not expressed in the retinal neurons; therefore, supernormal ERG amplitude in the retina of CRVO may be caused by a factor secondary to increased VEGF. In this regard, the results of a recent animal study are of interest. Clermont et al. reported that the amplitudes of the scotopic ERGs were increased at 48 hours after the intravitreal injection of VEGF in mice (Clermont, et al. *IOVS* 2018;59:ARVO E-Abstract 3463).

Table 2. Comparison of Various Clinical Factors Between Nonischemic CRVO Eyes With Supernormal Flicker ERG (Group A, $n = 10$), Nonischemic CRVO Eyes Without Supernormal Flicker ERG (Group B, $n = 28$), and Ischemic CRVO Eyes (Group C, $n = 10$)

	Nonischemic CRVO With Supernormal ERG (Group A)	Nonischemic CRVO Without Supernormal ERG (Group B)	Ischemic CRVO (Group C)	*P* Value	Multiple Comparison, *P* Value		
					(A) to (B)	(A) to (C)	(B) to (C)
Age (years)	68.4 ± 10.6	66.4 ± 13.5	74.1 ± 9.4	0.237	0.893	0.553	0.208
Sex				0.652	1.000	1.000	1.000
Male	7	18	8				
Female	3	10	2				
Hypertension (%)	7/3	17/11	7/3	0.802	1.000	1.000	1.000
Diabetes mellitus (%)	0/10	8/20	2/8	0.161	0.169	0.812	1.000
Period from symptom onset to initial visit to hospital, mean ± SD, weeks	3.8 ± 3.5	5.0 ± 4.4	8.2 ± 8.7	0.166	0.829	0.175	0.246
Visual acuity before treatment, mean ± SD, logMAR	0.32 ± 0.20	0.58 ± 0.32	1.35 ± 0.61	<0.001*	0.151	<0.001*	<0.001*
Visual acuity at 12 months, mean ± SD, logMAR	0.05 ± 0.15	0.42 ± 0.49	1.27 ± 0.41	<0.001*	0.058	<0.001*	<0.001*
Improvement of visual acuity during 12 months, mean ± SD, logMAR	0.27 ± 0.19	0.16 ± 0.50	0.08 ± 0.45	0.634	0.799	0.608	0.861
Central macular thickness before treatment, mean ± SD, μV	544 ± 123	650 ± 191	915 ± 386	0.002	0.444	0.003*	0.010*
Central macular thickness at 12 months, mean ± SD, μV	295 ± 32	372 ± 183	409 ± 297	0.413*	0.544	0.403	0.863
Panretinal laser photocoagulation during 12 months (+/-)	0/10	8/20	8/2	<0.001*	0.230	<0.001*	0.009*
Iris/angle neovascularization during 12 months (+/-)	0/10	0/28	1/9	0.143	1.000	0.352	0.172
Number of anti-VEGF drug injection during 12 months, mean ± SD	3.5 ± 1.4	3.9 ± 2.1	4.0 ± 1.6	0.828	0.868	0.828	0.978

A 1-way layout analysis of variance (ANOVA) with Tukey-type multiple comparison was used for quantitative variables, and a chi-square tests with Bonferroni-type multiple comparison was used for qualitative variables to determine whether the clinical factors were significantly different among the three groups.

* $P < 0.05$.

Clermont and colleagues suggested that VEGF may cause the extravasation and activation of the kallikrein-kinin system, resulting in an increase of ERG amplitudes.

Another hypothesis is that nitric oxide (NO) produced by endothelial cells in response to VEGF[24–26] might enhance the electrical activities of retinal neurons. It has been reported that the NO increased the ERG a- and b-waves, oscillatory potentials within a limited concentration range.[27,28] If this suggestion is correct, a question arises as to why the amplitude of the flicker ERG decreased in eyes with severely ischemic CRVO (Fig. 3, red dots), despite the higher levels of VEGF expression in these retinas. We suggest that the flicker ERG amplitudes were reduced in these eyes because the retinal function were badly damaged due to the severe retinal ischemia.

There are two limitations in this study. The first limitation is that we mainly measured the fundamental component of the flicker ERGs while more generally using measurements of the peak implicit times or peak-to-peak amplitudes in clinical situations. To overcome this limitation, we also measured the conventional peak-to-peak amplitudes and peak implicit times of the "raw" flicker ERGs, and we confirmed that the results by measuring conventional peak-to-peak amplitudes and peak implicit times were approximately the same to those of the fundamental component (Supplementary Fig. S1).

The second limitation is that we focused only on flicker ERGs. If we could record other ERG components—including rod responses, maximal combined rod-cone responses, and single-flash cone responses—then we could analyze the functional changes of different retinal cells in more detail.

In conclusion, we found that about one-fifth of CRVO eyes have supernormal flicker ERGs. All CRVO eyes with these supernormal flicker ERGs were the nonischemic type associated with better prognosis. Our results also suggest that the supernormal flicker ERG amplitude may be caused by elevated intraretinal VEGF levels because the supernormal flicker ERG amplitude was markedly reduced after a single injection of anti-VEGF drug. Further clinical and experimental studies are needed to clarify the exact mechanism for this unique electrophysiological phenomenon in retinal vascular disorders.

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