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Correlation of fundus autofluorescence with photoreceptor morphology and functional changes in eyes with retinitis pigmentosa

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ABSTRACT.

Purpose: **To assess and correlate fundus autofluorescence (FAF) characteristics with photoreceptor morphology and functional features in eyes with retinitis pigmentosa (RP).**

Methods: **Thirty-four eyes of 17 patients with RP were examined. We compared FAF images obtained by confocal scanning laser ophthalmoscopy with Spectral-domain optical coherence tomography (SD-OCT) and retinal function assessed by microperimetry.**

Results: **Normal FAF surrounded by a ring of increased FAF at the macular area was detected in 32 (94%) eyes. The diameter of the normal FAF was correlated significantly with the preserved area of the photoreceptor inner segment and outer segment (IS/OS) junction on SD-OCT ($R = 0.939$, $p < 0.001$). The area outside the ring was associated with loss of IS/OS junction and external limiting membrane (ELM). The ring of increased FAF demarcated the border between the central retina with preservation of the IS/OS junction and ELM, and the adjacent eccentric retina with loss of these bands. In two eyes of one patient, there was no preservation of normal FAF at the macula and the photoreceptor IS/OS junction was not detected on SD-OCT. The mean retinal sensitivity derived from microperimetry was correlated significantly with the area of normal FAF ($R = 0.929$, $p = 0.007$) and the preserved area of the IS/OS junction ($R = 0.851$, $p = 0.032$). Ten eyes had progressive reduction in size of the normal FAF inside the ring accompanied by decreased area of preserved IS/OS during 3.1 years.**

Conclusion: **FAF appears to reflect the integrity of the photoreceptor layer. It may serve as a secondary outcome measure for novel therapeutic strategies for RP.**

Key words: fundus autofluorescence – photoreceptor inner segment and outer segment – retinitis pigmentosa – Spectral-domain optical coherence tomography

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Introduction

Retinitis pigmentosa (RP) is a set of inherited disorders characterized by nyctalopia and progressive peripheral visual loss leading to central visual loss. Mutations in a number of photoreceptor-specific genes are associated with RP (Dryja et al. 1990; Brown et al. 2006), and those mutations cause primary degeneration of rod photoreceptors followed by degeneration of cone photoreceptors. Photoreceptor survival seems to correlate closely with visual function (Brown et al. 2006). Therefore, assessment of photoreceptor status may be the most important clinical aspect of evaluating disease progression.

Identification and characterization of photoreceptor degeneration have been well studied in animal models and post-mortem human eyes (Flannery et al. 1989; Portera-Cailliau et al. 1994; Milam et al. 1996; Li et al. 1998; Reme et al. 1998); however, those changes have not been well investigated *in vivo* because conventional examination such as slit-lamp biomicroscopy and binocular indirect ophthalmoscopy cannot identify those specific changes in detail.

Fundus autofluorecence (FAF) imaging is a noninvasive method of retinal imaging (Delori et al. 1995; von Ruckmann et al. 1995). Originally, the

FAF signal was thought to originate predominantly from lipofuscin in the retinal pigment epithelium (RPE) (Delori et al. 1995). Increased FAF indicates abnormally high metabolic activity, impaired function to degrade waste material from the metabolic activities in the RPE, or the absence of retinal tissue or luteal pigment attenuating the excitation light (Holz et al. 2001; Bessho et al. 2009). While decreased or absent FAF may indicate RPE atrophy, loss of photoreceptors, or the presence of materials that attenuate the signal (Holz et al. 2001; Wakabayashi et al. 2008; Bessho et al. 2009). However, recent studies have demonstrated that the FAF signals reflect not only lipofuscin distribution in the RPE but also other fluorophores in the photoreceptor layer (Sawa et al. 2008; Schmitz-Valckenberg et al. 2008). Because most inherited retinal diseases, including RP, affect mainly the photoreceptors, FAF imaging may provide additional insight into such disease condition.

Spectral-domain optical coherence tomography (SD-OCT) has axial imaging resolution of < 5 microns and offers more than 50-fold higher data-acquisition speed compared to conventional time-domain OCT systems (Alam et al. 2006). The improved resolution enhances visualization of intraretinal structures, particularly at the level of the external limiting membrane (ELM) and photoreceptor inner segment/outer segment (IS/OS) junction, which may indicate the integrity of the photoreceptor layer. SD-OCT also provides rapid sweep of serial OCT B-scan images to capture subtle microstructural changes in the area of interest (Hangai et al. 2007; Fujimoto et al. 2008).

The purpose of this study is to obtain FAF images in patients with RP and assess a potential correlation between FAF patterns and photoreceptor morphology obtained by SD-OCT imaging. Changes in the FAF and SD-OCT images over time after initial examination also were followed to explore the possibility of time-dependent changes in the pattern of FAF images and photoreceptor morphology. In addition, retinal function was assessed by microperimetry-1 (MP-1) in selected cases to explore the relation between FAF changes and retinal function.

Methods

This study was an observational case series of consecutive patients with RP who were examined at Osaka University Hospital. Diagnoses were based on a history of nyctalopia, characteristic pigmentary retinal changes, bone spicule pigmentation around the periphery, and ISCEV (International Society for Clinical Electrophysiology of Vision) standard full-field ERGs (Marmor et al. 2004). All patients underwent a comprehensive ophthalmologic examination including measurement of the best-corrected visual acuity (BCVA), binocular indirect ophthalmoscopy, contact lens slit-lamp biomicroscopy, and fundus photography. Fundus photographs were obtained using a standard fundus camera (TRC-50LX/ImageNet2000; Topcon, Tokyo, Japan). Patients provided written informed consent after a detailed explanation of the study was provided. Approval from the internal review board was not required for this retrospective study.

FAF images were obtained with a confocal scanning laser ophthalmoscope, Heidelberg Retina Angiograph 2 (HRA2; Heidelberg Engineering, Heidelberg, Germany) as previously described (Wakabayashi et al. 2008). The optically pumped solid state laser (488 nm) was used for excitation, and emission was detected with a barrier filter above 500 nm. The maximum horizontal and vertical diameters of the central normal autofluorescent area were measured by two masked observers with the software included in the HRA2 and the values of the measurements were averaged.

Retinal microstructural imaging was obtained using SD-OCT, RTVue (Optovue Inc., Fremont, CA) as previously described (Fujimoto et al. 2008). Multiple serial cross-sectional images were obtained using a three-dimensional raster scan protocol. The scan consisted of equally spaced 101-frame horizontal B-scans, each comprising 512 A-scans/frame in a transverse direction, covering 4 × 4 mm with a depth of 2 mm. A pair of high-definition 1024 A-scans/frame horizontal and vertical B-scans also was obtained. SD-OCT identifies three distinct lines corresponding to back reflection from the ELM, IS/OS junction, and RPE/Bruch's membrane. Microstructural changes in the photoreceptor layer were defined as loss of the back-reflection line corresponding to the photoreceptor IS/OS junction or ELM. The extent of the preserved photoreceptor IS/OS junction in the SD-OCT images was measured on the horizontal and vertical scans through the centre of the fovea by two masked observers using software in the RTVue and the values of the measurements were averaged. To assess the potential correlation of FAF images with photoreceptor morphology on SD-OCT, the virtual OCT fundus image created by the raster scan protocol was correlated with FAF image and used for the registration of each cross-sectional OCT image to the specific features on the FAF image.

Microperimetry was performed with automatic fundus-related perimetry (Nidek Technologies, Padova, Italy) in selected patients who agreed with this examination. Stimuli attenuation ranged from 0 to 20 decibels (dB). Stimulation size was equivalent to the Goldmann III spot size, with 200-ms projection; the fixation target was a 2-degree cross. The '4-2 strategy' default setting was applied, and 56 stimulus locations covering the central 16 degrees were examined.

After the initial enrolment, eyes with pre-existing macular disease, cystoid macular oedema (CMO), cataracts, or diabetes mellitus, which could affect VA measurement or interfere with image interpretation, were excluded from data analysis. For statistical analysis, the BCVA was measured using the Landolt C acuity chart and analysed on a logarithm of minimal angle of resolution (logMAR) scale; values of 2.0 and 3.0 indicated counting fingers and hand motions vision, respectively. To assess the inter-observer repeatability of the measurements of normal autofluorescent area on FAF images and preserved photoreceptor IS/OS area on SD-OCT images, the methods described by Bland and Altman was used (Bland & Altman 1986). The mean difference between two measurements (Observer1–Observer2) for each of the FAF and SD-OCT images represented the bias. The 95% limits of agreement (LoA), an expected difference between two measurements, was calculated as the mean of the differences ± 1.96 × standard deviation (SD) of the difference. The coefficient of

repeatability (1.96 × SD of the difference), an indicator of the amount of variation that can be attributed to measurement error, was also calculated. All analyses were conducted using SigmaStat software version 3.1 (SPSS Inc., Chicago, IL). $p < 0.05$ indicated statistical significance.

Results

Basic information

Forty-four eyes of 22 consecutive patients with RP underwent examinations. Ten eyes were excluded because of CMO (two eyes of one patient) and cataracts (eight eyes of four patients), leaving 34 eyes of 17 patients (9 men, 8 women; mean age, 39.8 ± 18.3 years; range, 12–80) for data analysis. The mean BCVA was 0.55 (range, hand motions-1.5). The patients had the typical bone spicule appearance in the peripheral retina. The posterior pole had a relatively normal appearance.

FAF images

The most prominent feature of FAF in most patients (32/34 eyes; 94%) was a ring of increased autofluorescence in macular area with different eccentricities (Figs 1–4). The normal FAF was observed inside the ring in those 32 eyes. Outside the ring, various FAF patterns were seen, i.e., mottled decreased FAF in eight eyes (4 patients) (Fig. 1), apparently normal FAF in four eyes (2 patients) (Fig. 4D), and apparently normal FAF surrounded by mottled decreased FAF in 20 eyes (10 patients) (Figs 2, 3 and 4A). The mean diameters of the normal FAF surrounded by the hyperfluorescent ring on horizontal and vertical sections through the fovea were 1.71 ± 1.29 (range, 0.43–5.60) mm and 1.23 ± 1.23 (range, 0.34–5.86) mm, respectively. The mean diameter of the normal FAF on horizontal and vertical sections was significantly associated with VA (linear regression analysis, $R = 0.37$, $p = 0.03$).

Two eyes of one patient did not have a ring as well as the normal FAF in the macular area, and a small area of increased FAF was seen centrally (diameters, 1340 μm and 1360 μm in the right eye, and 1310 μm and 1110 μm in the left eye on horizontal and vertical sections) (Fig. 4G). The VA levels in these eyes were the worst among the study eyes (0.2 in the right eye and hand motions in the left eye).

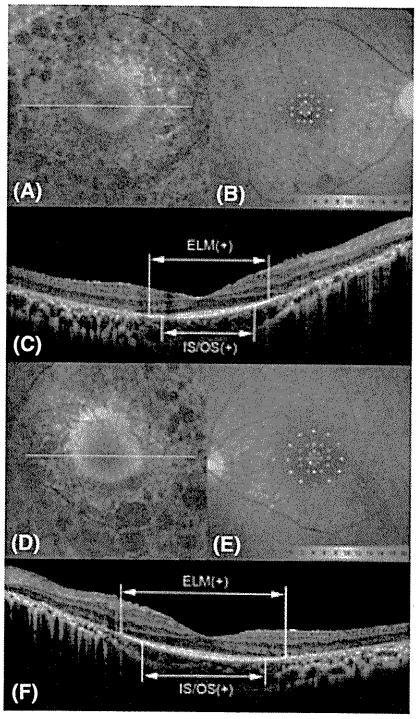

Fig. 1. The fundus autofluorescence (FAF), microperimetric results on microperimetry-1 (MP-1), and Spectral-domain optical coherence tomography (SD-OCT) images from a 31-year-old man with retinitis pigmentosa (RP). (A) A FAF image of the right eye shows normal FAF centrally surrounded by a ring of increased FAF. A mottled area of decreased FAF is present at the outermost lesion. The white line indicates the locations of SD-OCT image shown in panel C. (B) MP-1 shows preserved retinal sensitivity within the ring of increased FAF (seen in the colour-coded scale) and no retinal sensitivity in areas of mottled decreased FAF. (C) A horizontal cross-sectional SD-OCT image shows an intact photoreceptor inner segment and outer segment (IS/OS) junction in areas with normal FAF. The IS/OS junction and external limiting membrane (ELM) are absent outside the areas of a ring of increased FAF. (D) An FAF image of the left eye shows normal FAF centrally surrounded by a ring of increased FAF. The area of normal FAF is slightly larger than that in the right eye. A mottled area of decreased FAF is present at the outermost lesion. The white line indicates the locations of SD-OCT image shown in panel F. (E) MP-1 results show preserved retinal sensitivity within a ring of increased FAF and no retinal sensitivity in areas of mottled decreased FAF. (F) A horizontal cross-sectional SD-OCT image shows an intact IS/OS junction in areas with normal FAF. The transverse diameter of the intact IS/OS junction is larger than that of the right eye. The IS/OS junction and ELM are absent outside the area of a ring of increased FAF.

SD-OCT images

Based on multiple serial B-scans, the photoreceptor IS/OS and ELM were preserved in a different degree at the macular area in 32 eyes with hyperfluorescent rings. In two eyes of one patient, the photoreceptor IS/OS was not seen at the macular area. In 32 eyes with a hyperfluorescent ring, the interface between the preserved and lost IS/OS was seen clearly. The mean extent of the preserved IS/OS junction was 1.90 ± 1.23 mm (range, 0.49–5.26) and 1.73 ± 1.18 mm (range, 0.44–5.39) on horizontal and vertical B-scans, respectively. The transverse extent of the preserved ELM was larger than that of the IS/OS in the 32 eyes (0.83 ± 0.45 and 0.77 ± 0.39 larger, on horizontal and vertical sections, respectively). Outside the intact ELM, the photoreceptor IS/OS and ELM were not seen even in eyes in which the retina appeared relatively normal. The diameter of preserved IS/OS on the horizontal and vertical sections was significantly associated with VA ($R = 0.421$, $p = 0.013$ and $R = 0.399$, $p = 0.019$, respectively).

Repeatability of the analysis

The mean difference in horizontal and vertical diameters of the normal FAF area between observer1 and 2 were + 0.02 mm and + 0.03 mm, respectively. No significant bias was found between observers ($p = 0.953$, $p = 0.837$, respectively). The 95% LoA were between −0.23 and 0.27 mm in the horizontal measurement and −0.23 and 0.28 in the vertical measurement. Therefore, the coefficient of repeatability was 0.24 in the horizontal section and 0.25 in the vertical section. The bias of the measurements for preserved IS/OS area in the SD-OCT images was 0.00 mm with 95% LoA between −0.19 and 0.20 mm in the horizontal section and 0.02 mm with 95% LoA between −0.17 and 0.20 mm. No significant bias was found between observers ($p = 0.883$, $p = 0.352$, respectively). The coefficient of repeatability was 0.19 in the horizontal section and 0.18 in the vertical section.

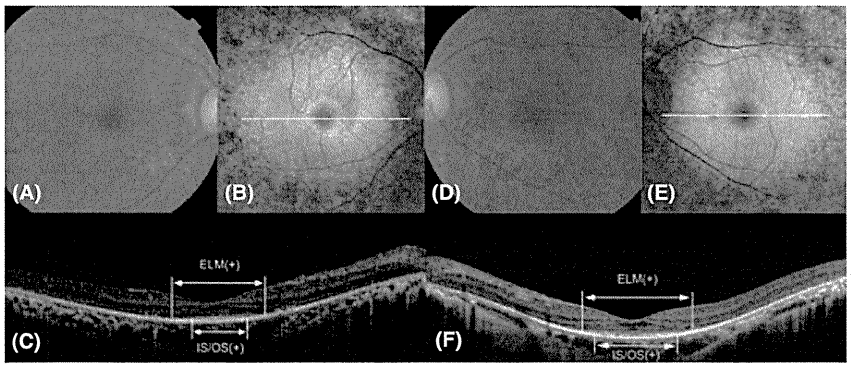

Fig. 2. Fundus photograph, fundus autofluorescence (FAF), and Spectral-domain optical coherence tomography (SD-OCT) images from a 54-year-old woman. (A) A fundus photograph of the right eye. (B) An FAF image of the right eye shows normal FAF at the macula. A ring of increased FAF around the normal FAF is seen. Outside this, apparently normal FAF and a surrounding area of decreased FAF with a mottled appearance are present. The white line indicates the locations of SD-OCT image shown in panel C. (C) A horizontal cross-sectional SD-OCT image shows an intact photoreceptor IS/OS junction in areas with normal FAF. The IS/OS junction and external limiting membrane (ELM) are absent outside the ring of increased FAF even in areas with apparently normal FAF. (D) A fundus photograph of the left eye. (E) An FAF image in the left eye shows normal FAF at the macula. A ring of increased FAF around the normal FAF is seen. Outside this, apparently normal FAF and surrounding area of decreased mottled FAF are present. The white line indicates the locations of SD-OCT image shown in panel F. (F) A horizontal cross-sectional SD-OCT image shows an intact IS/OS junction within areas with normal FAF. The IS/OS junction and ELM are absent outside the ring of increased FAF.

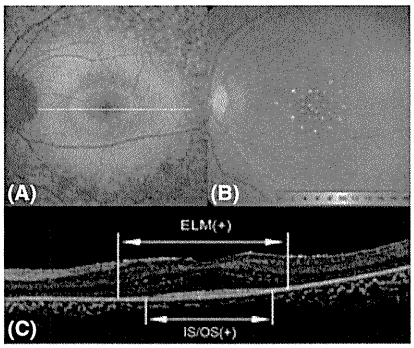

Fig. 3. The fundus autofluorescence (FAF), microperimetric results on microperimetry-1 (MP-1), and Spectral-domain optical coherence tomography (SD-OCT) images from a 32-year-old woman. (A) An FAF image of the left eye shows normal FAF centrally surrounded by a ring of increased FAF. Outside this, apparently normal FAF and surrounding area of decreased FAF with a mottled appearance are present. The white line indicates the locations of SD-OCT image shown in panel C. (B) MP-1 shows preserved retinal sensitivity within the ring of increased FAF (seen in the colour-coded scale) and no retinal sensitivity in areas of apparently normal FAF. (C) A horizontal cross-sectional SD-OCT image shows an intact photoreceptor IS/OS junction within areas with normal FAF. The IS/OS junction and external limiting membrane (ELM) are absent outside the ring of increased FAF even in areas with apparently normal FAF.

Correlation of FAF with SD-OCT images

The area of normal FAF surrounded by the hyperfluorescent ring was corresponded to the area of preserved IS/OS on SD-OCT (Figs 1–4). The diameter of the normal FAF at the macular area was significantly correlated to the preserved area of the IS/OS on SD-OCT horizontally and vertically (R = 0.939, $p < 0.001$ and R = 0.962, $p < 0.001$, respectively). No IS/OS was seen in areas with a ring of increased FAF; however, the ELM was preserved in those areas. Outside the areas with a ring of increased FAF, both IS/OS and ELM were not observed and there was gradual thinning of the outer nuclear layer towards the periphery. All eyes with hyperfluorescent rings had no IS/OS and ELM outside the ring, regardless of the FAF pattern outside the ring. Therefore, the ring of increased FAF demarcated the border between the central retina with preserved IS/OS and ELM and the adjacent peripheral retina with no IS/OS and ELM. In two eyes of one patient without normal FAF at the macula, the IS/OS was not seen on SD-OCT even at the fovea (Fig. 4C).

Microperimetry

Microperimetry was performed in six eyes. Retinal sensitivity was detected only within the hyperfluorescent ring on FAF (Figs 1 and 3); the mean retinal sensitivity within the examined area was 4.39 ± 2.40 dB (range, 1.04–6.64). The mean retinal sensitivity was correlated significantly with the area of normal FAF seen by HRA2 (R = 0.929, p = 0.007) and the preserved area of the IS/OS on SD-OCT (R = 0.851, p = 0.032).

Time-dependent changes in FAF and SD-OCT images

Of the 32 eyes with a hyperfluorescent ring, 26 eyes (81%) continuously underwent FAF and SD-OCT examinations during 3.1 (range, 3–3.5) years of follow-up to determine the possibility of time-dependent changes in FAF and SD-OCT images. Ten eyes from 5 patients had progressive reduction in size of the ring as well as the area of normal FAF inside the ring. Those changes were accompanied by decreased area of preserved IS/OS. One of these patients had disappearance of pre-existing normal FAF with concomitant loss of intact IS/OS (Fig. 5). In 16 eyes of 8 patients, the normal FAF inside the ring and associated preserved IS/OS showed no significant changes during the follow-up.

Discussion

In the current study, the FAF images in patients with RP showed various sizes of characteristic hyperfluorescent ring and the normal FAF inside the ring. The area of normal FAF measured with acceptable repeatability was significantly correlated with a preserved area of photoreceptor IS/OS junction on SD-OCT as previously reported (Murakami et al. 2008; Lima et al. 2009). The intact photoreceptor morphology with normal metabolic activities may lead to normal lipofuscin distribution in the RPE, resulting in normal intensity of the FAF. In addition, the area of normal FAF had preserved retinal functions based on the microperimetric examination. Therefore, the area of normal FAF inside the ring may be the key for evaluating the integrity of photorecep-

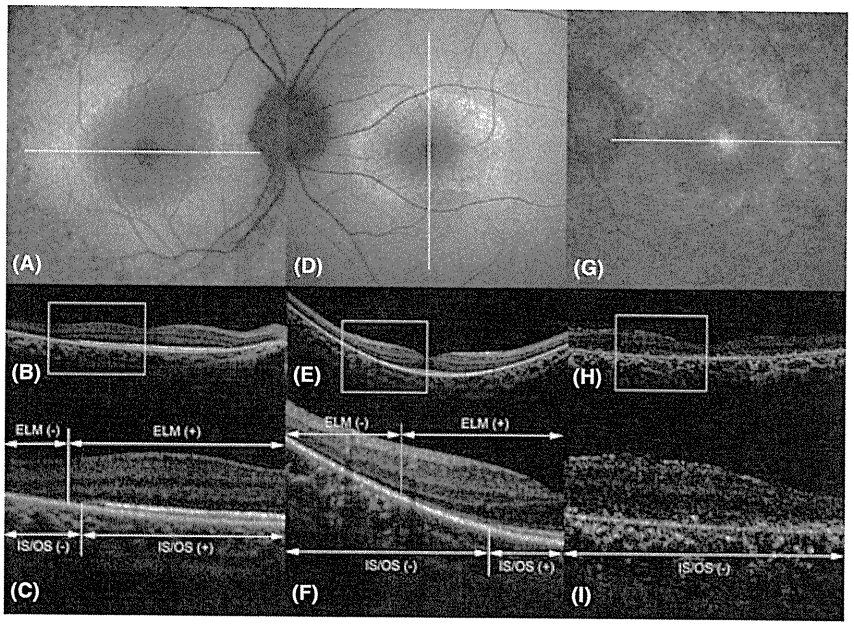

Fig. 4. Various patterns of fundus autofluorescence (FAF) and Spectral-domain optical coherence tomography (SD-OCT) images. (A) An FAF image from a 54-year-old woman shows an area of relatively large normal FAF including the macula. A ring of increased FAF around the normal FAF can be seen. Outside this, apparently normal FAF surrounded by an area of dark mottling is detected. The white line indicates the locations of SD-OCT image shown in panel B. (B) A horizontal cross-sectional SD-OCT image. (C) Magnification of the area outlined in B shows a largely preserved photoreceptor IS/OS junction within areas with normal FAF. A cross-sectional image scan shows the absence of the IS/OS junction and external limiting membrane (ELM) outside the ring with apparently normal FAF surrounded by dark mottling. (D) An FAF image from a 15-year-old girl shows normal FAF centrally surrounded by relatively large areas of increased FAF. Outside this, apparently normal FAF is seen. The white line indicates the locations of SD-OCT image shown in panel E. (E) A horizontal cross-sectional SD-OCT image. (F) Magnification of the outlined area in E shows a preserved IS/OS junction within areas with normal FAF. The IS/OS junction and ELM are lost outside area of increased FAF, although the FAF has apparently normal intensity. (G) An FAF image from a 35-year-old man shows increased FAF centrally without normal FAF at the macula. The white line indicates the locations of SD-OCT image shown in panel H. (H) A horizontal cross-sectional SD-OCT image. (I) Magnification of the area outlined in H shows loss of the IS/OS junction.

tor IS/OS as well as the visual function in RP (Robson et al. 2003, 2004, 2006; Popovic et al. 2005). The normal FAF with a larger diameter was also significantly associated with a better VA in our series.

Outside the ring of increased FAF, the FAF images showed various patterns, i.e., mottled decreased FAF in eight eyes (Fig. 1), apparently normal FAF in four eyes (Fig. 4D), and apparently normal FAF surrounded by mottled decreased FAF in 20 eyes (Figs 2, 3 and 4A). However, the patterns outside the ring were less relevant for evaluating the visual function because both photoreceptor IS/OS and ELM were completely lost and the retinal function was not maintained regardless of the pattern of FAF outside the ring. Only the area of normal FAF inside the ring seems to be clinically relevant for evaluating the visual function.

The reason why some eyes present normal intensities of the FAF despite photoreceptor loss outside the ring is not clear in the present study. However, these findings may be similar to the findings of normal-appearing FAF with photoreceptor loss outside the ring observed in patients with pigmented paravenous retinochoroidal atrophy which Fleckenstein et al. reported. They considered the normal-appearing FAF without intact photoreceptor layer represented surviving RPE containing lipofuscin granules that were formed prior to the occur-

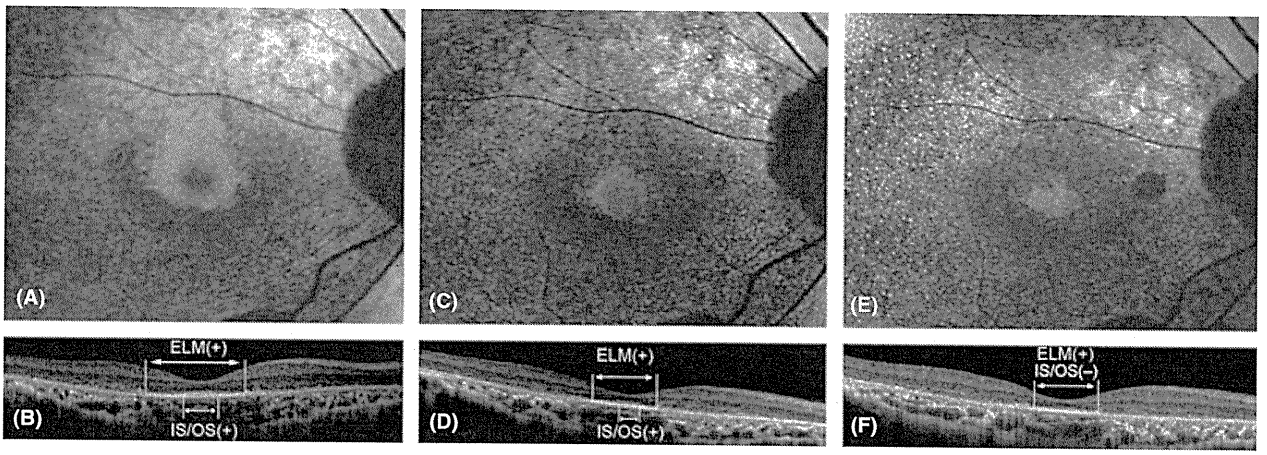

Fig. 5. Changes in the Fundus autofluorecence (FAF) and SD-OCT images of a 56-year-old man. (A) An FAF image of the right eye shows normal FAF centrally surrounded by a ring of increased FAF. Outside this, decreased FAF with a mottled appearance is present. (B) A horizontal cross-sectional SD-OCT image shows an intact photoreceptor IS/OS junction within areas with normal FAF. The IS/OS junction and external limiting membrane (ELM) are absent outside the ring. (C) After three years, the FAF image showed reduction in size of the ring as well as the area of normal FAF inside the ring. (D) The area of preserved IS/OS decreased. (E) After six months, the pre-existing normal FAF disappeared. (F) The SD-OCT image shows loss of intact IS/OS. The ELM seems to be preserved.

rence of photoreceptor impairment. Normal FAF in the presence of severe photoreceptor dysfunction also has been reported in patients with leber congenital amaurosis (Scholl et al. 2004a). Although photoreceptor morphology in the normal FAF were not investigated in their study, the normal metabolic activities of the photoreceptors and constant outer segment phagocytosis by the RPE may not be necessarily required for normal FAF in some patients with inherited retinal dystrophies.

The increased FAF has been reported to indicate dysfunction of the RPE cells and represent reduced retinal sensitivity. (Delori et al. 1995; von Ruckmann et al. 1995; Scholl et al. 2004b). A pathologic RPE might not digest visual pigment from the photoreceptors, resulting in excess accumulation of autofluorescent materials and subsequent increased FAF, as suggested in patients with age-related macular degeneration (AMD) (Holz et al. 1999). However, recent studies have reported that the FAF represents the condition of not only the RPE but also photoreceptors in various retinal diseases (Sawa et al. 2008; Schmitz-Valckenberg et al. 2008). Because the primary disease locus is the photoreceptors in most RP cases, the hyperfluorescent ring in patients with RP may also be related to the photoreceptors. In our series, IS/OS was not observed in areas with a ring, although the ELM was present. Those areas of hyperfluorescent ring might represent regional distribution of active photoreceptor degeneration and the increased rate of outer segment phagocytosis by the RPE.

Histopathologic studies of RP have shown that the shortening of the rod outer segments and reduction of the cell bodies indicative of photoreceptor cell death occur at the midperiphery and progress with time to involve the cone photoreceptors (Flannery et al. 1989; Portera-Cailliau et al. 1994; Milam et al. 1996; Li et al. 1998; Reme et al. 1998). These findings may be compatible with the time-dependent changes in the FAF and SD-OCT images in the current series, which Robson et al. 2006 first reported. They identified the constriction of the ring of increased FAF and associated worsening of macular function in RP. In the current series, ten eyes had reduction in size of the ring as well as the area of normal FAF inside the ring within three and a half years. Although the morphologic implications of the progressive ring constriction were not investigated further in their study, we newly found that the reduced size of normal FAF was accompanied by decreased area of intact photoreceptor IS/OS (Fig. 5). The photoreceptor death may progress to involve the outermost region of the normal FAF inside the ring, resulting in constriction of the ring and reduced retinal function. With the progressive replacement of normal FAF with the constricted ring, the island of normal FAF inside the ring eventually disappear, resulting in increased FAF centrally without normal FAF and IS/OS, as demonstrated in one patient at the initial examination with worst visual function (Fig. 4C) and another patient during the follow-up (Fig. 5).

In contrast to the eyes with ring constriction, sixteen eyes had no significant changes in the area of normal FAF inside the ring and IS/OS during the follow-up. This finding may suggest that the disease was stationary or had limited progression. The presence or absence of the ring constriction during the follow-up may reflect the difference in the rate of photoreceptor death in the studied eyes. Therefore, the areas of normal FAF inside the ring and the rate of subsequent ring constriction seems critical for predicting the visual prognosis. Further studies are needed to identify the possible factors that may influence the rate of ring constriction, such as patient age, mode of inheritance or gene mutation.

In summary, FAF and SD-OCT are useful objective tools for evaluating the photoreceptor status and the time course of progressive photoreceptor loss not seen on clinical examination in patients with RP. Normal FAF surrounded by a hyperfluorescent ring indicates the integrity of the photoreceptor IS/OS and the ELM with preserved retinal function. Long-term preservation of the normal FAF to maintain visual function may be an objective in the development of new therapeutic strategies in the future.

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Simultaneous intravitreal injection of triamcinolone acetonide and tissue plasminogen activator for central retinal vein occlusion: a pilot study

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ABSTRACT

Purpose To evaluate the efficacy and safety of simultaneous intravitreal injection of triamcinolone acetonide (TA) and tissue plasminogen activator (tPA) for macular oedema associated with central retinal vein occlusion (CRVO).

Methods Twenty eyes of 20 patients with CRVO were enrolled. A mixture of TA (4 mg) and tPA (25 μg) was injected into the vitreous of 20 eyes with CRVO. Best corrected visual acuity (BCVA) and macular thickness before and 1, 3, 6 and 12 months after the procedure were measured.

Results The BCVA improved three lines or more in 65%, 55%, 55% and 53% of eyes and the mean macular thickness decreased from 1072 μm to 455, 450, 480 and 409 μm ($p<0.001$) at 1, 3, 6 and 12 months, respectively. Fifteen (75%) of the 20 eyes required at least one additional injection to prevent a recurrence of macular oedema. The intraocular pressure increased in four eyes.

Conclusion Overall, intravitreal injection of the TA/tPA mixture improved the BCVA by three lines or more in at least 50% of eyes and decreased the mean macular thickness at four time points without serious side effects. A randomised clinical trial is necessary to evaluate the efficacy of this treatment.

INTRODUCTION

Central retinal vein occlusion (CRVO) is one of the most frequently occurring retinal vascular disorders that results in severe visual loss. The natural history of CRVO is associated with a final visual acuity (VA) of 20/200 or less in 93% of patients with ischaemic CRVO and in 50% of patients with non-ischaemic CRVO.[1] Eighty per cent of patients with poor VA (<20/200) at the first visit have a VA <20/200 at their final visit. Regarding neovascular glaucoma—which can cause legal blindness—iris neovascularisation, angle neovascularisation, or both, developed in 16% of eyes.[2]

Several treatments for CRVO have been developed, including laser photocoagulation,[3] laser-induced chorioretinal venous anastomosis,[4] intravenous injection of tissue plasminogen activator (tPA),[5] pars plana vitrectomy,[6 7] radial optic neurotomy,[8 9] retinal endovascular surgery[10–12] and sub-Tenon's injection of triamcinolone acetonide (TA).[13 14] However, no established treatment improves the VA, although panretinal laser photocoagulation reduces neovascular complications, and grid pattern photocoagulation reduces macular oedema.[3 15–17]

Intravitreal injection of TA is a common treatment for macular oedema associated with CRVO. TA downregulates the production of vascular endothelial growth factor (VEGF).[18 19] Many reports have shown that TA significantly decreases the macular oedema associated with CRVO.[20–25] However, TA does not directly relieve the venous occlusion, despite the fact that venous thrombi are presumed to play an important role in the pathogenesis of CRVO. Nevertheless, since corticosteroids decrease fibrinolytic activity,[26–28] blood flow may be compromised.

tPA has been used to treat cerebral infarction to lyse blood clots. Several reports have suggested that intravitreal tPA for CRVO with macular oedema is safe and effective.[29–32] tPA is administered intravitreally to avoid the serious complications associated with systemic administration.

We theorised that these two drugs, TA and tPA, used together might improve the status of CRVO, because both have different mechanisms and both partially improve the VA in patients with CRVO; tPA also can offset the tendency of TA to decrease fibrinolytic activity.[27 28] We conducted a prospective pilot study using simultaneous intravitreal injection of the mixture of TA and tPA for CRVO to determine the efficacy and safety of the treatment.

PATIENTS AND METHODS

Twenty eyes of 20 consecutive patients with CRVO who were referred to Osaka University Hospital were enrolled. The diagnosis of CRVO was confirmed using fluorescein angiography (FA). The principal inclusion criteria were CRVO with a VA of 20/50 or less and macular oedema exceeding 400 μm seen on optical coherence tomography (OCT).

Patients were excluded if vitreous haemorrhage or any other retinal pathology was present or if they had undergone previous treatments with an intravitreal drug injection for CRVO except for anticoagulant medication, or if they had undergone previous surgical or laser treatment for any reason, and/or if they were younger than 40 years. Patients were fully informed about the purpose of this study and the possible risks and benefits of the treatment. TA (Kenacort; Bristol-Myers Squibb Co., Tokyo, Japan) was purified by removing the vehicle and dissolving it in balanced saline solution at a concentration of 8 mg/0.1 ml.[33] tPA (Activacin; Kyowa Hakko Kogyo Co., Tokyo, Japan) was dissolved in sterile water at a concentration of

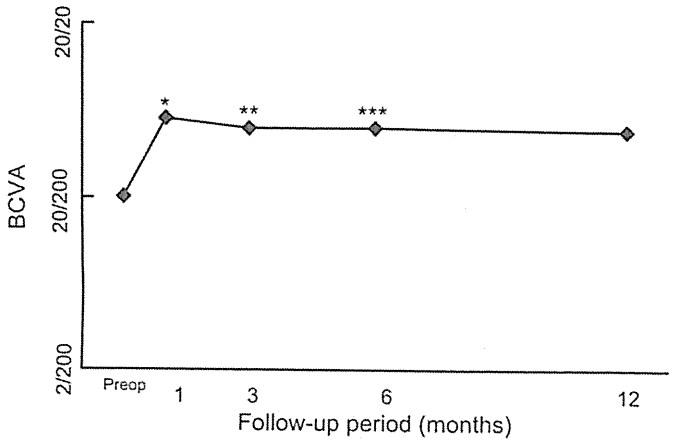

Figure 1 Changes in the mean best corrected visual acuity (BCVA) before and after the procedure. The BCVA significantly improved at 1, 3 and 6 months, although there was no significant difference at 12 months (p=0.098). *p<0.001, **p=0.006, ***p=0.009.

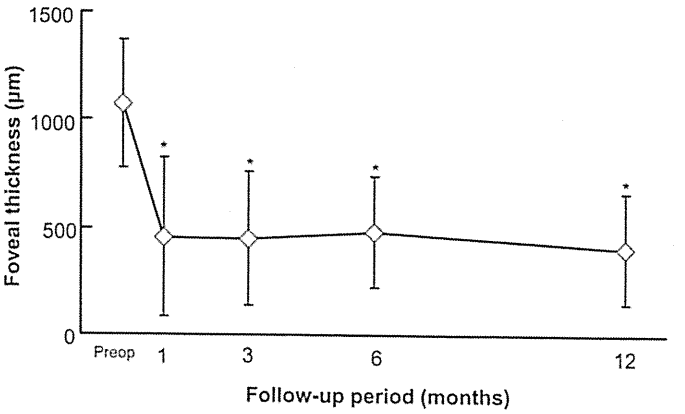

Figure 3 Changes in the mean foveal thickness before and after the procedure. The mean foveal thickness immediately decreased at 1 month and remained decreased at 12 months (*p<0.001). Preop, preoperatively.

25 μg/0.1 ml We mixed 0.05 ml (4 mg) of TA and 0.1 ml (25 μg) of tPA just before injection.

The conjunctiva was prepared with povidone-iodine solution, and lidocaine eye drops were administered for topical anaesthesia. The mixture was injected during an outpatient procedure using a 27-gauge needle through the pars plana. A paracentesis was performed to adjust the intraocular pressure (IOP). If macular oedema recurred during the follow-up period and exceeded 400 μm on OCT, an additional injection was administered.

Complete ophthalmological examinations were performed before intravitreal TA/tPA injection and at 1, 3, 6 and 12 months after treatment. These examinations included measurement of the BCVA, pneumatic tonometry, biomicroscopy, fundus examination, and OCT to measure the foveal thickness. The BCVA was converted to the logarithm of the minimum angle of resolution (logMAR). Results are expressed as the mean±SD. Data were analysed by t test, paired t test or Fisher's exact test using Sigma Stat 2.03 (Jandel Scientific Software, San Rafael, CA). p Values <0.05 were considered statistically significant.

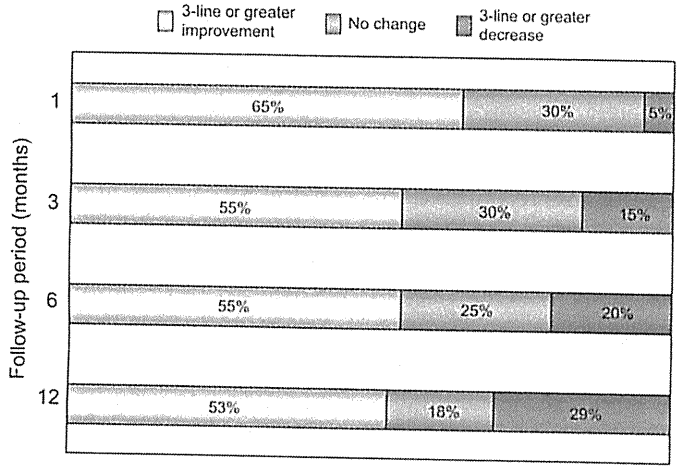

Figure 2 Change in the total lines of best corrected visual acuity at 1, 3, 6 and 12 months after the procedure. Thirteen (65%) of 20 eyes, 11 (55%) of 20 eyes, 11 (55%) of 20 eyes and nine (53%) of 17 eyes gained three or more lines at 1, 3, 6 and 12 months, respectively.

RESULTS

The mean patient age was 63.4±9.9 years. The mean duration from onset of symptoms to treatment was 7.4±6.9 weeks, and the mean follow-up period was 11.1±2.2 months.

Using FA, the status of perfusion was determined; an ischaemic eye has defined as having 30 disc areas or more of capillary non-perfusion. Of the 20 eyes, 12 were non-ischaemic and five were ischaemic; in three eyes the status was undetermined at baseline. At baseline, one non-ischaemic eye became ischaemic during the follow-up period. Three eyes of three patients were excluded from the 12-month analysis because two patients underwent surgery to treat an epiretinal membrane at 9 and 11 months, and one patient was treated with an intravitreal injection of bevacizumab (Avastin; Genentech, South San Francisco, California, USA) at 9 months.

The mean BCVA improved from 20/198 (±20/56) to 20/70 (±20/60), 20/81 (±20/59), 20/81 (±20/63) and 20/83 (±20/60) at 1, 3, 6 and 12 months after treatment, respectively. There were significant differences at 1, 3 and 6 months but not at 12 months (p<0.001, p=0.006, p=0.009 and p=0.098, respectively) (figure 1). The BCVA improved by three lines or more in 13 (65%) of 20 eyes, 11 (55%) of 20 eyes, 11 (55%) of 20 eyes, and nine (53%) of 17 eyes at 1, 3, 6 and 12 months after injection, respectively (figure 2).

The mean macular thickness decreased significantly from 1072±297 to 455±371 (p<0.001), 450±310 (p<0.001), 480±257 (p<0.001) and 409±256 (p<0.001) μm at 1, 3, 6 and 12 months after treatment, respectively (figure 3).

To evaluate the correlation between the BCVA at baseline and the final follow-up visit, we classified the eyes according to the baseline BCVA into two groups, that is, group 1 (n=9), BCVA less than 20/200; and group 2 (n=11) BCVA of 20/200 or better (figure 4). In group 1, the mean BCVA improved significantly (p=0.007) from 8/200±20/42 to 20/51±20/50. In group 2, the mean BCVA decreased slightly from 20/95±20/34 to 20/109±20/63, which did not reach significance (p=0.694). The mean final BCVA in group 1 was better than that in group 2, but the difference was not statistically significant (p=0.190).

We then classified the eyes according to the BCVA at the final follow-up visit into three groups: group A (n=6), BCVA less than 20/200; group B (n=8), BCVA between 20/200 and 20/50; and group C (n=6), BCVA of 20/40 or better. We evaluated the time course of the BCVA in these groups (figure 5). In group A, the mean baseline BCVA was poor (14/200±20/66); there was

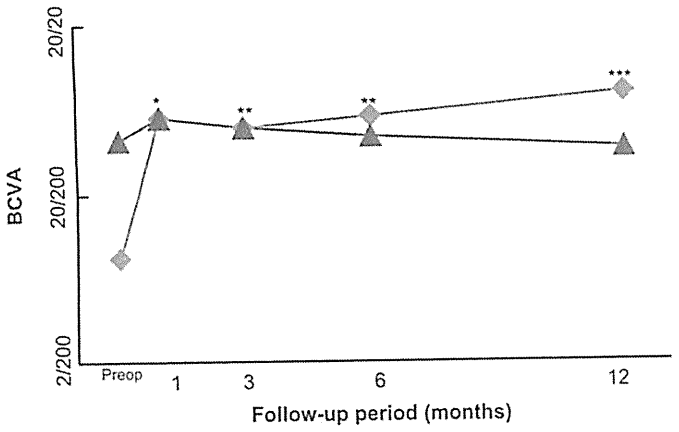

Figure 4 Changes in the mean best corrected visual acuity (BCVA) before and after the procedure in group 1 (baseline BCVA, <20/200) and group 2 (20/200≤baseline BCVA). The BCVA in group 2 significantly improved at 1, 3, 6 and 12 months. Diamonds, baseline BCVA <20/200; triangles, 20/200≤baseline BCVA, *p<0.001, **p=0.002, ***p=0.028. Preop, preoperatively.

no change at 1 month (17/200±20/62; p=0.693) or at any other time point. The mean BCVA at 12 months was 11/200±20/35 (p=0.188). In group B, the mean baseline BCVA was 20/116±20/45, which improved significantly to 20/41±20/34 at 1 month (p=0.033) but decreased gradually to 20/80±20/41 at 12 months (p=0.327). However, the mean BCVA at 12 months was still higher than the baseline BCVA. Surprisingly, the mean baseline BCVA in group C was also poor (14/200±20/54); however, the mean BCVA improved dramatically to 20/42±20/33 at 1 month (p=0.003) and reached a mean of 20/28±20/26 (p=0.012) at 12 months.

The patients received a mean of 2.5±1.1 (range 1–4) injections. Fifteen (75%) of the 20 eyes required additional injections (mean 2.9±0.8 times; range 2–4) to prevent recurrence of macular oedema. The mean time between injections was 3.6±1.8 (range 0.9–8.6) months. Complications are shown in table 1. The IOP increased in four (20%) of the 20 eyes. A patient who had been treated for glaucoma before the onset of CRVO underwent a trabeculotomy. Other patients were treated with a topical drug. Cataracts worsened in three (15%) of the 20 eyes, but none required surgery. No rubeosis or neovascular glaucoma developed and no eyes required laser photocoagulation. Non-infectious endophthalmitis developed in one eye (5%) and was treated with an intravitreal antibiotic injection. The inflammation resolved within 2 weeks.

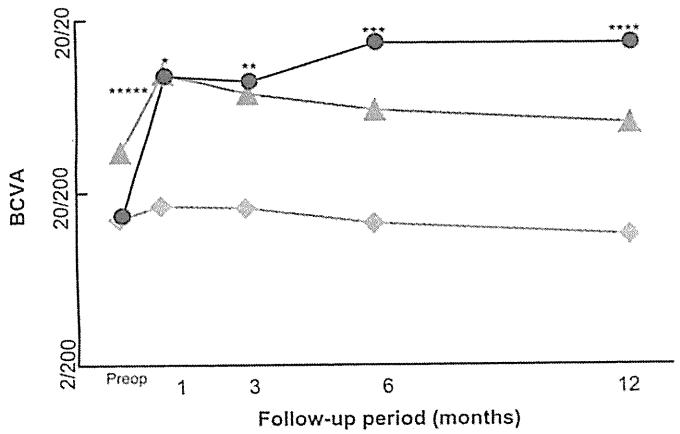

Figure 5 Changes in the mean best corrected visual acuity (BCVA) before and after the procedure in group A (final BCVA <20/200), group B (20/200≤final BCVA≤20/50) and group C (20/40≤final BCVA). The mean BCVA significantly improved at 1, 3, 6 and 12 months in group C and at 1 month in group B. Diamonds, final BCVA<20/200; triangles, 20/200≤final BCVA ≤20/50; circles, 20/40≤final BCVA. *p=0.003, **p=0.020, ***p=0.001, ****p=0.012, *****p=0.021. Preop, preoperatively.

Table 1 Complications

Complication	n (%)
Neovascular glaucoma	0
IOP elevation	4 (20)
Cataract	3 (15)
Retinal detachment	0
Vitreous haemorrhage	0
Non-infectious endophthalmitis	1 (5.0)

The clinical course of a patient is presented.

CASE

A 49-year-old woman presented with a 1-week history of CRVO (figure 6). The blood pressure was 116/60 mm Hg. The BCVA at baseline was 4/200. The anterior segment evaluation was unremarkable. Ophthalmoscopy showed a swollen optic nerve disc with flame haemorrhages, dilation and tortuosity of the retinal vein, and marked scattered cotton-wool patches. The macular oedema was marked (962 μm). FA showed non-perfused CRVO without neovascularisation. The BCVA improved to 20/50 and 20/20 at 1 and 3 months, respectively, and the macular oedema decreased to 167 μm and 175 μm. Ophthalmoscopy also showed a substantial reduction in macular oedema and optic disc oedema and improved dilation and tortuosity of the retinal vein. The cotton-wool spots had decreased dramatically. However, 6 months after treatment, the macular oedema and vein dilation and tortuosity recurred. The BCVA was 20/32 and the macular thickness increased to 641 μm. The patient received another injection, which reduced the oedema. The final BCVA and macular thicknesses were 20/25 and 150 μm, respectively.

DISCUSSION

The current study showed that simultaneous intravitreal injection of TA/tPA for CRVO achieved favourable visual outcomes and reduced macular oedema.

The natural course of CRVO is generally disappointing. Spontaneous improvement in the BCVA tends to be small and infrequent. The Central Vein Occlusion Study (CVOS) Group reported that only 6% of patients gained three or more lines of BCVA compared with the baseline VA within the first year of follow-up.[2] Conventional treatment with one intravitreal injection of TA improved the BCVA in 31% to 39% of patients with CRVO,[23 24] whereas one injection of tPA did so in 29% to 62% of patients.[29–31] In the Standard Care versus Corticosteroid for Retinal Vein Occlusion study, in which the efficacy and safety of 1 and 4 mg doses of TA for 271 eyes with perfused CRVO were compared, 27% and 26% of patients gained 15 letters or more in the 1 and 4 mg groups, respectively.[25]

While these monotherapies were efficacious to some degree, we hypothesised that a simultaneous intravitreal injection of TA/tPA could be more effective than one injection of TA or tPA. The current results showed that a simultaneous intravitreal injection of the mixture of TA/tPA for CRVO improved the BCVA by three lines or more in at least 50% of eyes in which the macular oedema decreased at 1, 3, 6 and 12 months without serious complications.

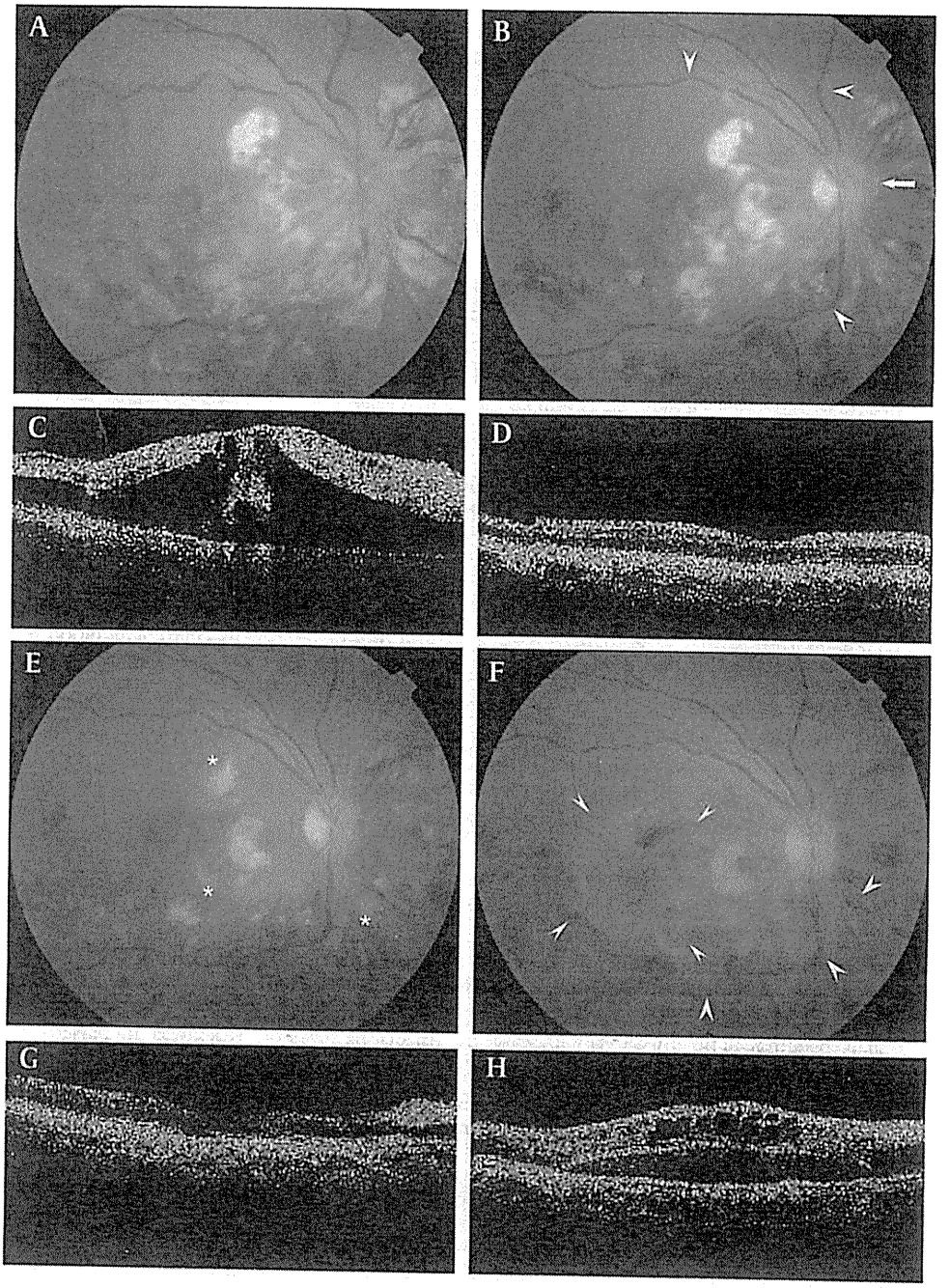

Figure 6 Images from case, a 49-year-old woman. (A, C) A colour fundus photograph and optical coherence tomography obtained before and 1 month (B, D), 3 months (E, G) and 6 months (F, H) after treatment. At 1 and 3 months, the dilation and tortuosity of the retinal vein have improved (white arrowheads). The oedema of the macula and optic disc has decreased markedly (white arrow). The number of cotton-wool spots has decreased (asterisks). The macular oedema and vein dilation and tortuosity have increased again at 6 months (yellow arrowheads).

This seems to be a more favourable result than the previous results using TA or tPA monotherapy[23–25 29–31]; however, a direct comparison is not possible because the patients' backgrounds and the treatment procedures differed. The injection also rapidly improved the haemorrhages, cotton-wool spots, optic nerve oedema, and venous dilation and tortuosity. We could not directly compare the efficacy of simultaneous intravitreal injection of TA/tPA and one injection of TA or tPA, because the current study was not a prospective comparison of these treatments. However, our results showed dramatic improvement.

When we classified the patients' eyes according to the baseline BCVA into two groups (figure 4), our data showed that the mean BCVA in group 1 improved significantly and was higher than in group 2 at 12 months, although there was no significant difference between the groups. The final BCVA after CRVO strongly depends on the baseline BCVA as reported by the CVOS group.[2] The current treatment may be especially effective in eyes with poor BCVA at baseline.

To review factors that may predict the visual prognosis, we classified the eyes according to the BCVA at the final follow-up into three groups (figure 5), which showed that the mean baseline BCVA in group A was less than 20/200. The baseline BCVA in group B was more than 20/200 and significantly improved at 1 month. The baseline BCVA in group C was the same as that in group A; however, the BCVA significantly improved at 1 month. Therefore, the BCVA at 1 month may be predictive of the final BCVA.

Intravitreal bevacizumab has been used recently to treat CRVO. The VA improved three lines or more in up to 44% of patients at the last follow-up visit.[34 35] However, more than one injection is usually needed. The mean number of injections reported in two studies was 2.8 (given over 3 months) and 5.8 (given over 12 months),[36 37] which differs from the 2.5 injections over 12 months in the current study.

Although most eyes responded rapidly to this treatment, that is, 65% of eyes gained three lines or more of vision 1 month after

the injection, macular oedema recurred in 15 (75%) eyes (case). However, the macular thickness decreased in most eyes with retreatment.

The expected mechanism of tPA is by lysis of clots and restoration of blood flow in the vessels.[38] tPA diffuses across the venous vessel wall and reaches the clot, which is damaged by CRVO, although intravitreal tPA did not diffuse through the intact neural retina in a rabbit model.[39] Meanwhile, TA is expected to downregulate VEGF production.[18 19] Although TA decreases fibrinolytic activity,[27 28] tPA could offset the limitations of TA. Thus, TA and tPA might complement each other.

Simultaneous injection of TA and tPA was especially effective for CRVO in patients with a relatively large number of cotton-wool spots and ischaemia (case). However, we did not compare them with a control group.

Non-infectious endophthalmitis developed in one eye and resolved within 2 weeks. The mechanism of inflammation in that case was unclear, but a previous report suggested that the vehicle used to deliver TA could cause ocular toxicity.[40] Other serious complications, such as retinal detachments, vitreous haemorrhages or endophthalmitis, did not develop. However, the IOP increased in four (20%) eyes and cataracts worsened in three (15%), which was similar to reports after one TA injection.[21 22]

In summary, simultaneous intravitreal injection of TA/tPA effectively reduced macular oedema and improved the BCVA in patients with CRVO without serious complications, although repeated treatments were required in 75% of eyes. The BCVA at 1 month may predict the final BCVA. A randomised clinical trial is needed to evaluate the efficacy of simultaneous intravitreal injection of TA/tPA.

Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Institutional Review Board of Osaka University. The study was performed according to the tenets of the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

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Efficacy of Intravitreal Bevacizumab Combined With Photodynamic Therapy for Polypoidal Choroidal Vasculopathy

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• PURPOSE: To compare the efficacy of photodynamic therapy (PDT) with or without intravitreal bevacizumab injection for polypoidal choroidal vasculopathy.
• DESIGN: Retrospective, comparative, interventional case series.
• METHODS: We included 146 eyes of 146 patients with treatment-naïve polypoidal choroidal vasculopathy including the subfoveal region treated with PDT monotherapy or combined with intravitreal bevacizumab injection. Treatments were chosen according to the time period. For eyes that received combination therapy, bevacizumab (1.25 mg) was administrated 1 day before PDT. All eyes were followed up for at least 12 months. Ophthalmic evaluations, including measurement of the best-corrected visual acuity and optical coherence tomography, were performed at every visit. Indocyanine green angiography and fluorescein angiography were performed every 3 months.
• RESULTS: Sixty-one eyes were treated with PDT combined with bevacizumab and 85 eyes were treated with PDT monotherapy. The mean best-corrected visual acuity was significantly better in the combined treatment group than in the monotherapy group at 3 months ($P = .0016$), 6 months ($P = .028$), 9 months ($P = .038$), and 12 months ($P = .048$). However, lesions resolved in 78.7% of eyes in the combined group and in 75.3% in the monotherapy group; the recurrence rates were 43.8% and 40.6%, respectively, and did not differ significantly. The rate of development of subretinal hemorrhage within 1 month from the initial treatment was significantly lower in the combined group than in the PDT monotherapy group (4.5% vs 17.7%; $P = .023$).
• CONCLUSIONS: Photodynamic therapy combined with bevacizumab injection offers significantly better early visual outcomes than PDT alone. Combined treatment did not affect the resolution and recurrence of lesions; however, it decreased the rate of development of PDT-related hemorrhages. (Am J Ophthalmol 2010;150:48–54. © 2010 by Elsevier Inc. All rights reserved.)

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POLYPOIDAL CHOROIDAL VASCULOPATHY (PCV) IS characterized by a complex of branching vascular networks terminating in aneurysmal or polypoidal lesions.[1–4] The prevalence of PCV is higher in Asian than in white individuals,[4,5] and recent studies have shown that PCV accounts for almost half of eyes presumed to have exudative age-related macular degeneration (AMD) in Japan.[6]

Photodynamic therapy (PDT) with verteporfin (Visudyne; Novartis Pharma AG, Basel, Switzerland), the first drug available for patients with subfoveal choroidal neovascularization (CNV) associated with AMD,[7] is more effective for treating PCV than CNV and can occlude the polypoidal vascular dilatations, resulting in stable or improved vision.[8–11] However, recurrent or newly developed polypoidal lesions may affect vision during a longer follow-up.[12–14] Beside the characteristic polypoidal dilatations, lacy CNV-like vessels can develop with exudation.[15] Unexpected subretinal hemorrhages after PDT are another serious problem in eyes with PCV.[16]

The efficacy of the anti–vascular endothelial growth factor (VEGF) drug bevacizumab (Avastin; Genentech, South San Francisco, California, USA) is well known for treating CNV secondary to AMD[17,18]; however, the efficacy for PCV seems limited.[19–21] We found that intravitreal injections of bevacizumab reduced exudative fluid but did not affect the original abnormal vasculatures and caused subsequent recurrence of fluid in most eyes.[10,19]

Combined PDT and intravitreal bevacizumab therapy was introduced recently as a treatment strategy for AMD.[22,23] It resulted in favorable outcomes, including improved visual acuity (VA), low rates of retreatment, and reduced fluorescein leakage, lesion size, and retinal thickness without new safety concerns.[24–26]

PDT monotherapy and anti-VEGF drugs have separate levels of efficacy and limitations. For PCV, PDT occluded polypoidal vascular lesions and anti-VEGF drugs resolved the associated fluid. If combining these therapies could result in additional or complementary effects, there may be further improvements in visual and anatomic outcomes in eyes with PCV compared with PDT monotherapy. The aim of the current study was to determine if the combined treatment of intravitreal bevacizumab and PDT for PCV is

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associated with additional efficacy during the 1-year follow-up.

METHODS

A TOTAL OF 146 JAPANESE PATIENTS WITH SYMPTOMATIC, treatment-naïve, subfoveal PCV were treated with PDT with or without an intravitreal injection of bevacizumab and followed up for more than 1 year. The initial treatments were chosen according to the time period. From March 2005 through September 2006, PDT monotherapy was administered and from October 2006 through August 2008, PDT combined with intravitreal bevacizumab injection was administered to consecutive patients who agreed to the use of bevacizumab. The data from patients who did not agree to bevacizumab treatment during the latter period were included as having been treated by PDT monotherapy.

The diagnosis of PCV was established based on the finding of polyplike choroidal vessel dilatation with or without a branching vascular network on indocyanine green angiography (ICGA). The ICGA images were obtained using a camera (TRC-50EX; Topcon, Tokyo, Japan) in all patients. A scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2; Heidelberg Engineering, Heidelberg, Germany) also was used beginning in June 2005. Eyes with either polypoidal lesions or regions of branching vascular networks in the subfovea were included. Eyes with coexisting CNV were excluded.

• TREATMENT AND FOLLOW-UP: Three authors (F.G., M.S., and M.T.) treated and followed up all patients. PDT with verteporfin was administrated in a full-fluence dose according to the Treatment of Age-Related Macular Degeneration with Photodynamic Therapy protocol[7] with 1 modification: the greatest linear dimension (GLD) of the lesion for laser exposure during PDT was determined based on ICGA findings in all patients as previously reported.[9] In eyes treated with combined therapy, 1.25 mg bevacizumab was injected intravitreously 1 day before PDT. Before the bevacizumab injections, the eyes and lids were disinfected with povidone iodine after topical anesthesia was administered. Bevacizumab was injected 3.5 to 4.0 mm posterior to the limbus using a 29-gauge needle. A topical antibiotic was applied 4 times daily for 1 week.

All patients underwent a comprehensive ocular examination, including measurement of the best-corrected visual acuity (BCVA) using Landolt C charts, fundus examinations, color fundus photography, and optical coherence tomography (OCT) using Stratus or Cirrus devices (Carl Zeiss Meditec, Dublin, California, USA) at least 1, 3, 6, 9, and 12 months after the initial treatments. If patients were not examined at those follow-up dates, the point closest to the defined date was selected. Additional intermediate visits were scheduled for patients with combined therapy at 4- to 6-week intervals. Fluorescein angiography and ICGA were performed at baseline and every 3 months to assess the resolution and recurrence of abnormal polypoidal lesions. Additional treatments were performed when abnormal vascular lesions associated with exudative fluid were seen on ICGA with the same therapy received at baseline. In the combined treatment group, intravitreal bevacizumab without PDT was administered when exudative fluid was seen on OCT during the intermediate visits.

The resolution of PCV lesions after the initial treatment was defined as the disappearance of symptomatic polypoidal vasculatures on ICGA at 3 months with complete resolution of exudative fluid on OCT. A recurrence was defined by the reappearance of abnormal vasculature on ICGA with exudative fluid on OCT.

The development of serious adverse events related to the initial treatment was recorded. Because a subretinal hemorrhage is the major adverse event associated with PDT for PCV, the incidence of new subretinal or subpigment epithelial hemorrhage of more than 1 optic disc diameter developed within 1 month after the initial treatment was assessed.

• STATISTICAL ANALYSIS: The BCVA was converted to the logarithm of the minimal angle of resolution (logMAR) equivalents. Missing data at intermediate time points were input using the method of last observation carried forward. Each 0.10-unit difference in logMAR VA was calculated as 1 line.

The changes in BCVA at 1, 3, 6, 9, and 12 months from baseline with each treatment were evaluated by analysis of variance repeated measures. The BCVA values at 1, 3, 6, 9, and 12 months were compared between treatments by analysis of covariance (ANCOVA) to rule out any bias correlated with the visual outcomes. The initial ANCOVA model included the treatment as a factor and the baseline age, gender, BCVA, GLD, presence or absence of pigment epithelial detachment (PED), and the presence or absence of subretinal or subpigment epithelial hemorrhages as covariates. A PED was defined as the presence of serous or hemorrhagic PEDs, or both, 1 disc area or larger on ICGA and OCT independent of the polypoidal lesions. Appropriate covariates were selected for the final ANCOVA model using the Akaike information criterion.[27] Stepwise regression analysis was performed to elucidate factors that affected the BCVA at 12 months among baseline age, gender, BCVA, GLD, PED, hemorrhage, and the treatment. To determine which factors were associated with a BCVA of 20/40 or better at 12 months, logistic regression analysis was performed using the same variables as the stepwise regression analysis. We also compared the rate of resolution and recurrence between treatments (Pearson chi-square test). The incidence of hemorrhage developing after PDT and other adverse events also were compared. Statistical analyses were performed with SAS software version 9.1 (SAS Institute,

TABLE. Baseline Characteristics of Patients with Polypoidal Choroidal Vasculopathy Treated by Bevacizumab Combined with Photodynamic Therapy or Photodynamic Therapy Monotherapy

	Combined Therapy (n = 61)	PDT Monotherapy (n = 85)
Sex, no. (%)		
Men	44 (72.1)	68 (80.0)
Women	16 (26.2)	17 (20.0)
Age (yrs)		
Mean ± SD	70.9 ± 7.1	70.9 ± 6.8
Range	54 to 83	53 to 85
Lesion GLD (μm)		
Mean ± SD	2626 ± 1138	2521 ± 996
Range	500 to 5800	800 to 5300
LogMAR visual acuity (Snellen)		
Mean	0.48 (20/60)	0.45 (20/56)
Median	0.40 (20/50)	0.40 (20/50)
Range	1.15 (20/286) to 0 (20/20)	1.05 (20/222) to 0 (20/20)
SRH, no. (%)	21 (34.4)	22 (25.9)
PED, no. (%)	30 (49.2)	36 (42.4)

GLD = greatest linear dimension; logMAR = logarithm of the minimal angle of resolution; PDT = photodynamic therapy; PED = pigment epithelial detachment; SD = standard deviation; SRH = subretinal and/or subpigment epithelial hemorrhage.

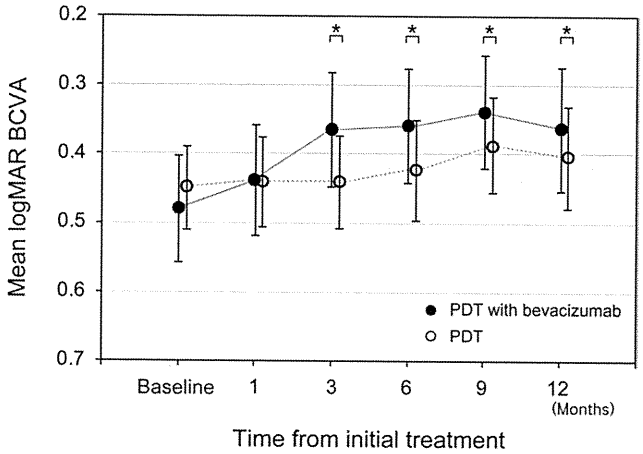

FIGURE 1. Graph showing changes in the best-corrected visual acuity (BCVA; mean with 95% confidence interval) in eyes with polypoidal choroidal vasculopathy treated with photodynamic therapy (PDT) with (solid circles) and without (open circles) intravitreal bevacizumab. The BCVA was converted to logarithm of the minimal angle of resolution (logMAR) units. The mean BCVA in the combined therapy group was significantly better than in the monotherapy group at 3, 6, 9, and 12 months. *$P < .05$.

Cary, NC). P values less than .05 were considered significant, and all tests were 2-sided.

RESULTS

A TOTAL OF 146 EYES OF 146 PATIENTS (113 MEN, 33 WOMEN) were included; 61 eyes of 61 patients (44 men, 16 women) were treated initially with combined therapy and 85 eyes of 85 patients (68 men, 17 women) were treated with PDT monotherapy. The patient characteristics in both groups are shown in the Table. The baseline factors, including BCVA, gender, age, GLD, and the rates of coexisting PED, hemorrhage, or both, did not differ significantly between treatments.

• VISUAL OUTCOMES: The mean BCVA scores over time are shown in Figure 1. The BCVA in the combined therapy group improved significantly from baseline at 1 month ($P = .016$), 3 months ($P < .0001$), 6 months ($P < .0001$), 9 months ($P < .0001$), and 12 months ($P = .0027$) after the initial treatment; in the PDT monotherapy group, the BCVA improved significantly at 9 months ($P = .011$) and tended to improve at 12 months ($P = .056$).

To compare the visual outcomes at various time points between treatments, we performed ANCOVA analyses. Because the initial ANCOVA analyses suggested that some baseline factors such as the BCVA, GLD, and the presence of PED were correlated with the posttreatment VA at each time point, those factors were adjusted and the mean logMAR VAs were compared between treatments. Ultimately, the mean BCVA in the combined therapy group was significantly better than that in the monotherapy group at 3 months ($P = .0016$), 6 months ($P = .028$), 9 months ($P = .038$), and 12 months ($P = .048$) from the initial treatment. The data showed that combined therapy had a maximum effect on VA improvement compared with PDT monotherapy at 3 months, and this effect gradually decreased with time but was still significant at 12 months.

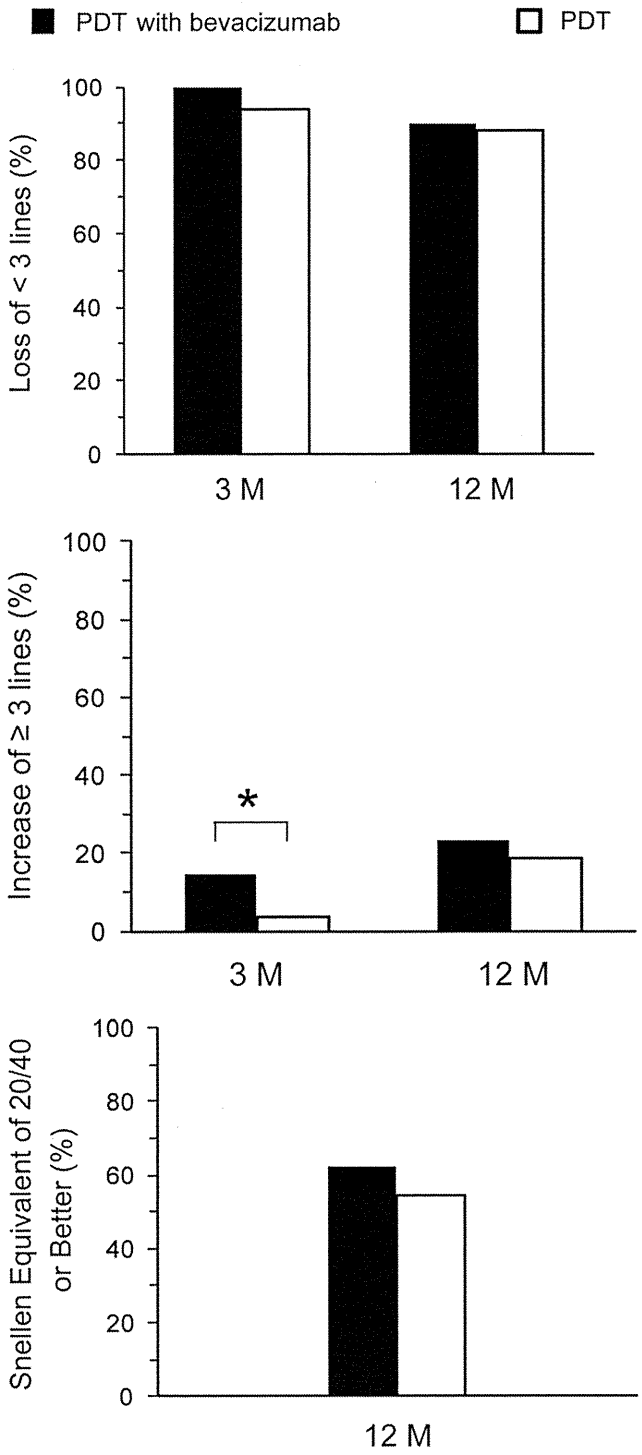

FIGURE 2. Bar graphs showing the rates of eyes with losses or gains in visual acuity at 3 and 12 months (M) and with a best-corrected visual acuity (BCVA) with a Snellen equivalent of 20/40 or better at 12 months associated with photodynamic therapy (PDT) combined with intravitreal bevacizumab compared with PDT monotherapy for polypoidal choroidal vasculopathy. (Top) Percentage of eyes in each group with improved or maintained BCVA (a loss of fewer than 3 lines of vision). The rates do not differ significantly between the groups. (Middle) Percentage of eyes with improved BCVA of 3 lines or more. The rate in the PDT combined with bevacizumab group is significantly ($P = .044$) higher than in the PDT group at 3 months. However, the rates do not differ significantly at 12 months. (Bottom) Percentages of eyes with a BCVA of 20/40 or better at 12 months. The rates do not differ significantly.

Eyes gained a mean of 0.12 logMAR unit (1.2 lines) in the combined therapy group and 0.04 logMAR unit (0.4 lines) in the PDT monotherapy group over 12 months. All eyes (100%) in the combined therapy group and 80 eyes (94.1%) in the PDT monotherapy group had improved or stable VA (defined as a loss of 3 or fewer lines of vision at 3 months); the rates were 90.2% (55/61) and 88.2% (75/85) at 12 months, respectively, in the treatment groups. An improvement in the BCVA of 3 lines or more was seen in 14.8% (8/44) in the combined therapy group and in 3.5% (4/79) in the PDT monotherapy group at 3 months, which was significant (Fisher exact test, $P = .044$); however, the respective rates were 23.0% and 18.8% at 12 months, which were not significant. The percentages of eyes with a BCVA of 20/40 or better at 12 months was 62.3% in the combined therapy group and 54.1% in the monotherapy group (Figure 2).

To elucidate factors associated with the long-term BCVA, we performed multivariate analyses. Based on stepwise regression analyses, the baseline BCVA ($P < .0001$), the GLD ($P = .0015$), PED ($P = .0022$), and treatment ($P = .048$) significantly affected the BCVA at 12 months. Therefore, the better BCVA at 12 months was correlated with a higher baseline BCVA, smaller GLD, no baseline PED, and the combined treatment. The combined therapy also was identified as a factor associated with a BCVA at 12 months of 20/40 or better from the logistic regression analysis (odds ratio, 2.66; $P = .040$), in addition to better baseline BCVA ($P < .0001$), no baseline PED ($P = .0072$), and smaller GLD ($P = .0094$).

• CHANGES IN LESIONS AND ADDITIONAL TREATMENTS: After the initial treatment, the resolution of lesions at 3 months was confirmed in 78.7% (48/61) of eyes in the combined therapy group and in 75.3% (64/85) of eyes in the monotherapy group. Among those eyes, the lesions recurred in 43.8% (21/48) of eyes in the combined therapy group and 40.6% (26/64) in the PDT monotherapy group during the 1-year follow-up. These rates did not differ significantly between treatments ($P = .63$, $P = .74$, Pearson chi-square test). During the 1-year follow-up, a mean of 0.43 PDT and 0.92 bevacizumab additional injections in the combined therapy group and a mean of 0.66 PDT retreatments performed in the PDT monotherapy group were administered.

• ADVERSE EVENTS: Subretinal hemorrhages are well-known adverse events associated with PDT for PCV. In the PDT monotherapy group, a subretinal or subpigment epithelial hemorrhage, or both, of 1 disc diameter or more developed in 15 eyes (17.7%) within 1 month after the initial treatment; however, this occurred in 3 eyes (4.9%) in the combined therapy group. The incidence of hemorrhages was significantly lower in the combined therapy group ($P = .022$, Fisher exact test). Hemorrhages seriously affected vision in 5 eyes in the PDT monotherapy group and in 1 eye in the combined therapy group. One eye with the combined therapy developed a retinal pigment epithelial tear within 1 month, which did not affect vision. No other serious adverse events were recorded.

DISCUSSION

THE PREVALENCE OF PCV IS HIGH IN PRESUMED EXUDATIVE AMD in Asian individuals,[4–6] and when considering the effective treatment for exudative AMD in this race, better management options for PCV should be explored.

PDT with verteporfin resulted in superior results for treating PCV compared with CNV secondary to AMD in the 1-year visual outcomes in Japanese patients.[9] The results may be satisfactory; however, in this era of anti-VEGF therapy, positive anatomic results such as resolution of fluid resulting from intravitreal bevacizumab also were observed in eyes with PCV,[19,28,29] suggesting that a certain amount of bevacizumab may act directly on the abnormal vasculature in the subretinal pigment epithelial spaces. However, limited efficacy of bevacizumab monotherapy was observed when treating the abnormal vasculature of PCV, and we suggest 2 possible reasons: that an insufficient amount of bevacizumab may reach the subretinal pigment epithelial spaces because of its larger molecular size or the VEGF-independent nature of PCV.[10,19] It seemed important to determine whether there was additional or complimentary efficacy of bevacizumab combined with PDT when treating PCV, especially during a longer follow-up.

In the current study, an additional positive effect of an intravitreal bevacizumab was observed in the visual outcomes through 1 year. During the early treatment period, the efficacy of bevacizumab for improving BCVA was apparent. ANCOVA showed that the combined therapy resulted in maximum improvement in the BCVA at 3 months, which was attributed primarily to rapid resolution of the exudative fluid and fibrin induced by bevacizumab, as we observed previously.[19] The leakage from abnormal vessels was treated effectively through the reduction of VEGF. Moreover, bevacizumab may prevent transient induction of VEGF after PDT[30] and subsequent reaction of vessels.

We also found a significantly lower rate of subretinal or subpigment epithelial hemorrhages, or both, associated with PDT in the combined treatment group. The rate of development of hemorrhage of 1 optic disc diameter within 1 month after PDT monotherapy was 17.7%, which was almost the same as the 19% that we reported previously.[9] However, hemorrhages developed in only 4.9% of the patients in the combined treatment group. The development of subretinal hemorrhages after PDT is a characteristic adverse event after PDT used to treat PCV, and something that physicians want to avoid. In addition to the decreased vascular permeability, the vasoconstrictive effect of bevacizumab[31,32] may result in suppression of hemorrhages. The reduced risk of hemorrhages induced by bevacizumab would encourage ophthalmologists to choose the combined therapy.

For the long-term VA, however, the impact of the combined therapy decreased possibly because of lesion recurrences and atrophic tissue changes related to persistent lesions. Resolution of the original PCV vasculature was observed at a similar rate between the treatments, and the recurrence rates also were similar. This seemed reasonable, because our previous studies showed that bevacizumab monotherapy did not cause regression of the abnormal PCV vasculature, and the resolution of polypoidal lesions may be attributed mainly to PDT alone.[9,10,19] However, analyses elucidating factors associated with a better 12-month VA selected combined therapy as significant in addition to better baseline VA, smaller lesion size, and the absence of PED. The vision in PCV after PDT beyond 1 year is reported to be not as good as within 1 year.[14] During longer follow-up periods, several additional sessions of PDT would be necessary for the recurrences of PCV; that means an increase of risks induced by PDT such as subretinal hemorrhages or ischemic damage of normal choroidal tissue.[33] PDT combined with an anti-VEGF drug can reduce those side effects of PDT. Also, injection of an anti-VEGF drug without PDT is an option for eyes with good vision or intermediate treatments for recurrent fluid or the CNV-like abnormal vasculature that can developed after PDT.[15] Thus, the appropriate combination of an anti-VEGF drug and PDT is important to maintain better VA for the long term.

Recently, Kokame and associates reported the 6-month interim results of monthly injections of ranibizumab (Lucentis; Genentech, South San Francisco, California, USA) in 12 eyes with PCV (PEARL Trial; clinicaltrials.gov identifier, NCT00424710).[34] The results showed that ranibizumab monotherapy improved the mean VA at 6 months by a mean of 7.2 letters and that 2 patients (17%) gained more than 15 letters. That seemed almost the same as the visual results in our combined therapy group at 12 months. In their manuscript, the polypoidal lesions seen on ICGA decreased in 4 eyes (33%) at 6 months, and the authors suggested that the smaller molecular size of ranibizumab compared with bevacizumab and monthly continuous injections may enable the drug to penetrate into the subretinal pigment epithelial space more efficiently. However, it meant difficulties in achieving complete resolution

of polypoidal lesions even after 6 monthly injections of ranibizumab. With the fact that the choroidal vascular networks persisted in all 12 eyes, ranibizumab monotherapy has the potential for continuous risk of recurrences. The pathogenesis of PCV is unknown, but the results suggested that VEGF may not be associated with the development and maintenance of abnormal vasculature of PCV.

The current study was limited because it was retrospective and not a randomized, comparative trial. However, the absence of bias in the choice of the initial treatment and a relatively large number of eyes that had not received any previous treatments compensated for these limitations. We believe the study results offer informative data to clinicians. From the results of the prospective randomized clinical trial comparing the 6-month results among PDT combined with intravitreal ranibizumab, PDT monotherapy, and ranibizumab for PCV (EVEREST Study; clinicaltrials.gov identifier, NCT00674323), it would be helpful to know which treatment should be chosen as the initial therapy, although the differences of efficacy between ranibizumab and bevacizumab remained to be clarified. Furthermore, improved methods to manage PCV are needed to preserve vision over the long term.

THE AUTHORS INDICATE NO FINANCIAL SUPPORT OR FINANCIAL CONFLICT OF INTEREST. INVOLVED IN DESIGN (F.G.) AND conduct (F.G., M.S., M.T.) of study; data collection (F.G., M.S., Y.S.); Analysis and interpretation of the data (F.G., T.W., Y.S.); and Writing (F.G., T.W.), critical revision (M.T., T.W.), and approval (F.G., M.S., M.T.) of the manuscript. The off-label use of bevacizumab and this retrospective study were approved by the Institutional Review Board of Osaka University Hospital, Osaka, Japan. All patients provided written informed consent. The study adhered to the tenets of the Declaration of Helsinki. The authors thank Dr Toshimitsu Hamasaki, Department of Biomedical Statistics, Osaka University Medical School, for performing the statistical analyses.

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