TABLE 2. Association of Single Nucleotide Polymorphisms With Visual Acuity and Visual Acuity Change After Intravitreal Ranibizumab Treatment for Neovascular Age-Related Macular Degeneration

	VA			VA Change	
	Baseline	3 Months	12 Months	3 Months	12 Months
Y402H-CC (n = 3, 4.0%)	0.52 ± 0.40	0.47 ± 0.42	0.51 ± 0.50	−0.04 ± 0.24	−0.01 ± 0.33
Y402H-CT (n = 19, 25.3%)	0.50 ± 0.39	0.49 ± 0.42	0.52 ± 0.46	−0.02 ± 0.12	0.03 ± 0.28
Y402H-TT(n = 53, 70.7%)	0.89 ± 0.77	0.93 ± 0.80	0.94 ± 1.09	0.04 ± 0.12	0.03 ± 0.33
P	NS	NS	NS	NS	NS
I62V-GG (n = 47, 65.3%)	0.49 ± 0.39	0.51 ± 0.46	0.52 ± 0.54	0.23 ± 0.19	0.05 ± 0.34
I62V-GA (n = 21, 29.1%)	0.58 ± 0.42	0.40 ± 0.37	0.49 ± 0.40	−0.18 ± 0.24	−0.09 ± 0.28
I62V-AA (n = 4, 5.6%)	0.53 ± 0.27	0.48 ± 0.17	0.43 ± 0.34	−0.05 ± 0.12	−0.10 ± 0.18
P	NS	NS	NS	.0009	NS
A69S-TT (n = 33, 44.0%)	0.58 ± 0.45	0.56 ± 0.50	0.61 ± 0.57	−0.01 ± 0.25	0.03 ± 0.33
A69S-TG (n = 26, 34.7%)	0.41 ± 0.34	0.38 ± 0.35	0.39 ± 0.41	−0.03 ± 0.19	−0.01 ± 0.27
A69S-GG (n = 16, 21.3%)	0.62 ± 0.40	0.55 ± 0.42	0.58 ± 0.53	−0.07 ± 0.20	−0.03 ± 0.36
P	NS	NS	NS	NS	NS

NS = not significant; VA = visual acuity.

TABLE 3. Association of Single Nucleotide Polymorphisms With Disappearance of Retinal Exudative Change After Intravitreal Ranibizumab Treatment for Neovascular Age-Related Macular Degeneration

	Retinal Exudative Change			
	3 Months		12 Months	
	Resolved	Remained	Resolved	Remained
Y402H-CC (n = 3, 4.0%)	2	1	3	0
Y402H-CT (n = 19, 25.3%)	14	5	10	9
Y402H-TT (n = 53, 70.7%)	35	18	34	18
P	NS	NS	NS	NS
I62V-GG (n = 47, 65.3%)	30	17	30	16
I62V-GA (n = 21, 29.1%)	16	5	12	9
I62V-AA (n = 4, 5.6%)	3	1	2	2
P	NS	NS	NS	NS
A69S-TT (n = 33, 44.0%)	23	10	18	14
A69S-TG (n = 26, 34.7%)	21	5	20	9
A69S-GG (n = 16, 21.3%)	7	9	9	7
P	NS	NS	NS	NS

NS = not significant.

month in both the typical neovascular AMD and PCV groups ($P < .0001$). Better baseline VA resulted in better VA outcome after ranibizumab treatment. Similarly, VA at twelfth month was significantly correlated with baseline VA ($P < .0001$).

In patients with typical neovascular AMD, patient age was significantly associated with VA change at the third month, VA at the twelfth month, and VA change at the twelfth month (Figure 1, $P = .040$, $P = .020$, and $P = .0014$, respectively). In contrast, the age of patients with PCV was not associated with VA or VA change at the third and twelfth months (Figure 2, $P > .22$).

The association of greatest linear dimension with VA or VA change showed a trend similar to that of the aforementioned age association. In patients with typical neovascular AMD, the greatest linear dimension showed a significant association with VA at the third month and twelfth month, and VA change at the twelfth month (Figure 3; $P = .015$, $P = .0004$, and $P = .0021$, respectively). In contrast, greatest linear dimension in patients with PCV was not associated with VA or VA change at either the third or twelfth month (Figure 4, $P > .63$).

Finally, we evaluated the association of genetic polymorphisms with the treatment response in AMD. For

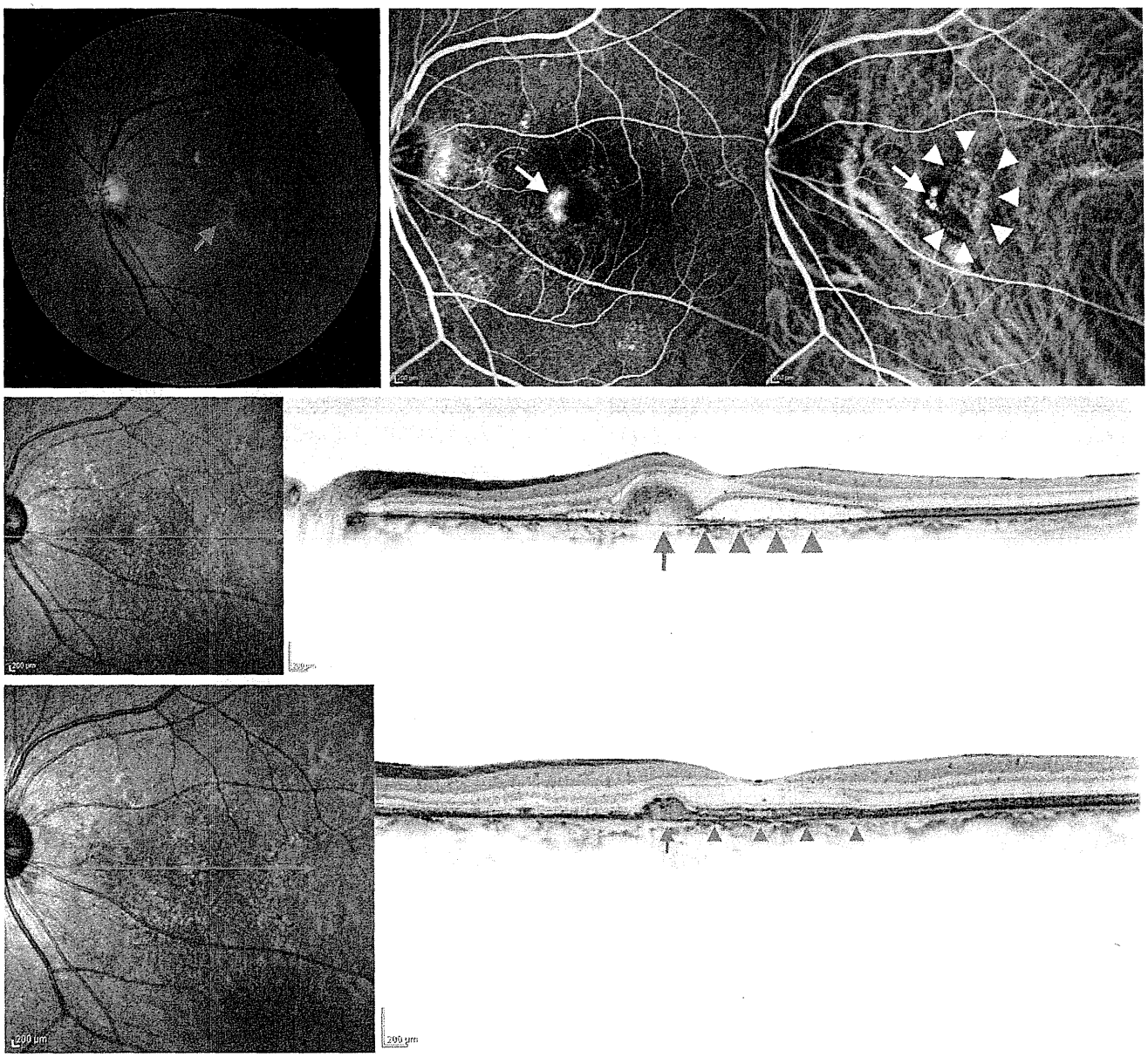

FIGURE 5. An eye with PCV at baseline and 3 months after the first ranibizumab injection. At baseline, (Top left) color photograph shows fibrin exudate (arrow) over the polypoidal lesion, (Top middle) fluorescein angiograph shows fluorescein leakage (arrow) from the polypoidal lesion, and (Top right) indocyanine green angiography shows polypoidal lesion (arrow) and network vessels (arrowheads) beneath the fovea. (Middle) Optical coherence tomography shows fibrin and retinal edema over the polypoidal lesion (arrow) and retinal detachment over the network vessels (arrowheads). The visual acuity was 18/20. (Bottom) At 3 months after the first ranibizumab injection, optical coherence tomography shows polypoidal lesion (arrow) and network vessels (arrowheads) without retinal detachment or retinal edema and the visual acuity improved to 24/20.

this analysis, typical neovascular AMD and PCV were first combined for evaluation and then separated for further evaluation. The associations of SNPs (CFH Y402H, I62V, and ARMS2 A69S) with VA and/or VA change are shown in Table 2. There was no significant association between these SNPs and baseline VA, VA or VA change at the third month, and VA or VA change at the twelfth month, except for the association between CFH I62V and VA change at the third month; eyes with a GA genotype had better VA change than those with a GG genotype ($P = .0009$). After Bonferroni's correction, the association of I62V with VA change at the third months became weaker ($P = .027$). Furthermore, there was not an association trend such as GG > GA > AA or GG < GA < AA. In the OCT findings, the 3 SNPs were not significantly associated with the resolution of retinal exudative change at either the third or twelfth month (Table 3). When patients with typical neovascular AMD and PCV were evaluated separately, the 3 SNPs did not show any association

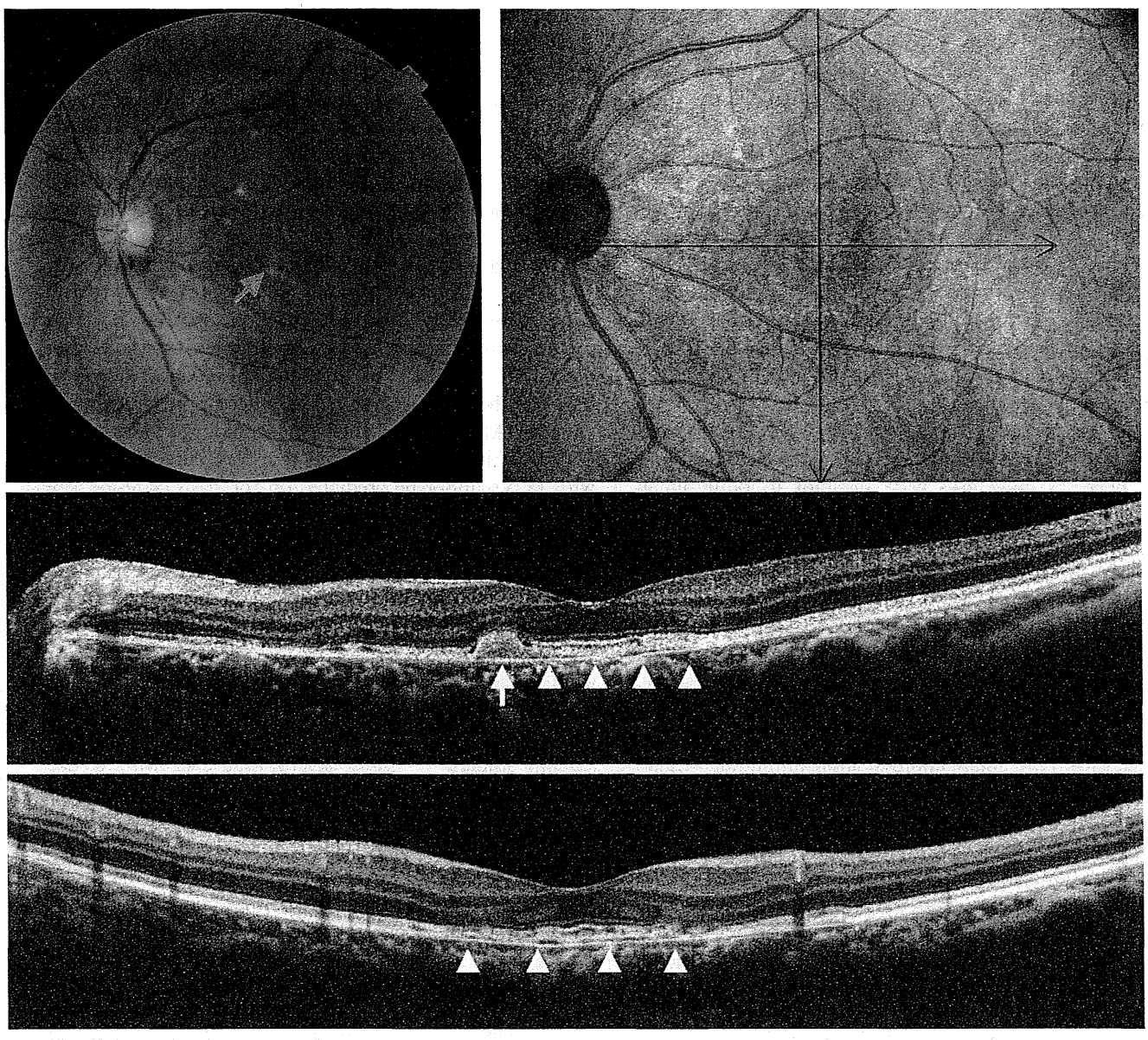

FIGURE 6. Twelve months after the first intravitreal injection of ranibizumab. (Top left and right) Color photograph shows disappearance of fibrin exudate, and (Middle) horizontal optical coherence tomography shows inactivated polypoidal lesion (arrow) without retinal detachment or retinal edema over the network vessels (arrowheads). (Bottom) Vertical optical coherence tomography shows no retinal detachment or retinal edema over the network vessels (arrowheads). The visual acuity was maintained at 24/20.

with VA, VA change, or the resolution rate of retinal exudation.

DISCUSSION

IN THE PRESENT STUDY, WE HAVE SHOWN THAT RANIBIzumab treatment equivalently reduced retinal exudative changes in patients with typical neovascular AMD or PCV. However, visual prognosis after treatment was better in patients with PCV compared to those with typical neovascular AMD. Furthermore, age and greatest linear dimension size were significantly associated with visual prognosis only in patients with typical neovascular AMD, not in those with PCV. SNPs in *CFH* and *ARMS2* genes were not associated with VA prognosis or resolution of retinal exudation in patients with either typical neovascular AMD or PCV after ranibizumab treatment.

Recently, it has been shown that ranibizumab can only maintain VA when used in AMD patients with relatively good VA.[19] Most studies that have shown VA improvements in AMD after ranibizumab treatment have only included patients with VA of 20/40 to 20/400.[20–23] Therefore, it is surprising that in our study, VA significantly

improved in patients with PCV, because we included patients with relatively good baseline VA. The baseline VA was better than 20/20 in 8 eyes (7.62%), better than 20/25 in 17 eyes (16.2%), and better than 20/40 in 45 eyes (42.9%). Including patients with good VA makes it more difficult to detect VA improvement after treatment. Indeed, the VA in eyes with typical neovascular AMD in the present study was only maintained and did not improve. However, patients with PCV showed significant VA improvement after ranibizumab treatment. Thus, a diagnosis of PCV is a favorable factor for VA outcome after ranibizumab treatment. Considering that retinal edema was observed more often in typical neovascular AMD than PCV, less retinal edema in PCV might lead to better VA outcome after treatment.

Seven eyes with typical neovascular AMD (14.3%) and 8 eyes with PCV (14.3%) received additional treatment with PDT, as these eyes were resistant to ranibizumab injections. Since the average VA in these eyes did not significantly change during follow-up, the aforementioned favorable visual prognosis in patients with PCV can be attributed to the effects of ranibizumab. Although early reports have suggested relatively poor results when ranibizumab is used to treat PCV, recent studies have shown that the VA in eyes afflicted with PCV improves after ranibizumab treatment.[3–8] Some of these studies have included patients with relatively good baseline VA.[5,8] Thus, the VA in eyes with PCV might improve after ranibizumab treatment, even if the eyes have a relatively good baseline VA. The rate of eyes resistant to ranibizumab (14.3%) seems to be higher than previously reported resistance rates (less than 5%). Previous treatment might increase the rate of eyes resistant to ranibizumab. In the present study, furthermore, additional PDT was performed when their retinal edema or subretinal fluid did not decrease after initial 3-loading-injection ranibizumab treatment. If we continue ranibizumab treatment after the 3 loading injections, the retinal edema or subretinal fluid might disappear.

The visual prognosis in eyes with PCV has been generally thought to be better than in those with typical neovascular AMD.[24,25] Visual outcomes after PDT were also reported to be better in PCV than in typical neovascular AMD.[10] Therefore, it should not be surprising that the VA prognosis after ranibizumab treatment was better in PCV than in typical neovascular AMD, because accumulating evidence has revealed substantial effects of anti-VEGF treatment on resolving retinal exudative changes in PCV.[3,4,6–8,26,27] Also in the present study, ranibizumab significantly reduced retinal exudative change in eyes with PCV (Figures 5 and 6). However, the polypoidal lesions of PCV are barely resolved following anti-VEGF treatment.[7,27–29] Although residual polypoidal lesions might easily cause a relapse in retinal exudative changes, the number of additional treatments was not significantly different between the typical neovascular AMD and PCV groups in the present study. Studies with follow-up periods of more than 1 year might reveal differences in the number of additional treatments required between patients with typical neovascular AMD and those with PCV.

Both the MARINA[17] and ANCHOR studies[18] have shown that important predictors of VA outcomes in AMD are baseline VA, CNV lesion size, and age at the time of ranibizumab treatment. The present study also confirmed that baseline VA is significantly correlated with VA outcomes in both typical neovascular AMD and PCV. However, the size of greatest linear dimension and age only correlated with VA outcome in patients with typical neovascular AMD and not in those with PCV. This inconsistency has also been reported in similar studies that used PDT; the size of greatest linear dimension was significantly correlated with VA outcome in typical neovascular AMD, while the same could not be said for PCV.[30] It has been suggested that VA outcome in eyes with PCV cannot be predicted on the basis of lesion size after any type of treatment.

Recently, the association between the response to treatments and polymorphisms in AMD/PCV susceptibility genes has been intensively explored. With respect to VA outcome after PDT, several studies have shown significant associations of *ARMS2/HTRA1* with AMD and PCV,[14–16] while *CFH* does not have a definite association. However, the association of *ARMS2/HTRA1* or *CFH* with visual outcome after ranibizumab treatment is still controversial. Several studies[31–33] have shown that eyes with a CC genotype in *CFH* Y402H have worse visual prognosis after anti-VEGF treatment, while a recent study[34] has shown that eyes with a TT genotype have worse visual prognosis. Lee and associates[35] have shown no association between Y402H and visual outcome after ranibizumab treatment in AMD. In general, genetic variations in *ARMS2/HTRA1* do not seem to be associated with response to anti-VEGF treatment;[31,32,34] however, 1 study[36] has reported that eyes with the TT genotype in *ARMS2* A69S have worse VA outcome. The present study did not reveal any associations of SNPs in *CFH* and *ARMS2* with VA outcome. Our negative findings are consistent with previous reports from Europe and the United States.[31,32,34] Also in East Asian populations, *ARMS2/HTRA1* could not be associated with response to ranibizumab treatment.

Regarding *CFH* Y402H, Imai and associates evaluated VA change in 83 Japanese patients with AMD for 3 months after bevacizumab treatment. In their study, they showed that eyes with the CT genotype have worse VA outcomes than eyes with the TT genotype, although there was no CC genotype in their cohort.[37] The frequency of the Y402H C allele is very low in East Asian populations; this often leads to false-negative results in association studies.[11,38] Although our findings suggest no association between *CFH* gene polymorphisms and response to ranibizumab treatment in both typical neovascular AMD and PCV, a potential association should be further evaluated using a larger cohort.

Limitations of this study include its retrospective nature and small sample size. In the present study, previous treatments had been performed in 25.7% of patients. It has been demonstrated that VA prognosis after PDT combined with anti-VEGF treatment is worse in eyes with PCV that recurred after PDT.[39,40] Although previous treatment did not affect the visual outcome in the present study (data not shown), increasing the cohort size might reveal a significant effect of previous treatments on the response to ranibizumab. Increasing the cohort size might also reveal associations between SNP variations and response to ranibizumab. Furthermore, our findings would have to be investigated with prospective studies since this study is retrospective. Recently, Yamaoka and associates have shown that hypertension is associated with the occurrence of PCV.[41] Although other study reported no association between hypertension and PCV[42] and the prevalence of hypertension was not significantly different between typical neovascular AMD and PCV in the present study (69% vs 66%, $P = .83$), examinations of the association between hypertension and treatment response might lead to further understanding of the pathophysiology of PCV.

In conclusion, we have shown that AMD subtype can be a predictive factor for response to ranibizumab treatment. VA outcome is better in patients with PCV compared to those with typical neovascular AMD, whereas the resolution of retinal exudative changes is similar in both groups. Lesion size and age can be used to predict the VA prognosis after ranibizumab treatment in typical neovascular AMD; however, these parameters cannot be used to predict the VA prognosis in PCV. Our study suggests that there is no association between SNPs in the *CFH* and *ARMS2* genes and responsiveness of typical neovascular AMD or PCV to ranibizumab treatment.

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RETINAL DISORDERS

Relationship between retinal morphological findings and visual function in age-related macular degeneration

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Abstract
Background We aimed to study the retinal morphological findings associated with exudative age-related macular degeneration (AMD) and their association with visual prognosis.
Methods We retrospectively reviewed the medical records of 96 consecutive patients (96 eyes) with exudative AMD. Retinal structural changes were examined using optical coherence tomography (OCT).
Results Initial OCT examination showed cystoid macular edema in 18 eyes (18.8%), fibrin exudate in 56 eyes (58.3%), and hyperreflective foci within the neurosensory retina in 78 eyes (81.3%). Upon initial examination, an external limiting membrane (ELM) line was detected under the fovea in 64 eyes (66.7%). Using Pearson's correlation analyses, final visual acuity (VA) was correlated with initial VA (r=0.61, p<0.001), age (r=0.34, p<0.001), initial total foveal thickness (r=0.41, p<0.001), presence of hyperreflective foci (r=0.40, p<0.001), and detection of a foveal ELM line (r=0.55, p<0.001). After multiple regression analysis, final VA correlated with initial VA (r=0.48, p< 0.001), initial presence of hyperreflective foci (r=0.23, p= 0.054), and detection of a foveal ELM line (r=0.36, p= 0.008).
Conclusions In eyes with exudative AMD, final VA was most correlated with initial VA. In addition, the initial integrity of the foveal outer retina was partially correlated with the visual prognosis. The initial ELM condition was associated with good final VA, while the initial presence of hyperreflective foci in the foveal neurosensory retina was associated with poor final VA.

Keywords Age-related macular degeneration · External limiting membrane · Hyperreflective foci · Optical coherence tomography · Polypoidal choroidal vasculopathy

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Introduction

Age-related macular degeneration (AMD) is a leading cause of blindness worldwide in individuals aged more than 50 years [1]. Although treatment for the disease has progressed dramatically since the development of anti-vascular endothelial growth factor (VEGF) therapy, not all patients achieve good vision [2–4]. A combination of photodynamic therapy (PDT) and anti-VEGF therapy is a promising option [5], but it might not markedly alter the prognosis of AMD [6]. Considering both the physical and financial costs of treatment [7], prediction of the visual prognosis at the patients' initial visits would be very useful to both patients and clinicians.

A more detailed evaluation of retinal structure and function is needed to predict visual prognosis [8]. For this purpose, we have been trying to make the best use of optical coherence tomography (OCT). Current models of commercially available spectral-domain OCT instruments provide fine images (up to 5-μm resolution) of the retinal structure [9, 10]. Using spectral-domain OCT, Hayashi et al. showed a correlation between retinal structure and function in eyes with AMD that had undergone PDT [11]. In short, the status of the junction between the inner and outer segments of the photoreceptors (IS/OS) [12, 13], which is considered to

reflect photoreceptor integrity, is associated with visual acuity (VA) [14]. Other authors have confirmed the association of IS/OS status with VA [15] and with retinal sensitivity as measured using microperimetry [16]. In addition to IS/OS, the status of the external limiting membrane (ELM) has also been reported to be a good indicator of visual function in eyes with AMD [17]. Evaluation of alterations in these retinal layer patterns at the time of the initial examination provides more detailed information about the retinal status [8, 18, 19].

In addition to the retinal layer pattern, the presence of hyperreflective foci represents another possible predictive factor of VA [18]. Hyperreflective foci are reported to be present in diabetic macular edema [20], retinal vein occlusion [21], and AMD [18], and have been suggested to be subclinical features of lipoprotein extravasation, which implies blood–retinal barrier impairment. Results consistent with this finding have been reported by other authors; the presence of hyperreflective foci is associated with hard exudate deposition [22]. The breakdown of the blood–retinal barrier, and the accumulation of hard exudates, would both be detrimental to eyes with AMD [18].

Based on this structure–function relationship, we hypothesized that there might be a correlation between the pretreatment structure of eyes with AMD and the visual prognosis. However, to date, limited information is available about predictive factors of visual prognosis among the features obtained from initial OCT sections in exudative AMD [23–25]. In the study reported herein, we longitudinally investigated OCT images of eyes with AMD throughout the course of anti-VEGF therapy, and examined the relationship between pretreatment features seen on OCT images and the visual prognosis.

Material and methods

For this observational study, we retrospectively reviewed the medical records of 96 consecutive patients (96 eyes) with exudative AMD who were seen by the Macula Service in the Department of Ophthalmology at Kyoto University Hospital between January 2008 and October 2009. Inclusion criteria included: (1) symptomatic subfoveal AMD, (2) the presence of choroidal neovascularization (CNV) beneath the foveal center, (3) the presence of macula-related exudative or hemorrhagic features, and (4) a minimum follow-up of 12 months after the initial visit. The current study included eyes with typical AMD and those with polypoidal choroidal vasculopathy (PCV). The diagnosis of PCV was based on indocyanine green angiography, which reveals a branching vascular network that terminates in polypoidal swelling. When both eyes met the inclusion criteria, only the eye with the more active lesion was included in the current study. Eyes with other macular abnormalities (i.e., pathologic myopia, retinal angiomatous proliferation, idiopathic CNV, presumed ocular histoplasmosis, angioid streaks, or other secondary CNV) and those with senile cataracts that resulted in poor-quality OCT images were excluded from the current study. This study was approved by the Institutional Review Board at the Kyoto University Graduate School of Medicine, and adhered to the tenets of the Declaration of Helsinki.

At the initial visit, each patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected VA (using the Landolt C test), determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), and fluorescein and indocyanine green angiography (HRA2; Heidelberg Engineering). At each scheduled follow-up visit, each patient underwent a complete ophthalmologic examination, including VA measurement, slit-lamp biomicroscopy, indirect fundus ophthalmoscopy, and OCT examination. Fluorescein and indocyanine green angiography were performed as deemed necessary.

After the initial visit, each eye was treated based on disease activity and VA. Eyes with exudative AMD were treated either with PDT combined with intravitreal injection of an anti-VEGF agent, or with intravitreal injections of anti-VEGF agents alone. After the initial combined therapy, each eye was considered for retreatment with the combined therapy every 3 months if the eye exhibited residual or recurrent polypoidal lesions upon indocyanine green angiography. In eyes treated with intravitreal injections of anti-VEGF agents alone, additional injections were administered on an as-needed basis.

In the current study, we evaluated the OCT images obtained from all eligible patients both quantitatively and qualitatively. In both the initial and final OCT images, we measured total foveal thickness, defined as the distance between the vitreoretinal interface and the retinal pigment epithelium (Fig. 1a). Furthermore, we examined the IS/OS line and the ELM to assess outer foveal photoreceptor layer integrity. The status of the IS/OS line and the ELM line under the fovea was defined as either complete or incomplete. OCT images in eyes with exudative AMD often exhibited subretinal fluid and hemorrhages beneath the neurosensory retina. In addition, most OCT images revealed morphological changes in the neurosensory retina. Cystoid spaces and hyperreflective foci were seen in many cases within the neurosensory retina. Fibrin derived from active CNV or polypoidal lesions was observed as amorphous hyperreflective material, not only in the subretinal space but also within the neurosensory retina. Using horizontal and longitudinal OCT sections, we evaluated whether cystoid macular edema, hyperreflective foci, and fibrin exudates were seen within the 1×1 mm area around the fovea (Fig. 1b).

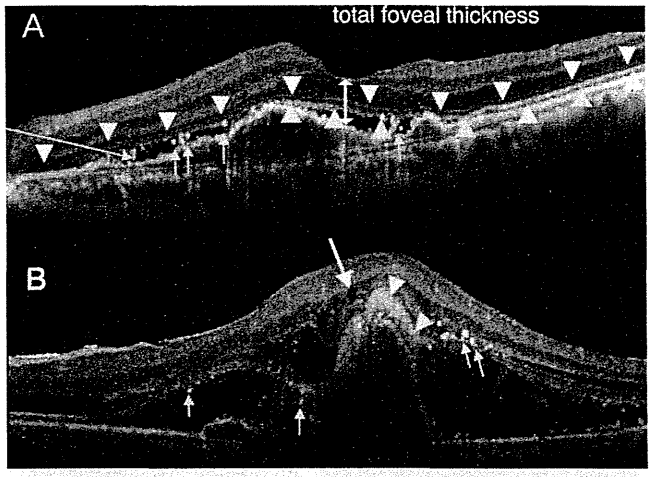

Fig. 1 **a** Horizontal image of the fovea obtained using optical coherence tomography (OCT) of an eye with active exudative age-related macular degeneration. Using an initial OCT image, three measurements were made in the fovea, including total foveal thickness, continuity of the external limiting membrane (ELM; *white arrowheads*), and continuity of the junction between the inner and outer photoreceptor segments (IS/OS; *yellow arrowheads*). **b** Cross-sectional image of the foveal region obtained using OCT. Using these OCT sections, we determined whether cystoid macular edema (*white arrow*), hyperreflective foci (*yellow arrows*), or fibrin exudate (*yellow arrowheads*) were seen within the 1×1 mm square area around the fovea

Statistical analysis was performed using software designed for this purpose (IBM SPSS Statistics Desktop, version 19.0.0; IBM Japan, Tokyo, Japan). All values are presented in terms of means and standard deviation. Best-corrected VA was converted to a logarithm of the minimum angle of resolution (logMAR) equivalent for statistical analysis. Values in typical AMD and PCV were compared using the Student's *t*-test. Bivariate relationships were analyzed using the Pearson's correlation coefficient or the Spearman rank correlation coefficient. Stepwise forward multivariate linear regression analyses were also performed to evaluate the contribution of each initially identifiable factor to final VA. A value of $p<0.05$ was considered statistically significant.

Results

In the current study, 96 eyes from 96 patients (72 men and 24 women) with exudative AMD who were 57–90 years of age (mean, 73.2±8.5 years) were examined (Table 1). Of these 96 eyes, 61 had PCV and 35 had typical AMD. The mean initial VA (logMAR) and foveal thickness were 0.58±0.49 and 422.5±255.9 μm respectively; there were no significant differences in these parameters between eyes with typical AMD or PCV ($p=0.889$, $p=0.787$). Active CNV lesions were treated with intravitreal injections of anti-VEGF agents in 38 eyes, and with PDT combined with anti-VEGF agents in 37 eyes. No treatments were performed in 21 eyes. The mean number of injections was 4.7±3.2 and mean number of PDT

Table 1 Characteristics of the study population

Number of eyes	96
Age (years; mean [SD[a]])	73.2 (8.5)
Gender (women/men)	24/72
Initial examination	
Visual acuity (logMAR[b]; mean [SD])	0.58 (0.49)
Total foveal thickness (m; mean [SD])	422.5 (255.9)
Detection of IS/OS[c] under the fovea (complete/incomplete)	22/74
Detection of ELM[d] under the fovea (complete/incomplete)	64/32
Cystoid macular edema, number of eyes (%)	18 (18.8)
Hyperreflective foci, number of eyes (%)	78 (81.3)
Fibrin, number of eyes (%)	56 (58.3)
Follow-up (months; mean [SD])	24.6 (5.7)
Treatment	
Photodynamic therapy (%)	37 (38.5)
Anti-vascular endothelial growth factor therapy (%)	38 (39.6)
Final examination	
Visual acuity (logMAR; mean [SD])	0.65 (0.61)
Total foveal thickness (m; mean [SD])	305.4 (224.9)
Detection of IS/OS under the fovea (complete/incomplete)	28/68
Detection of ELM under the fovea (complete/incomplete)	52/44
Cystoid macular edema, number of eyes (%)	11 (11.5)
Hyperreflective foci, number of eyes (%)	39 (40.6)
Fibrin, number of eyes (%)	22 (22.9)

[a] SD, standard deviation; [b] logMAR, logarithm of the minimum angle of resolution; [c] IS/OS, junction between the inner and outer photoreceptor segments; [d] ELM, external limiting membrane

treatments was 1.7±1.0. The mean duration from the final treatment to the final examination was 13.4±9.4 months. The mean follow-up period was 24.6±5.7 months.

At the initial visit, all eyes exhibited exudative changes beneath the neurosensory retina derived from active CNV (subretinal fluid and hemorrhage). In addition, most OCT images revealed morphological changes within the neurosensory retina. Cystoid macular edema was observed in 18.8% of eyes. Cystoid spaces were usually observed within neurosensory retinas that were in direct contact with the underlying type 2 CNV, whereas detached retinas were rarely seen in any of the cystoid spaces. In 58.3% of eyes, OCT revealed that the fibrin exudate was an amorphous hyperreflective material within the subretinal space. Fibrin, which was often seen just over the underlying active type 2 CNV or polypoidal lesions, often appeared to infiltrate the overlying neurosensory retina, especially in the outer aspect, resulting in a lack of IS/OS or ELM lines. In these eyes, the ELM seemed to work in some instances as a blocking agent against the exudates (Fig. 2). In 81.3% of eyes, intraretinal hyperreflective foci were seen within the neurosensory

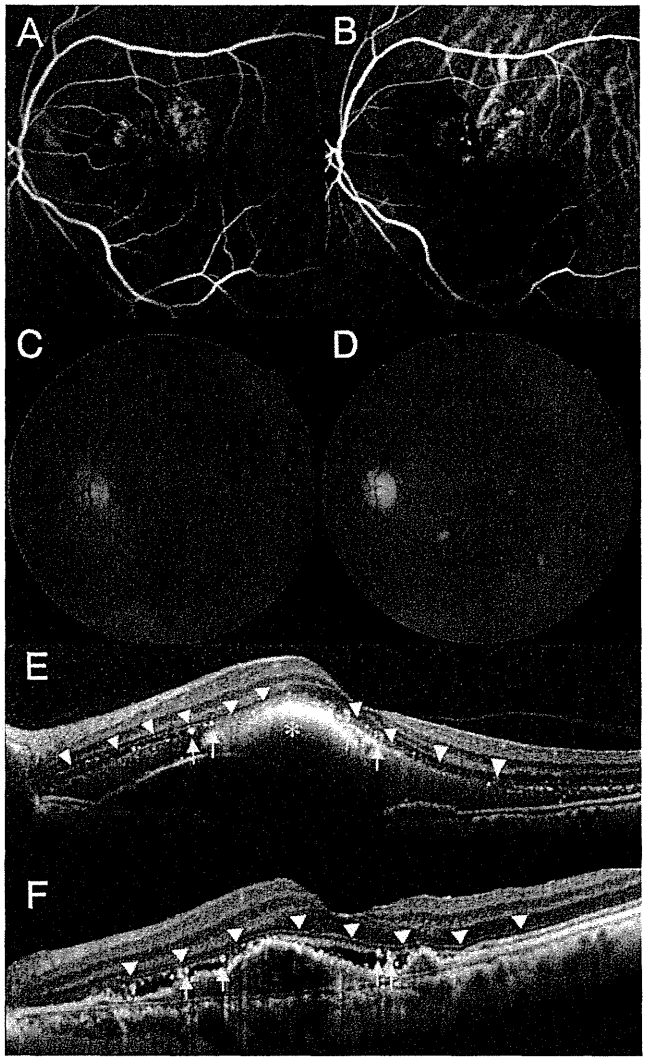

Fig. 2 Changes in retinal structure seen in polypoidal choroidal vasculopathy. **a** Initial fluorescein angiography. **b** Initial indocyanine green angiography shows numerous polypoidal lesions. **c** Initial fundus photograph exhibiting reddish-orange nodules and an adjacent detachment of the pigment epithelium. **d** Fundus photograph at final visit. **e** Sectional image obtained using optical coherence tomography (OCT) at the initial visit. Numerous hyperreflective foci (*yellow arrows*), as well as subretinal fluid/fibrin (*yellow asterisk*) are seen. The fibrin appears to have infiltrated the outer retina but appears to be blocked at the external limiting membrane (*white arrowheads*). The structure of the neurosensory retina seems to be relatively well-preserved. The line of the external limiting membrane is detectable under the fovea. The visual acuity was 0.3 by the Landolt chart. **f** Sectional image obtained using OCT at the final visit. The visual acuity was 0.9 by the Landolt chart

retina. Intraretinal hyperreflective foci were frequently seen throughout the entire outer retina, not only outside but also beyond the ELM (Figs. 1 and 2). In our patients, the integrity of the inner and outer segments of the foveal photoreceptor cells appeared to be compromised due to exudative change from the CNV. At the initial examination, foveal ELM was seen in 66.7% of the eyes examined, whereas foveal IS/OS was seen in only 22.9% of the eyes examined. Figure 3.

Table 2 shows the correlations of initial VA with other measurements obtained at the initial examination. Both the total foveal thickness and the presence of cystoid macular edema were correlated with initial VA (r=0.39, p<0.001; r=0.39, p< 0.001). In addition, foveal IS/OS and ELM were also correlated with initial VA (r=0.35, p<0.001; r=0.48, p<0.001).

At the final examination, the mean total thickness was significantly reduced to 305.4±224.9 μm (p<0.001), while

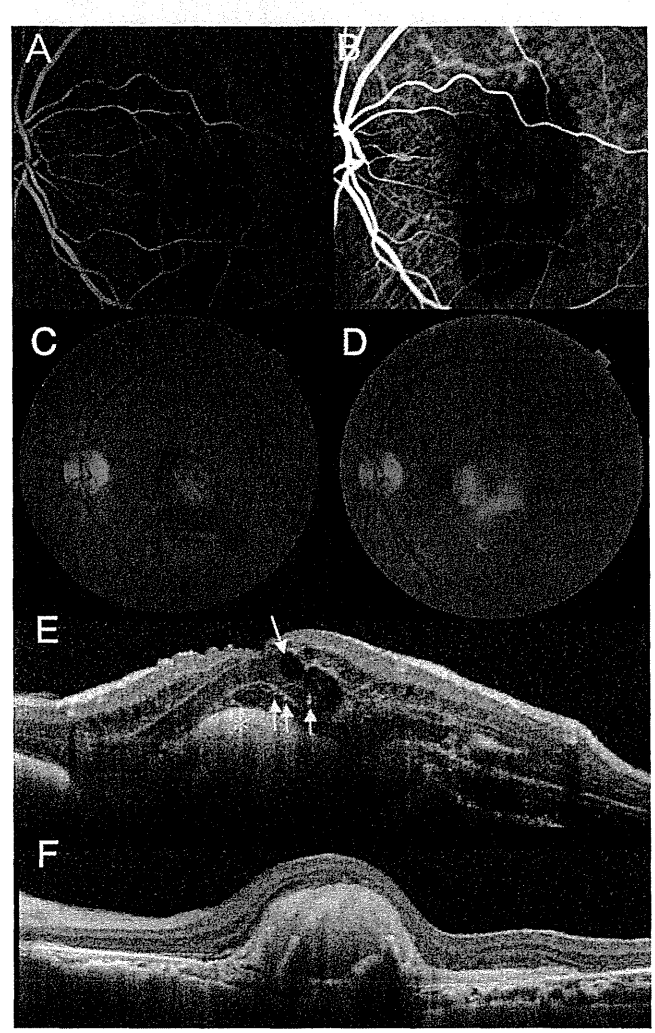

Fig. 3 Changes in retinal structure seen in typical age-related macular degeneration. **a** Initial fluorescein angiography shows minimally classic choroidal neovascularization (CNV). **b** Initial indocyanine green angiography. **c** Fundus photograph at initial visit shows subfoveal exudate with surrounding subretinal hemorrhage. **d** Fundus photograph at final visit shows subfoveal fibrous tissue. **e** Sectional image obtained using optical coherence tomography (OCT) at the initial visit. Hyperreflective foci (*yellow arrows*) and cystoid macular edema (*white arrow*) are seen within the neurosensory retina. Subretinal fluid/fibrin (*yellow asterisk*) is seen subfoveally. The line of the external limiting membrane is not detectable under the fovea. Visual acuity was 0.2 by the Landolt chart. **f** Sectional image obtained using OCT at the final visit. Sectional OCT image at 20 months shows thick subfoveal deposit. The visual acuity was 0.1 by the Landolt chart

Table 2 Association between initial visual acuity and other measurements obtained at initial examination

	R	*P* value
Age	0.20	0.048
Gender	0.03	0.782
Diabetes mellitus	0.04	0.720
Hypertension	0.08	0.413
Smoking	0.09	0.370
Type of disease	0.03	0.787
Total foveal thickness	0.39	<0.001
Cystoid macular edema	0.39	<0.001
Hyperreflective foci	0.11	0.261
Fibrin	0.05	0.603
Detection of IS/OS[a] under the fovea	0.35	<0.001
Detection of ELM[b] under the fovea	0.48	<0.001

[a] IS/OS, junction between the inner and outer photoreceptor segments; [b] ELM, external limiting membrane

cystoid macular edema was still seen in 11.5% of the eyes. Despite treatment, restoration of the integrity of the outer photoreceptor layer was limited. Complete detection of the IS/OS and ELM lines was achieved in only 29.1% and 54.2% of eyes respectively. Table 3 shows the correlations between final VA and other measurements obtained at the final examination. The total foveal thickness and presence of cystoid macular edema were correlated with poor final VA (r=0.27, p< 0.001; r=0.33, p<0.001), whereas detection of foveal IS/OS and ELM lines was correlated with good final VA (r=0.57, p<0.001; r=0.58, p<0.001). There were no differences in final VA between eyes with typical AMD and PCV (p=0.149).

Table 4 shows the correlations of final VA with measurements obtained at the initial examination (Pearson's correlation analyses); of these, initial VA showed the closest correlation with final VA (r=0.61, p<0.001). Age (r=0.34, p<0.001), total foveal thickness (r=0.40, p<0.001), and the presence of hyperreflective foci or cystoid macular edema (r= 0.26, p=0.012) at the initial visit also correlated with final VA. In addition, the initial detection of the foveal IS/OS and ELM lines was correlated with final VA (r=0.42, p<0.001 and r= 0.55, p<0.001 respectively). However, there were no differences in final VA between treatment types (p=0.637). Table 5 shows the correlations between VA and the measurements obtained at the initial examination in each group, stratified by treatment. In each group, while the correlations had similar tendencies, some were not statistically significant, perhaps due to the small number of eyes.

Table 6 shows the correlations between final VA and the measurements obtained at the initial examination after multiple regression analysis. By multiple regression analysis, final VA was correlated with initial VA (r=0.48, p<0.001) and the detection of a foveal ELM line (r=0.33, p=0.008). The initial presence of cystoid macular edema was associated with initial poor VA (r=0.39, p<0.001), but had no significant correlation with final visual function. On the other hand, the initial presence of hyperreflective foci had no significant correlation with initial VA (r=0.11, p=0.261), but showed a marginal correlation with final VA (r=0.23, p=0.054).

Table 3 Association between final visual acuity and other measurements obtained at final examination

	R	*P* value
Age	0.34	<0.001
Gender	0.03	0.770
Follow-up period	0.03	0.787
Diabetes mellitus	0.06	0.589
Hypertension	0.02	0.827
Smoking	0.01	0.908
Total foveal thickness	0.27	<0.001
Cystoid macular edema	0.33	<0.001
Hyperreflective foci	0.16	0.120
Fibrin	0.12	0.258
Detection of IS/OS[a] under the fovea	0.57	<0.001
Detection of ELM[b] under the fovea	0.58	<0.001

[a] IS/OS, junction between the inner and outer photoreceptor segments; [b] ELM, external limiting membrane

*Fisher's least significant difference test

Table 4 Associations between final visual acuity and measurements obtained at initial examination and during treatment

	R	*P* value
Age	0.34	<0.001
Gender	0.03	0.770
Diabetes mellitus	0.06	0.589
Hypertension	0.02	0.827
Smoking	0.01	0.908
Type of disease	0.15	0.149
Visual acuity (logMAR[a])	0.61	<0.001
Total foveal thickness	0.40	<0.001
Cystoid macular edema	0.34	<0.001
Hyperreflective foci	0.26	0.012
Fibrin	0.06	0.513
Detection of IS/OS[b] under the fovea	0.42	<0.001
Detection of ELM[c] under the fovea	0.55	<0.001
Treatment		0.637*

[a] logMAR, logarithm of the minimum angle of resolution; [b] IS/OS, junction between inner and outer segments of the photoreceptors; [c] ELM, external limiting membrane

*Fisher's least significant difference test

Table 5 Association between final visual acuity and other measurements obtained at initial examination in each group, stratified by treatment

	No treatment (n=21)		Photodynamic therapy with anti-VEGF agents (n=37)		Anti-VEGF[a] agents (n=38)	
	R	P value	R	P value	R	P value
Age	0.50	0.022	0.32	0.058	0.27	0.106
Gender	0.40	0.073	0.23	0.177	0.03	0.869
Diabetes mellitus	0.09	0.712	0.27	0.101	0.22	0.175
Hypertension	0.13	0.576	0.15	0.367	0.04	0.793
Smoking	0.02	0.926	0.12	0.481	0.14	0.397
Visual acuity (logMAR[b])	0.76	<0.001	0.48	0.003	0.62	<0.001
Total foveal thickness	0.45	0.042	0.36	0.029	0.40	0.013
Cystoid macular edema	NA	NA	0.26	0.117	0.49	0.002
Hyperreflective foci	0.38	0.090	0.01	0.968	0.28	0.093
Fibrin	0.01	0.957	0.07	0.666	0.12	0.469
Detection of IS/OS[c] under the fovea	0.47	0.034	0.32	0.055	0.46	0.004
Detection of ELM[d] under the fovea	0.71	<0.001	0.26	0.117	0.65	<0.001

[a] VEGF, vascular endothelial growth factor; [b] logMAR, logarithm of the minimum angle of resolution; [c] IS/OS, junction between inner and outer photoreceptor segments; [d] ELM, external limiting membrane

No eyes exhibited cystoid macular edema at the initial visit in the no-treatment group

Discussion

In this study, we evaluated the morphological findings of the retina associated with exudative AMD, and found that initial ELM status and the presence of hyperreflective foci are associated with the visual prognosis. Although the predictive power was inferior to that of initial VA, these parameters at the time of the initial examination can be of help in predicting the visual prognosis.

Table 6 Association between final visual acuity and measurements obtained at initial examination, evaluated by multiple regression analysis

	Partial regression coefficient	P value
Age	0.01	0.036
Gender	NA[a]	
Diabetes mellitus	NA	
Hypertension	NA	
Smoking	NA	
Type of disease	NA	
Visual acuity (logMAR[b])	0.48	<0.001
Total foveal thickness	<0.01	0.292
Cystoid macular edema	0.02	0.856
Hyperreflective foci	0.23	0.054
Fibrin	NA	
Detection of IS/OS[c] under the fovea	0.13	0.279
Detection of ELM[d] under the fovea	0.33	0.008

[a] NA, not applicable; [b] logMAR, logarithm of the minimum angle of resolution; [c] IS/OS, junction between inner and outer photoreceptor segments; [d] ELM, external limiting membrane

Although both ELM and IS/OS status were correlated with visual prognosis in bivariate relationship analysis, the multiple regression model showed that only ELM, and not IS/OS status, contributes to visual prognosis. This finding can be explained, at least in part, by the previously held notion that the IS/OS change is too sensitive for use in the evaluation of diseases that cause severe retinal damage, such as exudative AMD [17]. In the current study, of the 96 eyes, IS/OS and ELM lines just beneath the fovea were confirmed in 22 eyes (22.9%) and 64 eyes (66.7%) respectively, suggesting that the IS/OS is impaired relatively early in the course of AMD. In fact, the ELM line was always confirmed when an IS/OS line was detectable. This finding is consistent with the findings of previous studies that examined other macular diseases (e.g., macular hole [26], retinal detachment [27]). Taken together, these facts indicate that the IS/OS is more susceptible and is disrupted in earlier stages than is the ELM. Photoreceptor damage appears as disruption of the IS/OS at first and subsequently of the ELM in these conditions, so the evaluation of the ELM and of the IS/OS depends on the severity of the disease.

An intact ELM might indicate the preservation of the anatomic barrier as well as photoreceptor integrity. The ELM consists of the zonula adherens between the Müller cells and the photoreceptors at the base of the outer segments; this junction is not as tight as that of the zonula occludens, but it does limit the movement of large molecules [28]. As shown in Fig. 2, some eyes demonstrated

termination of subretinal fibrin at the ELM border, suggesting that the ELM actually acts as a barrier to subretinal proteins or lipids. The prevention of molecular invasion and the subsequent retinal fluid accumulation would have beneficial effects on visual prognosis, providing another reason to study ELM status and its relationship with visual prognosis.

The present study also showed that hyperreflective foci could possibly predict vision, as the presence of hyperreflective foci was negatively associated with the visual prognosis. Although hyperreflective foci are not a functional retinal component, and subfoveal hard exudate accumulation was not a cause of poor visual prognosis, we believe that the presence of hyperreflective foci are a hallmark of blood–retinal barrier function, and thus reflect visual outcome. Hyperreflective foci were visible before treatment in many eyes (81.3%) but tended to disappear as the exudative changes improved (40.6% at final examination). An earlier report also suggested that the presence of hyperreflective foci reflects blood–retinal barrier impairment [20]. It is possible that eyes with hyperreflective foci at the initial examination had more active CNV and more severe blood–retinal barrier damage. In other words, the small percentage of cases without hyperreflective foci had milder CNV and milder blood–retinal barrier damage; this difference in the degree of blood–retinal barrier damage might have affected visual prognosis.

There are many limitations to the present study, including the retrospective study design, the relatively small study population from a single institution, and the variety of treatment regimens used. While the heterogeneity in disease types might have affected the results, there were no differences in initial and final VA between eyes with typical AMD and PCV. In the current study, eyes with exudative AMD were treated with intravitreal injections of anti-VEGF agents with or without PDT. When stratified by treatment, the correlations showed similar tendencies, some of which were not statistically significant, perhaps due to the small number of eyes. Eyes treated with combination therapy showed the lowest association between the initial condition of the outer retina and final VA. While the phototoxic effect of PDT might be involved in this low correlation, the exact reason is unclear. It is possible that the treatment regimen used have some effect on the visual prognosis.

Another limitation is the very nature of OCT examinations. The device used depicts only a difference in light reflectance. Furthermore, we are not completely sure what the presence of an intact ELM or hyperreflective foci implies. Both of these are issues that need to be addressed in future studies. Although initial VA is most strongly associated with the visual prognosis, the initial condition of the outer retina may be of help in predicting the visual prognosis in eyes with AMD. This information will help clinicians provide appropriate information to their patients. A further prospective study is necessary to establish the factors that predict visual prognosis in eyes with exudative AMD.

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RETINAL DISORDERS

Two-year outcome of photodynamic therapy combined with intravitreal injection of bevacizumab and triamcinolone acetonide for polypoidal choroidal vasculopathy

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Abstract
Purpose To compare the 2-year results after photodynamic therapy (PDT) alone and PDT combined with intravitreal injections of bevacizumab and triamcinolone acetonide (triple therapy) for polypoidal choroidal vasculopathy (PCV).
Methods We retrospectively reviewed the medical records of 40 consecutive patients (40 eyes) with subfoveal PCV. Of these 40 eyes, 16 were treated with PDT alone and 24 were treated with triple therapy.
Results The change in visual acuity in the triple therapy group was significantly better than that in the PDT group ($P<0.001$). At 24 months, improvement in visual acuity was seen in only two eyes (12.5 %) of the PDT group, while it was seen in ten eyes (41.7 %) of the triple therapy group. Retreatment was given to 12 eyes (75.0 %) in the PDT group and to nine eyes (37.5 %) in the triple therapy group, although the retreatment-free period was significantly longer in the triple therapy group than in the PDT group ($P<0.001$). Post-treatment vitreous hemorrhage was seen in only two eyes (12.5 %), all of which were in the PDT group.
Conclusion Compared with PDT alone, triple therapy appears to reduce the postoperative hemorrhagic complications and recurrences of PCV and to improve the 2-year visual outcomes of PCV.

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Keywords Age-related macular degeneration · Bevacizumab · Photodynamic therapy · Polypoidal choroidal vasculopathy · Triamcinolone acetonide

Introduction

Previously, photodynamic therapy (PDT) with verteporfin was used primarily for the treatment of classic choroidal neovascularization (CNV) associated with age-related macular degeneration (AMD) [1], while today, intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents (bevacizumab or ranibizumab) have become the principal treatment for exudative AMD [2–4]. Three monthly injections of anti-VEGF agents often lead to visual acuity (VA) improvement, but after these initial injections, repeated injections are usually required in order to maintain initial visual recovery. However, repeated injections increase the risk of ophthalmic and systemic adverse events, such as endophthalmitis and stroke [5]. To achieve better visual outcomes with fewer treatments, several reports have shown promising short-term effects of PDT combined with intravitreal injection of bevacizumab and a steroid (triamcinolone acetonide [TA] or dexamethasone) for exudative AMD [6, 7].

Polypoidal choroidal vasculopathy (PCV) is now recognized as a distinct clinical entity, differing in many ways from exudative AMD [8]. PCV is characterized by a branching vascular network that terminates in polypoidal lesions seen on indocyanine green angiography (IA), and is more common in Asians than in Caucasians [9, 10]. It has been reported that the treatment effects of anti-VEGF agents on the vascular lesions of PCV are limited in short-term follow-up [11, 12]. In contrast, a number of studies have shown encouraging results of PDT for the treatment of PCV. A

small number of sessions of PDT causes regression of the polypoidal lesions, and often results in stable, or even improved VA [13–15]. Subsequent reports, however, have shown extensive hemorrhagic complications and recurrences of the polypoidal lesions after the initially successful treatment with PDT [16, 17].

Recently, PDT combined with anti-VEGF agents has been reported to improve the short-term visual outcome in PCV, compared with PDT alone [18–20]. In addition, it has been suggested that this combination therapy may reduce the risk of postoperative hemorrhagic complications [18]. Furthermore, it has been reported that TA suppresses the early proangiogenic response of retinal pigment epithelium (RPE) cells after PDT treatment [21], and that the intravitreal injection of TA per se has a suppressive effect on CNV [22]. For the treatment of PCV, when PDT is combined with an anti-VEGF agent and also with TA, this triple therapy might reduce postoperative complications and the recurrence rate, and lead to a better visual prognosis. To date, however, no information is available on the effects of this triple therapy for PCV. Accordingly, the study described herein aimed to evaluate the long-term results of this triple therapy on symptomatic subfoveal PCV by comparing it with PDT alone.

Patients and methods

For this retrospective study, we reviewed the medical records of 40 consecutive eyes (40 patients) with treatment-naïve subfoveal PCV, who were treated with PDT alone or with PDT combined with an intravitreal injection of bevacizumab and TA (triple therapy) at Kyoto University Hospital between September 2004 and December 2008. PDT alone was performed on 16 patients (PDT group) between September 2004 and December 2007 and PDT combined with bevacizumab and TA was performed on 24 patients (triple therapy group) between January 2008 and December 2008. Inclusion criteria of the study were: (1) symptomatic PCV in patients older than 50 years, (2) the presence of subfoveal vascular lesions, (3) best-corrected VA of 20/25 or worse, and (4) a minimum follow-up of 24 months after initial treatment. Exclusion criteria were: (1) eyes with other macular abnormalities (i.e., AMD, pathologic myopia, idiopathic CNV, presumed ocular histoplasmosis, angioid streaks, and other secondary CNV), (2) any contraindications for fluorescein angiography (FA), IA, or verteporfin, (3) the presence of an RPE tear, (4) any previous treatment for subfoveal PCV, (5) a history of previous vitrectomy, or (6) any other additional therapy during the study period (i.e., anti-VEGF therapy). This study was approved by the Institutional Review Board at Kyoto University Graduate School of Medicine, and adhered to the tenets of the Declaration of Helsinki.

The diagnosis of PCV was based on IA, which shows a branching vascular network terminating in polypoidal dilation. In the present study, pseudophakic eyes were included. When both eyes with PCV that were treated with PDT or triple therapy met the inclusion criteria, only the eye which was treated initially was included in the current study. Some patients in the PDT group were included in a previous study [17].

At the initial visit, each patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected VA with a Landolt chart, determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, optical coherence tomography examinations, and FA and IA (HRA-2, Heidelberg Engineering, Dossenheim, Germany).

In eyes of the PDT group, standard-fluence PDT was performed using a 689 nm diode laser unit (Visulas PDT system 690S; Carl Zeiss, Dublin, CA, USA) after an injection of verteporfin (Visudyne; Novartis Pharma AG, Basel, Switzerland), according to PDT guidelines for AMD [23]. In eyes that received triple therapy, injection of bevacizumab (1.25 mg) and TA (2 mg) was performed in a sterile manner, and prophylactic topical antibiotics were applied for 1 week after the injection. At 3–4 days after the intravitreal injection, standard-fluence PDT was performed according to PDT guidelines for AMD. The greatest linear dimension was calculated based on FA and IA, as described in detail previously [24]. All polypoidal lesions, the entire branching vascular network, and type 2 CNV detected by FA and IA were included. Serous pigment epithelial detachment was not included in the lesion area when the absence of underlying CNV was confirmed by IA.

After the initial treatment, each patient was scheduled to be seen at 3 months, at which time they again underwent a comprehensive ophthalmologic examination. When IA showed recurrent or residual polypoidal lesions and exudative change was seen on ocular coherence tomography (OCT), retreatment with PDT or the triple therapy was given according to the initial treatment. When residual polypoidal lesions were detected on IA but no exudative change was seen on OCT, no retreatment was given and the patient was reevaluated at the next visit.

All values are presented as mean ± standard deviation. For statistical analysis, best-corrected VA as measured with a Landolt chart was converted to a logarithm of the minimum angle of resolution (logMAR). VA was considered to be improved or deteriorated when the logMAR change was greater than 0.2. On OCT scans, foveal thickness was defined as the distance between the inner surface of the neurosensory retina and the RPE beneath the fovea. In each group, VA or foveal thickness after treatment was studied by one-way repeated measures analysis of variance with the

Table 1 Baseline characteristics of study population

	Photodynamic therapy group	Triple therapy group	*P* value
Number of patients	16	24	
Age (years)	73.3±9.9	73.7±5.8	0.887*
Gender (female/male)	5/11	6/18	0.665†
Initial visual acuity (logMAR)	0.36±0.13	0.50±0.31	0.119*
Initial foveal thickness (μm)	264.9±87.4	392.1±129.5	0.002*
Cystoid macular edema	4 (25.0 %)	11 (45.8 %)	0.182†
Serous retinal detachment	11 (68.8 %)	24 (100 %)	0.003†
Subretinal hemorrhage	8 (50.0 %)	7 (29.2 %)	0.182†
Pigment epithelial detachment	13 (81.3 %)	23 (95.8 %)	0.132†
Greatest linear dimension (μm)	2584±998	3193±1194	0.101*

logMAR, logarithm of the minimum angle of resolution

*Unpaired *t*-test

†Chi-squared test

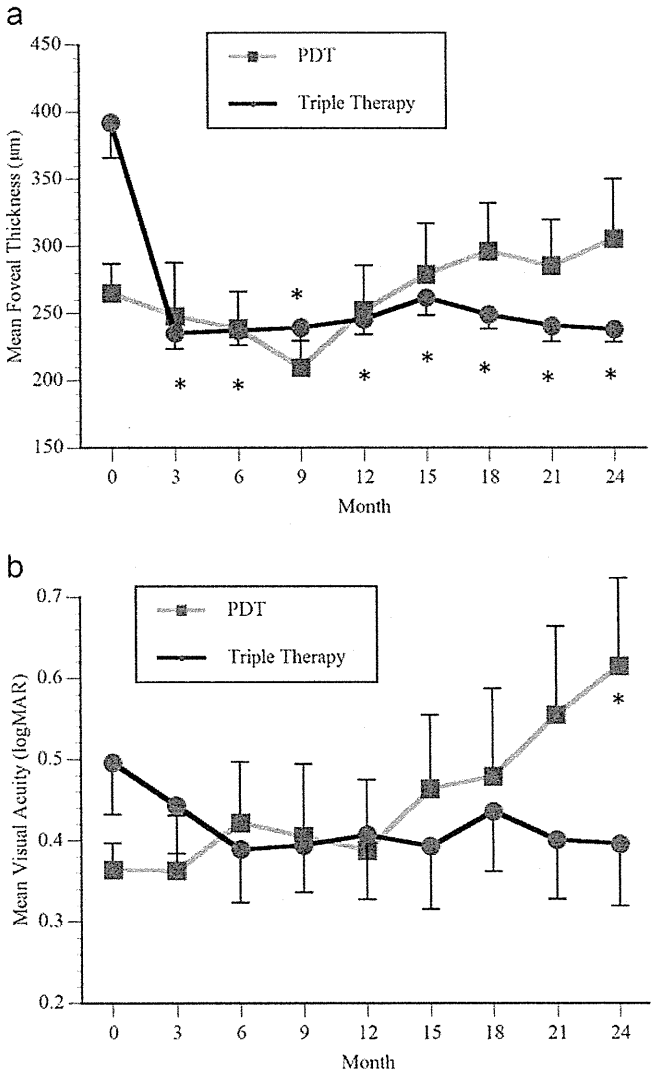

Fig. 1 Mean foveal thickness (**a**) and mean visual acuity (**b**) in eyes with polypoidal choroidal vasculopathy treated with photodynamic therapy (PDT group) or with PDT combined with intravitreal injections of bevacizumab and triamcinolone acetonide (triple therapy group). Visual acuity is shown in logMAR fashion. *$P<0.05$, compared with pretreatment values. *Error bars* represent the standard error

Dunnett test. To compare VA and foveal thickness between the PDT group and the triple therapy group, two-factor repeated measures analysis of variance was used. The retreatment-free period was calculated from the date of the initial therapy to the date when the treating physician determined the necessity of retreatment by PDT or by triple therapy. Survival analysis using Kaplan–Meier methods was used to compare the difference in the retreatment-free period after initial treatment between the PDT and the triple therapy groups. In patients who underwent no retreatment by either PDT or triple therapy, the retreatment-free period was established at 2 years of follow-up. Descriptive statistics for all demographic and clinical variables were calculated, and comparisons made using the unpaired *t*-test for means with continuous data (e.g., age) and the Chi-squared test for categorical data (e.g., gender). Statview version 5.0

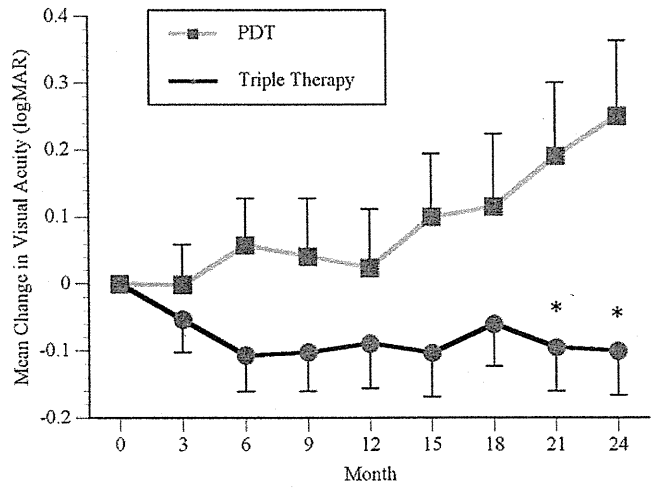

Fig. 2 Change in visual acuity in eyes with polypoidal choroidal vasculopathy treated with photodynamic therapy (PDT group) or with a combination of PDT and intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy group). Change in visual acuity in eyes treated with triple therapy was significantly better than that in eyes treated with PDT alone ($P<0.001$). Visual acuity is shown in logMAR fashion. *$P<0.05$, compared with values in the PDT group. *Error bars* represent the standard error

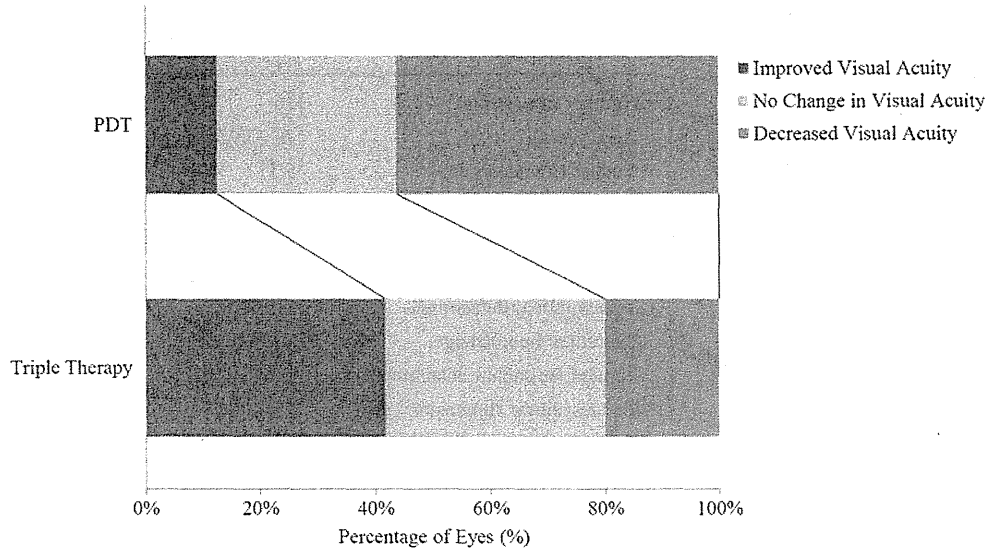

Fig. 3 Percentages of eyes with improved and deteriorated visual acuity at 24 months after treatment. All eyes with polypoidal choroidal vasculopathy were treated with photodynamic therapy (PDT group) or PDT combined with intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy group). Visual acuity was considered to be improved or deteriorated when the change in logMAR units was greater than 0.2. Improvement of visual acuity was seen more frequently in the triple therapy group (P 0.044)

software (SAS Institute, Inc., Cary, NC, USA) was used for statistical analyses. A P value<0.05 was considered to be statistically significant.

Results

In the study described herein, a total of 40 patients with treatment-naïve PCV were evaluated; 16 eyes which received only treatment with PDT (PDT group) and 24 eyes which received only triple therapy (triple therapy group) during the 24-month study period. Table 1 shows baseline characteristics of each group. There were no significant differences in age, gender, or baseline VA between the two groups. However, baseline foveal thickness of the triple therapy group was significantly larger than that of the PDT group, and the rate of serous retinal detachment was also higher in the triple therapy group.

Figure 1a shows the time-course in mean foveal thickness of each group. In the triple therapy group, the mean foveal thickness decreased immediately after initiation of treatment and remained throughout the 2-year follow-up period. Figure 1b shows the time-course of mean VA in each group. In the PDT group, there was no significant improvement of VA after initiation of treatment; in fact, mean VA was significantly decreased (+0.25±0.45) at 24 months (P 0.041). In contrast, VA somewhat improved after initiation of treatment in the triple therapy group, while the improvement was not statistically significant. Some improvement was maintained throughout the 2-year follow-up period.

Table 2 Final characteristics of study population and complications during study period

	Photodynamic therapy group	Triple therapy group	P value
Number of patients	16	24	
Final visual acuity (logMAR)	0.62±0.43	0.40±0.38	0.099*
Final foveal thickness (μm)	283±204	230±66	0.234*
Final conditions			
Cystoid macular edema	5 (31.3 %)	0 (0 %)	0.003†
Serous retinal detachment	5 (31.3 %)	0 (0 %)	0.003†
Subretinal hemorrhage	2 (12.5 %)	1 (4.2 %)	0.327†
Pigment epithelial detachment	11 (68.8 %)	5 (20.8 %)	0.002†
Polypoidal lesions	3 (18.8 %)	2 (8.3 %)	0.385†
Complications			
Cataract	2 (12.5 %)	1 (4.2 %)	0.327†
Suprachoroidal hemorrhage	1 (6.3 %)	0 (0 %)	0.215†
Vitreous hemorrhage	2 (12.5 %)	0 (0 %)	0.076†
Tear of retinal pigment epithelium	1 (6.3 %)	0 (0 %)	0.215†
Number of photodynamic therapy or triple therapy sessions (range)	2.19±0.91 (1–3)	1.50±0.78 (1–3)	0.015*
Retreatment-free period (months)	11.7±8.6	20.6±6.8	<0.001‡

logMAR, logarithm of the minimum angle of resolution; VEGF, vascular endothelial growth factor

*Unpaired t-test

†Chi-squared test

‡Survival analysis

Figure 2 shows the change in mean VA from baseline in each group. The mean change in VA in the triple therapy group was significantly better than that in the PDT group (P <0.001). At 24 months after initial treatment, mean change in VA in the triple therapy group (0.10±0.32) was significantly better than that in the PDT group (+0.25±0.45, P0 0.007). Figure 3 shows the percentage of eyes with improved or decreased VA at 24 months; improvement in VA was seen in two eyes (12.5 %) of the PDT group and in ten eyes (41.7 %) of the triple therapy group, and reduction in VA was seen in nine eyes (56.3 %) of the PDT group and in five eyes (20.1 %) of the triple therapy group. Thus, improvement in VA was seen more frequently in the triple therapy group (P0 0.044).

Table 2 summarizes final characteristics and complications during the study period. In both groups, the polypoidal lesions disappeared after treatment (Fig. 4). At 24 months, complete disappearance of the polypoidal lesions was confirmed in 13 eyes (81.3 %) in the PDT group and in 22 eyes (91.7 %) of the triple therapy group. Fifteen eyes (62.5 %) of the triple therapy group and four eyes (25.0 %) of PDT group underwent a single session of PDT during the 24-month study period (P0 0.020). Retreatment by PDT was done in 12 eyes (75.0 %) in that group, and nine eyes (37.5 %) in the triple therapy group received retreatment by triple therapy (Fig. 5). The mean numbers of treatment were 2.19±0.91 in the PDT group and 1.50±0.78 in the triple therapy group (P0 0.015). Figure 6 shows the overall survival analysis curve for the retreatment-free periods in each group, which was significantly longer in the triple therapy group (20.6±6.8 months) than in the PDT group (11.7±8.6 months, P<0.001).

In the current study, two eyes (12.5 %) in the PDT group developed cataract and underwent surgery during the study period; additionally, one eye (4.2 %) in the triple therapy group (P0 0.327) underwent similar surgery. In the PDT group, two eyes (12.5 %) developed a vitreous hemorrhage and one eye developed an RPE tear during the study period, while no eye in the triple therapy group developed a vitreous hemorrhage. No eye underwent glaucoma surgery for ocular hypertension after intravitreal injections of TA.

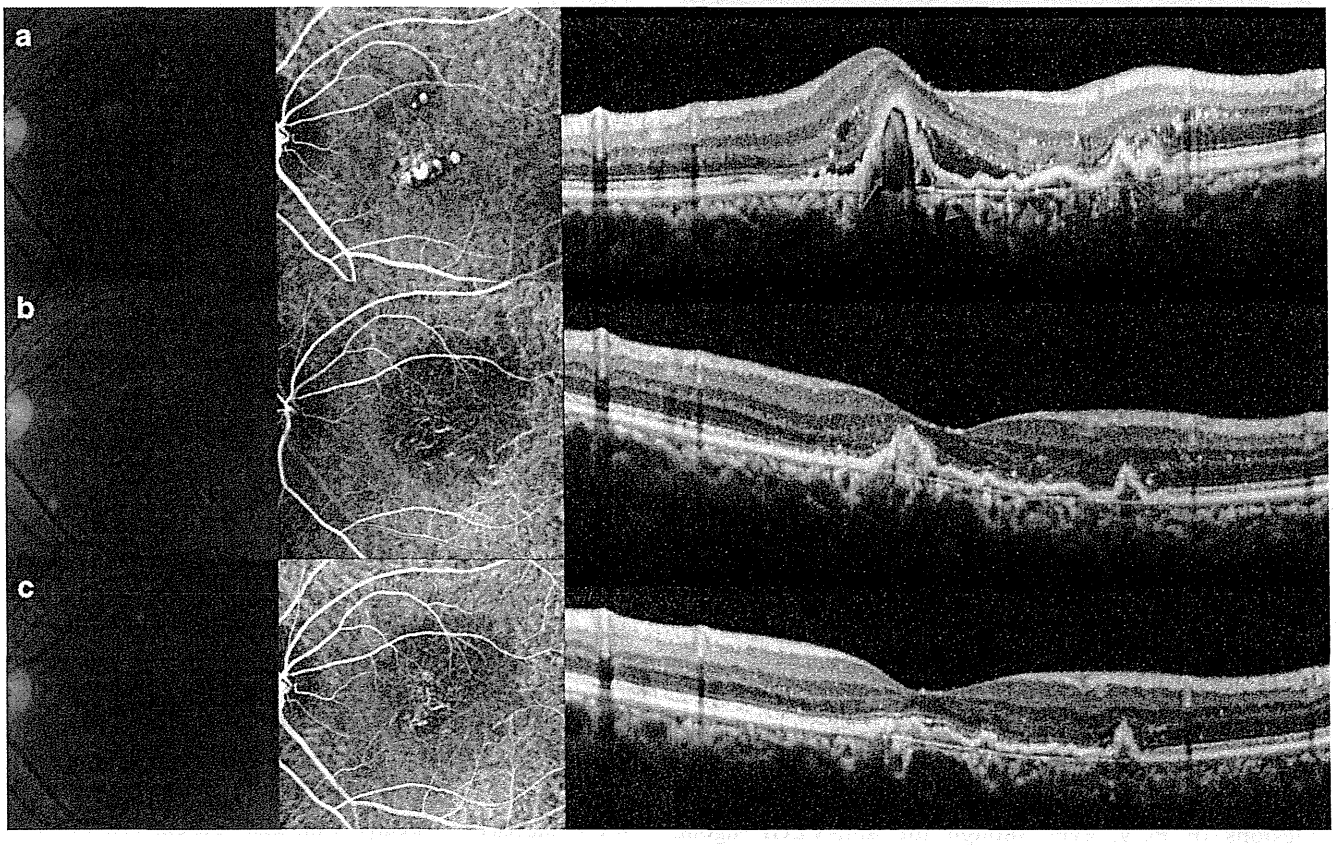

Fig. 4 Polypoidal choroidal vasculopathy successfully treated with photodynamic therapy combined with intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy). **a** Initial fundus photograph (*left*) shows reddish-orange nodules and fibrin exudate (20/50 OS). Indocyanine green angiography (*middle*) shows a branching vascular network that terminates in polypoidal lesions. A vertical optical coherence tomography section through the fovea (*right*) reveals sharp protrusions of the retinal pigment epithelium due to polypoidal lesions (*arrows*). A branching vascular network is seen as flat protrusions (arrowheads). **b** Three months after triple therapy. No polypoidal lesions are seen on indocyanine green angiogram. A branching vascular network is still seen. Protrusions due to polypoidal lesions have become flattened (20/60 OS). **c** 12 months after treatment, no recurrence is seen (20/50 OS)

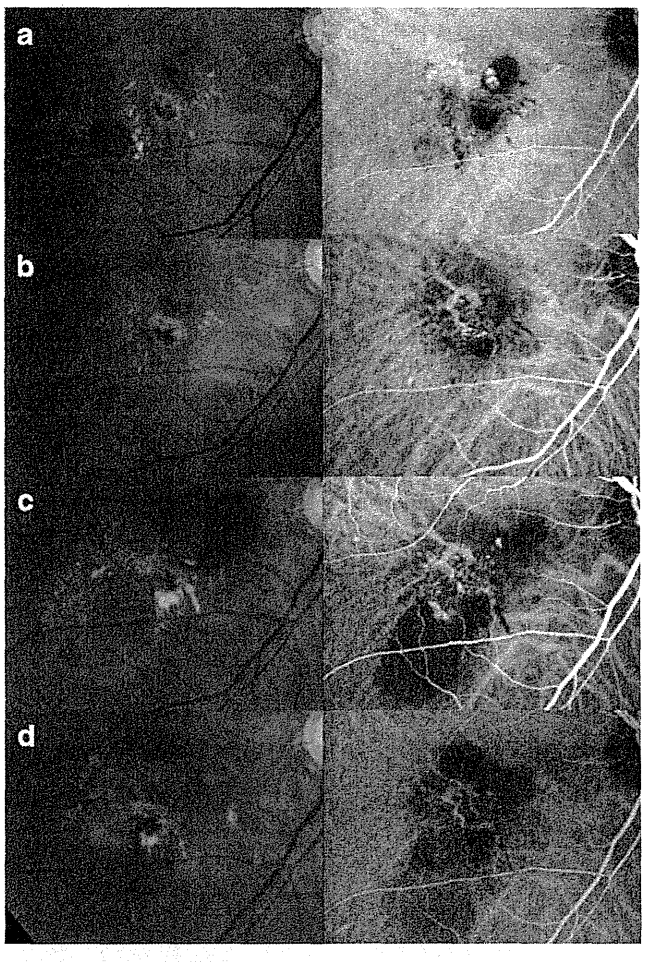

Fig. 5 Recurrence of polypoidal lesions after successful treatment with photodynamic therapy of polypoidal choroidal vasculopathy. **a** Initial fundus photograph (*left*) shows a reddish-orange nodule with a hard exudate and with a subretinal hemorrhage (20/40 OD). Indocyanine green angiography (*right*) shows a branching vascular network that terminates in polypoidal lesions. **b** Three months after photodynamic therapy. Reddish-orange nodules and polypoidal lesions have regressed (20/30 OD). **c** At 12 months after treatment, recurrence has occurred. Fundus photograph shows pigment epithelial detachments with surrounding subretinal hemorrhage and hard exudate (20/100 OD). Polypoidal lesions have recurred at the terminus of the remaining branching vascular network. **d** At 3 months after retreatment with photodynamic therapy, pigment epithelial detachments and polypoidal lesions have completely regressed (20/70 OD)

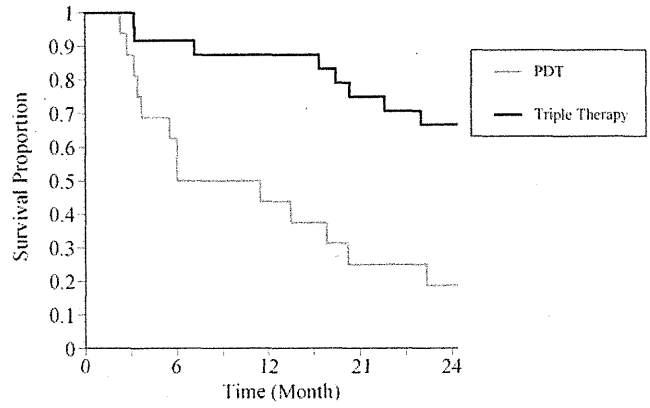

Fig. 6 Overall survival curve for the retreatment-free period in both treatment groups. Eyes with polypoidal choroidal vasculopathy were treated with either photodynamic therapy (PDT group) or PDT combined with intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy group). The period until the treating physician opted to perform additional treatment was significantly longer in the triple therapy group than in the PDT group ($P<0.001$)

Discussion

Several investigators have reported the short-term outcomes of anti-VEGF agents for the treatment of PCV, and have shown the limited effect of these agents on the vascular lesions of PCV, even though the anti-VEGF agents reduced the exudative change that was due to PCV. It has been reported that complete resolution of the polypoidal lesions was achieved in only 16.1 % of eyes, with a mean of 3.3 injections of bevacizumab over a 12 month period [25]. In an earlier report, although monthly injections of ranibizumab successfully reduced the exudative manifestations of PCV, a reduction in the polypoidal lesions was seen in only 33 % of eyes [11].

In contrast, a number of studies have shown encouraging results of PDT for the vascular lesions of PCV, with complete regression of the polypoidal lesions achieved in many cases with fewer sessions. In a report by Chan et al. [26], PDT led to complete regression of the polypoidal lesions in 95 % of eyes with PCV, and resulted in either stable or improved VA 1 year after treatment in 95 % of eyes. However, a year or more after successful treatment with PDT, recurrences of the polypoidal lesions sometimes cause a substantial decrease in VA. Using Kaplan–Meier methods, Yamashiro et al. reported that the recurrence of polypoidal lesions after successful PDT treatment was estimated to be 11.5 % at 15 months, 20.4 % at 18 months, and 38.8 % at 21 months [16]. Thus, the recurrence of polypoidal lesions after PDT is a major problem in the treatment of PCV.

In the combination therapy, anti-VEGF agents, which can cause rapid reduction of the exudative change, are thought to contribute to the visual recovery that is associated with regression of the polypoidal lesions induced by PDT. Previous experimental studies have shown increased expression of VEGF shortly after PDT treatment [27, 28], which suggests that an intravitreal injection of bevacizumab before PDT may well exert a protective effect. With regard to the injection of TA, Okubo and colleagues reported a case of PCV successfully treated with trans-Tenons retrobulbar injection of TA [29], and Mukai and colleagues reported the protective effects of TA against occlusion of the choriocapillaris which was induced by PDT [30]. However, Lai et al. reported that the adjunctive use of TA during PDT did not appear to result in additional benefit for the treatment of PCV [31], so the effect of TA on PCV remains controversial.