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LABORATORY INVESTIGATION

Correlation Between Indocyanine Green Angiographic Findings and Histopathology of Polypoidal Choroidal Vasculopathy

Masami Nakajima, Mitsuko Yuzawa, Hiroyuki Shimada, and Ryusaburo Mori

Department of Ophthalmology, Nihon University School of Medicine, Surugadai Hospital of Nihon University, Tokyo, Japan

Abstract

Purpose: To analyze the histopathology of polypoidal choroidal vasculopathy (PCV) and choroidal neovascularization (CNV) developing from PCV, the authors evaluated correlations between pathological findings and the findings of preoperative indocyanine green angiography (IA).

Methods: Two specimens were obtained during CNV excision associated with PCV. PCV tissue was excised with the CNV. The specimens were examined by light microscopy.

Results: In one case, IA revealed polypoidal lesions exhibiting hyperfluorescence in both the early and the late phase, and in the affected area, abnormally dilated vessels were identified histologically underneath relatively healthy retinal pigment epithelium (RPE). In the other case, the polypoidal lesions seen on IA showed early hyperfluorescence and late isofluorescence, and dilated vessels were observed under the RPE; perivascular amorphous material was present. The RPE adhered to the side of the choroid, and there was CNV under the neurosensory retina in both cases. The CNV had numerous vascular lumens, was not surrounded by the RPE, and exhibited few fibrous components.

Conclusions: IA findings vary depending on the condition of the RPE located above the PCV and the extent of amorphous material around the PCV. **Jpn J Ophthalmol** 2004;48:249–255 © Japanese Ophthalmological Society 2004

Key Words: choroidal neovascularization, histopathology, indocyanine green angiography, polypoidal choroidal vasculopathy

Introduction

Polypoidal choroidal vasculopathy (PCV) is a disease characterized by an abnormal network of vessels in the choroid and polypoidal lesions that protrude toward the retinal pigment epithelium (RPE). Many studies of PCV have been conducted since Yannuzzi and colleagues first described the disease in 1990.[1–3] Although indocyanine green angiography (IA) of the ocular fundus is considered to be extremely useful for the diagnosis of PCV,[4] there have been no studies comparing the histopathological and IA findings. Lafaut, Terasaki, and Rosa and colleagues have histopathologically analyzed polypoidal lesions associated with PCV,[5–7] but no studies have examined the individual components of PCV, namely, the polypoidal lesions, the network of vessels, and the choroidal neovascularization (CNV) that develops from PCV.

In this study, we performed CNV excision on both eyes of two patients with typical CNV that had developed under the neurosensory retina from accompanying PCV. PCV tissue was excised with the CNV, enabling us to carry out histopathological analysis of the excised specimens and a comparison of the histopathological and preoperative IA findings. We previously described the histopathological findings of CNV associated with age-related macular degener-

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Correspondence and reprint requests to: Masami Nakajima, Department of Ophthalmology, Surugadai Hospital of Nihon University, 1-8-13 Surugadai, Kanda, Chiyoda-ku, Tokyo 101-8039, Japan
e-mail: masamin@mail.med.nihon-u.ac.jp

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ation (AMD).[8] The present study demonstrates that the histopathological findings of CNV associated with PCV are different from those demonstrated in our previous investigation of AMD. The pathology of PCV is discussed herein.

Patients and Methods

Case 1

The patient was a 78-year-old man with no systemic disease other than hypertension. The patient had lost his right eye during World War II. Low-dose radiation therapy (total = 20 Gy) was performed on the left eye to treat foveal CNV, but the CNV recurred 19 months later. Preoperative visual acuity was 0.04 in the left eye. A white lesion in the macular region of the left eye, serous retinal detachment in the upper temporal region of the white lesion, and subretinal hemorrhage in the margin of the retinal detachment (Fig. 1a) were seen. Small orange lesions indicating PCV were confirmed by slit-lamp microscopic examination. IA and fluorescein fundus angiography (FA) were carried out, followed by CNV excision 1 week later. For IA, 25 mg of indocyanine green (ICG) was used as a fluorescent dye, and either TRC-50IA Plus Image Net (Topcon, Tokyo, Japan), or scanning laser ophthalmoscopy (Rodenstock, Nishinomiya, Japan) was used to capture the angiographic images. CNV excision was performed according to the method of Lambert and colleagues.[9]

Case 2

The patient was a 74-year-old man with no systemic disease other than hypertension. Preoperative visual acuity was 0.08 in the right eye and 0.2 in the left eye. Small orange lesions indicating PCV were confirmed by slit-lamp microscopic examination. CNV was excised from the right eye 1 week after FA and IA. Informed consent was obtained from both patients prior to surgery.

Histopathology

Each excised specimen was immediately fixed in 10% formaldehyde–phosphate buffer solution and dehydrated in an ethanol series according to conventional methods. The dehydrated sample was embedded in paraffin, and 4-μm serial sections were prepared and stained with H&E, periodic acid-Schiff, and immunohistochemical agents. The sections were then studied by light microscopy. Immunohistochemical analyses were conducted using antibodies against vascular endothelial growth factor (VEGF 1:100; Pharmingen, Tokyo, Japan). VEGF indicated the locations of factors facilitating neovascularization and vascular permeability. Histopathological findings were then compared to preoperative IA findings.

Results

Angiographic Findings

Case 1

FA revealed hyperfluorescence indicating classic CNV matching the periphery of the scar in the early phase, and leakage of fluorescent dye in the late phase. Polypoidal lesions were not clearly discernible (Figs. 1b,c). IA displayed hyperfluorescence indicative of PCV on the upper temporal side and inferior margin of the scar in both early and late phases. Furthermore, hyperfluorescence indicative of CNV was also observed (Figs. 1d,e).

Case 2

FA revealed hyperfluorescence indicative of classic CNV in the area above and nasal to the fovea in both early and late phases (Figs. 2a,b). IA showed hyperfluorescence indicative of parafoveal CNV in the early and late phases. On the upper temporal side of the parafoveal CNV, one area of the polypoidal lesion exhibited early hyperfluorescence and late isofluorescence. Other areas of the polypoidal lesion exhibited early and late hyperfluorescence. A network of vessels could be clearly visualized (Figs. 2c,d).

Histopathological Findings

Case 1

The polypoidal lesion comprised abnormally distended vessels, and the vascular walls of these vessels were thin (Figs. 3a,b). The RPE, which was located above the dilated vessels, appeared relatively healthy, with conservation of the layer-structure. Distended vessels were recognized in the choroid underneath the RPE (Fig. 3c). There was a relatively small amount of amorphous material around the polypoidal lesion. No VEGF-positive spots were seen in the PCV area (Fig. 3d).

In the CNV area, the RPE adhered to the side of the choroid, and CNV was present under the neurosensory retina. The CNV had numerous vascular lumens, was not surrounded by the RPE, and had a small amount of fibrous components (Fig. 3e). Many VEGF-positive spots were seen in vascular endothelial cells within the CNV area (Fig. 3f).

Case 2

Several dilated vessels were seen under the RPE corresponding to a polypoidal lesion with hyperfluorescence in the early phase and isofluorescence in the late phase of IA. Amorphous material was abundant around these vessels (Fig. 4a). In contrast, amorphous material around vessels in the area of the polypoidal lesion exhibiting

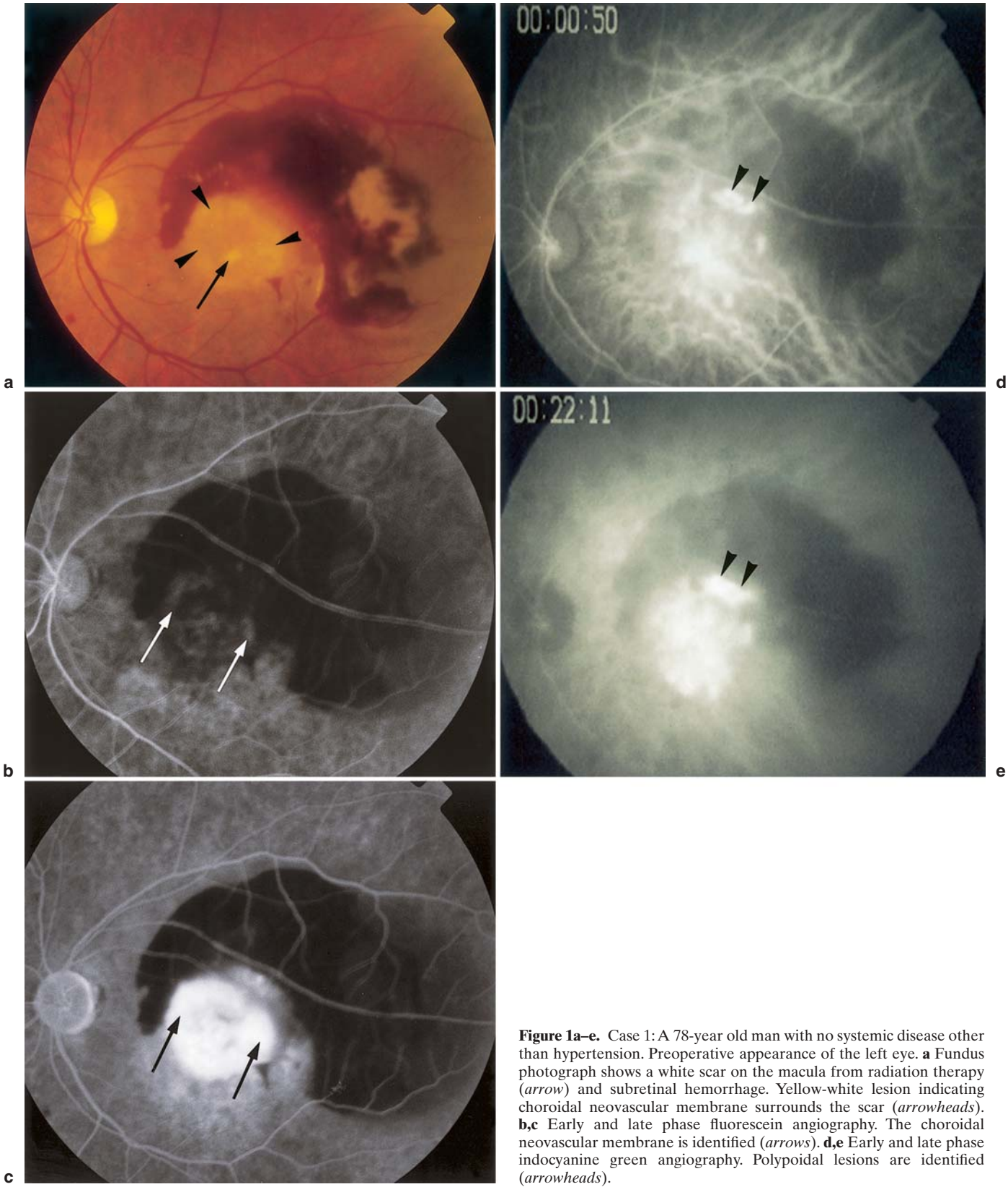

Figure 1a–e. Case 1: A 78-year old man with no systemic disease other than hypertension. Preoperative appearance of the left eye. **a** Fundus photograph shows a white scar on the macula from radiation therapy (*arrow*) and subretinal hemorrhage. Yellow-white lesion indicating choroidal neovascular membrane surrounds the scar (*arrowheads*). **b,c** Early and late phase fluorescein angiography. The choroidal neovascular membrane is identified (*arrows*). **d,e** Early and late phase indocyanine green angiography. Polypoidal lesions are identified (*arrowheads*).

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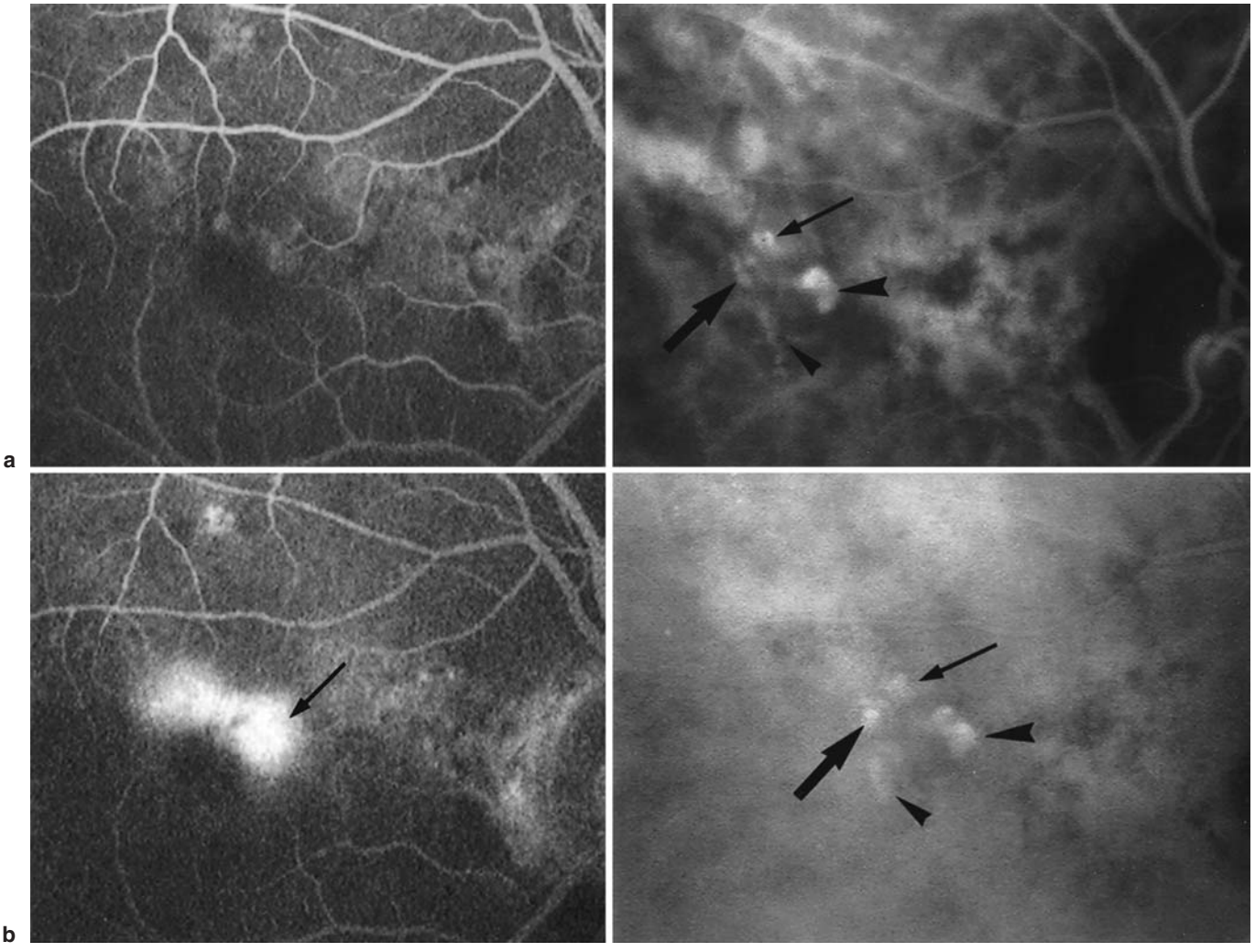

Figure 2a–d. Case 2: A 74-year-old man with no systemic disease other than hypertension. Preoperative appearance of the right eye. **a,b** Early and late phase fluorescein angiography. Fluorescein leakage from choroidal neovascular membrane is seen (*arrow*). **c,d** Early and late phase indocyanine green angiography. Several polypoidal lesions are identified, along with early and late phase hyperfluorescence (*large arrows*), and early phase hyperfluorescence and late phase isofluorescence (*small arrows*). A network of vessels is seen in the early phase, and indocyanine dye leakage from a network of vessels in the late phase (*small arrowheads*). Choroidal neovascular membrane is seen (*large arrowheads*).

hyperfluorescence in both the early and the late phase was minimal as compared with the above PCV (Fig. 4b). As in case 1, in the CNV area, there were numerous vessels with narrow lumen, and several VEGF-positive spots were recognized in vascular endothelial cells within the CNV (Fig. 4c).

Discussion

Uyama and colleagues[10] reported that in contrast to observations made in Caucasians, PCV often affects the macula of both eyes in relatively elderly Japanese men. The two patients described herein were also older (74 and 78 years), and in both patients PCV was localized within the macula, supporting the findings of Uyama's study. Two hypotheses have been introduced to explain the pathology of PCV: abnormally dilated existing choroidal vessels and CNV development under the RPE. Although the latter concept has had more support, histopathological findings have not been sufficient to confirm either hypothesis.

MacCumber and Spraul and colleagues investigated the pathology of PCV using enucleated eyes, and reported that fibrovascular tissue was seen in Bruch's membrane or between Bruch's membrane and the RPE.[11,12] However, they did not observe dilated polypoidal vessels. Shiraga et al.[13] histopathologically analyzed subretinal fibrovascular tissue that had been excised from PCV patients with subretinal hemorrhage, but did not find vessels resembling the dilated polypoidal vessels seen in our patients. In other words, it has been difficult to determine whether PCV constitutes abnormally dilated existing choroidal vessels or CNV developing under the RPE.

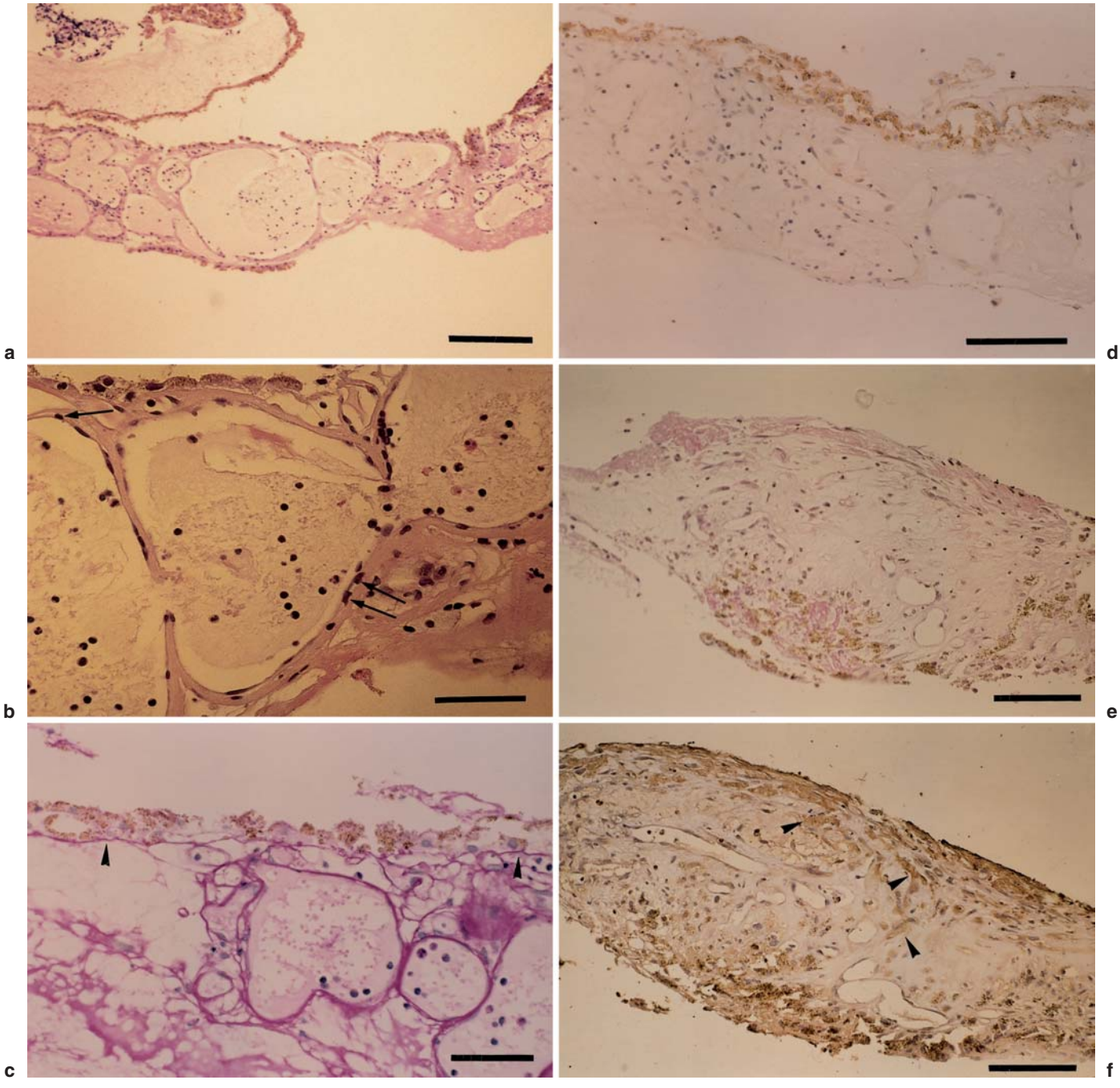

Figure 3a–f. Case 1: Histopathology. **a** Several dilated vessels are seen under the retinal pigment epithelium. H&E, Bar = 200 μm. **b** The vascular wall of vessels are thin and accompanied by pericytes (*arrows*). H&E, Bar = 50 μm. **c** Dilated vessels are seen under the Bruch's membrane (*arrowheads*). Periodic acid-Schiff, Bar = 50 μm. **d** Vascular endothelial growth factor positive spots are not seen in the polypoidal lesion. Immunohistochemical stain for vascular endothelial growth factor, Bar = 100 μm. **e** Many vascular lumens are seen in the choroidal neovascular membrane. H&E, Bar = 50 μm. **f** Many vascular endothelial growth factor-positive spots are seen in the choroidal neovascular membrane compared with in the polypoidal lesion (*arrowheads*). Immunohistochemical stain for vascular endothelial growth factor, Bar = 100 μm.

Lafaut et al.[5] first documented the pathology of dilated polypoidal lesions in Bruch's membrane and concluded, that since PCV was at this anatomical site, it represented a variant form of exudative AMD. Terasaki et al.[6] carried out macular translocation and collected PCV tissue that had developed under the neurosensory retina and was accompanied by CNV. Their histopathological analysis of the excised specimen revealed fibrovascular tissue underneath the RPE and in Bruch's membrane. Since immunohistological staining produced positive reactions in vascular

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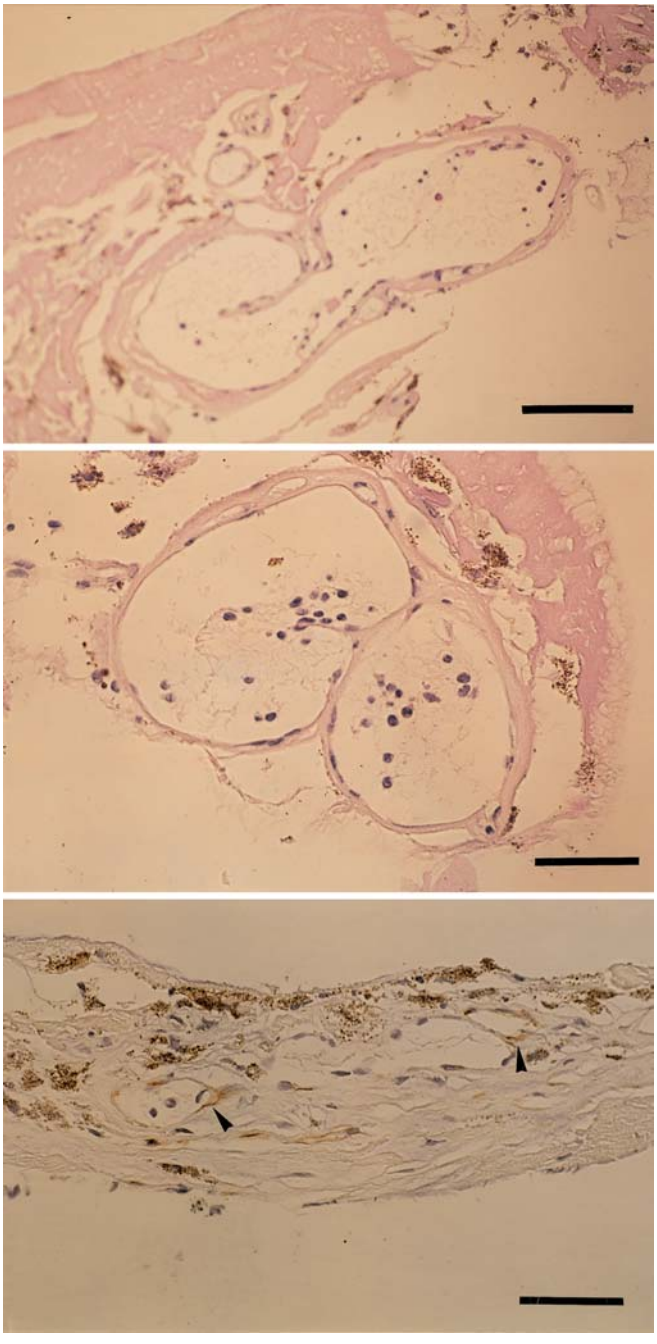

Figure 4a–c. Case 2: Histopathology. **a** Several dilated vessels and exudation are seen. H&E, Bar = 100 μm. **b** Exudation around dilated vessels is minimal. H&E, Bar = 50 μm. **c** Many vessels with narrow lumens are seen in the choroidal neovascular membrane. Vascular endothelial growth factor-positive spots are seen (*arrowheads*). Immunohistochemical stain for vascular endothelial growth factor, Bar = 25 μm.

endothelial cells and in the RPE, they concluded that the PCV was CNV. We have previously documented pathological findings of CNV associated with AMD.[8] However, the present histopathological study on PCV revealed vessels that were abnormally large, markedly exceeding ordinary choroidal vessels in size, and that these dilated vessels were interconnected and located under the RPE. Furthermore, pericytes were seen in these vessels, and the vessels were not surrounded by the RPE. In contrast to Terasaki's observations, in the present study, we found no VEGF-positive reactions in polypoidal lesions or in surrounding areas. These findings suggest that the histopathology of PCV differs from that of CNV. We consider PCV to represent abnormally dilated existing choroidal vessels.

In the present study, we compared preoperative IA findings and histopathological findings associated with PCV to ascertain the clinical significance of the IA findings. In Case 1, IA revealed a polypoidal lesion exhibiting hyperfluorescence in both early and late phases, and abnormally distended vessels were seen in the affected area under a relatively healthy RPE. In addition, there was little amorphous material around the polypoidal lesion, suggesting that the hyperfluorescence may be due to ICG dye filling the dilated vessels. In Case 2, IA demonstrated a polypoidal lesion with early hyperfluorescence and late isofluorescence. In this area, dilated vessels were observed under the RPE and perivascular amorphous material was abundant. These findings suggest that early hyperfluorescence is caused by ICG flowing into dilated vessels. It has previously been shown that the fluorescence of ICG is low, that it attenuates exponentially in the late phase, and that ICG readily produces blockage.[14] Thus, in this study, late isofluorescence was most likely attributable to blockage resulting from amorphous material surrounding the vessels.

Classic CNV associated with PCV that developed under the neurosensory retina had numerous vascular lumens and small amounts of fibrous components, and was not surrounded by the RPE. Since VEGF-positive reactions were seen in the vascular endothelial cells within the CNV areas, vascular permeability was probably elevated. These findings are in agreement with the histological findings of CNV associated with AMD, in that those lesions showed hyperfluorescence in both the early and the late phases.[15]

PCV was once reported to be a disease with a relatively favorable prognosis in terms of visual acuity, but if there is longstanding exudative sensory retinal detachment including the macula, or if massive subretinal hemorrhage occurs, the chance for the recovery of visual acuity is poor. Uyama et al.[16] followed PCV patients for long periods of time (mean: 39.9 months) and divided PCV into exudative and hemorrhagic types. In IA, the exudative type is solitary PCV, and although serous retinal detachment can be induced, the PCV is not highly active and eventually shrinks or disappears. Conversely, on IA, the hemorrhagic type exhibits a grape-like polypoidal lesion, and the PCV is highly active since hemorrhagic RPE detachment or subretinal hemorrhage can be induced. Case 1 had exudative or solitary PCV. Histopathological analysis demonstrated the RPE to be conserved, and the PCV was located underneath Bruch's membrane and the RPE. In addition, vessels were dilated but relatively maintained, and immunological staining revealed no VEGF-positive reaction around the polypoidal lesion, suggesting that the PCV was relatively

inactive. These findings agree with those of Uyama et al. However, CNV may develop from another area and reach the macula, thereby lowering visual acuity. We were not able to analyze the histopathology of hemorrhagic PCV in the present study. Further elucidation of the histopathological characteristics of both types of PCV is therefore necessary.

In conclusion, we have described herein two patients with PCV. As abnormally dilated polypoidal lesions were observed beneath the RPE, and the histopathological findings differed from those of CNV associated with AMD, the PCV was shown to represent abnormally dilated existing choroidal vessels. The results obtained from our present patients suggest that IA findings vary depending on the condition of the RPE located above the PCV and on the extent of amorphous material around the PCV.

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