Clinicopathologic Findings in Polypoidal Choroidal Vasculopathy

Hiroyuki Nakashizuka,¹ Masako Mitsumata,² Shigekuni Okisaka,³ Hiroyuki Shimada,¹ Akiyuki Kawamura,¹ Ryusaburo Mori,¹ and Mitsuko Yuzawa¹

PURPOSE. To elucidate the pathogenic mechanism of polypoidal choroidal vasculopathy (PCV) based on histopathologic findings.

METHODS. Specimens obtained by surgical excision of PCV from five eyes of five patients (mean age, 75.6 ± 3.1 years) were studied histopathologically. Immunohistochemical studies were also performed to identify CD34, vascular endothelial growth factor (VEGF), CD68, α-smooth muscle actin (α-SMA) and hypoxia-inducible factor (HIF)-1α.

RESULTS. Hyalinization of choroidal vessels and massive exudation of fibrin and blood plasma were observed in all the specimens of PCV lesions. Some blood vessels were located above the RPE in two of the five eyes. Immunohistochemically, CD68-positive cells were detected around the hyalinized vessels. There were no α-SMA-positive cells in the vessels of PCV. CD34 staining showed endothelial discontinuity. Vascular endothelial cells within the PCV specimens were negative for VEGF. HIF-1α positive inflammatory cells were located in the stroma of specimens.

CONCLUSIONS. Hyalinization of choroidal vessels, like arteriosclerosis, is characteristic of PCV. (Invest Ophthalmol Vis Sci. 2008;49:4729 – 4737) DOI:10.1167/iovs.08-2134

Polypoidal choroidal vasculopathy (PCV) is a disorder characterized by vascular networks and apical polypoidal lesions.¹ PCV is known to be more common in non-white populations (including blacks, Hispanics and Asians).² The incidence of PCV in Japanese has also been reported to be remarkably high.³,⁴ There are two opinions on the pathogenesis of PCV: inner choroidal vessel abnormalities¹⁵–¹² and variants in choroidal neovascularization (CNV).¹³–¹⁷ Although several pathologic studies have focused on PCV, the pathogenesis is still unclear.¹⁵–¹⁰,¹⁸

Recently, as PCV has become better recognized, it has become apparent that some specimens excised under a diagnosis of age-related macular degeneration (AMD) included PCV. We examined the histopathology of five PCV specimens from five eyes, the specimens having been excised between 2001 and 2003, with a diagnosis of neovascular AMD. Judged against the PCV diagnostic criteria which were subsequently introduced in 2005,¹⁹ these five specimens were obtained from definite cases of PCV. Our findings shed light on the pathogenesis of PCV.

MATERIALS AND METHODS

We studied specimens surgically extracted from five eyes of five patients (four men and one woman, 71–79 years of age, mean 75.6 ± 3.1) with PCV. A diagnosis of neovascular AMD was made based on fluorescein angiography (FA) and clinical findings between 2001 and 2003. Although indocyanine green angiography (IGA) showed polypoidal lesions in all five eyes, no typical network vessels were observed. However, PCV was diagnosed based on recently published criteria used to identify PCV¹⁹ and interpret FA findings.²⁰

Informed consent for the surgical procedure and for the use of excised tissue was obtained from all patients, in accordance with the tenets of the Declaration of Helsinki. Surgical excision of subfoveal CNV was performed according to the method of Lambet et al.²¹ The surgical specimens were immediately fixed in 10% formalin in phosphate-buffered solution (pH 7.4) and embedded in paraffin, and 4-µm serial sections were prepared and stained with hematoxylin and cosin (HE), periodic acid-Schiff (PAS) for basement membranes, phospho-tungstic acid hematoxylin (PTAH) for fibrin, and elastica van Gieson for elastic fibers.

For immunohistochemical studies, paraffin-embedded sections were deparaffinized, hydrated and rinsed in deionized water. Immunostaining was performed using an automated immunostaining machine (Ventana Medical Systems, Inc. Tucson, AZ) with Endogenous Biotin Blocking Kits (Ventana Medical Systems). The primary antibodies used were as follows. Anti-CD34 antibody (monoclonal mouse anti-human CD34, clone QBEnd-10; 1:20; Dako Cytomation, Carpintereia, CA) was used to confirm blood vessels in the specimens. Anti-α-smooth muscle antibody (Anti-actin, smooth muscle monoclonal clone; 1:15; Thermo Fisher Scientific Inc., Waltham, MA) was used to identify smooth muscle cells and myofibroblasts. Anti-vascular endothelial growth factor (VEGF) antibody (rabbit polyclonal antibody; 1:50; Santa Cruz Biotechnology, Santa Cruz, CA) was used to demonstrate localization of VEGF as an angiogenic factor. Anti-CD68 antibody (mouse monoclonal anti-human macrophage, clone PG-M1; 1:80; Dako Cytomation) was used to identify macrophages. Anti-hypoxia inducible factor (HIF)-1α antibodies (rabbit antibodies; 1:50; Chemicon International, Temecula, CA) were used to examine the oxidative states of tissues. Negative controls were obtained by omitting the primary antibodies. Subsequent reactions with secondary antibodies and visualization were achieved with DAB (DAB Universal Kit; Ventana Medical Systems). The sections were counterstained with hematoxylin and mounted (Permoun; Fisher Scientific, Pittsburgh, PA). All the stained slides were evaluated histologically by light microscopy (VANOX-S; Olympus, Tokyo, Japan).

From the ¹Division of Ophthalmology, Department of Visual Science, and the ¹¹Division of Pathology, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan; and the ²Laboratory of Ophthalmic Pathology Education, Tokyo, Japan.


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Corresponding author: Hiroyuki Nakashizuka, Division of Ophthalmology, Department of Visual Science, Nihon University School of Medicine, 1-8-13 Surugadai, Kanda, Chiyodaku, Tokyo 101-8309, Japan; shizuku@med.nihon-u.ac.jp.


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RESULTS

The clinical characteristics of the patients are summarized in Table 1. Three of the five patients had systemic diseases including hypertension and hyperlipidemia. In this study, PCV lesion sizes were relatively small in all cases; from 1.1 disc diameters (DD) to 2.2 DD, as measured on IGA photographs. The pathologic findings revealed little granulation tissue formation in any of the specimens. On the other hand, all the specimens exhibited massive exudative change and leak-
The continuity of the retinal pigment epithelium (RPE) above or beneath the vessels had been lost in most cases, and blood vessels were confirmed to be located above the RPE in two of the five specimens. Furthermore, all the vessels exhibited hyalinization, choriocapillaris had disappeared, even in the cases in which RPE had been preserved.

Immunohistochemically, CD68-positive foamy macrophages were detected around the hyalinized vessels, but α-smooth muscle actin (SMA)-positive cells were not. CD34 staining revealed discontinuity of the endothelial lining of these abnormal vessels.

Myofibroblasts, recognized by their fibroblast-like appearance and α-SMA immunoreactivity were seen in the stroma of several specimens. Although VEGF-positive cells were detected among CD68-positive foamy macrophages, fibroblast-like cells and RPE cells, vascular endothelial cells in the PCV specimens were negative for VEGF. HIF-1α-positive cells were located in the stroma of specimens.

Angiographic and histopathologic findings in three of our cases are presented in the following sections.

**Case 1**

The patient was a 77-year-old woman with hypertension and rheumatoid arthritis. Preoperative visual acuity was 0.3 in the right eye in December 2000. As the white lesion was situated above the RPE with subretinal hemorrhage (Fig. 1A), and classic CNV was identified on FA (Fig. 1B), the white lesion was thought to be subretinal CNV. Although IGA showed several small polypoidal lesions resembling clusters of grapes (Fig. 1C), a diagnosis of neovascular AMD was made based on ophthalmoscopic and FA findings. CNV excision was performed in June 2001.

The specimen contained abnormally dilated vessels beneath the RPE, and the vessel walls were thick and hyalinized, because of extravasation of plasma protein and deposition of basement membrane–like material (Fig. 2A). The RPE overlying the hyalinized vessels was obscured. There were numerous blood cells in the vascular cavity, and several neutrophils adhered to the inner vessel walls (arrow). (✱) Obstruction of hyalinized vessels. The diameter of the most dilated vessel exceeds 250 μm. (C) Bruch’s membrane is stained by PAS (arrowhead). A hyalinized vessel (asterisk) is located beneath Bruch’s membrane (periodic acid-Schiff). (D) Hyalinized vessels showing fibrosis (red) and exudative material (blue) (phosphotungstic acid hematoxylin).

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932946/)

**Figure 2.** (A) Photomicrograph showing abnormally dilated vessels (✱) beneath the RPE (arrowhead). The walls of these vessels were thickened and hyalinized, owing to extravasation of plasma protein and deposition of basement membrane–like material (hematoxylin-eosin). (B) Higher magnification of hyalinized vessels indicated in (A). There were numerous blood cells in the vascular cavity and several neutrophils adhered to the inner vessel walls (arrow). (✱) Obstruction of hyalinized vessels. The RPE overlying hyalinized vessels was obscured. The diameter of the most dilated vessel exceeds 250 μm. (C) Bruch’s membrane is stained by PAS (arrowhead). A hyalinized vessel (asterisk) is located beneath Bruch’s membrane (periodic acid-Schiff). (D) Hyalinized vessels showing fibrosis (red) and exudative material (blue) (phosphotungstic acid hematoxylin).

Scale bar, 200 μm; magnification: (A) ×25; (B) 50; (C, D) 100.
membrane (Fig. 2C). PTAH staining revealed significant hyalinization of these vessels with marked extravasated plasma protein (Fig. 2D). The abnormal vessels were lined with endothelium stained by CD34, which revealed discontinuity in the vascular endothelium (Fig. 3A). There were no α-SMA-positive cells in the hyalinized vessels (Fig. 3B). Macrophages staining positively for CD68 had infiltrated around the dilated vascular cavity, and foamy macrophages were occasionally seen within the vessel walls (Fig. 3C). HIF-1α-positive mononuclear cells were located in the stroma of specimens (Figs. 3D). Although immunoreactive deposits of VEGF were present at the macrophages, fibroblast-like cells, and RPE cells, the vascular endothelial cells were negative for VEGF (Fig. 3E).

**Case 2**

The patient was a 71-year-old man with no systemic disease. Preoperative visual acuity was 0.07 in the right eye in November 2002. Two orange lesions, presumably polypoidal, surrounded by a white lesion possibly indicating accumulation of fibrin material, were recognized in the macular area of the right eye (Fig. 4A). FA revealed two small hyperfluorescent areas and a hyperfluorescent area indicating pigment epithelial detachment (PED; Fig. 4B). The IGA revealed hyperfluorescent areas apparently representing polypoidal lesions and a hypo- or hypofluorescent area indicating PED (Fig. 4C). CNV excision was performed in March 2003. The specimen contained abnormally dilated vessels above the RPE, and these vessels were hyalinized. Exudative change around the vessels was significant. This lesion was thought to be a portion of a PCV. On the other hand, fibrovascular tissues with marked fibrosis, small vascular channels, and less exudative changes were identified beneath the RPE, and were thought to represent CNV (Fig. 5A). Although occult CNV was apparently not observed by FA, it was thought to have existed beneath the PED. This specimen was considered incidentally to include both PCV and CNV lesions. PTAH staining clarified the difference between PCV and CNV (Fig. 5B). In the PCV portion, defects in the vascular endothelium were revealed by CD34 staining (Fig. 6A), whereas the continuity of the endothelium was maintained in the CNV portion (Fig. 6B). Although no α-SMA-positive cells were detected in the hyalinized vessels located on the PCV portion (Fig. 6C), α-SMA-positive pericytes were observed in vascular channels located on the CNV por-

**Figure 3.** Immunohistochemistry of PCV. (A) Vascular endothelium expressing the CD34 marker. Note discontinuous endothelium stained with CD34 (arrow). (B) α-SMA staining was negative in the hyalinized vessels. (C) Macrophages expressing the CD68 marker. Note the presence of CD68-positive foamy macrophages around dilated vascular cavities and occasionally within vessel walls. (D) Immunohistochemistry for HIF-1α. There was distinct staining of nuclei, mainly in mononuclear cells (arrow). (E) Vascular endothelium is negative for VEGF staining. Magnification: (A, B, D, E) ×50; (C) ×100.
tion (Fig. 6D). Foamy macrophages stained positively for CD68 had infiltrated around the dilated vascular cavity in the PCV portion (Figure 7A) and in the stroma of the CNV portion (Fig. 7B). HIF-1α-positive cells were located in the stroma of both portions of the specimen (Figs. 7C, 7D). Although immunoreactive deposits of VEGF were present at macrophages, fibroblast-like cells, and RPE cells, vascular endothelial cells in both the PCV and the CNV portions were negative for VEGF (Figs. 7E, 7F).

Case 3

The patient was a 77-year-old man with hyperlipidemia. Preoperative visual acuity was 0.06 in the left eye in December 2000. IGA revealed a polyplike dilatation at the macula (Fig. 8A). Although the dilatation of vessels was not apparent in the specimen, many hyalinized vessels with surrounding exudative change were prominent above the RPE. The large number of vessels resembled a coil-like configuration. This lesion was thought to have a PCV portion. In contrast, fibrovascular tissues and small vascular channels were recognized beneath the RPE and were thought to represent occult CNV. Exudation and hyaline change of the vascular wall were not significant in this CNV portion. This specimen was considered incidentally to include PCV and CNV portions, as in Case 2. Furthermore, an RPE break was also identified in this specimen (Fig. 8B). PTAH staining clarified the difference between PCV and CNV (Fig. 8C). Immunohistopathologic findings were similar to those in case 1.

DISCUSSION

In this study, extensive exudative change and hyalinization of vessels were documented.

There are several reports suggesting the presence of hyalinized vessels in PCV specimens. Okubo et al.10 suggested histopathologic similarities between branched vein retinal occlusion and PCV, and the walls of the vessels in their cases had a hyaline-like appearance. Kuroiwa et al.11 also reported histopathologic features of surgically excised specimens from five patients with PCV. In their series, large choroidal arterioles with an inner elastic layer were described, and the walls of these arterioles were thick and showed sclerotic change associated with an increase in basement membrane-like materials together with collagen fibers. Terasaki et al.13 suggested the vascular components of PCV to represent subretinal neovascularization; however, they also noted massive leakage of fibrin material around the vessels. These reports were thought to suggest the insudative and transudative hyalinization of vessels in PCV specimens.

The term hyalinization refers to extensive replacement of the smooth muscle component by amorphous pseudocollagenous tissue of a poorly defined nature.22–25 Hyalinized vessels are characterized by extravasation of plasma protein and deposition of basement membrane-like material. In other words, hyalinization is one of the arteriosclerotic changes seen not only in choroids, but also in other parts of the body, for instance, the brain, kidneys, and pancreas.26,27

PCV is reportedly similar to retinal arterial macroaneurysm (RAM) as regards the epidemiologic associations in the following categories of patients: female, hypertensive, black, and elderly.28 Two cases of PCV with RAM have been reported by Ross et al.28

The histopathologic findings of retinal macroaneurysms have been extensively described by Fichte et al.29 Aneurysmal sites typically show thickening of the vessel wall secondary to a fibrin-laminated clot formation with accompanying hypertrophy of the muscle.29 Hyaline, hemorrhages, and, occasionally, foamy macrophages may be seen in the vessel wall.29 These findings are structurally similar to the histopathologic characteristics of PCV in our study. This may mean that PCV is closely associated with arteriosclerotic changes.

Pathologic comparison of CNV and PCV is important for understanding the pathogenesis of PCV. Case 2 included both PCV and CNV portions in the same specimen, and thus this case was thought to be appropriate for comparing the structural differences between PCV and CNV. The CNV portion
**Figure 5.** (A) Top: above the RPE (arrow), including abnormally dilated hyalinized vessels, thought to be a PCV specimen. Bottom: beneath the RPE which contained fibrovascular tissues with fibrosis, fibroblast-like cells, and small vascular channels, was thought to be a CNV specimen (hematoxylin-eosin). (B) Top: PCV staining (blue) indicated marked exudative change; bottom: CNV staining with PTAH (red) indicated no marked exudative change in fibrous tissue. Arrows: RPE. Original magnification, ×50.

**Figure 6.** Immunohistochemical comparison between the PCV (A, C) and CNV (B, D) portions. (A) Vascular endothelium expressing the CD34 marker in the PCV portion. Note the discontinuous endothelium stained with CD34 (arrow). (B) Vascular endothelium expressing the CD34 marker in the CNV portion. Note the continuous endothelium stained with CD34 (arrow). (C) α-SMA-positive cells were absent in the hyalinized vessels located on the PCV portion. (D) α-Smooth muscle actin-positive pericytes are present in vascular channels located on the CNV portion. Magnification: (A, B) ×50; (C, D) ×100.
showed granulation tissue proliferation, supporting the concept that CNV represents a stereotypic, nonspecific wound repair response. In contrast, little if any fibrosis or granulation tissue proliferation was observed in the PCV portion. The histopathologic characteristics of PCV include hyalinization of vessels, extravasation of plasma protein and deposition of basement membrane-like material. Hyalinized vessels in PCV were negative for \( \alpha \)-SMA expression, although pericytes of vessels in CNV were immunoreactive for \( \alpha \)-SMA. This finding may indicate that smooth muscle cells of choroidal vessels in PCV had disappeared due to the increased intraluminal pressure resulting from systemic hypertension. Furthermore, Kondo et al. reported that the elastin gene, which is a potent and specific regulator of the migration and proliferation of vascular smooth muscle cells, is a susceptibility gene for PCV. Therefore, elastin gene dysfunction may accelerate the disappearance of the smooth muscle cells of choroidal vessels in PCV.

Immunoreactive expressions of CD34 revealed discontinuity in the vascular endothelium in the PCV portion. This finding suggests that eddy diffusion of blood flow in hyalinized and dilated vessels causes sufficient damage to the vascular endothelium to cause sloughing. Recent investigations have demonstrated VEGF also to be expressed in vascular endothelial cells and RPE cells of surgically excised subfoveal fibrovascular tissues of human CNV tissue. In our study of PCV, VEGF positivity was recognized in macrophages, fibroblast-like cells and RPE cells, but not in vascular endothelial cells. Tong et al. reported that aqueous levels of VEGF in eyes with PCV were significantly lower than those in eyes with exudative AMD. These findings raise the possibility that these are distinct clinical entities with different pathogenic processes, and that VEGF may hardly contribute to the occurrence of PCV.

It was also reported that eyes with PCV sometimes had the appearance of classic CNV on FA and, as a result, the condition was wrongly attributed to type 2 CNV or to pure fibrinous tissue without CNV. In our study of PCV, three of the five eyes showed discontinuity in the RPE, and PCV portions including hyalinized vessels were found above the level of the RPE in two of the five cases. We speculated that the elevation of choroidal vessels through RPE breaks and the vulnerable Bruch’s membrane, as in our cases 2 and 3, may be attributable to rising intratissue pressure caused by massive exudation from hyalinized vessels, which would result in a classic CNV pattern on FA.

**Figure 7.** Immunohistochemical comparison between the PCV (A, C, D) and CNV (B, D, F) portions. (A) Macrophages expressing the CD68 marker. Note the presence of CD68-positive foamy macrophages around the dilated vascular cavity in the PCV portion. (B) Macrophages expressing the CD68 marker. A few CD68-positive cells are present in the stroma of the CNV portion (arrow). Arrowhead: RPE. (C, D) HIF-1α positive mononuclear cells are located in the stroma of specimens in both lesions (arrow). (E) Immunoreactive deposits of VEGF are present at macrophages (arrow), but not at vascular endothelial cells in the PCV portion (arrowhead). (F) Immunoreactive deposits of VEGF are present at macrophages and RPE cells (arrow), but not at vascular endothelium cells in the CNV portion (arrowhead). Original magnification: (A–F) ×50.
However, we cannot rule out the possibility that PCV and CNV can occasionally exist in the same eye simultaneously. Thus, there are two possibilities. The first is that PCV lesion and CNV associated with AMD are likely present together incidentally. The second possibility is that CNV may grow secondarily as a result of a wound repair reaction to a collapse of the RPE or Bruch’s membrane in advanced PCV. Furthermore, positivity for HIF-1α was observed in the nuclei of lymphocyte-like cells in the PCV. HIF-1α has been recognized as a transcription factor induced by hypoxia, and exposure of the cells to hypoxia (1% O₂) reportedly induced nuclear translocation of HIF-1α from the cytoplasm. It has also been shown to stimulate transcriptions of multiple genes that are upregulated by hypoxia, including VEGF. Thus, the environment in PCV specimens is thought to be hypoxic, and HIF-1α may accelerate the expression of VEGF which would stimulate CNV formation.

Yuzawa et al. have reported polypoidal lesions sometimes containing many vessels in various abnormal configurations shown on IGA with confocal scanning laser ophthalmoscopy. The present clinicopathologic study has demonstrated that the presence of many vessels in case 3 corroborates these IGA findings.

In this study, PCV was characterized by vascular hyalinization followed by massive extravasation of plasma protein, deposition of basement membrane-like material, and lack of granulation tissue proliferation. To our knowledge, hyalinization of CNV has not been reported. Furthermore, dilated hyalinized vessels were observed beneath but not within Bruch’s membrane in our case 1. These findings demonstrate that hyalinized vessels are the choroidal vasculature with arteriosclerotic changes similar to the hyalinization seen in other parts of the body (i.e., they do not represent neovascularization). Furthermore, massive exudation of fibrin and blood plasma from dilated hyalinized choroidal vessels may raise choroidal tissue pressure sufficiently to produce protrusion of choroidal tissues through the weakened or disrupted RPE and Bruch’s membrane.

The results in this study suggest that hyalinization of choroidal vessels is characteristic of PCV and that arteriosclerosis is an important pathologic feature.

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**References**


