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Indocyanine Green Angiography and Histopathology of Choroidal Neovascular Membrane in Age-Related Macular Degeneration

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Purpose: Histopathological investigation of the choroidal neovascular membrane (CNM) in age-related macular degeneration (AMD) patients who showed various findings in indocyanine green angiography (IA).

Methods: Before surgery, 20 eyes of 20 patients were classified into four types based on IA findings (Type I: both early and late phase hyperfluorescence; Type II: hyperfluorescence only in the early phase; Type III: hyperfluorescence only in the late phase; Type IV: virtually no hyperfluorescence in any phase). Seventeen surgically excised specimens stained with hematoxylin-eosin and azan, were examined by light microscopy. Three other specimens were examined by electron microscopy.

Results: Type I membrane showed many vascular channels not present in the surrounding retinal pigment epithelium (RPE) cells, and little fibrous tissue. Type II membrane had many vascular channels but RPE cells surrounded the CNM. Type III membrane showed few vascular channels and RPE cell proliferation. Type IV membrane showed dense fibrous tissue.

Conclusion: The IA findings in AMD agreed with the CNM membrane structure in regard to the number of vascular channels, maturity of vessels, the extent of envelopment of RPE cells and the amount of fibrous tissue. **Jpn J Ophthalmol 2000;44:360–367**

Key Words: Age-related macular degeneration, choroidal neovascular membrane, fibrous tissue, indocyanine green angiography, retinal pigment epithelium cell.

Introduction

In recent years, indocyanine green angiography (IA) has commonly been used to examine the choroidal circulation. Indocyanine green angiography is one of the most effective methods for detecting a choroidal neovascular membrane (CNM). In particular, several studies have shown that IA is also useful with fluorescein angiography (FA) for detecting the CNM caused by age-related macular degeneration (AMD).[1,2] If a CNM is not located at the fovea, laser treatment can be performed. For a CNM developing beneath the fovea, including the fovea and feeder vessels, laser treatment is also indicated. Recently, CNMs have been removed by submacular surgery, and a large number of histopathological investigations have been reported.[3–5] Although CNMs associated with AMD show various features, the reasons for these differences are still unclear. In this study, we classified IA findings in AMD into four types. The membranes removed by vitrectomy, and histopathological features were compared with the IA findings.

Materials and Methods

Informed consent was obtained from AMD patients (20 eyes) awaiting vitrectomy. One week before surgery, the patients underwent color fundus

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Table 1. Clinical Characteristics of Patients

Membrane Type	Mean Age (y)	Mean Preoperative Visual Acuity (log MAR)	Mean Follow-Up Interval (Mo)*
I	66.6	−1.22	9
II	54.5	−1.15	7
III	66.3	−1.34	6
IV	69.0	−1.03	15

*Follow-up interval is from first symptoms to surgery.

photography, FA, and IA. Indocyanine green dye (25 mg) was used for IA, and angiography was performed using a TRC-50IA (Topcon, Tokyo) or a scannning laser ophthalmoscope (SLO). Analysis was done with Topcon IMAGEnet. Table 1 shows the relationship of mean age, preoperative mean visual acuity, and duration of symptoms.

Indocyanine green angiography findings were classified into the following four types. Type I showed both early and late hyperfluorescence, Type II showed hyperfluorescence only in the early phase, Type III showed hyperfluorescence only in the late phase, and Type IV showed virtually no hyperfluorescence in any phase.

The CNMs were excised by the method of Lambert[6] after standard three-port vitrectomy was performed. The surgical specimens from 17 eyes were fixed in 10% neutral-buffered formalin (Type I: 10 eyes, Type II: 2 eyes, Type III: 3 eyes, Type IV: 2 eyes). Three eyes were fixed in 4% glutaraldehyde (Type I: 2 eyes, Type IV: 1 eye). These specimens were processed for light microscopic or transmission electron microscopic examination. For light microscopy, the specimens were dehydrated through increasing concentrations of alcohol, cleared with xylene, and embedded in paraffin. Then 4μm-thick sections were cut through the center of the CNM and stained with hematoxylin-eosin or azan. For transmission electron microscopy, the specimens were postfixed with 1% osmium tetroxide. After standard dehydration, the CNM sections were embedded in epoxy resin, and thin sections were cut and stained with uranyl acetate-lead citrate for electron microscopy.

Results

The CNM had grown into the fovea in 19 eyes, and 1 eye showed recurrence of proliferation at the fovea after laser photocoagulation. In FA, all types of CNM showed the classic pattern of hyperfluorescence.

Light Microscopic Findings

Type I (Figure 1). In 10 eyes, numerous vessels of various sizes were seen in the CNM. The retinal pigment epithelium (RPE) was poorly formed and did

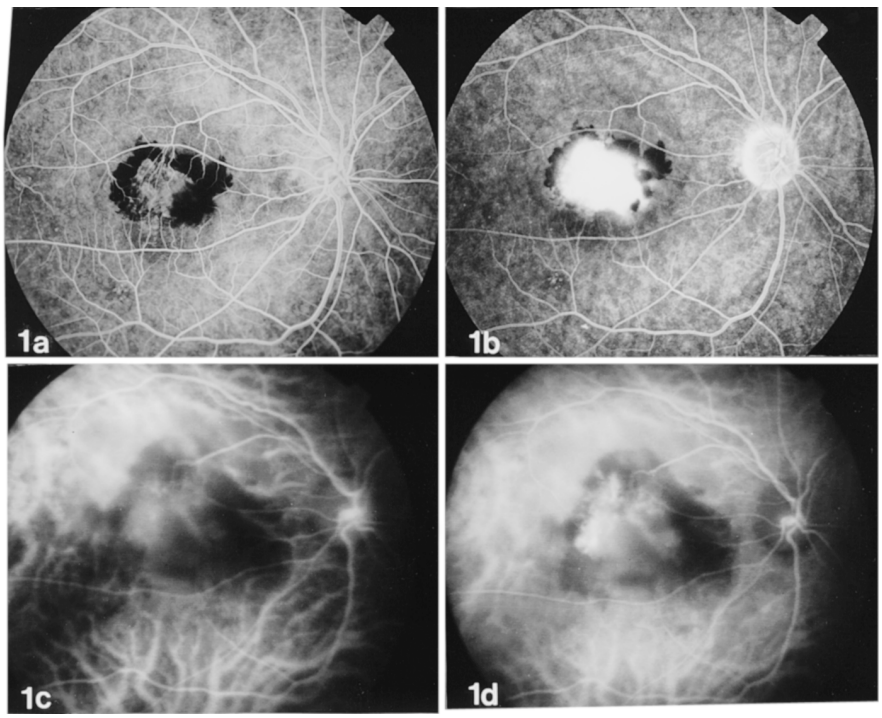

Figure 1. Fluorescein angiography (FA) and indocyanine green angiography (IA) of choroidal neovascular membrane (Type I membrane). FA: early phase (**1a**). FA: late phase (**1b**). IA: Hyperfluorescence can be seen in early phase (**1c**). IA: Indocyanine green dye leakage can be seen from neovascular membrane in late phase (**1d**).See text for explanation of membrane types.

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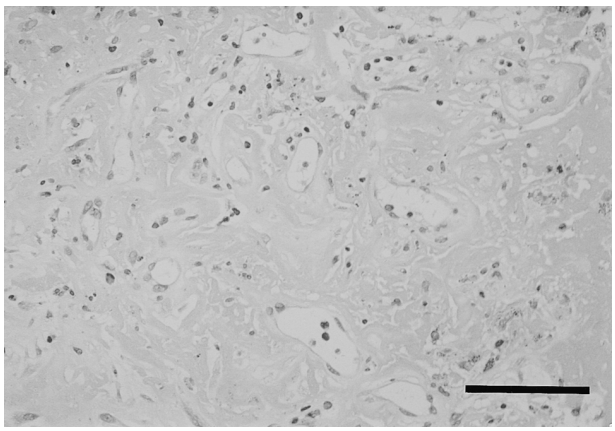

Figure 2. Light micrograph (hematoxylin-eosin staining) (Type I membrane). Numerous vascular channels can be seen. A few retinal pigment epithelium cells can be seen but surrounding neovascular membrane is difficult to distinguish. Bar = 100 μm.

not surround the vascular channels (Figure 2). Although fibrous tissue was seen, there was only a small amount (Figure 3).

Type II (Figure 4). This type of membrane contained a large amount of the RPE, and was surrounded by the RPE overall (Figure 5). In 1 eye, the RPE entered the CNM and surrounded the vascular channels (Figure 6). Many vascular channels were observed and fibrous tissue was more prominent than in Type I (Figure 7).

Type III (Figure 8). Vascular channels were less prominent than in Type I and the RPE proliferation was noted. There was also fibrous tissue (Figure 9).

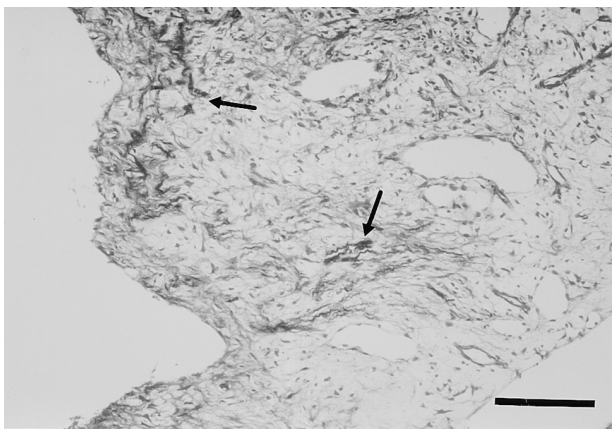

Figure 3. Light micrograph (azan staining) (Type I membrane). Small amount of fibrous tissue can be seen (arrows). Bar = 150 μm.

Type IV (Figure 10). Although neovascular channels were less prominent than in Type I, RPE partly surrounded the vascular channels (Figure 11). Abundant fibrous tissue was observed and it was closely packed (Figure 12).

Electron Microscopic Findings

Type I. The vascular endothelial cells had *thick* cell bodies that were rich in organelles and the vessel lumens were narrow (Figure 13). Most blood vessels in the CNM appeared immature. Some blood vessels in the CNM had wide lumen and thin cell bodies. They appeared fenestrated (Figure 14). It seemed that the blood vessels originated from the choroidal vessels. The RPE contained pigment granules and had a basement membrane that appeared thin in the CNM. Junctional complexes between RPE were immature (Figure 15).

Type IV. Cytoplasms of endothelial cells were thin and the vessels had wide lumens compared to Type I (Figure 16). The abundant fibrous tissue seen on light microscopy was fibrin and collagen fibers, arranged closely around the blood vessels.

Discussion

Changes in the RPE, Bruch's membrane, and choriocapillaris due to AMD cause a neovascular membrane to develop from the choroid. Irreversible secondary changes of the retina also occur. Although a large number of pathological studies have addressed the mechanism of neovascularization, it remains unclear. Several studies have shown clinically that IA along with FA is also useful for detecting the CNMs due to AMD. Indocyanine green angiography findings showed not only the classic type (both early and late hyperfluorescence), but also showed atypical findings. A comparison of dye leakage in IA and pathological data has been reported only by Chang[7] and Lee.[8] Chang found late hyperfluorescence of a CNM associated with AMD in 1 eye which did not show abnormal hyperfluorescence in FA. The eye was enucleated and examined histopathologically. Neovascularization was not observed by FA because it existed only beneath the RPE and there was no serous detachment at the circumference of the neovascularization. In IA, late phase hyperfluorescence was observed because indocyanine green (ICG) dye stained the fibrous tissue. Lee et al studied 15 eyes with CNM by IA and classified them as well-demarcated (a clear boundary of hyperfluorescence) or poorly demarcated (hyperfluorescense not observed).

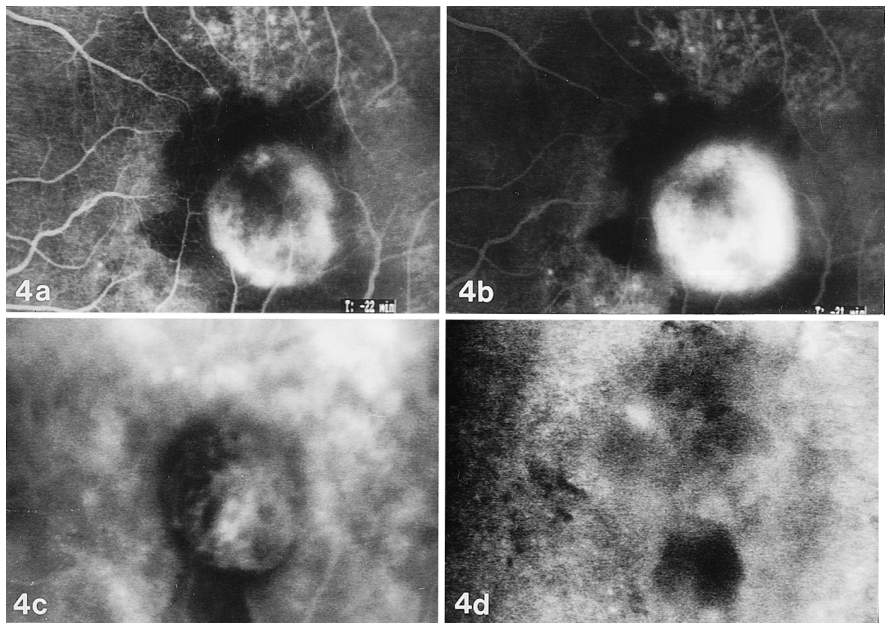

Figure 4. Fluorescein angiography (FA) and indocyanine angiography (IA) (Type II membrane). FA: early phase (**4a**). FA: late phase (**4b**). IA: Neovascular membrane shows hyperfluorescence and is surrounded by hypofluorescence in early phase (**4c**). IA: Site of hyperfluorescence in early phase is difficult to determine because of dye leakage in late phase (**4d**).

There were no significant pathological differences between the two types of eyes. In this study, we classified the IA findings in AMD into 4 types and made a comparison with the pathological data. Type I had numerous types of vascular channels and showed hyperfluorescense in the early phase. The RPE did not surround the vascular channels and there was little fibrous tissue. ICG dye leakage was observed in the late phase. Type I was the most common pattern in IA findings.

Electron microscopy showed that vascular endothelial cells were bulky and rich in mitochondria and rough endoplasmic reticulum, with a thin basement membrane. These findings suggested that the vessels were immature and ICG dye could leak out easily. Although a few RPE were observed, junctional complexes were not seen. Type II had many vascular channels, but less than Type I, and was surrounded by RPE, with resulting hypofluorescense surrounding hyperfluorescense in the early phase. Fibrous tissue was also abundant. The molecular weight of ICG dye is double that of fluorescein. The bulk of ICG dye is bound to plasma protein, making it less likely to spread to the extravascular space because of the surrounding RPE and a large amount of fibrous tis-

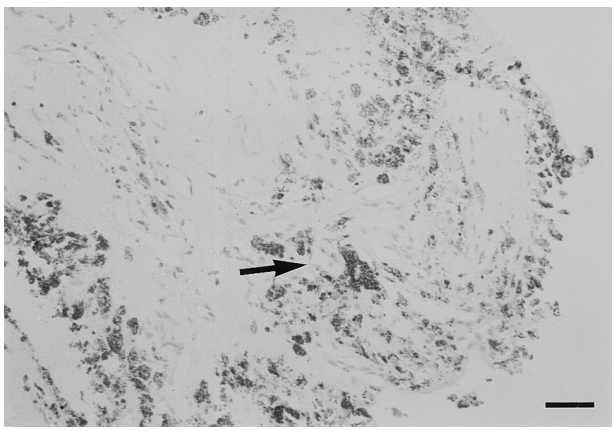

Figure 5. Light micrograph (hematoxylin-eosin staining) (Type II membrane). Cells containing pigment proliferate in or around neovascular membrane (arrow). Bar = 50 μm.

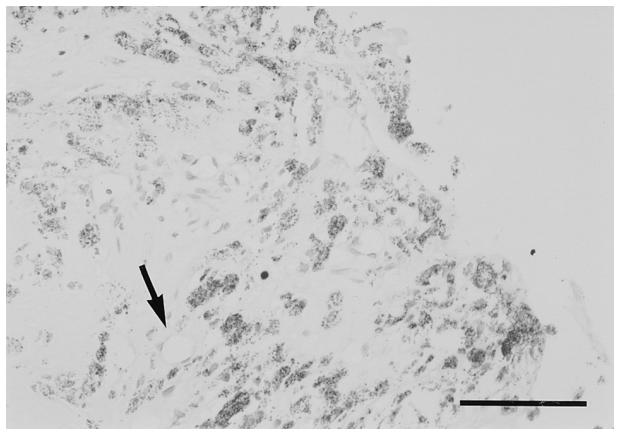

Figure 6. Light micrograph (hematoxylin-eosin staining) (Type II membrane). Retinal pigment epithelium cells proliferate in neovascular membrane and surround vascular channels (arrow). Bar = 100 μm.

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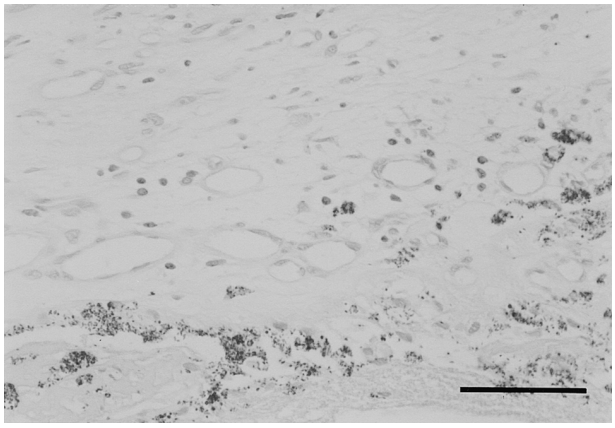

Figure 7. Light micrograph (hematoxylin-eosin staining) (Type II membrane). Many vascular channels can be seen. Bar = 100 μm.

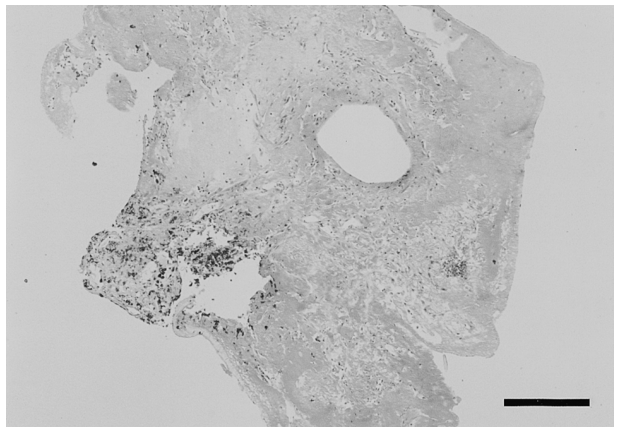

Figure 9. Light micrograph (hematoxylin-eosin staining) (Type III membrane). Vascular channels are few in contrast to type I membrane. High- and low-density areas of fibrous tissue are both seen. Outer segment is shown at bottom. Bar = 250 μm.

sue. This was the reason for hypofluorescense in the late phase. Type III had few vascular channels and ICG dye could not be detected in the early phase because of instrument limitations. This was the reason for hypofluorescence in the early phase. The RPE only partly surrounded vessels and had little relatively fibrous tissue, so ICG dye leaked from the vascular channels in the late phase, causing late hyperfluorescence. Type IV had few vascular channels, a large amount of fibrous tissue and RPE surrounding the CNM so that ICG dye was blocked and no hyperfluorescence was seen in the early phase. Most of the dye did not spread to the surrounding tissue in the late phase, so there was no late hyperfluorescence. By electron microscopy, Type IV CNM had wide neovascular channels and thin endothelial cells. These findings suggest that the vessels in the CNM were mature. Both Type I and Type IV had fenestrations within the endothelial cell membrane, suggesting that the origin of the proliferative vessels was the choroidal vessels.

Fukushima et al,[9] produced experimental choroidal neovascularization by strong laser photocoagulation in monkeys and studied the eyes. Neovascular-

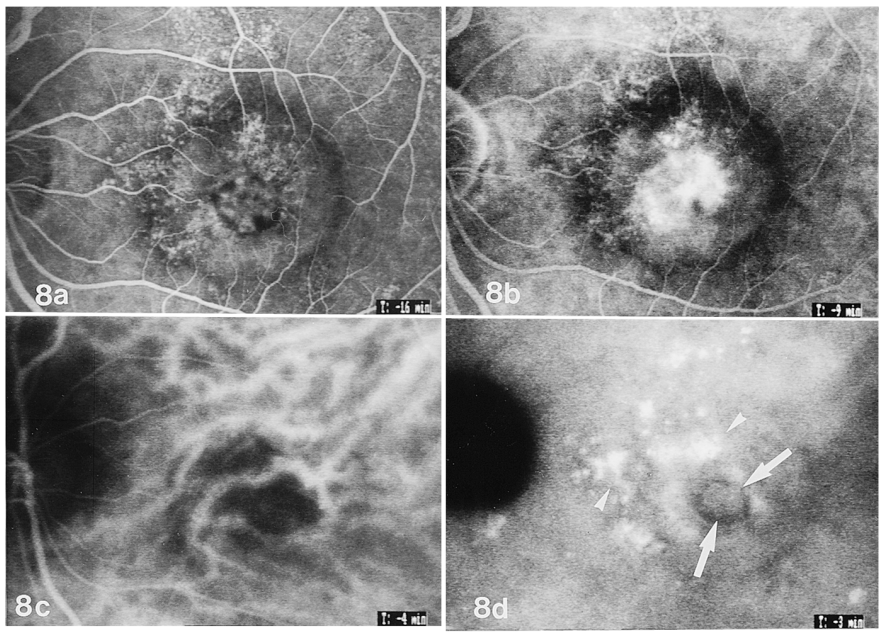

Figure 8. Fluorescein angiography (FA) and indocyanine green angiography (IA) (Type III membrane). FA: early phase (**8a**). FA: late phase (**8b**). IA: In early phase, abnormal hyperfluorescence is not detected (**8c**). IA: Hyperfluorescence suggests leakage from neovascular vessels (arrows). Drusen shows dot hyperfluorescence (arrowheads) (**8d**).

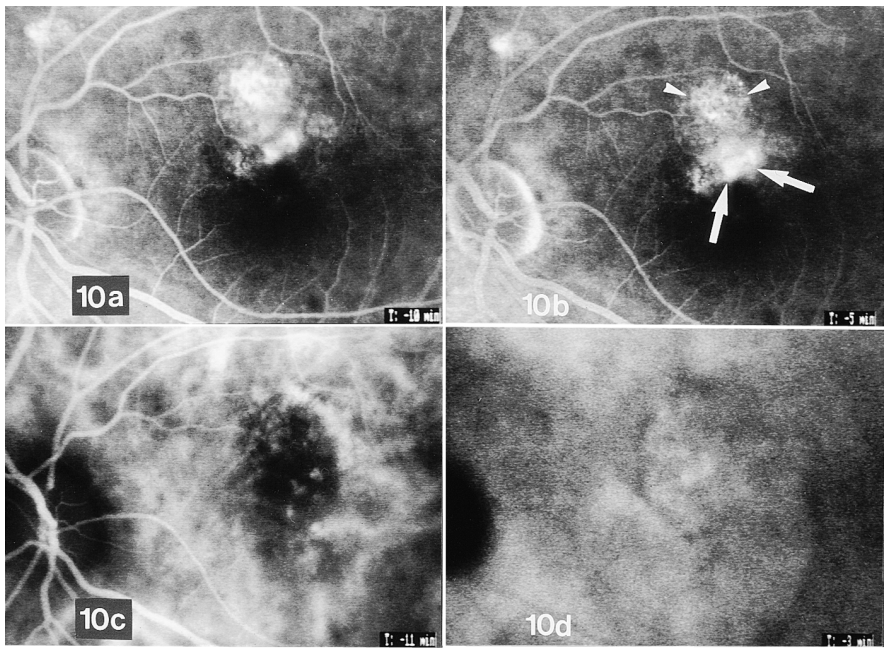

Figure 10. Flourescein angiography (FA) and indocyanine green angiography (IA) (Type IV membrane). FA: early phase (**10a**). FA: late phase. Indocyanine green dye leakage from neovascular membrane (arrow). Retinal pigment epithelium atrophy is observed above neovascular membrane (arrowhead) (*10b*). IA: Dye leakage is not seen in early and late phases (**10c,d**).

ization showing ICG dye leakage was immature and the RPE did not surround the vessels. Dye leakage did not occur when the immature vessels were surrounded by RPE or the vessels were mature. In this study, the Type I CNM was not surrounded by RPE, but Types II, III, and IV showed RPE surrounding all the membrane or proliferative RPE surrounding each vascular channel in the CNM. These results suggested that the amount of RPE surrounding the vessels is related to ICG dye leakage. Most vessels were immature in Type I CNMs. In contrast, there were mature vessels in Type IV CNMs, but only a few. We believe that ICG dye leakage was related to the maturity of vessels and the number of vascular channels in the CNM. Azan staining showed differences in the density and amount of fibrous tissue in each type. After immunohistochemical study, fibrous tissue at the circumference of neovascular vessels was reported to contain collagen, fibronectin, and laminin.[10–12] In Type IV CNM, fibrous tissue was abundant in light microscopy and surrounded the neovascular vessels closely in electron microscopy in our study. This suggested that fibrous tissue blocked visualization of the ICG dye during IA and blocked ICG dye leakage as well. Abundant fibrous tissue might obscure IA findings and thus modify findings.

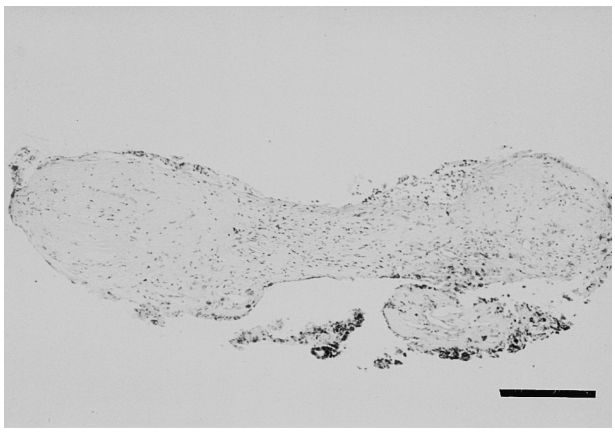

Figure 11. Light micrograph (hematoxylin-eosin staining) (Type IV membrane). Few vascular channels are seen. Retinal pigment epithelium cells partly surround neovascular channels. Bar = 200 μm.

In conclusion, IA findings are dependent on the number of vascular channels, the maturity of vessels in the CNM structure, the extent of envelopment by RPE cells, and the amount of fibrous tissue. Clinically, types II, III and IV showed atypical IA findings and required cautious reading of IA findings in AMD. Type I had many immature vessels and little fibrous tissue, suggesting a high level of activity. Type IV had few vascular channels and dense fibrous tissue, suggesting little activity.

Surgical removal of CNM in AMD causes RPE damage. After surgery, the defect in the RPE and choriocapillaris does not always correspond with the

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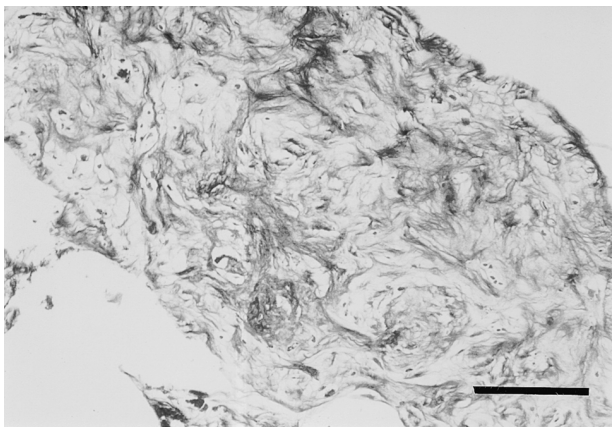

Figure 12. Light micrograph (azan staining) (Type IV membrane). Abundant fibrous tissue is seen and there are few vascular channels. Bar = 100 μm.

CNM. It appears larger than on preoperative FA. The postoperative visual outcome of AMD is not better than that of presumed ocular histoplasmosis.[13,14] Gass reported that CNMs in AMD usually grow within the subpigment epithelial space, and are excised with the RPE at surgery.[4] Visual acuity improvement is not expected after surgery in AMD patients. Some visual acuity improvement was noted in the present series.[15] Minimal damage to the RPE during surgical removal of the CNM is vital. Further studies are warranted to determine the status of the RPE and choriocapillaris in CNM, the extent of the defect of the RPE and choriocapillaris after surgery, and the changes in the visual field.

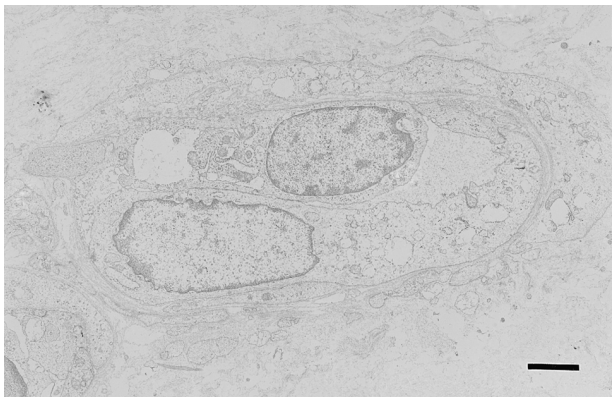

Figure 13. Electron micrograph (Type I membrane). Vascular endothelial cells are thick and have abundant organelles. Basement membrane is thin and lumen is narrow. Bar = 2 μm.

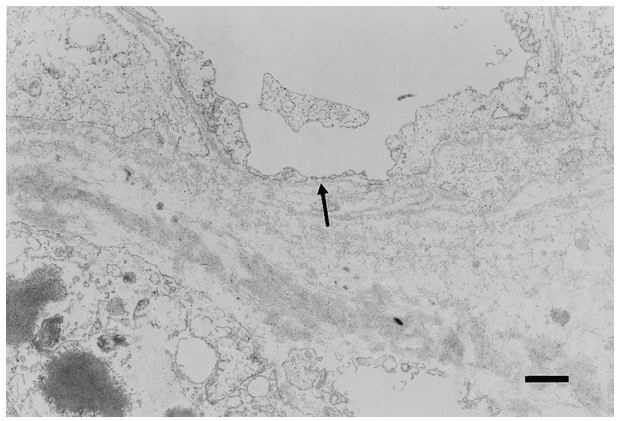

Figure 14. Electron micrograph (Type I membrane). Fenestration (arrow). Bar = 500 nm.

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References

1. Kawamura A, Yuzawa M, Shoda M, Matsui M. Infrared video angiography in age-related disciform macular degeneration. Jpn J Ophthalmol 1991;45:1355–9.
2. Regillo CD, Benson WE, Maguine JI, Annesley WH Jr. Indocyanine green angiography and occult choroidal neovascularization. Ophthalmology 1994;101:280–8.
3. Green WR, Enger C. Age-related macular degeneration, histopathologic studies. Ophthalmology 1993;100:1519–35.
4. Gass JDM. Biomicroscopic and histopathologic considerations regarding the feasibility of surgical excision of subfoveal neovascular membranes. Am J Ophthalmol 1994;118: 285–98.
5. Lopez PF, Grossniklaus HE, Lambert HM, Aaberg TM, Capone A Jr, Sternberg P Jr. Pathologic features of surgically excised subretinal neovascular membranes in age-related macular degeneration. Am J Ophthalmol 1991;112:647–56.
6. Lambert HM, Capone A Jr, Aaberg TM, Sternberg P Jr, Mandell BA, Lopez PF. Surgical excision of subfoveal membranes in age-related macular degeneration. Am J Ophthalmol 1992;113:257–62.
7. Chang TS, Freund KB, De La Cruz Z, Yannuzzi LA, Green WR. Clinicopathologic correlation of choroidal neovascularization demonstrated by indocyanine green angiography in a patient with retention of good vision for almost four years. Retina 1994;14:114–24.
8. Lee BL, Lim JI, Grossniklaus HE. Clinicopathologic features of indocyanine green angiograpy-imaged, surgically excised choroidal neovascular membranes. Retina 1996;16:64–9.
9. Fukushima I, Takahashi K, Ohkuma H, et al. Dye leakage from choroidal neovascularization with indocyanine green angiography. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1995;99:878–88.

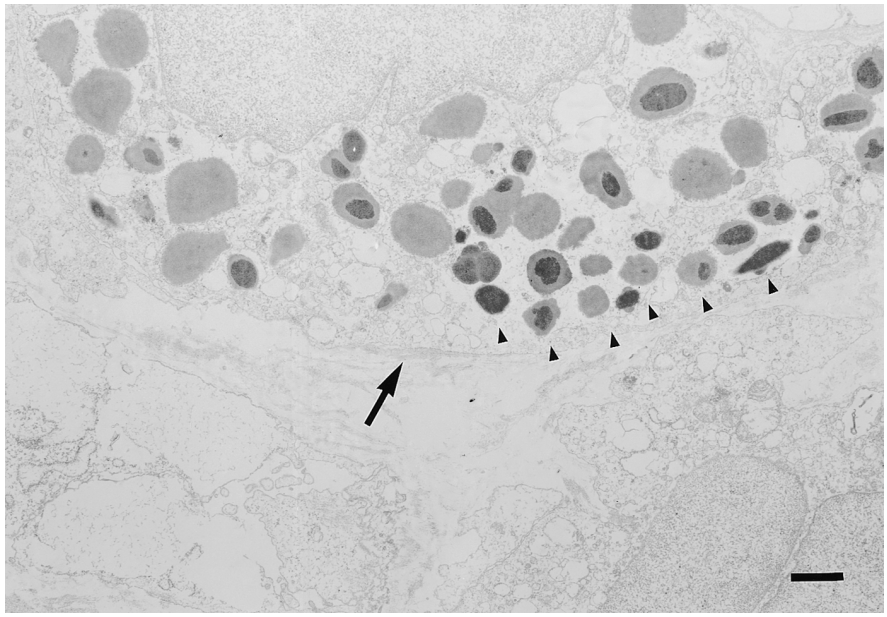

Figure 15. Electron micrograph (Type I membrane). Basement membrane (arrow). Melanin granules in cell body (arrowheads). Bar = 1 μm.

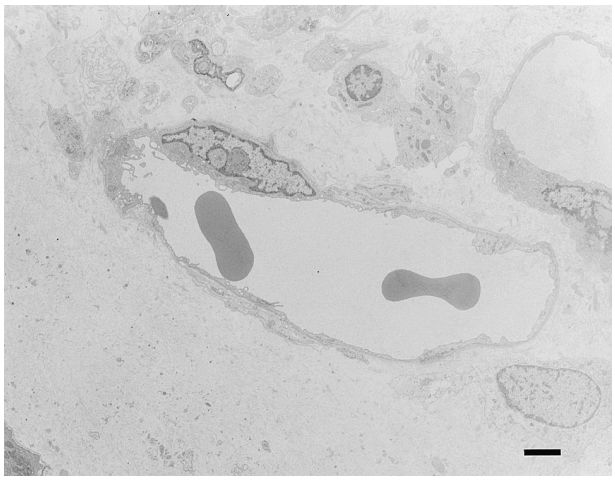

Figure 16. Electron micrograph. (Type IV membrane). Vascular endothelial cells lined wide vascular channel that seems mature. Bar = 2 μm.

10. Reddy VM, Zamora RL, Kaplan HJ. Distribution of growth factors in subfoveal neovascular memcranes in age-related macular degeneration and presumed ocular histoplasmosis syndrome. Am J Ophthalmol 1995;120:291–301.

11. Das A, Puklin JE, Frank RN, Zhang NL. Ultrastructural immunocytochemistory of subretinal neovascular membranes in age-related macular degeneration. Ophthalmology 1992;99:1368–76.

12. Grossmiklaus HE, Martinez JA, Brown V, et al. Immunohistochemical properties of surgically excised subretinal neovascular membranes in age-related macular degeneration. Am J Ophthalmol 1992;114:464–72.

13. Hsu JK, Thomas MA, Ibanez H, Green WR. Clinicopathologic studies of an eye after submacular membranectomy for choroidal neovascularization. Retina 1995;15:43–52.

14. Bynoe LA, Chang TS, Funata M, Del Priore LV, Kaplan HJ, Green WR. Histopathologic examination of vascular patterns in subfoveal neovascular membranes. Ophthalmology 1994; 101:1112–7.

15. Berger AS, Kaplan HJ. Clinical experience with the surgical removal of subfoveal neovascular membranes. Ophthalmology 1992;99:969–76.