Morphometric Analysis of the Choroid, Bruch’s Membrane, and Retinal Pigment Epithelium in Eyes With Age-Related Macular Degeneration

Christoph W. Spraul,* Gabriele E. Lang,† and Hans E. Grossniklaus*

Purpose. To quantify morphologic changes in the choroid, including choriocapillaris and larger choroidal vessels, Bruch’s membrane, and retinal pigment epithelium in eyes with age-related macular degeneration (AMD) and late ARM (age-related maculopathy).

Methods. The authors analyzed 40 eye bank eyes with late ARM (21 with the neovascular AMD and 19 with nonneovascular AMD) and compared them with 40 age-matched eyes without signs of late ARM (AMD). The eyes were processed for light microscopy, and seven variables were measured in the macular and peripheral regions with a digital filar micrometer.

Results. There were no statistically significant differences observed between eyes with neovascular versus nonneovascular AMD. The single most important difference between eyes with and without AMD was the amount of basal laminar deposit \( P < 0.001 \). Eyes with AMD displayed fewer large choroidal vessels in the submacular choroid than eyes without AMD \( (3.5 \pm 1.5 \text{ mm}^{-1} \text{ and } 5.7 \pm 1.6 \text{ mm}^{-1}; P < 0.001) \). The submacular choriocapillaris density was higher in eyes with AMD \( (0.62 \pm 0.06) \) than in eyes without AMD \( (0.51 \pm 0.08; P < 0.001) \). The diameter of the large choroidal vessels in the peripheral choroid was increased in eyes with AMD \( (30 \pm 8 \mu m) \) compared to eyes without AMD \( (21.4 \pm 6.2 \mu m; P < 0.001) \). The peripheral choriocapillaris density displayed the same pattern as in the macular region in eyes with and without AMD.

Conclusions. The amount of basal laminar deposit strongly correlates with the histologic presence of AMD. Eyes with AMD show differences of the density and diameter of choroidal blood vessels compared to eyes without AMD. Invest Ophthalmol Vis Sci. 1996; 37:2724–2735.

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in industrialized countries. Only 12% of patients have the exudative form, which accounts for 88% of patients with legal blindness caused by AMD. The International ARM Epidemiological Study Group recently suggested using the term ARM (age-related maculopathy) to describe age-related macular changes. Clinically, early ARM is defined by the presence of drusen and retinal pigment epithelium pigmentary abnormalities without the features of late ARM (similar to AMD), i.e., annular and geographic atrophy (dry AMD) and/or detachment of neurosensory retina and/or retinal pigment epithelium (RPE), hemorrhages, and fibrous scarring (wet AMD). The term AMD is used in this article interchangeably with late ARM changes. Histologically, AMD is characterized by the presence of atrophy of the retinal pigment epithelium and the photoreceptors, with possible associated choroidal neovascularization.

In 1973, Sarks described finely granular material in close association with degenerating pigment epithelium in eyes with AMD and termed it basal linear deposit. Ultrastructural examination displayed that this material can be observed internally or externally to the basement membrane of the RPE. Different terms have been applied for those deposits. Since the original description by Sarks, some authors still use the term...
basal linear deposit for material internal to the basement membrane of the RPE, whereas others8–14 prefer the term basal laminar deposit for this location. We use the same terminology as the latter authors and refer to a deposit localized between the basal cytoplasmic membrane of the RPE and its basement membrane as basal laminar deposit and to material between the RPE-basement membrane and the remainder of Bruch’s membrane as basal linear deposit. Frequently, drusen are found in the eyes of older patients, but their significance regarding the pathogenesis of AMD is unclear. Age-related changes of the RPE, Bruch’s membrane, choriocapillaris, and choroidal vessels have been morphologically described,13,15–22 although morphometric changes of choroidal vessels have not been studied systematically in different types of AMD. Clinical studies have shown that there is impairment of choroidal blood flow in patients with AMD.23–26 To study the relationship between the choroid and different stages of AMD, we investigated morphologic properties of the RPE, Bruch’s membrane, the choriocapillaris, and larger choroidal vessels in the macular and peripheral regions in a series of 40 eyes with either neovascular or nonneovascular AMD and compared our measurements with 40 age-matched eyes without AMD.

**MATERIALS AND METHODS**

All eyes used for this study were obtained from the Georgia Eye Bank, Atlanta, and were processed and histologically diagnosed in the L. F. Montgomery Eye Pathology Laboratory, Emory University Eye Center, for research purposes. All studies were approved by the Human Investigation Committee, Emory University. For processing, eyes were placed in 10% neutral buffered formalin, and, after fixation for 48 hours, they were opened horizontally, first with removal of the superior calotte. Internal examination with a dissection microscope (American Optics, Buffalo, NY) was performed, and special attention was given to diagnose hypertrophy and atrophy of the RPE, hemorrhages, and exudates in the macular area. Thereafter, the inferior calotte was removed, and a central section that included the pupil, optic nerve, and center of the macula (PO section) was processed routinely and dehydrated in increasing concentrations of ethyl alcohol. The PO section was embedded in paraffin, and 5-μm-thick step sections through the center of the macula were obtained and stained with periodic acid-Schiff and hematoxylin and eosin. The slides were examined by an Olympus BHTU microscope (Olympus Optical, Tokyo, Japan), and standard criteria were used to analyze for the presence and absence of drusen, hypertrophy, and atrophy of the retinal pigment epithelium, atrophy, and loss of photoreceptors, and choroidal neovascularization.29–27 No clinical data, such as visual acuity or results of fluorescein angiography, were available. Age-related macular degeneration, which is comparable to late ARM,3 was defined histologically as the presence of hypertrophy and atrophy of the RPE with associated photoreceptor loss with or without associated choroidal neovascularization.27 The presence or absence of AMD was determined by an ophthalmic pathologist (HEG). Eyes with neovascular (exudative, wet) AMD were characterized by the presence of a choroidal neovascular membrane. Eyes were classified as having the nonneovascular (nonexudative, dry) form of AMD when they exhibited hypertrophy and atrophy of the retinal pigment epithelium with associated atrophy of photoreceptors. In general, eyes with the neovascular form of AMD had more severe damage to the overlying retina than eyes with nonneovascular AMD on histologic examination. Eyes with drusen and/or basal laminar (linear) deposit only, without the above-described features and in contrast to the study group with a normal gross examination, were not classified as having AMD (or late ARM). Those eyes could not be classified as having early ARM because the histologically identified drusen in them had not caused identifiable changes of the overlying RPE on gross examination. However, we could not exclude that in the clinical situation or on fluorescein angiography, those changes might have been identified; eyes without AMD were called the comparison group.

For this study, we randomly selected five slides of each of 40 consecutive unpaired eyes histologically diagnosed with AMD and compared them with an age-matched, otherwise randomly chosen group of eyes from a group of approximately 1000 eye bank eyes without AMD changes. Both groups were collected during the same time period (1990 to 1995) and were processed by the same technician. Eyes with historical or morphologic evidence of ocular surgery, diabetic retinopathy, or photocoagulation were excluded. Eyes with choroidal neovascularization resulting from other possible causes, such as histoplasmosis and myopia, were excluded by the typical findings on gross and microscopic examination. The inclusion of eyes with choroidal neovascularization from factors other than AMD, such as punctate inner choroidopathy or Sorsby’s macular dystrophy, could not be excluded entirely, but they probably were not part of the study because of the younger age of onset and the rarity of those diseases.

For this study, the severity of basal laminar deposit (class 0 to 3) and drusen (class 0 to 3) were classified according to the scheme used by van der Schaft et al9 (Table 1). Basal laminar deposit was distinguished from basal linear deposit by the characteristic brushlike appearance with periodic acid-Schiff stain of the former. In our experience and in the experience of
TABLE 1. Classification of Basal Laminar Deposit and Drusen According to van der Schaft et al

<table>
<thead>
<tr>
<th>Class</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal laminar deposit</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No basal laminar deposit</td>
</tr>
<tr>
<td>1</td>
<td>Small solitary patches on the basal side of the RPE</td>
</tr>
<tr>
<td>2</td>
<td>A thin, continuous layer (&lt; 1/2 height of RPE)</td>
</tr>
<tr>
<td>3</td>
<td>A thick layer (≥ 1/2 height of the RPE)</td>
</tr>
<tr>
<td>Hard and soft drusen</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No drusen</td>
</tr>
<tr>
<td>1</td>
<td>1–3 drusen</td>
</tr>
<tr>
<td>2</td>
<td>4–10 drusen</td>
</tr>
<tr>
<td>3</td>
<td>&gt;10 drusen or confluent drusen</td>
</tr>
</tbody>
</table>

RPE = retinal pigment epithelium.

other investigators, many eyes with basal laminar deposit also have basal linear deposit. Because of this and the difficulty of distinguishing basal linear deposit from diffuse soft drusen by light microscopy, we did not separately analyze basal linear deposit. In addition to the assessment of the presence and severity of basal laminar deposit and drusen, seven other variables were measured (Fig. 1): the thickness of the RPE, Bruch's membrane, and choroid; the density and diameter of the capillaries of the choriocapillaris; and the luminal diameter and density of the choroidal vessels. All measurements were performed in the macular and peripheral regions and were made by one observer (CWS). The peripheral area was defined as a horizontal (tangential) zone of the temporal choroid extending from the equator to a point 4.5 mm posterior to the equator. The thickness of the choroid and the diameter and density of the choroidal vessels were assessed at ×250 magnification. All other variables were measured at ×640 magnification. Measurements were performed using a digital filar micrometer and a programmable scientific calculator (1602 N-10; Lasico, Los Angeles, CA) and a Zeiss (Oberkochen, Germany) microscope. The luminal diameter of all choroidal vessels was measured, and vessel number was counted in a zone of 4500 µm in the macular area, posterior to the equator, and the density of these vessels was calculated in vessels per 1000 µm (mm⁻¹). Arteries and veins were analyzed separately in the macular area, but not in the periphery, because it was not always possible to differentiate arteries from veins in the peripheral choroid. For statistical analysis, the mean of the five arteries and five veins with the widest diameter was used. This method was chosen because of its high reproducibility with a small standard deviation. All other parameters were measured in each histologic section in four randomly selected sites in the macular and peripheral zone, and the values were averaged. The thickness of Bruch's membrane was measured between the RPE side of the inner collagenous layer and the choriocapillaris side of the outer collagenous layer, between the intercapillary pillars. The capillary luminal diameter of the choriocapillaris was assessed perpendicular to Bruch's membrane, always in the area of its widest diameter. Choroidal thickness was defined as the distance between the internal border of the sclera and the external border of the outer collagenous layer of Bruch's membrane. The density of the choriocapillaris was expressed as a ratio between the sum of the lengths of the lumina of the capillaries (measured parallel to Bruch's membrane) divided by the length of the zone in which measurements were performed. This ratio can range from 0 to 1 and will be referred to as the choriocapillaris density. The length of the zone was 700 µm and was chosen because of practical and statistical reasons. Preliminary measurements of the choriocapillaris density, with calculation of the standard deviation, revealed a minimal measurement window of 700 µm to find a significant result for a 5% difference between groups.

To minimize bias, slides were chosen randomly in a masked fashion out of the pool of all slides selected for this study, and examination was begun in the periphery, where disease would be less obvious. This procedure precludes that the observer immediately could assign every case to a special group, and it minimizes subconscious bias in the measurement of the parameters. For assessment of intraobserver variability, 10 eyes from each group—neovascular, nonneovascular, and comparison—were selected randomly 1 month after the initial measurement. Slides of these eyes were evaluated again by the same investigator, and the result of this second measurement was compared to the result of the initial measurement.

For statistical analysis of the different parameters, we used analysis of variance (ANOVA) with Bonferroni correction for multiple tests. Depending on the distribution of the data, the parametric one-way ANOVA test or the nonparametric Kruskal-Wallis one-way analysis of ranks test was applied. The distribution was assessed by calculation of standardized coefficients of kurtosis. If the values for this parameter were outside the range of −2 to +2 (indicating significant deviation from a normal distribution), the nonparametric test was used. Spearman's rank-correlation test (correlation coefficient = r) was used to determine a monotonic relationship between any two of the measured variables. Intraobserver variability was analyzed with the Wilcoxon paired signed rank test and the paired Student's t-test. P ≤ 0.0025 (adjusted for multiple tests) for one-way ANOVA, P ≤ 0.01 for Spearman's rank-correlation, and P < 0.05 for the other tests were considered significant.
Morphometric Analysis of the Choroid in Age-Related Macular Degeneration

RPE

Choroid capillaris

Choroid

Choroidal artery

Choroidal vein

Sclera

RESULTS

Forty eyes with histologic signs of AMD were examined for this study. Nineteen were classified as nonneovascular and 21 as neovascular AMD. In the nonneovascular AMD group, 29% (5/17) of eyes were from male and 71% (12/17) were from female donors (mean age at time of death, 85.7 years; range, 65 to 91 years). In the neovascular AMD group, 33% (6/18) of eyes were from male and 67% (12/18) were from female donors (mean age at time of death, 82.8 years; range, 64 to 92 years). In addition, 40 eyes without AMD (mean age at time of death, 82.4 years; range, 66 to 101 years) were examined. In this group, 46% (16/35) of eyes were from men, and 54% (19/35) were from women. There was no statistically significant difference between these groups regarding age, sex, axial length, and laterality of the eyes (Table 2).

Analysis of the intraobserver differences with the Wilcoxon paired signed rank test and the paired Student’s t-test displayed P values between 0.20 and 0.95.

Comparison of the neovascular AMD group with the nonneovascular AMD group showed no statistically significant differences in all measured variables. Results of statistical analyses between the neovascular AMD group and the comparison group, the nonneovascular AMD group and the comparison group, and the combined neovascular and nonneovascular AMD groups and the comparison group are shown in Table 3 for the macular area and in Table 4 for the peripheral area.

Macular Area

The density of large choroidal vessels was reduced significantly in the neovascular and the nonneovascular AMD groups compared to the comparison group: 3.6 vessels mm\(^{-1}\), 3.5 vessels mm\(^{-1}\), and 5.7 vessels mm\(^{-1}\), respectively. This decrease of choroidal vessels...
TABLE 2. Comparison of Mean and Standard Deviation of Age, Sex, Laterality, and Axial Length of All Groups and Calculation of P Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Neovascular</th>
<th>Nonneovascular</th>
<th>Neovascular and Nonneovascular</th>
<th>Comparison Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes (n)</td>
<td>21</td>
<td>19</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>82.8 ± 6.9</td>
<td>85.7 ± 6.6</td>
<td>84.2 ± 6.8</td>
<td>82.4 ± 7.2</td>
<td>0.10–0.82</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>24.3 ± 1.3</td>
<td>23.8 ± 1.1</td>
<td>24.1 ± 1.2</td>
<td>24.1 ± 1.3</td>
<td>0.20–0.78</td>
</tr>
<tr>
<td>Male (%)</td>
<td>33.3</td>
<td>29.4</td>
<td>31.4</td>
<td>45.7</td>
<td>0.22–0.81</td>
</tr>
<tr>
<td>Right eye (%)</td>
<td>52.6</td>
<td>58.8</td>
<td>55.5</td>
<td>54.3</td>
<td>0.71–0.92</td>
</tr>
</tbody>
</table>

was only significant for the density of the veins (P < 0.001), not for the arteries. The diameter of choroidal arteries and veins displayed no significant difference among all groups. The thickness of the choroid, RPE, and Bruch’s membrane displayed no significant difference among all groups. ANOVA revealed no difference in the diameter of the capillaries of the choriocapillaris. Choriocapillaris density was increased significantly in the neovascular and nonneovascular AMD groups compared to the comparison group with 0.61, 0.63, and 0.51, respectively. The difference in the amount of basal laminar deposit between the neovascular (2.6) and the nonneovascular AMD groups (2.1) compared to the comparison group (0.8) was striking. Although 84% (18/21) of eyes in the neovascular AMD group had class 2 or class 3 basal laminar deposit (Table 1), this percentage decreased in the nonneovascular and comparison groups to 53% (10/19) and 19% (8/40), respectively. The number of drusen displayed no significant difference among all groups. Class 2 or class 3 drusen (Table 1) were found in the neovascular, nonneovascular, and comparison groups in 84% (18/21), 88% (17/19), and 81% (32/40) of eyes, respectively. Calculation of ratios between different variables showed that in relation to the thickness of the choroid, the mean diameter of the 10 largest choroidal vessels was higher in the neovascular (0.31) and the nonneovascular AMD groups (0.33) compared to the comparison group (0.26) (P < 0.0025). The ratio of choroidal arteries to veins displayed no significant difference among all groups. There was no significant difference for the ratios of the diameter of the capillaries of the choriocapillaris to RPE or choroid, thickness of Bruch’s membrane to the RPE, thickness of the RPE to the choroid, and diameter of veins with relation to the diameter of arteries among all groups.

Peripheral Area

In contrast to the macular area, there was no significant difference in the densities of the choroidal vessels among the groups (Table 4). The mean diameter of the vessels was increased in the neovascular (29.9 μm) and the nonneovascular AMD groups (30.1 μm) compared to that of the comparison group (21.4 μm). There was no difference in diameter of the capillaries of the choriocapillaris or in the thickness of RPE and Bruch’s membrane among all groups. The thickness of the choroid was decreased in both AMD groups, neovascular (78.2 μm) and nonneovascular (76.8 μm), compared to eyes without AMD (112.7 μm). As observed in the macular area, the density of the choriocapillaris displayed a similar pattern. The density was again increased in the neovascular (0.62) and nonneovascular AMD groups (0.67) compared to the comparison group (0.55).

Calculation of ratios between variables showed that the mean of the diameter of the choroidal vessels was increased significantly with relation to the thickness of the choroid in the neovascular and the nonneovascular AMD groups compared with the comparison group—0.41, 0.48, and 0.20, respectively (ANOVA, P < 0.001). The ratio of the diameter of the capillaries of the choriocapillaris to the thickness of the choroid displayed no significant differences among all groups. The ratio of the thickness of the RPE to the thickness of the choroid was increased in both groups with AMD (0.13 and 0.15) compared to the comparison group (0.09) (P = 0.0008). The ratio of Bruch’s membrane thickness to RPE thickness and the ratio of the diameter of capillaries of the choriocapillaris to RPE thickness was not significantly different among all groups.

To evaluate whether the morphologic changes of the choroid in the different groups were confined to the macular region, ratios between the macular and peripheral values of all variables, except for basal laminar deposit, were calculated. There was no significant difference in the ratios of the mean of the diameter of the 10 largest choroidal vessels, the thickness of the RPE, and the thickness of Bruch’s membrane. There was, however, a significantly higher ratio of macular to peripheral choroidal thickness in the AMD group compared with the comparison group—2.05 and 1.34, respectively (P = 0.002).

To estimate the actual capacity for blood delivery, the total luminal area of vessels was calculated. This was done by multiplying the frequency of vascular pro-
files (the vascular density) with the mean luminal area of the vessels. The calculation of the luminal area of the vessels was approximated by using the formula for calculating a sphere area ($\pi \times (\text{diameter}/2)^2$). To compare the different groups, the total luminal area was calculated in $\mu m^2$ per 1 mm choroid—that is, an area of full-thickness choroid with a length of 1 mm measured parallel to Bruch’s membrane. There was no difference in total luminal area for macular chorioidal vessels among all groups (Table 5). There was, however, a significant increase of total luminal area of capillaries of the macular choriocapillaris in eyes with AMD compared to the comparison group (46.2 $\mu m^2/mm^{-1}$ versus 29.9 $\mu m^2/mm^{-1}$). This increase reached statistical significance only in eyes with neovascular AMD (49.5 $\mu m^2/mm^{-1}$) but not in eyes with nonneovascular AMD (42.6 $\mu m^2/mm^{-1}$). In addition, this increase of total luminal area was observed only in the macular choriocapillaris, not in the peripheral choriocapillaris. The total luminal area of the peripheral vessels was increased significantly in the AMD group compared to controls (1590 $\mu m^2/mm^{-1}$ versus 955 $\mu m^2/mm^{-1}$).

Spearman’s rank correlation coefficients between measured variables for all 80 eyes were shown in Table 6. The amount of basal laminar deposit displayed a positive correlation with the macular ($r_s = 0.36$) and peripheral density ($r_s = 0.41$) of the choriocapillaris and diameter of peripheral choroidal vessels ($r_s = 0.43$) and a negative correlation with the density of larger macular choroidal vessels ($r_s = -0.44$) and the peripheral choroidal thickness ($r_s = -0.35$).

**DISCUSSION**

Results of this morphometric study demonstrate that the amount of basal laminar deposit strongly correlates with the histologic presence of neovascular and nonneovascular types of AMD, although there was no significant difference between those groups in the amount of this deposit. The amount of drusen in our study showed no significant difference among all groups. Van der Schaft et al. found in their study that 5/5 eyes with choroidal neovascularization displayed class 2 or class 3 basal laminar deposit in contrast to drusen, which were seen only in 2/5 eyes. Sarks found that in a group of 46 eyes with class 3 basal laminar deposit, six eyes (14.3%) displayed histologic evidence of early choroidal neovascularization. Two of these six eyes also displayed drusen. The RPE appeared as an attenuated layer, and there were areas of proliferative changes in most of those 46 eyes. In our study, we could not find a difference in thickness of the RPE in the macular or in the peripheral areas. Green et al. used histologic changes similar to our criteria to define AMD and found, in a series of 760 eyes with AMD,
TABLE 4. Mean, Standard Deviation, and P Values for All Variables Measured in the Peripheral Choroid

<table>
<thead>
<tr>
<th>Neovascular</th>
<th>Nonneovascular</th>
<th>AMD group†</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of Vessels $\bar{X}$ (mm$^{-1}$)</td>
<td>2.2 ± 1.2</td>
<td>1.9 ± 1.1</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>Diameter of Vessels $\bar{X}$ (μm)</td>
<td>29.9 ± 8.4</td>
<td>30.1 ± 7.6</td>
<td>30.0 ± 8.0</td>
</tr>
<tr>
<td>Thickness of Choroid (μm)</td>
<td>78.2 ± 31.1</td>
<td>76.8 ± 35.0</td>
<td>77.5 ± 32.6</td>
</tr>
<tr>
<td>Diameter of CC $\bar{X}$ (μm)</td>
<td>6.6 ± 1.7</td>
<td>7.3 ± 1.9</td>
<td>7.0 ± 1.8</td>
</tr>
<tr>
<td>Thickness of RPE (μm)</td>
<td>9.0 ± 1.8</td>
<td>9.3 ± 1.4</td>
<td>9.1 ± 1.6</td>
</tr>
<tr>
<td>Thickness of Bruch (μm)</td>
<td>2.4 ± 0.7</td>
<td>2.3 ± 0.3</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Density of CC (0–1)</td>
<td>0.62 ± 0.10</td>
<td>0.67 ± 0.05</td>
<td>0.65 ± 0.08</td>
</tr>
</tbody>
</table>

Analysis of variance with Bonferroni correction for multiple tests

<table>
<thead>
<tr>
<th>Neovascular</th>
<th>Nonneovascular</th>
<th>Neovascular</th>
<th>Nonneovascular</th>
<th>AMD group†</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NS</td>
<td>&lt;0.001</td>
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<tr>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CC = choriocapillaris; RPE = retinal pigment epithelium; AMD = age-related macular degeneration; NS = not significant.
* Significant.
† All eyes with neovascular or nonneovascular changes.
‡ All eyes with neovascular or nonneovascular changes.
§ Mean diameter of the ten largest choroidal vessels (five arteries and five veins).

TABLE 5. Mean, Standard Deviation, and P Values for Calculated Total Luminal Area of Choroidal Vessels

<table>
<thead>
<tr>
<th>Macular Arteries</th>
<th>Macular Veins</th>
<th>Macular Choriocapillaris</th>
<th>Peripheral Vessels</th>
<th>Peripheral Choriocapillaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>(μm$^2$/mm$^{-1}$)$\ddagger$</td>
<td>(μm$^2$/mm$^{-1}$)$\ddagger$</td>
<td>(μm$^2$/mm$^{-1}$)$\ddagger$</td>
<td>(μm$^2$/mm$^{-1}$)$\ddagger$</td>
<td>(μm$^2$/mm$^{-1}$)$\ddagger$</td>
</tr>
<tr>
<td>Neovascular</td>
<td>1429 ± 1059</td>
<td>5090 ± 8342</td>
<td>49.5 ± 19.9</td>
<td>1707 ± 1139</td>
</tr>
<tr>
<td>Nonneovascular</td>
<td>1760 ± 1502</td>
<td>6130 ± 7703</td>
<td>42.6 ± 25.4</td>
<td>1460 ± 1262</td>
</tr>
<tr>
<td>AMD group†</td>
<td>1588 ± 682</td>
<td>5581 ± 7969</td>
<td>46.2 ± 22.6</td>
<td>1590 ± 1187</td>
</tr>
<tr>
<td>Comparison</td>
<td>1367 ± 1095</td>
<td>6804 ± 18288</td>
<td>29.9 ± 17.7</td>
<td>955 ± 691</td>
</tr>
</tbody>
</table>

Analysis of variance with Bonferroni correction for multiple tests

<table>
<thead>
<tr>
<th>Neovascular</th>
<th>Nonneovascular</th>
<th>Neovascular</th>
<th>Nonneovascular</th>
<th>AMD group†</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>NS</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>0.0025</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

AMD = age-related macular degeneration; NS = not significant.
* Significant.
† All eyes with neovascular or nonneovascular changes.
‡ Total luminal area of vessels present in a full thickness choroidal area with a length of 1 mm measured parallel to Bruch's membrane.
Evidence for the existence of this interaction in vivo comes from experimental histopathologic studies that report sprouting of the choriocapillaris adjacent to selective damage of the RPE or containment and involution of choroidal neovascular membranes by proliferated RPE. On the other hand, widespread loss of RPE, as observed in geographic atrophy, is followed by secondary choriocapillaris atrophy, which in turn rarely is complicated by (further) choroidal neovascularization. This observation suggests that besides production of an endothelial cell growth inhibitor, trophic factors (e.g., vascular modulation factor) are released by the RPE and maintain the normal function of the choriocapillaris. This biochemical balancing act, which controls events at the RPE-choriocapillaris interface, may be disturbed by a thick layer of basal laminar deposit. Basal laminar deposits may interfere with the diffusion of those factors that maintain stability at that interface. One possibility is that this may be followed by a decrease in concentration of inhibitor of endothelial growth, which in turn is followed by proliferation of capillaries of the choriocapillaris. Furthermore, the normal inhibition of ingrowth of those new vessels through Bruch’s membrane, mediated by contact of the RPE with the neovascular tissue, is compromised by the layer of basal laminar deposit. In addition, the basal laminar deposit appears to be a good substrate for vascular growth and may serve as a cleavage plane. Another structure that may hamper diffusion between the choriocapillaris and RPE is the thickness and composition of Bruch’s membrane. The thickness was not significantly different in eyes with AMD than it was in the comparison group. Except for a thinned Bruch’s membrane in eyes with disciform scarring, Ramrattan et al found similar results. All maculae with disciform scarring were from pseudophakic eyes, and other factors, such as mechanical trauma of cataract extraction and the presence of a foreign body in the eye, might have been responsible for the discrepancy. Furthermore, most eyes in our neovascular AMD group displayed earlier stages of neovascular AMD (less disciform scarring) than did eyes of the disciform group in that study.

In our study, the diameter of the capillaries in the macular choriocapillaris in eyes of the AMD group showed an average, though not a statistically significant one, increase of 14% compared to eyes without AMD (Table 3). The density of the choriocapillaris displayed a statistically significant increase of 22% in eyes with AMD compared to eyes without AMD (Table 3). At least two possible ways of interpreting this asymmetric "engorgement" of capillaries of the choriocapillaris exist. One is that there may be an asymmetric engorgement of these capillaries more pronounced in the plane parallel to Bruch’s membrane than in
the plane perpendicular to Bruch’s membrane. The other, more likely, possibility is that a new formation of capillaries (i.e., intrachoroidal neovascularization) in the plane parallel to Bruch’s membrane has occurred in eyes with AMD, and this proliferation may be interpreted as secondary to a lack of inhibitor of endothelial cell growth (see previous page) or hypoxic-mediated stimulation (see next paragraph). The peripheral choriocapillaris displayed findings similar to those of the macular choriocapillaris, with the exception of a statistically insignificant decrease (7%) of the diameter of these capillaries associated with a statistically significant, but slightly lower, increase in density (18%) (Table 4). The peripheral findings may be interpreted similarly to the macular findings. These findings contrast with the results of Ramrattan et al,15 who found a decrease in the choriocapillaris density and diameter of the capillaries of the choriocapillaris in eyes with AMD, basal laminar deposit, or both. Ramrattan et al15 used multiple log-linear regression analysis with adjustment for age, and they did not use age-matched controls. It may be difficult to compare our results with those of Ramrattan et al15 because they investigated eyes with late-stage AMD—geographic atrophy and disciform scarring—conditions characterized by an extensive loss of RPE. Similar limitations may be applied for the study by Sarks,13 which found a decrease of choriocapillaris density in eyes with longstanding geographic atrophy and disciform scarring—conditions characterized by an extensive loss of RPE. This is further evidence that basal laminar deposits are involved in the pathogenesis of choroidal neovascularization, possibly by the induction of relative hypoxia in the overlying retina or by disinhibition of endothelial cell growth by modulation of the biochemical balancing act, which maintains stability at the RPE–choriocapillaris interface.

Sarks5,13 described thinning of the choroid with accentuation of the large choroidal vessels in eyes with AMD. Ramrattan et al15 could not find a significant difference in the choroidal thickness between eyes with and without AMD. In our study, the thickness of the macular choroid showed no significant difference among all groups; however, the peripheral choroid was significantly thinned in eyes with AMD. The luminal diameter of the large peripheral choroidal vessels was increased significantly in eyes with AMD. The ratio of the diameter of the choroidal vessels to the thickness of the choroid was increased in the macular and peripheral choroid, giving the impression of accentuated prominent choroidal vessels, a phenomenon observed by Sarks.5,13 There was a significant decrease in the density of large choroidal vessels observed in eyes of the AMD group in the macular choroid. Further analysis showed that it was only significant for the density of veins. Arnold et al19 found a decrease in the number of large choroidal veins that was more pronounced in AMD eyes with reticular pseudodrusen than in eyes without reticular pseudodrusen. The choriocapillaris displayed no significant changes in
those eyes. Friedman et al proposed that the sclera in eyes with AMD becomes increasingly rigid and noncompliant, increasing the resistance of venous outflow and inducing an elevated venous pressure with distension of choroidal veins. This phenomenon is consistent with our observation of an increase in individual and total luminal area of peripheral choroidal vessels. In patients with AMD, using color Doppler imaging, Friedman et al found decreased velocity and increased pulsatility in arteries that perfuse the eye. This was interpreted as an increased resistance of choroidal vessels in patients with AMD. The morphologic correlate may be the decreased density of choroidal vessels in the macular area observed in our study, although the total luminal area was not significantly different. In many studies, hyperopia has been associated with AMD; in one study, there was a slightly increased odds ratio for the prevalence of AMD with myopia, and in another, no significant association was observed with refractive status. One hypothesis is that increased scleral thickness in hyperopic eyes with an associated increase in scleral rigidity leads to an obstructed venous outflow. The study design in our investigation precludes a comparison of the axial length between different groups because this was a matched parameter. Within the groups, the axial length was closely related only to the degree of drusen in the neovascular AMD group (τ = 0.51; P = 0.01), although a valid postmortem assessment of axial length has limitations.

We propose that the primary mechanism in the pathogenesis of AMD is related to a dysfunction of the RPE with production of basal laminar deposit and other deposits, impairment of the diffusion of solutes, and communication between the RPE and choriocapillaris. Our study of earlier stages of neovascular and nonneovascular AMD and other studies of later stages of AMD show that the course of AMD can be interpreted as a dynamic process with early proliferation and later atrophy of capillaries of the choriocapillaris. We cannot determine from our study whether the observed vascular changes in the choroid are secondary phenomena or are independent primary pathogenic factors. Although the calculated total luminal area of choroidal vessels was not found to be decreased, significant changes of the vascular pattern in the choroid were observed—for example, the ratio between the diameter of the choroidal vessels to the thickness of the choroid was usually higher in the macular area than in the periphery, whereas this is reversed in eyes with AMD. In the macula, a negative correlation was observed between the density of the choroidal vessels and the density of the capillaries of the choriocapillaris, indicating a regulatory mechanism that provides blood supply to the photoreceptors with a decrease of density of the large choroidal vessels. On the other hand, basal laminar deposit was associated with decreased density of macular choroidal vessels; however, it is not possible to decide whether this is a consequence or a cause of basal laminar deposit. In the periphery, a positive correlation was observed between the density of the choroidal vessels and the thickness of the choroid, and the thickness of the choroid was in turn negatively correlated with the density of the choriocapillaris, indicating again a similar regulatory mechanism to supply blood for the continuing metabolic demand of the photoreceptors. Those correlations indicate that at least part of the observed choroidal vascular changes are secondary in nature.

This study cannot determine whether AMD is an exaggeration of normal aging changes or a fundamentally different disease process. It seems, however, that production of basal laminar deposit by compromised RPE characterizes the pathologic state of the aging process, although Curcio et al have shown that even in the presence of basal laminar deposit and diseased RPE, the foveal cone mosaic can be preserved with good visual acuity. On the other hand, formation of hard drusen (present in 83% of autopsy eyes with no documented ocular disease) seems to be a physiologic process of aging. Further evidence that drusen may represent normal expression of the metabolic failure of the aging RPE and is not related to the formation of choroidal neovascularization is that drusen were found not to be anatomically related to the site of choroidal neovascularization; however, the disappearance of drusen at this site of neovascularization cannot be ruled out.

We found morphologic choroidal changes associated with AMD in the peripheral and macular choroid. It has been shown that changes in the macular choroid are related closely to those in the peripheral choroid. Peripheral retinal function is not affected in patients with AMD. One possible explanation is that the macular area is proportionally more affected than the peripheral area in eyes with AMD. The ratios between the values of all measured variables in the macular and peripheral regions displayed no significant difference between groups, except that basal laminar deposit was present only in the macular area. This is further evidence that basal laminar deposit is related more closely to the pathogenic factors responsible for the development of AMD than the other variables we examined.

All measured variables displayed no significant differences between eyes with the neovascular and nonneovascular form of AMD. This is an indication that the underlying pathophysiologic mechanisms are not different in these two AMD groups.

We did not correlate age at the time of death with the measured variables in our study because of the high variation of morphologic parameters in a single
age group and the narrow range of ages at time of donor death. Sixty-seven percent of eyes were from donors between 77 and 89 years of age at the time of death, and statistical analysis would not have been meaningful.

Limitations of our study include artificial alteration by postmortem changes or processing artifacts. Our study provides evidence that basal laminar deposit is strongly associated with the development of AMD and that AMD is associated with many changes of the thickness, density, and diameter of large and small vessels in the choroid. This may result in changes of ocular hemodynamics as clinically described in patients with AMD.

Key Words
age-related macular degeneration, basal laminar deposit, choriocapillaris, drusen, morphometry

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