Quantifying Changes in RPE and Choroidal Vasculature in Eyes with Age-Related Macular Degeneration

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PURPOSE. An image-analysis technique was developed to quantify changes in the retinal pigment epithelium (RPE) and choriocapillaris in eyes of deceased donors with age-related macular degeneration (AMD).

METHODS. Both eyes of two donors with AMD and of one normal control donor were used to develop this technique. After removal of the anterior segments, the eyecups were hemisected through the macula, with the disc included in one half of the eyecup. The choroid with RPE cells was dissected from the sclera and incubated for alkaline phosphatase (APase) activity, and the pigment was partially bleached with H2O2. The APase-incubated choroid was flat embedded and sectioned after image and morphometric analyses. Quantitative computer-assisted morphometric analyses of the two AMD-affected eyes (cases 1 and 2) were compared with analysis of the normal eye of a 70-year-old control subject (case 3).

RESULTS. The right eye in case 1 had geographic atrophy (GA) and demonstrated a large area in the posterior pole with very few RPE cells (90% loss of RPE), but the border of the area of RPE atrophy was not well defined. The density of choroidal blood vessels in this area was reduced 30% to 50%, compared with the same regions in the control eye. No area was completely devoid of choriocapillaris. Clinically undetected choriocapillaris in eyes of deceased donors with age-related macular degeneration (AMD) is present in approximately 4% of the older population (≥75 years). Unlike neovascular AMD, which is characterized by choroidal neovascularization (CNV) and disorganization of the RPE, GA progresses slowly and eventually results in a well-defined round or oval area of hypopigmentation due to the loss of RPE. The atrophy tends to form around the central fovea in a horseshoe-like manner and usually spares the fovea until late in the course of disease.1-3

Histologic studies have described an absence of photoreceptors, RPE, and choriocapillaris within the area of atrophy, with the loss of RPE preceding loss of choroidal capillaries.2 Loss of choriocapillaris is followed by erosion of the intercapillary pillars.4 Histopathologic studies have shown that small, inactive choroidal neovascular membranes may be present in eyes with GA and that CNV is more frequently bilateral than clinical impression suggests.5,6 The purpose of this study was to develop a technique for quantifying pathologic changes in the RPE and choriocapillaris that could be applied to the study of AMD.

METHODS

Subjects

Case 1 involved an 84-year-old white woman with a history of hypertension and smoking, who had died of lung cancer. She had clinically documented GA in her right eye and a central macular disciform scar from exudative AMD in her left eye. She had been observed at the Wilmer Ophthalmological Institute for 10 years and had been enrolled in a GA natural history study for more than 5 years. The patient was last seen for fundus photography and fluorescein angiograms 6 months before her death, at which time her visual acuity was 20/213 in the right eye and 20/250 in the left eye. Case 2 involved a 95-year-old white man with a history of coronary artery disease, who had died of dilated cardiomyopathy. The subject’s oculist history noted that he was legally blind, and his vision was recorded as worse than counting fingers. No fundus photographs or fluorescein angiograms were available, and therefore we had no knowledge about prior choroidal vascular abnormalities in this subject. Case 3 involved a 70-year-old white man, an exsmoker with a history of prostate cancer, who had died of acute pulmonary embolism. This subject had no history of AMD.

The protocol of the study adhered to the tenets of the Declaration for Helsinki regarding research involving human tissue.

Tissue Preparation

The globes were opened at the limbus, the anterior segments removed, and the eyecups examined by stereomicroscope (Stemi 2000; Carl Zeiss, Inc., Thornwood, NY). Gross images (Fig. 1A) were obtained from a digital microscope camera (DMC; Polaroid Corp., Cambridge, Massachusetts). The right eye in case 2 had GA (aracinar RPE atrophy) and demonstrated a reduction in vascular density in the area from disc to macula that was even greater than that in the eye in case 1 (55% reduction in the submacular region). RPE atrophy between the disc and macula was almost complete. The border of the RPE defect was clearly delineated and coincided closely with the area of decreased vascular density. Surviving choriocapillaris in the area of RPE atrophy was significantly narrower than choriocapillaris in the control subject and in normal areas of the eyes with GA (P < 0.0001).

CONCLUSIONS. In these eyes with GA, RPE atrophy was more severe than loss of choriocapillaris. Surviving choriocapillaris in areas with complete RPE loss was highly constricted. The association of surviving RPE cells with CNV suggests that RPE cells may furnish a stimulus for new vessel formation or stabilization. (Invest Ophthalmol Vis Sci. 2002;43:1986-1993)

Age-related macular degeneration (AMD) is the leading cause of blindness in older persons. It can be classified as nonexudative (dry type) or neovascular (wet type). Geographic atrophy (GA) of the retinal pigment epithelium (RPE), also called areolar atrophy of the RPE, is the advanced form of dry AMD and is present in approximately 4% of the older population (≥75 years).1 Unlike neovascular AMD, which is characterized by choroidal neovascularization (CNV) and disorganization of the RPE, GA progresses slowly and eventually results in a well-defined round or oval area of hypopigmentation due to the loss of RPE. The atrophy tends to form around the central fovea in a horseshoe-like manner and usually spares the fovea until late in the course of disease.1-3

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MA) and imported directly (through a plug-in, version 2.0, Polaroid Corp.) into image-analysis software (Photoshop, ver. 5.0; Adobe Systems Inc., San Jose, CA) running on a PowerMac G3 (Apple Computer, Cupertino, CA). The globes were examined using both epi- and retro-illumination. A grid was placed over the image of the excised piece, with each *square* representing a field of analysis (1 × 1 mm). The coordinates of the grid were used for bookkeeping and to produce the data shown in Figure 3. The optic nerve was adjacent to area Aa and the submacular choroid at area Ac. The area of RPE atrophy appears *blue-green* from APase reaction product in the vessels (*bottom right corner*), whereas the area with RPE remaining appears *salmon pink*. Magnification: (B) ×1.5; (C) ×10.

After incubation, the choroids were washed and postfixed in 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4°C before being partially bleached in 30% hydrogen peroxide at 4°C. The tissue was inspected microscopically every 2 days during bleaching, to ensure that some pigment remained visible. Bleaching was halted when the pigment turned salmon pink and permitted visualization of the underlying choroidal vasculature. The choroids were washed extensively and stored in 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4°C until processed further.

**FIGURE 1.** (A) Gross photograph of right eyecup in case 2. (B) Flat-mounted right eye choroid in case 2, after incubation for APase and bleaching. Note the excised square piece that included peripapillary and submacular choroid. (C) Excised area shown in (B) at higher magnification. A grid was placed over the image of the excised piece, with each *square* representing a field of analysis (1 × 1 mm). The coordinates of the grid were used for bookkeeping and to produce the data shown in Figure 3. The optic nerve was adjacent to area Aa and the submacular choroid at area Ac. The area of RPE atrophy appears *blue-green* from APase reaction product in the vessels (*bottom right corner*), whereas the area with RPE remaining appears *salmon pink*. Magnification: (B) ×1.5; (C) ×10.

**FIGURE 2.** Two different methods of illumination for analysis of the APase-incubated choroids: transmitted and reflected light. (A) Transmitted light was used to visualize viable blood vessels that had *blue* APase reaction product. (B) Reflected light was used to visualize viable RPE, which was *salmon pink* because of the remaining pigment. (C) For blood vessels, the *blue* was selected in the software images captured under transmitted light and the *blue-area*-only image was converted into a binary image to determine density of vasculature, which is called percent vascular area (number of *black* pixels divided by the total number of pixels × 100). (D) Under reflected light, only the RPE’s color was selected in the images and converted into a binary image to determine the area of the Bruch’s membrane covered with RPE, which we called percent RPE area, or percentage of the Bruch’s membrane covered with RPE. Note that RPE cells remain over the most normal choriocapillaris; *bottom right* in all images. Magnification, ×100.
Wet choroids were placed on slides, and several radial cuts were made in the tissue to allow for flattening. The flat preparations were initially imaged on a stereomicroscope using the system described earlier. The submacular choroid, including the entire extent of the area with RPE atrophy and extending several millimeters into nonatrophic RPE temporally, was trimmed from the tissue (Fig. 1B) after low-magnification images were obtained with both transmitted- and reflected-light illumination.

**Tissue Analysis**

The wet excised choroid containing the lesion was placed on a slide with the RPE closest to the objective, coverslipped under buffer, and imaged at higher magnification with the digital color microscope camera on a photomicroscope (Carl Zeiss, Inc.). Red-green-blue (RGB) images (1600 × 1200 pixels) comprising areas of choroid equal to 1 × 1 mm in area were captured in a gridlike manner to map the entire piece of tissue for analysis (Fig. 1C). The coordinates of the grid were used for bookkeeping and for the presentation of the numerical data obtained from analysis of each field (see the Results section). Two different types of illumination were used for imaging each field. Transmitted light from the microscope base was used to image viable blood vessels, because it provided excellent visualization of the blue APase reaction product (Fig. 2A). Reflected light from fiber optic cables was used to image viable RPE, because it highlighted the remaining melanin granules within the cells (Fig. 2B). Images were captured and imported directly into the image-analysis program (Photoshop; Adobe Systems).

Using the color range command of the software’s select menu, the blue APase staining was sampled by using the eyedropper tool and vessels automatically selected in transmitted-light images. This method of selection provided a way of digitally isolating the blue choroidal vessels from other colored features within the image. The method therefore enabled sampling of both large and small blood vessels in a field. By selecting the salmon pink of the pigment in reflected-light images, we used the same process to digitally isolate the RPE. The blood vessel selection and the RPE selection were then copied and pasted into new gray-scale documents (Figs. 2C, 2D). The image size of the new documents was reduced to 640 × 480 pixels, saved in tagged information file format (TIFF) and subsequently imported into NIH Image software (version 1.62; NIH Image: provided in the public domain by the National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/nih-image/). Thresholding was performed and images were converted to binary mode. The percent vascular area or percentage of Bruch’s membrane with RPE was measured on binary images (black vessels or RPE on a white background) using the Compute Percent Black and White command in the measurement macros. This process was repeated for all fields within the excised choroidal tissue. Before thresholding, the images were saved to disc and used to make morphometric measurements of choriocapillary diameters at a later date, as previously described.

The diameter of capillaries in the choriocapillaris was measured from high-magnification, transmitted-light digital images. Using the measuring tool in NIH Image on the calibrated images, the diameters of 15 capillaries in five random fields were determined in five grid areas with RPE atrophy, five areas at the border of atrophic and nonatrophic RPE, and five areas with no RPE atrophy. Only the portion of the capillary distant from any branches, bifurcations, or arteriolar or venular connections was measured.

After the wet-preparation analysis was completed, the excised choroidal tissue containing the lesion was flat embedded in glycol methacrylate (JB-4; Polysciences Inc., Warrington, PA), as described previously, and sectioned for histologic analysis. Cross sections were cut in an orientation that corresponded to the mapping grid and included areas with complete RPE atrophy, the border region of the lesion and unaffected choroid (Fig. 1C, areas a-f). Sections were stained with periodic acid-Schiff and hematoxylin.

**Statistical Analysis**

Statistical evaluation of the choroidal capillary diameter (independent variable) data involved calculating probabilities with the unpaired Students t-test for two samples assuming unequal variances. The null hypothesis was that the means of the two data sets were equal. P ≤ 0.05 was considered significant. Linear regression analysis of the distribution curves was used for comparing the percent vascular area with the percentage of RPE in the posterior pole, resulting in a curve equation and a bivariate correlation coefficient (r²). A computer program (InStat, ver. 2.03; San Diego, CA) was used to determine a best-fit line and the 95% confidence interval of that line and to run an F test to determine the probability for the significance of the line fit.

**RESULTS**

Gross examination of the eyes in case 1 revealed peripheral reticular degeneration and depigmentation, with GA in the macular region of the right eye and advanced AMD with a large, long-term submacular disciform scar in the left eye. Similarly, in case 2 there was advanced nonexudative AMD with the appearance of GA in the right eye (i.e., well demarcated areas of RPE atrophy in the macula and no apparent neovascularization). No peripheral degeneration was seen. Advanced AMD with a large submacular disciform scar was observed in the left eye in case 2. Based on the severity of disease in the left eyes in both cases, these choroids were not analyzed further. The eyes in case 3 had unremarkable fundi.

Stereomicroscopic examination of the APase-incubated choroid from case 1, demonstrated RPE atrophy in the posterior pole with choriocapillaris degeneration and a small isolated CNV lesion. Drusenoid deposits were observed in the submacular region and throughout the postequatorial and peripheral choroid. Their appearance was yellow and refractile in flatmounts of APase-incubated choroid. These deposits consisted of typical nodular drusen and diffuse, confluent drusen. Areas of RPE atrophy were present throughout the equatorial and peripheral choroid. In most cases, these regions demonstrated choroidal vascular changes ranging from loss of some individual capillary segments to widespread capillary rarefaction and disappearance. Microscopic examination of specimens in case 2 revealed a large, well-defined area of RPE atrophy in the posterior pole with choriocapillaris degeneration. Few drusenoid deposits were observed. The equatorial and peripheral regions had intact RPE and were unremarkable. Close visual inspection of the choriocapillaris at the border of the RPE atrophy in both GA specimens demonstrated that, in these cases, RPE atrophy was more advanced than was choriocapillaris degeneration (Fig. 2). The specimen in case 3 had no obvious RPE atrophy, drusenoid deposits, or choriocapillaris degeneration.

Distribution graphs showing the percentage of Bruch’s membrane with RPE and the percent vascular area for different regions within the two choroids of GA-affected eyes and the choroid in a normal eye are shown in Figure 3. In the normal eye (Fig. 3A), where 99% to 100% of the RPE was intact, the percent vascular area was highest in the posterior pole (areas a–e, 87.2% ± 5.3%) and declined somewhat in the equator (areas f and h, 78.9% ± 8.1%). The graphs from the eyes with GA (Figs. 3B, 3C) appeared to demonstrate a correlation between the percentage of surviving RPE in a given region, with the percent vascular area decline (all areas with <100% RPE in Fig. 3D). This was especially true in the eye in case 2, where the border of the lesion was well defined (Fig. 3B). Because the eye in case 1 had peripheral reticular degeneration with diffuse RPE atrophy, this correlation was not as striking as in case 2 (Fig. 3C). Regions of eyes with GA with complete RPE atrophy demonstrated a substantial loss of choroidal blood vessels. For
example, in posterior pole regions (a–e), with complete RPE atrophy, the percent vascular area was 42.7% ± 8.2%. When the data from cases 1 and 2 were analyzed by linear regression analysis, there was a significant linear relationship between the percentage of RPE covering an area and the density of choroidal blood vessels in that region (Fig. 3D, $r^2 = 0.79$). In Figures 3B and 3C, 100% loss of RPE in eyes with GA was associated with a 51% mean decrease in vascular area compared with the control subject (data not shown).

Although most of the changes in percent vascular area in GA-affected eyes could be attributed to loss of vascular channels, the capillaries that remained in regions of RPE atrophy had severely constricted lumens. Capillary diameters were smallest in regions with total RPE atrophy (Fig. 4, A1, A2), were less constricted at the border of the lesion (B1, B2), and were similar to the normal control in nonatrophic regions (V1, V2). Using Student’s t-test, we found that the differences in capillary diameter between areas with viable RPE and areas of RPE atrophy and between areas with viable RPE and border areas were statistically significant ($P < 0.0001$ for all paired comparisons). This was clearly demonstrated in sections where the lumen of choroidal capillaries in regions of complete RPE atrophy were collapsed and appeared to be pulled away from the intercapillary septa (Fig. 5, top). Basal laminar deposits (BLDs) were located in some atrophic regions, at the border of RPE atrophy, and, to a lesser degree, in nonatrophic regions (Fig. 5, middle). Areas of long-standing RPE atrophy had few BLDs.

CNV was easily recognized in the flat perspective by the intense APase reaction product and abnormal vascular pattern.7,9 The eye in case 1 had a small neovascular formation in the region of RPE atrophy that was undetected clinically by fluorescein angiography (Fig. 6). The formation was identified in flat perspective by its unusual vascular pattern and intense APase reaction product. Sections through the CNV lesion demonstrated that the new vessels were highly constricted, similar to viable choriocapillaris vessels in the area of RPE atrophy. Nine small CNV lesions were observed in the postequatorial choroid in case 1. They were located at the border of RPE–choriocapillaris degeneration and generally had viable RPE overlying the neovascularization. Sections through the nine small CNV lesions in postequatorial choroid (Fig. 7) showed

**Figure 3.** (A–C) Graphs of percent RPE area (area of the Bruch’s membrane covered with RPE) and percent vascular area. The x- and y-axes indicate the areas of choroid (coordinates) that match the 1 × 1-mm areas on original maps, as shown in Figure 1. The z-axis indicates percentages. The RPE is the *darker solid color*. In all three choroids, the optic nerve was adjacent to area Aa and the submacular choroid at area Ac. (A) In the control eye (case 3, 70-year-old donor), the percent RPE areas were almost 100% in all areas in this graph, and the percent vascular areas were between 70% to 90%. (B) The right eye in case 2 (95-year-old donor) had a large area in the posterior pole, which included macula, with very little RPE. There was a good correlation between RPE loss and reduction in percent vascular area. Note that there was no complete loss of choriocapillaris in the areas of total RPE atrophy. (C) Percent vascular area and percent RPE area in the posterior pole of the right eye in case 1 (84-year-old donor). (D) Linear regression analysis of the correlation between percent RPE and percent vascular area in cases 1 and 2. There is a significant linear correlation between percentages (x-axis; correlation coefficient $r^2 = 0.7511$, $P < 0.0001$).
the lesions to be adjacent to areas with atrophic RPE and choriocapillaris (Figs. 7A, 7B). However, viable RPE remained over the CNV lesions, unlike the subfoveal CNV lesion observed in this case.

**DISCUSSION**

During normal aging, progressive morphologic changes occur in the choroid, Bruch’s membrane, RPE, and photoreceptors. In macular degeneration, these processes become pathologic and result in a myriad of clinical and histologic changes. Decreased blood flow has been attributed to changes in chorioidal blood volume in the normal aged choroid. Prolonged chorioidal filling times using both fluorescein and indocyanine green (ICG) angiography have been noted in eyes with early AMD and may be an indicator of loss of choriocapillaris. Histologic studies have shown that the density of chorioidal capillaries and medium-sized chorioidal blood vessels decreases considerably with age. Inconsistencies have been reported with regard to histologic changes in choriocapillaris in early forms of AMD, with some investigators reporting a decrease in density of choriocapillaris and others reporting an increase. The technique presented herein avoids the sampling limitations and errors involved in cross-sectional analysis and dem-

**FIGURE 4.** Mean capillary diameters (in micrometers) ± SD in the choriocapillaris, as measured in the flat perspective in different areas: atrophic (A), border (B), and completely viable or nonatrophic RPE (V), in cases 1, 2, and 3 (normal). In the areas with RPE atrophy and in border areas, capillary diameters were significantly smaller than those in nonatrophic areas in both cases 1 and 2 ($P < 0.0001$). There was a significant difference between diameters in the atrophic region and border region in case 2 ($P < 0.0001$) but the difference was not significant in case 1. Capillary diameters in nonatrophic areas in both cases of GA were comparable to those in the normal control eye (case 3) in the same area of choroid.

**FIGURE 5.** Left: Transmitted-light images of the specimen in case 2 taken en bloc in three areas: an area of complete RPE atrophy (top), an area at the border of atrophic and viable RPE (middle), and a nonatrophic area (bottom). In the area with no RPE, there was viable choriocapillaris (APase-positive vessels), but the density was greatly reduced when compared with an area fully covered with RPE. The border area had a very sparse vasculature where RPE was absent (top left of middle micrograph) and many more blood vessels in the area covered with RPE (bottom right of middle micrograph). This region of the choroid is shown in Figure 2, which clearly demonstrates where RPE are present and absent in the field. Right: Sections through the areas shown on the left. Sections from two areas are shown in each panel. Sections were stained with PAS and hematoxylin, making the viable vessels bluegreen (APase reaction product), viable nuclei dark blue, and basement membranes and glycogen salmon pink. Top: sections from the RPE atrophy area. Arrowheads: atrophic choriocapillaris, with no APase reaction product; arrows: constricted capillaries. The capillary lumens were so constricted that they pulled away from the intercapillary septa. Middle: sections from the border area shown at left and in Figure 2 (i.e., between the RPE atrophic area and nonatrophic area). Arrowheads: edge of surviving RPE; arrows: BLDs that were PAS positive. Note that there were normal-looking choriocapillaris vessels ($) in the area adjacent to the remaining RPE (lower section). Bottom: sections from the nonatrophic area. The choriocapillaris in these areas appeared normal. Both areas were covered with viable RPE, but there were areas with and without BLDs in the upper section. Magnification: (left) ×100; (right) ×300.
impaired choroidal perfusion may be responsible for dysfunction of the RPE in AMD. Which of these theories, if any, is correct may be elucidated by the ability to examine relationships and quantify pathologic changes in the RPE-Bruch’s membrane-choriocapillaris complex in AMD. The technique described permits these relationships to be quantified.

We found good correlation between loss of choriocapillaris and diffuse deposits beneath the RPE in a previous study. Unfortunately, the RPE in those specimens had been removed and, therefore, the relationship between RPE and choriocapillaris could not be examined. By modifying that technique and using computer-assisted image processing and analysis, we have developed a method for accurately measuring pathologic changes in the RPE-Bruch’s membrane-choriocapillaris complex in human tissue. Application of this technique to eyes with GA, as presented herein, demonstrates several advantages of this method over standard histologic techniques.

Histologic studies examining changes associated with the RPE-Bruch’s membrane-choriocapillaris complex in normal aged and AMD-affected eyes have relied primarily on morphometric measurements, performed tediously by hand on cross sections of paraffin-embedded tissue. Although the results are undoubtedly accurate, the time and labor required to perform this type of analysis on a large scale can be prohibitive. The technique presented herein provides a method for accurately mapping an entire area of submacular choroid and quantifying RPE atrophy and blood vessel loss with relative speed and ease. The ability to section the tissue after flat, wet-preparation analysis provides additional data on the structural changes that occur in specific geographic locations within the lesion. Moreover, the bird’s eye view afforded by this technique offers visualization of RPE-choriocapillaris relationships that cannot be appreciated in tissue sections. One drawback to the method is that the retina must be removed from the eyecup and the choroid dissected from the sclera before incubation for APase reaction, which could cause mechanical loss of RPE cells. Eyes with extended death-to-enucleation times (greater than 3 hours) or excessive postmortem times (greater than 20 hours) may have artifactual loss of RPE cells if processed with this technique (McLeod DS, Lutty GA, unpublished data, 2000). Even with these restrictions, it is possible that loss of RPE may have occurred in the eyes with GA because the RPE may be unhealthy and less adherent. Similarly, it is possible that the percent RPE area may be underestimated in the subjects with GA because unhealthy RPE may have no pigment and, therefore, would have been excluded from this analysis.

Although limited to only two specimens with GA, this study provides some interesting observations about this form of AMD. Our data show a linear relationship between the percentage of viable RPE and percentage of viable vessels in a given region (Fig. 3D). We were somewhat surprised to find that areas of choroid completely devoid of RPE (and which obviously had had RPE atrophy for many years, based on clinical observations) remained somewhat well vascularized, in that viable choriocapillaries persisted. The literature is inconsistent regarding state of the choriocapillaris in GA; however, our results are in good agreement with Ramrattan et al. who found a 54% decrease in density of choriocapillaris in eyes with GA. If ICG angiography were available for the subjects analyzed, it could be determined whether the highly constricted, yet viable choriocapillary lumens actually had reasonable blood flow. The technique is strictly histologic and therefore represents a snapshot of disease in the RPE-choriocapillaris complex in the eyes with GA included in this study. Although this time point suggests that degeneration of the RPE is more advanced than loss of choriocapillaris, the results cannot be used to draw conclusions about the sequence in which degeneration occurred in both cell types.

![Figure 6](image-url)

**Figure 6.** CNV in posterior pole of the eye in case 1. The CNV in the submucosa had the darkest blue APase reaction product (arrows) and a very abnormal vascular pattern (top). There was no RPE around this CNV lesion, and the choriocapillaris that remained in the area was sparse (right portion of micrograph). Lines: areas shown in (A) and (B). (A) Area marked with line A in the top micrograph. (Arrow, top and in A) A single new vessel projecting through a break in the Bruch’s membrane. (Double arrow, top and in B) Edge of the CNV lesion, which had several new capillaries, above the Bruch’s membrane. The new capillaries were highly constricted, but their viability was shown by the presence of APase reaction product. Magnifications: (top) ×150, (A) ×300, (B) ×180.

Based on clinical and histologic studies, two basic theories have evolved concerning the pathogenesis of AMD. One view is that the primary insult occurs at the level of the RPE and secondary changes occur in the choroidal blood vessels. RPE is thought to modulate the viability of choroidal capillaries through the secretion of growth factors. Degeneration of the RPE or limited diffusion from RPE to choriocapillaris because of deposits that accumulate at the level of the Bruch’s membrane reduces growth factor availability and results in choriocapillary atrophy. However, increased pulsatility and decreased velocity of blood in the short posterior ciliary arteries, as demonstrated in color Doppler imaging studies, suggest that...
The technique devised in this study offers the ability to observe subclinical pathologic changes and place them in relationship to the whole choroid. Submacular and peripheral CNV lesions in the eyes in case 1 had escaped clinical detection by fluorescein angiography. Not only were the formations clearly observed with our technique but also the postequatorial CNV lesions were shown to be intimately associated with surviving RPE cells. CNV has previously been observed histopathologically in approximately one third of subjects with GA.\(^5\)\(^6\) As in the study by Schatz and McDonald,\(^18\) in which areas with CNV had surviving choriocapillaris, we found very few areas, if any, without viable (APase-positive) choriocapillaris. Sunness et al.\(^19\) reported a high risk for CNV in GA-affected eyes if the fellow eye had CNV. This was true in our study in both cases, but the eye in case 1 had many peripheral CNV lesions whereas that in case 2 had only a singular subfoveal CNV lesion.

In summary, the technique presented herein permits the relationship of RPE to the Bruch’s membrane and choriocapillaris to be examined and the changes in the components of this complex to be quantified. The technique has been applied in a limited number of subjects at this point but, in eyes with GA, it has demonstrated that atrophy of the RPE is more severe than loss of choriocapillaris. There was surviving choriocapillaris in areas with complete RPE loss, but the capillaries were highly constricted (Figs. 4, 5). The association of surviving RPE cells with CNV suggests that RPE cells may furnish a stimulus for new vessel formation or stabilization. This technique should contribute to our further understanding of the differences between exudative and nonexudative forms of AMD.

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References


