Morphometric Analysis of Angiograms of Exudative Lesions in Age-Related Macular Degeneration

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Objectives: To analyze the morphometric composition of lesion components in exudative age-related macular degeneration and to study the relationships between individual lesion components and choroidal neovascularization (CNV) subtype, at 2 time points.

Methods: Morphometric analysis of 98 sets of angiograms separated by an interval of at least 3 weeks, with no treatment delivered in the intervening period between angiograms. Area measurements of individual lesion components were made from digitally captured angiograms. Choroidal neovascularizations were classified into subtypes based on the proportions of classic CNV. Fully corrected distance visual acuity measured on logMAR Early Treatment of Diabetic Retinopathy Study charts was available at baseline and at a subsequent visit in 78 subjects. Data were analyzed using parametric and nonparametric tests, linear regression, and McNemar test of equal proportions.

Results: Wholly and predominantly classic CNVs were significantly smaller at initial presentation than minimally classic or occult with no classic CNVs. Lesions containing blood and lipid were also significantly larger than lesions not exhibiting these features. Lesions containing any classic CNV expanded at a significantly greater rate than lesions without classic CNV. Approximately 40% of lesions categorized as wholly classic CNV converted to predominantly classic CNV between baseline and the next follow-up visit.

Conclusion: The presence of classic leakage in exudative age-related macular degeneration is the most important risk factor for rapid expansion of CNV.


The Development of Choroidal Neovascularization (CNV) is a devastating complication of age-related macular degeneration (AMD) and leads to serious losses of central vision. Longitudinal cohort studies and the early randomized controlled clinical trials undertaken by the Macular Photocoagulation Study Group have added to our understanding of the natural history of CNV expansion. These studies have determined that patterns of visual loss are affected by clinical and angiographic features of the exudative macular lesion at presentation. It is widely accepted that angiograms performed using a standardized protocol and subjected to a methodical grading process yield robust information on visual and morphological outcomes. This type of analysis and interpretation of fluorescein angiograms has led to the classification of CNV into distinct morphological subtypes. The classification is based on the spatial and temporal characteristics of fluorescein leakage, which is seen as hyperfluorescence, and the relative areas occupied by the different lesion components. Recent clinical trials vindicate such detailed classification, as treatment effects have been shown to be dependent on the morphological composition of the CNV. Some angiographic studies have documented growth rates in AMD lesions. In these studies, linear measurements on lesion diameter were made, but analyses of the effect of lesion components on lesion growth and subclassification based on proportions of CNV were not undertaken. Scrutiny of the literature yielded no information on expansion rates of components of the neovascular lesion or the effect of lesion components on overall changes in lesion size.

The present study was therefore undertaken to analyze the morphometric composition of lesion components in exudative AMD at 2 time points. Furthermore,
we examined the relationships between individual lesion components and CNV subtype at these time points.

**METHODS**

**ANGIOGRAMS**

Angiograms were selected from an image database spanning January 1, 1995, to June 30, 2001. Criteria for selection were as follows:

1. An angiographic diagnosis of exudative AMD with evidence of classic or occult (late leakage of indeterminate origin) CNV was required.
2. Two angiograms a minimum of 3 weeks apart on the same eye of any one patient were needed. If additional angiograms were available, a third was also selected for analysis.
3. Angiograms were required to have been performed according to a standardized protocol that included capture of stereocolor and angiographic frames on the selected eye, with late frames of both eyes.
4. No therapeutic intervention may have occurred in the interval between angiograms.

When features suggesting chronicity (such as fibrosis involving ≧50% of the lesion or large lesions [≧6000 µm in greatest linear dimension]) were seen on the first angiogram, these were considered exclusion criteria, as it was our clinical policy not to repeat angiography in such cases. We also did not include angiograms of eyes in which the primary lesion was a fibrovascular pigment epithelial detachment, as our experience suggested that, when present, it often represented end-stage CNV. Most patients included in the image database did not have indocyanine green angiography; therefore, we could not comment on the other phenotypes with features best recognized on indocyanine green testing, such as idiopathic polypoidal choroidopathy.

Our target sample was sets of angiograms from 100 subjects. The angiograms from 2 subjects were rejected after initial inclusion, because there was no leakage due to CNV in one and the lesion extended beyond the vascular arcades in the other, which suggested chronicity.

Following selection of angiograms, the clinical notes were scrutinized, and if visual acuity had been measured within 1 week of the angiogram, this was entered into the database. Where visual acuity data were available, this had been performed according to a standardized protocol using Early Treatment of Diabetic Retinopathy Study logMAR charts under set conditions of illumination.

**Equipment**

Fundus color and angiographic images were obtained using 2 digital capture systems. Before April 2000, fundus photography and angiography were performed using the Ophthalmic Imaging System (Ophthalmic Imaging Systems Inc, Sacramento, Calif) equipped with a Topcon 5LTF fundus camera (Topcon, Surrey, Great Britain) and a black-and-white charge-coupled device camera (Sony Corp, New York, NY), with each image occupying 512 × 512 × 16-bit pixels, where 1 pixel length was equivalent to 17.6 µm. Accompanying color photographs were captured on 35-mm film. From April 2000 onward, images were captured on the Topcon retinal camera (model 50IX; Topcon) with IMAGEnet 2.11 software (IMAGEnet, Melbourne, Victoria). The high analogue definition charge-coupled device camera (HAD3CCD; Sony Corp), with 800 × 600-pixel resolution per chip for each of the 3 colors, and a Kodak Megaplus camera (model 1.4i/10; Redlake, Vianen, the Netherlands) were used for the capture of color and angiographic images, respectively. Images from angiograms captured on the Ophthalmic Imaging System were imported into an Image Tools program (a free software program for image analysis) before grading. Images captured on the Topcon digital system were analyzed using IMAGEnet software.

Each angiogram, which generally consisted of some 40 frames, was viewed in its entirety on-screen. Selected angiographic frames were then magnified and viewed singly or as pairs using stereoviewers. Stereoviewing was necessary to detect elevated lesion components. Total lesion area was defined as all CNV (classic or occult), plus any feature obscuring the boundaries of the lesion and thus contiguous to the area of fluorescein leakage. The definitions used to assign lesions to specific categories were based on the proportions of classic CNV to occult CNV (Table 1) and conformed to the definitions previously described for grading and analyzing angiograms.

**Classic leakage** was defined as clearly delineated hyperfluorescence occurring early in the angiographic sequence, with blurring of the margins in the later frames. **Late leakage of indeterminate origin** (a form of occult CNV) was defined as hyperfluorescence with indistinct margins, generally seen after 30 seconds had elapsed, which persisted into late frames. **Fibrovascular pigment epithelial detachment** was said to be present when elevated speckled hyperfluorescence with a dome-shaped distribution was seen early in the angiographic series. The presence or absence of lipid exudation was also noted.

**Measurements**

The planimetric area occupied by leakage from classic CNV and occult CNV (late leakage of indeterminate origin) was measured on selected angiographic frames in which these features were most clearly seen, by tracing around the borders of the hyperfluorescent region with the appropriate tool. The contributions of blocked fluorescence due to blood and other components were distinguished and the areas outlined, when present. When remotely present to the lesion, or when small specks of blood were noted within the lesion, area measurements were not made. The size of the images was precalibrated in both systems, and area measurements were generated in square millimeters. Other associated features of the CNV, such as subretinal fibrosis, atrophy, and subretinal fluid, were estimated using a graded categorical approach, as the diffuse nature of these com-

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**Table 1. Definitions of Lesion Categories Based on Proportions of Classic Choroidal Neovascularization (CNV)**

<table>
<thead>
<tr>
<th>Descriptive Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Lesion</td>
<td>Leakage caused by classic and occult CNV plus features obscuring the boundaries, including blocked fluorescence due to blood, thick exudate, hypertrophic retinal pigment epithelium, and associated pigment epithelial detachment</td>
</tr>
<tr>
<td>Wholly classic CNV</td>
<td>≧50% Of the lesion is composed of classic CNV</td>
</tr>
<tr>
<td>Predominantly classic CNV</td>
<td>≧48% But &gt;49% of lesion is composed of classic CNV</td>
</tr>
<tr>
<td>Minimally classic CNV</td>
<td>But ≦49% of lesion is composed of classic CNV</td>
</tr>
<tr>
<td>Occult with no classic CNV</td>
<td>No classic CNV is present within the lesion; although this definition is not synonymous with occult CNV only, eyes with occult only could be assigned to this category</td>
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components often precluded detailed delineation of the edges. Therefore, the contributions of each of these were estimated as accounting for less than 25% of the lesion, 25% to 49%, 50% to 74%, or 75% or greater.

The position of the CNV with respect to the fovea was also recorded, and any change in position in the subsequent angiogram was noted. Two graders (F.A. and K.A.M.) who were trained in recognition of angiographic criteria undertook the gradings. Grading was performed by both graders on every fifth angiogram to ensure interobserver reproducibility. All discrepancies in grading were resolved by one of us (U.C.).

STATISTICAL ANALYSIS

All data were analyzed using the Statistical Packages for Social Sciences (SPSS Inc, Chicago, Ill), version 10. Summary statistics on CNV category at baseline and subsequent visits and planimetric area measurements of lesion components were generated. Only 38 subjects had been seen on 3 occasions; therefore, the final visit was designated as the first visit after baseline if only 2 visits had taken place, or the second visit if 3 visits had taken place. To test for differences in lesion size by CNV subtype, we used the nonparametric Mann-Whitney test.

Linear regression analysis was used to examine the effect of baseline lesion components on change in lesion area at follow-up. A regression model was also used to analyze the effect of lesion components on change in distance visual acuity. To facilitate comparison with the studies by Vander and coworkers, we examined lesion expansion in 58 eyes in which the interval between angiograms was 120 days or less. The mean area increased from baseline to the first visit by CNV subtype are shown in Table 2 and Table 3. When grouped by the presence or absence of classic CNV, lesions containing any classic CNV were significantly smaller at baseline and at the first visit than lesions without classic CNV. Similarly, when grouped by the presence or absence of occult CNV, lesions in which occult CNV was present were significantly larger than those in which occult CNV was absent. Lesions that were composed of classic CNV only (100% classic) had the smallest mean area and were significantly smaller than lesions composed of 99% classic CNV or less. Lesions that consisted entirely of occult CNV (100% occult) were not significantly different from those lesions composed of 99% occult CNV or less.

When grouped by the presence or absence of blood at baseline, lesions with blood were significantly larger at baseline (5.99 vs 3.25 mm², P = .01), the first visit (8.17 vs 5.52 mm², P = .04), and the final visit (8.56 vs 6.27 mm², P = .06). When the relationship at baseline between CNV subtype and the presence of blood was examined, the mean ± SD proportion of classic CNV was higher in lesions without blood (blood present, 51.3% ± 38.2% vs blood absent, 34.1% ± 31.2%; P = .02, independent samples t test). When grouped by the presence or absence of lipid exudates, lesions with exudate were larger (5.55 vs 1.96 mm², P = .08, nonparametric Mann-Whitney test) and more likely to contain some occult CNV.

RELATIONSHIPS BETWEEN LESION COMPONENTS, SIZE, AND SUBTYPE

The mean areas of the lesions at baseline and at the first visit by CNV subtype are shown in Table 2 and Table 3. When grouped by the presence or absence of classic CNV, lesions containing any classic CNV were significantly smaller at baseline and at the first visit than lesions without classic CNV. Similarly, when grouped by the presence or absence of occult CNV, lesions in which occult CNV was present were significantly larger than those in which occult CNV was absent. Lesions that were composed of classic CNV only (100% classic) had the smallest mean area and were significantly smaller than lesions composed of 99% classic CNV or less. Lesions that consisted entirely of occult CNV (100% occult) were not significantly different from those lesions composed of 99% occult CNV or less.

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EFFECT OF LESION SUBTYPE ON LESION EXPANSION

The mean lesion size increased from baseline to the first visit, as did the mean area occupied by individual lesion components. A comparison of the mean lesion size at base-
line and the first visit by lesion subtype at baseline showed that increasing amounts of classic CNV generally resulted in proportionally greater expansion of the lesion (Figure 1). Figure 2 illustrates the relationship between change in area of the lesion and classic and occult leakage with increasing interval size. The trend lines show that the change in area of classic CNV is steeper than that of occult CNV.

Table 3 shows the effect of lesion composition on lesion area. When grouped by the presence or absence of classic CNV, the expansions in area of the lesion from baseline to the first visit (3.45 vs 1.60 mm², \( P = .03 \)) and from baseline to the final visit (3.78 vs 2.15 mm², \( P = .04 \)) were significantly different. When grouped by the presence or absence of blood at baseline, eyes without blood did not exhibit significantly different expansion rates compared with eyes with blood (data not shown).

Linear regression analysis, with change in area of the lesion at the final visit as the dependent variable, and with baseline lesion components, age, sex, and interval between angiograms as independent variables, showed that the area of classic CNV at baseline significantly affected lesion expansion (\( t = 3.52, P < .001 \)).

**CHANGE IN LINEAR DIMENSIONS OF LESIONS BY CNV SUBTYPE**

Table 4 shows the mean lesion area at baseline and the first visit in eyes with baseline values of 100% classic (A), 50% to 99% classic (B), 1% to 49% classic (C), and 0% classic (D) choroidal neovascularization (CNV).

Figure 1. Bar graphs showing the mean area of lesion components at baseline and the first visit in eyes with baseline values of 100% classic (A), 50% to 99% classic (B), 1% to 49% classic (C), and 0% classic (D) choroidal neovascularization (CNV).

Figure 2. Graph demonstrating the relationship between area of lesion, leakage due to classic choroidal neovascularization (CNV), and leakage due to occult CNV and the interval between angiograms at baseline and the first visit. Dashed lines represent trend.

Regression analysis with change in distance visual acuity as the dependent variable showed that the model that explained most of the variation included change in the of 6.57 µm/d, compared with 3.92 µm/d when classic CNV was absent.

**RELATIONSHIP BETWEEN BASELINE LESION VARIABLES AND CHANGES IN VISUAL ACUITY**

Regression analysis with change in distance visual acuity as the dependent variable showed that the model that explained most of the variation included change in the
area occupied by classic CNV at the final visit, distance visual acuity at baseline, and duration of follow-up.

**CATEGORIZATION OF CNV AT BASELINE AND FOLLOW-UP**

The distribution of eyes by CNV category at baseline and the first visit is shown in Table 5. At the baseline assessment, only 12% of eyes were graded as having wholly classic CNV. Examination of the distribution of eyes by CNV subtype at the first visit showed that most eyes remained in the category to which they were assigned at baseline. Conversions were noted in a small number of eyes, with 5 eyes with wholly classic CNV at baseline having converted to predominantly classic CNV. There were no conversions from any of the other categories to wholly classic CNV during the interval spanning baseline and the first visit. A change in category from predominantly classic CNV to minimally classic or occult CNV, with noclassic CNV between baseline and the first visit, occurred in a small proportion of eyes. Three of 20 eyes initially graded as occult with no classic CNV had developed a classic component, which led to a revised classification of minimally classic CNV at the first visit. We also examined the change in categorization between baseline and the final visit (Table 6). Most eyes remained in the categories to which they were assigned at baseline. Of the 24 changes noted, 12 were in the direction of increasing classic CNV, while 12 were in the opposite direction (P = .99, McNemar test).

The present study used a systematic morphometric approach to quantify the size of the exudative lesion and its constituents and made several important observations. We confirmed that wholly classic and predominantly classic CNVs are smaller at initial presentation than lesions with a lesser proportion of classic CNVs. We found that the rate of expansion of classic CNV is faster than that of occult CNV, which would account for the more rapid increase in lesion size in the former.

Vander and Klein and coworkers have reported mean daily growth rates for CNV membranes of 10 µm and 9 µm, respectively. Both of these studies used unidimensional measures of expansion. The present study performed area measurements that detect change in all the boundaries of the lesion. To facilitate comparison between these previous studies and the present work, we estimated a linear growth rate of lesion expansion using angiograms in which the interval between angiograms was 120 days or less. The growth rate of the lesion ranged from approximately 6.5 µm/d when classic CNV was present to 3.9 µm/d when classic CNV was absent, with a mean growth rate of 5.5 µm/d. The slightly lower rate of linear expansion in the present study may reflect differences in the methods used (we did not measure linear diameters) or the longer intervals between angiograms in the present study, compared with that of Klein et al, in which the interval separating the angiograms was shorter, with a mean of 13 days.

Our findings support previous observations that the presence of occult leakage is associated with larger lesion size. Recent clinical trials using verteporfin photodynamic therapy attributed the treatment benefit seen in eyes with predominantly classic without occult CNV to the smaller size of the lesions in that subgroup. The slower growth rate of the lesions in eyes containing occult CNV and a corresponding slower decline in visual function may permit such patients to remain eligible for entry into clinical trials, despite long-standing disease. However, the data from the present study were from patients at first consultation. Therefore, the larger lesion

![Table 4. Estimated Linear Change From Baseline to the First Visit in Eyes in Which the Interval Between Angiograms Was 120 Days or Less](image)

<table>
<thead>
<tr>
<th>Choroidal Neovascularization Subtype</th>
<th>Area, mm²</th>
<th>Diameter, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic present (n = 24)</td>
<td>2.95</td>
<td>1938</td>
</tr>
<tr>
<td>Classic absent (n = 16)</td>
<td>4.09</td>
<td>2281</td>
</tr>
<tr>
<td>All (n = 40)</td>
<td>3.40</td>
<td>2080</td>
</tr>
</tbody>
</table>

*Table 5. Distribution of Eyes at Baseline and the First Visit*

<table>
<thead>
<tr>
<th>Distribution at Baseline</th>
<th>0% Classic</th>
<th>1%-49% Classic</th>
<th>50%-99% Classic</th>
<th>100% Classic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Classic (n = 21)</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1%-49% Classic (n = 33)</td>
<td>3</td>
<td>26</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>50%-99% Classic (n = 37)</td>
<td>0</td>
<td>7</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>100% Classic (n = 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

*Table 6. Distribution of Eyes at Baseline and the Final Visit*
size of occult CNV may simply reflect better levels of visual function in eyes with occult CNV than those with classic CNV, which would be compatible with later presentation.

Although not reaching statistical significance (P = .08), our data suggest that the presence of lipid exudates was associated with larger lesion size and occult leakage. These findings are in accord with the observations of Lozano-Rechy et al, who reported that subretinal membranes in eyes with lipids tended to be ill defined. The present study suggests that blood is a risk factor for more rapid expansion of the lesion in eyes with occult CNV, which is consistent with the findings of Stevens et al, in which variable growth rates in eyes with occult CNV were noted.

The present study demonstrated changes in lesion classification over time. We accept that grader variability may account for some of the changes in lesion classification. However, the appearance of classic CNV when none was present or the conversion of wholly classic CNV into a predominantly classic subtype is unlikely to be influenced by subjective factors. Furthermore, the present study found fewer than expected changes in classification of lesions. Notwithstanding a mean follow-up in excess of 4 months between baseline and the first visit, only 13% of eyes developed a classic component when none was present at baseline. Stevens et al described changes in lesion size and lesion components in a subgroup of 40 subjects enrolled in a preliminary randomized controlled trial of the macular grid laser. Among 35 subjects with 9 months’ follow-up, they found that 32% of occult lesions had doubled in size and that classic CNV had developed in 52% of eyes. The present study is not in complete accord with these previous observations, as we found that classic CNV only developed in some 15% of eyes that have no classic CNV at initial presentation, nor did we find a doubling in size of occult lesions. The discordant findings suggest that the participant profiles in the 2 studies were different. The study by Stevens et al excluded subjects if the amount of hemorrhage or blocked fluorescence was greater than the area of visible CNV. The present study did not exclude such subjects, and in 13% of eyes, the amount of visible CNV was smaller than the area occupied by other lesion components. The median change in distance visual acuity in the study by Stevens et al was 2.5 lines. In the present study, the mean change in distance visual acuity in eyes with occult with no classic CNV was 1.7 lines. It is therefore possible that the selection criteria used in the macular grid laser trial resulted in the recruitment of a group of patients with a particularly worse natural history, whereas the present study did not seek to exclude subjects on the basis of angiographic or visual acuity criteria. This may have resulted in the inclusion of angiograms from subjects with occult CNV without features that predispose to rapid growth and vision loss.

A small number of eyes in which classic CNV accounted for 100% of the lesion were reclassified as predominantly classic or minimally classic CNV at a subsequent visit because of the appearance of occult leakage or other lesion components. These findings emphasize the importance of early diagnosis and fast-tracking of patients with wholly classic or classic with no occult CNV, in whom visual outcomes have been shown to be improved with treatment such as photodynamic therapy.

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