Age-related macular degeneration (AMD) is a leading cause of vision loss in elderly people worldwide. The prognosis for patients with advanced AMD is generally not good; therefore, early detection and intervention of AMD are very important. Drusen are landmark lesions of early AMD. With the development of multimodal imaging technology, combined with histopathological studies, our understanding of drusen and their clinical significance has become more comprehensive. In addition, subclinical deposits, including basal linear deposit (BlamD), have been demonstrated as very important early pathological changes of AMD. The BlinD is one piece of AMD pathology, and its specific role in progression beyond being associated with drusen is not yet clear. The BlinD and soft drusen are specific for early AMD and may confer a risk of progression to advanced AMD. Thus far, as a subclinical deposit, the BlinD has not been visualized in vivo. In the present study, we propose for the first time that ASHS-LIA might represent the BlinD or pre-BlinD (lipid wall).

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PurPOSE. Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) might represent fundus aging and neutral lipid accumulation. The present study was conducted to determine the association between drusen and ASHS-LIA and to provide further evidence for our hypothesis.

METHODS. Patients who underwent indocyanine green angiography (ICGA), fundus fluorescein angiography (FFA), and spectral-domain optical coherence tomography (SD-OCT) at the Zhongshan Ophthalmic Center from January 2014 to March 2018 were reviewed. Patients with soft drusen or ASHS-LIA in the fellow eyes were included in this study.

RESULTS. A total of 345 patients aged 33 to 87 years (mean: 66.1 ± 8.4 years) were included in this study. Results showed that all patients had ASHS-LIA, among them, 70 patients (20.3%) had concurrent soft drusen, while 156 patients (45.2%) had concurrent hard drusen. Furthermore, the incidence of soft drusen was 8.9% (10/112), 21.2% (41/193), and 47.5% (19/40), respectively, in the different grades of ASHS-LIA and significantly increased with the grade of ASHS-LIA (all P < 0.01). In addition, soft drusen were located inside the ASHS-LIA in all cases and both soft drusen and ASHS-LIA presented as hypofluorescence on late-phase ICGA, while hard drusen were located outside the ASHS-LIA and presented as hyperfluorescence on late-phase ICGA.

Conclusions. Soft drusen might occur in the presence of ASHS-LIA, and were correlated with the grade of ASHS-LIA. Based on the association between drusen and ASHS-LIA and their ICGA characteristics, we hypothesized that ASHS-LIA might be a candidate correlate of lipid accumulation in Bruch’s membrane.

Keywords: age-related, hypofluorescent spots, indocyanine green angiography, drusen, lipid accumulation
and both are located in precisely the same plane.\textsuperscript{16,19} The purpose of the current study was to evaluate the association between ASHS-LIA and drusen (especially soft drusen) and to provide further evidence for our hypothesis that ASHS-LIA might represent the BlinD or pre-BlinD (lipid wall).

**MATERIALS AND METHODS**

This is a hospital-based retrospective study. Patients who were referred to the Zhongshan Ophthalmic Center for ICGA, color FP, FFA, and SD-OCT examination between January 2014 and March 2018 were reviewed. The study was conducted according to the Declaration of Helsinki and was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. Potential risks associated with the ICGA and FFA examination were fully discussed with the patients, and written informed consent was obtained from all included patients.

To prevent lesions from interfering with the assessment of fluorescence, only fellow eyes with normal fundi or with soft or hard drusen were screened for the study, although ASHS-LIA always appeared in both eyes of any patient. The inclusion criterion was soft drusen or ASHS-LIA in the study eye. And the right eye was included in the study when both eyes of the patient met the inclusion criteria. Patients with bilateral lesions or inflammatory conditions involving the choroid, such as multifocal choroiditis, and patients who had a previous ophthalmological intervention procedure, such as laser coagulation, vitrectomy, anti-VEGF injection, or photodynamic therapy in the study eye, were excluded. Patients with evidence of significant cataracts or vitreous opacity, which influences imaging quality and fluorescence judgment, were also excluded from the study. After these criteria were applied, 345 fellow eyes with normal fundi or with drusen were eligible for inclusion in this study.

ASHS-LIA were determined based on the following criteria according to our previous study:\textsuperscript{6} (1) the presence of hypofluorescent spots on late-phase ICGA (20–40 minutes after dye injection), (2) primary distribution in the macular region, (3) regular arrangement pattern with possible confluence, and (4) no corresponding abnormalities on color FP, SW-FAF, FFA, and SD-OCT (Fig. 1). Furthermore, according to the region of involvement, ASHS-LIA were divided into three grades: grade 1, macular region; grade 2, macular region and around the optic disc; and grade 3, throughout the posterior pole. Drusen were determined based on multimodal imaging.\textsuperscript{20} Hard drusen were defined as discrete yellow-white deposits situated underneath the RPE with diameters less than 63 \( \mu \)m, and more compact structures than soft drusen. Soft drusen were defined as mound-like deposits situated between the reflective band representing the RPE and its basal lamina and the remainder of BrM, with diameters usually \( \leq 125 \) \( \mu \)m and less well demarcated structures than hard drusen.

Subretinal drusenoid deposits (SDDs) (originally called reticular pseudodrusen) also presented as hypofluorescent spots on late-phase ICGA but had different distribution patterns and locations compared with ASHS-LIA, and SDDs could be detected via other multimodal imaging techniques, including color FP, infrared reflectance, and SD-OCT.\textsuperscript{21–23} A striking characteristic of SDDs is that they seem to follow the topography of rod photoreceptors, starting in the perifovea, sweeping around the optic nerve head, and encroaching on the optic nerve head.
the fovea only in late stages. Therefore, SDDs can be easily differentiated from ASHS-LIA, and patients with SDDs have been excluded as ASHS-LIA in this study. In addition, hypofluorescence on late-phase ICGA could be observed in patients with disturbance of the choroidal circulation, such as chronic central serous chorioretinopathy (CSC), and in patients with inflammatory diseases, such as punctate inner choroidopathy (PIC) and multiple evanescent white dot syndrome (MEWDS), which have also been excluded as ASHS-LIA in the present study.

Demographic information, medical records, and multimodal imaging data were reviewed. The best-corrected visual acuity (BCVA) was measured with Snellen charts, and a comprehensive ophthalmological examination including slit-lamp examinations and dilated funduscopy was conducted. Color FP was performed with a Zeiss FF450 plus fundus camera (Carl Zeiss, Inc., Jena, Germany). ICGA, FFA, and SW-FAF were performed with a Heidelberg retina angiogram (Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany). SD-OCT was performed with an OCT Spectralis (Heidelberg Engineering). Two retina physicians (Ling Chen and Xiongze Zhang) independently evaluated and diagnosed the patients, evaluated the ASHS-LIA and the grades, and assessed the soft or hard drusen and the association between ASHS-LIA and drusen. When the two retina physicians disagreed, discrepancies in their findings were referred to a third retina specialist (Feng Wen) for final determination.

Statistical analyses were performed with SPSS Version 21.0 software (SPSS, Inc., Chicago, IL, USA). Descriptive statistics (means ± SDs) of normally distributed variables and geometric means with 95% confidence intervals (CIs) of nonnormally distributed variables were calculated. Comparisons were evaluated by independent samples t-test, 1-way ANOVA (normal data) or χ² test (nonnormal data). Significant differences were defined as P values < 0.05.

RESULTS

A total of 345 fellow eyes from 345 patients were included in this study. The patient ages ranged from 33 to 87 years (mean: 66.1 ± 8.4 years), and the male to female ratio was 2.2:1. The mean BCVA was a Snellen equivalent of 20/25 (range: 20/16–20/50). Table 1 shows the diagnostic information and the demographic characteristics of the included patients. Among the 345 included patients, 228 patients had polypoidal choroidal vasculopathy (PCV); 41 patients had neovascular AMD (nAMD); 17 patients had dry AMD; 11 patients had CSC; 9

<table>
<thead>
<tr>
<th>Age Range, y</th>
<th>31–40</th>
<th>41–50</th>
<th>51–60</th>
<th>61–70</th>
<th>71–80</th>
<th>81–90</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1</td>
<td>8</td>
<td>72</td>
<td>164</td>
<td>83</td>
<td>17</td>
<td>345</td>
</tr>
<tr>
<td>ASHS-LIA, n/%</td>
<td>1/100</td>
<td>8/100</td>
<td>72/100</td>
<td>164/100</td>
<td>83/100</td>
<td>17/100</td>
<td>345/100</td>
</tr>
<tr>
<td>SD, n/%</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
<td>23/14.0</td>
<td>35/42.2</td>
<td>12/70.6</td>
<td>70/20.3</td>
</tr>
<tr>
<td>HD, n/%</td>
<td>0/0</td>
<td>0/0</td>
<td>15/20.8</td>
<td>73/44.5</td>
<td>53/63.9</td>
<td>15/88.2</td>
<td>156/45.2</td>
</tr>
</tbody>
</table>

Data represent n or %. SD, soft drusen; HD, hard drusen.
patients had retinal vein obstruction (RVO); 9 patients had idiopathic pigment epithelium detachment (IPED); and 30 patients had other fundus diseases, including pathological myopia, retinal macular edema, epiretinal membrane, focal choroidal atrophy, choroidal hemangioma, Coats' disease, optic neuropathy, and syphilitic retinitis.

All 345 patients had ASHS-LIA, among them, 70 patients (20.3%) had concurrent soft drusen, and 156 patients (45.2%) had concurrent hard drusen. Table 2 shows the ASHS-LIA and drusen among the different age groups by decade. The findings showed that among the 345 patients with ASHS-LIA, only one patient was in the age group of 31 to 40 years. Furthermore, soft drusen were only detected in patients over 60 years of age, including 23 of 164 (14%) patients aged 61 to 70 years, 35 of 83 (42%) patients aged 71 to 80 years, and 12 of 17 (71%) patients aged 81 to 90 years. The incidence of soft drusen increased significantly with age ($\chi^2$ test, all $P < 0.001$). Moreover, hard drusen were detected in only patients over 50 years of age, including 15 of 72 (21%) patients aged 51 to 60 years, 73 of 164 (45%) patients aged 61 to 70 years, 53 of 83 (64%) patients aged 71 to 80 years, and 15 of 17 (88%) patients aged 81 to 90 years. The incidence of hard drusen increased significantly with age ($\chi^2$ test, all $P < 0.001$).

Table 3 shows patients with different grades of ASHS-LIA. Our findings showed that the mean age of patients was significantly increased with the grade of ASHS-LIA (1-way ANOVA, all $P < 0.001$). Nevertheless, there was no significant correlation between gender and the grade of ASHS-LIA. In addition, 10 of 112 (8.9%) patients with grade 1 ASHS-LIA, 41 of 193 (21.2%) patients with grade 2 ASHS-LIA, and 19 of 40 (47.5%) patients with grade 3 ASHS-LIA had soft drusen. The incidence of soft drusen was significantly increased with the grade of ASHS-LIA ($\chi^2$ test, all $P < 0.01$). Additionally, 38 of 112 (33.9%) patients with grade 1 ASHS-LIA, 86 of 193 (44.6%) patients with grade 2 ASHS-LIA, and 32 of 40 (80%) patients with grade 3 ASHS-LIA had hard drusen. The incidence of hard drusen in patients with grade 3 ASHS-LIA was significantly higher than that in patients with grade 1 and grade 2 ASHS-LIA (both $P < 0.001$).

Figure 1 shows the multimodal imaging of ASHS-LIA. ASHS-LIA presented as hypofluorescence on late-phase ICGA were mainly located in the macular region and could be confluent (Fig. 1E, red arrow). No corresponding abnormalities were detected by other multimodal imaging techniques, including color FP, SW-FAF, FFA, early-phase ICGA, and SD-OCT.

Figure 2 shows a representative example of soft drusen and ASHS-LIA. Both ASHS-LIA (Fig. 2C, red arrow) and soft drusen (Fig. 2C, yellow arrow) presented as hypofluorescence on late-phase ICGA, and were mainly located in the macular region. Soft drusen was located within the bounds of the region with ASHS-LIA in all cases, with lower fluorescence than ASHS-LIA on late-phase ICGA. Figure 3 shows a representative example of hard drusen and ASHS-LIA. Hard drusen were mainly distributed in the peripheral retina, but there were 32 cases with hard drusen distributed in the posterior pole, even in the macular region. Furthermore, hard drusen (Fig. 3C, yellow arrow) presented as hyperfluorescent spots on late-phase ICGA and were always located outside the ASHS-LIA (Fig. 3C, red arrow).

We also display other conditions with hypofluorescence that could be distinguished from ASHS-LIA. Figure 4 shows a representative example of hypofluorescence resulting from disturbance of the choroidal circulation in CSC patients. This kind of hypofluorescence was caused by focal filling defects in the choriocapillaris, so it could be an irregular shape and might occur at any position in the fundus. Moreover, it was better demarcated than ASHS-LIA and commonly surrounded by

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**Table 3. Demographic Characteristics and Drusen Incidence of Patients With Different Grades of ASHS-LIA**

<table>
<thead>
<tr>
<th></th>
<th>Grade 1 ($n = 112$)</th>
<th>Grade 2 ($n = 193$)</th>
<th>Grade 3 ($n = 40$)</th>
<th>$P$ Values</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>61.9 ± 8.1</td>
<td>67 ± 7.2</td>
<td>73.7 ± 8.1</td>
<td>$P &lt; 0.001$</td>
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<tr>
<td>Male, %</td>
<td>67.0</td>
<td>69.4</td>
<td>67.5</td>
<td>0.655</td>
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<tr>
<td>SD, n (%)</td>
<td>10 (8.9)</td>
<td>41 (21.2)</td>
<td>19 (47.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>HD, n (%)</td>
<td>38 (35.9)</td>
<td>86 (44.6)</td>
<td>32 (80)</td>
<td>0.068</td>
</tr>
</tbody>
</table>

*Data represent n, mean ± SD, or %, Grade 1, ASHS-LIA in the macular region; grade 2, ASHS-LIA in the macular region and around the optic disc; grade 3, ASHS-LIA throughout the posterior pole; SD, soft drusen; HD, hard drusen.*
hyperfluorescent rings (Fig. 4D). Furthermore, abnormalities corresponding to the area of hypofluorescence in this case could be detected by color FP, FFA, early-phase ICGA, and SD-OCT. Figure 5 shows a representative example of hypofluorescent spots on late-phase ICGA in patients with PIC. These hypofluorescent spots were uneven in size, were irregular in distribution, and had lower hypofluorescence than ASHS-LIA (Fig. 5E). In addition, abnormalities corresponding to the hypofluorescent spots could be detected by color FP, SW-FAF, FFA, early-phase ICGA, and SD-OCT.

**Figure 4.** Multimodal imaging of disturbance of choroidal circulation in CSC. The left eye (BCVA: 20/40) from a 50-year-old man; he was diagnosed with central serous chorioretinopathy 2 years ago. (A) Color FP showed irregular lesions and pigment derangement in the macular region. (B) FFA revealed hyperfluorescence due to depigmentation. (C) Early-phase ICGA showed choroidal vascular dilation and hyperpermeability in the macular region. (D) Late-phase ICGA showed irregular hypofluorescence that was well demarcated and surrounded by hyperfluorescent rings. (E) SD-OCT (the green line in D) showed increasing subfoveal choroidal thickness (428 μm) and abnormalities of the ellipsoid zone and RPE.

**Figure 5.** Multimodal imaging of PIC. The left eye (BCVA: 20/32) from a 28-year-old woman; she was diagnosed with binocular PIC. (A) Color FP showed yellow-white punctate lesions in the macular region. (B) SW-FAF showed hypoautofluorescent spots of PIC (white arrow) and hyperautofluorescent spots of MEWDS (yellow arrow). (C) FFA revealed hyperfluorescent spots of lesion staining. (D) Early-phase ICGA revealed hypoautofluorescent spots. (E) More hypofluorescent spots were indicated in the late-phase ICGA than in the early-phase ICGA, with inhomogeneous enhancement of background fluorescence. (F) Several active PIC lesions were detected on SD-OCT (the green line in E).
DISCUSSION

In the present study, we evaluated the association between drusen and ASHS-LIA and hypothesized that ASHS-LIA might represent the BlinD or pre-BlinD (lipid wall). Our findings showed that all eyes with soft drusen had concurrent ASHS-LIA and that the incidence of soft drusen significantly increased with the grade of ASHS-LIA. Both soft drusen and ASHS-LIA presented as hypofluorescence on late-phase ICGA, and soft drusen were located within the bounds of the region with ASHS-LIA in all cases. In contrast, hard drusen presented as hyperfluorescence on late-phase ICGA, and were located outside the region with ASHS-LIA.

Our recent study, with a large sample size and a large age range, demonstrated that the occurrence and grade of ASHS-LIA increased with age. Therefore, ASHS-LIA might represent the aging of the fundus. Based on the age correlation, distribution, confluent features, ICGA, and other multimodal imaging features, we speculated that ASHS-LIA might represent neutral lipids accumulating in BrM. BrM is the innermost 2 to 4 μm of the choroid (subjacent to the RPE), consisting of five layers of connective tissue. Lipoproteins begin to accumulate within BrM by the fourth decade of life, slowly increasing and eventually leading to the formation of a “lipid wall,” a precursor of the BlinD. The BlinD was described as membranous debris in previous studies due to its ultrastructural features, but with lipid-preserving ultrastructural techniques, Curcio et al. demonstrated that the BlinD was in fact a thin, tightly packed layer (0.4–2 μm) of lipoprotein-derived debris containing neutral lipids. The BlinD and soft drusen are diffuse and focal deposits, respectively, of the same lipoprotein-derived debris, and both are located in precisely the same plane. It has been demonstrated that the BlinD and soft drusen are specific for early AMD and may be significantly related to the progression to advanced AMD. Nevertheless, the BlinD, as one piece of
characteristics, is a likely candidate for histologic correlate of features, relationship with soft drusen, and possible ICGA BlinD, with similar age correlation, distribution, confluent fluorescence was observed on late-phase ICGA. Therefore, homogeneous background extravasate into the choroidal stroma, pass through BrM and eventually be taken up by the RPE. Therefore, homogeneous background fluorescence was observed on late-phase ICGA. (B) With aging, neutral lipids accumulate in BrM, reducing ICG dye through BrM into the RPE. Hence, hypofluorescent spots were observed in the macular region. (C) Neutral lipids gradually increased and became confluent; hence, confluent hypofluorescence were observed in the macular region. (D) Soft drusen formed in the presence of diffuse deposits of neutral lipids and contained mainly neutral lipids. Hence, soft drusen presented as even lower hypofluorescence than ASHS-LIA on late-phase ICGA.

AMD pathology, cannot yet be detected in vivo, and thus, its specific role in progression beyond being associated with drusen is not yet clear.

In the current study, we reviewed patients who underwent multimodal imaging examination, and we found that all eyes with soft drusen had concurrent ASHS-LIA and that the incidence of soft drusen increased with the grade of ASHS-LIA. This means that soft drusen may occur in the presence of ASHS-LIA. A previous clinicopathological study demonstrated that soft drusen were found in only eyes with diffuse deposits (BlinD). Therefore, it provided evidence for the correspondence between ASHS-LIA and BlinD. Moreover, both soft drusen and ASHS-LIA presented as hypofluorescence on late-phase ICGA, and soft drusen were located inside the region with ASHS-LIA and had lower fluorescence than ASHS-LIA. As we know, indocyanine green dye is water soluble and does not bind to neutral lipids, such as esterified and unesterified cholesterol, which can explain the hypofluorescence of soft drusen on late-phase ICGA and give us sufficient reason to speculate that the BlinD, containing the same lipoprotein-derived debris as in soft drusen, might also present as hypofluorescence on late-phase ICGA.

Therefore, the BlinD, with similar age correlation, distribution, confluent features, relationship with soft drusen, and possible ICGA characteristics, is a likely candidate for histologic correlate of ASHS-LIA. Then, how did soft drusen and the BlinD (ASHS-LIA) lead to varying degrees of hypofluorescence on late-phase ICGA? A previous study demonstrated that ICG dye was located within the retinal and choroidal vessels in the early phase after injection, but it was extravasated into the choroidal stroma and accumulated within the RPE over time. An in vitro experiment demonstrated that cultured human RPE cells took up ICG dye. Recently, Tam et al. demonstrated that RPE cells contributed to the fluorescence signal observed in the late phase of ICGA. Both the local and diffusing deposition of neutral lipids between BrM and the RPE (soft drusen and the BlinD) would impede hydrophilic moieties (ICG dye) through BrM into the RPE. Figures 6 and 7 show the proposal as to how the BlinD and soft drusen can present as varying degrees of hypofluorescence on late-phase ICGA. Under normal conditions, ICG dye could extravasate into the choroidal stroma, pass through BrM, and eventually be taken up by the RPE. Therefore, homogeneous background fluorescence was observed on late-phase ICGA (Figs. 6A, 7A). With age, the BlinD formed between the inner collagenous layer of BrM and the RPE basal lamina, which could reduce ICG dye through BrM into the RPE and present as hypofluorescent spots on late-phase ICGA (Figs. 6B, 7B). When the BlinD became confluent, confluent hypofluorescence was observed on late-phase ICGA (Figs. 6C, 7C). In addition, soft drusen would have even lower hypofluorescence than the BlinD because of thicker deposits of neutral lipids (Figs. 6D, 7D).

Additionally, our findings showed that hard drusen were always located outside the ASHS-LIA, regardless of the peripheral retina or the macular region. Hard drusen, consisting of homogeneous hyalinized material, manifested hyperfluorescence on ICGA because the high content of phospholipids binds more ICG dye. Hard drusen are considered a normal consequence of age; however, why they are always located outside the ASHS-LIA is still unclear. We inferred that this might be due to the differences in composition and polarity between the hard drusen and ASHS-LIA material (BlinD), considering that hard drusen, soft drusen, and BlinD were located in the same sub-RPE space. This finding provides further evidence for our hypothesis, but further research is needed.

This study has limitations. As a clinic-based retrospective study, selection bias might occur. No longitudinal observation was made because ICGA is an invasive examination. Furthermore, we have no direct clinicopathologic correlation evidence, and we cannot explain why ASHS-LIA present as regular scattered spots before confluence. Nevertheless, previous pathological findings about lipid deposition, the BlinD, and soft drusen provided us with a great deal of information. We included a large number of patients who underwent multimodal imaging examinations.

In conclusion, our study indicated that soft drusen might occur in the presence of ASHS-LIA and that the incidence of soft drusen significantly increased with the grade of ASHS-LIA. Furthermore, soft drusen were located inside the ASHS-LIA in all cases, and both the soft drusen and ASHS-LIA presented as hypofluorescence on late-phase ICGA. In contrast, hard drusen were always located outside the ASHS-LIA and presented as hyperfluorescence on late-phase ICGA. We hypothesized that ASHS-LIA might be a candidate correlate of lipid accumulation in BrM, and might represent histopathological BlinD/pre-BlinD.
ASHS-LIA, a Candidate Correlate of Lipid Accumulation

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