Age-Related Scattered Hypofluorescent Spots on Late-Phase Indocyanine Green Angiography as Precursor Lesions of Polypoidal Choroidal Vasculopathy

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PURPOSE. Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) might represent lipid accumulation in Bruch’s membrane in the form of basal linear deposits (BLinD). The present study was conducted to describe the clinical characteristics of polypoidal choroidal vasculopathy (PCV) associated with ASHS-LIA.

METHODS. Consecutive patients with treatment-naïve PCV who underwent color fundus photography (FP), fundus fluorescein angiography (FFA), indocyanine green angiography (ICGA), spectral-domain optical coherence tomography (SD-OCT) at the Zhongshan Ophthalmic Center from June 2016 through May 2018, were reviewed. ASHS-LIA and choroidal vascular hyperpermeability (CVH) were evaluated by ICGA. Subfoveal choroidal thickness (SFTC) was assessed by SD-OCT.

RESULTS. A total of 187 patients were eligible for inclusion in this study (mean, 63.2 ± 7.6 years; range, 41–85 years). Of these patients, 117 (62.6%) showed ASHS-LIA, 57 (30.5%) had bilateral lesions and 70 (37.4%) showed CVH. Moreover, compared with patients without ASHS-LIA, PCV patients with ASHS-LIA were older (P = 0.001), more frequently had bilateral lesions (P = 0.001), and less frequently showed CVH (P = 0.006). SFTC in eyes with ASHS-LIA was significantly greater than that in eyes without ASHS-LIA after adjusting for age, sex, and CVH (P = 0.026). Nevertheless, there was no significant difference in best-corrected visual acuity or lesion characteristics between the two groups.

CONCLUSIONS. ASHS-LIA, which is very common in PCV patients, might be involved in the pathogenesis of PCV. PCV with ASHS-LIA was more frequently associated with bilateral involvement, less CVH, and a thicker choroid than PCV without ASHS-LIA.

Keywords: polypoidal choroidal vasculopathy, age-related scattered hypofluorescent spots, indocyanine green angiography, ASHS-LIA, basal linear deposits, lipid accumulation

AMD is a leading cause of visual loss in the elderly worldwide. Polypoidal choroidal vasculopathy (PCV), a common subtype of neovascular AMD in the Asian populations, is characterized by branching vascular networks (BVNs) with aneurysmal dilations referred to as polyps. Recently, the term aneurysmal type 1 neovascularization (AT1) was introduced to describe lesions with type 1 NV and aneurysmal dilations. Based on histopathology studies, BVNs are located in the sub-RPE basal lamina space, which reveals that PCV is essentially type 1 NV. PCV and typical AMD share many common clinical features, genetic variants, and risk factors, but they also have distinct pathophysiologic processes, natural histories, and treatment outcomes.

Recent studies have indicated that different subtypes of neovascular AMD have different precursor lesions. Eyes with both pigmentary abnormalities and large drusen were deemed more likely to develop typical AMD, whereas pigmentary abnormalities without large drusen were associated with PCV. Choroidal vascular hyperpermeability (CVH) might be associated with the occurrence of PCV as well as the treatment response to intravitreal injections of anti-VEGF; however, inconsistent outcomes were obtained in two studies. Recently, a new kind of drusen-like deposit called pachydrusen was reported in PCV patients. The study of precursor lesions and risk factors in different subtypes of neovascular AMD would help to elucidate the pathogenesis of the disease and improve the visual prognosis of patients. However, thus far, precursor lesions that have been reported in PCV patients mainly include CVH and a thicker choroid, which cannot explain the pathogenesis of PCV well because many PCV patients have a thin choroid.

Our recent study reported age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) and revealed that ASHS-LIA, more frequent in patients with PCV than in patients with typical AMD, might represent lipid accumulation in Bruch’s membrane (BrM) in the form of a basal linear deposit (BLinD). BLinD is a very important early change in AMD and represents a thin layer of soft druse material between the RPE basal lamina and the inner collagenous layer of BrM. Soft drusen is associated with progression to typical neovascular AMD, and subretinal drusenoid deposits (SDD) confer a high risk for developing type 3 neovascularization. We propose that ASHS-LIA (BLinD) might be associated with PCV.

The aims of the current study are to investigate the association between ASHS-LIA and the clinical characteristics...
of PCV and to provide new insights into the pathogenesis of PCV.

**Materials and Methods**

We retrospectively reviewed the medical records and multimodal images of patients who were diagnosed with PCV at the Zhongshan Ophthalmic Center from June 2016 through May 2018. All patients underwent comprehensive ophthalmologic examinations, including best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, binocular ophthalmoscopy, color fundus photography (FP), fundus fluorescein angiography (FFA), indocyanine green angiography (ICGA), and spectral-domain optical coherence tomography (SD-OCT). The study was approved by the institutional review board of the Zhongshan Ophthalmic Center and complied with the tenets of the Declaration of Helsinki. Possible risks associated with invasive examination, including FFA and ICGA, were fully discussed with the patients, and all patients signed written informed consent forms.

PCV was diagnosed based on the ICGA findings showing characteristic polypoidal (aneurysmal) structures at the border of the BVN and the presence of an orange-red retinal protrusion through fundus examination.\(^2\) The inclusion criterion was treatment-naïve PCV in at least one eye. The treatment-naïve PCV eye was designated as the study eye. When a patient had bilateral treatment-naïve PCV, the eye with the newest onset was designated as the study eye. Patients who had a history of ophthalmologic intervention (cataract operation within 3 months, vitrectomy, anti-VEGF injection, and laser coagulation) and patients with poor-quality images due to media opacity were excluded from this study. Patients with a spherical equivalent of -6 diopters (D) or less and patients with systemic diseases such as diabetes mellitus or malignant hypertension were also excluded.

ASHS-LIA was judged according to the following criteria\(^16\): hypofluorescent spots on late-phase ICGA (20–40 minutes after dye injection); spots mainly distributed in the macular region; regulated distribution and could be confluent; and no corresponding abnormalities on other forms of multimodal imaging, including color FP, FAF, FFA and SD-OCT. ASHS-LIA always occurred binocularly and the observation of ASHS-LIA could be interfered by lesions in the macular region; thus, we evaluated the late-phase ICGA images of both eyes to determine whether ASHS-LIA existed. CVH presented as multiple choroidal vascular dilatation in early-phase ICGA and usually hyperfluorescent foci in the middle and late phases of ICGA.\(^22\) However, CVH of a long duration might present with hypofluorescent foci due to the disturbance of the choroidal circulation.\(^22\) Subfoveal choroidal thickness (SFCT) was defined as the distance between the hyper-reflective line representing BrM and the inner surface of the sclera measured using the caliper function of enhanced depth imaging (EDI) OCT.\(^23\)

The angiograms of the contralateral eyes of study eyes were also carefully evaluated to investigate the bilateral involvement of the neovascular membrane. Definite PCV lesions, BVN or fibrous scar formation in the contralateral eyes were considered indicative of bilateral involvement.\(^15\) In addition, we assessed the number of polyps; the size of the largest polyp; the polyp lesion area; the greatest linear dimension (GLD) of PCV lesions, including the BVN and polyps; and the total lesion area (TLA) based on ICGA.

**Figure 1.** Multimodal imaging of unilateral PCV with ASHS-LIA. Left eye of a 61-year-old man diagnosed with PCV. His right eye exhibited no abnormality in (A) color FP, (B) FFA, and (C) early-phase ICGA; (D) yellow arrowheads) ASHS-LIA on late-phase ICGA were distributed in the macular region with partial confluence; (E) SD-OCT (green line in D) shows that the retinal structure was generally normal, and the RPE band was intact and smooth, but the choroid was thick (431 \(\mu\)m). Color FP of his left eye (F) shows hemorrhage, exudation, and edema in the macular region; (G) FFA shows fluorescein leakage and staining; (H) early-phase ICGA shows a BVN (teal arrowheads) and polyps (white arrow); (I) late-phase ICGA shows hyperfluorescence of PCV lesions and surrounding ASHS-LIA (yellow arrowheads); (J) SD-OCT (green line in I) shows an irregular neovascular pigment epithelial detachment (PED; teal arrowhead), subretinal fluid (red arrow) and intraretinal hyperreflective material. The choroid was thick (423 \(\mu\)m).
Demographic information was collected. Multimodal imaging and medical records were reviewed. The BCVA was measured with Snellen charts. Color FP was examined with a fundus camera (Zeiss FF450 Plus; Carl Zeiss, Inc., Jena, Germany). FFA and ICGA were examined with an angiogram (Heidelberg Retina Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany). OCT was examined with an HRAþOCT Spectralis. Two experienced retina physicians (LC, XZZ) diagnosed and assessed the patients independently. The mean of measurements performed by the retina physicians (LC, XZZ) was used for analysis of quantitative data such as SFCT. When discrepancies existed, a third retina specialist (FW) made the final decision.

Statistical analyses were performed using commercial software (SPSS Version 21.0; SPSS, Inc., Chicago, IL, USA). The descriptive statistics of normally distributed variables (means ± standard deviations) and geometric means of nonnormally distributed variables were calculated. Independent sample t-tests (normal data) or $\chi^2$ tests (nonnormal data) were used to compare statistical differences. Differences in SFCT between patients with and without ASHS-LIA were evaluated by multiple logistic regression analyses, with adjustments for age, sex, and CVH. Significant differences were defined as values of $P < 0.05$.

**RESULTS**

As Table 1 shown, a total of 187 patients with treatment-naive PCV in at least one eye were included in this study. The average age was 63.2 ± 7.6 years (range, 41 to 85 years), with 125 men and 62 women. Among these patients, ASHS-LIA was observed in 117 of 187 patients (62.6%), and CVH was detected in 70 of 187 patients (37.4%). A total of 57 patients (30.5%) had bilateral lesions according to our criteria, including 29 patients and 28 women. Among these patients, ASHS-LIA was observed in 117 of 187 patients (62.6%), and CVH was detected in 70 of 187 patients (37.4%). A total of 57 patients (30.5%) had bilateral lesions according to our criteria, including 29 patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
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<tbody>
<tr>
<td>N</td>
<td>187 patients</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.2 ± 7.6 (41–85)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>125 (66.8)</td>
</tr>
<tr>
<td>ASHS-LIA, n (%)</td>
<td>117 (62.6)</td>
</tr>
<tr>
<td>CVH, n (%)</td>
<td>70 (37.4)</td>
</tr>
<tr>
<td>Affected eye, n (%)</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>57 (30.5)</td>
</tr>
<tr>
<td>PCV/PCV</td>
<td>29 (15.5)</td>
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<tr>
<td>PCV/BVN</td>
<td>16 (8.6)</td>
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<tr>
<td>PCV/fibrous scar</td>
<td>12 (6.4)</td>
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<tr>
<td>Unilateral</td>
<td>130 (69.5)</td>
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<tr>
<td>Right eye only</td>
<td>67 (35.8)</td>
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<tr>
<td>Left eye only</td>
<td>63 (33.7)</td>
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<tr>
<td>n</td>
<td>187 eyes</td>
</tr>
<tr>
<td>Lesion location, n (%)</td>
<td></td>
</tr>
<tr>
<td>Macula region</td>
<td>178 (95.2)</td>
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<tr>
<td>Peripapillary</td>
<td>9 (4.8)</td>
</tr>
<tr>
<td>BCVA (logMAR)</td>
<td>0.7 ± 0.3 (0.1–1.6)</td>
</tr>
<tr>
<td>SFCT (µm)</td>
<td>290.4 ± 99.1 (122–562)</td>
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Data represent $n$, means ± SD or %. CVH, choroidal vascular hyperpermeability.
TABLE 2. Demographic and Clinical Characteristics of PCV Patients With and Without ASHS-LIA

<table>
<thead>
<tr>
<th></th>
<th>ASHS-LIA (+)</th>
<th>ASHS-LIA (−)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>117</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Age, y</td>
<td>64.6 ± 7.1</td>
<td>60.9 ± 7.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>84 (71.8)</td>
<td>41 (58.60)</td>
<td>0.063</td>
</tr>
<tr>
<td>Bilateral, n (%)</td>
<td>46 (39.3)</td>
<td>11 (15.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>CVH, %</td>
<td>35 (29.9)</td>
<td>35 (50)</td>
<td>0.006</td>
</tr>
<tr>
<td>SFCT, µm</td>
<td>293.8 ± 100.4</td>
<td>284.7 ± 97.3</td>
<td>0.541 (0.026*)</td>
</tr>
<tr>
<td>BCVA (logMAR)</td>
<td>0.73 ± 0.33</td>
<td>0.68 ± 0.28</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Data represent n, means ± SD or %.

* Adjusted for age, sex and CVH.

TABLE 3. Lesion Characteristics of PCV Patients With and Without ASHS-LIA

<table>
<thead>
<tr>
<th></th>
<th>ASHS-LIA (+)</th>
<th>ASHS-LIA (−)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>117</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Number of polyps, n</td>
<td>2.7 ± 1.9</td>
<td>2.7 ± 1.8</td>
<td>0.76</td>
</tr>
<tr>
<td>Size of the largest polyp, µm</td>
<td>266 ± 161</td>
<td>245 ± 130</td>
<td>0.37</td>
</tr>
<tr>
<td>Polyp lesion area, mm²</td>
<td>0.27 ± 0.38</td>
<td>0.20 ± 0.29</td>
<td>0.18</td>
</tr>
<tr>
<td>Greatest linear dimension, µm</td>
<td>3189 ± 1228</td>
<td>3087 ± 1092</td>
<td>0.57</td>
</tr>
<tr>
<td>Total lesion area, mm²</td>
<td>8.4 ± 6.4</td>
<td>7.4 ± 5.6</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Data represent n or means ± SD.
late-phase ICGA (Figs. 1D, 1I). The right eye was more suitable for the observation of ASHS-LIA because there was no PCV lesion in the macular region. No abnormalities corresponding to ASHS-LIA were detected by color FP, FFA, early-phase ICGA, and SD-OCT (Figs. 1A–C, E). The PCV lesion was located in the macular region of the left eye (Figs. 1F–J), within the bounds of the region with ASHS-LIA (Fig. 1I). SD-OCT showed a thick choroid in both eyes (Figs. 1E, 1J). Figure 2 shows representative multimodal imaging of a patient with bilateral PCV who had ASHS-LIA without CVH. The PCV lesions were located in the macular region of both eyes (Figs. 2A–D, 2F–J), and were situated within the bounds of ASHS-LIA (Figs. 2D, 2I). SD-OCT showed PCV lesions in the macular region and a thick choroid (Figs. 2E, 2J).

Figure 3 shows representative multimodal imaging of a patient with bilateral PCV who had concurrent CVH and ASHS-LIA. PCV lesions were located in the macular region of both eyes (Figs. 3A–D, 3F–J) within the bounds of ASHS-LIA (Figs. 3D, 3I). CVH appeared as focal choroidal vascular dilatation in early-phase ICGA (Figs. 3C, 3H, pink arrowhead) and irregular hyperfluorescence in late-phase ICGA (Figs. 3D, 3I, pink arrowhead). SD-OCT showed PCV lesions and a remarkably thick choroid (Figs. 3E, 3J). Figure 4 shows representative multimodal imaging of a patient with unilateral PCV who had concurrent CVH but no ASHS-LIA. PCV lesions were observed in the macula and peripapillary of the right eye (Figs. 4A–E) but no PCV lesion in the left eye (Figs. 4F–J). CVH was observed in the posterior pole of both eyes (Figs. 4C, 4D, 4H, pink arrowhead), while no ASHS-LIA has been observed in the late phase ICGA of both eyes (Figs. 4D, 4I). SD-OCT revealed PCV lesions in the right eye and a remarkably thick choroid in both eyes (Figs. 4E, 4J).

DISCUSSION
ASHS-LIA were age related, mainly located in the macular region, and manifested as hypofluorescent spots on late-phase ICGA that could be scattered or confluent. No corresponding abnormalities on other multimodal imaging, including color FP, FFA, FAF, and SD-OCT, were observed, and the incidence of ASHS-LIA in PCV patients was significantly higher than that in patients with typical AMD or other retina diseases. In our recent study on ASHS-LIA and drusen, both soft drusen and ASHS-LIA presented as hypofluorescence on late-phase ICGA. ICG dye does not stain soft drusen; thus, soft drusen is displayed as hypofluorescence on late-phase ICGA. Therefore, ICG should not stain BLinD either because the two entities have the same composition. Therefore, with a similar age correlation, distribution, confluency, possible ICGA, and multimodal imaging features with BLinD, we inferred that ASHS-LIA might represent lipid accumulation in BrM in the form of BLinD. In addition, our previous studies indicated that PCV pathogenesis might be involved in the lipid metabolism pathway, which was also reported by other groups. Thus, we speculate that diffuse lipid accumulation in BrM (BLinD) might be associated with the pathogenesis of PCV.

In the current study, we analyzed the association between ASHS-LIA and the clinical characteristics of PCV. Our findings revealed that 62.6% of PCV patients had concurrent ASHS-LIA, and PCV patients with ASHS-LIA were older than those without ASHS-LIA, more frequently showed bilateral involvement and less frequently showed CVH. In addition, PCV eyes with ASHS-LIA showed greater SFCT than did those without ASHS-LIA after adjusting for age, gender and CVH. Nonetheless, there was no
significant association between ASHS-LIA and lesion characteristics of PCV.

In the present study, 62.6% of PCV patients had concurrent ASHS-LIA, which was higher than the percentage in our previous study,\textsuperscript{16} because we included both unilateral and bilateral PCV patients in this study. Moreover, in this study, 30.5% of PCV patients had bilateral lesions, which is comparable to a study from another research group.\textsuperscript{13} PCV patients with ASHS-LIA showed significantly more bilateral involvement. Therefore, ASHS-LIA might be an important precursor lesion of PCV and might confer a risk of bilateral involvement. Furthermore, 37.4% of PCV patients showed CVH in this study, which was also comparable to other studies.\textsuperscript{13,51} PCV patients with ASHS-LIA showed significantly less CVH, suggesting that compared with CVH, ASHS-LIA might represent a different pathway in PCV pathogenesis. In addition, the mean SFCT in PCV eyes with ASHS-LIA was significantly higher than that in those without ASHS-LIA after adjustment for age, sex and CVH, which means that ASHS-LIA might be associated with a thickened choroid in PCV patients without CVH.

Recent research reported risk factors or precursor lesions related to PCV, including CVH, a thicker choroid, pachydrusen and pigmentary abnormalities.\textsuperscript{10–12,15,31,52} PCV was considered to be within the pachychoroid spectrum of conditions because of the thicker choroid.\textsuperscript{53–54} but there is controversy due to the racial difference and pronounced interindividual variability in choroidal thickness in PCV patients.\textsuperscript{3,5} In addition, recent studies classified PCV as different subtypes and suggested that different subtypes of PCV might have a different pathogenesis, treatment, and prognosis.\textsuperscript{55–57} Numerous genetic studies have been conducted on PCV patients,\textsuperscript{28,38–41} and a recent meta-analysis revealed that PCV was associated with genetic variants of major pathways implicated in lipid, inflammation, and complement cascades.\textsuperscript{50} Nevertheless, the specific pathogenesis of PCV remains unclear; pachychoroid may not be underlying the PCV pathogenesis but just a coexisting manifestation of this disease. Our study might provide insights into the pathogenesis of PCV and indicated that the proposed histologic correlate of ASHS-LIA (BLinD) might be associated with PCV and confer a risk of bilateral involvement.

Pathology studies have demonstrated that lipid deposition in BrM increases with age and gradually forms a “lipid wall” between the RPE basal lamina and the inner collagenous layer (ICL) of BrM,\textsuperscript{62–64} which is attributed to the constitutive and physiologic secretion of apolipoprotein B- and E-containing lipoprotein particles by the RPE and the slowing of their exit to the circulation by aging of BrM and choriocapillaris.\textsuperscript{15} With degradation of the apolipoprotein component, individual particles are thought to fuse and form BLinD.\textsuperscript{46,47} Soft drusen and BLinD are focal and diffuse deposits, respectively, of the same lipoprotein-derived debris.\textsuperscript{20,47} The association between soft drusen and typical AMD has been established, but soft drusen has not been associated with PCV.\textsuperscript{11} It has been speculated that Asian populations prone to neovascularization without much drusen might have BLinD that escaped clinical detection.\textsuperscript{45} Whether PCV is a subtype of AMD remains controversial.\textsuperscript{4,48} Our findings might contribute to clarifying the association between PCV and typical AMD and reveal the role of BLinD in PCV pathogenesis. In addition, more lipids accumulate in BrM in the macular region than in the periphery,\textsuperscript{42,49} which can explain why most PCV lesions are located in the macular region. Additionally, PCV patients with ASHS-LIA were older than those without ASHS-LIA, which indicated that ASHS-LIA might be an important factor of PCV development in older people.
Figure 5 shows our proposal regarding the development of PCV in the presence of BLMd (ASHS-LIA). Under normal circumstances, nutrients and oxygen can freely pass from the choroidal circulation through the BrM to reach the RPE and photoreceptors. Thus, the retina is healthy (Fig. 5A). With age, BLMd forms between the RPE basal lamina and the ICL of BrM (Fig. 5B). Neutral lipid accumulation in BrM impedes the transport of nutrients across BrM. Therefore, the outer retina is in a state of hypoxia. To provide necessary nutrients and oxygen for the RPE and photoreceptors, together with some growth factors produced under hypoxia, choroidal vessels are in a state of hypoxia. To provide necessary nutrients and oxygen for the RPE and photoreceptors, together with some growth factors produced under hypoxia, choroidal vessels grow, break though BrM and dilate locally (Fig. 5C). These immature blood vessels continue to grow between the RPE and BrM, which might cause retinal edema, hemorrhage, and exudation. Our findings can explain why BNVs and aneurysms are always observed in the sub-RPE-basal laminar space.

This study has limitations. First, selection bias may have occurred because this study had a clinic-based retrospective design. Second, the incidence of ASHS-LIA in bilateral PCV patients might be underestimated because PCV lesions might block visualization of ASHS-LIA. Third, without longitudinal observation, the relationship between ASHS-LIA and PCV is correlative and not causal. Therefore, prospective studies with a large sample size are needed. Finally, we still have no direct histopathologic evidence that ASHS-LIA represents lipid in BrM. Despite these limitations, our study provides new insights into the pathogenesis of PCV and might contribute to the prevention and treatment of PCV.

In conclusion, ASHS-LIA, which occurs very frequently in PCV patients, might be involved in the pathogenesis of PCV. The patients with PCV associated with ASHS-LIA more frequently demonstrated bilateral involvement, less CVH and a thickened choroid than did those without ASHS-LIA.

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References


