Association Between Hyperreflective Dots on Spectral-Domain Optical Coherence Tomography in Macular Edema and Response to Treatment

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PURPOSE. To investigate the association between hyperreflective dots (HRDs) on spectral-domain optical coherence tomography (SD-OCT) and response to intravitreal bevacizumab (IVB) or dexamethasone injection in eyes with diabetic macular edema (DME) or macular edema due to retinal vein occlusion (RVO).

METHODS. A retrospective review was conducted involving patients with DME or macular edema due to RVO. Patients with treatment-naïve macular edema were initially treated with three consecutive IVB injections and classified based on the treatment response to bevacizumab. After three consecutive IVB injections, bevacizumab nonresponders were treated using dexamethasone implants and classified based on the treatment response. The best-corrected visual acuity, number of HRDs, and outer plexiform layer (OPL) disruptions were analyzed according to the treatment response.

RESULTS. Eighty-two eyes with DME and 68 eyes with RVO were included in this study. Thirty-six (43.9%) eyes with DME and 22 (32.4%) eyes with RVO were bevacizumab nonresponders. The number of baseline HRDs in bevacizumab nonresponders (16.06 ± 6.60 in DME, 14.23 ± 4.09 in RVO) was significantly greater than that in responders (11.26 ± 3.64, P < 0.001 in DME, 11.17 ± 4.83, P = 0.015 in RVO), and it did not decrease after IVB injections. Unlike the response to bevacizumab, eyes that responded to dexamethasone implant but not to IVB had significantly more HRDs (19.56 ± 6.75) than eyes that did not respond (11.50 ± 3.78, P = 0.006). The OPL disruption rate was significantly higher in bevacizumab nonresponders than in responders (P < 0.001 in DME and P = 0.001 in RVO).

CONCLUSIONS. In patients with DME or macular edema due to RVO, the number of HRDs on SD-OCT may be a predictive indicator of the response to IVB injection or dexamethasone implant. In bevacizumab responders, the number of HRDs on SD-OCT was small. In contrast, more HRDs, which might reflect increased inflammation in the retina, were observed in dexamethasone responders. Therefore, dexamethasone implants might be more effective in DME or RVO eyes with multiple HRDs and OPL disruption on SD-OCT.

Keywords: hyperreflective dot, macular edema, diabetic retinopathy, retinal vein occlusion, dexamethasone implant
Hyperreflective Dots and Their Response to Bevacizumab in ME secondary to DR and RVO. We also analyzed the association response to intravitreal bevacizumab (IVB) injections in ME patients with ME. Therefore, in our present study, we aimed to

**METHODS**

A retrospective review was conducted involving patients who received IVB injection for DME and ME due to RVO at the Chungbuk National University Hospital, Cheongju, Korea, between November 2013 and August 2016. The primary objective of this study was to analyze the differences in number of HRDs on SD-OCT according to treatment response to IVB injection or dexamethasone implant. The secondary objectives were to analyze the differences in the outer plexiform layer (OPL) disruptions and the ellipsoid zone (EZ) and external limiting membrane (ELM) defects on SD-OCT, according to the treatment response to IVB or dexamethasone implant. This study was approved by the institutional review board of Chungbuk National University Hospital and followed the tenets of the Declaration of Helsinki.

The inclusion criteria were patients with treatment-naive DME and ME due to RVO, and patients with ME that was initially treated with IVB. The exclusion criteria were patients with high myopia (>8 diopters), glaucoma, media opacities due to cataract or corneal disease, vitreous hemorrhage, combined retinal disease, and those whose SD-OCT images were of poor quality. At the initial visit, all patients underwent a comprehensive ophthalmic examination, including best-corrected visual acuity (BCVA) using the Snellen chart, IOP measurement, slit-lamp examination, color fundus photography, fluorescein angiography, and SD-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany). During each visit, ophthalmic examinations, including the assessment of BCVA, applanation tonometry, slit-lamp examination, dilated fundus examination, fundus photography, and SD-OCT were performed.

**Treatment of ME**

All patients with ME were treated with three monthly consecutive IVB injections. Subsequently, the eyes were divided based on the treatment response to IVB. A patient was classified as a bevacizumab responder if his or her central subfield thickness (CST) became less than 300 μm or reduced by more than 50 μm after three consecutive IVB injections. After three consecutive bevacizumab injections, those with a poor response to IVB were treated with dexamethasone implants (Ozurdex; Allergan, Inc., Irvine, CA, USA). One month after dexamethasone implants, the bevacizumab nonresponders were subsequently reclassified according to the treatment response to the dexamethasone implant.

**OCT Examination and Interpretation**

For SD-OCT images, a Spectralis OCT (Heidelberg Engineering) was used and a custom 20° × 20° volume acquisition protocol, which consisted of 49 sections, was used. The integrated follow-up mode of the device was used to acquire scans of the same retinal areas at each visit. The SD-OCT images obtained before and 1 month after each treatment (bevacizumab or dexamethasone implant) were used for interpretation. The CST was automatically calculated as the average retinal thickness within the central circle of a 500-μm radius. We also analyzed the OPL disruptions, EZ defects, and ELM defects within a radius of 1500 μm centered on the fovea.

**Hyperreflective Dots on SD-OCT**

HRDs were defined as discrete and well-circumscribed particles 20 to 40 μm in diameter, and of equal or higher reflectivity than the RPE band on SD-OCT. The number of HRDs within an area of 1500-μm radius centered on the fovea on the fovea-spanning horizontal raster scan was manually counted by a masked retina specialist (J.B. Chae and J.Y. Kim) using the ImageJ software (http://image.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The HRDs were subdivided according to the retinal layers: inner retina (from internal limiting membrane to outer nuclear layer), outer retina (from ELM to RPE), and subretinal fluid (SRF) (Fig. 1).
responders, but no improvement in the BCVA and CST was noted in bevacizumab nonresponders. Figures 2 and 3 show representative cases of bevacizumab responders and nonresponders, respectively.

Of the 68 eyes with ME due to RVO, 46 eyes (67.65%) were classified as bevacizumab responders and 22 eyes (32.35%) as bevacizumab nonresponders. As in the eyes with DME, the BCVA, CST, EZ disruption, and ELM disruption at baseline did not significantly differ between the bevacizumab responders and nonresponders. However, the number of total HRDs, outer retinal HRDs, and OPL disruptions were significantly higher in bevacizumab nonresponders. As was the result in eyes with DME, there was a decrease in the number of HRDs after bevacizumab injection in bevacizumab responders; however, there was no decrease in eyes with bevacizumab nonresponders (Table 3; Fig. 1).

Among the patients with DME, 24 eyes (66.67%) of 36 bevacizumab nonresponders received intravitreal dexamethasone implants. Eighteen eyes (75%) had a good response and six eyes (25%) had a poor response to intravitreal dexamethasone implant. The number of bevacizumab injections, logMAR BCVA, CST, EZ disruption, and ELM disruption at baseline did not significantly differ between the intravitreal dexamethasone responders and nonresponders. In contrast to the response to bevacizumab, the number of total HRDs, inner retinal HRDs, and outer retinal HRDs were significantly higher in intravitreal dexamethasone responders. Even in eyes with OPL disruption, dexamethasone injections showed a good therapeutic response (Table 4; Fig. 4).

To evaluate the predictive accuracy of the number of HRDs and OPL disruptions regarding the response to bevacizumab, ROC curve analysis was performed (Fig. 5). In eyes with DME, the areas under the ROC curve (AUROC) for the number of HRDs and OPL disruptions were 0.687 (0.572–0.801; \( P = 0.004 \)) and 0.715 (0.597–0.833; \( P = 0.001 \)), respectively. When both parameters were used simultaneously, the AUROC increased to 0.796 (0.699–0.893; \( P < 0.001 \)). In eyes with ME due to RVO, the AUROC for the number of HRDs and OPL disruptions were 0.688 (0.556–0.820; \( P = 0.013 \)) and 0.713 (0.585–0.842; \( P = 0.005 \)), respectively. When both parameters were used simultaneously, the AUROC increased to 0.778 (0.659–0.896; \( P < 0.001 \)).
We analyzed the effect of ME duration on SD-OCT findings such as OPL disruption and the HRD numbers. The duration of ME was indirectly confirmed through symptom duration. We found that symptom duration was not different according to the OPL disruption. In DME patients, symptom duration with or without OPL disruption was 2.74 ± 1.83 or 3.00 ± 2.00, respectively (P = 0.573). In RVO patients, symptom duration with or without OPL disruption was 1.58 ± 1.78 or 1.28 ± 1.59, respectively (P = 0.465). Pearson correlation analysis was also performed to evaluate the relationship between symptom

TABLE 2. HRDs and OCT Findings at Baseline and Final Visit in DME Patients

<table>
<thead>
<tr>
<th></th>
<th>Bevacizumab Responder, n = 46, 56.10%</th>
<th>Bevacizumab Nonresponder, n = 36, 43.90%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After Bevacizumab</td>
</tr>
<tr>
<td>Age, y</td>
<td>53.61 ± 12.11</td>
<td>56.56 ± 8.90</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>26/20</td>
<td>19/17</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>14/32 (30.43)</td>
<td>14/22 (38.89)</td>
</tr>
<tr>
<td>Renal disease (%)</td>
<td>12/34 (26.09)</td>
<td>4/32 (11.11)</td>
</tr>
<tr>
<td>Symptom duration, mo</td>
<td>2.63 ± 1.76</td>
<td>3.01 ± 2.01</td>
</tr>
<tr>
<td>logMAR BCVA</td>
<td>0.56 ± 0.33</td>
<td>0.57 ± 0.30</td>
</tr>
<tr>
<td>No. of HRDs</td>
<td></td>
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<tr>
<td>Total</td>
<td>11.26 ± 3.64</td>
<td>16.06 ± 6.60</td>
</tr>
<tr>
<td>Inner</td>
<td>7.46 ± 2.74</td>
<td>11.50 ± 4.95</td>
</tr>
<tr>
<td>Outer</td>
<td>3.59 ± 1.67</td>
<td>4.42 ± 2.13</td>
</tr>
<tr>
<td>Subretina</td>
<td>0.22 ± 0.66</td>
<td>0.14 ± 0.59</td>
</tr>
<tr>
<td>CST, μm</td>
<td>484.22 ± 131.17</td>
<td>506.89 ± 135.51</td>
</tr>
<tr>
<td>EZ disruption (%)</td>
<td>19/46 (41.30)</td>
<td>24/46 (66.67)</td>
</tr>
<tr>
<td>EZ disruption length, μm</td>
<td>113.15 ± 263.94</td>
<td>216.06 ± 376.54</td>
</tr>
<tr>
<td>ELM disruption (%)</td>
<td>15/46 (32.61)</td>
<td>15/46 (32.61)</td>
</tr>
<tr>
<td>ELM disruption length, μm</td>
<td>121.20 ± 280.64</td>
<td>208.78 ± 422.64</td>
</tr>
<tr>
<td>OPL disruption (%)</td>
<td>25/46 (54.55)</td>
<td>35/46 (91.67)</td>
</tr>
</tbody>
</table>

* Determined using the independent t-test.
† Determined using the Pearson χ² test.
‡ Determined using the paired t-test.
§ Determined using the McNemar test.

We analyzed the effect of ME duration on SD-OCT findings such as OPL disruption and the HRD numbers. The duration of ME was indirectly confirmed through symptom duration. We found that symptom duration was not different according to the OPL disruption. In DME patients, symptom duration with or without OPL disruption was 2.74 ± 1.83 or 3.00 ± 2.00, respectively (P = 0.573). In RVO patients, symptom duration with or without OPL disruption was 1.58 ± 1.78 or 1.28 ± 1.59, respectively (P = 0.465). Pearson correlation analysis was also performed to evaluate the relationship between symptom

FIGURE 2. Representative case of DME (bevacizumab responder). Baseline SD-OCT image showing a relatively small number of HRDs and intact OPL (A). After IVB injection (B), number of HRDs are fewer and the ME has improved.
duration and the number of HRDs on SD-OCT; however, we found that there was no significant correlation between symptom duration and HRDs (DME, Pearson correlation coefficient; -0.122, \(P = 0.275\)/RVO, Pearson correlation coefficient; 0.223, \(P = 0.067\)).

**DISCUSSION**

In this study, we investigated the association between the number of HRDs on SD-OCT and their response to IVB or dexamethasone injection in eyes with DME or ME due to RVO. The primary finding of this study was that the number of baseline HRDs in bevacizumab nonresponders (16.06 ± 6.60 in DME, 14.23 ± 4.09 in RVO) was significantly more than that in responders (\(P < 0.001\) in DME, \(P = 0.013\) in RVO). Moreover, unlike the response to bevacizumab, eyes that responded to dexamethasone implants but did not respond to bevacizumab had significantly more number of HRDs (20.78 ± 3.34) than the eyes that did not respond to dexamethasone implants (14.00 ± 3.85, \(P = 0.001\)).

Chatziralli et al.\textsuperscript{28} reported that hyperreflective foci are associated with poorer visual outcome in patients with ME due to retinal vascular diseases. We conducted the generalized least squares linear regression analysis, examining the factors associated with visual acuity (logMAR BCVA) (Supplementary Table S1). We also found that hyperreflective foci are associated with poor visual prognosis in DME and RVO patients. The difference between the current study and the research by Chatziralli et al.\textsuperscript{28} is that this current study evaluated the correlation between the number of HRDs and the therapeutic responsiveness of bevacizumab or dexamethasone implant. We found therapeutic responsiveness of certain treatments might be different according to the numbers of...
HRDs. We thought that this different therapeutic responsiveness is due to the origin of HRDs on SD-OCT.

The origin of the HRDs remains unclear. Bolz et al.29 reported that HRDs are the morphologic manifestations of lipid extravasation in DME. Well-demarcated, hyperreflective foci tend to be located within the walls of intraretinal microaneurysms and scattered throughout all retinal layers, forming confluent plaques in the OPL.29 Comparison with histologic studies suggests that these foci may represent subclinical extravasations of lipoproteins and/or proteins being a byproduct of lipoprotein extravasation after breakdown of the inner blood-retina barrier. Therefore, they concluded that the foci represent extravasated lipoproteins and/or proteins being a very early subclinical barrier breakdown sign in DME.29 In contrast, several other studies have stated that HRDs are associated with inflammatory responses in the retina.30–35 As the retinal inflammation increases, microglial cells are transformed into an activated state, increasing in number and translocating through the retina.35 When microglial cells are activated, their morphologies change and they aggregate.35 Vujosevic et al.36 reported that in the early stage of DR, HRDs are mainly located in the inner retina, where the resident microglia are present. With progressive retinopathy, HRDs reach the outer retinal layer. HRDs may represent a surrogate of microglial activation in diabetic retina. These activated and aggregated microglia cells appear as HRDs on SD-OCT.34 Therefore, because aggregated microglia reflect increased inflammation in the retina and HRDs show aggregated microglia, an increased number of HRDs in SD-OCT may indicate an activated inflammatory process in the retina.

The cause of ME is multifactorial, complex, and incompletely understood. Over the past few years, various treatments have been advocated in relation to the pathogenesis of the ME. Especially, increased VEGF expression is an important causative factor for ME; hence, anti-VEGF agents, such as bevacizumab, ranibizumab, and aflibercept have been widely used for the treatment of ME secondary to RVO or DR.8–13 Along with an increased VEGF expression, the inflammatory pathway also indicates an activated inflammatory process in the retina.30–35 As contrast, several other studies have suggested that HRDs are formed into an activated state, increasing in number and the retinal inflammation increases, microglial cells are trans-activated, their morphologies change and they aggregate.33 In our study, in both DME and ME due to RVO with a poor response to IVB, the number of HRDs was higher than in those with a good response to IVB. Eyes that responded poorly to IVB were treated with dexamethasone implants; 75% of such eyes showed a good response. In these dexamethasone responders, the number of HRDs was higher than in poor responders to dexamethasone. Regarding the point of view that HRDs on SD-OCT might represent inflammation in the retina, these findings suggest that in eyes with several HRDs, the inflammatory pathway might contribute more to the pathogenesis of ME than the VEGF pathway. Therefore, in a case of ME with many HRDs, we may infer in advance that anti-inflammatory drugs, such as dexamethasone implants, might be more effective than intravitreal anti-VEGF injections.
<table>
<thead>
<tr>
<th></th>
<th>Dexamethasone Responder, ( n = 18 ), 75.00%</th>
<th></th>
<th>Dexamethasone Nonresponder, ( n = 6 ), 25.00%</th>
<th></th>
<th>( P ) Before Dexamethasone</th>
<th>( P ) After Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>( 55.44 \pm 7.62 )</td>
<td>( 62.47 \pm 8.80 )</td>
<td>( 6.057^\ast )</td>
<td>( 1.000^\dagger )</td>
<td></td>
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<tr>
<td>Sex, male/female</td>
<td>( 10/8 )</td>
<td>( 6/2 )</td>
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<td>Hypertension (%)</td>
<td>( 6/12 ) (33.33)</td>
<td>( 3/3 ) (50.00)</td>
<td>( 0.035^\dagger )</td>
<td>( 0.005^\ast )</td>
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<tr>
<td>Renal disease (%)</td>
<td>( 2/16 ) (11.11)</td>
<td>( 1/5 ) (16.67)</td>
<td>( 1.000^\dagger )</td>
<td>( 0.005^\ast )</td>
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<tr>
<td>Symptom duration, mo</td>
<td>( 2.83 \pm 1.79 )</td>
<td>( 4.00 \pm 2.68 )</td>
<td>( 0.454^\ast )</td>
<td>( 0.454^\ast )</td>
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<tr>
<td>No. of bevacizumab injections</td>
<td>( 4.17 \pm 1.67 )</td>
<td>( 3.83 \pm 2.04 )</td>
<td>( 0.378^\ast )</td>
<td>( 0.378^\ast )</td>
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<tr>
<td>LogMAR BCVA</td>
<td>( 0.65 \pm 0.37 )</td>
<td>( 0.99 \pm 0.65 )</td>
<td>( 0.088^\dagger )</td>
<td>( 0.280^\ast )</td>
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<tr>
<td>No. of HRDs</td>
<td></td>
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<tr>
<td>Total</td>
<td>( 20.78 \pm 3.34 )</td>
<td>( 14.78 \pm 3.92 )</td>
<td>( &lt;0.001^\dagger )</td>
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<tr>
<td>Inner</td>
<td>( 15.33 \pm 2.47 )</td>
<td>( 10.72 \pm 3.34 )</td>
<td>( &lt;0.001^\dagger )</td>
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<tr>
<td>Outer</td>
<td>( 5.38 \pm 1.97 )</td>
<td>( 4.06 \pm 1.92 )</td>
<td>( &lt;0.001^\dagger )</td>
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<tr>
<td>Subretina</td>
<td>( 0.06 \pm 0.23 )</td>
<td>( 0.00 )</td>
<td>( 0.35^\dagger )</td>
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<td></td>
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<tr>
<td>CST, ( \mu ) nm</td>
<td>( 559.06 \pm 95.77 )</td>
<td>( 301.22 \pm 59.16 )</td>
<td>( &lt;0.001^\dagger )</td>
<td></td>
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<tr>
<td>EZ disruption (%)</td>
<td>( 12/18 ) (66.67)</td>
<td>( 11/18 ) (61.11)</td>
<td>( 1.000^\dagger )</td>
<td>( 1.000^\dagger )</td>
<td></td>
<td></td>
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<tr>
<td>EZ disruption length, ( \mu ) m</td>
<td>( 307.17 \pm 532.33 )</td>
<td>( 283.44 \pm 415.12 )</td>
<td>( 0.61^\dagger )</td>
<td>( 0.494^\ast )</td>
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<tr>
<td>ELM disruption (%)</td>
<td>( 11/18 ) (61.11)</td>
<td>( 11/18 ) (61.11)</td>
<td>( 1.000^\dagger )</td>
<td>( 0.494^\ast )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELM disruption length, ( \mu ) m</td>
<td>( 356.56 \pm 459.66 )</td>
<td>( 326.76 \pm 467.17 )</td>
<td>( 0.696^\dagger )</td>
<td>( 0.378^\ast )</td>
<td></td>
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</tr>
<tr>
<td>Opt. disruption (%)</td>
<td>( 17/18 ) (94.44)</td>
<td>( 16/18 ) (88.89)</td>
<td>( 1.000^\dagger )</td>
<td>( 1.000^\dagger )</td>
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</table>

* Determined using the Mann-Whitney U test.
† Determined using the paired t-test.
‡ Determined using the Fisher's exact test.
§ Determined using the McNemar test.
Lee et al. reported that OPL disruption in SD-OCT corresponded well with the extent of deep capillary plexus loss in eyes with DME and might be useful predictors of response to anti-VEGF treatment. Moreover, eyes with OPL disruption and deep capillary plexus loss did not respond to the anti-VEGF treatment but responded well to the dexamethasone implant. Similarly, in our study, the rate of OPL disruption was higher in eyes with a poor response to IVB.

To evaluate the predictive accuracy of the number of HRDs and OPL disruptions regarding the response to bevacizumab, the ROC curve analysis was performed. We found that the AUROC for the number of HRDs and OPL disruptions were 0.687 and 0.715 in DME, and 0.688 and 0.713 in ME due to RVO, respectively. When both parameters were used simultaneously, the AUROC increased to 0.796 in DME, and 0.778 in ME due to RVO. Based on these findings, along with HRDs on SD-OCT, OPL disruption may also be used as a predictive factor regarding the treatment response to bevacizumab.

The strength of the current study is that it provides new insights into the association between HRDs on SD-OCT and its response to IVB and dexamethasone implant in eyes with DME or ME due to RVO. However, our present study also had some notable limitations that were inherent to its retrospective design. Compared with the number of bevacizumab-treated eyes, the number of dexamethasone-treated eyes was relatively small. Moreover, we counted the number of HRDs manually. Although two masked retinal specialists performed the counting, this method inevitably resulted in counting errors. Therefore, further large-scale prospective studies with an automatic quantification system for HRDs will be needed.

In conclusion, in patients with DME or ME due to RVO, the number of HRDs on SD-OCT can be a predictive indicator of the treatment response to IVB injection or dexamethasone implant. A higher number of HRDs and higher rate of OPL disruptions was observed on SD-OCT in bevacizumab nonresponders than in responders. In contrast, more HRDs were observed in dexamethasone responders than in nonresponders, and OPL disruptions did not affect the response to dexamethasone. Therefore, dexamethasone implant may be more effective in treating DME or RVO eyes with many HRDs and OPL disruptions on SD-OCT.
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References


