Diabetic retinopathy (DR) is the most common retinal vascular disease and the most common cause of moderate and severe vision loss in individuals of working ages. Retinal vein occlusion (RVO) is the second-most common type of retinal vascular disease. Macular edema (ME) secondary to DR and RVO is considered the leading cause of central vision loss in the developed world, and is therefore of enormous medical and socioeconomic importance. Various treatments, such as intravitreal anti-VEGF injections and intravitreal dexamethasone injections, have shown good visual and anatomic outcomes. However, some ME cases show suboptimal response to these treatment modalities.

If we could know in advance the treatment response to a specific therapeutic option, this would not only reduce unnecessary treatment, but also improve the visual outcome. Determination of the morphologic and topographic characteristics of ME may help in elucidating the pathophysiology of the disease, which in turn helps predict the treatment response. The morphologic features of ME on spectral-domain optical coherence tomography (SD-OCT) might be classified into three major types: diffuse retinal thickening (DRT), cystoid ME (CME), and serous retinal detachment. Seo et al. reported that DRT showed a particularly good response to ranibizumab with fewer injections. In contrast, Wu et al. and Roh et al. reported that patients with CME showed a greater improvement in visual acuity and macular thickness after bevacizumab injections than those with other types of diabetic ME (DME). Therefore, the SD-OCT findings of ME that predict the treatment response are still unclear.

The hyperreflective dots (HRDs) on SD-OCT are well-circumscribed particles that are 20 to 40 μm in diameter, and are of equal or higher reflectivity than the RPE band.
Hyperreflective Dots and Their Response to Bevacizumab in ME

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-naïve 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 diopeters), 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.23,24 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. Cha 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).

Statistical Analysis
The SPSS version 22.0 software (SPSS, Inc., Chicago, IL, USA) was used to perform the statistical analyses and a P < 0.05 was considered statistically significant. Differences in the parameters, including the number of HRDs and BCVA, between the baseline and each visit were evaluated using the paired t-test. The independent t-test was used to analyze the follow-up period, number of IVB injections, number of HRDs, and CST, according to the treatment response to IVB or dexamethasone implant. The Pearson χ² test was used to analyze the differences in OPL disruptions and EZ and ELM defects between IVB or dexamethasone implant responders and nonresponders. To evaluate repeatability of hyperreflective foci counting by two different observers, the intraclass correlation coefficient (ICC) analysis was performed. To determine the accuracy of prediction of the number of HRDs and OPL disruption regarding the response to IVB, receiver operation characteristic (ROC) curve analysis was performed.

RESULTS
A total of 82 eyes of 65 patients with DME and 68 eyes of 68 patients with ME due to RVO were included in this study. There were no significant differences in the age, sex, refractive error, and follow-up duration in the DME and RVO groups, but the baseline CST was more in the RVO group (Table 1) and the ICC of two HRDs (counting by J.B. Cha and J.Y. Kim) was 0.935.

Of the 83 eyes with DME, 46 eyes (56.10%) were classified as bevacizumab responders and 36 eyes (43.90%) as bevacizumab nonresponders. Symptom duration, presence of hypertension, and accompanying kidney disease in both groups were not significantly different. The baseline BCVA, CST, EZ disruption, and ELM disruption did not significantly differ between bevacizumab responders and nonresponders. However, the number of total HRDs, inner retinal HRDs, and OPL disruption at baseline were significantly higher in bevacizumab nonresponders. Moreover, there was a decrease in the number of total HRDs and inner retinal HRDs after IVB injection in bevacizumab responders; however, no decrease in the number of HRDs after treatment was observed in bevacizumab nonresponders (Table 2; Fig. 1). After IVB injection, there was an improvement in the BCVA and CST in bevacizumab responders; however, no decrease in the number of total HRDs, inner retinal HRDs, and OPL disruption was observed in bevacizumab nonresponders (Table 2; 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 is observed in bevacizumab nonresponders.

In eyes 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 0.778 (0.659–0.896; \( P < 0.001 \)).

### Table 1. Basic Characteristics of the Patients in This Study

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients (eyes)</th>
<th>Age, y</th>
<th>Sex, male/female</th>
<th>Right/Left</th>
<th>Refractive error, SE</th>
<th>Baseline CST, µm</th>
<th>Follow-up duration, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>DME Group</td>
<td>63 (82)</td>
<td>55.13 ± 11.16</td>
<td>35/28</td>
<td>45/37</td>
<td>−0.69 ± 1.35</td>
<td>494.17 ± 132.75</td>
<td>6.04 ± 4.72</td>
</tr>
<tr>
<td>RVO Group</td>
<td>68 (68)</td>
<td>59.49 ± 13.40</td>
<td>34/34</td>
<td>38/30</td>
<td>−0.20 ± 1.68</td>
<td>551.06 ± 156.84</td>
<td>5.34 ± 4.75</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>0.052*</td>
<td>0.525†</td>
<td>0.052*</td>
<td>0.017**</td>
<td>0.370*</td>
<td></td>
</tr>
</tbody>
</table>

SE, spherical equivalent.
* Determined using the independent t-test.
† Determined using the Pearson \( \chi^2 \) 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 duration and HRD numbers.
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 \((P < 0.001 \text{ in DME, } P = 0.013 \text{ in RVO})\). Moreover, unlike the response to bevacizumab, eyes that responded to dexamethasone implants 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.\(^{28}\) 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.\(^{28}\) 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 features of lipoprotein extravasation after breakdown of the inner blood-retina barrier. Therefore, they concluded 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 formed 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 progressing 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 plays an important role in the pathogenesis of ME.35-39 Funatsu et al.40 reported that increased inflammatory cytokine levels in the vitreous were related to the changes in the retinal vascular permeability and severity of DME. The intravitreal concentrations of proinflammatory cytokines have been found to be higher in patients with DME than in controls.41 Therefore, inhibiting inflammatory pathway mediators could be one of the therapeutic options for the ME and the use of intravitreal steroid therapy, including the dexamethasone implant, has been increasingly used for the treatment of ME in DR and RVO patients.5-7,42,43

In a patient with ME, a certain mechanism (VEGF pathway or inflammatory pathway) might contribute more to the development of ME. This eventually affects the treatment response to a certain treatment modality. If we can know in advance the pathogenic mechanism that contributes more to the development of ME, we can use a specific treatment modality for targeting the pathogenic mechanism. Such targeted treatment not only improves the visual outcome, but also reduces ineffective treatment. However, unfortunately, we do not know in advance the exact pathogenic mechanism (VEGF versus inflammation) contributing to the development of ME.

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 responders 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 inflammation mainly contributed to the development of ME and that anti-inflammatory drugs, such as dexamethasone implants, might be more effective than intravitreal anti-VEGF injections.
|                          | Dexamethasone Responder, n = 18, 75.00% |      | Dexamethasone Nonresponder, n = 6, 25.00% |      | P Before Dexamethasone | P After Dexamethasone |
|--------------------------|----------------------------------------|--|--|------------------------------------------|--|--|---|--|
| Age, y                   | 55.44 ± 7.62                           |       | 62.47 ± 8.80                           |       | 0.057*                          |       |
| Sex, male/female         | 10/8                                   |       | 6/2                                     |       | 1.000†                          |       |
| Hypertension (%)         | 6/12 (33.33)                           |       | 3/3 (50.00)                             |       | 0.655†                          |       |
| Renal disease (%)        | 2/16 (11.11)                           |       | 1/5 (16.67)                             |       | 1.000†                          |       |
| Symptom duration, mo     | 2.83 ± 1.79                            |       | 4.00 ± 2.68                             |       | 0.454*                          |       |
| No. of bevacizumab injections | 4.17 ± 1.67                         |       | 3.83 ± 2.04                             |       | 0.378*                          |       |
| LogMAR BCVA              | 0.65 ± 0.37                            | 0.51 ± 0.22 | 0.046†                      |       | 0.99 ± 0.65 | 0.65 ± 0.39 | 0.088†                      |       | 0.280*                          | 0.454*                          |
| No. of HRDs              |                                       |       |                                         |       |                                |       |                                |       |                                |       |
| Total                    | 20.78 ± 3.34                           | 14.78 ± 3.92 | <0.001†                       |       | 14.00 ± 3.85 | 13.00 ± 4.38 | 0.530†                       |       | 0.001‡                          | 0.378*                          |
| Inner                    | 15.33 ± 2.47                           | 10.72 ± 3.34 | <0.001†                       |       | 10.00 ± 3.52 | 8.67 ± 4.18 | 0.408†                       |       | 0.003‡                          | 0.310†                          |
| Outer                    | 5.38 ± 1.97                            | 4.06 ± 1.92 | <0.001†                       |       | 4.00 ± 1.55 | 4.17 ± 2.14 | 0.741†                       |       | 0.104‡                          | 0.974†                          |
| Subretina                | 0.06 ± 0.25                            | 0.00 | 0.35†                       |       | 0.00 | 0.17 ± 0.41 | 0.363†                       |       | 0.871*                          | 0.581†                          |
| CST, μm                  | 559.06 ± 95.77                         | 301.22 ± 59.16 | <0.001†                       |       | 567.83 ± 201.75 | 469.83 ± 159.43 | 0.011†                       |       | 0.721*                          | <0.001†                         |
| EZ disruption (%)        | 12/18 (66.67)                          | 11/18 (61.11) | 1.000‡                       |       | 5/6 (83.33) | 4/6 (66.67) | 1.000§                       |       | 0.629‡                          | 1.000§                          |
| EZ disruption length, μm | 307.17 ± 532.33                        | 283.44 ± 415.12 | 0.61†                       |       | 532.33 ± 582.85 | 539.00 ± 627.62 | 0.884†                       |       | 0.494*                          | 0.581*                          |
| ELM disruption (%)       | 11/18 (61.11)                          | 11/18 (61.11) | 1.000‡                       |       | 5/6 (83.33) | 4/6 (66.67) | 1.000§                       |       | 0.621‡                          | 1.000‡                          |
| ELM disruption length, μm| 356.56 ± 459.66                        | 326.76 ± 467.17 | 0.696†                       |       | 534.00 ± 615.64 | 472.83 ± 612.16 | 0.048‡                       |       | 0.378*                          | 0.581*                          |
| OPL, disruption (%)      | 17/18 (94.44)                          | 16/18 (88.89) | 1.000§                       |       | 6/6 (100.00) | 6/6 (100.00) | 1.000§                       |       | 1.000‡                          | 1.000‡                          |

* 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. Moreover, eyes with ME associated with a high number of HRDs and OPL disruptions may be predicted to respond poorly to anti-VEGF treatment.

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


