Algorithm for the Measure of Vitreous Hyperreflective Foci in Optical Coherence Tomographic Scans of Patients With Diabetic Macular Edema

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IMPORTANCE Developing a noninvasive measure of diabetic retinopathy disease progression may provide physicians with information needed for patient-specific intervention.

OBJECTIVE To develop an algorithm to measure vitreous hyperreflective foci (VHRF) from standard, 3-dimensional optical coherence tomographic (OCT) images in an unbiased manner.

DESIGN, SETTING, AND PARTICIPANTS We retrospectively analyzed OCT scans from 97 patients who were evaluated at the Kellogg Eye Center, University of Michigan. Patients with diabetes mellitus without signs of retinopathy (n = 29) and patients with diabetic macular edema (DME) (n = 31) were compared with healthy control participants (n = 37). The algorithm was used to determine whether the VHRF score is associated with DME and may serve as a noninvasive measure of inflammation. The study was conducted from November 14, 2011, to August 5, 2015. Data analysis was performed from May 15, 2014, to August 13, 2015.

MAIN OUTCOMES AND MEASURES An algorithm was developed to enhance the vitreous imaging from OCT to allow automated quantification of VHRF and calculation of a VHRF score. This score was compared between the healthy control, diabetes without retinopathy, and DME groups.

RESULTS In the 97 scans evaluated, VHRF scores, reported as mean (SD), were increased in patients with DME by 2.95-fold (5.60 [8.65]) compared with healthy controls (1.90 [3.42]; 95% CI, 0.75-7.45; P = .012) and by 6.83-fold compared with patients with diabetes without retinopathy (0.82 [1.26]; 95% CI, 1.46-8.82; P = .005).

CONCLUSIONS AND RELEVANCE Scores obtained using the VHRF algorithm may be obtained from OCT images that include the vitreous and could provide a rapid, noninvasive clinical correlate for ocular inflammation. Higher VHRF scores in patients with DME compared with controls and diabetic patients without retinopathy warrant further population-based and longitudinal studies to help determine the value of the VHRF score in selecting therapeutic intervention.
The incidence of diabetes mellitus is rising, with the total number of people affected worldwide projected to increase to 366 million by 2030. The most common cause of vision impairment in patients with diabetic retinopathy is macular edema. Macular edema is believed to result from a loss of the normal blood-retinal barrier with an increase in vascular permeability. It may also include impaired removal of proteins and fluid, with subsequent accumulation of fluid and cystoid formation. Vascular endothelial growth factor contributes to this process, and therapies targeting vascular endothelial growth factor have proved effective in improving visual acuity.

In addition to vascular endothelial growth factor, mounting evidence suggests that neuroinflammation contributes substantially to the development and progression of diabetic retinopathy. Inflammatory mediators found in the vitreous fluid are associated with the severity of diabetic macular edema (DME). Evidence suggests that inflammatory cells are present in patients with diabetic retinopathy requiring vitrectomy and in those with vitreous hemorrhage. However, these findings were limited to advanced stages of diabetic retinopathy with confounding factors that necessitated the vitrectomy procedure. The contribution of neuroinflammation to the induction and progression of diabetic retinopathy remains an area of active investigation.

Optical coherence tomographic (OCT) scans are used in the diagnosis of ocular disease. Some investigators have noted the presence of hyperreflective foci in OCT scans of the vitreous of patients with inflammatory conditions, including uveitis. These foci have been proposed to represent inflammatory cells. Saito et al characterized hyperreflective foci as dots that were larger and denser than the “usual background speckle” and noted increased spot density closer to areas of retinitis. In addition, the authors noted a decreased number of spots as vitritis resolved with treatment. Gallagher et al similarly described OCT findings, characterized vitreous spots in patients with uveitis, and suggested that the spots or foci represented inflammatory cells migrating into the vitreous. Furthermore, other authors have proposed using OCT to analyze anterior chamber inflammation by quantifying these hyperreflective spots and have compared them with clinical grades of inflammation. Keane et al quantified the total OCT vitreous signal and compared it with clinical markers of inflammation. However, to our knowledge, no method has been developed to quantify the vitreous hyperreflective foci (VHRF), which may represent a more specific and accurate method to assess infiltrating cells.

To objectively characterize these VHRF, we have created an algorithm to analyze and quantify OCT scans, thereby providing a VHRF score. This algorithm has the benefits of analyzing the total volume of a scan in 3 dimensions (3-D) and controlling for variability in the vitreous volume scanned by use of a spots-per-volume metric. This algorithm was applied to OCT scans from patients with diabetes to determine whether the VHRF score may serve as a noninvasive measure of diabetic retinopathy disease progression and potential ocular inflammation. This algorithm was used to compare the incidence of VHRF in healthy controls, diabetic patients without retinopathy, and patients with DME. We observed increased VHRF scores in patients with DME compared with both healthy controls and diabetic patients without retinopathy. Collectively, this study demonstrates an algorithm to analyze OCT scans and rapidly quantify VHRF. The VHRF scores increased with DME, and quantification of a VHRF score may act as a noninvasive measure of the progression of diabetic retinopathy.

Methods

Patients

We retrospectively analyzed the OCT scans from 97 patients who were evaluated at the Kellogg Eye Center, University of Michigan. Patient characteristics, including demographics, duration of disease, retina volume, and hemoglobin A1C level closest to the date of the scan, were recorded. The OCT scans had been obtained from 37 healthy control individuals, 29 diabetic patients without retinopathy, and 31 patients with DME. The OCT scans were obtained as dense, 20 × 20°, high-speed, automatic, real-time spectral-domain images (Spectralis HRA+OCT; Heidelberg Engineering). Optical coherence tomographic cube scans (512 A-scans in each B-scan, and 3.87-μm axial resolution, automatic real-time noise reduction of 16 scans per line over the macula with 97 sections) were obtained using the standard OCT acquisition window. The scans were deidentified before being exported from the OCT machines, and the image analysis protocol described below was conducted for each scan. Each study was approved by the University of Michigan institutional review board. Participants had provided written informed consent. The investigation was conducted from November 14, 2011, to August 5, 2015; data analysis was performed from May 15, 2014, to August 13, 2015.

VHRF Algorithm

Complete algorithm details are available in the eAppendix in the Supplement. Each OCT scan was imported into ImageJ software as a raw .vol file via the Open Hyex Raw plugin. A median filter was then applied to all 97 sections to reduce signal noise, and the file was converted into individual 16-bit .tif format images. These images were imported into IMARIS software (Imaris X64; Bitplane Scientific Software) and reconstructed in 3-D (Figure 1B). The 3-D renderings were then cropped to include the area of interest of vitreous and retina.
removing the subretinal tissues from analysis. Next, the retinal volume was automatically mapped by our algorithm and subtracted from the image analysis field, leaving only the vitreous for spot detection. The normalize layers function, which sets the contrast of each section equal to the mean contrast for the 3-D rendering, was used to automatically adjust layers with aberrantly high background signal that may affect analysis. We then applied an algorithm (eAppendix in the Supplement) to identify and quantify VHRF (Figure 1C). Our algorithm accounted for VHRF size (estimated diameter, 4.0 μm) and the IMARIS proprietary quality thresholds (quality >30), which selectively identify spots based on signal intensity with respect to the background, as seen in the 3-D–rendered algorithm (Video). The same algorithm was applied to all scans. The volume of the vitreous and retina was then calculated.

We calculated the ratio of spots per volume of vitreous by using the following formula: 

\[(\text{Total Spots}/\text{Total Vitreous Volume Imaged}) \times 10^5.\]

We defined the resulting value as the VHRF score and designated it as our primary outcome. Owing to the limitations of OCT, including space in the z-axis between sections and limited resolution in the horizontal axis, “volume” is in fact rendered volume in IMARIS and should not be interpreted as the true value of vitreous volume analyzed.

Repeatability

We performed 2 studies to validate the repeatability of this algorithm in the identification of VHRF, one comprising 5 individuals (4 healthy controls and 1 diabetic patient without retinopathy) who received 2 successive OCT scans within a session and completed 3 sessions within a 2-week period. The second study included 12 patients with DME who received 2 successive OCT scans within a single session. In each session, OCT scans were conducted with acquisition variables identical to those of the main study and were performed by the same technician (T.S.). Our VHRF identification algorithm was subsequently applied to all scans.

Statistical Analysis

Statistical analysis was done using PRISM, version 6 (GraphPad Software Inc). One-way analysis of variance with posttest Bonferroni multiple comparison was used to compare means, and differences were considered statistically significant at \( P < .05. \) The Grubbs test (http://graphpad.com/quickcalcs/grubbs1/) for outliers was used in each group analysis.

A total of 97 OCT scans were analyzed from 37 healthy controls, 29 diabetic patients without retinopathy, and 31 patients with DME. Significant artifacts were identified in the OCT scans of 1 healthy control, 3 diabetic patients without retinopathy, and 1 patient with DME; these scans were excluded from analysis. After the algorithm was applied, 1 outlier in the VHRF score was identified in each of the control, diabetes without retinopathy, and DME groups through the use of a Grubbs test, and these scans were excluded from analysis.

Vitreoretinal separation (VRS) subgrouping was defined by OCT images according to grading by a retinal specialist (G.C.) as to whether the patients had full or partial posterior vitreous detachment or no posterior vitreous detachment and was completed in a masked fashion. After the algorithm was applied, 1 outlier in the VHRF score was identified in a control with no VRS, 1 in a control with VRS, and 1 in a patient with diabetes but without DME or VRS; these OCT scans were excluded from analysis.

Statistical analysis for the repeatability study was done using the variance components procedure of SAS, version 9.4 (SAS Institute Inc), which estimates the contribution of each effect to the variance of the VHRF score. The intraclass correlation coefficient (ICC) was then calculated for each source of variability. Nonparametric bootstrapping was used to calculate 95% CIs for the ICCs. The original sample of 12 participants with diabetes (each person with 2 VHRF measures on a single day) was resampled with replacement to obtain 1000 bootstrap samples, each with 12 patients. The unit of selection was the patient to preserve the nesting of measures. The ICCs were then calculated on each resample to obtain the distributions of the ICC statistics. The 2.5- and 97.5-percentiles were used to create 95% CIs. All analysis was performed with SAS, version 9.4).

Results

Application of the algorithm to 97 OCT scans from healthy controls and patients with diabetes, with or without DME–identified VHRF, led to the development of a VHRF score. The Table provides relevant patient data for the OCT scans used.
Table. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group, Mean (SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Age, y</td>
<td>48.1 (17.6)</td>
</tr>
<tr>
<td>Duration of diabetes mellitus, y</td>
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</tr>
<tr>
<td>Hemoglobin A1c level, %</td>
<td>NA</td>
</tr>
<tr>
<td>Initial retinal volume, mm³</td>
<td>8.7 (0.3)</td>
</tr>
</tbody>
</table>

Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; NA, not applicable.

Figure 2. Vitreous Hyperreflective Foci (VHRF) Scores for Comparison Groups

![VHRF Scores Graph](image)

DME indicates diabetic macular edema; DR, diabetic retinopathy. Horizontal bars represent mean values; error bars, SD.

Figure 1 depicts a typical OCT scan from a patient with DME. Hyperreflective foci are present in the vitreous of the patient (Figure 1A). Image stacks were imported into IMARIS software and cropped to illustrate VHRF (Figure 1B), followed by contrast enhancement and application of the spot detection algorithm that accounted for signal in relation to the background and contained a defined minimum and maximum spot size. The VHRF were readily detected and quantified in an unbiased manner using IMARIS (Figure 1C).

The algorithm was applied to controls, diabetic patients without retinopathy, and patients with DME. Diabetic macular edema was associated with a clear increase in the VHRF score. Bonferroni analysis yielded statistical significance in the VHRF score between the control without VRS and DME with VRS groups (Figure 3) (P < .0001), between the control with VRS and the DME with VRS groups (P = .002), between the diabetes without retinopathy or VRS and the DME with VRS groups (P < .0001), and between the DME without VRS and the DME with VRS groups (P = .001). No significant difference was observed for VRS as an independent factor for VHRF score by post-test analysis of variance between the control and VRS group. However, the clear increase in VHRF score between the DME without VRS and DME with VRS groups suggests that VRS, at a minimum, acts with DME as a risk factor for a high VHRF score. Collectively, these results demonstrate increased VHRF scores in patients with DME compared with controls or patients with diabetes without retinopathy and suggest increased inflammation in individuals with DME. Furthermore, increased VHRF scores were associated with patients with DME who had VRS, which may reflect increased neuroinflammation in these patients but may also represent increased cell debris.

To determine whether patient age affected the VHRF score, regression analysis was performed. In a model of age predicting VHRF score, age is a predictor: for every 1-year increase in age, the VHRF score increases by 0.12 units (P = .0028). In a model including age and both diabetes groups, after adjustment for age, there was still an effect of DME on the VHRF score (overall P = .0364). Specifically, the DME group showed larger VHRF scores than the diabetes without retinopathy group (P = .0340) and the control group (P = .0165). The control group did not show a significant difference in VHRF score from the diabetes without retinopathy group (P = .8236). There was no interaction between group and age (P = .6654).

The difference in VHRF score was not the result of variations in measures. To determine repeatability, the variance of 2 successive OCT scans and the VHRF scores for each scan was calculated within a session. There were 4 controls and 1 patient with diabetes without diabetic retinopathy, for which 3 sessions were completed during a 2-week period (Figure 4). The variation between the 2 scans in each session accounted for 13% of the variation in the data (ICC, 0.13), and the variation within a patient over different sessions accounted for 29% of the variation in the data (ICC, 0.29). The variation in the group as a whole over different days was approaching 0, suggesting that the variation in scans from day to day was nearly undetectable compared with differences among individuals. We performed a separate repeatability study of patients with DME. There were 12 patients who had 2 successive OCT scans within a single session. The variation between the 2 scans in each session accounted for 38% of the variation in the data (ICC, 0.38).

Discussion

The institution of spectral-domain OCT in most ophthalmology clinics provides a direct, noninvasive means to assess retinal structure associated with disease processes. Previous studies¹⁰⁻¹²,¹⁵ have observed VHRF associated with inflammat-
tion; in the present study, we developed an algorithm to quantify, in an unbiased manner, the VHRF and provide a score for use in patients with diabetic retinopathy.

We observed an increased VHRF score in spectral-domain OCT images from patients with DME compared with both healthy control individuals and diabetic patients without retinopathy. This finding suggests an increase in VHRF at some point in the progression of the disease to DME. If these VHRF represent inflammatory cells, additional questions are raised regarding the contribution of vitreous inflammatory cells in the pathogenesis and progression of DME that warrant further investigation.

The repeatability study demonstrated the fidelity of our algorithm as observed over successive OCT scans within the same day, as well as additional scans during a short period (2 weeks). The mean VHRF score variance in healthy controls of 0.13 between successive scans is minor compared with the mean VHRF values for our main study groups. A nontrivial amount of variance in the VHRF score was seen within participants in our healthy repeatability study between different days as well as in patients with DME who underwent successive scans on the same day. The vitreous is a fluid structure with likely nonhomogeneous distribution of VHRF, and our scan window is restricted to the posterior portion; therefore, some variation may be expected. Thus, a mean of successive VHRF scores in a session may be more accurate when used in a clinical setting. Still, the variation of the control group as a whole on different days was approaching 0, suggesting that the variation in scans from day to day had little effect on the VHRF score compared with individual differences. Together, these outcomes suggest acceptable reliability and repeatability of our algorithm for use as a clinical diagnostic tool.

Certain limitations exist within this proof-of-concept study, which we look to address in our continuing work through more expansive prospective studies. First, the nature of VHRF is not known. As described in the introduction, VHRF are associated with inflammation and may represent inflammatory cells, but whether they also represent cell debris or other factors is unknown. The VHRF score may become an important biomarker, but it is unknown whether the foci precede DME, contribute a causative role, or resolve with treatment. Finally, the role of VRS and age as contributors to higher VHRF scores needs to be explored further.

Studies using larger populations and longitudinal treatment response analysis may determine whether the use of the VHRF score will provide a means of characterizing disease progression for patients with diabetic retinopathy. Furthermore, the application of the algorithm to existing OCT images may allow larger cross-sectional and longitudinal studies to identify independent risk factors for the VHRF score. When patient OCT images were subgrouped for VRS in the present study, differences between the DME and DME with VRS groups were observed, suggesting that VRS was associated with neuroinflammation and might help explain the development of epiretinal membranes in this setting. We suspect that VRS is an independent risk factor for a higher VHRF score. With larger groups and longitudinal studies, we hope to further elucidate the risk factors for a high VHRF score and determine the precise stages of disease progression that may be associated with increasing scores.
Conclusions

The present studies demonstrate the development and use of an algorithm to quickly and easily quantify VHRF in OCT scans. Even in these limited studies, the VHRF score demonstrated a correlation with the presence of DME and likely represents an inflammatory component of the disease process. Applying this algorithm to OCT imaging may help to quantify disease progression and could identify individual patients with an inflammatory process associated with diabetic retinopathy.

References