Characterization of Vitreoretinal Interface Disorders Using OCT in the Interventional Phase 3 Trials of Ocriplasmin

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PURPOSE. We determined the reproducibility of a novel optical coherence tomography (OCT) protocol designed to evaluate formally vitreoretinal interface abnormalities on scans obtained during two phase 3 studies of intravitreal ocriplasmin to treat symptomatic vitreomacular adhesion with or without macular hole.

METHODS. Certified technicians obtained time-domain OCT scans that included a macular thickness map (MTM), Fast MTM, and three high resolution linear scans: one 10 mm horizontal and one 10 mm vertical through the optic nerve head (ONH), and one 10 mm 5-degree-offset through the ONH and fovea. Reading Center teams graded all 3695 scans from 652 study eyes for pre-established quantitative and morphologic features. Grading reproducibility at baseline and follow-up visits was tested for presence of vitreomacular adhesion (VMA), width of vitreous adhesion (focal <1500 μm versus broad >1500 μm), presence and minimum width of full thickness macular hole (FTMH), and presence of epiretinal membrane (ERM).

RESULTS. Team grading reproducibility for VMA (kappa 0.91, 95% confidence interval [CI] 0.81–1.00), broad versus focal width of vitreous adhesion (kappa 0.87, 95% CI 0.78–0.95), FTMH (kappa 0.87, 95% CI 0.78–0.95), and ERM (kappa 0.87, 95% CI 0.78–0.95) was high. Percent agreement was 97%, 92%, 95%, and 82% for VMA, vitreous adhesion width, FTMH, and ERM, respectively. For repeated measurements of FTMH width, the intraclass correlation was 0.89 (95% CI 0.85–0.93), and the mean paired difference between grading team measurements was 34.4 μm (95% limits of agreement –149.5–218.2 μm).

CONCLUSIONS. Quantitative and morphologic vitreoretinal interface features were assessed reproducibly using a newly developed OCT scan acquisition and grading protocol. This protocol will be useful to evaluate OCT endpoints in future clinical trials, and can facilitate identification of vitreoretinal interface pathology during care of individual patients. (ClinicalTrials.gov number, NCT00781859 and NCT00798317.)

Vitreous adhesion to the retinal internal limiting membrane (ILM) is mediated in part by laminin and fibronectin.1,2 Ocriplasmin (Microplasmin; ThromboGenics, Leuven, Belgium) is a recombinant truncated human plasmin with intact protease activity. When given as an intravitreal injection, ocriplasmin degrades fibronectin and laminin,3 and is a promising method to induce posterior vitreous detachment (PVD).4–7

The Microplasmin IntraVitreal Injection for Traction Release without Surgical Treatment (MIVI-TRUST) program investigated the safety and efficacy of ocriplasmin to treat symptomatic vitreomacular adhesion with or without macular holes. To be enrolled, patients were required to have vitreomacular adhesion (VMA), defined as focal adherence of the posterior hyaloid to the macula with detached vitreous on both sides of the adhesion on at least one optical coherence tomography (OCT) image. VMA was considered symptomatic if, in the opinion of the trial investigator, it caused decreased visual function such as reduced visual acuity, metamorphopsia, or other visual complaint. Nonsurgical resolution of VMA on OCT was the primary ocriplasmin study endpoint, and closure without vitrectomy of preexisting macular hole, as determined by OCT was a secondary endpoint. The Duke Reading Center independently determined both endpoints through masked grading of images generated during the study.

OCT was used to determine primary and secondary study endpoints. This imaging modality was suited ideally to evaluate vitreomacular adhesion in this trial, as it displays spatial relationships between the posterior vitreous and inner retina in a manner that otherwise is unachievable with a noninvasive technique. Likewise, OCT has proven valuable to demonstrate pathologic conditions associated with incomplete PVD, such as epiretinal membrane (ERM),8,9 macular hole,10,11 and tractional macular edema.12 Incomplete separation of the posterior vitreous from the macula also may exacerbate diseases, such as diabetic macular edema,13,14 retinal schisis in high myopes,15 and neovascular age-related macular degeneration.16–18

While OCT undoubtedly is helpful to characterize vitreoretinal interface disorders, no consensus exists for a standardized vitreoretinal interface classification scheme based on OCT. In a study of 209 healthy eyes, Uchino et al. proposed a 5-stage scale that ranged from “no PVD” to “complete PVD.”19 This protocol was modified by Johnson and applied to 43 eyes with various vitreoretinal interface disorders.20 More recently, one group has proposed a 3-category scale to correlate vitreous morphology with post-vitrectomy outcomes,12 while others have described a system that includes width of vitreomacular adhesion and presence of morphologic findings on OCT.8 From the varied classification methods described in the literature, it is clear that a standardized imaging protocol to depict and analyze systematically vitreoretinal interface disorders is needed, and would be useful especially when OCT is used to evaluate treatment effects in interventional clinical trials for vitreoretinal interface disorders.

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Since reproducibility of prior protocols for OCT-based analysis of vitreoretinal disorders is not well known, it would be important to determine the precision of any proposed method to acquire and analyze OCT scans in eyes with vitreoretinal interface pathology. This analytical precision is of particular importance to the MIVI-TRUST program, since baseline VMA was an eligibility requirement for entry into the ocriplasmin phase 3 trials and resolution of VMA as determined by OCT was a primary study endpoint. OCT also may yield important prognostic information as ocriplasmin was more likely to resolve VMA and macular hole in eyes without ERM and in those with focal vitreous adhesion, again emphasizing the need for reliable OCT grading.\textsuperscript{4,21} We describe a method to acquire and evaluate systematically OCT images of eyes with vitreoretinal interface disorders, and also determine the reproducibility of this OCT grading protocol as used during two pivotal phase 3 interventional trials.

**METHODS**

**Trial Overview**

The MIVI-TRUST program consists of two pivotal multicenter, randomized, phase 3 trials that investigated the safety and efficacy of ocriplasmin compared to placebo injection for the treatment of symptomatic vitreomacular adhesion with or without macular hole (in the public domain, ClinicalTrials.gov identifier: NCT00781859 and NCT00798317). For this study, VMA was denoted when the posterior hyaloid adhered to the macula with vitreous separation on both sides of the adhesion on at least one OCT image. The MIVI-TRUST program included eyes with VMA resulting in decreased visual function and visual acuity worse than or equal to 20/25. For the 6-month follow-up duration, post-injection eyes followed with serial OCT scans at each study visit. Additionally, study-site echographers performed B-scan ultrasonography during the MIVI-TRUST program, however these images were not evaluated by the Reading Center. All experimental procedures adhered to the tenets of the Declaration of Helsinki, appropriate Institutional Review Board (IRB) approval was obtained, and all participants engaged in an informed consent process and signed a written consent document before enrollment in the MIVI-TRUST program.

**OCT Acquisition**

OCT scans were acquired in a standardized fashion as follows. All scans were acquired in dilated eyes by certified technicians with Stratus OCT machines using software version 4.0 or greater. To obtain certification, technicians were required to submit 52 OCT scans depicting partial or complete vitreous separation for feedback by the Reading Center regarding scan quality and avoidable artifacts. To obtain optimal visualization of the vitreoretinal interface and retina, the scan protocol also emphasized appropriate OCT scan focus, saturation, and line placement. For the duration of the study, an automated system relayed Reading Center feedback to all OCT technicians for each graded image through reports that detailed scan placement, quality, and individually identified submissions of concern.

At each study visit, technicians performed 5 distinct scan protocols on each study eye. All eyes were imaged using the Stratus fast macular thickness map (FMTM) and macular thickness map (MTM) protocols. These protocols each included 6 radial lines of 6 mm length placed at the foveal center at 30-degree rotational increments. Three custom, high-resolution (512 A-scans per tomographic line) line scans that each were 10 mm in length also were obtained on all eyes; two that produced a “cross-hair” pattern were performed through the optic nerve, one vertically at 90 degrees and the other horizontally at 180 degrees. The third “offset” scan through the optic nerve center and foveal center was angled 5 degrees inferiorly from the optic nerve, yielding a 5-degree angle for the right eye and a 355-degree angle for the left eye (Fig. 1). Scans were de-identified and labeled with a unique code, and then submitted to the Reading Center. Scans were submitted from the 7 study visits and included OCT images recorded at baseline; injection day; post-injection days 7, 14, and 28, and post-injection months 3 and 6.

**OCT Grading**

The 3695 Stratus OCT scans from 652 study eyes were graded by certified readers at the Duke Reading Center. For this study, the fovea on any individual OCT line scan was defined as the horizontal region 1 mm in width with midpoint at the foveal center. First, quality of each OCT scan was determined. Scan quality was termed “acceptable” if the scan was available, positioned correctly, and adequately saturated. The designation “not interpretable” was applied if one or more line images were missing, positioned incorrectly, or very poorly saturated and prevented grading a feature as present or absent.

Our protocol sought to identify reproducibly vitreoretinal interface findings of potential clinical relevance. To this end each OCT scan was analyzed for presence of full thickness macular hole (FTMH) and ERM (Fig. 2). Each of 15 line scans (6 from the FMTM protocol, 6 from the MTM protocol, and 3 from custom 10 mm protocols) was evaluated, and then a grade was assigned for each predetermined morphologic feature. For each morphologic feature analyzed, one of the following terms was assigned: feature present, feature absent, or not interpretable (due to poor saturation, incorrect placement, or scan absence). Standardized OCT images were chosen to reference all morphologic characteristics of interest.

Morphologic feature characteristics were subcategorized further during grading. Retinal deformation at any vitreous attachment sites or ERM and whether the central 1 mm (horizontal dimension) of the retina was deformed were recorded. In addition, a notation was made to indicate whether ERM was located at any point of vitreous adhesion. If a macular hole was present, the presence of intraretinal fluid at the macular hole lateral borders and/or vitreous adherent to the macular hole lateral borders also was recorded.

FTMH was characterized by two measurements performed with software-based calipers on images derived from the MTM protocol. All 6 radial images were reviewed, and the maximum FTMH width at the level of the RPE was recorded (Fig. 3a). Similarly, the minimum FTMH...
width at any location along the hole was determined on all 6 radial images. The largest of these minimum widths was recorded (Fig. 3b). Eyes with minimum FTMH width measurement greater than 400 μm at the baseline visit were not eligible for this trial.

Our grading system categorized the interaction between the posterior hyaloid and inner retina. VMA was denoted when the posterior hyaloid adhered to the macula with vitreous separation on both sides of the adhesion on at least one of the radial line scans. Subtypes of VMA included vitreous attachment at the fovea and optic nerve head (ONH) with intervening separation (Fig. 4a), or vitreous attachment only at the fovea (Fig. 4b). Posterior vitreous interaction with the retina could be classified only after reviewing all scans from a study visit. A reader could classify scans from a study eye as depicting VMA only after observing at least one image depicting posterior vitreous attachment to the macula with separation on both sides of the attachment (Figs. 4a, 4b). The vitreoretinal interface configuration could not be termed VMA based only on a single image depicting no visible vitreous separation between the fovea and ONH, but visible vitreous separation outside this zone (Fig. 4c), or an image depicting vitreous attachment between the fovea and ONH with intervening separation, but with no vitreous separation visible temporal to the fovea (Fig. 4d).

Several vitreous states other than VMA were observed during the trial. Upon review of the complete set of scans, a lack of visible vitreous adhesion to the macula or optic nerve was categorized as no visible vitreous separation or adhesion (Figs. 5a, 5b). In the MIVI-TRUST trials, eyes initially had vitreomacular adhesion, with incomplete vitreous separation. Accordingly, in subsequent visits, if OCT scans appeared as in Figure 5b (no visible vitreous attachment or separation), then by definition the vitreous had separated and retracted out of the field of view. Another vitreous state observed during the course of the trial was vitreous attached at the ONH only (Fig. 5c). Finally, if the scan position, lack of availability, or poor saturation precluded evaluation of vitreomacular adhesion, then “not interpretable” was recorded.

For all OCT scans that had VMA, the width (horizontal dimension) of the vitreous adhesion was measured for each line scan using software-based calipers. For multifocal attachments with intervening separations, each of the individual attachment widths was summed to arrive at the final total attachment width. Vitreous adhesion width greater than 1500 μm on one or more image was termed “broad,” while vitreous adhesion width less than or equal to 1500 μm on all images was denoted “focal.” Vitreous adhesion width was measured preferentially from images produced by the MTM protocol, but available FMTM images could be substituted when MTM images were not interpretable.

Team-Based OCT Evaluation

Two masked readers first graded all scans independently. Next, a separate data specialist identified discrepant values between the paired readers’ grades. Manual measurement of morphologic features was deemed discrepant if there was more than 60 μm difference between vertical dimension measurements or 100 μm difference in horizontal dimension measurements. Caliper-based FTMH width grading was more stringent, and values were considered discrepant between readers if a disparity greater than 30 μm in horizontal dimension was observed. All graded scan pairs with discrepant data then were presented to a senior reader for arbitration. During the arbitration process, a senior reader reconciled all discrepancies between the initial reader pair and recorded the final arbitrated values. A senior reader additionally arbitrated all OCT scans for the presence of VMA, since this was an endpoint of the MIVI-TRUST program. Any finding or measurement that remained controversial after arbitration was forwarded to the Director of Grading for final decision.

OCT Grading Reproducibility

From the 3695 scans obtained during the MIVI-TRUST program, 100 scans were selected randomly to determine Reading Center team grading reproducibility. The computerized scan selection algorithm was designed so that only one scan per individual study subject was included. The reproducibility testing was performed 5 weeks or more after initial grading to minimize the chance that readers would
Of the 100 scans selected initially, 10 contained FTMH. This number was below the minimal sample size \((n = 22)\) determined to be necessary for meaningful statistical analysis. Therefore, 100 additional scans were selected randomly from the 587 scans that depicted FTMH to quantify effectively reproducibility of FTMH width measurements.

For all reproducibility studies, a repeat grading team was defined as 2 readers chosen at random from the pool of 5 readers who performed the initial grading, matched with the same arbitrator who had performed the initial grading. For the reproducibility analysis, readers repeated grading of images in a number proportional to the number of images that they graded initially. Readers were masked to the results of the initial grading. After a second grading value was determined for a particular finding or measurement, this value was compared to the initial grade (Fig. 6). Reproducibility was determined for the categorical variables presence of VMA, broad versus focal width of vitreous adhesion, presence of FTMH, and presence of ERM. Quantitative grading reproducibility was analyzed for the minimum FTMH width measurement.

### Statistical Analysis

For categorical variables, the concordance, or percent agreement, was computed as a measure of reproducibility for repeated grading. The Kappa statistic, a measure of agreement, also was computed. All analyses were performed with SAS 9.2 software (SAS, Cary, NC).

For continuous values, paired differences between Reading Center teams were computed. The significance of the paired difference was assessed using a test of equality to the value 30.0 \(\mu m\), the maximal limit of equivalence for FTMH width specified in the grading protocol. The intraclass correlation with confidence intervals (CI) was used to assess the agreement of continuous measurements.

### RESULTS

We evaluated initial reader pair agreement, before arbitration on VMA presence or absence, and on VMA width, FTMH, and ERM. Of 100 randomly selected scans, during initial grading, reader agreement was 77% for scans with final grading as “VMA absent” \((n = 22)\) and 80% for scans with final grading as “VMA present” \((n = 78)\), respectively. During initial grading, the primary reader pair agreed on 78%, 91%, and 75% of scans independently evaluated in parallel for the parameters “broad versus focal width of vitreous adhesion,” “FTMH,” and “ERM,” respectively. Accordingly, arbitration was not performed for these parameters on the corresponding scans. In the remaining scans, the initial reader pairs disagreed with one another, and arbitration was required on 22%, 9%, and 25% of scans for the parameters “broad versus focal width of vitreous adhesion,” “FTMH,” and “ERM,” respectively.

Reading Center team percent grading agreement was 97% for presence of VMA, 92% for broad versus focal width of vitreous adhesion, 95% for presence of FTMH, and 82% for presence of ERM. Reading Center team grading reproducibility was high for VMA, broad versus focal width of vitreous adhesion, FTMH, and ERM. Reading Center team qualitative grading reproducibility is shown in Table 1.

For repeated Reading Center measurements of minimum FTMH width, the intraclass correlation was 0.89 (95% CI 0.85–0.93). For this measurement, the mean paired difference between grading team measurements was 34.4 \(\mu m\) (95% limits of agreement –149.5–218.2 \(\mu m\), \(P = 0.643\), Table 2).

During repeat grading, the primary reader pair agreed on 65%, 92%, and 77% of scans evaluated independently in parallel for the parameters “broad versus focal width of vitreous adhesion,” “FTMH,” and “ERM,” respectively. Accordingly, arbitration was not performed for these parameters on the corresponding scans. In the remaining scans during repeat grading, the initial reader pairs disagreed with each other, and arbitration was required on 37%, 8%, and 23% of scans for the parameters “broad versus focal width of vitreous adhesion,” “FTMH,” and “ERM,” respectively.

### DISCUSSION

In our study, we developed a scan protocol to evaluate vitreoretinal interface disorders, and studied the ability of...
Our protocol was designed to depict optimally the relationship among foveal, retinal, and optic nerve vitreous adhesions with a 10 mm line scan that passed through the optic nerve and fovea. Also, our protocol that required review of all 6 radial line images allowed readers to identify associated pathologic features that may not have been demonstrated on a horizontal or vertical line scan. The use of 10 mm radial line scans obviated the need for manual image compilation in our study.

We likewise adapted the categorization scheme proposed by Uchino et al. to categorize comprehensively the various vitreous morphologies observed in this trial. Uchino’s stage 0 was defined as absence of PVD, and stage 4 was defined as complete PVD out of range of OCT and verified by biomicroscopy. The ability of slit-lamp biomicroscopy to identify reliably vitreous separation remains to be determined. Regardless, since our study did not incorporate biomicroscopy, Uchino’s stages 0 and 4 correlated to our “no visible vitreous” category. However, we included a “complete vitreous separation” category for those cases where complete macular PVD could be confirmed with OCT.

The primary morphologic feature of interest during this trial, VMA, most closely corresponded to Uchino’s stage 2, vitreous detachment with attachment at the optic nerve, fovea, and midperipheral retina. Uchino et al. did not describe our VMA subcategory of “vitreous adhesion to the fovea only.” In contrast, the two categories of partial vitreous separation with no visible separation elsewhere used in our study roughly correlated with Uchino’s Stage 1 classification, defined as perifoveal PVD. Uchino’s Stage 3 classification corresponded exactly to the “vitreous attached at the ONH and temporal macula with intervening separation” category from our study, though no mention was given to cases of “vitreous adhesion to the optic nerve only” as observed during our study. Finally, poor OCT scan quality precluding vitreomacular adhesion evaluation was not addressed by Uchino et al.19 In anticipation of the several thousand scans to be generated during this clinical trial, we included a grading category for scans in which the quality was insufficient to interpret imaging parameters.

Prior series detailing OCT grading protocols have used individual examiners,27–29 and multiple grader pairs in parallel.30 We chose a team-based grading approach with two primary readers and a senior reader who arbitrated scans to maximize grading consistency and to provide feedback between the senior reader and primary readers during the course of the study. In addition, this method allowed a senior reader to review more efficiently scans with subtle pathology, as opposed to more obvious abnormalities that can be graded effectively by primary readers without the need of senior reader arbitration. For example, the majority of grading performed in parallel by readers did not require arbitration in our study as demonstrated by the 8% to 37% range of arbitration frequencies. Our study demonstrates that team-based grading leads to increased levels of reproducibility when compared to agreement rates between independent primary readers.

The protocol used in the ETDRS study for certain fundus photographs has most similarity to our team-based protocol for grading OCT scans. In the ETDRS study, baseline fundus photos were reviewed only by a pair of readers, and a subset of discrepancies were arbitrated by a senior reader.31 Our protocol differed in that independent reading teams graded baseline and follow-up OCT scans, and a senior reader

Figure 5. OCT images depicting vitreous morphologies other than vitreomacular adhesion observed during the MIVI 3 trial. (a, b) No visible vitreous attachment or separation. (c) Vitreous attached at the ONH only.
reconciled all discrepancies between the initial reader pairs. We believe that it was important to maintain this team analysis approach at each study visit, since consistent grading was important to evaluate reliably the primary study endpoint, which was a change in the OCT appearance of VMA from baseline to follow-up visits.

Readers also generally were able to measure reproducibly minimum FTMH width. Repeated minimum FTMH width measurement resulted in narrower mean width, although this difference was not significantly different from the pre-specified 30 µm equivalence limit. The width differences from one evaluation to the next in our study may be due to measurement disparities among individual readers who made manual measurements at slightly different locations on the same radial line scans. Very small variations in Stratus on-screen caliper placement can cause a 30 µm or greater difference in width measurement. These measurement disparities were minimized by a grading protocol that required evaluation of all radial line images before macular hole width was determined for an individual patient with a FTMH.

The primary question to be answered from the current report was whether the methods that we used to describe vitreoretinal interface abnormalities and the associated Reading Center team-based grading methodology could evaluate reproducibly the presence or absence of VMA and macular hole, and therefore determine reliably the key MIVI-TRUST endpoints, resolution of symptomatic VMA, and macular hole closure. At the time the MIVI-TRUST studies were initiated, it was not possible to obtain spectral domain (SD-OCT) data from most subjects enrolled in these studies, because SD-OCT was not available to the majority of study centers. Nonetheless, the key result from our study is that the methods used herein to characterize and analyze Stratus time domain OCT (TD-OCT) images are suitable to identify reproducibly initial presence and subsequent resolution of VMA and macular hole.

Since the MIVI-TRUST studies were initiated, we anticipated that many clinicians would transition to SD-OCT in their practices. Accordingly, we performed an SD-OCT MIVI-TRUST substudy to compare morphologic and quantitative variables on eyes imaged with both TD-OCT and SD-OCT (data presented at the American Society of Retinal Specialists

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**Table 1.** Team Reproducibility for Qualitative OCT Evaluation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Agree</th>
<th>Total</th>
<th>Percent Agreement</th>
<th>Kappa Statistic</th>
<th>Kappa Statistic Lower 95% CL</th>
<th>Kappa Statistic Upper 95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMA</td>
<td>97</td>
<td>100</td>
<td>97</td>
<td>0.91</td>
<td>0.81</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitreous adhesion width (broad vs. focal)</td>
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<td>100</td>
<td>92</td>
<td>0.87</td>
<td>0.78</td>
<td>0.95</td>
</tr>
<tr>
<td>FTMH</td>
<td>95</td>
<td>100</td>
<td>95</td>
<td>0.87</td>
<td>0.78</td>
<td>0.95</td>
</tr>
<tr>
<td>ERM</td>
<td>82</td>
<td>100</td>
<td>82</td>
<td>0.87</td>
<td>0.78</td>
<td>0.95</td>
</tr>
<tr>
<td>Any retinal deformation at ERM</td>
<td>85</td>
<td>100</td>
<td>85</td>
<td>0.87</td>
<td>0.78</td>
<td>0.95</td>
</tr>
<tr>
<td>Fovea deformed at ERM</td>
<td>82</td>
<td>100</td>
<td>82</td>
<td>0.87</td>
<td>0.78</td>
<td>0.95</td>
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<tr>
<td>ERM at vitreomacular adhesion</td>
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<td>100</td>
<td>80</td>
<td>0.61</td>
<td>0.46</td>
<td>0.75</td>
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CL, confidence limit.
min, median, max 69, 337, 1590 50, 296.5, 1559
Mean (SD) 375.0 (212.2) 340.3 (221.2) 34.4* (93.8)

99 100 99

References


