MICROPERIMETRIC ASSESSMENT OF RETINAL SENSITIVITY IN EYES WITH DIABETIC MACULAR EDEMA FROM A PHASE 2 STUDY OF INTRAVITREAL AFLIBERCEPT

VICTOR H. GONZALEZ, MD,* DAVID S. BOYER, MD,† URSULA SCHMIDT-ERFURTH, MD,‡ JEFFREY S. HEIER, MD,§ CARMELINA GORDON, MD,¶ MATTHEW S. BENZ, MD,** DENNIS M. MARCUS, MD,†† NELSON R. SABATES, MD,‡‡ ROBERT VITTI, MD,§§ HUSAIN KAZMI, MD,§§ ALYSON J. BERLINER, MD, PHD,§§ YUHWEN SOO, PHD,§§ XIAOPING ZHU, PHD,§§ HADI MOINI, PHD,§§ OLIVER ZEITZ, MD,¶¶ RUPERT SANDBRINK, MD,¶¶ DIANA V. DO, MD‡‡‡

Purpose: To evaluate retinal sensitivity in patients with diabetic macular edema who received intravitreal aflibercept injection (IAI) or laser.

Methods: A substudy included 46 patients from DA VINCI (a randomized, double-masked Phase 2 study) receiving either laser, 0.5 mg IAI every 4 weeks, 2 mg IAI every 4 weeks, 2 mg IAI every 8 weeks after 3 monthly doses (2q8), or 2 mg IAI as-needed after 3 monthly doses for 52 weeks. Retinal sensitivity was measured in one (central), five (one central and four inner), and eight (four inner and four outer) optical coherence tomography subfields.

Results: Mean best-corrected visual acuity improvement in the subgroup at Week 52 was 3.3 letters with laser and ranged from 5.4 to 16.3 letters in the IAI groups. Retinal sensitivity of laser patients at Week 52 was comparable with baseline in the central optical coherence tomography subfield but decreased in the five and eight optical coherence tomography subfields. Compared with laser, retinal sensitivity significantly increased with IAI in the 2q8 and pooled IAI groups in the 5 and 8 optical coherence tomography subfields at Week 52 (P < 0.05).

Conclusion: Intravitreal aflibercept injection improved best-corrected visual acuity and retinal sensitivity in this subgroup of patients. Laser may cause a deterioration of macular function that is not detectable with best-corrected visual acuity testing.

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Diabetic macular edema (DME) is a major cause of vision loss among working-age individuals in developed countries.1–3 Based on results from the Early Treatment Diabetic Retinopathy Study (ETDRS), focal laser photocoagulation reduces the risk of vision loss (≥15 letters) because of DME by 50% at 3 years after treatment and has been the standard of care.4 However, laser photocoagulation minimally improves visual acuity and is associated with destruction of retinal photoreceptors, progressive enlargement of laser retinal scars, and development of choroidal neovascularization and subfoveal fibrosis.5–8 Over the past several years, anti-vascular endothelial growth factor agents have been developed, and multiple studies have shown that intravitreal vascular endothelial growth factor blockers are superior to laser photocoagulation for the treatment of DME.9 Recently, the DA VINCI study compared the efficacy and safety of four different dosing regimens of intravitreal aflibercept injection (IAI), an anti-vascular endothelial growth factor agent also known as VEGF Trap-Eye, with laser photocoagulation in patients with DME.10,11 The DA VINCI study demonstrated that IAI significantly improved best-corrected visual acuity (BCVA) and central retinal thickness compared with laser at Weeks 24 and 52 in patients with DME.10,11 High-contrast ETDRS BCVA is currently considered as a primary endpoint for assessing the functional impact of DME on the macula and measuring efficacy.
of new therapies.11–13 Conventional BCVA testing may, however, underestimate both initial macular impairment and subsequent macular response to a treatment, because it predominantly reflects foveal function.14 Best-corrected visual acuity testing may also fail to detect small scotomas.12,15 Microperimetric measurement of retinal sensitivity assesses a larger macular area than conventional BCVA testing, and therefore may be a better indicator of changes in macular function. In the DA VINCI study, a subset of patients with DME was evaluated by microperimetry to assess the changes in retinal sensitivity in response to laser photocoagulation and IAI. In this report, we present retinal sensitivity results of patients in the microperimetry substudy.

Methods

Design of the Primary Study

The DA VINCI study was a 52-week, multicenter, randomized double-masked Phase 2 clinical study comparing the efficacy and safety of four different dosing regimens of IAI with laser photocoagulation. The study protocol was approved by the institutional review board at each participating institution. All patients signed a written consent form before initiation of the study-specific procedures. The study was registered with ClinicalTrials.gov (identifier no. NCT00789477) and was conducted in compliance with regulations of the Health Insurance Portability and Accountability Act and the Declaration of Helsinki.

As described previously, the DA VINCI study randomized 221 patients with Type 1 or 2 diabetes mellitus who were older than or equal to 18 years, had clinically significant DME with central involvement defined as central retinal thickness ≥250 μm in the central subfield on optical coherence tomography (OCT), and had BCVA of 24 to 73 letters (Snellen equivalent, 20/320–20/40).10,11 Only one eye from each patient was included in the study. Eyes were randomly assigned in a 1:1:1:1 ratio to one of the 5 treatment regimens: 1) Laser photocoagulation using a modified ETDRS protocol at baseline and then as needed per prespecified criteria at a minimum of 16 weeks of intervals, 2) 0.5 mg IAI once every 4 weeks (0.5q4), 3) 2 mg IAI once every 4 weeks (2q4), 4) 2 mg IAI once every 8 weeks after 3 initial monthly doses (2q8), and 5) 2 mg IAI as-needed per prespecified criteria after 3 initial monthly doses (2PRN).10,11 Starting at Week 24, eyes treated with IAI could receive laser rescue treatment at 16-week intervals if they met prespecified criteria.11 Laser photocoagulation was applied according to a commonly used modified ETDRS treatment protocol using a 50-μm spot size and green to yellow laser light applied for 0.05 seconds to 0.1 seconds to create barely visible (light gray) burns in the areas of retinal edema.
Eight sites were chosen to participate in the protocol specified substudy based on the availability of the fundus-monitored MicroPerimeter 1 (MP-1; Nidek, Gamagori, Japan). All patients randomized at these sites were evaluated for retinal sensitivity with microperimetry.

**Outcome Measures**

The primary efficacy endpoint of the DA VINCI study was the change in BCVA from baseline at Week 24. The secondary efficacy endpoints were the change in BCVA from baseline at Week 52, the proportion of patients who gained at least 15 ETDRS letters in BCVA from baseline to Weeks 24 and 52, the change in central retinal thickness from baseline to Weeks 24 and 52, and the number of laser treatments. An exploratory endpoint of the DA VINCI study was the change in retinal sensitivity, which was measured by microperimetry in the substudy. The microperimetry results of the substudy are reported here.

Visual acuity and retinal sensitivity measurements were carried out at baseline and Weeks 4, 12, 24, 36, and 52. Visual acuity was measured using the ETDRS protocol. Retinal sensitivity was measured by the fundus-monitored MicroPerimeter 1 (MP-1; Nidek), which automatically tracks fundus movements to ensure that the anatomical landmarks revealed in the fundus photographs are aligned with the sensitivity maps generated by the perimeter. A 4-2-1-strategy with Goldmann III size stimulus was used, and a radial grid of 29 stimulus locations covering central 16° in diameter (centered onto the fovea) was applied (Figure 1). The stimuli were projected on a white background. The starting stimulus light attenuation was set at 10 dB. The duration of the stimulus was 200 milliseconds, and a red 1° radius circle was used as the fixation target. After pupil dilation, pretest training was performed in each patient, and a 5-minute visual adaptation was allowed before initiation of the test. Fixation for each patient was categorized as “stable,” “relatively unstable,” or “unstable.” In addition, the percentage of fixations within a 2° and 4° circle was collected. The mean retinal sensitivities were evaluated in the central OCT subfield (1) alone, the central OCT subfield along with 4 inner OCT subfields (1 through 5), and the 4 inner OCT subfields along with 4 outer OCT subfields without including the central subfield (2 through 9) (Figure 1).

Microperimetry datasets were sent to the Vienna Reading Center, Medical University of Vienna, Austria.

**Statistics**

The last observation carried forward approach was used to account for missing data. Between-group differences in retinal sensitivity at baseline were evaluated by analysis of covariance. Change in retinal sensitivity from baseline was analyzed using Student’s paired t-test. Differences between the IAI and laser groups in change from baseline in retinal sensitivity and BCVA were analyzed using analysis of covariance.

**Results**

A total of 46 patients were evaluated by microperimetry, of these, 37 patients (80.4%) completed all 52 weeks. Demographics and baseline characteristics of these patients are presented in Table 1. Across treatment groups, patients had similar BCVA and diabetic retinopathy severity scores at baseline. Over the 52 weeks of study, the mean (standard deviation) number of IAI injections was 11.1 (3.3) in the 0.5q4 group, 12.0 (1.5) in the 2q4 group, 8.0 (0.0) in the 2q8 group, and 6.1 (3.9) in the 2PRN group. The IAI groups received an average of 1.4 to 1.8 laser treatments out of 2 laser treatments allowed between Months 6 and 12. Patients in the laser group received a mean (standard deviation) of 2.8 (0.8) laser treatments over the 52 weeks of study.

**Visual Acuity**

The range of BCVA improvements in the IAI groups was 2.5 to 13.1 letters at Week 24, and 5.4 to 16.3 letters at Week 52 in patients who were in the microperimetry substudy (Figure 2). Best-corrected visual acuity was improved with laser by 0.9 and 3.3 letters, respectively, at Weeks 24 and 52 in the...
substudy patients (Figure 2). The differences between the IAI and laser groups in BCVA changes were not statistically significant in these subgroup of patients (Figure 2), likely because of small sample size.

Retinal Sensitivity

Retinal sensitivity was determined in 3 different settings: the central OCT subfield alone, the central OCT subfield along with 4 inner OCT subfields (1 through 5), and 4 inner and 4 outer OCT subfields without including the central subfield (2 through 9). Baseline retinal sensitivities were similar across treatment groups in each setting (Table 2).

When retinal sensitivity was analyzed in the central OCT subfield alone, the increase from baseline with IAI was significant for the 2PRN and pooled IAI groups at Week 24 and for the 2q8, 2PRN, and pooled IAI groups at Week 52 (Figure 3A). Central retinal sensitivity of patients treated with laser was comparable with baseline at Weeks 24 and 52. The mean retinal sensitivity change from baseline in the 2PRN and pooled IAI groups was significantly greater than that in the laser group at Week 24 (Figure 3A).

When retinal sensitivity was analyzed in central and inner OCT subfields (1 through 5) or 4 inner and 4 outer OCT subfields (2 through 9), the IAI groups had an increase in retinal sensitivity while the laser group had a decrease in retinal sensitivity (Figure 3, B and C). In both analyses, the increase from baseline in retinal sensitivity with IAI was significant for the 2q8 and pooled IAI groups at Weeks 24 and 52 (Figure 3, B and C). An additional significant change from baseline was observed for the 2PRN group at Week 24 only in the central and inner OCT subfields (1 through 5) analysis (Figure 3B). In both analyses, the mean retinal sensitivity change from baseline in the 2q4 and pooled IAI groups was significantly greater than that in laser group at both Weeks 24 and 52 while the mean change from baseline in the 2PRN group was significantly greater than that in laser only at Week 24 (Figure 3, B and C). Additional between-group differences compared with laser were observed for the 2q4 and 0.5q4 groups at Week 52, respectively, in the central and inner OCT subfields (1 through 5) and 4 inner and 4 outer OCT subfields (2 through 9) analyses.

Fixation Stability and Location

In the laser group, the number of patients who were categorized as “stable” was 6 of 10 (60%) at baseline and 5 of 10 (50%) at Week 52. In the pooled IAI group, the number of patients categorized as “stable”

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Table 1. Demographics and Baseline Characteristics of Patients with DME in the Microperimetry Substudy

<table>
<thead>
<tr>
<th>IAI Treatment Groups</th>
<th>Laser (n = 11)</th>
<th>0.5q4 (n = 10)</th>
<th>2q4 (n = 7)</th>
<th>2q8 (n = 8)</th>
<th>2PRN (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>65.0 (6.7)</td>
<td>64.7 (7.9)</td>
<td>57.7 (5.4)</td>
<td>57.5 (11.9)</td>
<td>58.8 (10.2)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>9 (81.8)</td>
<td>6 (60.0)</td>
<td>3 (42.9)</td>
<td>3 (37.5)</td>
<td>6 (60.0)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>9 (81.8)</td>
<td>10 (100.0)</td>
<td>7 (100.0)</td>
<td>7 (87.5)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Black</td>
<td>1 (9.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other*</td>
<td>1 (9.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mean diabetic retinopathy severity score (SD)†</td>
<td>3.4 (0.5)</td>
<td>3.7 (0.7)</td>
<td>3.4 (0.5)</td>
<td>3.5 (0.8)</td>
<td>3.4 (0.5)</td>
</tr>
<tr>
<td>Mean BCVA, ETDRS letters (SD)</td>
<td>60.2 (7.8)</td>
<td>57.0 (15.1)</td>
<td>60.4 (13.6)</td>
<td>63.6 (9.8)</td>
<td>66.3 (10.9)</td>
</tr>
</tbody>
</table>

*Asian for the laser group and multi-racial for the 2q8 group.
†Severity of diabetic retinopathy was graded as none = 1, mild = 2, moderate = 3, severe = 4, or proliferative = 5.
0.5q4, 0.5 mg monthly; 2q4, 2 mg monthly; 2q8, 2 mg every 2 months after 3 initial monthly doses; SD, standard deviation.

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Fig. 2. Mean changes from baseline in BCVA of patients with DME in the microperimetry substudy. For missing data, the last observation was carried forward. Error bars indicate 95% confidence intervals. The number of patients in each treatment group is as indicated in the legend except for Weeks 4 and 12 in which a few patients missed BCVA assessment. Baseline assessments cannot be carried forward using last observation carried forward. At Week 4, the number of patients in the laser, 0.5q4, 2q4, 2q8, 2PRN, and pooled IAI groups was 8, 7, 5, 7, 8, and 27, respectively. At Week 12, the number of patients in the laser, 0.5q4, 2q4, 2q8, 2PRN, and pooled IAI groups was 8, 9, 5, 8, 9, and 31, respectively. 0.5q4, 0.5 mg monthly; 2q4, 2 mg monthly; 2q8, 2 mg every 2 months after 3 initial monthly doses.
Safety quantifying imaging with automatic perimetry has enabled the introduction of a microperimetry system combining digital fundus morphologic and functional changes. 

In the microperimetry substudy, the number of patients who experienced at least 1 ocular adverse event was 7 (of 11 patients) in the laser group, 8.2% and −1.2%, respectively. Percentage of fixations within a 2° circle remained essentially unchanged in both pooled IAI and laser groups with a mean change from baseline of 2.0% and −0.6% at Week 52, respectively.

Safety

The safety profile of IAI in all patients who participated in the DA VINCI study has been reported previously.10,11 In the microperimetry substudy, the number of patients who experienced at least 1 ocular adverse event was 7 (of 11 patients) in the laser group, and for patients treated with IAI, ranged from 6 patients (of 10 patients) in the 2PRN group to 9 patients (of 10 patients) in the 0.5q4 group. Serious ocular adverse events occurring during the substudy were reduced visual acuity, uveitis, and angle closure glaucoma, which were reported each for 1 patient in the laser (1 of 11 patients), 0.5q4 (1 of 10 patients), and 2q4 (1 of 7 patients) groups, respectively.

Discussion

Macular diseases are assessed on the basis of morphologic and functional changes. Introduction of a microperimetry system combining digital fundus imaging with automatic perimetry has enabled the quantification of retinal sensitivity.16 Accumulating evidence suggests that microperimetric assessment of retinal sensitivity may provide additional useful information over that obtained from BCVA testing, regarding the degree and pattern of changes in macular function, scotoma size, and the response to a treatment intervention.12,17,18

The results of this study demonstrate that, in parallel with the improvements in BCVA, patients treated with intravitreal aflibercept had increased macular sensitivity from baseline, mostly ranging from 1 dB to 4 dB. The magnitude of increases in retinal sensitivity after IAI was consistent with previous studies of retinal sensitivity, which defined a significant improvement as an increase in retinal sensitivity by ≥1 dB, ≥2 dB, or >2 dB.14,19,20

In contrast to treatment with intravitreal aflibercept, patients treated with laser did not demonstrate an increase in retinal sensitivity. Retinal sensitivity measurements with and without including the central OCT subfield allowed for the evaluation of the contribution of the central OCT subfield, which is typically not exposed to laser because of its close proximity to the foveal center. The loss of retinal sensitivity in patients treated with laser was more prominent (mostly >1 dB) when additional OCT subfields were included along with the central OCT subfield in the microperimetric analysis (central and inner OCT subfields, 1 through 5) or when the central OCT subfield was excluded from the analysis (4 inner and 4 outer OCT subfields, 2 through 9), indicating that the loss of retinal sensitivity was mostly confined to inner and outer OCT subfields where laser was originally applied. Given the marginal improvements in mean BCVA of the substudy patients, the findings suggest that laser may cause a deterioration of macular function that is not detectable with BCVA testing.

Several studies have investigated the changes in retinal sensitivity of patients with DME after treatment with laser photocoagulation, intravitreal triamcinolone acetonide, and ranibizumab. Consistent with our findings, modified ETDRS laser photocoagulation was shown to reduce retinal sensitivity.6,21 After intravitreal triamcinolone acetonide, retinal sensitivity

Table 2. Baseline Retinal Sensitivities in the Microperimetry Substudy

<table>
<thead>
<tr>
<th>Subfield</th>
<th>Mean ± SD Baseline Retinal Sensitivity, dB (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser (n = 10)</td>
<td>5.65 ± 5.18*</td>
</tr>
<tr>
<td>IAI 0.5q4 (n = 9)</td>
<td>6.14 ± 4.90</td>
</tr>
<tr>
<td>IAI 2q4 (n = 7)</td>
<td>8.79 ± 5.68</td>
</tr>
<tr>
<td>IAI 2q8 (n = 7)</td>
<td>4.25 ± 3.77</td>
</tr>
<tr>
<td>IAI 2PRN (n = 7)</td>
<td>7.93 ± 5.14</td>
</tr>
<tr>
<td>IAI 0.5q4 (n = 9)</td>
<td>6.14 ± 4.90</td>
</tr>
<tr>
<td>IAI 2q4 (n = 10)</td>
<td>7.93 ± 5.14</td>
</tr>
<tr>
<td>IAI 2q8 (n = 10)</td>
<td>4.25 ± 3.77</td>
</tr>
<tr>
<td>Pooled IAI group (n = 33)</td>
<td>6.84 ± 5.00</td>
</tr>
</tbody>
</table>

Mean ± SD baseline retinal sensitivities are shown for central OCT subfield, central and 4 inner OCT subfields (1 through 5), and 4 inner and 4 outer OCT subfields (2 through 9).

*P < 0.2005 versus IAI (single or pooled group).
0.4q4, 0.5 mg monthly; 2q4, 2 mg monthly; 2q8, 2 mg every 2 months after 3 initial monthly doses; SD, standard deviation.
Fig. 3. Mean changes from baseline in retinal sensitivity of patients with DME in the microperimetry substudy. Retinal sensitivities were evaluated in central OCT subfield (A), central and 4 inner OCT subfields (1 through 5) (B), and 4 inner and 4 outer OCT subfields (2 through 9) (C) over 52 weeks. For missing data, the last observation was carried forward. *P < 0.05 versus baseline; †P < 0.01 versus baseline; ‡P < 0.05 versus laser; ††P < 0.01 versus laser. The number of patients in each treatment group is as indicated in the legend except for Weeks 4 and 12 in which a few patients missed retinal sensitivity assessment. Baseline assessments cannot be carried forward using last observation carried forward. At Week 4, the number of patients in the laser, 0.5q4, 2q4, 2q8, 2PRN, and pooled IAI groups was 8, 7, 5, 7, 8, and 27, respectively. At Week 12, the number of patients in the laser, 0.5q4, 2q4, 2q8, 2PRN, and pooled IAI groups was 8, 8, 5, 7, 9, and 29, respectively. 0.5q4, 0.5 mg monthly; 2q4, 2 mg every 2 months after 3 initial monthly doses.
showed no improvement at 1 week, despite a significant improvement in BCVA at 1 week, but was significantly improved at 1 month. In contrast, a single ranibizumab administration significantly improved retinal sensitivity as early as 1 hour after the injection, lasting for 56 days postinjection. The findings of this study provide evidence for sustained improvements in retinal sensitivity after repeated IAI over a period of 52 weeks.

In the DA VINCI study, the exploratory analysis of retinal sensitivity was limited by the small number of patients in the microperimetry sub-study. The relationship between retinal sensitivity, fixation stability, and BCVA in DME remains unclear. Although one study found no relationship between macular sensitivity and fixation stability, another study found that fixation impairment was negatively associated with central retinal sensitivity and BCVA. In this study, patients in the pooled IAI group had an increased retinal sensitivity and relatively more stable fixation compared with patients in the laser group at Week 52. Similarly, a number of studies reported no relationship between retinal sensitivity and BCVA, whereas others found a positive correlation between these two variables.

A recent study suggested that retinal sensitivity may be a predictor of visual outcomes after interventions in eyes with DME. Our findings indicate that microperimetric assessment of retinal sensitivity may provide additional information to more sensitively identify functional and non-functional areas of the retina when compared with BCVA testing alone. Larger clinical studies are warranted to explore the relationship between visual function, retinal sensitivity, and fixation stability, and the potential value of microperimetric assessment of retinal sensitivity and fixation stability in predicting visual outcomes in response to a treatment intervention in patients with DME.

**Key words:** DA VINCI, DME, intravitreal aflibercept injection, laser, macular function, microperimetry, retinal sensitivity, VEGF Trap-Eye.

**References**


