Effects of Intravitreal Ranibizumab on Retinal Hard Exudate in Diabetic Macular Edema

Findings from the RIDE and RISE Phase III Clinical Trials

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Purpose: To evaluate the effect of monthly intravitreal ranibizumab on hard exudate (HE) area and the impact of HE on visual acuity (VA) outcomes in diabetic macular edema (DME) patients using data from 2 phase III clinical trials.

Design: Exploratory analyses of phase III, randomized, double-masked, sham-controlled, multicenter clinical trials.

Participants: Adults with DME, baseline best-corrected VA 20/40 to 20/320 Snellen equivalent, and central foveal thickness of ≥275 μm.

Methods: Between the 2 studies, 759 patients with DME were randomized to receive monthly 0.3 or 0.5 mg intravitreal ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA) or sham injections.

Main Outcome Measures: Hard exudate area was assessed from color fundus stereophotographs both on an ordinal scale and using continuous estimates of areas within the Early Treatment Diabetic Retinopathy Study grid.

Results: Data from 739 eyes were available for analysis. Mean baseline HE area was similar across treatment groups, ranging from 0.65 to 0.82 mm². Through month 24, the percentage of eyes without HE increased from 20.9% to 36.3% in the sham group and from 22.1% to 61.3% and 23.6% to 62.0% in the ranibizumab 0.3-mg and 0.5-mg groups, respectively. Resolution of HE became apparent sometime after month 6 in ranibizumab-treated eyes. At baseline, there was no meaningful correlation between VA and presence or absence of HE. After baseline, there also was no consistent correlation between presence or absence of HE and change in VA over time.

Conclusions: In this exploratory analysis, monthly intravitreal ranibizumab resulted in significantly greater reduction of HE area compared with sham (P < 0.0001). In contrast to the rapid effects of ranibizumab on macular edema, changes in HE area were more gradual. Contrary to prior expectations, the presence and area of HE did not increase as DME resolved (either in the ranibizumab or sham groups). Importantly, baseline VA was not correlated with presence of HE, nor was the therapeutic benefit of ranibizumab on VA affected negatively by the presence of HE. These data suggest that in the context of intravitreal anti–vascular endothelial growth factor therapy, the presence of HE is not a prognostic indicator of poor visual outcomes. Ophthalmology 2015;122:779-786 © 2015 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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visual acuity outcomes. In recent years, intravitreal therapy with anti-vascular endothelial growth factor (VEGF) has emerged as the treatment of choice for DME, either independently or in combination with macular laser. In phase III trials, intravitreal injections of ranibizumab (Lucentis; Genentech, Inc., South San Francisco, CA), an anti-VEGF agent, have been shown to improve visual acuity in patients with DME, to improve retinal anatomic features (both macular edema and retinopathy severity), and to reduce the risk of subsequent vision loss.

The purpose of the current analysis was to assess the effect of intravitreal anti-VEGF therapy on retinal HE in patients with DME and to assess the impact of retinal HE on DME treatment outcomes. The timing and degree of resolution and the effect of HE on visual function also were assessed. Data collected as part of phase III randomized studies of ranibizumab in patients with center-involved DME, which included frequently acquired fundus photographs throughout a 24-month double-masked treatment period, provides a rich data set for exploration of this topic.

Methods

Clinical Trial Design

The RIDE and RISE studies are methodologically identical phase III, double-masked, sham injection-controlled, randomized clinical trials of ranibizumab in center-involved DME. Details of the methodology and key visual acuity and safety findings have been described previously. Study protocols were approved by institutional review boards and ethics committees, and participants provided written informed consent. The 2 completed studies are registered on clinicaltrials.gov (RIDE identifier, NCT00473382; and RISE identifier, NCT00473330).

Briefly, individuals 18 years of age or older with decreased vision resulting from DME (study eye best-corrected visual acuity of 20/40 to 20/320 approximate Snellen equivalent) and central subfield thickness 275 μm or more on time-domain OCT were eligible for enrollment. Study inclusion criteria are detailed elsewhere.

One eye per patient was randomized to monthly sham injections or monthly intravitreal injections of 0.3 or 0.5 mg ranibizumab through month 24. Macular laser was available to all patients per protocol-specified criteria, beginning at month 3. Although the phase III program continued to follow up and treat patients beyond month 24, the data derived from the first 24 months of the RIDE and RISE studies are the focus of this report.

Grading Protocol

Stereoscopic 7-field dilated color fundus photographs were obtained at each patient’s screening visit and at months 3, 6, 12, 18, and 24. Photographs were evaluated at the University of Wisconsin Fundus Photograph Reading Center (Madison, WI) by trained evaluators masked to both treatment assignment and images from previous visits. Evaluation included assessment of severity of diabetic retinopathy and features of macular edema. Macular edema evaluation included a detailed grading of HE within the modified ETDRS grid. The presence of HE within the grid and at the center of the macula was assessed. The area of HE in each subfield is estimated using standard drusen circles, similar to drusen area assessment. Cumulative areas of HE were derived for the central and inner subfields and for the entire grid (Fig 1). The ETDRS classification based on a set of standard photographs was used for categorical assessment of HE. Based on these standard photographs, the area of HE was classified as definite (>0–0.1 mm²), obvious (>0.1–0.5 mm²), moderate (>0.5–2.5 mm²), or severe (>2.5 mm²). Reproducibility of HE evaluation was assessed by periodic re-evaluation of the images (see details in “Results”).

Statistical Analysis

The percentage of patients with HE was compared across the 3 arms. Cross-tabulations were used to compare change in HE status over time. For the purposes of this report, a change of 2 steps in the HE category was considered a clinically significant outcome. Pearson correlation coefficients were used to compare baseline HE with change in visual acuity. Reproducibility was assessed with percentage agreement and κ statistics for the ordinal scale and Bland-Altman plots for the area measurements.
Results

A total of 759 patients with DME were randomized to sham or 1 of the 2 active ranibizumab treatment arms. Baseline fundus photographs were available for 746 patients, of which 739 (97%) could be evaluated. The number of study eyes with HE at baseline was similar across all groups: 200 (79.1%) in the sham group, 187 (77.9%) in the 0.3-mg ranibizumab group, and 188 (76.4%) in the 0.5-mg ranibizumab group. The mean area standard error of HE at baseline was $0.82 \pm 0.06$, $0.65 \pm 0.06$, and $0.68 \pm 0.06$ mm$^2$ for the sham, 0.3-mg ranibizumab, and 0.5-mg ranibizumab groups, respectively. The approximately 0.1-mm$^2$ difference in mean HE area across the groups is too small to be relevant in the scheme of the classification, considering the magnitude of the differences in HE severity categories and the range of areas observed. At baseline, the distribution of ETDRS classification of HE also was similar in the 3 groups (Table 1).

Over time, resolution of HE area was significantly more common in the ranibizumab-treated groups, although some improvement of HE area was observed in the sham group as well (Fig 2). A difference in the presence of HE between the sham and ranibizumab-treated groups was evident at month 12, with approximately 40% to 43% of ranibizumab-treated eyes having no HE compared with 26% of eyes in the sham injection group. Through 24 months, the percentage of study eyes in the HE absent category increased from 20.9% to 36.3% in the sham arm and from 22.1% to 61.3% and 23.6% to 62.0% in the 0.3- and 0.5-mg ranibizumab arms, respectively. Thus, more than 60% of ranibizumab-treated eyes experienced complete resolution of HE compared with 36% of eyes in the sham group ($P < 0.0001$). Figure 3 shows a cross-tabulation between HE category at baseline compared with the category at month 24. Hard exudate area improved in most eyes, with a higher proportion of patients demonstrating a 2-step improvement (e.g., HE area reduction from moderate [$>0.5-2.5$ mm$^2$] to definite [$>0-0.1$ mm$^2$]) in the ranibizumab arms compared with sham. At month 24, 2-step improvement in HE category occurred in 24% of eyes in the sham group and 39% in the 2 ranibizumab treatment arms ($P = 0.001$, chi-square test). In eyes with severe HE at baseline ($n = 21$), 11 eyes treated with ranibizumab demonstrated a 2-step or more improvement in HE category compared with 3 eyes in the sham arm.

There was no correlation between baseline visual acuity and the total area of HE in any of the 3 treatment groups (Fig 4); Pearson correlation coefficients were $-0.16$ (sham), $-0.01$ (0.3 mg ranibizumab), and $-0.18$ (0.5 mg ranibizumab). The presence of central HE (i.e., in the center of the fovea) also was not correlated meaningfully with baseline visual acuity (Pearson correlation coefficient, $-0.1153; P = 0.0056$). Figure 5 shows the change over time in visual acuity in patients in the ranibizumab and sham groups, categorized according to the status of central HE at baseline. In the ranibizumab groups, eyes with central HE at baseline seemed to trend toward greater improvements in visual acuity compared to eyes without central HE.
HE. However, the trend was statistically significant in the 0.3-mg ranibizumab arm only.

In patients randomly assigned to sham injections, mean improvements in OCT central foveal thickness from baseline after 24 months were statistically significantly greater in patients with HEs present at baseline ($109.41 \pm 82.25 \mu m$; $P = 0.0231$). The mean reduction in OCT central foveal thickness from baseline at month 24 was not statistically different in eyes with or without baseline HE in patients treated with 0.3 or 0.5 mg ranibizumab ($P = 0.3779$ and $P = 0.6041$, respectively; Table 2, available online at www.aaojournal.org).

To validate further the HE grading metric used in this report, repeat grading was performed on 117 eyes. Intergrader concordance was 92.5% in the 1-step improvement group and 99.6% in the 2-step group on the ordinal scale, with a weighted $k$ of 0.73 (95% confidence interval, 0.7–0.77). Intraclass correlation coefficient for the area measurement was 0.87, with a mean difference of 0.04 $mm^2$ (95% confidence interval, −0.71 to 0.63).

**Discussion**

Monthly intravitreal ranibizumab has a beneficial effect on reduction of HE area, as shown in this exploratory analysis from phase III randomized clinical trials in patients with DME. Most eyes (>75%) had HE at baseline. There was a significant resolution of HE in more than 60% of eyes in the ranibizumab groups compared with only 36% in the sham group at the end of the 24-month follow-up period. Very few eyes in any treatment group had a worsening of HE at any time during the study.

It has been hypothesized previously that treatment for DME (with laser photocoagulation, for example) can leave residual HE as an extracellular precipitate within the retina as the macular edema resolves.25,26 In contrast, the current study shows that neither the presence nor overall area of HE increases as DME resolves. This was observed across all treatment groups. Note that approximately 72% of patients in the sham group received macular laser treatment during the RIDE and RISE studies.22 Images were obtained at baseline and every 3 to 6 months thereafter, so it is possible, but unlikely, that the hypothesized phenomenon of increased HE coinciding with edema resolution could have occurred within this duration and was missed because of the timing of image capture. This is unlikely
because the HE area would have had to increase within the initial 3-month period and then decrease again to baseline levels because the area of HE was very similar at baseline and month 3. A detailed examination of the categorical shift in HE over time shows that the resolution of HE in both ranibizumab arms is not evident before 6 months of treatment. In contrast, the RIDE and RISE trials showed that resolution of edema and improvement in vision in the ranibizumab group started as early as day 7 after the first intravitreal treatment. In an assessment of pooled treatment groups of the Diabetic Retinopathy Clinical Research Network Protocol I interventional study of DME, Bressler et al. showed that the presence of retinal HE seen on color photographs was associated with more favorable OCT outcomes (i.e., better reduction in thickness) after adjusting for baseline central subfield thickness. The authors hypothesized that presence of HE may indicate retinal microvascular hyperpermeability as the pathological mechanism for edema, which is amenable to the pharmacologic inhibition of VEGF with ranibizumab. Hard exudates could be indicative of a nonischemic process and therefore could respond to anti-VEGF therapy. In the RIDE and RISE studies, although eyes with HE at the center tended toward larger gains in visual acuity from baseline, no statistically significant differences in visual gain or central retinal thickness change were observed in patients who did or did not have exudate at the center before starting treatment with ranibizumab. By contrast, eyes in the sham injection group with HE present at baseline demonstrated a significantly greater reduction in central retinal thickness after 24 months of observation (plus macular laser in approximately 75% of patients), compared with eyes having no HE at baseline. Other possible mechanisms of action are direct effect and clearance by phagocytic inflammatory cells such as macrophages.

The main weakness of the current study is the exploratory (post hoc) nature of the analysis. The RIDE and RISE phase III studies primarily were designed to evaluate the efficacy of ranibizumab on visual acuity, not the effect

Figure 4. Scatterplot showing baseline visual acuity versus baseline total area of hard exudate (HE) in the study eye by baseline center HE status. Horizontal rules are trendlines. ETDRS = Early Treatment Diabetic Retinopathy Study; VA = visual acuity.
of ranibizumab on the presence or extent of retinal HE. However, there are several strengths to the current analysis. These include the relatively large number of eyes with evaluable photographs (739 eyes total between the 2 studies) and the fact that the distribution of HE area was similar in the 3 treatment arms at baseline. Additionally, the fundus photographs were obtained by certified photographers, obtained serially at protocol-prescribed intervals throughout the 24-month study period, and evaluated by trained and masked evaluators at an
independent reading center with specific expertise in diabetic retinopathy imaging. Finally, the cohort studied is one of a very few with exposure to monthly ranibizumab for 24 months. This allows for an assessment of the effect of anti-VEGF therapy with ranibizumab on area of HE in the absence of potentially confounding circumstances, such as adherence to a re-treatment protocol, or concomitant use of other therapies, such as corticosteroids or other anti-VEGF drugs.

In conclusion, monthly intravitreal ranibizumab results in a significant reduction of the area of HE compared with sham therapy, rescue macular laser, or both. The effect was profound, with most eyes treated with ranibizumab having an absence of HE at the end of the study period. In contrast to the rapid effect of ranibizumab on visual acuity and retinal thickness, the effect of ranibizumab on HE area seems to be more gradual, with a detectable effect no earlier than 6 months after treatment is initiated. In the current study, baseline HE was not meaningfully correlated with baseline visual acuity or with change in visual acuity after treatment with ranibizumab, although those with central HE at baseline trended toward better visual acuity outcomes. These findings suggest that in eyes with concomitant DME and moderate areas of HE at baseline, it can be expected that the area of HE will be reduced gradually with ranibizumab therapy. Importantly, the use of ranibizumab should not be discouraged in eyes with HE at baseline because the presence of HE does not predict an inferior visual outcome compared with eyes that do not have HE at presentation.

References


**Footnotes and Financial Disclosures**

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Abbreviations and Acronyms:
**DME** = diabetic macular edema; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **HE** = hard exudate; **OCT** = optical coherence tomography; **VEGF** = vascular endothelial growth factor.

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