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Predictive imaging biomarkers relevant for functional and anatomical outcomes during ranibizumab therapy of diabetic macular oedema

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ABSTRACT

Background/aims The objective is to identify imaging biomarkers in optical coherence tomography predicting functional/anatomical outcomes in diabetic macular oedema (DMO).

Methods The presented study is a post hoc analysis of the RESTORE/RESTORE-extension studies. Best-corrected visual acuity (BCVA) was analysed using general estimating equation models using treatment group/morphological features as predictor variables. In addition, linear multiple regression models analysed BCVA gain up to 12 and 36 months with BCVA/morphological baseline characteristics as independent predictor variables. The correlations between central retinal thickness (CRT)/BCVA were calculated as Spearman's/Pearson's correlation coefficients.

Results A weak negative linear correlation between CRT/BCVA was observed in all study arms at baseline (r=−0.34, p<0.001) and at month 36 (r=−0.26, p<0.001). Patients with baseline height of intraretinal cystoid fluid (IRC) ≤380 µm had better baseline BCVA compared with patients with IRC height >380 µm (64.84±10.63 vs 61.66±9.92 letters; p=0.0071, respectively), which was maintained until the end of month 12 (70.5±12.33 vs 67.0±14.09 letters; p=0.0252, respectively). With laser, there was a trend for patients with subretinal fluid (SRF) at baseline to lose BCVA letters at month 12 (−5.38±16.54 vs 2.49±9.72 letters; p=0.1038), whereas ranibizumab patients trended towards higher BCVA gains (10.28±7.14 vs 6.76±7.67; p=0.0563), compared with those without SRF. With combined therapy, all patients had similar BCVA gains regardless of SRF (p=0.3768).

Conclusion With ranibizumab treatment, the height of IRC spaces at baseline was a better predictor of functional/anatomical improvement than CRT alone. There was also a trend for SRF to show a positive impact on ranibizumab therapy response and a negative impact on laser therapy response.

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INTRODUCTION

Diabetic macular oedema (DMO) is a leading sight-threatening complication in diabetes, especially in industrial countries. In the USA, 25.9% of the population aged 65 or older are diagnosed with diabetes. About every 4th person (28.5%) with diabetes will be diagnosed with diabetic retinopathy during the course of their disease and about every 20th person (4.4%) will have advanced diabetic retinopathy, such as DMO, that can lead to severe vision loss.[1]

Antivascular endothelial growth factor (anti-VEGF) agents, such as ranibizumab, approved for intravitreal injection, have been routinely used for the treatment of DMO in most European industrialised countries since its European approval in 2011. Studies have since shown a large variability in a patient's anatomic and functional outcomes in response to therapy.[2–9] It is therefore of substantial scientific interest to identify if there are baseline or other characteristics observable on optical coherence tomography (OCT) images of individual patients early in the disease course that could potentially predict outcomes of DMO therapy. Imaging, especially with OCT, has been proven to deliver predictive biomarkers in prior investigations.[2–9]

Imaging in ophthalmology has allowed the precise morphological evaluation of the microstructural features of DMO. Whereas the ETDRS relied on two-dimensional colour fundus photographs, the three-dimensional evaluation of OCT provides additional depth information of the oedema. Morphological features such as intraretinal cystoid fluid (IRC), subretinal fluid (SRF) or pigment epithelial detachments have already been identified as predictive factors for visual and anatomical outcomes during anti-VEGF therapy in other retinal diseases such as neovascular age-related macular degeneration (nAMD).[10]

In all major trials investigating anti-VEGF therapy in DMO, the only morphological study end point was central retinal thickness (CRT).[11–15] A defined CRT cut-off point has been used previously to distinguish between clinically significant macular oedema and a retina considered 'healthy' for these trials. However, there are many more morphological features such as IRC, with different localisation in various retinal layers such as the outer nuclear layer, inner nuclear layer or ganglion cell layer; or SRF that also plays a major role in DMO, besides known parameters from two-dimensional analysis, such as vascular leakage on fluorescein angiography. Small-scale scientific studies have investigated the role of other potential markers for therapy outcomes in DMO. A disruption of the external limiting membrane, the photoreceptor layers,[2] the inner retinal layers[3 4] or the thinning

of layers, such as the ganglion cell layer,[5] have been shown to be relevant biomarkers for worse functional outcomes in DMO treated with anti-VEGF agents.

The primary objective of this study was to perform a post hoc analysis of the RESTORE and RESTORE-extension studies to evaluate several morphological features with the potential to be used as predictive biomarkers for visual and anatomical outcomes during ranibizumab, combined or retinal laser photocoagulation therapy.

METHODS

Study design

This study is an exploratory post hoc analysis of the randomised, prospective 1-year RESTORE and 2-year follow-up RESTORE-extension studies, which were conducted in accordance with the Declaration of Helsinki and the standards of Good Clinical Practice. The institutional review boards or ethics committees at each participating centre approved the RESTORE and RESTORE-extension studies. Prior to the RESTORE studies, each participant gave written informed consent.

Detailed information on the RESTORE programme has been published previously.[11 16 17] The RESTORE core trial was a randomised, double-blind, laser-controlled, multicentre, phase III study in patients with visual impairment due to DMO. Patients (n=345) were randomised 1:1:1 to receive either intravitreal ranibizumab (0.5 mg) injection plus sham retinal laser photocoagulation (subsequently referred to as the ranibizumab group), intravitreal ranibizumab (0.5 mg) injection plus active retinal laser photocoagulation (subsequently referred to as the combined group) or sham injection plus active retinal laser photocoagulation (subsequently referred to as the laser group). Out of the cohort of 345 patients from the RESTORE core study (months 0–12), 240 patients were included in the 13–36-month extension study. In the RESTORE-extension study, all patients could receive individualised ranibizumab according to prespecified stability-based best-corrected visual acuity (BCVA) and disease progression re-treatment criteria. All patients were eligible to receive laser pro re nata (PRN) in accordance with ETDRS guidelines at the investigators' discretion. The RESTORE study aimed to demonstrate superiority of ranibizumab or combined therapy over laser therapy only. Details on patients, demographic data and the inclusion and exclusion criteria for the study are provided in the original study publications.[11 16 17] The RESTORE and RESTORE-extension studies are registered with www.clinicaltrials.gov as NCT00687804 and NCT00906464.

This post hoc analysis of RESTORE and RESTORE-extension study data aimed to measure and assess morphological characteristics (CRT, IRC and SRF) in patients with DMO and to evaluate any potential relationship between BCVA outcomes and CRT, IRC or SRF at baseline, 3 months, 1 year and 3 years.

Visual acuity testing and (re-)treatment

BCVA using ETDRS study charts was assessed at every study visit. Demographic data (age, gender, diabetes type, duration of diabetes, duration of DMO) and treatment parameters (laser, ranibizumab, concomitant medication) were evaluated for each patient.

In year 1, patients in the ranibizumab and combined groups received three initial monthly injections of ranibizumab 0.5 mg. Patients were treated monthly until stable vision was achieved, defined by either an observed BCVA letter score of ≥84 or if the investigator felt no further BCVA improvement had been achieved with the previous two consecutive treatments. Once stability was reached, treatment was administered PRN (as required with monthly visits). If a decrease in BCVA due to DMO was observed by the investigator, monthly ranibizumab treatment was resumed until stable BCVA was again reached. Patients in the laser therapy groups received an active laser treatment on day 1 of the study. This treatment was either performed at once or split into two sessions 4 weeks apart. Re-treatment was performed according to ETDRS guidelines no more often than every 3 months. In the combined group, re-treatment of ranibizumab and laser was done independently of the other treatment.

In years 2 and 3, all patients received 0.5 mg ranibizumab injections with PRN re-treatment criteria from year 1 (months 3–11) and were eligible to receive laser treatment according to ETDRS re-treatment criteria. Further detail on the treatment regimens can be found in the original study publications.[11 16 17]

Standardised image acquisition and evaluation

Each patient was imaged monthly by operators certified by the Vienna Reading Center (VRC) using Stratus OCT devices V.4.0 (Carl Zeiss, Meditec, Dublin, California, USA). The OCT imaging protocol consisted of a fast macular thickness map with six radial cross-sectional images, each scan consisting of 128 a-scans; and a 6-mm-crosshair scan with two orthogonal b-scans with 512 a-scans each. Scans were anonymised and exported as digital raw data sets.

All images were independently evaluated at the VRC. The reading centre performed a masked, standardised evaluation on every image using a predefined evaluation protocol and custom software (with high-quality control and grading reproducibility standards, reported previously[18]). The six radial scans allowed quantitative measurement of retinal thickness in all ETDRS subfields and the foveal centrepoint and the crosshair scans were used to qualitatively evaluate retinal morphology, most importantly IRC and SRF. IRC was defined as round, minimally reflective spaces within the neurosensory retina. The height of IRC was measured at the foveal centre. If more than one IRC lesion was present in the centrepoint, all individual IRC heights were summed to a total height value. IRC was divided by height into two groups, small and large IRC, as defined in the following paragraph. The position of the IRC with regards to retinal layers (outer nuclear layer, inner nuclear layer, ganglion cell layer) was identified. Height was chosen as measurement of IRC volume in time-domain OCT is not convincing as only six radial cuts are available. SRF was identified as a non-reflective space between the posterior boundary of the neurosensory retina and the retinal pigment epithelium reflection and its mean height at the foveal centrepoint, maximum height and mean width were measured.[18]

Statistical analysis

All statistical analyses were performed on the full analysis set defined in the study (115 patients in the ranibizumab arm, 118 patients in the combined arm, 110 patients in the laser arm). BCVA outcomes were analysed using general estimating equation models with compound symmetry for correlation matrix using treatment group and morphological features (presence of SRF, IRC height) at baseline as predictor variables. In addition,

Table 1 Morphological fluid features and their measurement parameters at baseline, month 3, month 12 and month 36

	IRC at CP (%)	IRC anywhere (%)	IRC mean height at CP (µm)	SRF at CP (%)	SRF anywhere (%)	SRF mean height at CP (µm)	SRF max. height (µm)	SRF mean width (µm)
Baseline								
All arms combined (n=329)	75.5	95.7	427±226	20.8	22.8	118±103	533	1182±960
Ranibizumab (n=112)	74.3	95.4	394±197	22.9	26.9	108±86.9	374	1110±663
Combined (n=114)	75.0	94.6	425±214	20.5	21.4	134±113.8	483	1232±1226
Laser (n=103)	77.4	97.2	461±262	18.9	20	116±117.3	533	1223±1019
Month 3								
All arms combined (n=323)	54.1	83.0	390±229	6.1	7.0	46±29.8	125	734±579
Ranibizumab (n=112)	42.9	79.5	405±245	8.0	8.0	46±37.2	125	771±692
Combined (n=114)	50.0	79.0	343±193	1.8	1.8	49±n/a	49	260±214
Laser (n=103)	70.8	91.2	418±243	8.7	11.7	45±25.9	83	597±530
Month 12								
All arms combined (n=289)	52.9	79.5	366±218	4.2	5.9	58±16	175	658±500
Ranibizumab (n=96)	44.8	71.9	363±229	7.3	8.3	53±52.6	175	828±637
Combined (n=100)	53.0	79.2	287±168	2.0	3	54±29.7	75	489±536
Laser (n=93)	61.3	87.9	440±228	3.2	6.5	38.6±55	133	515±174
Month 36								
All arms combined (n=205)	32.7	62.9	275±222	1.5	2.4	45±6	58	643±789
Ranibizumab (n=70)	34.3	62.9	299±228	2.9	4.3	29±41.0	58	902±994
Combined (n=72)	40.3	68.1	241±192	1.4	2.8	33±n/a	33	254±85
Laser (n=63)	22.2	57.1	307±272	0.0	0	–	–	–

At the 36-month time point all patients were receiving pro re nata ranibizumab. Mean height and width of SRF was calculated from measurements on horizontal and vertical line scans.
CP, centrepoint; IRC, intraretinal cystoid fluid; n/a, not available; SRF, subretinal fluid.

BCVA gain up to months 3, 12 and 36 of treatment were analysed using linear multiple regression models within treatment groups with baseline BCVA letter score, CRT and morphology as independent variables. The correlations between CRT and BCVA letter score were calculated as Spearman's and Pearson's correlation coefficients.

The relationship between height of IRC at baseline and BCVA is described by a Lowess function (stiffness 0.5). The cut point was defined as the point where the Lowess function cuts the mean BCVA. Thus, patients were divided into groups of small IRC and large IRC. This analysis was purely exploratory in nature and no adjustments for multiplicity were made.

RESULTS

Patient population

Of 345 patients randomised to the three study arms, 303 patients completed year 1. There were 116 patients in the ranibizumab arm (102 by the end of year 1), 118 (103) patients in the combined arm and 111 (98) patients in the laser arm. Baseline demographics and diabetes characteristics were comparable in patients in all three arms.[11] Patients treated with ranibizumab and combined therapy had a significantly higher gain in visual acuity at the end of year 1 than patients receiving laser therapy only ($p<0.001$). There was no difference in the ranibizumab or combined therapy arms in terms of visual acuity at the end of year 1 ($p=0.61$, Cochran-Mantel-Haenszel test).[11]

In the RESTORE-extension study, 303 patients were eligible to enter, of which 240 were enrolled (prior ranibizumab group n=83, prior combined group n=83, prior laser group n=74). The second year of the study was completed by 220 patients and 208 patients completed the third year of the study. Patient demographics and disease characteristics throughout the extension study were comparable to those observed in the first year and have been described previously.[16 17]

BCVA and treatment frequency

In year 1, mean BCVA at baseline was 64.8±10.11 letters in the ranibizumab arm, 63.4±9.99 letters in the combined arm and 62.4±11.11 letters in the laser arm; at month 12 there was a change of +6.1±6.43 letters and −118.7±115.07 µm of CRT

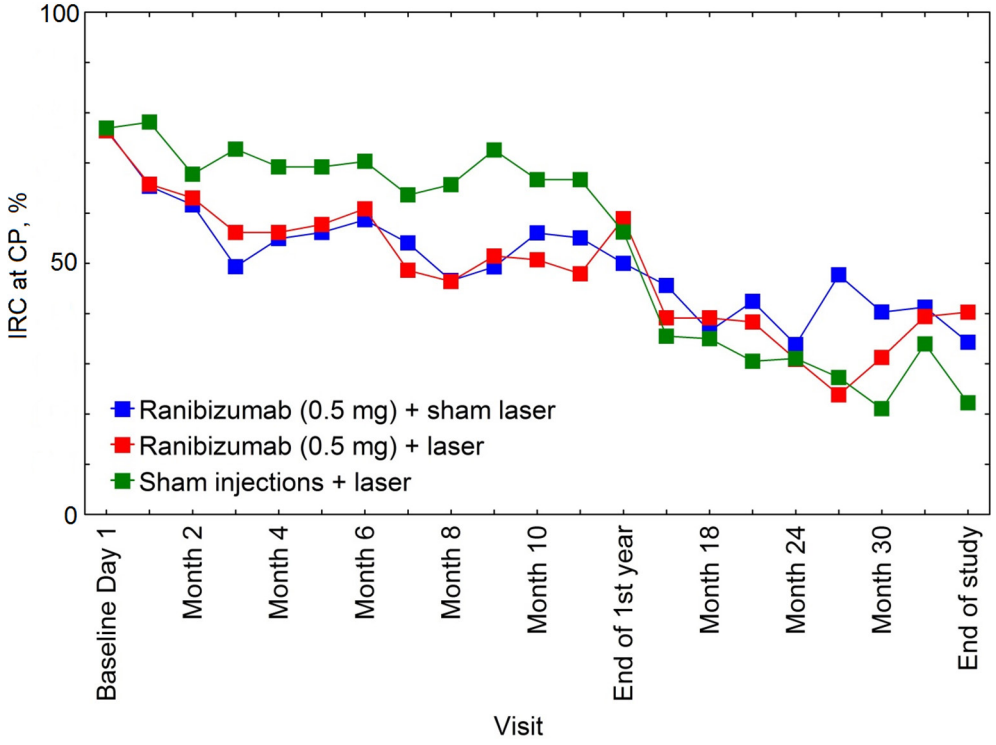

Figure 1 Presence of intraretinal cystoid fluid (IRC) at the centrepoint (CP) at different time points in the three treatment groups. About 80% of patients had IRC including the CP at baseline. IRC reduced to a large extent in all treatment arms by month 12, although the level of IRC reduction was lower in the laser arm than in the ranibizumab and combined treatment arms (both ranibizumab groups p<0.001; laser group p=0.095). By 36 months, under ranibizumab pro re nata treatment, levels of IRC had reduced by the same extent in all treatment groups (p<0.001).

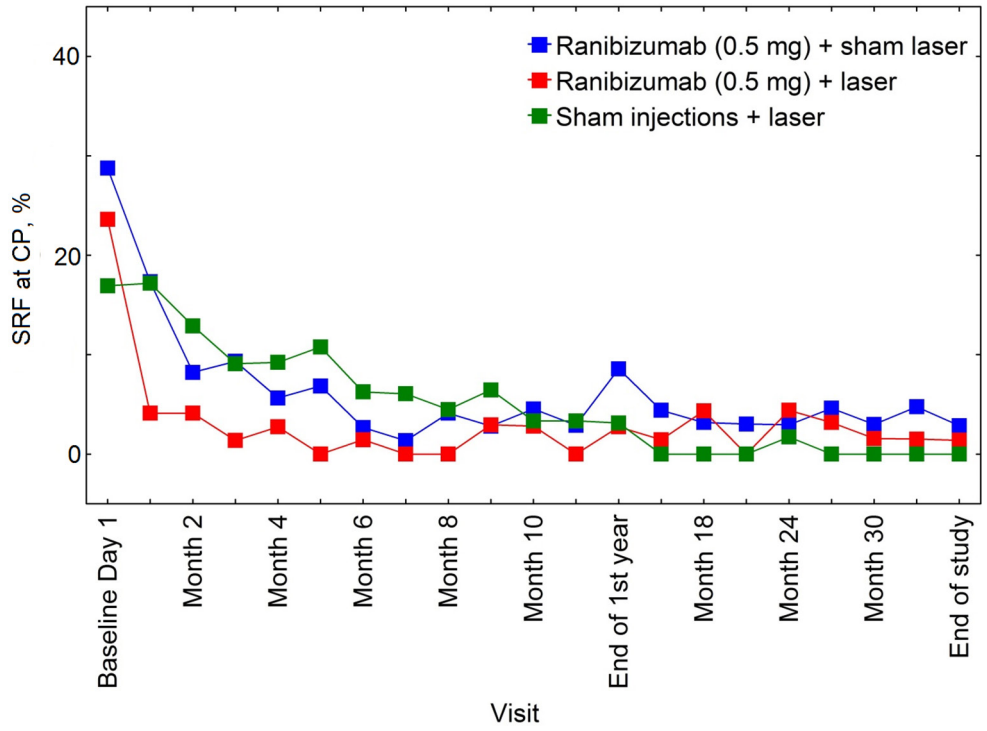

Figure 2 Presence of subretinal fluid (SRF) at the centrepoint (CP) at different time points in the three treatment groups. About 25% of patients had SRF including the CP at baseline. There was a large reduction in SRF in all treatment arms to month 3 regardless of the therapy and further reduction to months 12 and 36 (all patients were receiving pro re nata ranibizumab between months 12 and 36).

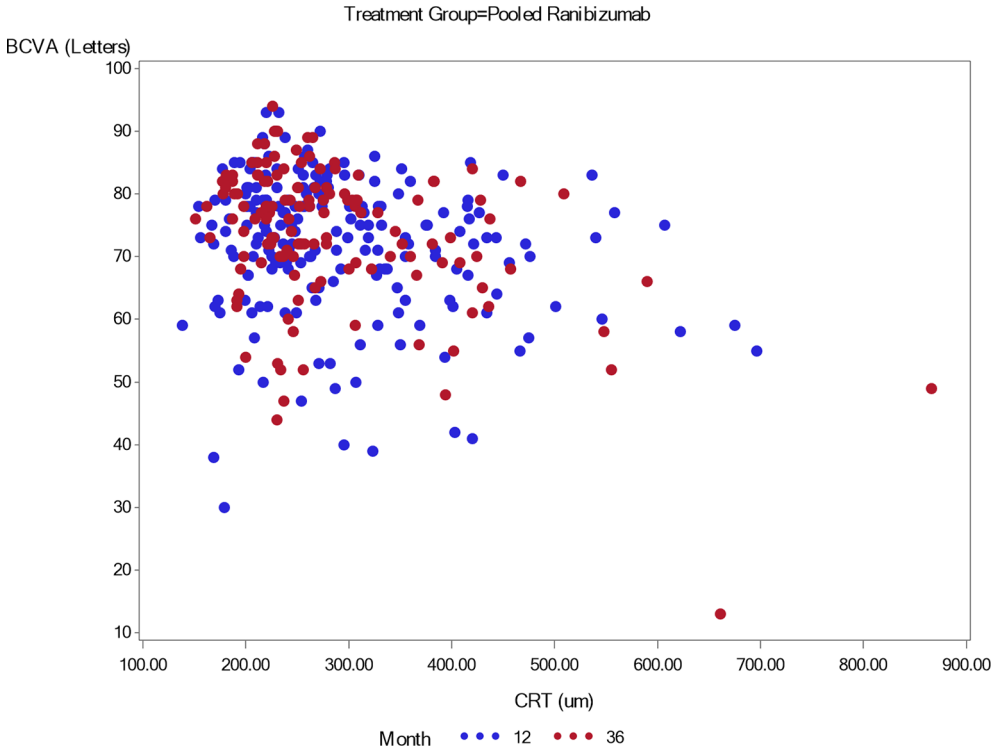

Figure 3 Correlation between central millimetre retinal thickness and best-corrected visual acuity (BCVA) letter score is weak at baseline and reduces further over time. At baseline it was r=–0.34 (p<0.001) and at the end of the study (month 36) it was r=–0.26 (p<0.001) (Pearson's correlation coefficients). The figure shows the correlation example for pooled ranibizumab groups for the time points months 12 and 36. At the 36-month time point, all patients were receiving pro re nata ranibizumab. CRT, central retinal thickness.

in the ranibizumab arm with a mean of 7.0±2.81 injections, a change of +5.9±7.92 letters and −128.3±114.34 µm of CRT in the combined arm with a mean of 6.8±2.95 injections and a mean of 1.7±0.89 laser treatments and a change of +0.8±8.56 letters and −61.3±132.29 µm in the laser arm with a mean of 2.1±1.04 laser treatments.[11]

In the extension study, when all patients could receive individualised ranibizumab treatment after month 12, mean BCVA and CRT changes were maintained until the end of year 2 in the ranibizumab and the combined therapy arms with a mean of 3.9±3.4 and 3.5±3.4 injections, whereas patients in the prior laser monotherapy arm gained 5.4 letters in BCVA and lost 126.6 µm of CRT from the end of year 1 to the end of year 2 with a mean of 4.1±3.6 injections.[16] In the third year of the study, the mean number of injections was low (2.4–2.9) across all three study arms, while BCVA and CRT were maintained at the same levels.[17]

Retinal morphology

At baseline and month 3, 329 and 323 patients had available OCT images of sufficient quality for evaluation, while at 12 and 36 months, 289 and 205 patients had evaluable OCT scans. From month 12, all patients were eligible to receive individualised ranibizumab. The mean central millimetre retinal thickness reduced from baseline to month 36 in all three treatment arms (from 428±116 to 294±109 µm, 417±121 to 277±101 µm and 410±125 to 251±79 µm in the prior ranibizumab, prior combined and prior laser groups, respectively). Retinal thickness values of all time points evaluated in all ETDRS grid subfields can be found in online supplementary table 1.

At baseline, IRC involving the centrepoint was found in 75.5% of patients, while IRC anywhere in the macular region was found in 95.7% of patients (table 1 and figure 1). IRC decreased from baseline in all treatment arms and there was a large reduction in IRC by month 36, by which point all patients were under the same treatment regimen (table 1). IRC levels were higher in the laser arm than in the ranibizumab and combined treatment arms at months 3 and 12. The IRC reduction from baseline to month 12 was statistically significant for both ranibizumab groups (p<0.001), but not for laser therapy (p=0.095). By month 36, the reduction in IRC from baseline was statistically significant for all three treatment arms (p<0.001).

SRF at the centrepoint was found in 20.8% of patients (ranibizumab 22.9%, combined 20.5% and laser 18.9% of patients) at baseline and was found in substantially fewer patients at 3 months (6.1% of patients). There was a reduction in levels of SRF measurements in all treatment arms from baseline that continued until month 36 (table 1 and figure 2).

BCVA as only predictor at month 3

The effect in BCVA change from baseline seen in the first three months of the study is maintained throughout the first year. When investigating predictive values at month 3, BCVA letter score at 3 months is the only predictive value for the BCVA outcome at month 12, across all three treatment arms.

Correlation of CRT with BCVA

There was a weak, negative correlation between CRT and BCVA letter score of r=−0.344 (p<0.001) at baseline, which weakened over time to r=−0.259 (p<0.001) by the end of the study at month 36 (Pearson's correlation coefficients, figure 3).

Table 2 Correlation between central millimetre retinal thickness and best-corrected visual acuity

			Spearman's correlation		Pearson's correlation					Spearman's correlation		Pearson's correlation	
Treatment group at baseline	Analysis visit	n	Coefficient	p Value	Coefficient	p Value	Treatment group at baseline	Analysis visit	n	Coefficient	p Value	Coefficient	p Value
Ranibizumab	Baseline	107	−0.392	**<0.001**	−0.370	**<0.001**	Pooled ranibizumab	Baseline	219	−0.373	**<0.001**	−0.356	**<0.001**
	Month 3	109	−0.167	0.082	−0.178	0.064		Month 3	221	−0.190	**0.005**	−0.177	**0.008**
	Month 6	107	−0.266	**0.006**	−0.400	**<0.001**		Month 6	209	−0.286	**<0.001**	−0.354	**<0.001**
	Month 9	97	−0.199	0.050	−0.283	**0.005**		Month 9	192	−0.236	**<0.001**	−0.287	**<0.001**
	Month 12	96	−0.198	0.053	−0.169	0.099		Month 12	196	−0.186	**0.009**	−0.190	**0.008**
	Month 24	71	−0.103	0.391	−0.168	0.161		Month 24	141	−0.171	**0.043**	−0.262	**0.002**
	Month 36	68	−0.306	**0.011**	−0.505	**<0.001**		Month 36	136	−0.261	**0.002**	−0.420	**<0.001**
Combined	Baseline	112	−0.364	**<0.001**	−0.351	**<0.001**	All arms combined	Baseline	322	−0.355	**<0.001**	−0.344	**<0.001**
	Month 3	112	−0.221	**0.019**	−0.187	**0.048**		Month 3	323	−0.311	**<0.001**	−0.348	**<0.001**
	Month 6	102	−0.297	**0.002**	−0.318	**0.001**		Month 6	305	−0.352	**<0.001**	−0.404	**<0.001**
	Month 9	95	−0.281	**0.006**	−0.295	**0.004**		Month 9	282	−0.366	**<0.001**	−0.405	**<0.001**
	Month 12	100	−0.188	0.061	−0.224	**0.025**		Month 12	289	−0.304	**<0.001**	−0.353	**<0.001**
	Month 24	70	−0.281	**0.019**	−0.364	**0.002**		Month 24	201	−0.160	**0.024**	−0.231	**<0.001**
	Month 36	68	−0.250	**0.039**	−0.356	**0.003**		Month 36	196	−0.124	0.082	−0.259	**<0.001**
Laser	Baseline	103	−0.329	**<0.001**	−0.336	**<0.001**							
	Month 3	102	−0.398	**<0.001**	−0.398	**<0.001**							
	Month 6	96	−0.289	**0.004**	−0.335	**<0.001**							
	Month 9	90	−0.483	**<0.001**	−0.471	**<0.001**							
	Month 12	93	−0.451	**<0.001**	−0.453	**<0.001**							
	Month 24	60	−0.179	0.171	−0.240	0.064							
	Month 36	60	0.118	0.370	0.125	0.342							

After month 12, all patients were receiving pro re nata ranibizumab.
Bold values are statistically significant correlations.

Table 2 shows the different correlation coefficients for each arm at baseline through to month 36.

Visual acuity in patients with IRC

In total, 325 of the evaluated 329 patients at baseline showed IRC in the centre; therefore, these patients were included in this analysis and divided by the height of IRC at the centrepoint. In <1% of cases, more than one cystoid space could be measured at the centrepoint, therefore, we are talking about height instead of summative height. The Lowess curve cuts the mean BCVA of 63.5 BCVA letters at 380 µm. Thus, all patients were divided into two groups: small IRC≤380 µm and large IRC>380 µm. For the total population, an IRC≤380 µm compared with an IRC>380 µm at baseline showed significantly better BCVA letter scores (64.8±10.63 vs 61.7±9.92, p=0.0071) and maintained significantly better BCVA letter scores than patients with IRC>380 µm through to month 12 (70.5±12.33 vs 67.0±14.09, p=0.0252) and trended towards significance at month 36 of the study (73.2±11.49 vs 70.4±11.27, p=0.0871), with about the same difference of BCVA letters at all time points in the total population. Online supplementary table 2 shows the BCVA letter scores in patients with small and large IRC in each treatment arm separately. Figure 4 shows the difference between BCVA letter scores in patients with large and small IRC at baseline from baseline throughout year 1.

Visual acuity in patients with SRF

There was a trend for SRF at baseline to be associated with different functional response patterns in the different treatment regimens. With laser monotherapy, patients with SRF at

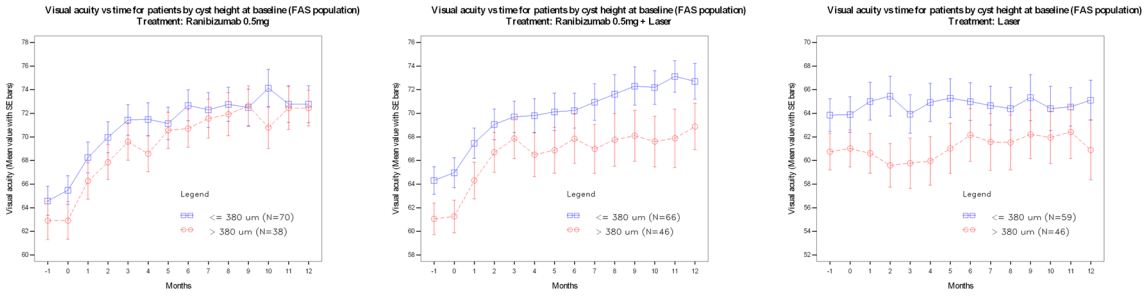

Figure 4 Visual acuity in patients with intraretinal cystoid fluid (IRC) of summative height of ≤380 µm versus >380 µm. Patients were divided by the height of IRC at the centrepoint. Patients with IRC of summative height ≤380 µm at baseline show significantly higher best-corrected visual acuity (BCVA) at baseline and maintain higher BCVA until the end of the study. This difference is not significant (from baseline onwards) in the ranibizumab arm (left) but in the combined (middle) and laser (right) arms. Supplementary file 2 provides quantitative results. FAS, full analysis set.

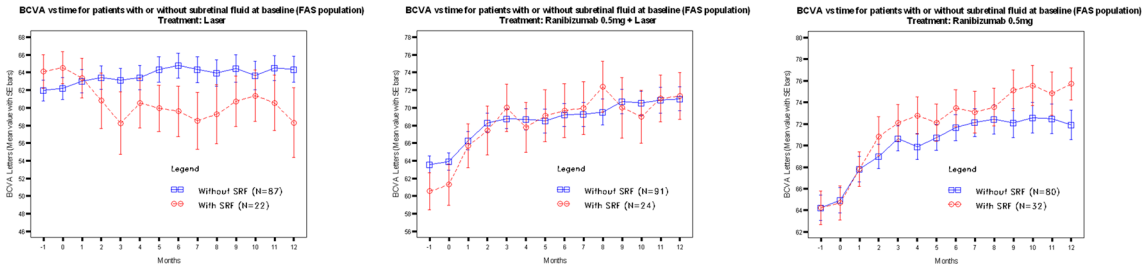

Figure 5 Influence of the presence of subretinal fluid (SRF) at baseline at the centrepoint on best-corrected visual acuity (BCVA) in the three treatment groups in the first study year. With laser monotherapy (left), patients with SRF at baseline show a trend to lose visual function in the first year (−5.38±16.54 BCVA letter scores), whereas patients without SRF remain somewhat stable (2.49±9.72 BCVA letter scores) (p=0.1038). In the combined therapy arm (middle), all patients gain visual function to the same extent regardless of their state of SRF at baseline (9.38±7.91 BCVA letter scores with SRF, 6.94±9.48 BCVA letter scores without SRF, p=0.3768). Under ranibizumab monotherapy (right), all patients gained visual function and improved anatomically but patients with SRF at baseline had a trend to respond with higher BCVA letter score gains (10.28±7.14 vs 6.76±7.67 BCVA letter scores without SRF, p=0.0563).

baseline showed a trend to lose visual function in the first year (−5.38±16.54 BCVA letter scores), whereas patients without SRF remained somewhat stable (2.49±9.72 BCVA letter scores; p=0.1038, figure 5). In the combined therapy arm, all patients showed a trend to gain visual function to the same extent regardless of their state of SRF at baseline (9.38±7.91 BCVA letter scores with SRF vs 6.94±9.48 BCVA letter scores without SRF, p=0.3768, figure 5). Under ranibizumab monotherapy, all patients gained visual function and improved anatomically, but patients with SRF at baseline trended towards higher BCVA letter score gains (10.28±7.14 vs 6.76±7.67 without SRF, p=0.0563, figure 5).

Patients without SRF at baseline had lower CRT values than patients with SRF at baseline when observing the total population (395.9±108.86 vs 510.6±108.47 µm, p<0.0001) or the separate treatment arms (table 3). At month 12, patients with SRF at baseline had higher CRT values in the ranibizumab (p=0.0201) and laser arms (p=0.0254), but CRT is statistically the same in the presence or absence of SRF at baseline in the combined therapy arm (p=0.1376). In the combined therapy arm, levels of CRT show a maximum level of reduction and stabilisation by month 1, regardless of the presence or absence of SRF at baseline (p=0.6599) (table 3 and figure 6).

DISCUSSION

The overall benefit of ranibizumab therapy shown in the pivotal trials of RIDE/RISE and RESTORE has revolutionised the treatment of DMO and started a new era beyond laser therapy.[11 15] However, the response of individual patients can be highly variable. The identification of predictive biomarkers may help determine patients' responses to treatment in terms of gaining vision, remaining stable or not responding to ranibizumab therapy. Some biomarkers have been identified in the past: poor baseline visual acuity or macular atrophy with loss of photoreceptors are known to have an unfavourable prognosis for functional outcome after ranibizumab therapy.[6] On the other hand, good baseline visual acuity, young age and the presence of hard exudates in the macula at the time of treatment initiation have been identified as predictors for favourable OCT and visual acuity changes after 1 year of treatment with ranibizumab and laser photocoagulation.[7]

This post hoc analysis of the RESTORE and the RESTORE-extension studies has particular value for identifying biomarkers for therapeutic efficacy because it allows the comparison of ranibizumab to sham ranibizumab plus active laser in the first year. A limitation of our study is the use of time-domain OCT in this study. Compared with the possibilities in spectral-domain and swept-source OCT, the number of features that can be evaluated in such OCT scans is low and the potential of a study evaluating features in high-quality OCT scans in the future is very promising. Nevertheless, different morphological parameters were evaluated from OCT imaging of patients with DMO. Three major morphological findings could be identified from this broad, exploratory search for biomarkers. First, CRT cannot serve as an imaging biomarker in DMO. Second, the size of IRC was found to be an important factor associated with functional outcomes. Eyes with larger IRC (>380 µm) demonstrated poorer baseline BCVA compared with eyes with smaller (<380 µm) IRC spaces. The difference in the gain in visual acuity between patients with smaller IRC compared with larger IRC spaces persisted through the entire study and could not be compensated for over time, suggesting that there is a need to treat patients as early as possible. Third, there was a trend for eyes with SRF at baseline to respond better to ranibizumab therapy and worse to laser therapy than eyes without SRF at baseline. These data suggest that further investigations of the relationship between SRF and response to laser therapy or ranibizumab combined with laser therapy in a prospective trial would be worthwhile.

CRT has already been shown in other diseases, such as nAMD or retinal vein occlusion (RVO), to be a poor morphological marker for functional outcomes. In nAMD, the correlation between CRT and BCVA letter score is very weak and is lost shortly after the start of therapy.[18 19] In central RVO, CRT serves as a prognostic factor only when the change of CRT from baseline to month 3 is evaluated, but there is no correlation in patients with branch RVO.[20] This study demonstrates similar findings in DMO. The correlation is stronger than in nAMD, but still weak, and by 3 years of ranibizumab therapy there is no longer any correlation between CRT and BCVA. This disparity can be partly explained by the morphology of oedema in the different diseases. In nAMD, approximately 50% of patients show IRC at baseline and approximately 60%–70% of patients show SRF at baseline,[18] in branch RVO 98.7% show IRC at baseline and about 45% of patients show SRF at baseline, in central RVO 98.5% show IRC and about 57% of patients show SRF,[20] whereas in DMO almost all patients show IRC (95.7% in our analysis, 94.1% in DRCR.net protocol I[7] at baseline) but only 25% of patients show SRF at baseline (in our analysis; 22.5% in DRCR.net protocol I).[7] Therefore, the different composition of oedema in different conditions leads to different

Table 3 Central retinal thickness (CRT) at baseline, month 1, month 12 and month 36 with and without subretinal fluid (SRF) at baseline

Treatment group at baseline	Baseline				Month 1				Month 12				Month 36			
	SRF	n	CRT± SD	p Value	SRF	n	CRT± SD	p Value	SRF	n	CRT± SD	p Value	SRF	n	CRT± SD	p Value
Ranibizumab	No	78	395.1±103.06	<0.0001	No	79	313.1±83.34	0.0003	No	79	289.7±96.42	0.0201	No	56	293.9±118.32	0.8611
	Yes	29	521.3±102.83		Yes	29	388.8±109.24		Yes	29	354.1±144.25		Yes	23	299.2±90.24	
Combined	No	88	396.6±114.34	0.0003	No	88	344.4±99.99	0.6599	No	88	278±79.15	0.1376	No	57	263.6±65.85	0.1743
	Yes	23	497.2±116.09		Yes	24	333.5±123.25		Yes	23	312.7±134.37		Yes	20	305.6±182.43	
Laser	No	83	390.9±115.56	0.0024	No	84	394±122.52	0.0158	No	84	333.5±126.79	0.0254	No	57	243±76.59	0.0702
	Yes	20	483.1±131.2		Yes	21	470.5±120.91		Yes	21	414.8±154.2		Yes	14	291.6±87.19	
Ranibizumab combined	No	166	395.9±108.86	<0.0001	No	167	329.4±93.45	0.0403	No	167	283.6±87.79	0.0058	No	113	277.7±94.66	0.2608
	Yes	52	510.6±108.47		Yes	52	362.8±118.13		Yes	52	333.4±139.27		Yes	43	301.8±132.74	
All arms combined	No	249	394.3±110.89	<0.0001	No	251	350.7±108.04	0.0065	No	251	300.2±104.76	0.0009	No	170	266.5±90.44	0.0570
	Yes	72	503±114.95		Yes	73	392.9±127.64		Yes	73	356.8±147.14		Yes	57	299.2±121.8	

At the 36-month time point, all patients were receiving pro re nata ranibizumab. p Value comes from analysis of variance model.

pathophysiological responses to anti-VEGF therapy. SRF, if present in nAMD, usually responds very well to ranibizumab—out of 60%–70% of patients with SRF, following ranibizumab treatment, SRF remains in about 20% of total patients. The same can be seen with IRC—out of 50% of patients with IRC at baseline, only 15% of total patients show IRC after the loading phase.[18 19] In RVO, about 50% of ranibizumab-treated patients have resolution of IRC and almost all patients have resolution of SRF.[20] In DMO, this process is different—out of a total of 25% of patients with SRF, only 5% of patients show SRF after a loading phase; and out of almost 100% of patients with IRC, IRC remains in approximately 50% of patients after a loading phase. These differences in anatomical responses could also lead to different correlations of CRT and function.

Our analysis revealed that smaller IRC at the foveal centre had a better functional prognosis than larger IRC. This finding is supported by an earlier study of Pelosini *et al*. There, a linear relationship between the volume of remaining tissue between inner and outer retinal layers on OCT C-scans and visual acuity could be shown.[8] The larger the IRC, the less tissue was visible in the C-scan sections. Pelosini *et al* postulated that this tissue, connecting the retinal photoreceptors to ganglion cells, consists of bipolar cells and Müller fibres. The displacement of the Müller fibres might cause damage to bipolar axons and therefore permanent vision loss. This supports also the finding that vision cannot be restored in patients with extensive IRC changes even if the retina dries out with aggressive therapy. It is therefore logical that the patient group with larger IRC will never gain visual function to the same level of the patient group with small IRC. A recent post hoc analysis of the RIDE/RISE studies by Sophie *et al* also supports that an increased size of IRC increases the likelihood for a poor visual outcome. Here, this effect was only seen in sham-treated patients, whereas in patients treated with ranibizumab, the size of macular IRC did not predict a worse functional outcome.[9] This could be explained by the different analysis of the reading centres. In our analysis, the height was a vertical measurement at the foveal centrepoint; this might be different in the RIDE/RISE subanalysis (eg, maximum extension of cystoid space).

There was a trend for patients with SRF at baseline to gain more function under ranibizumab therapy and lose function under laser therapy compared with those without SRF at baseline in our study. This finding is also supported by the study of Sophie *et al*. Here, patients treated with ranibizumab and with SRF at baseline were more than twice as likely to gain vision to 20/40 or better or to have an improvement in BCVA letter score of >15 letters (p=0.0004, OR 2.43, 95% CI 1.49 to 3.98) or more compared with those without SRF at baseline (p=0.0002, OR 2.88, 95% CI 1.65 to 5.03).[9] In our analysis, laser therapy seems to have a negative effect on the treatment of patients with SRF. In a subanalysis of the DRCR.net protocol I, there was no difference in visual acuity at 1 year of treatment when comparing patients with and without SRF at baseline. Patients in this study were treated with ranibizumab and underwent either prompt or deferred laser treatment, therefore there is no ranibizumab monotherapy arm available for comparison. It is possible that the negative effect of the laser treatment and the positive effect of the ranibizumab treatment cancelled each other out, as we hypothesise from our findings.[7]

In conclusion, this post hoc analysis of the RESTORE studies increases our understanding of imaging biomarkers derived from OCT that may have utility in the prediction of treatment response at baseline in patients with DMO. The size of IRC and, potentially, the presence of SRF at baseline were identified as

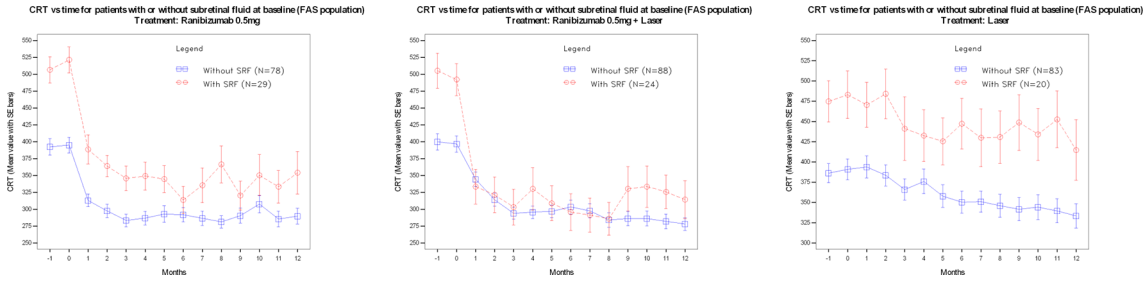

Figure 6 Decrease of central retinal thickness (CRT) from baseline to the end of the study in patients with and without subretinal fluid (SRF) at baseline. Patients without SRF at baseline have lower CRT values than patients with SRF at baseline when looking at all arms combined (393.86±111.41 µm vs 502.58±114.96 µm, p≤0.0001). The difference in CRT persists over time in the ranibizumab (left) and laser (right) arms, although SRF is only rarely present afterwards (ranibizumab 3.3%, laser 7.3%), whereas CRT decreases more with baseline SRF in the combined arm (middle). After month 1, no difference in CRT can be observed between the presence and absence of SRF at baseline in the combined therapy arm.

relevant factors for functional and anatomical outcomes during ranibizumab therapy of DMO. CRT was not, however, found to be a reliable marker for visual function. A more precise understanding of the role of retinal morphology and biomarkers may provide guidance to caregivers, offer patients a more realistic prognosis and guide the development of future study end points. Further studies using spectral-domain OCT will allow a deeper insight into the pathophysiology of DMO.

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Patient consent Obtained.

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Predictive imaging biomarkers relevant for functional and anatomical outcomes during ranibizumab therapy of diabetic macular oedema

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Notes