# The Association of Fluid Volatility With Subretinal Hyperreflective Material and Ellipsoid Zone Integrity in Neovascular AMD

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**Purpose.** To evaluate the association of fluid volatility with ellipsoid zone (EZ) integrity and subretinal hyperreflective material (SHRM) volume during anti–vascular endothelial growth factor (VEGF) therapy in neovascular age-related macular degeneration (nAMD).

**Methods.** This study was a post hoc analysis of the OSPREY study. Retinal volatility was quantified as the standard deviation across weeks 12 to 56 for six optical coherence tomography (OCT) metrics: central subfield thickness (CST), total fluid (TF) volume, subretinal fluid (SRF) volume, intraretinal fluid (IRF) volume, macular total retinal fluid index (TRFI), and central macular TRFI. Eyes with volatility $\leq$ 25th or $\geq$ 75th percentile values were compared.

**Results.** Eyes with low volatility in several exudative metrics showed greater change from baseline in SHRM volume at week 12 than eyes with high volatility. During the maintenance phase (weeks 12–56), eyes exhibiting high SRF volatility demonstrated increased SHRM volume compared to eyes with low SRF volatility ($P = 0.027$). Eyes exhibiting high volatility in CST, TF, and SRF demonstrated less improvement in EZ total attenuation ($P < 0.001$, $P = 0.033$, and $P = 0.043$, respectively) than eyes with low volatility. Early exudative instability (i.e., between weeks 4–8 or weeks 8–12) in multiple parameters (i.e., CST, TF, IRF, macular TRFI, or central macular TRFI) was associated with greater volatility during the maintenance phase ($P < 0.05$).

**Conclusions.** Greater volatility in exudative OCT metrics, particularly SRF volatility, was associated with a greater increase in SHRM and less improvement in EZ integrity, suggesting that volatility is detrimental to multiple anatomic features in nAMD. Early exudative instability during the loading phase of treatment was associated with longer-term volatility in exudation.

Keywords: fluctuation, intraretinal fluid, subretinal fluid, neovascular age-related macular degeneration, optical coherence tomography

Neovascular age-related macular degeneration (nAMD) is characterized by the development of new blood vessels that proliferate into the subretinal pigment epithelium (sub-RPE) space and/or subretinal space, resulting in exudation of fluid into surrounding tissue.[1,2] Choroidal neovascularization and fluid leakage may lead to the development of subretinal fibrosis and atrophic retinal changes.[3–5] Anti–vascular endothelial growth factor (anti-VEGF) therapy is the current standard of care for nAMD, with drug delivery provided through regular intravitreal injections.[6,7] Visual acuity improvement with anti-VEGF therapy is associated with reductions in retinal fluid and retinal thickness as measured by optical coherence tomography (OCT).[8,9] Despite the efficacy of anti-VEGF therapy, there is variability in the extent to which patients respond to therapy.[4,6] Elucidating the disease processes associated with poor outcomes could help to identify patients who may benefit from additional care.

Several studies have examined retinal thickness and fluid as imaging biomarkers of visual acuity and outcomes.[8–11] A reduction in central retinal thickness has been found to weakly correlate with improvement in visual acuity over the first year of treatment.[10] Additionally, the presence of intraretinal fluid (IRF) has been associated with






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poorer visual acuity,[11–14] whereas presence of subretinal fluid (SRF) has been associated with better visual acuity.[12,13,15,16] Although assessment of retinal fluid presence has led to insights into disease features relevant to functional outcomes, the impact of variations in retinal fluid over time, also referred to as volatility, is not well understood. Volatility of retinal thickness or fluid is thought to represent unstable disease activity.[17,18] Recently, post hoc analyses of the CATT/IVAN and HAWK/HARRIER studies reported that greater variation over time in retinal thickness is associated with poorer visual acuity outcomes[19,20]; however, it is unknown which specific underlying fluid compartments may be causing the variation in retinal thickness and how these may affect various anatomic features important for visual acuity.

Advancements in spectral-domain OCT (SD-OCT) image analysis allow for detailed assessment of anatomic features.[21–23] These analysis techniques enable the evaluation of volatility of retinal fluidics and the association of this volatility with anatomic features. Several studies have established that integrity of the outer retina on OCT, including the ellipsoid zone (EZ), is important for visual acuity.[5,24] Furthermore, the presence of subretinal hyperreflective material (SHRM) on OCT is associated with poorer preservation of the EZ and poorer visual acuity.[5,25] These findings suggest that EZ integrity and SHRM volume are important OCT outcome measures for understanding processes affecting visual acuity. The goal of this post hoc analysis of the OSPREY study was to assess the association of retinal fluid and thickness volatility with alterations in SHRM volume and EZ integrity.

## Methods

### OSPREY Study Design

This study was a post hoc analysis of the OSPREY study (ClinicalTrials.gov identifier NCT01796964).[7] The methods of the OSPREY study have been previously described. Briefly, the OSPREY study was a 56-week, prospective, randomized, phase 2 trial conducted at 41 investigational centers in the United States. The protocol was approved by all institutional review boards and complied with the tenets of the Declaration of Helsinki. All participants provided written informed consent prior to participation. Ninety participants with untreated active choroidal neovascularization due to AMD were randomized to receive intravitreal injections of brolucizumab (6 mg/50 µL) or aflibercept (2 mg/50 µL). Dosing regimens consisted of three treatment periods: three loading doses on a 4-week cycle, an 8-week dosing cycle with assessment up to week 40, and extension to a 12-week dosing cycle (injection at week 44) for the brolucizumab group or continuation of the 8-week dosing cycle (injections at weeks 40 and 48) for the aflibercept group. There were no criteria for reducing the treatment interval in the OSPREY study. SD-OCT macular cube scans centered on the foveal center point were obtained at each visit, which occurred every 4 weeks, using a 49-line scan covering a 20° × 20° area of the macula with the SPECTRALIS imaging system (Heidelberg Engineering, Heidelberg, Germany) or a 512 × 128 macular cube covering a 6-mm × 6-mm area of the macula with the CIRRUS imaging system (Carl Zeiss Meditec, Dublin, CA, USA).

### Higher-Order OCT Analyses: Fluid, SHRM, and EZ

SD-OCT raw data generated for the OSPREY study were transferred to the Cleveland Clinic's Cole Eye Institute for further analyses. Of the 89 study eyes that received treatment in the OSPREY study, 81 (41 in the brolucizumab arm and 40 in the aflibercept arm) were included in the present analysis. Seven eyes were excluded because they did not have sufficient OCT scans (i.e., OCT scans at baseline and additional time points for evaluating volatility and early instability, as described below). One additional eye was excluded because of poor OCT scan quality at baseline, which interfered with the ability to identify retinal layers or boundaries of fluid or SHRM. The image quality exclusion was based on insufficient quality to allow for accurate segmentation (i.e., automated with manual correction). Macular cube scans were imported for higher-order OCT analysis using a proprietary machine-learning enhanced segmentation and feature extraction tool (Cleveland Clinic, Cleveland, OH, USA).[21–23] Each macular scan was automatically segmented to identify fluid boundaries, SHRM boundaries, and relevant retinal layers (i.e., internal limiting membrane, EZ, and RPE). SHRM was defined as the hyperreflective material bound anteriorly by the posterior-most aspect of visible retinal tissue and the anterior aspect of the RPE. The zonal boundary of the retina varied based on the amount of outer retinal atrophy present. The boundary line for the anterior border was defined by the transition zone between hyperreflective material and the more hyporeflective tissue of the retina.

Two masked trained expert readers sequentially evaluated the identification and segmentation accuracy of the features of interest and manually corrected any segmentation errors, as needed. The center point, which was defined initially through automated placement was also evaluated and confirmed by the expert readers in all scans. All readers received the same training for the OCT analysis, and the reading environment was standardized based on location, computer configuration, monitor settings, and lighting configuration. The same trained readers reviewed and corrected the segmentation lines for all time points for every B-scan for every volume scan of a single participant to minimize inter–time point and inter-reader variability. Following the initial reader reviews, a senior project lead who had extensive experience in OCT interpretation (including complex pattern recognition, segmentation adjudication, and volumetric feature characterization) reviewed all corrected scans for consistency and accuracy of segmentation.[23,26] If needed, additional segmentation corrections were performed. Following segmentation, en face maps of macular thickness from the EZ to the RPE (EZ-RPE, a surrogate for photoreceptor outer segment length) were generated to visualize the extent of loss of outer retinal integrity.

Multiple metrics were exported for analysis, including IRF and SRF volume across the entire macular cube and the central macula, SHRM volume across the entire macular cube, and the percentage of the macular cube showing total absence of the EZ band (reported as percentage of total EZ attenuation). The central macula was defined as the circular area with a 1-mm radius from the fovea. A total retinal fluid index (TRFI) was calculated and defined as the percentage of fluid (IRF and SRF) within the total retinal and fluid compartments (i.e., percentage of fluid among the tissue and fluid between the internal limiting membrane and the RPE). The TRFI was calculated and evaluated for two regions: (1) the central macula (circular area with a 1-mm radius), referred to

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as central macular TRFI (CM-TRFI); and (2) across the entire macular cube, referred to as total macular TRFI (TM-TRFI). The accuracy of the metrics was limited by inter-individual variability in axial length, which affect lateral measurement due to ocular magnification.[27,28]

### Volatility Assessment: Weeks 12 to 56

Volatility was quantified using six parameters: central subfield thickness (CST, obtained from the original study); total fluid (TF) volume (SRF volume + IRF volume); SRF volume; IRF volume; TM-TRFI; and CM-TRFI. TF volume, SRF volume, IRF volume, and TM-TRFI were pan-macular measurements, whereas CM-TRFI was confined to the central macula. For each parameter, volatility was defined as the standard deviation (SD) of the parameter over weeks 12 to 56 for any given eye. This period was chosen because it excluded the loading-dose period, during which rapid improvements in parameters were expected. For each measure of volatility from weeks 12 to 56, eyes that showed volatility less than or equal to the value corresponding to the lower quartile (Q1) were categorized as having low volatility. Eyes that showed volatility greater than or equal to the value corresponding to the upper quartile (Q3) were categorized as having high volatility.

### Early Instability Assessment: Weeks 4 to 12

As a potential predictor of volatility from weeks 12 to 56, a measure of early instability was evaluated for each eye with each of the six parameters (CST, TF volume, SRF volume, IRF volume, TM-TRFI, and CM-TRFI). Eyes were categorized as having high early instability if they demonstrated an increase in the parameter greater than or equal to a cutoff value from weeks 4 to 8 or weeks 8 to 12. Eyes were categorized as having low early instability if they showed change less than the cutoff value from weeks 4 to 8 and weeks 8 to 12. Cutoff values were selected for each parameter based on previous determination of visually meaningful changes and confirmation based on reviewing patient-level data of change from weeks 4 to 8 and weeks 8 to 12, with the aim of allowing only non-negligible increases to be considered high early instability. A cutoff of 10 µm was used for CST; 0.001 $mm^3$ for TF volume, SRF volume, and IRF volume; 0.01% for TM-TRFI; and 0.05% for CM-TRFI.[29]

### Statistical Analysis

The analysis included participants from both treatment groups (brolucizumab and aflibercept) of the OSPREY study. Change from week 12 in each outcome measure (i.e., SHRM volume, percentage of total EZ attenuation, and best-corrected visual acuity [BCVA]) was analyzed using an analysis of covariance model with volatility group (low or high) and treatment as factors, and week 12 values as a covariate. Change from baseline at week 12 was similarly analyzed but with baseline values as a covariate. $P < 0.05$ was considered statistically significant. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Analyses were post hoc, and adjustments for multiple comparisons were not made because the study was intended to be for hypothesis generation. Therefore, all findings should be considered preliminary and in need of further validation with larger datasets.

## Results

### Participant Characteristics

Baseline characteristics of the participants ($N = 81$) are shown in Table 1. The distribution of volatility across eyes for each parameter from weeks 12 to 56 is shown in Supplementary Figure S1. The overlaps in participants across the parameters for the low- and the high-volatility groups are shown in Supplementary Tables S1 and S2, respectively.

### Low Versus High Volatility

**Change From Baseline During the Loading Phase (Weeks 0–12).** For each of the low- and the high-volatility groups, there was a significant change from baseline at week 12 for SHRM volume and total EZ attenuation (Table 2). For SHRM volume, significant differences between the low- and high-volatility groups in change from baseline at week 12 were observed with each volatility parameter except for IRF, with the low-volatility group showing a greater decrease in SHRM volume than the high-volatility group for the TF, SRF, TM-TRFI, and CM-TRFI volatility parameters. No significant differences in change from baseline at week 12 were observed between the low- and high-volatility groups for total EZ attenuation.

**Change From Week 12 During the Maintenance Phase (Weeks 12–56).**

*Subretinal Hyperreflective Material.* Mean change from week 12 in SHRM volume is shown in Figure 1 for each visit from weeks 12 to 56. Each panel shows the low- and high-volatility groups as determined by a different volatility parameter. Select high-volatility groups demonstrated significant increases from week 12 in SHRM volume at week 56, including the high-volatility groups based on the SRF, TM-TRFI, and CM-TRFI volatility parameters ($P < 0.05$ for each parameter). Conversely, the low-volatility groups generally showed small numeric decreases from week 12 in SHRM volume at week 56, but these were not statistically significant. Interestingly, high volatility in IRF volume did not appear to affect SHRM volume. These results are further illustrated in Figure 2, which shows SHRM volume at each visit from baseline through week 56 for the low- and high-volatility groups based on the SRF and CM-TRFI volatility parameters. A significant difference between the

**Table 1.** Participant Baseline Characteristics

| Characteristic | All Participants ($N = 81$) |
|---|---|
| Age (y), mean (SD) | 78.1 (9.4) |
| Female, *n* (%) | 51 (63.0) |
| BCVA, ETDRS letters, mean (SD) | 55.4 (12.5) |
| Lesion type, *n* (%) | |
| Predominantly classic | 37 (45.7) |
| Minimally classic | 19 (23.5) |
| Occult | 25 (30.9) |
| Presence of hyperreflective material, *n* (%) | 67 (82.7) |
| Presence of subretinal fluid, *n* (%) | 72 (88.9) |
| Presence of intraretinal fluid, *n* (%) | 70 (86.4) |
| SHRM volume ($mm^3$), mean (SD) | 0.38 (0.34) |
| Total EZ attenuation (%), mean (SD) | 31.66 (20.62) |

BCVA, best corrected visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; EZ, ellipsoid zone; SD, standard deviation; SHRM, subretinal hyperreflective material.

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**Table 2.** Outcome Measures at Baseline and Mean Change From Baseline at Week 12 for Low- and High-Volatility Groups

| Volatility Parameter | Low-Volatility Group, Mean (SD) at BL | High-Volatility Group, Mean (SD) at BL | Low-Volatility Group, Mean Change From BL (SD) at Week 12 | High-Volatility Group, Mean Change From BL (SD) at Week 12 |
|---|---|---|---|---|
| SHRM volume ($mm^3$) | | | | |
| CST | 0.31 (0.32) | 0.45 (0.36) | −0.27 (0.29)*† | −0.29 (0.22)*† |
| TF | 0.41 (0.37) | 0.38 (0.27) | −0.36 (0.33)**† | −0.27 (0.22)**† |
| SRF | 0.35 (0.34) | 0.32 (0.23) | −0.30 (0.31)*† | −0.23 (0.19)*† |
| IRF | 0.24 (0.32) | 0.46 (0.38) | −0.20 (0.29)† | −0.31 (0.28)† |
| TM-TRFI | 0.41 (0.36) | 0.39 (0.27) | −0.36 (0.33)**† | −0.27 (0.22)**† |
| CM-TRFI | 0.43 (0.38) | 0.40 (0.32) | −0.37 (0.34)*† | −0.30 (0.28)*† |
| Macular percentage of total EZ attenuation (%) | | | | |
| CST | 24.64 (18.55) | 36.82 (18.27) | −10.06 (12.05)† | −18.91 (15.09)† |
| TF | 34.13 (24.84) | 36.71 (18.96) | −10.84 (15.37)† | −17.95 (14.89)† |
| SRF | 29.64 (22.91) | 34.77 (20.50) | −9.77 (13.36)† | −18.47 (14.88)† |
| IRF | 28.23 (24.33) | 36.29 (18.81) | −10.13 (12.52)† | −15.99 (16.49)† |
| TM-TRFI | 32.45 (24.40) | 36.50 (18.53) | −12.29 (15.49)† | −17.78 (14.54)† |
| CM-TRFI | 32.34 (22.74) | 35.37 (18.61) | −10.60 (13.44)† | −18.20 (13.67)† |

Volatility was calculated for each eye as the SD of the parameter across weeks 12 to 56. *P* values were from an analysis of covariance model with volatility group (low or high) as a factor and baseline value and treatment (brolucizumab or aflibercept) as covariates. BL, baseline.

* $P < 0.05$ for low versus high volatility.

** $P < 0.01$ for low versus high volatility.

† $P < 0.05$ versus baseline.

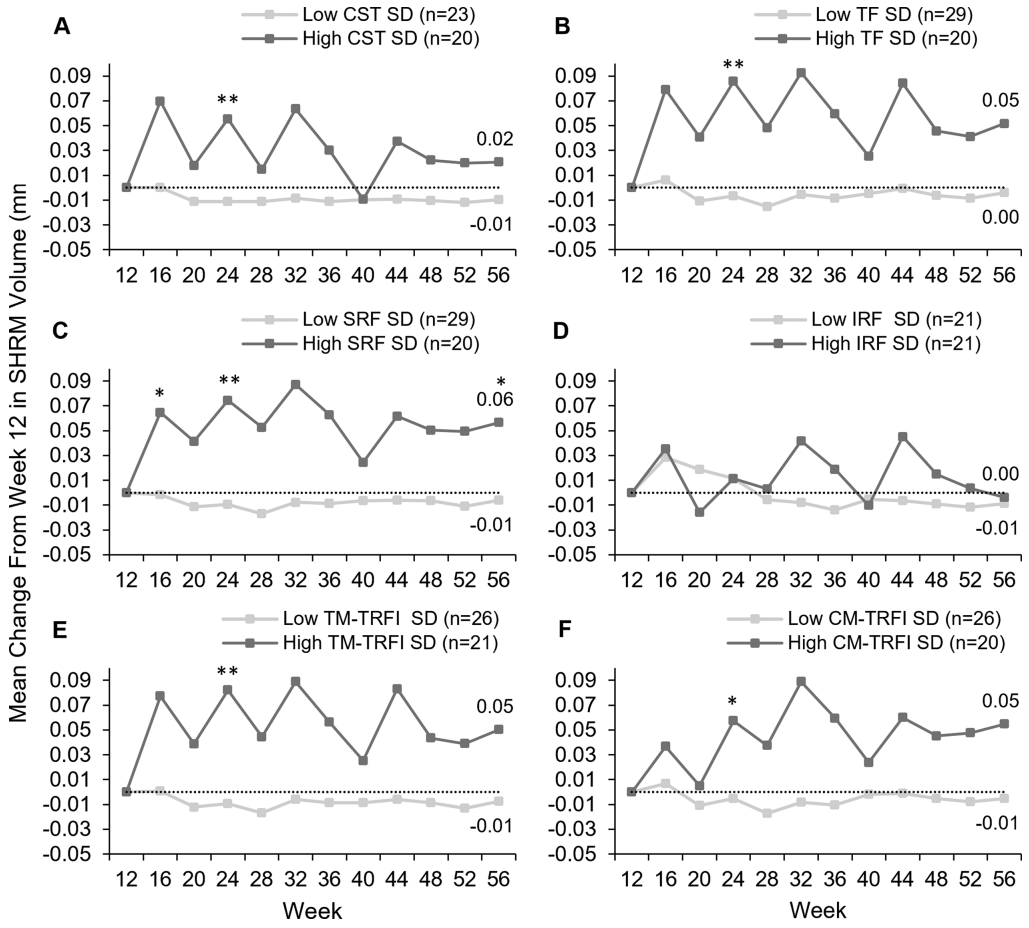


**Figure 1.** (**A–F**) Mean change from week 12 in SHRM volume at visits from weeks 12 to 56 for a group with low volatility (*light gray*) and one with high volatility (*dark gray*) in a specific parameter (i.e., CST, TF, SRF, IRF, TM-TRFI, or CM-TRFI, respectively). Volatility was calculated for each eye as the SD of the parameter across weeks 12 to 56. Values are specified at weeks 40 and 56. Asterisks indicate significant differences between low- and high-volatility groups (*$P < 0.05$; **$P < 0.01$).

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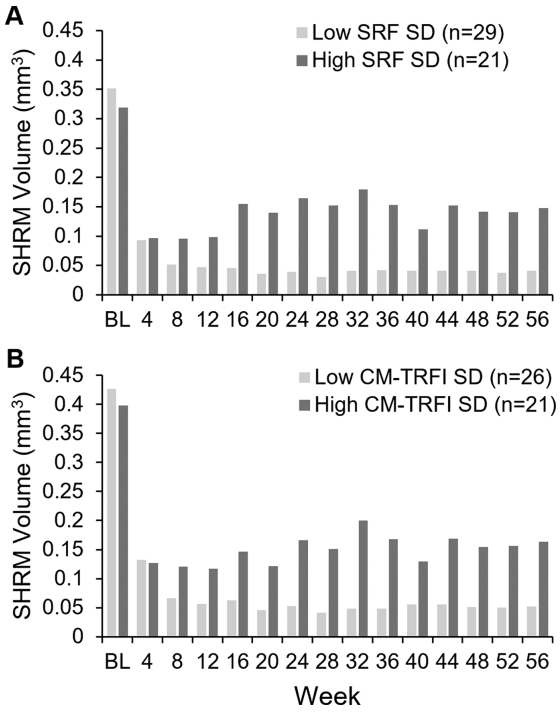


**Figure 2.** SHRM volume from baseline to week 56. Each panel shows the SHRM volume at each visit for the low- and high-volatility groups. The volatility groups were determined by the SRF and CM-TRFI volatility parameter in the top and bottom panels, respectively. Volatility was calculated for each eye as the SD of the parameter across weeks 12 to 56. BL, baseline.

low- and high-volatility groups in change from week 12 in SHRM volume was observed with the SRF volatility parameter at week 56 ($P = 0.027$), indicating a greater increase for the high-volatility group than the low-volatility group (Fig. 1). Figure 3 shows example OCT B-scans of SHRM for a participant in the low SRF volatility group and a participant in the high SRF volatility group.

*Total EZ Attenuation.* The low-volatility groups demonstrated significant decreases in total EZ attenuation from week 12 with each volatility parameter at week 56 ($P < 0.05$ for each parameter), indicating improvement in EZ integrity (Fig. 4). In contrast, total EZ attenuation showed increases from week 12 for the high-volatility groups at week 56 with all volatility parameters except IRF volume, but the increases were not statistically significant. At week 56, there was a significant difference between low- and high-volatility groups in the change in total EZ attenuation with the CST ($P < 0.001$), TF ($P = 0.033$), and SRF ($P = 0.043$) volatility parameters (Fig. 4), indicating greater improvement in EZ integrity from week 12 for the low-volatility groups than for the high-volatility groups. Figure 5 shows example OCT B-scans and EZ topographical thickness maps for a participant from the low CST volatility group and a participant from the high CST volatility group.

*Functional Outcomes.* Although the majority of visual acuity gain with anti-VEGF treatment in nAMD is typically obtained in the first 12 weeks of treatment,[7,8] the low-volatility groups showed a significant increase from week 12 in BCVA at specific time points with select volatility parameters (at week 28 with the TF volatility parameter, $P = 0.039$; at week 52 with the IRF volatility parameter, $P = 0.018$; at week 28 and week 32 with the TM-TRFI volatility parameter, $P = 0.015$ and $P = 0.020$, respectively). Conversely, no significant gain in BCVA was observed at any time points in the high-volatility group with any of the volatility parameters.

### Early Instability as a Predictor of Later Volatility

The percentage of patients who were categorized as having high early instability, based on change from either weeks 4 to 8 or weeks 8 to 12, ranged from 8.75% to 23.75% depending on the parameter (Table 3). Volatility from weeks 12 to 56 was greater for eyes with high early instability than for eyes with low early instability. The difference in mean volatility between high and low early instability groups was significant ($P < 0.05$) for all parameters except for SRF (CST volatility: 32.3 µm vs. 19.0 µm, $P = 0.038$; TF volatility: 0.047 $mm^3$ vs. 0.014 $mm^3$, $P = 0.0089$; SRF volatility: 0.051 $mm^3$ vs. 0.014 $mm^3$, $P = 0.055$; IRF volatility: 0.012 $mm^3$ vs. 0.003 $mm^3$, $P = 0.010$; TM-TRFI volatility: 0.46% vs. 0.15%, $P = 0.014$; CM-TRFI volatility: 1.51% vs. 0.51%, $P = 0.0033$).

## Discussion

This post hoc analysis of the OSPREY study examined volatility in retinal fluid and retinal thickness as predictors of change in anatomic features in patients with nAMD after the loading-dose period of anti-VEGF treatment. The results showed that lower volatility of SRF volume was associated with reduced increase in SHRM volume. Furthermore, lower volatility of CST, TF volume, or SRF volume was associated with greater improvement in total EZ attenuation. These findings suggest that higher volatility of retinal fluid or thickness is associated with poorer recovery of disease features in nAMD.

The finding that higher volatility of SRF was associated with poorer anatomic outcomes contrasts with findings that the presence of SRF is associated with better visual acuity outcomes.[12,13,15,16] However, it is consistent with a report that the recurrence of SRF is associated with a greater likelihood of visual acuity loss.[30] Possibly, the fluctuation or volatility of SRF is detrimental to visual acuity or is associated with anatomic disease processes that are detrimental to visual acuity, whereas static SRF is benign or beneficial. Conversely, the presence of IRF has been associated with poorer visual acuity outcomes.[3,9,11] The present study did not find a relationship between IRF volatility and anatomic outcomes, suggesting that, although the presence of IRF is detrimental, the fluctuation of IRF may not be as important. This may be explained in that eyes with low IRF volatility may nevertheless have persistent IRF. Notably, for each anatomic outcome measure, similar patterns were observed in change from week 12 for both the low- and high-volatility groups across the volatility parameters (Figs. 1, 4). Further analysis and validation with a larger dataset would be beneficial to enhance our understanding of the effects of the volatility of different retinal fluid measures.

Given that retinal fluid and thickness are markers of disease activity, high volatility may be thought of as high variation in disease activity. Variation in disease activity may occur because of natural disease processes and because of undertreatment. Specifically, when dosing intervals are too long, disease activity may initially decrease but reoccur prior to the subsequent injection. These changes in disease activity have been observed with CST in stud-

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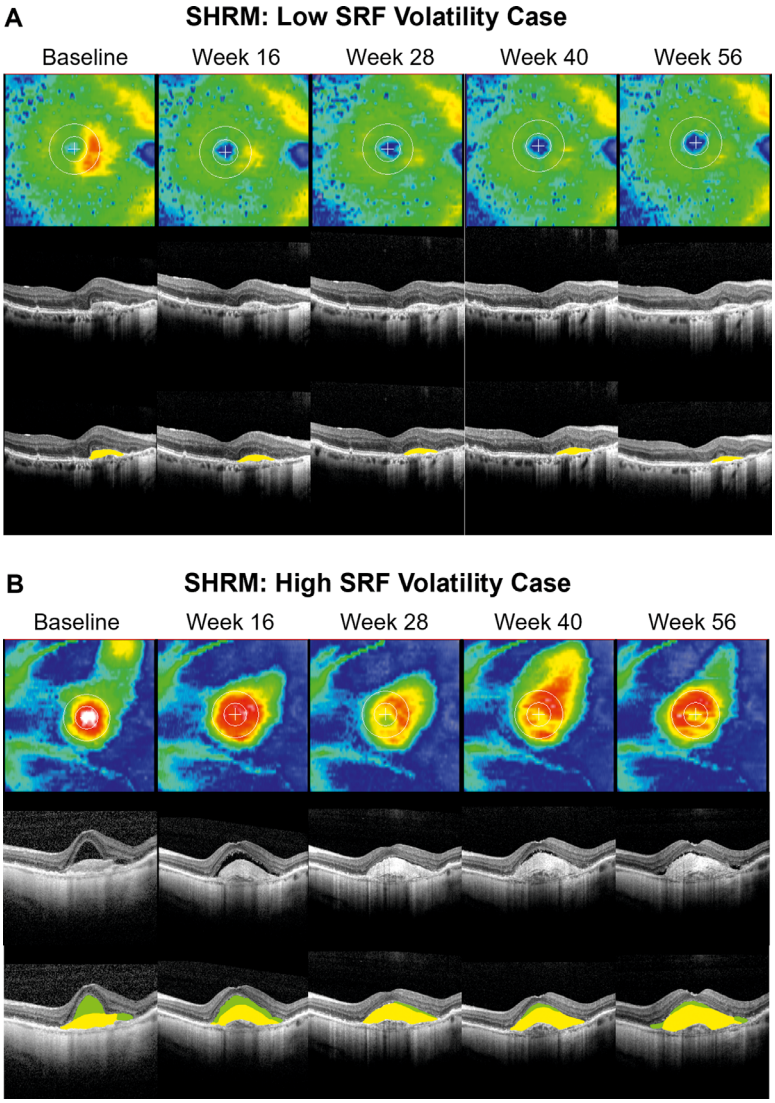


**Figure 3.** Examples of SHRM. En face SHRM thickness maps (*top row*) and B-scans (*middle* and *bottom rows*) showing SHRM at baseline and at weeks 16, 28, 40, and 56 for (**A**) a participant with low SRF volatility and (**B**) a participant with high SRF volatility across weeks 12 to 56. The *bottom row* is the same as the *middle row* but with SHRM shown in *yellow* and SRF shown in *green*.

ies using fixed-dosing regimens.[17,31,32] Unstable disease activity due to undertreatment may also occur with pro re nata dosing regimens but may be less likely with treat-and-extend regimens. If high volatility is representative of undertreatment, our results suggest that undertreatment resulting in instability of SRF or CST may be associated with negative anatomic outcomes in nAMD. The finding that high exudative volatility is associated with poorer anatomic outcomes may also have clinical implications for small amounts of "persistent" fluid in the management of nAMD. It may be important for clinicians to determine whether there is fluid resolution or recurrence, which may have different implications for outcomes than persistent fluid.

The anatomic findings of the present study are consistent with reports that lower CST volatility predicts greater BCVA gain in nAMD,[19,20] as greater EZ integrity and the absence of SHRM are associated with better visual acuity.[24,25] The present study did not find an effect of volatility on change in BCVA, which may be explained by limitations in sample size. Previous studies that found an association between CST volatility and BCVA analyzed more than 1700 eyes, whereas the present study analyzed 81 eyes.[19,20] Furthermore, although both this study and previous studies used quartiles to determine low- and high-volatility groups, further research is required to identify optimal volatility thresholds for determining low- and high-volatility groups. Notably, differences between low- and high-volatility groups in EZ integrity and SHRM volume were found as early as week 16 (Figs. 1, 4). This is similar to previous findings with BCVA outcomes, suggesting that cumulative volatility is not required for a detrimental effect.

With most of the fluid volatility parameters, the high-volatility group showed a smaller early reduction from baseline in SHRM volume than the low-volatility group (Table 2, Fig. 2). This may suggest that eyes that show limited early response in SHRM volume may be more susceptible to later retinal fluid fluctuations. Further investigation is needed to determine whether early persistent SHRM is a predictor of greater fluctuations in retinal fluid. The present study also found that early increases in CST or reti-

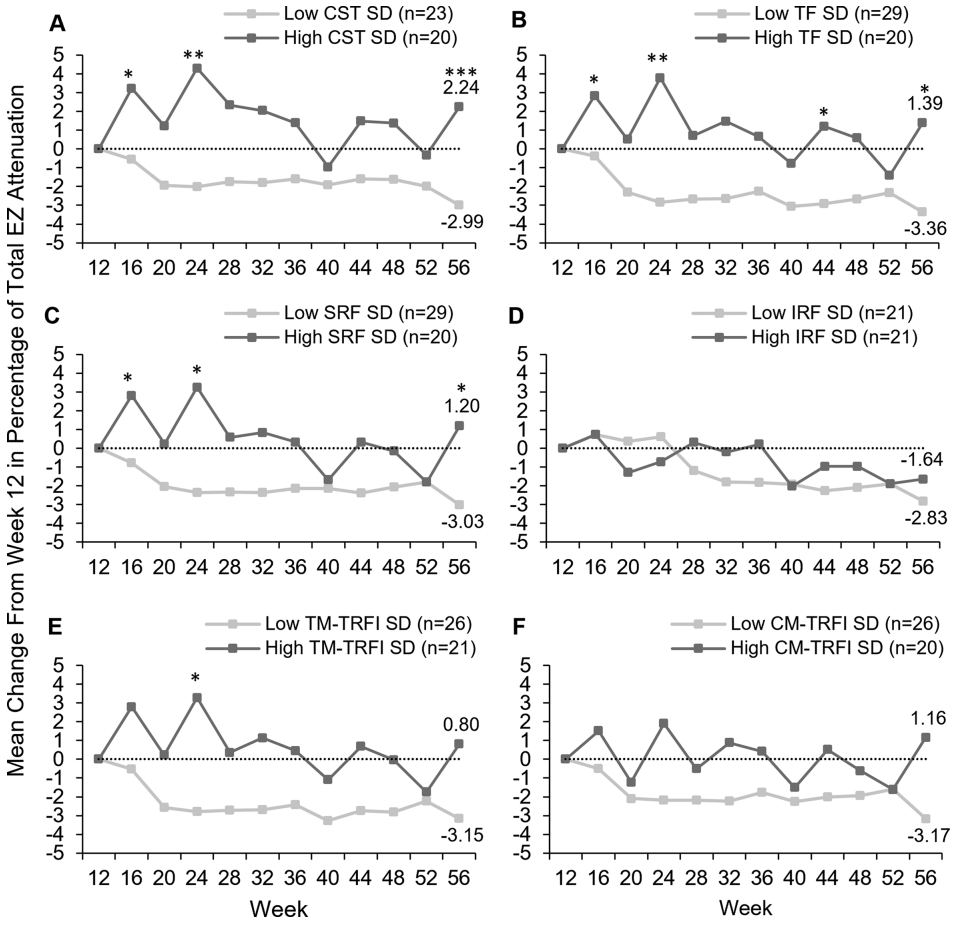


**Figure 4.** **(A–F)** Mean change from week 12 in percentage of total EZ attenuation at visits from weeks 12 to 56 for a group with low volatility (*light gray*) and high volatility (*dark gray*) in a specific parameter (i.e., CST, TF, SRF, IRF, TM-TRFI, or CM-TRFI, respectively). Volatility was calculated for each eye as the SD of the parameter across weeks 12 to 56. Values are specified at weeks 40 and 56. Asterisks indicate significant differences between low- and high-volatility groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

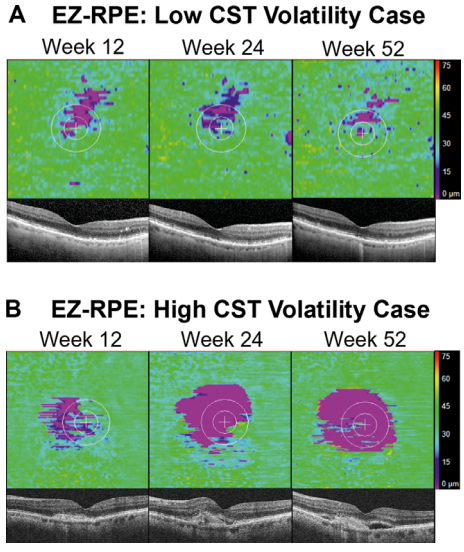


**Figure 5.** Examples of EZ integrity. En face EZ thickness maps (*top row*) and B-scans (*bottom row*) showing EZ integrity at weeks 12, 24, and 52 for (**A**) a participant with low CST volatility and (**B**) a participant with high CST volatility across weeks 12 to 56.

nal fluid between visits (weeks 4 to 8 or weeks 8 to 12) was associated with greater volatility during the maintenance phase (Table 3), suggesting that early retinal fluid increases are a predictor of later retinal fluid fluctuations. Further investigation is needed to determine the strength of the association and optimal thresholds for identifying patients with early retinal fluid instability.

There are important limitations that should be acknowledged regarding this study. This study was an exploratory analysis of the OSPREY study. All significant findings were post hoc and not corrected for multiple comparisons. Furthermore, power was limited because of the small sample size. This study is intended to be a guide for planned analyses in larger datasets on the relationship between retinal fluid and thickness fluctuations with respect to anatomic features in nAMD.

Overall, this analysis suggests that lower volatility in SRF is associated with a smaller increase in SHRM and greater improvement in EZ integrity after the loading-dose period. Similar trends were seen with macular and central macular TRFI for both the SHRM and EZ integrity measures. Greater CST volatility was also associated with less EZ integrity recovery. These findings suggest that volatility in disease activity is detrimental to optimal anatomic recovery. Further research will focus on larger dataset validation and in-depth

**Table 3.** Mean Volatility for Patients With Low Versus High Early Instability From Weeks 4 to 8 or Weeks 8 to 12

| Early Instability Group[†] | Volatility,[‡] Mean (SD) |
|---|---|
| CST (µm) | |
| Low ($n = 68$) | 19.0 (19.6) |
| High ($n = 12$) | 32.3 (26.0)[*] |
| TF ($mm^3$) | |
| Low ($n = 65$) | 0.014 (0.035) |
| High ($n = 15$) | 0.047 (0.066)[**] |
| SRF ($mm^3$) | |
| Low ($n = 73$) | 0.014 (0.041) |
| High ($n = 7$) | 0.051 (0.052) |
| IRF ($mm^3$) | |
| Low ($n = 69$) | 0.003 (0.008) |
| High ($n = 11$) | 0.012 (0.022)[*] |
| TM-TRFI (%) | |
| Low ($n = 65$) | 0.15 (0.36) |
| High ($n = 15$) | 0.46 (0.65)[*] |
| CM-TRFI (%) | |
| Low ($n = 61$) | 0.51 (0.99) |
| High ($n = 19$) | 1.51 (1.98)[**] |

[†] Eyes were categorized as having high early instability if they showed change in the parameter (CST, TF volume, SRF volume, IRF volume, TM-TRFI, or CM-TRFI) greater than or equal to a cutoff value from weeks 4 to 8 or weeks 8 to 12. All other eyes were categorized as having low early instability. A cutoff of 10 µm was used for CST; 0.001 $mm^3$ for TF volume, SRF volume, and IRF volume; 0.01% for TM-TRFI; and 0.05% for CM-TRFI.

[‡] Volatility was calculated for each eye as the SD of the parameter across weeks 12 to 56.

[*] $P < 0.05$ for high versus low early instability.

[**] $P < 0.01$ for high versus low early instability.

exploration of the specific volatility metrics that may be most important for outcomes. Future availability of automated fluid detection and characterization may allow for the clinical application of these measurements and a personalized approach to dosing to minimize volatility in disease activity in nAMD.

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