Identification of Optical Density Ratios in Subretinal Fluid as a Clinically Relevant Biomarker in Exudative Macular Disease

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PURPOSE. To investigate the potential role of optical density ratios (ODRs) obtained from subretinal fluid analysis in exudative macular disease and to identify the predictive role of ODRs under therapy in comparison to conventional morphometric measurements (CMMs).

METHODS. Fifteen patients with neovascular age-related macular degeneration (nAMD) and 15 with acute central serous chorioteretinopathy (CSC) were included in this prospective comparative and interventional case series. High-definition optical coherence tomography (SD-OCT) was performed according to a standardized protocol. nAMD patients received a standard treatment consisting of three monthly doses of intravitreous ranibizumab. Best corrected visual acuity (BCVA) was assessed at baseline (BSL) and weeks 2, 4, and 12. SD-OCT parameters were compared between CSC and nAMD at baseline. Predictive factors for functional recovery under ranibizumab treatment were identified in patients with nAMD.

RESULTS. ODR showed highly significant differences between CSC and nAMD, whereas it was not possible to differentiate between these diseases on the basis of CMM. During follow-up, CMM correlated with BCVA at BSL only, whereas ODR showed a significant correlation with BCVA at week 4 and 12 during antiangiogenic therapy.

CONCLUSIONS. Results suggest that CMM may correlate with BCVA at BSL, but has limited predictive value regarding recovery of visual function. Most interesting, ODR correlated with BCVA under therapy and was the only parameter that was pathognomonic for nAMD in contrast to CSC in this study. ODR may reflect the status of the blood–retina barrier and may be used for pathophysiologic differentiation and prognostic purposes in exudative macular disease. (Invest Ophthalmol Vis Sci. 2009;50:3417–3424) DOI:10.1167/iovs.08-2759

Age-related macular degeneration (AMD) is the leading cause of severe and irreversible vision loss in developed countries.1,2 Even though neovascular (nAMD) occurs at an incidence rate of only 10% to 20% of cases, this entity is responsible for most cases of severe vision loss.3 Fortunately, identification of vascular endothelial growth factor (VEGF) as a relevant factor for development and progression of nAMD offered an opportunity to design new therapeutic drugs that selectively target this critical factor.4,5

Since then, anti-VEGF strategies have increasingly been used and have reduced the use of competing strategies.6 As a consequence, treatment indications as well as monitoring efforts have markedly increased because the number of patients is rising, and frequent injections are often needed to maintain the initial recovery of visual function once therapy has been initiated.7,8 Thus, intravitreous strategies have a major socioeconomic impact in the field of ophthalmology.9 Individualized, flexible treatment regimens are expected to reduce this burden, but the objective evidence for relevant retreatment criteria needed for adequate guidelines is still limited.10

Parallel to these developments, use of optical coherence tomography (OCT) and, more recently, spectral domain OCT (SD-OCT) was increasingly widespread, because it allows for noninvasive imaging and quasi-histologic cross sectioning of the macular region. Small amounts of intra- or subretinal fluid (SRF) could be detected precisely.11–13 Consequently, Fung et al.14 were the first defining retreatment criteria for anti-VEGF therapy, mostly on a morphologic OCT basis. The authors significantly reduced the number of retreatments compared with a fixed regimen, whereas BCVA outcomes were comparable to the fixed regimen of monthly injections used in phase III trials. Trying to find relevant prognostic factors for nAMD in SD-OCT, our group recently identified SRF as a potentially relevant retreatment parameter and a valuable prognostic factor.15

However, other retinal disorders such as acute central serous chorioretinopathy (CSC) also show the presence of SRF, but usually present a fundamentally different BCVA and time course and much better prognosis, suggesting that factors other than the sole existence of SRF alone may play a role for visual function.16–18

To further investigate the characteristics of SRF, which has meanwhile become a widely accepted factor for retreatment in current anti-nAMD strategies, a prospective study was initiated. Mean reflectivity of the SRF compartment was measured and expressed as optical density ratios (ODRs) between SRF and OCT signal strength, media opacities, and other potentially biasing factors in our study. Doing so, we used a different approach than Barthelmes et al.,19 who used time domain Stratus OCT (Carl Zeiss Meditec, Inc., Dublin, CA) imaging and a different software solution in an interesting study published recently. ODRs were compared in patients with nAMD and CSC, taking advantage of the novel SD-OCT imaging technology. To investigate the prognostic value of ODR in nAMD and highlight the clinical potential of this new biomarker, we correlated baseline ODRs with follow-up BCVA.
METHODS

Patient Characteristics and Antiangiogenic Therapy

Thirty consecutive patients were included if SRF due to nAMD (n = 15) or acute-onset CSC (n = 15) was present in standard Stratus OCT. A standardized ophthalmic examination procedure including BCVA, SD-OCT (Cirrus; Carl Zeiss Meditec), and fluorescein angiography (FA, HRA2; Heidelberg Engineering, Heidelberg, Germany) was performed. SD-OCT examinations included a five-line high-resolution raster scan of the central macular region as well as a 512 × 128 raster scan of the entire macula.

In nAMD cases, the lesion size had to be less than 12 disc areas with the choroidal neovascularization (CNV) covering more than 50% of the lesion size. All nAMD patients were older than 50 years; six patients were women. Former treatment with anti-VEGF drugs, corticosteroids, or photodynamic therapy was an exclusion criterion, as was previous vitrectomy, aphakia, or any surgical procedure performed within the past 6 months before inclusion. After inclusion, nAMD patients received a loading dose consisting of three consecutive injections of 0.5-mg intravitreous ranibizumab in monthly intervals (baseline, weeks 4 and 8). During follow-up, patients received BCVA and SD-OCT examinations before the injection. At month 3, all patients were excluded and received further interventions if needed.

Fifteen CSC eyes of 15 patients were included after they were classified as acute-onset CSC (between 2 and 10 days since the first symptoms were recognized). Four patients were women. Patients with chronic or recurrent CSC were not included. None of the CSC patients received an intervention after the initial visit. A control visit was scheduled 3 months after the initial symptoms, to allow spontaneous remission of the SRF and symptoms. Because no intervention was performed, patients with CSC underwent no follow-up examinations.

A written informed consent after an extensive discussion of the perspectives and results was obtained from all patients before inclusion into the study. Recent disease onset or progression was documented in all patients with CSC and treatment-naïve nAMD. The institutional ethics committee approved the procedures of the study, including the treatment and diagnostic examinations. The protocol complied with the standards of the Declaration of Helsinki. All examinations were performed at the Department of Ophthalmology, Medical University of Vienna, Austria.

Visual Acuity Testing

BCVA was measured after a standardized refraction procedure at 4 m with standard Early Treatment Diabetic Retinopathy Study (ETDRS) charts. If a patient was not able to read four or more letters of the first line at 4 m, the chart was moved to 1 m and +0.75 D were added to the patient’s prior refraction. Thirty letters were added to the letters identified at 4 m, if a patient was able to read more than the first line at 4 m.

Conventional Morphometric SD-OCT Analysis

Automatic retinal thickness analysis was based on the standard, commercially available procedures provided with the Cirrus SD-OCT (software version, 2.0.1.3; Carl Zeiss Meditec, Inc.; Fig. 1). The algorithms are able to automatically identify the retinal surface and the retinal pigment epithelium (RPE), before an estimated RPE-fit is applied to the dataset. This RPE fit was introduced to act as a reference line representing an estimated physiologic course of the RPE. Retinal thickness measurements reflect the distance between the retinal surface and the RPE, whereas pigment epithelial detachment (PED) volumes are obtained by calculating the difference between RPE and RPE fit. In this study, we chose central retinal thickness (CRT) and retinal volume (RV) as parameters to image both, central and peripheral changes in retinal thickness. To include data of the RPE, we compared the difference in sub-RPE values between CSC and nAMD. We did not compare the volume of the “neovascular tissue”; since, in our experience, this parameter is often difficult to assess exactly.

Manual volumetric analysis of SRF was performed by applying 3D-Doctor software (Able Software Corp., Lexington, MA) to SD-OCT datasets. 3D-Doctor is approved by the United States Food and Drug Administration (FDA) for medical imaging and 3D visualization applications. It offers the opportunity to generate 3D models based on a sequence of B-scan images and has recently been used in the field of ophthalmology. Therefore, SRF has to be delineated manually within each of the 128 B-scans contained in a complete macular raster scan. Volumes were calculated for the segmented SRF once segmentation was completed (Fig. 2). OCT graders were masked to the subject’s identity and diagnosis, were supervised by an experienced, masked, and certified supervisor (IG) at the Vienna Reading Center, and had successfully completed an OCT grading training before segmenting datasets for this study.

OD Measurements of the SRF

OD measurements were performed with ImageJ software, a public-domain, Java-based program (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). Analyses were performed in original bitmap files which were generated from *.img files in a commercial program (Image Viewer; Labview 7 Express; National Instruments, Austin, TX). These
*.img files can be directly exported from the Cirrus OCT with research browser software (distributed by Carl Zeiss Meditec, Inc.). No further image manipulation was performed in the OCT data before the ODR analyses were performed. Three B-Scans scans showing significant amounts of SRF were chosen for the analysis of each case. In CSC, the central scan was selected in addition to one scan superior and one scan inferior to the central scan. Scans showing significant shadowing due to retinal vessels were avoided.

The B-scan demonstrating the maximum area of SRF was selected as the central scan in nAMD cases. As in CSC two additional scans were evaluated inferior and superior to the position of the central scan (Fig. 3).

To quantify the OD of SRF, mean pixel intensities were calculated for a defined ellipsoid location within the center of the SRF and an area with the same area and dimensions in the vitreous cavity 200 pixels above the inner limiting membrane (ILM; Fig. 4). ODRs were calculated by dividing the mean pixel intensity of the SRF by the mean pixel intensity of the vitreous. This measurement procedure was called the strict modality.

In a second setting, the ratios were calculated between an undefined and largest possible form within the SRF and an undefined form at roughly the same x coordinate but at a randomly chosen y coordinate more than 150 pixels above the level of the ILM. This second measurement procedure was called the flexible modality (Fig. 5).

Both measurements were applied to all CSC and nAMD cases in the same manner. Mean values of three measurements from three different B-scans within one raster scan were used for statistical analysis. Examiners performing the analysis were blinded to patients’ identity, primary disease, and BCVA.

Diagnostic Procedures and Follow-up in Patients with nAMD

To investigate the relation between visual function and SRF in nAMD, we compared four parameters at baseline and weeks 2, 4, and 12: BCVA, automatic retinal thickness and RPE segmentation, manual segmentation of SRF volume, and ODRs.

Statistics and Software

Database software (Excel; Microsoft, Redmond, WA) was used for evaluation and documentation of masked patient data. Statistical analysis was performed with another commercial program (SPSS for Windows; ver., 15.0.1; SPSS Inc., Chicago, IL). P ≤ 0.05 was considered statistically significant. Scatterplot and box plot analyses were used to demonstrate the results of different measurement modalities. Two-tailed t- and Pearson tests were used to identify significant differences and correlations between morphometric data and time course of BCVA.

RESULTS

Patients’ Characteristics and Possible Confounders

The following lesion types were present in the patients with nAMD: six occult, four minimally classic, three predominantly classic, and two classic. Retinal angiomatous proliferations (RAPs) were not present. Intra- or subretinal bleeding (ISB) was present in three cases. ISB, however, was discrete, with a total area affecting less than one fourth of a disc area in each case.
ODRs in patients with ISB (mean, 4.14; SD, 1.47) were similar to the ODRs in patients without ISB (mean, 4.72; SD, 2.75). Patients with nAMD were significantly older than those with CSC (Table 1).

Comparison of Morphometric Data between nAMD and CSC

Mean CRT was slightly higher but still comparable in CSC, whereas the mean RV was higher in nAMD eyes, but not significantly (in CSC: mean CRT, 410.53; SD, 88.45; in nAMD: mean CRT, 388.33; SD, 137.12; in CSC: mean RV, 10.96; SD, 0.77; in nAMD: mean RV, 10.92; SD, 1.21). Automatic segmentation of the RPE to RPE-fit volume showed a wider spectrum in values for nAMD than for CSC. Again, this difference was not statistically significant ($P > 0.11$).

Manual segmentation values for SRFV demonstrated consistent findings with slightly higher but also comparable values for CSC (Fig. 6).

OD Measurements

In contrast to the morphometric data of manual and automatic segmentation procedures, ODRs were significantly different in CSC and nAMD ($P < 0.0016$ for the strict; $P < 0.0004$ for the flexible modality; Fig. 7). Both measurement modalities
showed very similar outcomes, and no statistically significant
difference ($P_{\text{H}11022} < 0.6$ for nAMD; $P_{\text{H}11022} < 0.3$ for CSC) within an
entity. Most interesting is that the third quartile is clearly
located below an ODR of 2 for CSC, whereas the first quartile
for nAMD is located above 2.5 for both measurement modalities.

The strict modality led to slightly higher mean values, but a
similar range in nAMD. Scatterplot analysis of the ODR results
showed a high consistency between the two modalities ($P <
0.001$, $R^2 = 0.57$; Fig. 8, Table 2).

**Correlation of Baseline SD-OCT Parameters with
Visual Function during Follow-up**

RV and CRT values identified by automatic segmentation at
baseline correlated well with baseline visual acuity at the same
day in nAMD patients ($P = 0.01$ for RV; $P = 0.04$ for CRT), but
did not correlate with BCVA during the subsequent treatment
and follow-up phase.

As for RV and CRT, which were obtained by automated
algorithms, a significant correlation was found for manual seg-
mentation results of SRFV and BCVA at baseline. Unlike RV and
CRT, the amount of SRF at baseline showed a significant cor-


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<th>Mean, nAMD group</th>
<th>Signal Strength</th>
<th>Age (y)</th>
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<td>39.47*</td>
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<td>SD, CSC group</td>
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<td>1.41</td>
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OD in the vitreous and signal strength were comparable, even
tough the mean age was significantly different between both groups.
OD of the vitreous was assessed similar to the procedure performed to
identify ODRs. A large area was marked within the vitreous and the
mean pixel intensity was calculated for the area with ImageJ software.
Steps for data processing were identical to those described in context
with ODR measurements.

* Significant difference in ages.

**FIGURE 6.** Comparison of morphometric data between nAMD and CSC. None of the conventional morphometric OCT parameters allowed for a
safe differentiation between nAMD and CSC. Retinal volume (A) and central retinal thickness (B) were comparable, but slightly higher in CSC than
in nAMD. The difference was not statistically significant. Segmentation of the RPE deviation volume (C) showed a broader spectrum in nAMD cases.
Outliers in the CSC were due to errors in automatic segmentation. Differences between manual segmentation of SRFV (D) were minor and
statistically irrelevant.
relation with the relative change in BCVA at weeks 2 ($P < 0.0004$), 4 ($P < 0.009$), and 12 ($P = 0.33$).

RPE segmentation values comprising a PED with sub-RPE fluid showed no significant correlation to BCVA at any time point. A trend was shown between BCVA at baseline and sub-RPE volume ($P = 0.052$).

Noteworthy, baseline ODRs measured with the flexible modality did not correlate with visual function at this visit, but correlated significantly with BCVA at week 4 ($P = 0.01$) and BCVA at week 12 ($P = 0.03$). The product from SRFV and ODR correlated with BCVA at BSL ($P = 0.004$), week 4 ($P = 0.049$) and week 12 ($P = 0.015$).

![Figure 7](https://iovs.arvojournals.org/figure/7.png)

**Figure 7.** Difference between ODRs of SRF in nAMD and CSC. The ODR allowed differentiation of CSC and nAMD. Left: results of ODR measurements in patients with nAMD; right: ODRs in patients with CSC. The boxes are located below values of 2.0 for acute CSC, whereas values are significantly higher for nAMD. No significant difference was demonstrated for the two presented methods of obtaining the ODR in CSC, whereas the strict modality led to slightly higher mean values in nAMD.

![Figure 8](https://iovs.arvojournals.org/figure/8.png)

**Figure 8.** Analysis of two different modalities of assessing ODRs. Two different graders assessed the ODR measurements by using different modalities. The results of the strict measurement modality are plotted on the x-axis, the results of the flexible regimen are plotted on the y-axis. Both investigators analyzed the same scans and were blinded to the patient’s name, disease entity, BCVA, and ODR results obtained by the second reading.
most interesting, ODR measurements also correlated with week-4 and -12 BCVA in our study, implying that a nonmorphometric parameter shows substantial differences between CSC and nAMD and correlate well with the functional outcome of an anti-VEGF therapy.

Although the outcomes of ODR measurements were very informative, our study has several limitations. One is the small sample size and the limited follow-up. However, we believe that the numbers of patients is high enough to show the distinct differences between ODR in CSC and nAMD. The 3-month interval was selected because SRF has usually resolved and most of the treatment effect has taken place at the end of this “loading” regimen. Larger studies including more data of more patients have been initiated to show whether the correlation between BCVA and ODR could be proven in other settings. Moreover, only three scans out of a raster scan containing 128 images were analyzed for ODR. Automated segmentation for SRF as discussed earlier would clearly enhance the reflectivity pattern of SRF in OCT. This effect has not been described in the literature before.

BCVA showed a step-wise increase in this study. Most of the absolute treatment benefit was gained within the first 2 weeks of treatment. Two more booster doses further increased mean BCVA up to month 3.

A strong correlation was also shown between BCVA at baseline and visual function at all subsequent follow-up visits ($P < 0.022$).

**DISCUSSION**

A prospective study was performed to further investigate the specific properties of SRF and to compare a new biomarker to well-known SD-OCT parameters in two diseases with the presence of SRF. Measurements of the OD within the SRF were performed and ratios were calculated between the OD of SRF and the vitreous. Correlations between BCVA and SRF-related SD-OCT parameters were studied in detail to identify the prognostic potential for current anti-VEGF strategies. The results showed that standard CRT and RV are useful descriptive parameters correlating with baseline BCVA in nAMD only. Therewith, results of this report support the findings of earlier studies identifying similar correlations for central retinal thickness and retinal volume values and demonstrate the limited prognostic value of these parameters.

Automated RPE analyses also provided valuable data, but were compromised by segmentation errors. A trend but no statistically significant correlation was found between RPE deviation values and baseline BCVA, indicating that RPE deviations may have a minor impact on central visual function and a subordinated role as a predictive factor in nAMD.

In contrast to conventional retinal thickness or volume parameters based on automated algorithms, SRFV obtained by manual segmentation correlated with a relative change of BCVA at weeks 2, 4, and 12 when most of the beneficial anti-VEGF effect has already been gained. Therefore, baseline SRFV measurements appear to be useful to predict the visual prognosis of the initial treatment phase. Having a predictive factor for this first and most important phase of intra-vitreous anti-CNV therapy is of great clinical importance, as it may facilitate optimized patient counseling and guidance as well as individualized treatment regimens. Since segmentation analysis of the SRFV is not included in current standard SD-OCT reports, OCT manufacturers should be encouraged to develop algorithms capable of assessing SRFV by an automated process. This approach would clearly be clinically more practical than manual delineation of SRFV in multiple consecutive B-scans, which implies substantial effort. The impact of SRF on BCVA, however, appears to be strongly disease-specific, as the volume of this parameter at baseline did not allow discrimination between CSC and nAMD in this study.

ODRs were assessed by using two different measurement modalities in this study. The difference between the ODR results in SRF associated with CSC and those in nAMD were significant. The good correlation between the two measurement modes of assessing ODR suggests that there is a realistic discrepancy and eventually a pathophysiologic difference in

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**TABLE 2. Mean BCVA during Follow-up in Patients with nAMD within the First 3 Months of Treatment**

The results showed that standard CRT and RV are useful descriptive parameters correlating with baseline BCVA in nAMD only. Therewith, results of this report support the findings of earlier studies identifying similar correlations for central retinal thickness and retinal volume values and demonstrate the limited prognostic value of these parameters. The results showed that standard CRT and RV are useful descriptive parameters correlating with baseline BCVA in nAMD only. Therewith, results of this report support the findings of earlier studies identifying similar correlations for central retinal thickness and retinal volume values and demonstrate the limited prognostic value of these parameters. The results showed that standard CRT and RV are useful descriptive parameters correlating with baseline BCVA in nAMD only. Therewith, results of this report support the findings of earlier studies identifying similar correlations for central retinal thickness and retinal volume values and demonstrate the limited prognostic value of these parameters.
risk by avoiding scans with relevant shadowing due to overlying vessels for ODR measurements.

Data of ODR suggest that SD-OCT images may offer additional, relevant information, which is neglected by today’s standard OCT analysis. Researchers may consider investing efforts to optimize and further promote the identification of relevant novel parameters in SD-OCT datasets.

In summary, this study shows that conventional morphometric analyses correlate with baseline BCVA in nAMD, whereas SRFV may be a predictive parameter for initial functional treatment response in anti-VEGF treatment strategies. Conventional morphometric measurements, however, may be supplemented by other new parameters such as ODR, which may reflect pathophysiologically relevant conditions like the level of the blood–retina barrier breakdown or other biological differences regarding the pathogenesis of the disease in future. Analysis of reflectivity patterns from distinct compartments as they were performed in this study and in a study of Barthelmes et al.19 in a different setting appears to be a promising option. These parameters may lead to a better correlation between OCT and central visual function in the long term and will further improve our understanding of exudative macular disease.

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References