Identification and Quantification of the Angiofibrotic Switch in Neovascular AMD

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PURPOSE. We quantify volumetric changes of subretinal hyperreflective material (SHRM) and determine the conversion toward subretinal fibrosis, the angiofibrotic switch, under anti-VEGF therapy using polarization-sensitive optical coherence tomography (PS-OCT).

METHODS. A total of 50 eyes of 50 patients with treatment-naive neovascular age-related macular degeneration (AMD) were included in this prospective observational study: 26 diagnosed with type 1 choroidal neovascularization (CNV), seven with type 2 CNV, 11 with mixed type CNV, three with a retinal angiomatous proliferation (RAP) lesion and three with a polypoidal choroidal vasculopathy (PCV). Patients were imaged at baseline and at the end of the loading phase (after treatment with three intravitreal anti-VEGF injections) using a PS-OCT system with a scanning angle of 30° × 30° and a scan pattern of 1024 × 250 A-scans. The device is capable of detecting fibrosis based on birefringence and the RPE based on depolarization. The volume of SHRM was quantified by manual delineation in each PS-OCT B-scan and interpolation between B-scans using proprietary reading center certified software. The occurrence of fibrosis detected by PS-OCT was compared to the clinical presentation of subretinal fibrosis.

RESULTS. Of 50 eyes, 28 had SHRM at baseline. Seven of these eyes had subretinal fibrosis within 3 months, six of which could be detected unambiguously based on PS-OCT imaging. SHRM thickness and volume at month 3 (P = 0.001 and P = 0.02) were significantly larger and the reduction of SHRM thickness and volume (P = 0.002 and P = 0.027) in response to therapy were significantly less pronounced in eyes with fibrosis.

CONCLUSIONS. SHRM volume decreases significantly under anti-VEGF therapy. However, lesions unresponsive to therapy may progress to fibrosis as early as 3 months. Reduction in SHRM thickness may be a prognostic marker for treatment response.

Keywords: age-related macular degeneration, optical coherence tomography, subretinal fibrosis, choroidal neovascularization, anti-VEGF

Fibrosis is one of the most important threats to vision in neovascular age-related macular degeneration (AMD) and, despite optimal treatment, develops in almost half of all eyes after 2 years.1–4 Subretinal hyperreflective material (SHRM) on optical coherence tomography (OCT) has been identified as a risk factor for fibrosis and may be observed in up to three quarters of eyes with treatment-naive choroidal neovascularization (CNV) secondary to AMD.3–6 SHRM has had a negative impact on best-corrected visual acuity (BCVA) and eyes showing reduction of SHRM with anti-VEGF treatment have a better visual prognosis than eyes with SHRM resistant to treatment.1,3,4,7 One hypothesis states that anti-VEGF therapy may cause a decrease in vascular components and an increase in fibrous components, thus promoting the transition from angiogenesis to fibrosis, a reaction termed the angiofibrotic switch.7,8 SHRM may be composed of different types of tissue, such as neovascular tissue, fibrosis, fibrin, lipid, blood, exudation, or other AMD-specific material, which cannot be differentiated by SD-OCT alone.3,5,7,9,10 Type 2 (subretinal) CNV lesions, which have been identified as major risk factors for subretinal fibrosis, typically appear as SHRM on SD-OCT and are associated with a co-localized area of classic leakage in fluorescein angiography (FA).1,2 A differentiation between SHRM and the RPE is often challenging and in some cases not possible because of similar reflectivity of the two types of tissue in spectral domain (SD)-OCT B-scans.4,11,12 Polarization-sensitive optical coherence tomography (PS-OCT), a functional extension of SD-OCT, can segment fibrosis as well as the RPE based on their birefringent and depolarizing properties, respectively.13

We used PS-OCT to quantify the volume of SHRM in eyes with neovascular AMD before and after initiation of anti-VEGF therapy and to determine the early switch from nonfibrotic SHRM to subretinal fibrosis.

METHODS

Study Participants

The study protocol and procedures adhered to the ethics tenets of the Declaration of Helsinki and were approved by the ethics committee of the Medical University of Vienna. Informed consent was obtained from all participants. The study protocol and methods adhered to the tenets of the Declaration of Helsinki and were approved by the ethics committee of the Medical University of Vienna.
First, a cornea compensation algorithm was applied before 250 A-scans was chosen, centered on the fovea. Then, birefringent tissue of the posterior pole was segmented. 15 Then, the degree of polarization uniformity (DOPU) is calculated from the backscattered probing light beam, which has been described previously. 16–17 DOPU values <0.75 were considered depolarizing and highlighted in red in PS-OCT B-scans, allowing automated segmentation of pigmented tissue, such as the RPE.

For segmentation of the RPE, the degree of polarization uniformity (DOPU) is calculated from the backscattered probing light beam, which has been described previously. 16–17 In eyes with subretinal fibrosis, however, areas with random and abrupt changes of axis orientation, a “column-like” pattern, can be noticed as previously shown by our group in end stage neovascular AMD (Fig. 2). 13

**Image Analysis**

FA evaluation (CNV type and leakage area) was performed by two retina specialists and certified readers (PKR and MR) as a consensus grading. Type 1 CNV was diagnosed when a sub-RPE fibrovascular complex was present on OCT imaging and poorly defined or occult leakage was noted in FA. Type 2 CNV was diagnosed when sub-RPE hyperreflective material was noted colocalized with an area of classic leakage on FA. In cases of types 1 and 2 CNV, a mixed type CNV was diagnosed. A RAP lesion was diagnosed in the presence of an intraretinal hyperreflective area overlying an irregular PED, colocalized with an area of poorly defined intraretinal staining and leakage in FA. We diagnosed a PCV when sub-RPE polyps were seen on SD-OCT in close vicinity to a shallow PED with heterogeneous reflectivity, as described previously. 20–21 In these eyes, indocyanine green angiography (ICGA) showed polyps as hyperfluorescent dots in the late phase images. The area of fluorescein leakage was measured in mm² based on late phase (5 minutes after dye injection) FA images. The area of leakage was marked manually using the built-in annotation software for area measurement in the Heidelberg Spectralis device.
SHRM volume was assessed independently by two graders (PKR and MR) on PS-OCT volumetric scans at baseline and after three anti-VEGF injections. For the volumetric quantification of SHRM, we used custom, validated Vienna Reading Center software ("OCTAVO"), described in detail previously by Waldstein et al.22 In short, SHRM was marked manually on each of the 250 B-scans of every volume scan. SHRM was defined as hyperreflective material located between the neuroretina and the anterior border of the RPE (Fig. 4). Since PS-OCT offers automated RPE segmentation based on depolarization, we used the intensity B-scans with the RPE overlaid in red for manual segmentation. The program then calculated SHRM area and volume by interpolation between adjacent B-scans with marked SHRM. Furthermore, the program calculated maximum thickness of SHRM as well as mean signal intensity of SHRM scaled by maximum intensity.

Statistics
For statistical analysis we used commercial software (SPSS Statistics, version 21; IBM Corp., Armonk, NY, USA). BCVA was converted to logMAR for statistical analysis. Paired t-tests were performed to test for a change in BCVA and SHRM characteristics (volume, area, maximum thickness, mean intensity) between baseline and 3 months. We used nonparametric testing (Fisher’s exact test and independent samples Mann-Whitney U test) to analyze the difference in age, sex, leakage area on FA, type of CNV, and BCVA between patients with and without SHRM at baseline. We also used nonparametric testing to analyze the difference in BCVA, SHRM volume, SHRM area, SHRM maximum thickness, and SHRM mean intensity between eyes with and without subretinal fibrosis. No adjustment for multiple testing has been performed as the goals of the study are exploratory rather than confirmatory.

RESULTS
We included 50 eyes of 50 patients (36 female/14 male) with treatment-naive CNV secondary to AMD: 26 had type 1 CNV, seven type 2 CNV, 11 mixed type CNV, three with a RAP lesion, and three with a PCV. At baseline, 22 eyes (44%) had no SHRM on OCT imaging and 28 (56%) had SHRM, two of which (4%) had a fibrous component within the neovascular complex at baseline. At month 3, the number of eyes with SHRM decreased to 21 (42%), one-third of which (7 eyes, 14% of all study eyes) had subretinal fibrosis based on dilated fundus examination, SD-OCT, and PS-OCT imaging. Detailed patient characteristics are presented in Tables 1 and 2.

There was no significant difference in age between patients with and without SHRM ($P = 0.815$). No eye without SHRM at baseline had subretinal fibrosis at month 3, compared to seven of the 28 eyes with SHRM at baseline. Figure 2 shows a representative example of an eye with subretinal fibrosis development. Three eyes with fibrosis had a mixed type CNV, two had type 2 CNV and two had type 1 CNV. Fibrosis was...
FIGURE 3. PS-OCT imaging of a left eye with treatment-naïve type 1 CNV at baseline (a–d) and after three anti-VEGF injections (e–h). Resolution of SHRM (white dashed line) can be noted in RPE segmentation B-scans (a, e), axis orientation B-scans (b, f), and retardation B-scans (c, g), respectively. Note the decrease in segmented SHRM volume and area in en face pseudo-SLO images between baseline (d, color scale = 0–222 μm) and month 3 (h, color scale = 0–29 μm). Color scale = 0°–180° for axis orientation (b, f) and 0°–90° for retardation (c, g).

FIGURE 4. PS-OCT B-scans of a left eye with treatment-naïve mixed type CNV and SHRM are shown. The RPE and SHRM cannot be differentiated on intensity-based imaging (a); however, a clear differentiation is possible with automated RPE segmentation (b). Manually segmented SHRM is highlighted in yellow in (c).
unambiguously detected by the presence of birefringence within SHRM by PS-OCT in six of these seven eyes. In one eye, fibrosis was detected at baseline and at month 3 clinically, but was not clearly identifiable on PS-OCT. In one eye the presence of fibrosis within SHRM was detected at baseline on PS-OCT, but was not detected clinically (Supplementary Fig. S1). At month 3, subretinal fibrosis was detected clinically and by PS-OCT imaging and an increase in the volume of birefringent material was observed on PS-OCT imaging. In seven eyes with SHRM at baseline, SHRM resolved completely between baseline and month 3. There was no significant change in BCVA over time in the SHRM group, whereas in the no SHRM group the difference in BCVA (logMAR) between baseline and month 3 was statistically significant. A paired samples t-test was performed comparing SHRM characteristics and BCVA at baseline and at 3 months follow-up. SHRM intensity is given as arbitrary unit.

## DISCUSSION

In this prospective observational study we tested whether PS-OCT could be used to detect fibrous tissue within SHRM. We included 50 eyes of 50 consecutive patients with treatment-naïve neovascular AMD and evaluated the change in polarization characteristics of SHRM. SHRM volume, area, thickness, and intensity between baseline and month 3. Previous studies already showed a high prevalence of subretinal fibrosis in eyes with neovascular AMD after 1 and 2 years. However, since we were interested in detection of the early angiofibrotic switch after treatment initiation, changes after 3 months were analyzed. Since the composition of SHRM cannot be identified by SD-OCT alone and a differentiation between RPE and SHRM may not be possible, we chose PS-OCT, which offers clear and objective RPE segmentation based on depolarization and enables identification of fibrosis based on birefringence. We quantitatively analyzed the volume of SHRM based on PS-OCT scans with a dense scan pattern using proprietary software.

Our group recently showed that subretinal fibrosis can be detected automatically by PS-OCT in eyes with end-stage CNV and extensive scarring. To analyze whether the early change from nonfibrotic to fibrotic material can be detected in neovascular AMD, we conducted this prospective short-term follow-up study. We observed a change in SHRM composition from polarization preserving to birefringent material on PS-OCT in six eyes, marking the angiofibrotic switch. In one eye, fibrosis was detected clinically, but not by PS-OCT. In this eye,
birefringence was detected in the area of fibrosis, but the pattern of birefringence in en face view closely resembled that of Henle’s fiber layer. Thus, we could not unambiguously determine whether birefringence originated from SHRM or from the overlying Henle fiber layer. Segmentation algorithms that subtract Henle’s fiber layer could compensate for this ambiguity. In one eye fibrosis was detected at baseline by PS-OCT, but not clinically. Subsequently, fibrosis was detected at month 3 clinically and by PS-OCT with an increase of the birefringent component (Supplementary Fig. S1). This finding may suggest the ability of PS-OCT to detect even subclinical accumulations of fibrosis.

BCVA at baseline and at month 3 was worse in eyes with than in those without SHRM. However, in nonparametric testing there was no significant difference in BCVA change over time between the groups (Table 1). In the paired t-tests there was no significant change in BCVA in the SHRM group, whereas a significant increase in BCVA was observed in the group without SHRM. These results suggested that patients in both groups benefit from anti-VEGF therapy; however, the prognosis in regards to absolute BCVA may be limited in eyes with SHRM.

Table 3. Comparison of SHRM Characteristics and BCVA Between Eyes With and Without Fibrosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No Fibrosis Detected</th>
<th>Fibrosis Detected</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>21 (75%)</td>
<td>7 (25%)</td>
<td></td>
</tr>
<tr>
<td>BCVA (logMAR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCVA baseline</td>
<td>0.4 (0.1 to 1.3)</td>
<td>0.8 (0.4 to 1.3)</td>
<td>0.113</td>
</tr>
<tr>
<td>BCVA 3 months</td>
<td>0.5 (0.0 to 1.8)</td>
<td>0.8 (0.3 to 1.5)</td>
<td>0.090</td>
</tr>
<tr>
<td>BCVA change</td>
<td>-0.1 (-0.5 to +0.8)</td>
<td>0.0 (-0.1 to +0.5)</td>
<td>0.249</td>
</tr>
<tr>
<td>SHRM volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHRM volume baseline, mm³</td>
<td>0.32 (0.02 to 2.15)</td>
<td>0.48 (0.28 to 0.66)</td>
<td>0.101</td>
</tr>
<tr>
<td>SHRM volume 3 months, mm³</td>
<td>0.04 (0 to 0.49)</td>
<td>0.28 (0.03 to 0.37)</td>
<td>0.020*</td>
</tr>
<tr>
<td>SHRM volume change, %</td>
<td>-82% (-100% to +17%)</td>
<td>-48% (-92% to -2%)</td>
<td>0.027*</td>
</tr>
<tr>
<td>SHRM area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHRM area baseline, mm³</td>
<td>4.79 (0.70 to 24.12)</td>
<td>5.92 (4.34 to 12.82)</td>
<td>0.113</td>
</tr>
<tr>
<td>SHRM area 3 months, mm³</td>
<td>0.88 (0 to 9.55)</td>
<td>4.74 (0.63 to 8.78)</td>
<td>0.071</td>
</tr>
<tr>
<td>SHRM area change, %</td>
<td>-70% (-100% to +40%)</td>
<td>-35% (-92% to +5%)</td>
<td>0.062</td>
</tr>
<tr>
<td>SHRM thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHRM max. thickness baseline, μm</td>
<td>189 (83 to 338)</td>
<td>198 (126 to 294)</td>
<td>0.796</td>
</tr>
<tr>
<td>SHRM max. thickness 3 months, μm</td>
<td>87 (0 to 222)</td>
<td>146 (79 to 235)</td>
<td>0.001*</td>
</tr>
<tr>
<td>SHRM max. thickness change, %</td>
<td>-52% (-100% to -6%)</td>
<td>-19% (-58% to +17%)</td>
<td>0.002*</td>
</tr>
<tr>
<td>SHRM intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHRM intensity baseline</td>
<td>0.0035 (0.0005 to 0.0075)</td>
<td>0.0026 (0.0013 to 0.0092)</td>
<td>0.499</td>
</tr>
<tr>
<td>SHRM intensity 3 months</td>
<td>0.0064 (0.0 to 0.0266)</td>
<td>0.0081 (0.0060 to 0.0175)</td>
<td>0.548</td>
</tr>
<tr>
<td>SHRM intensity change, relative</td>
<td>1.3085 (-1.0 to 6.9)</td>
<td>2.7129 (0.5513 to 4.1)</td>
<td>0.113</td>
</tr>
</tbody>
</table>

* P < 0.05.

Independent samples Mann-Whitney U test was used. SHRM intensity is given as arbitrary unit.

These results may suggest that SHRM contracts in response to anti-VEGF therapy regardless of SHRM composition. However, contraction in the vertical dimension is less pronounced in eyes with than in those without fibrosis. Since SHRM volume is essentially a derivative of SHRM area and thickness, thickness may be the most important SHRM characteristic in intensity-based OCT imaging. One explanation may be that SHRM starts to develop anterior to the CNV and extends laterally only slowly. SHRM borders, therefore, are “younger” and, hence, may be more susceptible to anti-VEGF treatment than the central SHRM component. As observed in optical coherence tomography angiography (OCTA), CNV responds to anti-VEGF therapy with a budding of the small peripheral vessels but with a preservation of central larger vessels.25-27 Similar treatment response may happen within SHRM. Thus, adding OCTA to PS-OCT analysis of SHRM could strongly improve our understanding of pathophysiologic mechanisms in neovascular AMD.
In line with previous studies, we observed SHRM in the majority of study eyes even though with 28 of 50 eyes at baseline (56%) the percentage of SHRM eyes was a little lower than in most previous studies.1–3,4,28 Approximately half of eyes in the SHRM group were diagnosed with a subretinal CNV composed of type 2 or mixed type CNVs, suggesting that in a large proportion of eyes, SHRM is composed of neovascular tissue, in line with the results from CATT.1 Similarly, type 2 CNV has been identified as a risk factor for subretinal fibrosis in a study by Bloch et al.2 Interestingly, in their study most cases of fibrosis developed within the first 6 months after diagnosis. However, the first follow-up examination after baseline in their study was scheduled 16 weeks after the first injection and so fibrosis development within the first 3 months may have been missed.

In multiple studies, SHRM was analyzed quantitatively and has been shown to affect BCVA during follow-up in eyes receiving anti-VEGF therapy for neovascular AMD.1–3,29 Lee et al.28 recently showed that quantitative evaluation of SHRM is superior to qualitative evaluation. In their study, they used manual segmentation to quantify the volume of different AMD-specific pathologic findings, such as subretinal or intraretinal fluid, SHRM, or pigment epithelial detachment (PED) within the central 1 mm². They found SHRM volume to be the only significant volumetric factor associated with BCVA change.28 Our study showed that eyes with subretinal fibrosis show significantly less reduction of SHRM volume compared to eyes without subretinal fibrosis and, thus, may also explain the outcomes of previous studies.

A widely accepted explanation for the formation of subretinal fibrosis is that the vascular component of the CNV lesion regresses while the fibrous component increases when the lesion becomes less active.4,5,9 Willoughby et al.,3 who examined SHRM characteristics in CATT, as well as other groups, hypothesized that anti-VEGF treatment reduces the fluid component within SHRM.5,4,29 With continued anti-VEGF injections, the fibrous components may increase relative to fluid and further reduction in SHRM thickness may not be observed during follow-up, fitting well the significant difference in SHRM thickness at baseline and at 3 months and change in SHRM thickness over time between eyes with and without fibrosis in our study.

SHRM intensity has previously been identified as another important characteristic with clinical value.3,29,30 With anti-VEGF treatment, an increase in reflectivity of SHRM can be observed. We hypothesized that eyes with subretinal fibrosis would show higher reflectivity of SHRM than eyes without subretinal fibrosis. Since reflectivity of OCT volume scans varies significantly between different follow-up visits and is influenced by a number of different factors during image acquisition, we chose to calculate the mean reflectivity of SHRM scaled by maximum reflectivity to accurately assess signal intensity in a standardized manner. We did not observe a significant difference in maximum reflectivity in nonparametric testing between the groups with and without fibrosis at baseline, at month 3, or in change over time. However, in the paired t-test, mean intensity increased significantly from baseline to month 3 in all eyes with SHRM. Hence, our data suggested that increasing intensity of SHRM material on SD-OCT is not sufficient to identify fibrosis vs. nonfibrotic SHRM. Different types of SHRM may react to anti-VEGF therapy with condensation of fibrous material and formation of fibrosis, as previously shown in proliferative diabetic retinopathy.8,54 Combination of PS-OCT and OCTA could allow quantification and an exact spatial analysis of the relationship between vascular and fibrous components of SHRM longitudinally.

The efficacy of anti-VEGF treatment as well as that of (adjunct) medications directed at fibrous components of SHRM may be objectively assessed by PS-OCT. A recent publication by Lechner et al.35 suggests an important role of the complement system in the development of fibrosis in AMD, which may represent a promising target for antifibrosis medication. There are certain limitations of this study. Apart from the short follow-up time, the relatively small number of seven study eyes with fibrosis deserves mentioning. A longitudinal study with a longer follow-up would likely show a higher rate of fibrosis. However, we were interested only in the development of early fibrosis and whether it is detectable by PS-OCT. Another limitation is that we did not use a PS-OCT device with an integrated eye-tracker; however, by using a wide scan angle of 30° × 30°, centering volume scans on the fovea and excluding eyes with obvious motion artifacts in PS-OCT volume scans, we compensated for the missing eye-tracking and managed to cover the same macular area in every volume scan. The strengths of our study include a meticulous quantitative analysis of SHRM volume as well as the advantage of PS-OCT imaging, offering tissue segmentation based on polarization as opposed to mere signal intensity as in conventional SD-OCT.

In conclusion, we showed by novel PS-OCT imaging that in our patient cohort 25% of eyes with SHRM at baseline have subretinal fibrosis after the first three anti-VEGF injections. Development of subretinal fibrosis is associated with thicker SHRM as well as larger SHRM volume and less reduction of SHRM thickness and volume in response to anti-VEGF therapy. PS-OCT offers detection of minute, perhaps even subclinical amounts of fibrosis based on tissue-specific birefringence. Future long-term follow-up studies are required to confirm our results and demonstrate quantification of area and volume of subretinal fibrosis longitudinally.

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