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ORIGINAL RESEARCH

Analysis of Choriocapillaris Reperfusion Topography Following Faricimab Treatment for Neovascular Age-Related Macular Degeneration in Therapy-Naïve Patients

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ABSTRACT

Introduction: To assess changes in choriocapillaris (CC) vascular density surrounding macular neovascularization (MNV) in treatment-naïve age-related macular degeneration (AMD) after faricimab application using optical coherence tomography angiography (OCTA).

Methods: Twenty-five eyes of 25 treatment-naïve individuals who underwent intravitreal faricimab injections for neovascular AMD (nAMD) with type 1 MNV were included. Spectral-domain optical coherence tomography (SD-OCT) images and en-face swept-source OCTA images were analyzed, and the percentage of CC flow deficit (FD%), FD average area (FDa) and FD number (FDn) in five progressive 20.0-µm-wide concentric rings (R1, R2, R3, R4 and R5) surrounding the dark halo around the MNV were calculated. Image acquisition was carried out prior to the first faricimab injection (T0) and 1 month after the injection (T1).

Results: The topographical sub-analysis revealed noteworthy changes in all rings at T1 compared to T0. There was a notable progressive reduction in FD% at T1 compared to T0 values across all rings, indicating a gradual CC reperfusion following anti-VEGF treatment. Additionally, the average size of FD decreased after the loading phase. Although not reaching statistical

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significance, there was a progressive reduction in the FDa across all rings.

Conclusion: Our study highlights a CC FD reduction following the administration of three consecutive faricimab injections. This effect was detected in all rings surrounding the dark halo. These observations suggest a partial CC reperfusion surrounding the MNV, potentially serving as an indicator for disease regression.

Keywords: Faricimab; Choroid; OCTA; Anti-VEGF; Angiopoietin 2; AMD

Key Summary Points

Why carry out this study?
There is convincing evidence emphasizing the role of the choriocapillaris in the setting of age-related macular degeneration.
The aim of the study was to analyze the influence of faricimab treatment on choriocapillaris perfusion.
What was learned from the study?
Analysis of optical coherence tomography angiography images demonstrated a significant reduction in flow deficit within the choriocapillaris surrounding the dark halo around the macular neovascularization following faricimab treatment.
Faricimab treatment seems to initiate reperfusion of the choriocapillaris surrounding the macular neovascularization membrane in the setting of neovascular age-related macular degeneration.

INTRODUCTION

Age-related macular degeneration (AMD) is a significant cause of blindness affecting > 6 million individuals globally [1, 2]. Compelling evidence points to the crucial role of impaired choroidal flow signal in the development of AMD. Recent histopathologic examinations have revealed instances of choroidal dysregulation even in the early and intermediate stages of AMD, promoting angiogenesis and continuous vascular remodeling [3–6]. Notably, a heightened impairment in choroidal and particularly choriocapillaris (CC) flow is significantly associated with eyes affected by AMD compared to eyes without AMD [7]. This impairment is particularly pronounced in neovascular AMD (nAMD), where irregularities in the spatial distribution of the CC align with the development of abnormal blood vessels in the sub-retinal pigment epithelium (RPE) cell spaces, leading to the occurrence of macular neovascularization (MNV) [5, 8]. The spatial distribution of CC impairment surrounding MNV can be quantified by delineating the MNV and measuring the flow deficit (FD) at specific distances from the MNV outline [5, 9].

The RPE secretes vascular endothelial growth factor (VEGF), which plays a crucial role in the formation of MNV. Historically, VEGF has been the primary protein targeted in various diseases associated with MNV formation [10, 11]. However, our understanding of the effects of anti-VEGF drugs on the choroid is limited and subject to controversy. Some studies have suggested that prolonged use of anti-VEGF agents may lead to a reduction in vascular density, particularly in the CC [12, 13]. Utilizing optical coherence tomography (OCT) and OCT angiography (OCTA), previous research has demonstrated that intravitreal administration of anti-VEGF agents does influence the choroid, affecting the CC flow signal [14–16]. This impact was investigated by Viggiano et al., revealing a decrease in FD and reperfusion of the CC surrounding MNV following anti-VEGF therapy [16].

In 2022, faricimab was approved by the Food and Drug Administration (FDA) for the treatment of nAMD and diabetic macular edema [17]. It is the first intraocular drug approved by the FDA to target both VEGF and angiopoietin2 (Ang2). Ang2, a crucial component of the Ang/Tie pathway, plays a multifaceted role in vascular homeostasis, influencing vascular permeability and participating in neoangiogenic and proinflammatory processes [18, 19]. Despite its recognized impact on these processes, the specific influence of Ang2 on choroidal flow signal remains unexplored in vivo. This aspect is particularly intriguing, given Ang2's role in

regulating vascular homeostasis. As a result, this research aims to assess the choroidal flow signal both before and after the intravitreal injection of faricimab, utilizing OCTA.

METHODS

Study Participants

In this retrospective study, we assessed 25 eyes from 25 individuals who received intravitreal faricimab injections for neovascular AMD and type 1 MNV at the Department of Ophthalmology, Klinikum Klagenfurt, Austria. The period of investigation spanned from April 2023 to January 2024. None of the study eyes had previously received any intravitreal injection. The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the local review board (Ethikkommission Kärnten, S2023-13). All subjects provided informed consent prior to treatment. As data were deidentified, consent for publication was not required. Each patient underwent a comprehensive ophthalmologic assessment, encompassing the measurement of best-corrected visual acuity (BCVA), intraocular pressure (IOP) and dilated ophthalmoscopy. Only individuals with IOP levels falling within the normal range (10–21 mmHg) were considered for inclusion. Exclusion criteria comprised: (1) significant cataract; (2) myopia > 3.00 diopters; (3) occurrence of myocardial infarction or cerebrovascular disease within the preceding 6 months; (4) presence of infection or inflammation in both eyes; (5) existence of other concurrent retinal and/or macular conditions (e.g., diabetic retinopathy and retinal venous occlusion); (6) any form of optic neuropathy, including glaucoma; (7) presence of type 2 or type 3 MNV; (8) previous intravitreal medical treatment.

Imaging Acquisition

All imaging was performed by well-trained ophthalmologists (M.B. and T.M.). High-resolution SD-OCT images (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) taken before and after a series of three faricimab injections were analyzed. For each OCT scan, a pattern of B scans ($n = 19$), horizontally oriented and centered over the fovea in a 20° × 15° (5.7 × 4.2 mm) area, were acquired. The central retinal thickness (CRT) was determined using the macular thickness map tool in the Heidelberg software (Heidelberg Eye Explorer, Heidelberg Engineering, Heidelberg, Germany).

Zeiss PLEX Elite 9000 Swept-Source OCTA (Carl Zeiss AC, Jena, Germany) was used to capture imaging data for all patients. OCTA imaging occurred at two specific time points: 0 to 3 days before the initial injection of faricimab (T0) and 1 month after completion of a series of three consecutive faricimab injections (T1). Time span between the injections was 6 weeks.

En-face OCTA 6 × 6-mm volume scans were obtained. Each imaging session comprised OCTA volumetric scans of the posterior pole. The device's follow-up mode ensured consistent measurements at identical locations across all time points. OCTA scans with a strength index below eight out of ten or exhibiting significant motion artifacts or shadowing effects were excluded from the analysis [20]. As part of our clinic's protocol, all medical retina patients underwent routine assessments during a specified time frame (between 08:00 and 12:00 a.m.), mitigating potential bias arising from physiologic diurnal changes in ocular flow signal [21, 22]. Manufacturer default settings were employed for the automatic segmentation of OCTA images in all B scans [23]. Automated segmentation facilitated the extraction of 10-µm slabs of the CC. CC areas beneath major superficial retinal vessels were excluded from the analysis to prevent potential shadows or projection artifacts [24] (Fig. 1). Additionally, for inclusion in the study, all edges of the MNV were required to be localized at least 1 mm away from the scan edge.

Image Analysis

A subsequent analysis was conducted following a pre-established protocol designed for the quantification of flow metrics [16, 25]. En-face

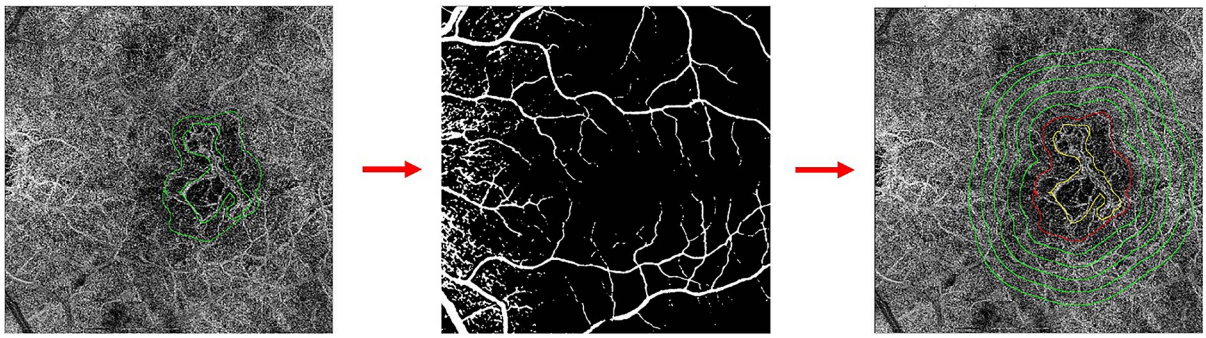

Fig. 1 Analysis of choriocapillaris (CC) using optical coherence tomography angiography en-face imaging. Automated segmentation was employed to extract 10-μm slabs of the choriocapillaris, excluding CC areas beneath major superficial retinal vessels to mitigate potential shadows or projection artifacts. The 6 × 6-mm en-face CC image showcases subfoveal treatment-naïve macular neovascularization (MNV, highlighted in green). The perilesional dark halo, denoted by a low-flow area surrounding the MNV, is encircled in red

OCTA images of the CC were imported into Fiji ImageJ (software version 2.0.0; National Institute of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). Each CC image underwent compensation to eliminate retinal vessel projection artifacts and adjustment for shadowing artifacts (Fig. 1) [25, 26]. A low-flow signal area surrounding each MNV, known as the dark halo (DH), was identified [16]. The borders of the MNV lesion and the associated DH were manually outlined by two masked expert graders (P.V. and G.B.) in each en-face CC OCTA scan. Subsequently, five progressive 200-μm-wide concentric rings from the DH edge were generated using the "Distance Map" function in ImageJ, which automatically creates a border that follows the contour of the perilesional halo (Fig. 1) [27]. Each ring (R1, R2, R3, R4 and R5) was added to the region of interest (ROI) manager for CC flow analysis. The custom configuration, unique for each patient, consisting of these rings was applied to the CC en-face image at T0 and T1 at the same size and position (Fig. 2) [27]. Following this, the resulting CC images were binarized using the Phansalkar method for the quantitative measurement of the FD in each ring using a radius of 15 pixels (Fig. 2) [28]. The "Analyze Particles" command was utilized to calculate the CC FD, and the "Analyze Particles" tool provided by ImageJ was employed to quantify CC flow in each ring area (R1, R2, R3, R4 and R5). Specifically, the following metrics were quantified: (1) the FD percentage (FD%), representing the percentage of flow deficits within the analyzed area; (2) the FD average area (FDa), representing the average size of the flow deficits within the analyzed region; (3) the FD number (FDn), quantifying the number of flow deficits in the ROI. Histologic studies with direct measurements of intercapillary distances indicate that 76.8% are < 24 μm [29]. Thus, we did not enforce a minimum cutoff [30].

Statistical Analysis

Statistical calculations were conducted using the Statistical Package for Social Sciences (SPSS IBM Statistic 25, Chicago, IL, USA). The data distribution was assessed using the Shapiro-Wilk test. Paired *t*-tests were used for analyzing quantitative data across the study visits. A significance level of $p < 0.05$ was chosen to determine statistical significance.

RESULTS

After initial screening, 29 eyes which had received OCTA imaging at T0 and T1 were identified for this study. After further analysis

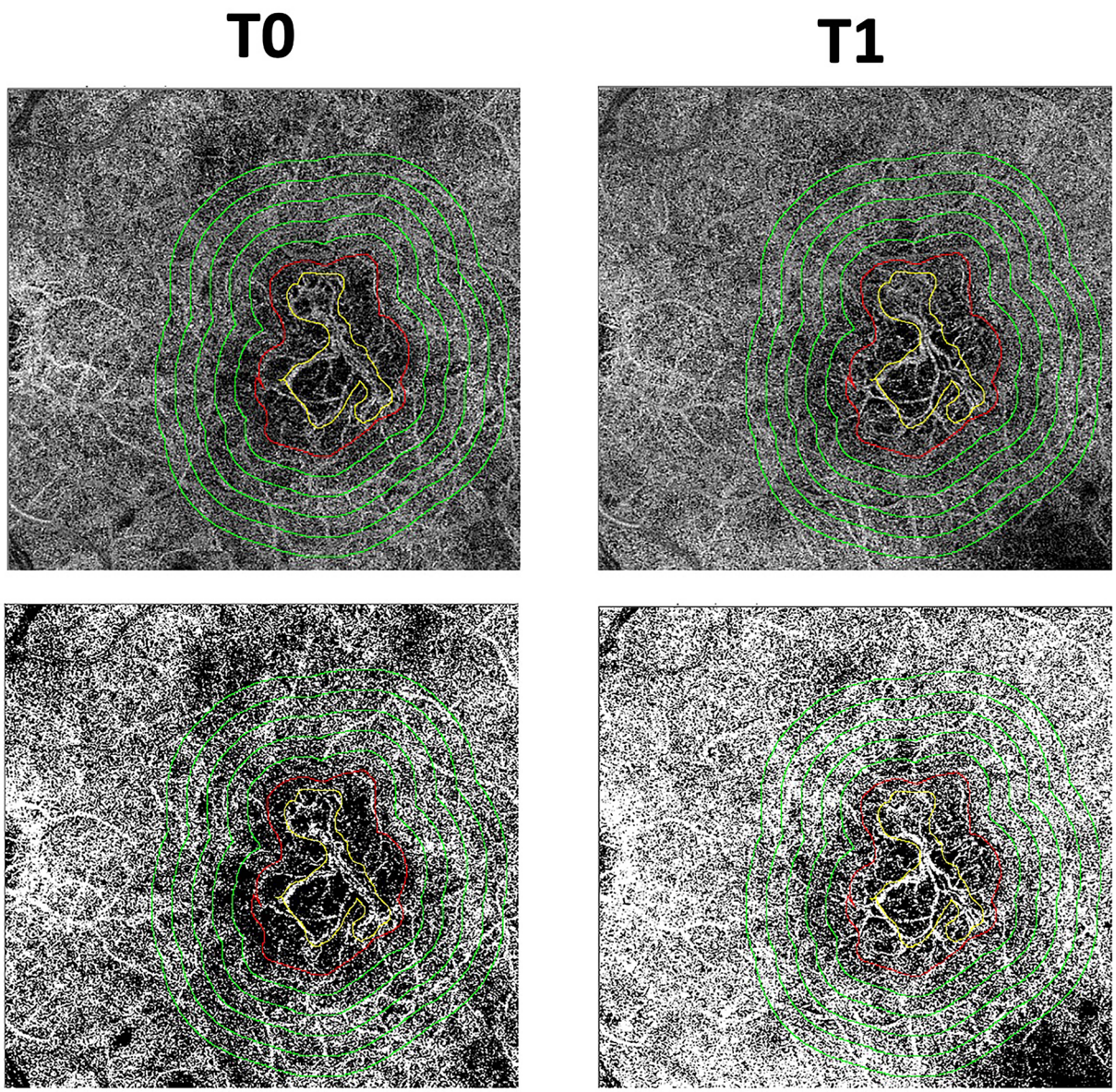

Fig. 2 En-face choriocapillaris (CC) optical coherence tomography angiography images are employed for the manual delineation of borders for both the macular neovascularization type 1 lesion and its associated dark halo. Subsequently, utilizing the "Distance Map" function in ImageJ, five progressive concentric rings, each 200 μm wide, are generated from the periphery of the dark halo's edge. This function in ImageJ automatically establishes a border that follows the contour of the perilesional halo. Every ring (R1, R2, R3, R4 and R5) was incorporated into the Region of Interest manager for CC flow analysis. This custom configuration, unique to each patient, was then implemented on the CC en-face images at T1, maintaining consistent size and position for comparative analysis. Subsequently, the obtained CC images underwent binarization for the quantitative assessment of the flow deficit within each ring. The Phansalkar method was employed for this purpose

of the OCTA images, four eyes were excluded because of poor imaging quality or presence of type 2 MNV. Twenty-five eyes of 25 patients of European descent receiving a series of three faricimab injections for the treatment of nAMD were finally included in the study. Seventeen patients were female; eight were male (Table 1). The cohort consisted of 11 right and 14 left eyes. The mean age was 76.6 ± 7.2 years (range = 64–88 years; ± SD, Table 1). Mean BCVA was 0.57 ± 0.34 (range 0.2–1.0 LogMAR, ± SD) at T0 and 0.55 ± 0.35 (range 0.2–1.0 LogMAR, ± SD) at T1, showing no significant change before and after treatment ($p = 0.59$, Table 1).

Mean CRT was 322.4 ± 102.1 µm before and 258.6 ± 50.0 µm after faricimab treatment ($p = 0.002$, Table 1). In the subjects included in the analysis, the MNV lesion displayed an average mean area of 0.68 ± 0.57 mm^2 at T0 and 0.44 ± 0.47 mm^2 at T1 ($p = 0.007$, Table 1). Inter-observer agreement (average) was found to be excellent in the MNV assessment [0.90 (confidence interval, 0.86–0.93)]. Subretinal fluid (SRF) was detected in 14 eyes (56%), intraretinal fluid (IRF) in 15 eyes (60%) (Table 1). Pigment epithelial detachments (PEDs) were present in nine eyes (36%) (Table 1). Alle PEDs were located within a radius of 2000 µm from the foveal center.

The sub-analysis of topographic CC revealed statistically significant changes in all analyzed rings. Specifically, there was a notable progressive reduction in FD% at T1 compared to T0 values across all rings, indicating a gradual CC reperfusion following faricimab treatment (Table 2). Additionally, the average size of FD showed a decrease after the loading phase. Although not reaching statistical significance, there was a progressive reduction in the FDa across all rings (Table 3). Conversely, at T1, a significant increase in FDn was observed only in ring 1 and ring 2 compared to T0 ($p < 0.034$; Table 4).

DISCUSSION

The primary objective of this study was to assess CC flow changes after faricimab treatment for nAMD in treatment-naïve eyes. Significant

Table 1 The clinical characteristics of subjects included in the analysis

MNV type 1 eyes		**N = 25**
Age (years)		76.6 ± 7.2
Gender (female, %)		18 (72%)
Right eye, n		11
Subretinal fluid		14 (56%)
Intraretinal fluid		15 (60%)
Pigment epithelial detachment		9 (36%)
	T0	**T1**
BCVA logMAR	0.57.50 ± 0.343	0.5547 ± 0.353 $p = 0.59$
CRT (µm)	322.4 ± 102.1	258.6 ± 50.0 $p = 0.002$*
MNV area (mm^2)	0.68 ± 0.57	0.44 ± 0.47 $p = 0.007$*

Data are presented as mean ± SD. *MNV* macular neovascularization, *BCVA* best-corrected visual acuity, *CRT* central retinal thickness, *T0* before, *T1* 4 weeks after three consecutive faricimab injections. Paired test, *$p < 0.05$ comparison with T0

Table 2 OCT angiography FD% data and comparisons

	CC FD (%) T0	CC FD (%) T1	*p* value
Ring 1 (R1)	45.7 ± 8.71	40.3 ± 7.33	0.003*
Ring 2 (R2)	40.7 ± 8.02	36.1 ± 6.31	0.005*
Ring 3 (R3)	38.1 ± 6.44	34.4 ± 6.36	0.009*
Ring 4 (R4)	35.7 ± 5.56	33.2 ± 6.17	0.043*
Ring 5 (R5)	34.4 ± 4.94	31.8 ± 5.48	0.027*

Data are presented as mean ± SD. *OCT* optical coherence tomography, *CC* choriocapillaris, *FD%* flow deficit percentage, *T0* before, *T1* 4 weeks after three consecutive faricimab injections, *SD* standard deviation. Paired test, *$p < 0.05$ comparison with T0

Table 3 OCT angiography FDa, data and comparisons

	CC FDa T0	CC FDa T1	*p* value
Ring 1 (R1)	77.1 ± 10.84	42.9 ± 27.52	0.107
Ring 2 (R2)	48.1 ± 6.24	29.1 ± 15.64	0.121
Ring 3 (R3)	33.7 ± 25.32	24.7 ± 12.63	0.086
Ring 4 (R4)	27.1 ± 15.82	22.5 ± 11.53	0.219
Ring 5 (R5)	24.3 ± 13.41	19.8 ± 8.75	0.159

Data are presented as mean ± SD. *OCT* optical coherence tomography, *CC* choriocapillaris, *FDa* flow deficit average area, *T0* before, *T1* 4 weeks after three consecutive faricimab injections, *SD* standard deviation. Paired test

Table 4 OCT angiography FDn, data and comparisons

	CC FDn T0	CC FDn T1	*p* value
Ring 1 (R1)	751.5 ± 604.84	1024.8 ± 729.41	0.008*
Ring 2 (R2)	895.4 ± 571.53	1096.4 ± 613.54	0.034*
Ring 3 (R3)	1065.2 ± 547.71	1226.9 ± 536.14	0.063
Ring 4 (R4)	1224.8 ± 473.11	1353.1 ± 501.11	0.131
Ring 5 (R5)	1331.9 ± 398.12	1475.1 ± 437.22	0.063

Data are presented as mean ± SD. *OCT* optical coherence tomography, *CC* choriocapillaris, *FDn* flow deficit number, *T0* before, *T1* weeks after three consecutive faricimab injections, *SD* standard deviation. Paired test, *$p < 0.05$ compared with T0

changes in choroidal perfusion following intravitreal injection therapy were observed across different regions, indicating a notable reperfusion of the CC. A significant decrease in perilesional CC FD was measured following faricimab treatment. At T1, there was a noteworthy increase in perilesional CC flow signal indicating reperfusion in all five rings. Specifically, our results demonstrate a significant reduction in CC FD and a reduction of FDa.

Prior research has underscored the significance of the choroid, particularly the CC, in the context of AMD [3, 6, 31, 32]. It is hypothesized that dysfunction of the CC plays a pivotal role in the initiation and progression of AMD [6, 32, 33]. Numerous studies have provided evidence supporting CC impairment in the context of AMD. Histologic findings indicate that CC degeneration has an impact on the viability of the RPE [32]. Biesemeier et al., using light and electron microscopy, observed that CC breakdown is a normal part of aging but becomes significantly more pronounced when AMD develops [3]. They further noted that CC alterations precede RPE loss, leading to the conclusion that AMD can be characterized as a vascular disease [3]. Spaide, in an examination involving 104 eyes of 80 patients with AMD using OCTA, described significant alterations in the flow pattern [34]. In a separate cohort of 42 eyes with intermediate AMD, Borrelli et al. later discovered that eyes with intermediate AMD in patients with nAMD in the fellow eye exhibited an increased average size of choriocapillaris signal voids compared to eyes without nAMD in the fellow eye [6]. Previous reports have documented a compromise in the CC adjacent to the dark halo and MNV. McLeod et al. substantiated this observation by demonstrating a 50% reduction in CC circulation surrounding MNV in postmortem nAMD eyes even in the presence of a structurally intact overlying RPE [32]. Recent advancements in OCTA have confirmed that CC hypoperfusion is most pronounced in the vicinity of the MNV membrane [5]. Treister et al., defining a 200-µm ring as the "halo" zone, showcased significantly greater CC non-perfusion adjacent to MNV [35]. In a prospective case series involving 80 eyes, Coscas et al. linked the presence of a dark halo to the shadowing effect

caused by blood or intraretinal and subretinal fluids, interpreting it as a sign of active MNV that warrants treatment [36]. The true nature of the dark halo, whether it signifies genuine CC ischemia or is a result of a shadowing effect, remains uncertain [37]. To address this ambiguity, our investigation focused on CC flow outside the dark halo, excluding the halo itself.

Considering that faricimab is the first approved drug targeting not only VEGF but also Ang2, investigating its effects on CC flow signal holds particular interest. Ang2 is a component of the Ang/Tie pathway, participating in the regulation of vascular homeostasis, modulation of vascular permeability and involvement in neoangiogenic and proinflammatory processes [18]. The angiogenic or anti-angiogenic activity of Ang2 depends on the context, with one of the determining factors being the expression of other angiogenic growth factors, notably VEGF [38]. In vitro studies have indicated that Ang2 induces permeability and angiogenesis on the pupillary membrane when VEGF is present. However, in the absence of VEGF, Ang2 leads to vessel regression and endothelial cell death [39]. While caution is warranted in extrapolating

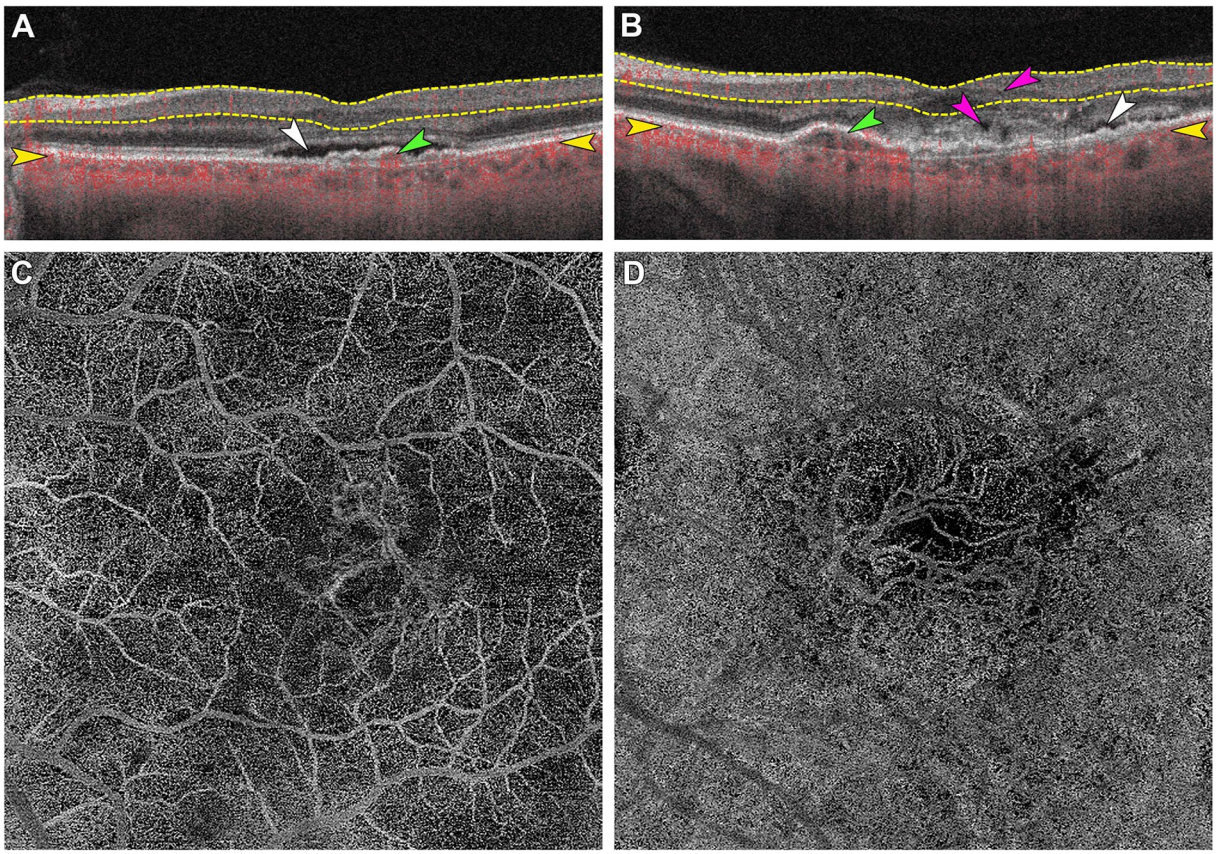

Fig. 3 **A** and **B** Display central optical coherence tomography angiography (OCTA) B scans of three different eyes of three different individuals from our cohort. Detected flow signals are displayed as red dots according to the standard setting of the utilized OCTA machine. **A** Flow signal is clearly detectable at choriocapillaris (CC) layer (red dots between yellow arrowheads) despite presence of subretinal fluid (SRF, white arrowhead) and pigment epithelial detachment (PED, green arrowhead) containing the macular neovascularization (MNV) type I membrane. **B** Flow signal is clearly detectable at CC layer (red dots between yellow arrowheads) despite presence of SRF (white arrowhead), intraretinal fluid (pink arrowheads) and PED (green arrowhead) containing MNV type I membrane. **C** and **D** Corresponding en-face angiography images on the CC layer

these findings to clinical scenarios, it is conceivable that blocking Ang2, especially in conjunction with VEGF depletion, could impact the permeability of choroidal vessels, indirectly influencing CC perfusion. Razavi et al. have reported a similar observation, speculating that in neovascular AMD, anti-VEGF treatment may not only positively affect retinal exudation but also underlying choroidal exudation by reducing choroidal vascular hyperpermeability [40].

No notable change in BCVA was observed in our study. This lack of significant change is likely attributed to the small number of injections that had been applied. This constitutes a noteworthy limitation of our study.

As of our current understanding, this study represents the initial exploration into the influence of intravitreally administered faricimab on CC flow in treatment-naïve eyes. It is essential to acknowledge the limitations inherent in the retrospective design and the relatively small sample size of the study. Measuring CC flow and FD using OCTA is a challenging task especially in the setting of AMD. There is an ongoing discussion about potential errors caused by technical and anatomical conditions [41]. We have addressed this issue by applying an up-to-date acquisition and image processing method, which has proven to be robust with excellent repeatability in a number of published studies [25, 30]. This method has been shown to be effective in compensating for the signal loss caused by retinal vessels as well as the RPE/Bruch's membrane complex. It was especially tested to account for the shadowing effect from the elevated RPE caused by drusen in the setting of AMD [25]. Adaptive local thresholding was performed to obviate small regional variations in image brightness, and the Phansalkar method was used because it was designed to select darker regions in potentially low-contrast images [28]. Moreover, we used a swept-source OCTA device that allowed for deeper light penetration into the choroid, thereby obtaining higher quality images with outstanding repeatability [25, 42]. Figure 3 displays three different eyes of three different individuals from our cohort. CC flow signal was clearly detectable despite presence of SRF/IRF as well as PEDs.

CONCLUSIONS

In conclusion, our study reports a decrease of CC FD after the administration of a series of three faricimab injections. This effect was significant in all rings surrounding the dark halo around the MNV. This was accompanied by a decrease in CC FDa in all rings. These findings indicate reperfusion of the CC surrounding the MNV, which may be an indicator for disease regression. This is of particular interest as OCTA-based parameters gain prominence in the management of nAMD. In the future, larger prospective studies are needed to support our preliminary results.

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Author Contributions. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Max Brinkmann, Pasquale Viggiano, Giacomo Boscia and Tom Müller. The methodology was provided by Pasquale Viggiano, Giacomo Boscia and Francesco Boscia. Formal anaylsis was performed by Pasquale Viggiano and Giacomo Boscia. Investigation was performed by Tom Müller and Max Brinkmann. Statistical analysis was performed by Pasquale Viggiano and Max Brinkmann. Visualization was performed by Niccolò Castellino, Pasquale Viggiano and Max Brinkmann. The first draft of the manuscript was written by Max Brinkmann, Pasquale Viggiano and Jakob Schweighofer. Resources and supervision were provided by Francesco Boscia, Mario Damiano Toro and

Yosuf El-Shabrawi. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability. The datasets generated and analysed during the current study are not publicly available due to patient confidentiality.

Declarations

Conflict of Interest. Max Brinkmann, Pasquale Viggiano, Giacomo Boscia, Tom Müller, Niccolò Castellino, Jakob Schweighofer, Francesco Boscia, Mario Damiano Toro and Yosuf El-Shabrawi have nothing to disclose. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval. The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the local review board (Ethikkommission Kärnten, S2023-13). All subjects provided informed consent prior to treatment. As data were deidentified, consent for publication was not required.

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