Retinal Macrophage-Like Cells as a Novel Clinical Biomarker in Retinal Vein

Dmitrii S. Maltsev (glaz.med@yandex.ru)
Kirov Military Medical Academy
Alexey Kulikov
Kirov Military Medical Academy
Yaroslava Volkova
Kirov Military Medical Academy
Maria Burnasheva
Kirov Military Medical Academy
Alexander Vasiliev
Kirov Military Medical Academy

Research Article

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Abstract

Aim: To study the macrophage-like cells (MLC) of the inner retinal surface in eyes with retinal vein occlusions (RVO) and association of MLC with clinical characteristics of RVO.

Methods: In this retrospective cross-sectional study, for treatment-naïve patients with unilateral RVO and no abnormalities of vitreoretinal interface electronic medical records and multimodal imaging data were reviewed and analyzed.To visualize MLC, structural projections of optical coherence tomography (OCT) angiography scans within a slab between two inner limiting membrane segmentation lines (with 0 and -9 µm offset) were evaluated. The density of MLC was calculated and compared between affected and fellow eye regarding different OCT and clinical characteristics of RVO.

Results: Thirty-six eyes (28 branch RVO and 8 central RVO of 36 patients (21 males and 15 females, mean age 48.9 ± 9.8 years) were included. The density of MLC in affected eye was statistically significantly higher than that in fellow eye, 8.5 ± 5.5 and 4.0 ± 3.6 cells/mm^2, respectively ($P < 0.001$). The MLC number in the affected eye correlated statistically significantly that of the fellow eye ($r = 0.76$, $p = 0.0001$) but with none of OCT and clinical characteristics of the affected eye except for the presence of subfoveal fluid. Eyes with subfoveal fluid had statistically significantly higher mean number of MLC that that in eyes without subfoveal fluid, 12.6 ± 6.3 and 6.9 ± 4.0 cells/mm^2, respectively ($P = 0.009$).

Conclusion: The number of MLC on the inner retinal surface increases in RVO eyes and may reflect activation of inflammatory reactions.

Introduction

Although retinal vein occlusion is a common retinal disorder affecting a significant proportion of the population, the pathophysiology of this condition is still not fully understood. Basic steps in the occlusion described by the Virchow's triad include venous congestion, alteration of endothelial sheet, and blood hypercoagulability[1]. However, clinically significant complications of the RVO, namely macular edema and neovascularization, are driven by the changes in specific molecular signaling pathways.

From studies, which analyzed vitreous samples, it is known that RVO is followed by the increase of intraocular concentrations of vascular endothelial growth factor (VEGF) and several proinflammatory cytokines controlling the integrity of the inner blood-retinal barrier[2,3]. This leads to an increase of vascular permeability, exudation, macular edema, and to further neovascular complications. Any study of the relationship between angiogenic and proinflammatory cytokines in RVO will be of high practical value as it will help to optimize the therapeutic approach. In cases where VEGF plays a leading role, patients benefit from anti-VEGF treatment. Where inflammatory reactions play a greater role, corticosteroids may be a more favorable approach[4].

Several studies have considered clinical biomarkers indicating the role of inflammation in RVO pathophysiology, including the types of fluid associated with macular edema, choroidal thickness[5],

intraretinal hyperreflective foci[4], and anterior chamber flare[6]. However, several recent studies have described a new biomarker with the potential to characterize intraocular inflammation, macrophage like-cells of the inner limiting membrane[7,8]. These cells demonstrated some changes in diabetic retinopathy[9] driven by ischemia and inflammation, which also play an important role in RVO. We may therefore expect some changes of MLC in RVO that may also be used as a biomarker for inflammation in RVO.

The aim of this study was to investigate changes of the density of MLC in eyes with RVO and the association of the density of MLC with the clinical and OCT characteristics of RVO.

Methods

This was a retrospective cross-sectional study. The study followed the ethical standards stated in the Declaration of Helsinki and was approved by the Local Ethics Committee. All participants signed informed consent for the use of their clinical data for investigation. Only treatment naïve unilateral RVO patients were included in this study. Exclusion criteria were the presence in either eye of any abnormalities of vitreoretinal interface, including any stage of posterior vitreous detachment detected using optical coherence tomography (OCT), diabetes mellitus, glaucoma, history or presence of active intraocular inflammation, retinal vascular occlusion in the fellow eye, or any concurrent ocular condition impeding OCT imaging.

For all patients, electronic medical records and multimodal imaging data were reviewed and analyzed. All patients received OCT and OCT angiography (OCTA) (RTVue-XR, Optovue, Fremont, CA, software version 2017.1.0.150), green scanning laser ophthalmoscopy (F-10, NIDEK, Gamagory, Japan), fluorescein angiography (F-10, Nidek or Visucam 524, Carl Zeiss Meditec AG, Jena, Germany), and color fundus photography (AFC-330, NIDEK or Visucam 524, Carl Zeiss Meditec AG). All imaging procedures were performed after medically induced mydriasis. OCTA scans with a 6 mm × 6 mm field of view centered on the center of the fovea were obtained for extracting structural and vascular parameters, including the density of MLC, central retinal thickness (CRT), vessel density in superficial capillary plexus (SCP) and deep capillary plexus (DCP). The presence of subfoveal fluid was defined on structural scans crossing the central subfield as a hyporeflective space between the photoreceptor outer segment layer and the retinal pigment epithelium (RPE). The subfoveal choroidal thickness (SCT) was measured, using a caliper tool, as the distance from RPE to choroidal-scleral junction beneath the center of the fovea. The mean of three measurements was taken for analysis.

To visualize MLC, structural projections of OCTA scans within a slab between two inner limiting membrane segmentation lines (with 0 and − 9 μm offset) were evaluated for both the affected and fellow eyes (Fig. 1). Using a cell counter tool, the density of MLC was calculated in ImageJ (NIH, Bethesda, CA) by two experienced masked graders as the number of MLC per mm^2. Correction of image magnification for myopic eyes was performed using Bennet's formula before calculating MLC. For BRVO eyes the density of MLC was calculated separately for affected and unaffected area. Based on *en face* OCTA projections, the area of RVO was manually delineated in ImageJ, and measured and converted to a mask,

which was further used to calculate MLC outside the affected area. The number of MLC within the affected area was calculated as a difference between the total number of MLC and the MLC number outside the affected area (Fig. 2). Finally, the MLC density was calculated for affected and unaffected area of branch RVO (BRVO) eyes.

Statistical analysis was performed in MedCalc 18.4.1 (MedCalc Software, Ostend, Belgium). The Kolmogorov-Smirnov test was used to check normality. The paired t-test was used to compare MLC density between the affected and fellow eyes of RVO patients as well as MLC density between the affected and unaffected area within BRVO eyes. The Wilcoxon test was used to compare MLC density between ischemic and non-ischemic RVO eyes as well as between central RVO (CRVO) and BRVO eyes. To define the factors associated with the density of MLC, correlation coefficient was calculated for the density of MLC and age, clinical, retinal structural, and vascular parameters. To assess the interrater repeatability of MLC density calculation, the intraclass correlation coefficient was calculated; $P < 0.05$ was considered statistically significant.

Results

Thirty-six eyes of 36 patients (21 males and 15 females, mean age 48.9 ± 9.8 years) were included. The mean LogMAR best corrected visual acuity (BCVA) was 0.51 ± 0.27 (20/63 Snellen equivalent). The mean period after RVO onset was 25 days (ranging from 5 days to 3 months). There were 28 BRVO and 8 CRVO cases. Twelve BRVO and two CRVO cases were considered ischemic based on the FA data.

The mean CRT and SCT was 466.3 ± 193.0 µm and 345.5 ± 105.4 µm, respectively. The density of MLC in the affected eye was statistically significantly higher than in the fellow eye, 8.5 ± 5.5 and 4.0 ± 3.6 cells/mm^2, respectively ($P < 0.001$). This difference remained statistically significant in CRVO and BRVO separately ($p < 0.05$) (Fig. 3). There was a strong correlation of MLC density between both eyes of each patient ($r = 0.76$, $P < 0.001$).

Although the density of MLC was higher in non-ischemic RVO compared to ischemic RVO eyes this difference was not statistically significant ($P = 0.38$). The mean density of MLC within the affected area of BRVO eyes was statistically significantly lower compared to that of the unaffected region, 6.3 ± 5.3 cells/mm^2 and 10.5 ± 6.2 cells/mm^2 ($P = 0.009$), respectively. The intraclass correlation coefficient values for MLC density in affected and fellow eyes were 0.94 (95% confidence interval (CI) 0.93–0.97) and 0.98 (95% CI 0.97–0.99). Age, time after occlusion, gender, BCVA, CRT, SCT, the area of the affected region, vessel density in SCP or DCP had no association with MLC density in the affected eye ($P > 0.05$).

Twelve eyes (4 CRVO and 8 BRVO) had subfoveal fluid. Eyes with subfoveal fluid had statistically significantly higher density of MLC than the eyes without subfoveal fluid, 12.6 ± 6.3 and 6.9 ± 4.0 cells/mm^2, respectively ($P = 0.009$) (Fig. 4). Regarding density of MLC in the fellow eye, there was a statistically significant difference between RVO eyes with and without subfoveal fluid, 5.8 ± 5.0 and 3.0 ± 2.3 cells/mm^2, respectively ($p = 0.04$).

Discussion

In this study we showed that MLC on the inner retinal surface more densely populate the macula in eyes with RVO compared to healthy unaffected eyes of unilateral RVO patients. The density of MLC in RVO eyes varies significantly between individuals but has a strong correlation with the density of MLC in the fellow eye of each patient. Even though these cells spare affected areas in eyes with BRVO they seem not to be related to ischemic status of RVO eyes and rather have a correlation with the presence of subfoveal fluid. At the same time MLC show no correlation with other clinical and OCT characteristics of RVO.

Retinal macrophage-like cells were first described using clinical OCT and image averaging by Castanos and coauthors[7]. Later this finding was confirmed by adaptive optics imaging[8]. Both these studies demonstrated dendriform morphology and motility of the cells on the inner limiting membrane. Among retinal cells, macrophages, glial cells, hyalocytes, and leukocytes may represent motile randomly distributed cells. However, leukocites are normally absent in the healthy retina, while the cells on the inner limiting membrane are present in a high number in healthy eyes[7,8]. On the other hand, other candidates for these cells all have macrophage origin and without histopathological studies it appears to be justified to characterize them without precise differentiation as the macrophage-like cells[10]. This term also allows us to define a particular cellular pool on the inner limiting membrane imaged with clinical OCT. Changes of MLC distribution in various posterior eye segment disorders have been proposed. However, MLC were studied only in diabetic retinopathy where they demonstrated growth of the population during the conversion of non-proliferative retinopathy to PDR[9], and in multiple evanescent white dots syndrome where they showed increased density in the acute stage of the disease[11]. Another feature registered in PDR was the accumulation of these cells along to the large retinal vessels, avoiding avascular regions. This may suggest the involvement of these cells in vascular remodeling and retinal ischemia.

Retinal vein occlusions are associated with overexpression not only of VEGF, but of many proinflammatory cytokines, including IL-6, IL-8, PlGF, MCP-1, ICAM-1 which increase retinal vessel permeability, leukocytes rolling and slow down the blood flow[3,6,12–14]. Activation of inflammatory signaling pathways may explain a relatively poor response to intravitreal anti-VEGF therapy in some cases. Such cases may benefit from corticosteroids therapy. However, identification of RVO cases where inflammation plays a leading role remains challenging. Inflammation biomarkers in RVO include subfoveal fluid and intraretinal hyperreflective foci[12,15] which have an association with MCP-1 which in turn is responsible for the recruitment of monocytes and macrophages[16]. MCP-1 was shown to be significantly overexpressed in RVO and we therefore may expect activation of the MLC pool in this condition.

The problem of studying of molecular signaling pathways in RVO results from the invasive character of the procedures required to obtain aqueous humor or vitreous tape. Therefore, direct measuring of the intraocular level of different inflammatory factors in RVO is not justified outside of clinical studies. This highlights the importance of studying clinical biomarkers which indicate the role of inflammation in each particular RVO case.

In this study we found a significant increase of MLC population in eyes with RVO compared to fellow unaffected eyes. As was previously shown in eyes with diabetic retinopathy, MLC in RVO eyes spared the retinal regions which had decreased perfusion or non-perfused areas. However, the density of MLC was still higher in affected eyes. We may therefore conclude that MLC not only migrate from the area affected by the occlusion to unaffected areas but also some additional cells may be recruited to the inner retinal surface as was seen in multiple evanescent white dots syndrome. The density of MLC does not correlate with the area of the occlusion or the ischemic character of the occlusion or vessel density in SCP or DCP and therefore cannot be used as a biomarker for the ischemic status of the RVO. No other parameters of the RVO showed any correlation with MLC density, including CRT, SCT or visual acuity. Only the presence of subfoveal fluid demonstrated an association with the density of MLC in RVO eyes. Subfoveal retinal fluid is a known biomarker of inflammation in RVO which was shown to be correlated with the levels of IL-6 and MCP-1[12,17]. This may in turn indicate the possible application of corticosteroids in the treatment of RVO.

Since the high density of the MLC in both affected and fellow eyes was associated with subfoveal fluid in RVO eyes and had a high interindividual difference, we may suggest that baseline density of the MLC may indicate predisposition to the activation of inflammatory reactions in RVO. In other words, if a patient had a high density of MLC, the inflammation may play a greater role in RVO, if any occurs in that patient.

The limitations of these study are the strict exclusion criteria. Firstly, we avoided any cases with vitreoretinal interface abnormalities, including any stage of posterior vitreous detachment, since there is no data on the effects of changes in posterior vitreous on visualization of MLC. This resulted in inclusion of relatively young patients and the mean age of our study group was 49 years while the mean age of RVO patients in other studies is about 65 years. Another consequence of applying strict inclusion criteria is the low number of cases included. Therefore, further studies with a larger and more diverse population of unilateral RVO patients is required. Finally, studies which measure vitreous and aqueous levels of proinflammatory mediators with regard to the density of MLC, as well as dynamic changes of MLC with the course of the disease are required.

In conclusion, this study revealed the potential role of MLC on the inner retinal surface as a novel biomarker in RVO, indicating the activation of inflammatory reactions. MLC density increases in eyes with BRVO and CRVO and is associated with the accumulation of subfoveal fluid, another biomarker of inflammation in RVO.

Declarations

Competing Interests

The authors declare that they have no conflict of interest.

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Author contribution

MDS, VYV, ANK — conception and design of the study; DSM, MAB, VAS — acquisition, analysis, and interpretation of data; MDS, VYV, ANK, VAS — drafted the work or revised it critically for important intellectual content, MDS, VYV, ANK, MAB, VAS —approved the final version of the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author (DSM) on request.

Animal Research (Ethics)

Not applicable.

Consent to Participate (Ethics)

Informed consent was not obtained from individual participants included in the study since this was a retrospective review of electronic medical records. The study was conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Local Ethics Committee approved this study.

Consent to Publish (Ethics)

Not applicable.

Clinical Trials Registration

Not applicable.

Gels and Blots/ Image Manipulation

Not applicable.

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Figures

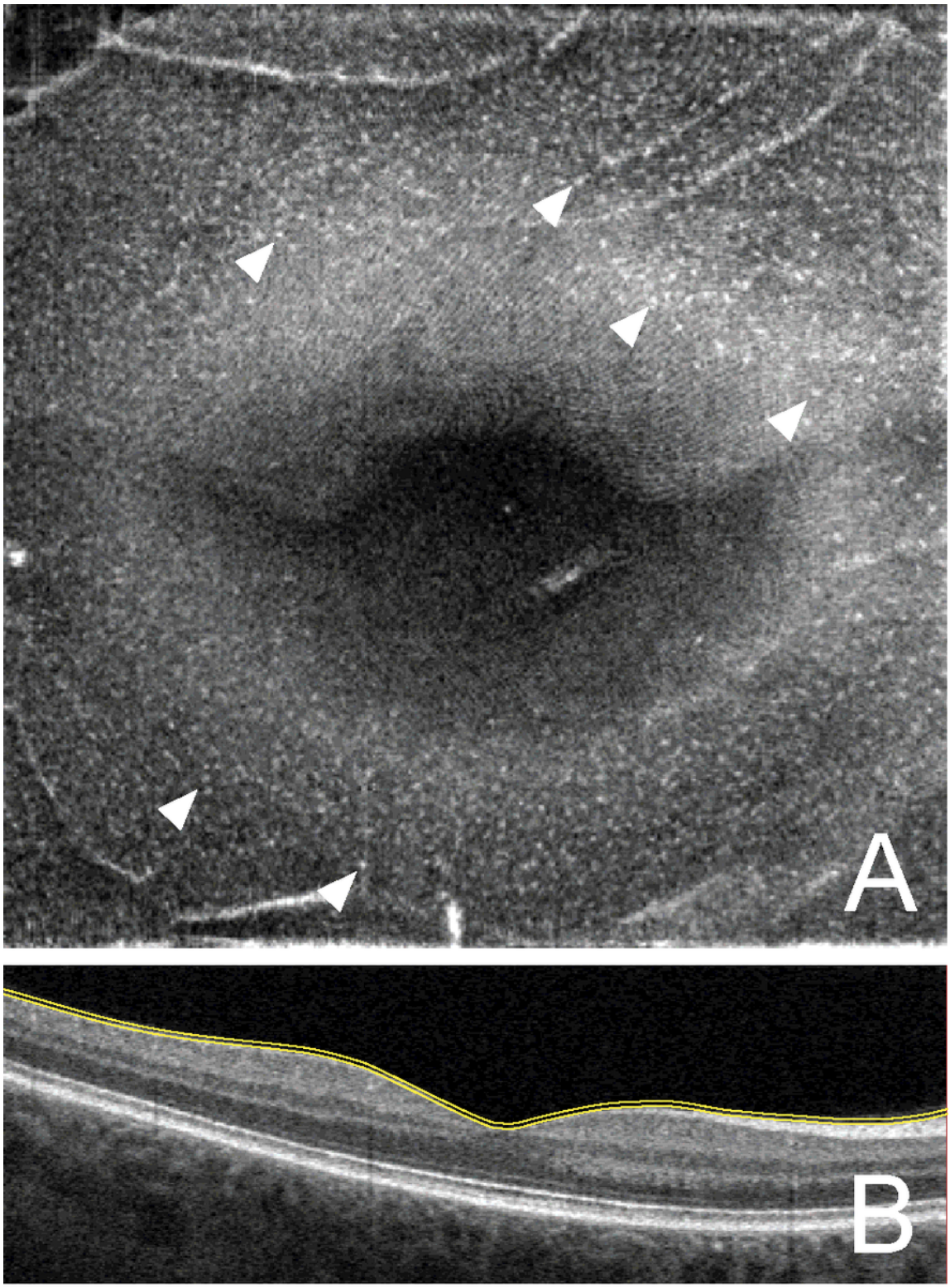

Figure 1

Representative example of visualization of retinal macrophage-like cells using 9-µm structural *en face* projection between two segmentation lines of the inner limiting membrane with -9 µm and 0 µm offset. Arrowheads indicate individual macrophage-like cells.

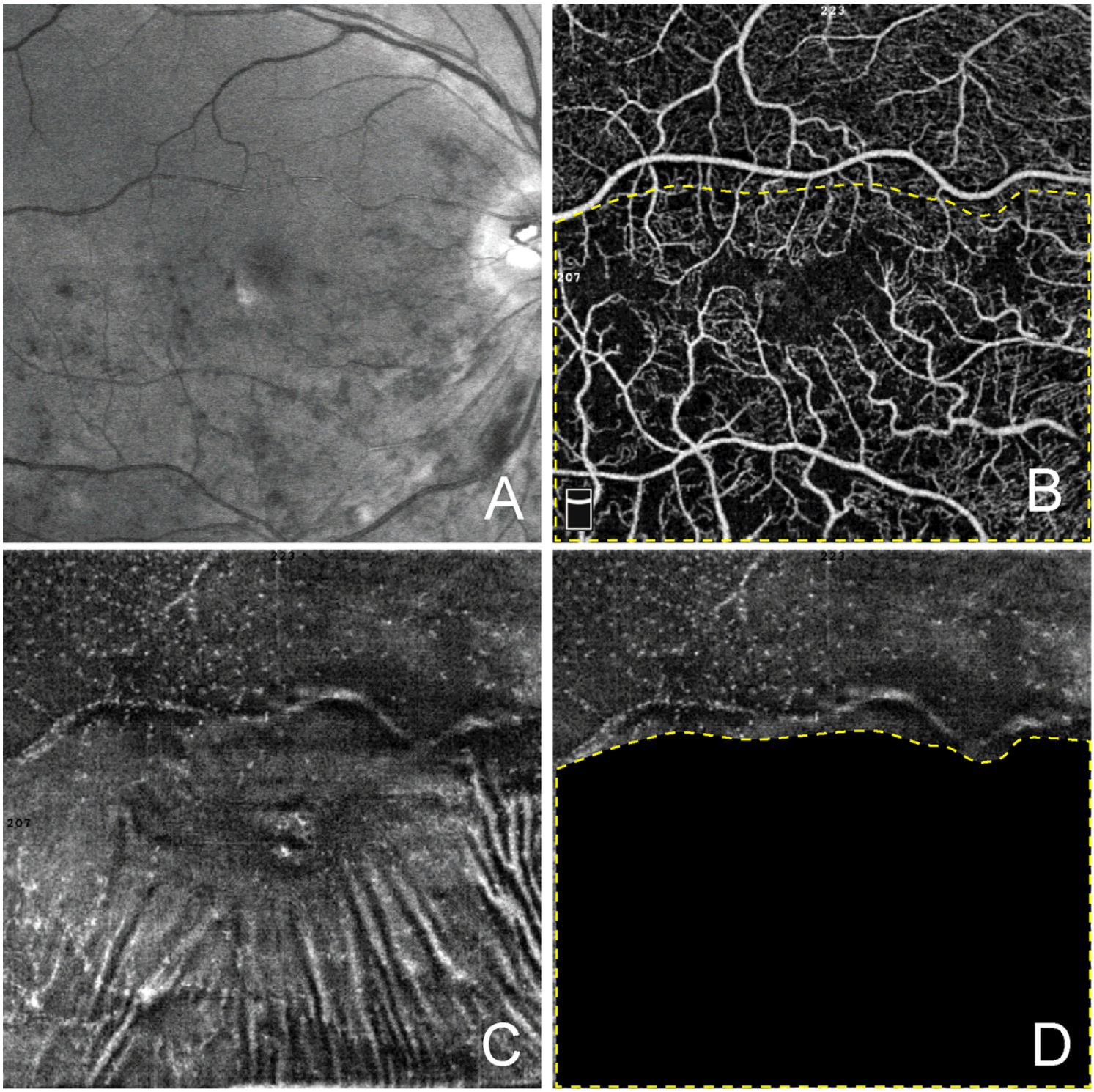

Figure 2

Counting of retinal macrophage-like cells in branch retinal vein occlusions. A. Green scanning laser ophthalmoscopy showing area of occlusion. B. *En face* optical coherence tomography angiography (OCTA) projection in the eye with branch retinal vein occlusion (BRVO). The yellow dashed line delineates the area of BRVO. C. Structural *en face* OCTA projection displaying MLC. C. Structural *en face* OCTA projection with the area affected by RVO masked to calculate MLC in unaffected area.

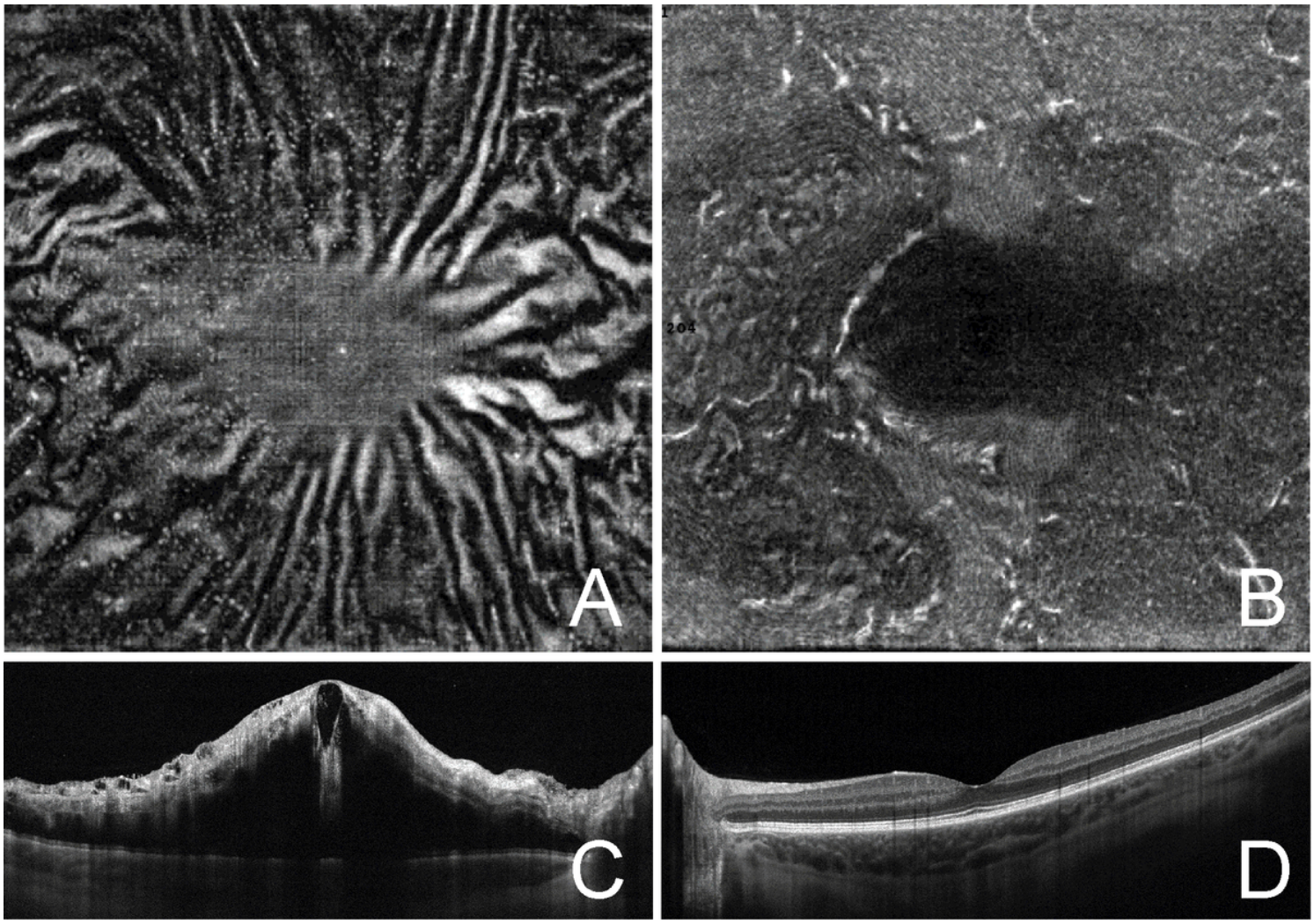

Figure 3

Optical coherence tomography angiography imaging of retinal macrophage-like cells in retinal vein occlusion with and without subfoveal fluid. A. Structural *en face* optical coherence tomography angiography (OCTA) projection in the RVO eye with macular edema and subfoveal fluid. B. Structural *en face* OCTA projection in fellow unaffected eye. C. Cross-sectional OCT image showing macular edema and subfoveal fluid in the RVO eye. D. Cross-sectional OCT image showing normal macula in the fellow eye. E. Structural *en face* optical coherence tomography angiography (OCTA) projection in the RVO eye with macular edema and no subfoveal fluid. F. Structural *en face* OCTA projection in fellow unaffected eye. G. Cross-sectional OCT image showing macular edema without subfoveal fluid in the RVO eye. H. Cross-sectional OCT image showing normal macula in the fellow eye.

Figure 4

Optical coherence tomography angiography imaging of retinal macrophage-like cells in central retinal vein occlusion. A. Structural *en face* optical coherence tomography angiography (OCTA) projection

displaying macrophage-like cells. B. Structural *en face* OCTA projection in fellow unaffected eye. C. Cross-sectional OCT image showing severe macular edema in the affected eye. D. Cross-sectional OCT image showing normal macula in the fellow eye.