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Autofluorescence indexes as biomarkers for antiangiogenic loading dose outcome in diabetic macular edema

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Abstract

Purpose: To evaluate the combination of fundus autofluorescence results with several clinical and structural variables into mathematical indexes to enhance their ability to predict visual and anatomical changes after the antivascular endothelial growth factor loading dose.

Methods: Patients with diabetic macular edema were enrolled. Each patient had a comprehensive ophthalmological examination, contrast sensitivity, optical coherence tomography, and fundus autofluorescence assessment. All patients received three monthly doses of ziv-aflibercept and were followed each month for response assessment. Autofluorescence was classified according to its level into five grades. The grades were combined with other variables (best-corrected visual acuity, contrast sensitivity, central macular thickness, macular cube volume, and macular cube average thickness) into normalized indexes. Statistical assessment was done using a Spearman's rank correlation coefficient, linear regression, and interobserver-agreement analysis.

Results: There was a strong correlation between the fundus autofluorescence/baseline best-corrected visual acuity index and the fundus autofluorescence/contrast-sensitivity index at baseline with the best-corrected visual acuity after the third dose of ziv-aflibercept ($r_s = -0.78$, $p = .000$ and $r_s = -0.68$, $p = .0009$ respectively). The fundus autofluorescence/baseline best-corrected visual acuity index and the fundus autofluorescence/contrast-sensitivity index, both at baseline had a mild correlation with the macular volume at 1 month of follow-up ($r_s = 0.56$, $p = .008$ and ($r_s = 0.64$, $p = .002$, respectively).

Conclusion: This study suggests that it is possible to combine fundus autofluorescence results with functional and structural variables into normalized indexes that could potentially predict outcomes after antivascular endothelial growth factor loading dose in patients with diabetic macular edema.

Keywords: antivascular endothelial growth factor therapy, diabetic macular edema, fundus autofluorescence, optical coherence tomography, ziv-aflibercept

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Introduction

Diabetic macular edema (DME) is the main cause of severe visual loss in diabetic patients.[1,2] It affects 30% of all patients after 20 years of suffering the disease.[3] The diagnosis is mainly clinical, but fluorescein angiography (FA) and optical coherence tomography (OCT) are diagnostic tests proven to be valuable tools in the characterization and treatment follow-up of the disease.[4,5] However, their ability to predict the clinical response to antivascular endothelial growth factor (anti-VEGF) therapy and final visual acuity is still limited.

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Monotherapy with anti-VEGF drugs is currently the gold standard of treatment for DME. Although it is highly effective, the patient's response could vary widely depending on several factors.[6] Unfortunately, there is still a need to find imaging biomarkers that help the physician to detect poor or better responders in advance.

Fundus autofluorescence (FAF) is a non-invasive retinal imaging modality used for the detection of ocular fluorophores in the retinal pigment epithelium. The resulting images have been used in the assessment of several retinal diseases such as geographic atrophy, age-related macular degeneration, and macular dystrophies.[7,8]

In the case of DME, the presence of intraretinal cysts and spongiform patterns are associated with changes in the FAF signal.[9–17] However, the lack of standardization of the technique has prevented the use of FAF as an accurate outcome biomarker.[9,10]

It is possible to overcome this limitation if we combine FAF results with other structural and functional variables into a mathematical index/quotient. This combination is a frequently used strategy in biostatistics that allows the aggregation of multiple variables into a single index that significantly improves its power to detect changes.[18] A prime example is the use of macular hole indexes to predict the anatomical and visual outcomes after macular surgery.[19]

Evidence suggests that the visual and anatomical results after the three initial intravitreal doses (loading dose) correlate highly with the patient's best-corrected visual acuity (BCVA) and macular thickness after 12 months of follow-up.[20–22]

A biomarker to help optimize resources, or choose an alternative more effective treatment, is highly desirable. Therefore, the objective of this study is to assess the correlation between several new FAF indexes and the visual outcomes after anti-VEGF therapy loading dose with ziv-aflibercept, in patients with DME and to propose new potential biomarkers that serve to predict patient's response to treatment.

Methods

Retrospective, case series

The study was reviewed and approved by the Hospital's Internal Review Board (IRB approval number: 002-180829). The study was conducted according to the tenets of the Declaration of Helsinki and Good Clinical Practices guidelines. All sensitive data were managed according to the Federal Law for the Protection of Personal Data in Possession of Individuals (NOM-024-SSA3-2010), and the Health Insurance Portability and Accountability Act (HIPAA) rules. Due to its retrospective nature, an informed consent form was not necessary at this time.

We included all consecutive patients seen in the retina department of Clinica David Ophthalmological Unit, who had a clinical diagnosis of DME and were candidates for anti-VEGF therapy (center subfield thickness of $>260\,\mu m$). We excluded patients with a medical history of laser treatment (focal or grid) within 1500 µm from the center of the fovea, vitreoretinal surgery within 6 months prior to enrollment, and incomplete medical records or medical records lacking baseline FAF.

All patients had a comprehensive ophthalmological examination at baseline, which included BCVA assessment, slit-lamp examination, fundus examination, and ancillary tests such as stereoscopic fundus photographs, contrast-sensitivity assessment, FAF, and SD-OCT.

After DME diagnosis, all patients received monthly anti-VEGF therapy with ziv-aflibercept (Zaltrap; Sanofi-Aventis, Paris, France). All study procedures were repeated monthly during a 3-month follow-up.

BCVA for each eye was assessed using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol at a distance of 4 m with a modified ETDRS distance chart (Precision Vision, La Salle, IL). BCVA was defined as the total number of letters correctly seen by each eye. For the contrast-sensitivity (CS) assessment, we used the Hamilton–Veale CS test chart (Hamilton Veale, Canterbury, New Zealand). The test was scored as the total number of paired letters correctly seen at 1 m on each eye.

Stereoscopic fundus photographs and FAF images were obtained with a fundus camera (VISUCAM®NM/FA; Carl Zeiss Meditec Inc, Oberkochen, Germany) using an excitatory wavelength of 510–580 nm and emitted light detection above 640 nm. FAF images were graded by two independent masked observers (F.B.C. and

S.H.D.). FAF patterns were classified into five different stages or grades based on the modification of two separate autofluorescence classification systems published elsewhere.[9,11]

The classification was defined as follows: grade 1: decreased autofluorescence (dFAF). Grade 2: normal autofluorescence (nFAF). Grade 3: single-spot increased autofluorescence (single-spot iFAF). Grade 4: multiple-spot increased autofluorescence (multiple-spot iFAF). Grade 5: plaque-like or confluent multiple-spot increased autofluorescence (plaque iFAF) (Figure 1).

SD-OCT images were obtained with a Cirrus 5000 SD-OCT (Carl Zeiss Meditec Inc, Oberkochen, Germany) using a macular cube of 512 × 128, automatic segmentation and metrics provided by the software. Central macular thickness (CMT) in µm, macular cube volume (MCV) in mm^3, and macular cube average thickness (MCAT) in µm were the main variables assessed at this time.

Intravitreal injections were performed according to the American Academy of Ophthalmology guidelines and general recommendations.[23] All patients received a loading dose of an anti-VEGF drug, consisting of a minimum of three monthly intravitreal injections of 0.05 ml ziv-aflibercept (25 mg/mL). A lid speculum, 5% povidone-iodine into the conjunctival cul-de-sac, facemasks, and topical anesthesia with 0.5% tetracaine hydrochloride (Ponti-ofteno; Laboratorios Sophia, Guadalajara, Mexico) were used in all cases.

Statistical analysis was done using GraphPad Prism 8 for macOS, version 8.0.2. (GraphPad Software Inc, San Diego, California). FAF grades were transformed into their corresponding logarithmic value: the logarithmic values of numbers 1 to 5 are 0, 0.3, 0.47, 0.6, and 0.69, respectively. Hence, grade 1 = 0, grade 2 = 0.3, grade 3 = 0.47, grade 4 = 0.6, grade 5 = 0.69). Standardized-normalized indexes were obtained by dividing the baseline FAF logarithmic value with each of the other baseline variables (BCVA, CS, CMT, MCV, and MCAT). For example, if a patient had a grade 2 (or 0.3 logarithmic value) baseline FAF, and a 45 letter score of baseline BCVA, the baseline FAF/BCVA index was 0.3/45 = 0.006.

A Friedman test was used to analyze repeated FAF measurements, functional (BCVA and CS) and structural (CMT, MCV, and MCAT) variables during follow-up. A correlation analysis (Spearman's rank correlation coefficient), linear regression analysis, and interobserver-agreement analysis (Cohen-Kappa) between different variables and the standardized-normalized indexes were also assessed, with an alpha value of 0.05 for statistical significance.

Results

We included 29 eyes from 15 patients (10 males, 5 females) who fulfilled all inclusion and exclusion criteria. A total of 14 patients had bilateral eligible eyes, while only one patient had only one eligible eye. The mean age was 61.8 ± 6.2 (range: 53–74) years. General demographic data are summarized in Table 1.

Classification of FAF at baseline was as follows: grade 1 (dFAF): 5 eyes (17.24%). Grade 2 (nFAF): 11 eyes (37.93%). Grade 3 (single-spot

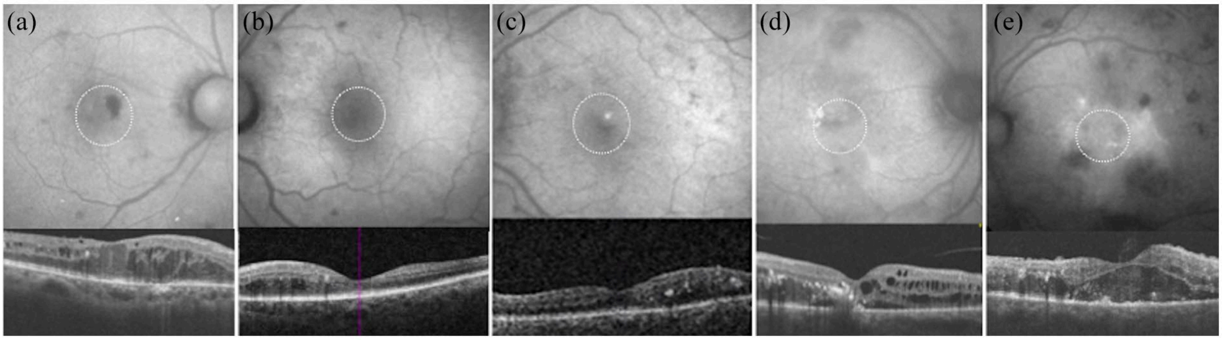

Figure 1. Grading of foveal fundus autofluorescence (FAF, dotted circle line) pattern in DME: (a) represents grade 1, decreased foveal FAF; (b) represents grade 2, normal foveal FAF; (c) represents grade 3, single-spot increased FAF; (d) represents grade 4, multiple-spot increased FAF, and (e) represents grade 5, plaque-like increased FAF.

Table 1. Baseline characteristics of patients with DME.

Sex	***n* (%)**
Male	10 (75)
Female	5 (25)
Age	**Years**
Mean ± SD	61.8 ± 6.2
Range, years	53–74
Eligibility	***n* (%)**
Unilateral eligible	1 (6.6)
Bilateral eligible	14 (93.4)
BCVA, number of letters on ETDRS chart	32.3 ± 16.3
CS, number of pair of letters	6.8 ± 3.7
Central subfield thickness, μm	390 ± 118.8
Macular cube volume, mm^3	11.2 ± 3.2
Macular cube average thickness, μm	383.8 ± 95.7
FAF pattern	***n* (%)**
Grade 1 (decreased)	6 (20)
Grade 2 (normal)	11 (36.7)
Grade 3 (increased single-spot)	4 (13.3)
Grade 4 (increased multiple-spot)	6 (20)
Grade 5 (increased plaque-like)	3 (10)
OCT edema patterns	***n* (%)**
Cystoid	7 (22.9)
Non-cystoid (sponge-like)	17 (60)
Subfoveal serous neuroretinal detachment	5 (17.1)

BCVA, best-corrected visual acuity; CS, contrast sensitivity; ETDRS, Early Treatment Diabetic Retinopathy Study; FAF, fundus autofluorescence; OCT, optical coherence tomography.

iFAF): 4 eyes (13.79%). Grade 4 (multiple-spot iFAF): 6 eyes (20.69%). Grade 5 (plaque-like iFAF): 3 eyes (10.34%). According to the structural OCT analysis, DME was classified at baseline as follows: cystoid (22.9%), sponge-like (60%), and serous neuroretinal detachment (17.1%). The FAF and OCT interobserver-agreement (Cohen–Kappa) were 0.806 ($p < .01$) at baseline, 0.828 ($p = .000$) at 1 month, and 0.763 ($p = .000$) at 2 months follow-up (high level of agreement).

Follow-up values of functional, OCT, and FAF values are summarized in Table 2. No adverse effects were noted at 3 months of follow-up.

Correlation and linear regression analysis

There was a significant correlation between the baseline FAF's standardized-normalized indexes and some of the assessed variables:

Baseline FAF/BCVA index and MCV at 1 month ($r_s = 0.56$, $p = .004$), (95% confidence interval (CI), 0.15–0.80) ($r^2 = 0.3$, $p = .02$), baseline FAF/BCVA index and BCVA at 2 months ($r_s = -0.78$, $p = .0003$) (95% CI, –0.92 to –0.44; $r^2 = 0.35$, $p = .016$; Figure 2). Baseline FAF/CS index and BCVA at 2 months ($r_s = -0.6$, $p = 0.008$; 95% CI, –0.86 to –0.13; $r^2 = 0.61$, $p = .001$), baseline FAF/CS index and MCV at 1 month ($r_s = 0.64$, $p = .001$; 95% CI, 0.27–0.85; $r^2 = 0.32$, $p = .009$; Figure 3). Baseline FAF/CMT index and MCV at 2 months ($r_s = 0.4$, $p = .02$; 95% CI, 0.01–0.68; $r^2 = 0.17$, $p = .04$), and baseline FAF/CMT index and CS at 1 month ($r_s = 0.44$, $p = .02$; 95% CI, –0.05 to 0.70; $r^2 = 0.26$, $p = .015$; Figure 4).

Discussion

FAF's assessment has proven to be a valuable tool for the diagnosis and follow-up of various retinal diseases. Regarding DME, previous studies by Calvo-Maroto and colleagues,[12] Shen and colleagues,[13] and Vujosevic and colleagues[14] have described several macular findings and proposed a classification based on FAF patterns. In this study, the authors used mathematical indexes composed by the index of the logarithmic transformation of the FAF patterns, and several structural and functional variables, to increase their predictive value regarding visual outcome after a loading dose with intravitreal ziv-aflibercept.

Our study results demonstrate that there is a potential benefit in applying some of these standardized-normalized indexes as predictive biomarkers in patients with DME. The FAF/BCVA index demonstrated a significant correlation with the BCVA after 2 months ($r_s = -0.78$, $p = .0003$). This correlation suggests that FAF might be directly proportional to BCVA loss. At the same time, baseline BCVA is inversely proportional to the latter, after the three loading doses

Table 2. Follow-up values of functional, OCT variables, and FAF grade of DME-treated patients.

	Baseline	1-month	2-month	*p* (Friedman test)
Functional variables				
BCVA (number of letters)	32.3 ± 16.3	36.7 ± 15.8	39.2 ± 15.7	.001
CS (pairs of letters)	6.8 ± 3.7	8.5 ± 2.4	7.9 ± 3	.89
OCT variables				
CST (μm)	390 ± 118.8	326.4 ± 107.1	302.2 ± 56	.000
MCV (mm^3)	11.2 ± 3.2	10.7 ± 2.6	10.1 ± 2.2	.001
MCAT (μm)	383.8 ± 95.7	329.5 ± 57.5	327.2 ± 67.5	.000
FAF				
FAF (grade)	2.53 ± 1.1	2.4 ± 0.9	2.2 ± 0.8	.16

BCVA, best-corrected visual acuity; CS, contrast sensitivity; CST, central subfield thickness; MCV, macular cube volume; MCAT, macular cube average thickness; FAF, fundus autofluorescence.

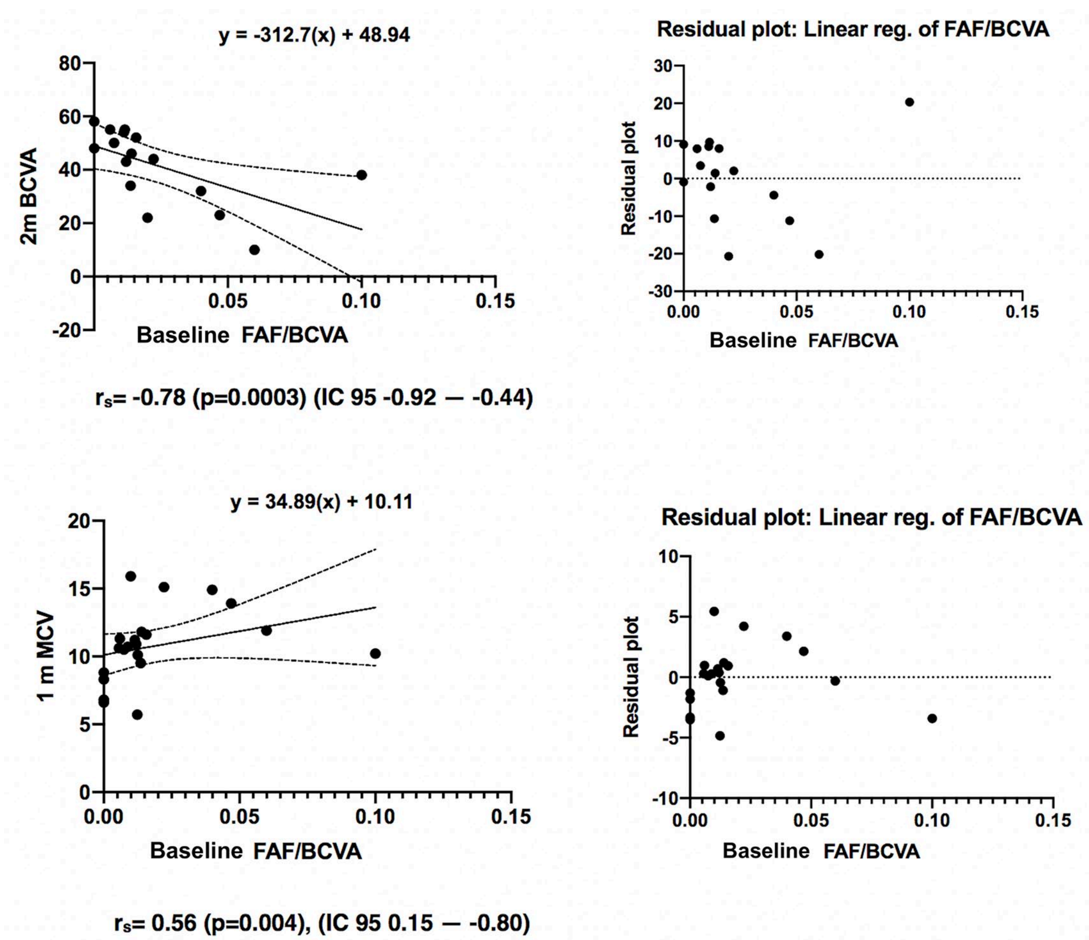

Figure 2. Fitted line and residual plots showing the relationship between baseline FAF/BCVA (fundus autofluorescence/best-corrected visual acuity) index and 1 month MCV (macular cube volume) and 2 months BCVA.

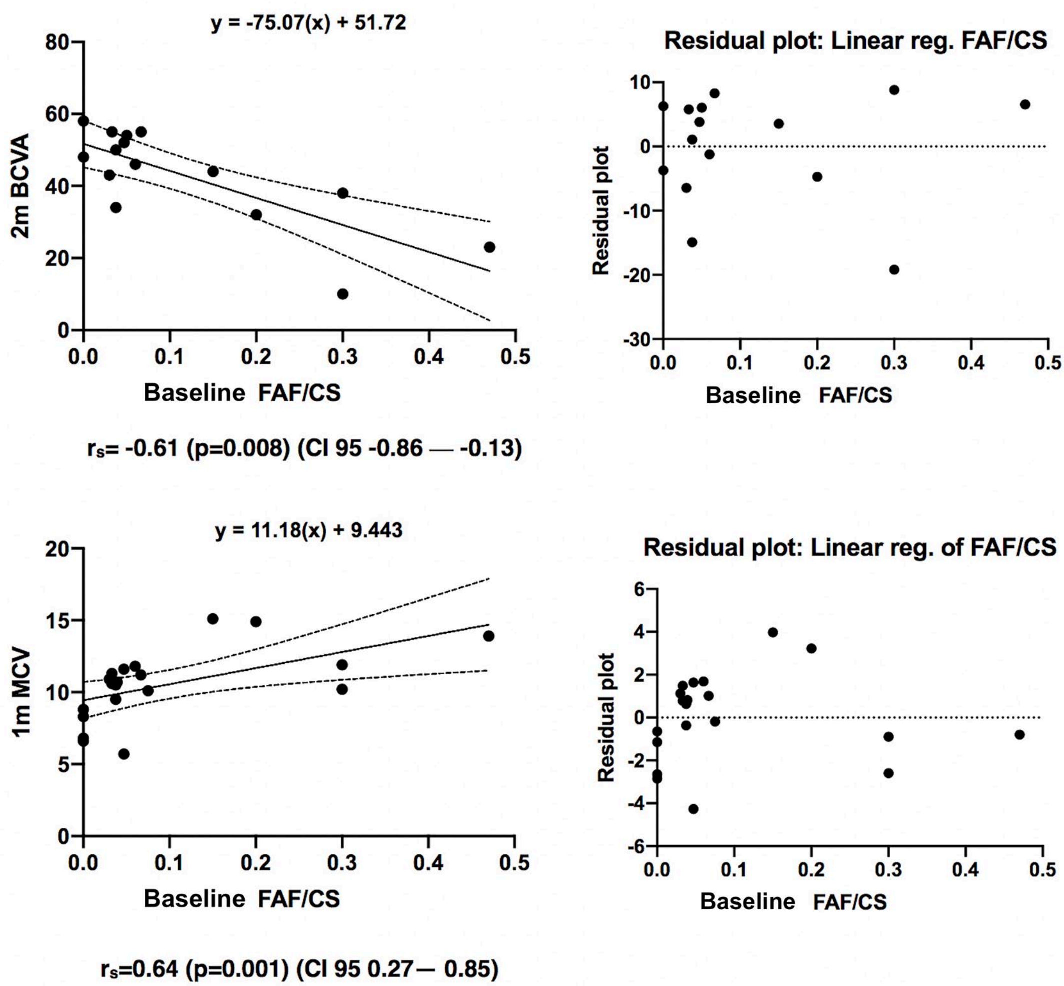

Figure 3. Fitted line and residual plots showing the relationship between baseline FAF/CS (fundus autofluorescence/contrast sensitivity) index and 1 month MCV (macular cube volume) and 2 months BCVA (best-corrected visual acuity).

of intravitreal anti-VEGF therapy. The result is consistent with the data published by Chung and colleagues.[15] In their study, patients with increased FAF were 4.2 times more likely to be associated with DME, especially if the edema had a cystic configuration. Moreover, for each 0.1 increase on the baseline BCVA logMAR, FAF increased by a factor of 1.7.

Vujosevic and colleagues[11] graded FAF images for different foveal patterns (normal, single-spot increased, and multiple-spot increased FAF). Mean retinal sensitivity over areas with iFAF was significantly different from that of normal FAF in both single- and multiple-spot iFAF groups (ANCOVA, $p = .0002$). Mean retinal sensitivity progressively decreased in these three groups from 15.1 ± 3.9 to 10.3 ± 5.2 dB.

Our results could be explained mainly by the added power conferred by the BCVA measurement to the proposed index. The use of this variable for this purpose seems natural because there is strong evidence that suggests that BCVA at baseline can be used as a predictive biomarker of final BCVA in several macular diseases.[20,22] Furthermore, an increased signal of FAF has been associated with increased macular thickness as well.[9,11] When an intraretinal cyst forms in the fovea, the fluid contained within displace laterally the retinal tissue and the macular pigments. This retinal tissue displacement enables the detection of the FAF signal coming from the retinal pigment epithelium, enhancing its detection despite the presence of DME.[12] If a direct relationship between increased macular thickness and BCVA loss does exist, the authors speculate

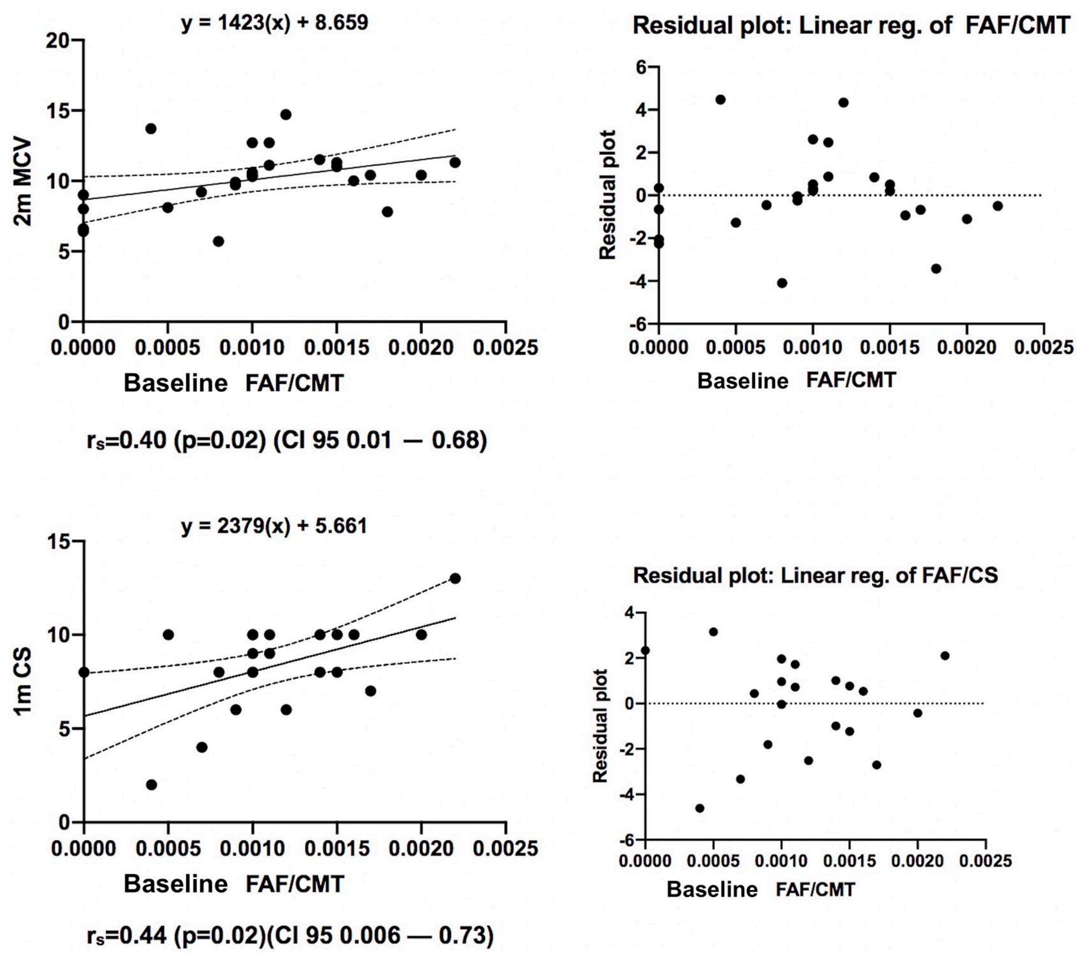

Figure 4. Fitted line and residual plots showing the relationship between baseline FAF/CMT (fundus autofluorescence/central macular thickness) index and 1 month CS (contrast sensitivity) and 2 months MCV (macular cube volume).

that an increase FAF might precede the loss of vision due to DME.

Several imaging OCT biomarkers have been described that correlated well with visual function.[24,25] Boiko and Maltsev,[24] investigated the relationship between baseline OCT biomarkers (retinal tissue area, RTA; optical density in the central subfield, ODRT), and post-anti-VEGF treatment variables (CMT and BCVA). They found that baseline RTA was strongly correlated with post-anti-VEGF treatment CMT ($r_s = 0.76$, $p = .001$) and BCVA ($r_s = 0.67$, $p = .001$). Baseline ODRT was moderately correlated with post-anti-VEGF treatment CMT ($r_s = -0.26$, $p = .049$) and BCVA ($r_s = -0.48$, $p = .001$). Furthermore, baseline RTA/ODRT index was strongly correlated with post-anti-VEGF treatment CMT ($r_s = 0.75$, $p = .001$) and BCVA ($r_s = 0.85$, $p = .001$).

In this study, the maximum level of FAF considered (grade 5, plaque-like or confluent multiple-spot increased autofluorescence), was significantly associated with large areas of subfoveal serous retinal detachment. It is also possible that the opposite phenomenon could be observed in the case of a spongiform pattern of the DME without central involvement.[9] A decrease in the FAF signal (grade 1: decreased autofluorescence) was associated with better visual acuity after the loading dose of anti-VEGF drugs.

The mathematical index composed by the combination of FAF and CS also showed a moderate correlation with MCV at 1 month of follow-up ($r_s = 0.64$, $p = .001$). Although the significance of this association is not very well understood, the authors believe that a possible explanation is that CS is more susceptible to changes in the macular thickness than the visual acuity.[26] Therefore, the correlation between FAF/BCVA index and MCV at 1 month was weaker ($r_s = 0.56$, $p = .004$) but still statistically significant.

Besides the small sample and short follow-up, this study has several other limitations that the authors would like to address. The use of a flash fundus camera in our research may artificially enhance the FAF signal by the phenomenon of pseudo-autofluorescence, which may increase the strength of the association observed.[7] Moreover, fundus cameras produce low-contrast images that could lead to a misinterpretation of uncertain FAF patterns.[7]

The use of Scanning Laser Ophthalmoscopy (SLO) and quantitative FAF, as described by Delori and colleagues,[27,28] could potentially solve this issue. Finally, the lack of standardization of the technique and the absence of a normative database regarding FAF values prevent us from drawing more definitive conclusions.

In conclusion, the results observed in this study may be relevant. It combines clinical variables with a non-invasive test that could potentially predict the initial visual outcome after the anti-VEGF loading dose. Applying these indexes could help physicians select alternative treatments with better chances of success from the beginning (intravitreal steroids or combined therapy) and before initial treatment failure.

Future studies that compare these indexes with other baseline imaging biomarkers that have been described are warranted to establish further their role in predicting anti-VEGF treatment response in both the short and long terms in patients with DME.

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Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The authors state that they have full control of all primary data, and they agree to allow *Therapeutic advances in Ophthalmology* to review their data upon request.

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