

Intravitreal Methotrexate in Extensive Macular Atrophy with Pseudodrusen- like Appearance: A Pilot Study

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Abstract

Purpose To report the clinical outcomes of off-label intravitreal methotrexate treatment in patients with extensive macular atrophy with pseudodrusen-like appearance (EMAP) over a 12-month follow-up period.

Methods In this non-randomized, fellow-eye–controlled pilot study, eight patients (16 eyes; mean age 59.5 ± 3.78 years) diagnosed with EMAP received monthly intravitreal methotrexate ($400 \mu\text{g}/0.1 \text{ mL}$) in one eye for 12 months; the fellow eye remained untreated. Best-corrected visual acuity (BCVA), fundus autofluorescence (FAF), and spectral-domain optical coherence tomography were assessed serially. Atrophic area was quantified on FAF and square-root transformed; the primary endpoint was the within-patient difference in 12-month progression between treated and fellow eyes.

Results Baseline atrophic area was comparable between eyes (24.70 ± 13.53 vs $25.62 \pm 15.40 \text{ mm}^2$; $P = 0.84$). Mean 12-month progression was $5.04 \pm 4.01 \text{ mm}^2$ in treated and $5.88 \pm 5.45 \text{ mm}^2$ in fellow eyes, 14.3% lower in treated eyes. The mean within-patient difference in square-root progression was $-0.080 \sqrt{\text{mm}^2}$ (95% CI, -0.259 to $+0.099$), favoring the treated eye but not statistically significant ($P = 0.46$). Median BCVA declined from 0.55 to 0.80 logMAR in treated and 1.45 to 1.65 logMAR in fellow eyes; baseline BCVA was better in treated eyes ($P = 0.03$), and the between-eye difference in change was not significant ($P = 0.30$). No serious adverse events occurred; two patients had mild, self-limited anterior chamber reactions.

Conclusion Intravitreal methotrexate showed a favorable safety profile and was associated with a trend toward reduced atrophic progression in treated eyes compared with untreated fellow eyes. Given the aggressive natural course of EMAP, these findings support pursuing larger, controlled studies to further evaluate immunomodulatory therapy for this disease.

Key Message

What is known:

Extensive macular atrophy with pseudodrusen-like appearance (EMAP) is a rare, progressive, bilateral retinal dystrophy with a proposed post-streptococcal autoimmune pathogenesis and no currently approved treatment.

What is new:

- In this fellow-eye–controlled pilot study, monthly intravitreal methotrexate over 12 months showed a favorable safety profile in patients with EMAP, with no serious adverse events.
- Treated eyes showed a 14.3% lower mean atrophic area progression than untreated fellow eyes, a difference that did not reach statistical significance in this small cohort ($n = 8$).

- These preliminary anatomical findings support pursuing larger, controlled trials of immunomodulatory therapy for EMAP.

Introduction

Extensive macular atrophy with pseudodrusen-like appearance (EMAP) is a rare, progressive bilateral retinal dystrophy first described by Hamel et al. in 2009 [1]. The condition is characterized by bilateral and symmetrical macular atrophy accompanied by pseudodrusen-like deposits, typically manifesting in middle-aged individuals [2, 3]. EMAP exhibits distinctive clinical features including vertically oriented atrophic progression, peripheral paving stone degeneration, and characteristic multimodal imaging findings that differentiate it from age-related macular degeneration (AMD) [4, 5].

Although EMAP has gained greater acknowledgment and benefits from enhanced diagnostic standards, the underlying causes and disease mechanisms remain predominantly unclear [3]. Emerging research indicates that processes involving the immune system may be fundamental to how the disease develops. A particularly convincing theory proposes that there is molecular resemblance between proteins found in the outer retina and antigens from bacteria, especially M proteins from streptococcus [6]. This hypothesis gains support from a significant French case-control investigation conducted by Douillard and colleagues, which revealed that EMAP patients showed notable changes in immune system indicators. The alterations encompassed increased plasma complement C3 concentrations, diminished CH50 activity, and raised erythrocyte sedimentation rates, collectively indicating persistent immune system stimulation [2]. These observations suggest the potential for a delayed immune hypersensitivity response that occurs after previous streptococcal infections, which might initiate an autoimmune attack targeting retinal components.

Several case series have documented an association between EMAP and a history of rheumatic fever during childhood, providing additional support for the autoimmune theory [2, 4, 7].

Currently, there is no approved treatment for EMAP. Management remains limited to visual rehabilitation, low-vision aids, and clinical monitoring of disease progression [3, 4]. Given the proposed autoimmune pathophysiology and the documented immune dysregulation in affected patients, immunomodulatory therapy represents a rational therapeutic approach. Methotrexate, a folate antagonist with well-established immunomodulatory and anti-inflammatory properties, has been successfully used in various autoimmune and inflammatory ocular conditions [8]. Its mechanism of action includes inhibition of T-cell proliferation, reduction of pro-inflammatory cytokine production, and modulation of complement activation—pathways potentially relevant to EMAP pathogenesis [9].

In light of these considerations and the absence of alternative therapeutic options, our group initiated off-label use of intravitreal methotrexate in patients with EMAP, aiming to attenuate disease progression through local immunomodulation. This study reports the clinical outcomes, structural changes, and safety profile of monthly intravitreal methotrexate injections over a 12-month period in eight patients with EMAP, using the untreated fellow eye as an internal control.

Methods

Study Design and Patient Selection

This was a single-arm, non-randomized, interventional pilot study including eight patients diagnosed with EMAP who underwent off-label treatment with intravitreal methotrexate at the Ophthalmology Division of Ribeirão Preto Medical School, University of São Paulo, Brazil, between 2023 and 2025. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the institutional review board. Written informed consent was obtained from all participants for off-label intravitreal methotrexate administration and for the use of their clinical data for research purposes.

Diagnostic Criteria

The diagnosis of EMAP was established based on clinical examination and multimodal imaging findings, according to recently proposed diagnostic criteria [3]. Eligible patients presented with bilateral and relatively symmetrical macular atrophy with a characteristic vertically-predominant distribution, pseudodrusen-like deposits on fundus examination, and peripheral paving stone degeneration. Fundus autofluorescence demonstrated confluent areas of hypoautofluorescence in the posterior pole, frequently with relative foveal sparing, corresponding to retinal pigment epithelium disorganization and atrophy. Spectral-domain optical coherence tomography showed outer retinal disruption and splitting of the retinal pigment epithelium–basal lamina–Bruch's membrane complex, often associated with overlying hyperreflective material. Patients with alternative diagnoses, including other inherited macular dystrophies, age-related macular degeneration, high myopia greater than 6 diopters, or any other retinal disorder that could confound the diagnosis or outcome assessment, were excluded.

Treatment Protocol

Treatment Protocol

Each patient received monthly intravitreal injections of methotrexate at a dose of 400 µg/0.1 mL in one eye for 12 months, totaling 12 injections. The fellow eye remained untreated and served as an internal control. The treated eye was selected through shared decision-making between the investigator and the patient, respecting the patient's preferences and interests. All injections were performed in an outpatient setting under sterile conditions, following standard intravitreal injection protocols.

Clinical Assessment

Patients underwent comprehensive ophthalmic examinations at baseline and at scheduled follow-up visits during the 12-month observation period, including assessments at approximately 3, 6, 9, and 12 months. Best-corrected visual acuity was measured using Snellen charts and subsequently converted to logMAR values for analysis. The ophthalmic evaluation also included slit-lamp biomicroscopy, indirect

fundus examination, color fundus photography, fundus autofluorescence, near-infrared reflectance imaging, and spectral-domain optical coherence tomography. Structural assessment focused on the integrity of the external limiting membrane, ellipsoid zone, outer retinal layers, retinal pigment epithelium, and the retinal pigment epithelium–basal lamina–Bruch's membrane complex.

Quantitative Analysis

Quantitative analysis of atrophic progression was performed using fundus autofluorescence images. Areas of hypoautofluorescence corresponding to retinal pigment epithelium atrophy were manually delineated by two masked observers using a computer mouse, a touch pen, and an external pad connected to the computer, via the built-in measurement tools of the imaging software on the Spectralis® HRA + OCT system (Heidelberg Engineering, Germany). A representative example of these measurements is illustrated in Fig. 1.

Measurements were expressed in square millimeters, and atrophic progression was calculated as the difference between baseline and 12-month measurements. For each eye, the average value obtained from the two observers was used for analysis.

Safety Monitoring

Safety was assessed throughout follow-up by monitoring local and systemic adverse events. Ocular events of interest included endophthalmitis, intraocular inflammation, increased intraocular pressure, cataract progression, retinal tear, and retinal detachment. Systemic safety was evaluated based on patient-reported symptoms and complementary laboratory testing when clinically indicated.

Statistical Analysis

Descriptive statistics were used to summarize demographic characteristics, clinical findings, imaging outcomes, and safety data. Continuous variables were reported as mean $\pm$ standard deviation or median values, as appropriate. Comparisons between treated eyes and untreated fellow eyes were performed using paired analyses when applicable.

Atrophic areas were square-root transformed to stabilize variance. The primary comparison was the within-patient difference in 12-month progression ($\Delta\sqrt{\text{area}}$) between the treated and untreated fellow eye, tested against zero with the exact Wilcoxon signed-rank test, with the mean within-patient difference reported alongside a 95% confidence interval. Baseline atrophic areas were compared between eyes to confirm allocation balance. As a sensitivity analysis, the comparison was repeated on the untransformed scale and with a paired t-test.

Best-corrected visual acuity was converted to logMAR for analysis, with hand motion assigned a value of 2.0 logMAR. Baseline BCVA was compared between treated and fellow eyes with the exact Wilcoxon signed-rank test to assess allocation balance, and the 12-month change from baseline was tested within

each group using the same test. The between-eye comparison was the within-patient difference in logMAR change (treated minus fellow eye), also tested with the exact Wilcoxon signed-rank test and summarized as the mean difference with a 95% confidence interval. Because hand motion was assigned a fixed value, eyes already at this measurement floor at baseline could not demonstrate further measurable decline; a sensitivity analysis therefore repeated the between-eye comparison after excluding patients in whom either eye was at this floor. All tests were two-sided ($\alpha = 0.05$), performed in Python v3.11. Given the exploratory nature of this study and the limited sample size, the results were interpreted primarily as descriptive findings.

Use of Artificial Intelligence

During the preparation of this work the authors used ChatGPT®, Google Gemini®, and Claude® to assist with text writing and revising. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the manuscript.

Results

This study included eight patients with EMAP, comprising five men (62.5%) and three women (37.5%), with a mean age of 59.5 ± 3.78 years. All patients presented with bilateral, progressive visual loss, with symptoms lasting a mean of 6.75 ± 2.76 years before diagnosis. All patients had a documented history of childhood rheumatic fever, occurring between 3 and 7 years of age, and had received prolonged benzathine penicillin prophylaxis for a mean duration of 11.1 ± 2.8 years. Systemic comorbidities included dyslipidemia, arterial hypertension, diabetes mellitus, cardiac arrhythmia, and hemophilia, while ocular history included previous cataract surgery, refractive surgery, and pterygium surgery.

At baseline, best-corrected visual acuity varied substantially among patients, reflecting different stages of disease severity. Treated eyes showed BCVA ranging from 20/32 to hand motion, whereas untreated fellow eyes ranged from 20/25 to hand motion. Anterior segment examination was unremarkable in most cases, except for incipient cataract in three patients. Fundus examination consistently demonstrated bilateral macular atrophy associated with pseudodrusen-like deposits and peripheral paving-stone degeneration (Fig. 2a, b; Fig. 3a, b). Fundus autofluorescence revealed small, round, confluent areas of hypoautofluorescence with a predominantly vertical distribution in the posterior pole, corresponding to retinal pigment epithelium disorganization and atrophy, with relative foveal preservation in most cases (Fig. 2g, h; Fig. 3g, h). Spectral-domain OCT showed variable preservation of the external limiting membrane and ellipsoid zone at the foveal center, with perifoveal disruption extending nasally and temporally (Fig. 2c, d, e, f; Fig. 3c, d, e, f). Splitting of the retinal pigment epithelium–basal lamina–Bruch's membrane complex was consistently observed, frequently associated with overlying hyperreflective material and variable choroidal hypertransmission in areas of retinal pigment epithelium and outer retinal atrophy.

Atrophic Area Progression

Quantitative analysis of atrophic area progression on FAF imaging revealed ongoing disease activity in both treated and untreated eyes (Table 1 and Fig. 4).

Table 1
Atrophic Area Progression Over 12 Months – Two Masked Observers Average

Case	Treated Eye	Baseline Treated (mm ²)	Final Treated (mm ²)	Progression Treated (mm ²)	Baseline Untreated (mm ²)	Final Untreated (mm ²)	Progression Untreated (mm ²)
1	OD	17.23	24.90	7.67	16.44	24.43	7.99
2	OS	7.60	9.32	1.72	8.57	10.78	2.21
3	OS	24.57	29.24	4.67	19.02	22.56	3.54
4	OS	26.29	38.66	12.37	31.30	49.53	18.23
5	OS	47.65	50.98	3.33	43.09	44.28	1.19
6	OD	41.02	49.05	8.03	52.97	58.93	5.96
7	OS	14.88	16.80	1.92	17.43	22.47	5.04
8	OS	18.34	18.98	0.64	16.13	18.98	2.85
Mean ± SD		24.70 ± 13.53	29.74 ± 15.25	5.04 ± 4.01	25.62 ± 15.40	31.50 ± 17.06	5.88 ± 5.45
OD = right eye; OS = left eye; SD = standard deviation.							

At baseline, the mean atrophic area was 24.70 ± 13.53 mm² in treated eyes and 25.62 ± 15.40 mm² in untreated fellow eyes, with no difference between the two eyes (exact Wilcoxon P = 0.84), confirming that they were well balanced at baseline. After 12 months of follow-up, the mean atrophic area increased to 29.74 ± 15.25 mm² in treated eyes and 31.50 ± 17.06 mm² in untreated fellow eyes. The mean absolute enlargement was 5.04 ± 4.01 mm² in treated eyes and 5.88 ± 5.45 mm² in untreated fellow eyes, corresponding to a 14.3% lower mean progression in treated eyes compared with untreated eyes. In 5 of 8 patients, the treated eye demonstrated less atrophic enlargement than the untreated fellow eye.

Mean 12-month progression was 0.478 √mm² in treated eyes and 0.559 √mm² in fellow eyes (14.4% lower in treated eyes), yielding a mean within-patient difference of – 0.080 √mm² (95% CI, – 0.259 to + 0.099) in favor of the treated eye (Fig. 4b); this difference was not statistically significant (exact Wilcoxon signed-rank test, P = 0.46). Results were unchanged on the untransformed scale and when analyzed with a paired t-test (P = 0.32). The confidence interval spanned from no effect to a clinically meaningful benefit, consistent with the limited statistical power of an eight-patient design.

Structural Changes on SD-OCT

Serial SD-OCT imaging demonstrated progressive disruption and loss of the EZ and ELM in both treated and untreated eyes throughout the 12-month follow-up period (Fig. 2c, d, e, f; Fig. 3c, d, e, f). Based on qualitative assessment, we observed that the outer retinal layers in the treated eyes were generally better preserved than those in the untreated control eyes in the majority of cases.

Progressive enlargement of zones with signal hypertransmission was noted bilaterally, reflecting ongoing expansion of RPE and outer retinal atrophy. The characteristic splitting of the RPE–BL–BrM complex persisted throughout follow-up, with continued presence of overlying hyperreflective material.

Visual Acuity Changes

Individual case-by-case Snellen and logMAR equivalents for treated and untreated fellow eyes over the 12-month follow-up period are summarized in Table 2.

Table 2
BCVA Changes in Treated and Untreated Eyes over the 12-Month Follow-up Period

Case	Group	Baseline (Snellen)	12 Months (Snellen)	Baseline (logMAR)	12 Months (logMAR)	Change (logMAR)
1	Treated	20/32	20/32	0.20	0.20	0.00
2	Treated	20/40	20/50	0.30	0.40	+ 0.10
3	Treated	20/50	20/100	0.40	0.70	+ 0.30
4	Treated	20/100	20/160	0.70	0.90	+ 0.20
5	Treated	20/400	20/1200	1.30	1.78	+ 0.48
6	Treated	HM	HM	2.00	2.00	0.00
7	Treated	20/100	20/400	0.70	1.30	+ 0.60
8	Treated	20/40	20/40	0.30	0.30	0.00
Median	Treated	20/70	20/125	0.55	0.80	+ 0.15
1	Fellow	20/25	20/40	0.10	0.30	+ 0.20
2	Fellow	20/400	20/400	1.30	1.30	0.00
3	Fellow	20/200	20/200	1.00	1.00	0.00
4	Fellow	HM	HM	2.00	2.00	0.00
5	Fellow	HM	HM	2.00	2.00	0.00
6	Fellow	HM	HM	2.00	2.00	0.00
7	Fellow	20/400	20/400	1.30	1.30	0.00
8	Fellow	20/800	HM	1.60	2.00	+ 0.40
Median	Fellow	20/565	20/895	1.45	1.65	+ 0.00
<i>HM = hand motion. Visual acuity values were converted to logMAR for analysis, with hand motion converted to logMAR 2.0. Positive change values indicate worsening visual acuity.</i>						

Visual outcomes varied among patients and reflected the heterogeneous baseline severity of EMAP. In treated eyes, the median baseline BCVA was 0.55 logMAR, corresponding approximately to 20/70 Snellen equivalent, declining to a median of 0.80 logMAR (approximately 20/125) at 12 months. In untreated fellow eyes, baseline visual acuity was considerably worse, with a median of 1.45 logMAR (approximately 20/565), declining further to a median of 1.65 logMAR (approximately 20/895) at 12 months. Baseline BCVA was significantly better in treated eyes than in fellow eyes (median 0.55 versus 1.45 logMAR; exact Wilcoxon signed-rank $P = 0.03$), reflecting the non-randomized selection of the treated eye.

Three treated eyes (Cases 1, 6, and 8) maintained stable visual acuity, while the remaining five treated eyes (Cases 2, 3, 4, 5, and 7) showed some degree of decline, ranging from 0.10 to 0.60 logMAR. Case 7 had the largest decline among treated eyes (20/100 to 20/400; +0.60 logMAR), and Case 5 the second largest (20/400 to 20/1200; +0.48 logMAR). In contrast, six untreated fellow eyes (Cases 2, 3, 4, 5, 6, and 7) remained visually stable, while two (Cases 1 and 8) declined, by 0.20 and 0.40 logMAR, respectively. The mean logMAR change was 0.21 ± 0.23 in treated eyes versus 0.08 ± 0.15 in untreated fellow eyes, indicating that, in absolute terms, treated eyes lost more visual acuity on average than untreated eyes over the follow-up period. Neither the within-group change from baseline reached statistical significance (treated eyes, median + 0.15 logMAR, $P = 0.06$; fellow eyes, median 0.00 logMAR, $P = 0.50$), nor did the within-patient difference in logMAR change between eyes (mean + 0.14 logMAR, 95% CI - 0.14 to + 0.41; exact Wilcoxon signed-rank $P = 0.30$; paired t-test $P = 0.29$). Interpretation of this comparison is constrained by a measurement floor: three fellow eyes (Cases 4, 5, and 6) were already at hand motion at baseline and could not decline further on the logMAR scale, compared with one treated eye, and six of eight fellow eyes had baseline acuity of 20/400 or worse versus two of eight treated eyes. When the three patients with either eye at this floor were excluded, the between-eye difference attenuated further (mean + 0.08 logMAR; exact Wilcoxon signed-rank $P = 0.81$). As discussed below, this pattern is possibly attributable to the more advanced baseline disease and reduced functional reserve already present in most untreated fellow eyes, rather than to a harmful effect of treatment.

Safety Profile

The 12-month treatment using intravitreal methotrexate resulted in no serious adverse events. We observed no instances of endophthalmitis, severe intraocular inflammation, retinal detachment, or spikes in intraocular pressure. The only issues observed were mild, temporary reactions in the anterior chamber of two patients after injection, resolving within 48 hours without intervention. No systemic adverse effects attributable to methotrexate were reported.

Discussion

This study represents the use of intravitreal methotrexate as an immunomodulatory therapy for EMAP, regarding which we are unaware of any prior reports of this procedure and found no reference in a computerized search of PubMed. Our findings demonstrate that monthly intravitreal methotrexate injections over 12 months were well-tolerated with a favorable safety profile but showed limited efficacy in halting disease progression. Treated eyes exhibited modestly reduced atrophic area expansion compared to untreated fellow eyes, with better functional outcomes observed in approximately half of the cases. However, this difference did not reach statistical significance in paired analysis, and the 95% confidence interval for the within-patient difference in square-root progression included zero.

Notably, a recent natural history study by Audi et al. characterized the visual outcomes and clinical features of EMAP, further consolidating the phenotypic description of this entity and providing a contemporary reference cohort against which future interventional studies, including the present series, can be contextualized [4]. The association between EMAP and childhood rheumatic fever supports the

hypothesis of post-streptococcal autoimmune mechanisms [2, 4]. The rationale for using methotrexate in EMAP is based on accumulating evidence suggesting immune-mediated pathogenesis. Douillard et al. demonstrated significant immunological alterations in EMAP patients, including elevated complement C3 levels and reduced CH50 activity, indicating chronic complement activation [2]. These findings parallel the immune dysregulation observed in other autoimmune retinal diseases. Molecular mimicry between retinal antigens and streptococcal M proteins provides a plausible mechanism for autoimmune targeting of the outer retina [6], potentially explaining the typical age of onset and progressive nature of EMAP.

Methotrexate blocks dihydrofolate reductase, reduces T-cell proliferation, decreases pro-inflammatory cytokine output (TNF- α , IL-1, IL-6), and modulates complement activation [8, 9]; anti-rheumatic drugs, including methotrexate, have been shown to lower complement activation markers in rheumatoid arthritis [10], supporting biological plausibility for its use in EMAP.

The 14.3% reduction in raw atrophic progression observed in treated eyes should be interpreted cautiously given the small sample size. Because only eight patients were studied, this comparison was statistically underpowered, and the treated-versus-fellow-eye difference did not reach significance, with a confidence interval spanning from no effect to a benefit of clinically meaningful magnitude. Notably, this magnitude falls within the range reported for approved complement inhibitors in geographic atrophy secondary to AMD: pegcetacoplan reduced GA lesion growth by 11–22% in the OAKS/DERBY trials [11], and avacincaptad pegol by 18–28% in GATHER1/GATHER2 [12, 13]. This comparison, however, is only contextual—EMAP and AMD-related geographic atrophy are distinct entities differing in age of onset, growth pattern, and presumed mechanism (post-streptococcal autoimmunity versus complement dysregulation)—and should not be taken as evidence of comparable efficacy. It is plausible that EMAP represents a more treatment-resistant process than dry AMD, particularly given the advanced, longstanding atrophy already present at enrollment (mean symptom duration 6.75 years) in most of our cohort; even a partial anatomical effect may therefore represent a meaningful proof-of-concept signal.

Several factors may explain the limited efficacy observed. First, disease stage: once extensive RPE and photoreceptor loss has occurred, immunomodulation alone may be insufficient to arrest progression, which raises the possibility that earlier intervention could yield better results. Second, the treatment regimen (400 μ g monthly for 12 months) was extrapolated from other ocular inflammatory conditions rather than EMAP-specific pharmacodynamic data [14, 15]; higher doses, greater frequency, longer duration, or combination therapy may be needed for a more robust effect, though this remains speculative at this point. Third, disease heterogeneity was evident: Cases 4, 7, and 8 showed marked anatomical benefit, while Cases 1 and 2 showed minimal benefit and Cases 3, 5, and 6 showed greater atrophic progression in the treated eye than in the untreated control, possibly reflecting differences in immune activity, genetics, or timing of treatment.

Visual acuity results require cautious interpretation. Although fewer treated eyes (3/8) than untreated eyes (6/8) remained stable, this likely reflects baseline disease imbalance rather than a harmful

treatment effect: treated eyes generally had more preserved foveal function at baseline and thus greater functional reserve to lose, whereas untreated eyes had less room for further measurable decline. These impressions are supported by the paired analysis: baseline BCVA was significantly better in treated eyes ($P = 0.03$), the between-eye difference in 12-month logMAR change was not statistically significant ($P = 0.30$), and excluding patients whose fellow eye was already at hand motion attenuated that difference further ($P = 0.81$). The greater mean acuity loss recorded in treated eyes should therefore not be interpreted as treatment-related harm. This underscores that visual acuity alone may be an insensitive endpoint in advanced, asymmetric cohorts; future studies should incorporate low-luminance acuity, microperimetry, reading speed, and patient-reported outcomes. Importantly, the favorable safety profile—no endophthalmitis, severe inflammation, or systemic toxicity—is consistent with prior reports of intravitreal methotrexate in other ocular conditions [14, 15] and supports feasibility of chronic use, pending larger, longer-term safety data.

This study has important limitations: small sample size ($n = 8$) limiting statistical power, such that the primary anatomical comparison did not reach statistical significance despite a point estimate favoring the treated eye; non-randomized design using fellow eyes as controls, partially mitigated by the characteristic bilateral symmetry of EMAP; a 12-month follow-up that may be insufficient for a slowly progressive disease; heterogeneous baseline severity and reliance on manually delineated, and thus partly subjective, atrophic area measurements despite masked grading.

These preliminary findings do not support routine use of intravitreal methotrexate for EMAP but provide a preliminary signal that immunomodulation warrants further investigation. Priorities for future research include adequately powered randomized controlled trials with longer follow-up, earlier-stage intervention, dose optimization, combination regimens (e.g., with complement inhibitors or corticosteroids), biomarker discovery to predict treatment response, and genetic studies to clarify disease mechanisms and identify novel therapeutic targets.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

This study was performed in line with the principles of the 1964 Declaration of Helsinki and its later amendments. The study protocol was approved by the Institutional Review Board of Ribeirão Preto Medical School, University of São Paulo [Ethics committee approval number: 97849826.3.0000.5440].

Consent to participate

Written informed consent was obtained from all individual participants included in the study, for off-label intravitreal methotrexate administration and for the use of their clinical data for research purposes.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy considerations but are available from the corresponding author on reasonable request.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by A.S.F.C.J., V.B. and L.O.A. Image grading was performed by V.R.S and C.M.M.R. The first draft of the manuscript was written by A.S.F.C.J. and all authors commented on previous versions of the manuscript. Study supervision was provided by L.H.L. and R.J. All authors read and approved the final manuscript.

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Figures

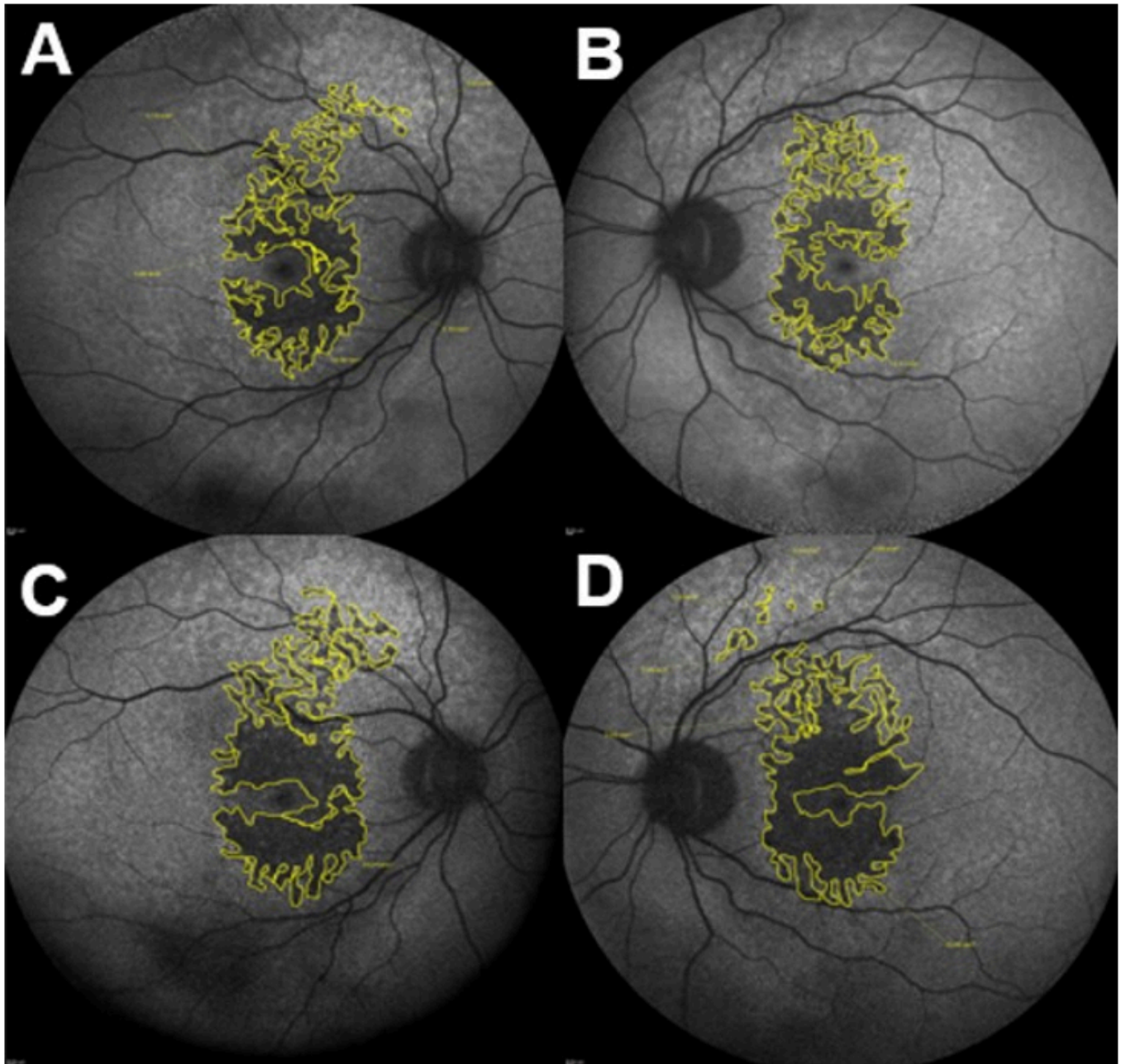


Figure 1

Fundus autofluorescence imaging of Case 1 shows the demarcated area of vertical hypoautofluorescence (which matches the atrophic area of the ellipsoid zone shown in the SD-OCT b-

scans in Fig. 2) of the OD and OS at baseline (a, b) and over the 12-month follow-up period (c, d), showing that the area of atrophy progressed slightly less in the treated eye (OD) during the period – progression of 7.67 mm² in the treated eye (OD) and 7.99 mm² in the untreated eye (OS)

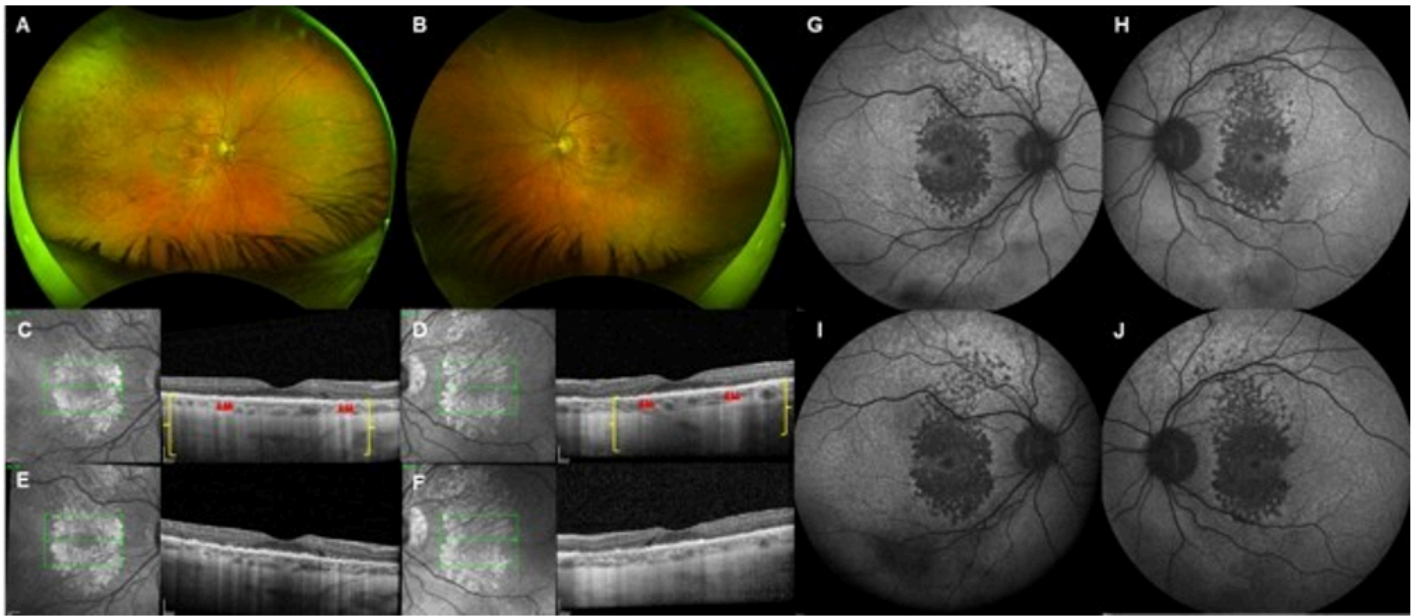


Figure 2

Multimodal imaging of Case 1: ultra-wide fundus photographs of the OD (a) and OS (b) show central macular atrophy, with vertical axis greater than the horizontal, pigmentary changes, diffuse pseudodrusen-like deposits in the posterior pole and subtle peripheral paving-stone like degeneration OU. SD-OCT b-scans show sparse subretinal drusenoid deposits (red arrowheads), diffuse ellipsoid zone attenuation, complete retinal pigment epithelium and outer retinal atrophy (cRORA) (yellow brackets), and diffuse choroidal thinning of the OD and OS at baseline (c, d) and over the 12-month follow-up period (e, f). Fundus autofluorescence imaging revealed small, round, and confluent areas of hypoautofluorescence vertically distributed in the posterior pole, corresponding to zones of retinal pigment epithelium (RPE) disorganization and atrophy, with relative preservation of autofluorescence at the foveal center at baseline (g, h) and over the 12-month follow-up period (i, j)

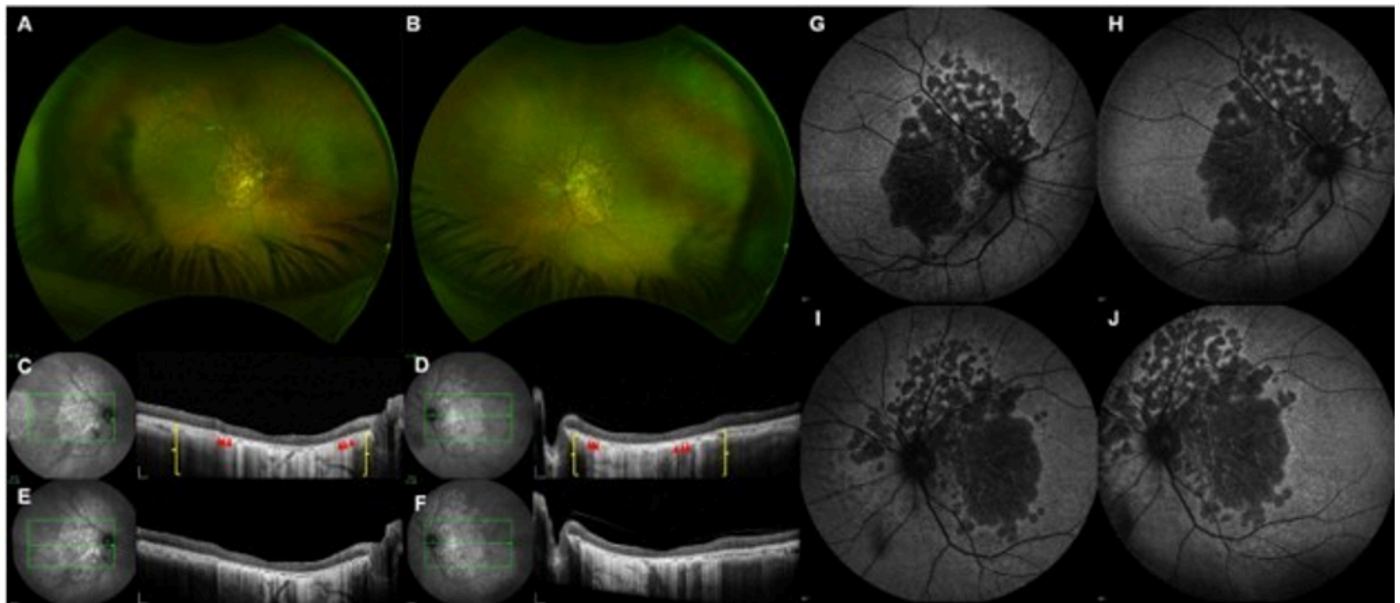


Figure 3

Multimodal imaging of Case 6: ultra-wide fundus photographs of the OD (a) and OS (b) show central macular atrophy, with vertical axis greater than the horizontal, pigmentary changes, diffuse pseudodrusen-like deposits in the posterior pole and subtle peripheral paving-stone like degeneration OU. SD-OCT b-scans show sparse subretinal drusenoid deposits (red arrowheads), diffuse ellipsoid zone attenuation, complete retinal pigment epithelium and outer retinal atrophy (cRORA) (yellow brackets), and diffuse choroidal thinning of the OD and OS at baseline (c, d) and over the 12-month follow-up period (e, f). Fundus autofluorescence imaging revealed small, round, and confluent areas of hypoautofluorescence vertically distributed in the posterior pole, corresponding to zones of retinal pigment epithelium (RPE) disorganization and atrophy at baseline (g, h) and over the 12-month follow-up period (i, j), showing that the area of atrophy progressed more in the treated eye (OD) than in the untreated fellow eye (OS) during the period – progression of 8.03 mm² in the treated eye (OD) and 5.96 mm² in the untreated eye (OS)

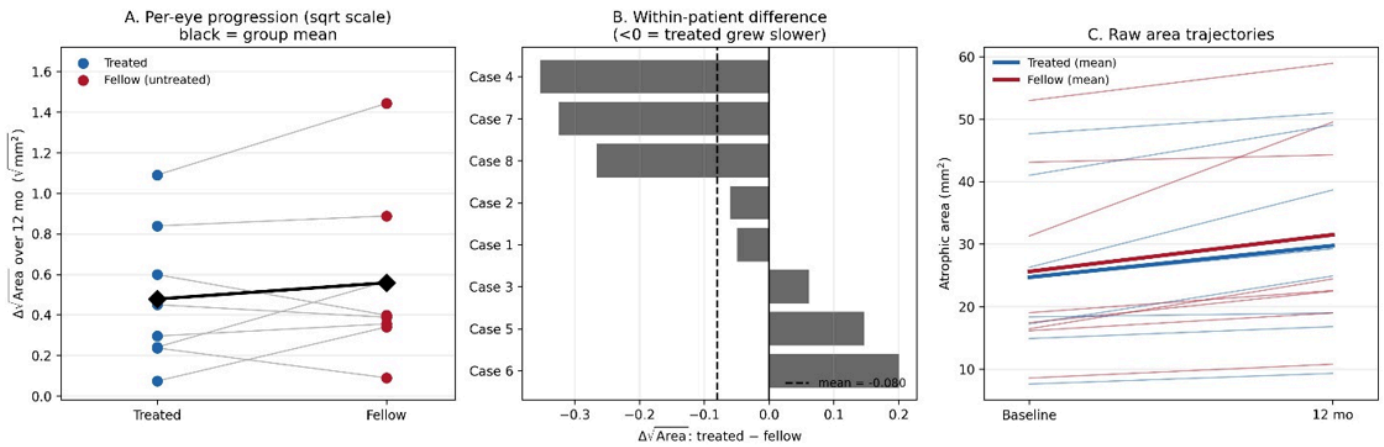


Figure 4

Paired analysis of atrophic area progression over 12 months. (a) Change in square-root transformed atrophic area ($\Delta\sqrt{\text{area}}$) in each eye; grey lines connect the treated and untreated fellow eye of the same patient, and black diamonds joined by a black line indicate the group means (0.478 $\sqrt{\text{mm}^2}$ in treated eyes and 0.559 $\sqrt{\text{mm}^2}$ in untreated fellow eyes). (b) Within-patient difference in $\Delta\sqrt{\text{area}}$ (treated minus untreated fellow eye) for each case, ordered from the greatest relative benefit in the treated eye (top) to the greatest relative progression in the treated eye (bottom). Negative values indicate slower atrophic enlargement in the treated eye, which was observed in five of eight patients; the dashed line marks the mean within-patient difference (-0.080 $\sqrt{\text{mm}^2}$; 95% CI, -0.259 to +0.099; exact Wilcoxon signed-rank $P = 0.46$). (c) Untransformed atrophic area at baseline and at 12 months for individual treated (blue) and untreated fellow (red) eyes, with thick lines indicating group means