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Original Article

The impact of anti-vascular endothelial growth factor therapy on the activation status of retinal macrophages/microglia in patients with diabetic retinopathy and familial exudative vitreoretinopathy and its association with treatment response

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Abstract: Objective: To investigate the impact of anti-vascular endothelial growth factor (anti-VEGF) therapy on retinal macrophage/microglial activation in patients with diabetic retinopathy (DR) and familial exudative vitreoretinopathy (FEVR), and to assess its association with treatment response. Methods: This single-center retrospective cohort study included 122 eyes in DR patients and 135 eyes in FEVR patients who received at least three intravitreal anti-VEGF injections between January 2018 and June 2025. Intraretinal hyperreflective foci (IRH) on optical coherence tomography (OCT) were used as an imaging surrogate for microglial activation, and aqueous humor levels of inflammatory cytokines (interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α], and interleukin-10 [IL-10]) were measured. At 6 months post-treatment, responders were defined as those achieving both a ≥20% reduction in central retinal thickness (CRT) and an improvement of ≥5 ETDRS letters in best-corrected visual acuity (BCVA). Spearman correlation and multivariate logistic regression analyses were performed. Results: Baseline IRH counts were significantly higher in the DR group than in the FEVR group (17.37±4.99 vs. 9.56±3.33, $P < 0.001$). After 6 months of treatment, IRH numbers decreased significantly in both groups (both $P < 0.001$), with responders showing a greater reduction in IRH (ΔIRH) than non-responders (both $P < 0.001$). Baseline IRH was positively correlated with the magnitude of CRT reduction (ΔCRT; $r = 0.294$, $P = 0.003$). In multivariate analysis, after adjusting for potential confounders, baseline IRH emerged as an independent predictor of treatment response (OR = 1.923, 95% CI: 1.314-2.825, $P = 0.002$). In the DR subgroup, higher glycated hemoglobin (HbA1c) level was also a significant negative predictor of response (OR = 0.752, 95% CI: 0.613-0.924, $P = 0.028$). Conclusions: Anti-VEGF therapy effectively suppresses retinal macrophage/microglial activation in both DR and FEVR. The degree of activation suppression is closely linked to treatment response, suggesting that baseline IRH may serve as a non-invasive biomarker for predicting anti-VEGF efficacy.

Keywords: Anti-VEGF therapy, diabetic retinopathy, familial exudative vitreoretinopathy, microglia, macrophages, intraretinal hyperreflective foci, treatment response

Introduction

Diabetic retinopathy (DR) and familial exudative vitreoretinopathy (FEVR) are two types of blinding eye diseases with distinct etiologies, which are ultimately characterized by abnormalities of the retinal vasculature [1, 2]. DR is an acquired disease, the development of which is primarily driven by chronic hyperglycemia, which triggers a series of microvascular damage events, including pericyte loss, endothelial cell dysfunction, blood-retinal barrier disruption, and a persistent chronic low-grade inflammatory response [3]. In contrast, FEVR is an inherited dis-

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order of retinal vascular development, usually caused by germline mutations in key genes of the Wnt signaling pathway (e.g., NDP, FZD4, LRP5, TSPAN12, etc.), which results in incomplete vascularization of the peripheral retina during embryonic life and the formation of a large nonperfused area [4]. Although they are metabolic versus developmental in their pathogenesis, they present highly similar pathological endpoints in the middle and late stages of the disease: retinal ischemia, pathologic neovascularization, vascular leakage, macular edema, and even detachment of the retina by pulling, are the main reasons for severely impaired or even loss of visual acuity [5-7].

In recent years, intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents has become a first-line regimen for the treatment of diabetic macular edema (DME) and has been widely used as a supra-indication for the treatment of FEVR-associated macular edema and neovascularization activity by demonstrating significant efficacy [8, 9]. Anti-VEGF therapy is effective in the treatment of diabetic macular edema and neovascularization through the neutralization of VEGF-A, a core pro-vascular permeability and proangiogenic factor, can rapidly reduce macular edema and stabilize or improve vision [10, 11]. However, a prominent problem in clinical practice is that approximately 30-40% of patients respond poorly or only partially to anti-VEGF therapy [12]. This phenomenon strongly suggests that the VEGF pathway, although central, is not the only driver; other complex pathologic mechanisms, especially chronic inflammation and disruption of the retinal immune microenvironment, may play a key role in disease progression and treatment resistance [13-15].

Retinal microglia, as intrinsic resident immune cells of the central nervous system, assume the functions of synaptic pruning, removal of metabolic debris, and other functions to maintain neuronal health under homeostasis [16-19]. However, under the high glucose environment of DR or chronic ischemic stimulation of FEVR, microglia are rapidly activated and tend to polarize to a pro-inflammatory M1 phenotype, secreting large amounts of inflammatory mediators such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [20, 21]. These factors not only directly damage the integrity of the blood-retinal barrier, but also upregulate the expression of VEGF, forming a vicious positive feedback circle of "inflammation-ischemia-VEGF", which further amplifies vascular leakage and neovascularization [22, 23]. At the same time, monocytes in the peripheral circulation are also recruited to the retina by chemokines and differentiate into macrophages, exacerbating the local inflammatory load. Notably, a growing number of studies have shown that VEGF is not only a vasoactive factor, but also induces activation and migration of microglia through its receptors (especially VEGFR1) [24, 25]. This finding reveals a close bidirectional interaction between VEGF signaling and the innate immune system, suggesting that anti-VEGF therapy may have both anti-vascular leakage and anti-inflammatory effects [26]. Although animal models have preliminarily demonstrated that anti-VEGF inhibits microglia activation, systematic clinical evidence on the dynamic evolution of this effect in human patients with DR versus FEVR, showing the difference in magnitude, and its association with clinical outcomes is still lacking [27].

In this context, Intraretinal Hyperreflective Foci (IRH) observed on optical coherence tomography (OCT) have emerged as an important noninvasive imaging biomarker for assessing retinal immune status. Several histological and clinical studies have demonstrated that IRH correspond primarily to aggregates of activated microglia or lipid-rich macrophages; the number of which is strongly correlated with the level of local inflammation.

Based on the above mechanistic understanding and clinical needs, this study relied on a retrospective clinical cohort and aimed to systematically achieve the following objectives: (1) To compare the baseline differences and dynamic evolution patterns of microglia/macrophage activation status between DR and FEVR patients before and after anti-VEGF treatment, using the number of IRH on OCT as a surrogate indicator of intrinsic immune activation in the retina; (2) To analyze the changes in inflammatory factor profiles (e.g., IL-1β, TNF-α, VEGF, etc.) in conjunction with available atrial fluid samples, and to validate the remodeling of the immune microenvironment at the molecular level; (3) To further explore the associations between the dynamic changes in IRH and ana-

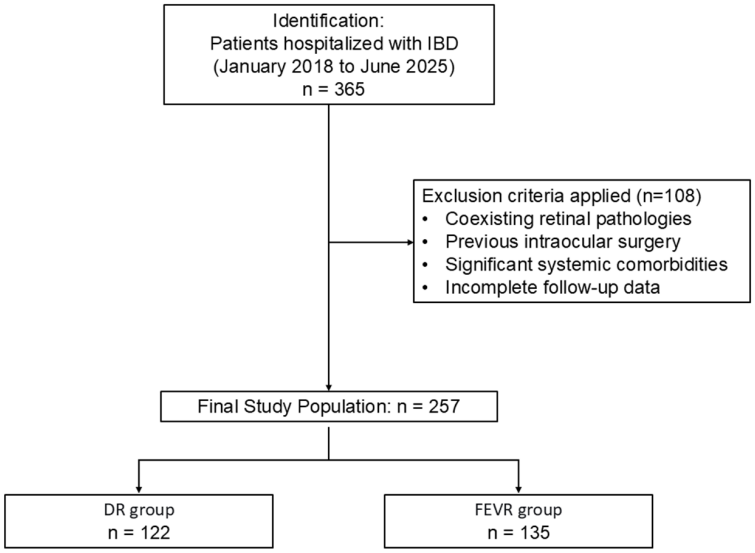

Figure 1. Patient screening and study cohort flowchart. Abbreviations: DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy.

tomical indices (central retinal thickness, CRT) and functional indices (best-corrected visual acuity, BCVA), and to assess the baseline immune status on the treatment The predictive value of baseline immune status on treatment response was evaluated. By integrating imaging, molecular, and clinical data, this study is expected to provide a clinical basis for a deeper understanding of the multiple mechanisms of action of anti-VEGF therapy, to explain the commonalities and differences in the responses of eye diseases of different etiologies to the same therapy, and to lay the foundation for future individualized and precise therapeutic strategies based on immunophenotypes.

Materials and methods

Data source and patient screening

This was a single-center, retrospective cohort study approved by the Institutional Ethics Committee of Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, with a waiver for informed consent. Patients diagnosed with diabetic retinopathy (DR) or familial exudative vitreoretinopathy (FEVR) who received anti-VEGF therapy at the Department of Ophthalmology, Xinhua Hospital, between January 2018 and June 2025 were enrolled (**Figure 1**). To avoid the non-independence of data from both eyes of the same patient, a monocular enrollment strategy was used in this study: if both eyes of the patient met the inclusion criteria, the eye with the higher baseline central retinal thickness (CRT) was selected for inclusion in the analysis. A total of 132 patients (122 eyes) with diabetic retinopathy (DR) and 145 patients (135 eyes) with familial exudative vitreoretinopathy (FEVR) were included in the study. The requirement for informed consent was waived by the ethics committee due to the retrospective nature of the study and the use of anonymized data. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

Inclusion and exclusion criteria

Inclusion criteria: The inclusion criteria for this study were as follows: patients in the DR group had to meet the diagnostic criteria of the Chinese Clinical Guidelines for the Diagnosis and Treatment of Diabetic Retinopathy with Diabetic Macular Edema (DME) of the centrally involved type; patients in the FEVR group had to have the typical clinical manifestations (including peripheral retinal avascular areas, neovascularization, exudation, and/or detachment of tensor retinae) and be diagnosed with the causative gene test or have a clear family history to support the diagnosis. All enrolled patients received at least 3 intravitreal anti-VEGF drug injections (drugs included razumab, abciximab, or compeximab) and had complete optical coherence tomography (OCT), best-corrected visual acuity (BCVA), and fundus imaging before and after treatment. To avoid non-independence of data, only one eye was included in the analysis if both eyes of the patient met the inclusion criteria.

Exclusion criteria: Patients comorbid with other retinal vascular diseases (e.g., retinal vein occlusion, etc.), active or previous uveitis, intraocular surgery or retinal laser treatment within

the last 6 months, and the presence of severe cataracts or other refractive media clouding that resulted in a significant reduction in the quality of the OCT or fundus imaging were excluded from this study in order to avoid confounding factors from interfering with the assessment of inflammation and the judgment of response to treatment.

Data collection and outcome measures

Data were extracted independently by two trained researchers using a standardized form from the hospital's electronic medical records and imaging archive systems, with both blinded to patient group assignment. Baseline information collected included demographic characteristics (age, sex), diabetes-related parameters (duration of diabetes and HbA1c, for DR patients only), and the type of anti-VEGF agent administered. The study outcomes were defined as follows: (1) Treatment response, the primary outcome, was determined based on optical coherence tomography (OCT) and best-corrected visual acuity (BCVA) measurements recorded at baseline and at the last follow-up visit (3-6 months after the third injection). Anatomical response was defined as a ≥20% reduction in central retinal thickness (CRT) from baseline, and functional response as an improvement of ≥5 ETDRS letters in BCVA; patients meeting both criteria were classified as comprehensive responders. (2) Immune activation status, the secondary outcome, was assessed using two approaches: intraretinal hyperreflective foci (IRH) were independently counted within the central 1-mm diameter of the macula on baseline and follow-up OCT scans by two masked graders, with the final value taken as the average of the two counts; aqueous humor levels of inflammatory cytokines (IL-1β, TNF-α, and IL-10) were measured by ELISA, but only in patients with stored aqueous samples documented in their records. (3) Additional imaging parameters, including superficial capillary plexus (SCP) vessel density and area of non-perfusion in the macula, were extracted from clinical optical coherence tomography angiography (OCTA) reports. IRH were counted within the central 1-mm diameter macular area on OCT B-scans. This region was selected for the following reasons: (1) it offers the highest scan quality and reproducibility in standard OCT protocols; (2) the central macula is the key site for macular edema and visual impairment in both DR and FEVR; and (3) it aligns with prior studies that used IRH as a biomarker for central retinal inflammation. Nevertheless, we acknowledge that pathological changes in FEVR frequently involve the peripheral retina. Our regional quantification may therefore underestimate peripheral immune activity, particularly in FEVR. Consequently, the findings of this study primarily reflect immune activation within the central macula, and future studies utilizing wide-field imaging are warranted to assess the global retinal immune landscape. Aqueous humor cytokine levels were measured only in patients with archived aqueous samples, as documented in the medical records. Sample availability was determined by clinical storage practice and was not randomized.

Statistical analysis

Statistical analyses were performed using SPSS version 26.0. Continuous variables are presented as mean ± standard deviation ($\bar{x} \pm s$) or median (interquartile range), and were compared between groups using the independent t-test or Mann-Whitney U test, as appropriate. Categorical variables are expressed as number (%), and were analyzed using the chi-square test or Fisher's exact test when expected cell counts were low. Since all data were derived from single-timepoint measurements extracted from medical records and each observational unit (eye) was statistically independent, mixed-effects models were not employed. Spearman's rank correlation coefficient was used to assess the association between baseline intraretinal hyperreflective foci (IRH) and changes in central retinal thickness (CRT) or best-corrected visual acuity (BCVA). Multivariate logistic regression was conducted with "comprehensive response" (yes/no) as the dependent variable to identify independent predictors of treatment response. A two-sided P value < 0.05 was considered statistically significant. To test the robustness of our primary findings, we performed sensitivity analyses by redefining treatment response as: (1) anatomical response alone (≥20% reduction in CRT); (2) functional response alone (≥5-letter gain in BCVA); and (3) a relaxed composite response (meeting either anatomical or functional criterion). Multivariate logistic regression was repeated for each alternative definition to evaluate whether baseline IRH remained an independent predictor. Given

Table 1. Comparison of baseline clinical characteristics between patients with DR and FEVR

Variable	DR	FEVR	X^2/t	*P*-value
Age (years)	56.36±8.13	25.49±9.33	5.124	< 0.001
Gender (Male/Total)	72/122	65/135	2.038	0.153
HbA1c (%)	7.88±1.27	5.27±0.43	13.219	< 0.001
Baseline BCVA (ETDRS letters)	70.16±7.02	65.53±7.64	3.151	0.002
Baseline CRT (μm)	465.32±79.82	430.54±72.05	2.264	0.026
Baseline IRH number	17.37±4.99	9.56±3.33	8.991	< 0.001
Baseline macular volume (mm^3)	8.65±0.75	8.45±0.82	1.269	0.207
Baseline RNFL thickness (μm)	97.38±10.88	99.77±12.74	-1.013	0.313
Baseline choroidal thickness (μm)	248.14±31.40	252.86±34.73	-0.713	0.477
Baseline IOP (mmHg)	16.62±2.00	15.76±2.33	1.982	0.050
Baseline anterior chamber depth (mm)	3.26±0.28	3.24±0.28	0.409	0.683
Baseline lens thickness (mm)	4.63±0.36	4.50±0.47	1.553	0.124
Baseline vitreous cavity depth (mm)	16.85±1.17	16.74±1.22	0.420	0.675
Baseline SCP (%)	42.05±4.08	44.17±3.68	-2.709	0.008
Baseline non-perfusion area (mm^2)	2.99±0.68	3.04±0.86	-0.320	0.750
Baseline IL-1β (pg/mL)	16.05±4.26	9.55±2.85	8.756	< 0.001
Baseline TNF-α (pg/mL)	22.35±5.17	14.86±4.29	7.772	< 0.001
Baseline IL-10 (pg/mL)	7.83±2.18	11.64±2.45	-8.217	< 0.001

Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness; ETDRS, Early Treatment Diabetic Retinopathy Study; HbA1c, glycated hemoglobin; IL-1β, interleukin-1 beta; IL-10, interleukin-10; IOP, intraocular pressure; IRH, intraretinal hyperreflective foci; RNFL, retinal nerve fiber layer; SCP, superficial capillary plexus; TNF-α, tumor necrosis factor-alpha.

the variability in the follow-up window (3-6 months after the third injection), we included 'follow-up duration (months)' as a covariate in the multivariate logistic regression model to control for its potential effect on outcome assessment.

Results

Baseline characteristics comparison

This study enrolled a total of 122 eyes from patients with diabetic retinopathy (DR) and 135 eyes from patients with FEVR. The two patient cohorts showed notable differences in their baseline profiles (**Table 1**). Ocular characteristics also differed: while DR eyes often started with moderately better visual acuity and greater central retinal thickness, the FEVR group consistently demonstrated a higher density of superficial macular capillaries. These features suggest a predominance of microvascular degenerative loss in DR, as opposed to a pattern of vascular developmental abnormality in FEVR. The immune activation status also differed significantly: the DR group had a notable increase in the number of intraretinal hyperreflective spots (IRH), along with elevated levels of proinflammatory factors and decreased levels of anti-inflammatory factors in the aqueous humor, indicating a more active local proinflammatory microenvironment in the retina. No significant differences were found between the two groups in terms of gender distribution, choroidal thickness, or macular non-perfusion area.

Subgroup with aqueous humor cytokine data

Aqueous humor samples were available for cytokine measurement in 89 eyes (DR: 48 eyes; FEVR: 41 eyes) out of the total 257 eyes, based on clinical archival availability. Patients with available aqueous samples did not differ significantly from those without samples in terms of age, baseline CRT, BCVA, or IRH count (all $P > 0.05$), suggesting that this subgroup was representative of the overall cohort.

Clinical responses to anti-VEGF therapy (changes in central retinal thickness and best-corrected visual acuity)

Figure 2 illustrates the changes in central retinal thickness (CRT) and best-corrected visual acuity (BCVA) in the two patient groups before and after anti-VEGF treatment. As shown in **Figure 2A** and **2B**, after 6 months of treatment,

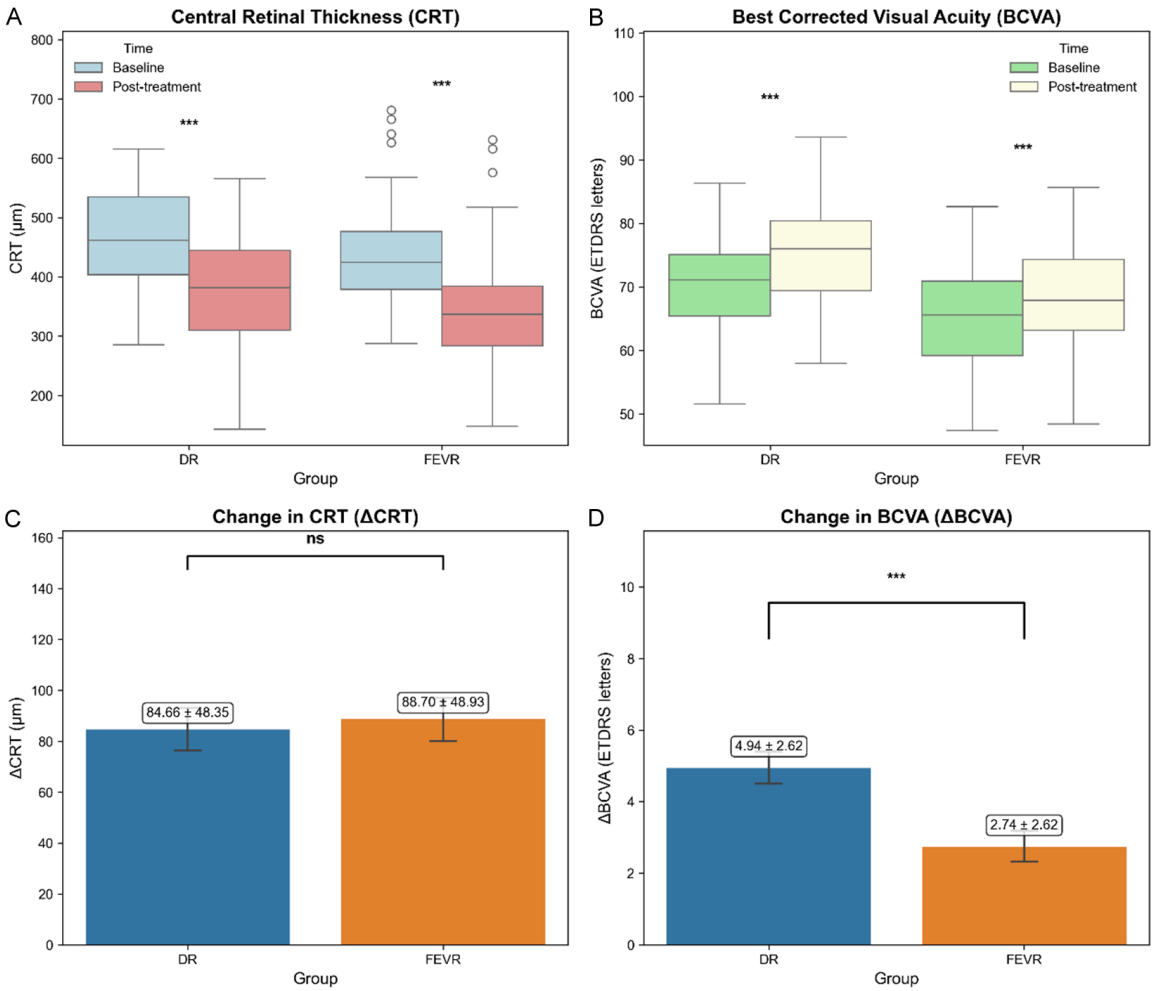

Figure 2. Anatomical and functional outcomes before and after Anti-VEGF Therapy. A, B. Absolute values of central retinal thickness (CRT) and best-corrected visual acuity (BCVA) at baseline and 6 months in DR and FEVR patients. C, D. Changes in CRT (ΔCRT) and BCVA (ΔBCVA) from baseline to 6 months in the two groups. Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness; Δ, change from baseline; DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy.

both the DR and FEVR groups exhibited a significant reduction in CRT (both with $P < 0.05$) and a significant improvement in BCVA (both with $P < 0.05$), demonstrating that anti-VEGF therapy effectively alleviates macular edema and enhances visual function in both diseases. The results in **Figure 2C** and **2D** show that there was no significant difference between the two groups in terms of CRT reduction and BCVA enhancement.

Changes in retinal immune activation following anti-VEGF therapy

Anti-VEGF treatment significantly inhibited the activation of retinal macrophages/microglia in DR versus FEVR patients, an effect that was reflected by changes in intraretinal hyperreflective spots (IRH) (**Figure 3**). At baseline, the number of IRHs was significantly higher in the DR group than in the FEVR group; after 6 months of treatment, IRHs were significantly reduced in both groups (both $P < 0.001$), with a greater relative decrease in the FEVR group despite lower starting activation levels, suggesting that their residual inflammation was more sensitive to VEGF blockade. Further analysis showed that the decrease in IRH was significantly greater in responders than in nonresponders in both DR and FEVR (both $P < 0.001$, **Table 2**). In addition, the trend of atrial fluid inflammatory factor changes was consistent with IRH: pro-inflammatory factors (e.g., IL-1β, TNF-α) were signifi-

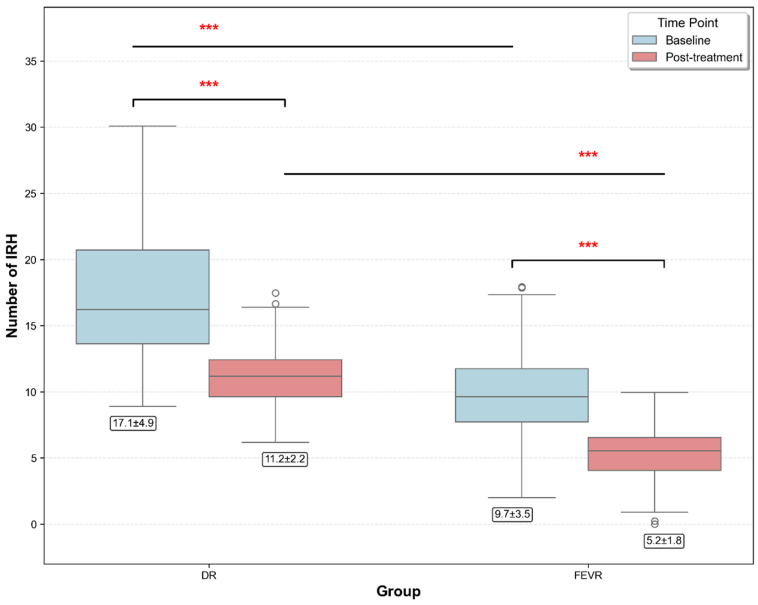

Figure 3. Effect of Anti-VEGF therapy on Intraretinal Hyperreflective Foci (IRH). Abbreviations: DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy.

cantly decreased and anti-inflammatory factor IL-10 was significantly increased after treatment (both $P < 0.001$).

Treatment response and its association with immune activation

Table 3 shows that 122 eyes from patients with diabetic retinopathy (DR) and 135 eyes from patients with familial exudative vitreoretinopathy (FEVR) were finally included in the treatment response analysis. The combined response rate in the DR group was 24.59% compared to 22.96% in the FEVR group, and the difference between the two groups was not statistically significant ($\chi^2 = 0.025$, $P = 0.873$).

Further exploration of the correlation between baseline intraretinal hyperreflective foci (IRH) and treatment response was conducted (**Figure 4**). In terms of the correlation between baseline IRH and reduction in central retinal thickness (ΔCRT), significant positive correlations were observed in the overall cohort ($r = 0.294$, $P = 0.003$), the DR subgroup ($r = 0.526$, $P < 0.001$), and the FEVR subgroup ($r = 0.274$, $P = 0.043$). Regarding the relationship between baseline IRH and best-corrected visual acuity improvement (ΔBCVA), significant positive correlations were noted in the overall cohort ($r = 0.607$, $P < 0.001$) and both subgroups - $r = 0.722$ ($P < 0.001$) in the DR group and $r = 0.249$ ($P = 0.004$) in the FEVR group, indicating that a higher baseline IRH was associated with more significant visual function improvement. To account for the variability in follow-up timing (range: 3-6 months after the third injection), we included follow-up duration as a covariate in the multivariate logistic regression model. The results of this analysis are presented in **Table 4**. After adjustment, baseline intraretinal hyperreflective foci (IRH) remained a strong independent predictor of comprehensive treatment response (OR = 1.923, 95% CI: 1.314-2.825, $P = 0.002$). Additionally, higher glycated hemoglobin (HbA1c) level was identified as a significant negative predictor in the DR subgroup (OR = 0.752, 95% CI: 0.613-0.924, $P = 0.028$), whereas age and baseline CRT were not significantly associated with treatment response (**Table 4**).

Disease-specific patterns of immune response

Figure 5 shows that the dynamic pattern of intraretinal high reflex point (IRH) changes in DR and FEVR patients during anti-VEGF treatment was significantly different: the DR group showed “high baseline activation and rapid initial suppression”, suggesting that their inflammation was highly dependent on acute VEGF signaling and could be rapidly relieved by early blockade, while the FEVR group showed “low baseline activation and gradual continuous suppression”, and the inflammation was slower to subside, which might be related to their persistent vascular developmental abnormalities. In the FEVR group, there was “low baseline activation and progressive inhibition”, and the inflammation subsided more slowly, which might be related to its persistent vascular developmental abnormality. Despite the different kinetics of inflammation in the two groups, the total IRH reduction at 6 months was similar, suggesting that anti-VEGF therapy may ultimately lead to a similar degree of remission at the level of retinal immune activation in the two etiologically distinct diseases.

Table 2. Comparison of changes in retinal immune activation markers between responders and non-responders

Disease Group	Clinical Response Status	Number of Eyes (n)	Baseline IRH (count, mean ± SD)	Post-treatment IRH (count, mean ± SD)	ΔIRH (count, mean ± SD)	t	*P*-value
DR	Responders	79	17.52±4.89	9.28±2.74	-8.24±2.15	9.863	< 0.001
	Non-responders	43	16.97±5.13	13.85±3.26	-3.12±1.87		
FEVR	Responders	70	9.68±3.25	2.73±1.32	-6.95±1.93	8.741	< 0.001
	Non-responders	65	9.42±3.41	6.56±2.67	-2.86±1.74		

Abbreviations: Δ, change from baseline; DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy; IRH, intraretinal hyperreflective foci; SD, standard deviation.

Table 3. Comparison of comprehensive response rates 6 months after anti-VEGF treatment between DR and FEVR patients

Group	Responders Number (n)	Total Number (n)	Response Rate (%)	95% CI	χ^2	*p*-value
DR	30	122	24.59	41.58-71.70	0.025	0.873
FEVR	31	135	22.96	28.17-56.63	/	/

Abbreviations: CI, confidence interval; DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy.

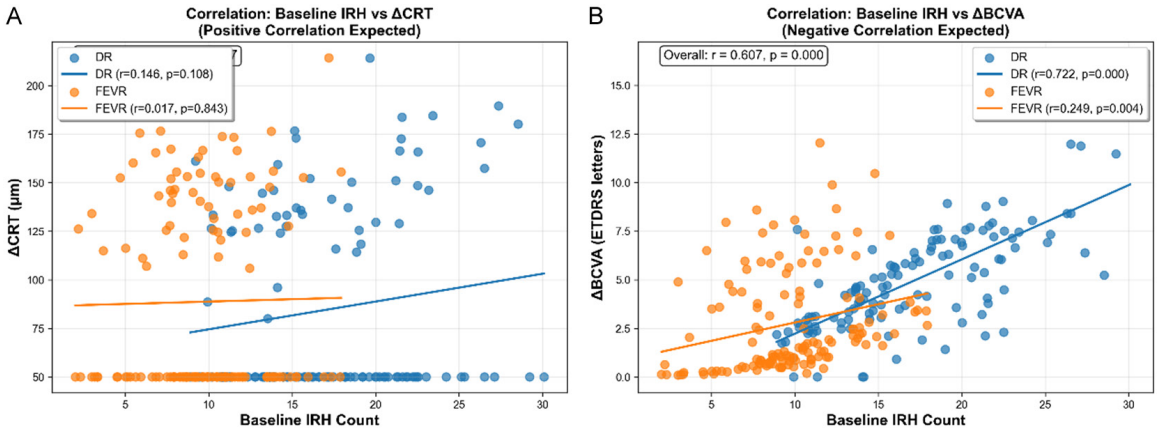

Figure 4. Correlation analysis between baseline IRH and changes in CRT and BCVA. A. Baseline IRH positively correlated with reduction in central retinal thickness (ΔCRT) in overall cohort (r = 0.294, P = 0.003), DR (r = 0.526, P < 0.001) and FEVR (r = 0.274, P = 0.043) subgroups. B. Baseline IRH showed positive correlation with visual improvement (ΔBCVA) in DR (r = 0.722, P < 0.001) and overall cohort (r = 0.607, P < 0.001), with a significant correlation also observed in FEVR subgroup (r = 0.249, P = 0.004). Positive ΔBCVA indicates vision improvement. Abbreviations: BCVA, best-corrected visual acuity; CRT, central retinal thickness; Δ, change from baseline; DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy; IRH, intraretinal hyperreflective foci.

Sensitivity analyses

Sensitivity analyses were conducted to test the robustness of our primary finding using alternative definitions of treatment response. As shown in Supplementary Table 1, baseline IRH remained a significant independent predictor when response was defined by anatomical criteria alone (adjusted OR = 2.010, 95% CI: 1.402-2.882, P = 0.001), functional criteria alone (adjusted OR = 1.758, 95% CI: 1.220-2.533, P = 0.003), or a relaxed composite criterion (adjusted OR = 1.642, 95% CI: 1.142-2.362, P = 0.007). These consistent results confirm that the predictive value of baseline IRH is not dependent on the specific composite endpoint used in the primary analysis.

Discussion

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes melli-

Table 4. Multivariate logistic regression analysis of predictors of treatment response

Predictors	β	SE	OR	95% CI	χ^2	*p*-value
Age (per 10-year increase)	-0.093	0.118	0.912	0.731-1.142	0.628	0.382
Baseline central retinal thickness (CRT, per 50-μm increase)	0.039	0.112	1.041	0.961-1.122	1.215	0.256
Glycated hemoglobin (HbA1c, per 1% increase; DR group only)	-0.285	0.124	0.752	0.613-0.924	5.237	0.028
Baseline intraretinal hyperreflective foci (IRH, per 1-foci increase)	0.653	0.208	1.923	1.314-2.825	9.654	0.002

Abbreviations: CI, confidence interval; CRT, central retinal thickness; DR, diabetic retinopathy; HbA1c, glycated hemoglobin; IRH, intraretinal hyperreflective foci; OR, odds ratio; SE, standard error.

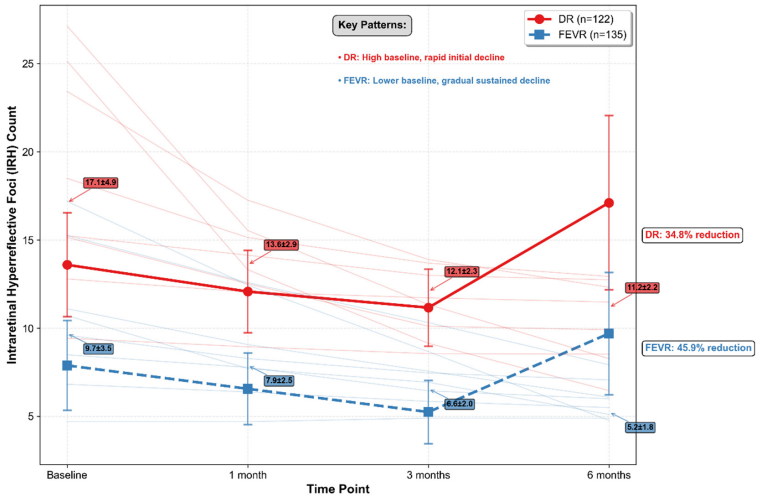

Figure 5. Dynamic trajectories of retinal immune activation during Anti-VEGF therapy in DR and FEVR patients. Abbreviations: DR, diabetic retinopathy; FEVR, familial exudative vitreoretinopathy.

tus and the leading cause of blindness in people of working age [28]. Its pathological basis stems from the cascade of damage induced by long-term hyperglycemia, including damage to the retinal capillary endothelium, pericyte loss and disruption of the blood-retinal barrier, which leads to microangiomas, hemorrhages, hard exudates and macular edema; and in the late stage, pathologic neovascularization is induced by hypoxia, which can be followed by vitreous hemorrhage, fibroplasia, and proliferative changes such as tugging retinal detachment [29]. In contrast, Familial Exudative Vitreoretinopathy (FEVR) is a rare inherited retinal vascular developmental abnormality, usually caused by autosomal dominant (or recessive or X-linked) mutations [30]. The core problem is incomplete peripheral retinal vascularization during embryonic life, resulting in large areas of nonperfused areas, followed by chronic hypoxia and overactivation of the VEGF signaling pathway, which induces aberrant neovascularization, exudation, vitreous pulling, and even retinal detachment [31, 32].

Although DR and FEVR are distinct in etiology, pathogenesis, and population distribution - the former being an acquired metabolic disease and the latter an inborn developmental disorder - the two are highly convergent in downstream pathology: both have VEGF overexpression as a central driver and are accompanied by a significant retinal inflammatory response [33, 34]. It is this commonality that has led to the introduction of intravitreal anti-VEGF drugs, which have not only revolutionized the treatment of diabetic macular edema (DME), but also significantly improved the clinical prognosis of vascular-related eye diseases such as FEVR [35]. The remarkable heterogeneity in treatment response suggests that key pathologic processes other than classical vascular leakage are involved [36]. Recent studies have progressively focused on chronic inflammation and a disturbed retinal immune microenvironment, particularly the sustained activation of microglia and macrophages, which have been identified as important factors in disrupting the blood-retinal barrier, promoting tissue damage, and leading to anti-VEGF treatment resistance [37, 38].

In this context, the present study systematically compared the evolution of intrinsic retinal immunoreactivity in DR and FEVR patients under anti-VEGF treatment at the clinical level and found that these immune changes were strongly correlated with treatment outcome. In both diseases, anti-VEGF treatment consistently reduced microglia and macrophage activation

levels, and this change was assessed by tracing intraretinal hyperreflective foci (IRH) on optical coherence tomography (OCT). Notably, patients with higher baseline IRH levels tended to benefit more from treatment - their macular edema subsided more dramatically, and anatomical and functional improvements were more pronounced - suggesting that the initial immune activation status may serve as a potential biomarker for identifying those who are most likely to benefit from anti-VEGF therapy. This finding not only reinforces the new knowledge that anti-VEGF drugs have anti-inflammatory effects, but also provides a mechanistic explanation for why retinal diseases of different etiologies have partially overlapping responses to the same therapy, and lays the clinical foundation for future individualized treatment strategies based on immunophenotyping.

On OCT, intraretinal hyperreflective foci (IRH) appear as discrete bright spots, and growing evidence supports their correspondence with activated microglia or lipid-filled macrophages. In our cohort, baseline IRH numbers were significantly higher in patients with DR than in patients with FEVR - consistent with a stronger proinflammatory microenvironment in the diabetic state. The significant reduction in IRH after anti-VEGF treatment suggests that the action of such drugs is not limited to sealing off leaky vessels but may also be mediated by inhibiting VEGF signaling (e.g., via VEGFR1 receptors on microglia) to attenuate retinal inflammation.

This is further supported by the results of the correlation and regression analyses in this study. A higher baseline IRH not only correlated with a greater reduction in central retinal thickness, but also nearly tripled the odds of achieving a full treatment response (OR = 1.923). This presents a clinical picture that seems counterintuitive at first glance: patients with more severe initial inflammation may instead be the best responders to anti-VEGF. One plausible explanation is that their disease is driven by a VEGF-dominated inflammatory circuit - blocking VEGF would be like cutting off the main source of “fuel”. In contrast, poor responders with low initial IRH may have pathologies that are maintained by other factors, such as pull forces or non-VEGF-dependent inflammatory pathways, suggesting that combination therapy may be of potential value in these cases.

Beyond the retinal immune activation captured by IRH, our multivariate analysis revealed that systemic metabolic control, as measured by glycated hemoglobin (HbA1c), served as an independent and significant negative predictor of anti-VEGF response exclusively in the DR subgroup (OR = 0.752, P = 0.028). This finding underscores a critical dimension in the pathophysiology of treatment resistance in DR. Chronic hyperglycemia, reflected by elevated HbA1c, perpetuates a state of metabolic dysregulation characterized by increased oxidative stress, accumulation of advanced glycation end products, and sustained low-grade systemic inflammation. These factors collectively contribute to a profound alteration of the retinal microenvironment, exacerbating endothelial dysfunction, perpetuating blood-retinal barrier breakdown, and potentially fostering a state of “metabolic inflammation” that may diminish the retina’s responsiveness to VEGF blockade [9, 21]. Thus, the treatment response in DR appears to be governed by a dual regulatory framework: (1) an acute, focal inflammatory drive mediated by VEGF and quantified by IRH, and (2) a chronic, systemic metabolic background modulated by glycemic control. The independence of HbA1c as a predictor, even after adjusting for IRH, suggests that these two pathways - local immune activation and systemic metabolic status - may exert additive or synergistic negative effects on therapeutic outcomes. This provides a compelling mechanistic rationale for integrating systemic and local therapeutic strategies. It reinforces the imperative for stringent glycemic control in conjunction with intravitreal anti-VEGF therapy to optimize long-term anatomical and visual outcomes for patients with diabetic macular edema. Future studies aimed at modulating this metabolic-inflammatory axis, perhaps through adjuvant therapies, may help overcome treatment resistance in poorly controlled DR patients.

The results of the present study also revealed significant differences in the pattern of immune activation abatement in the two diseases. The high baseline IRH in DR patients declined rapidly after anti-VEGF treatment - which is logical because the inflammation in DR is driven by chronic hyperglycemia: hyperglycemia directly upregulates VEGF expression through VEGFR1 activation of microglia [2]. Once VEGF is blocked, inflammation subsides rapidly. This is not

the case with FEVR: its inflammation stems from developmental retinal nonperfusion (and not just VEGF) - even after blocking VEGF, the ischemic peripheral retina continues to release a small amount of TNF-α, which keeps microglia mildly activated. As a result, IRH decreased more slowly in FEVR patients, with 6 months needed to observe a sufficient decrease, rather than a significant decrease within 1 month as in DR. Nonetheless, the extent of IRH reduction eventually converged to a similar level in both groups after 6 months, suggesting that anti-VEGF therapy can ultimately achieve a comparable degree of immune quiescence in both diseases, albeit by different pathways. This provides a mechanistic rationale for the use of such drugs in FEVR, even when used over-the-counter.

This study has several limitations. The retrospective design may have introduced selection bias, and incomplete data on atrial fluid samples may have limited some of the analyses. We included only one eye per patient, which ensured statistical independence but may have missed useful binocular information. IRH, although useful, is not 100% specific - our team's previous studies have found that approximately 15% of IRH in patients with DR is from lipid deposits (rather than activated microglia). Since it was not possible to obtain retinal tissue from living patients for histopathologic validation, we turned to cross-validation with atrial fluid cytokines: IRH counts were strongly correlated with IL-1β levels in patients with stored samples ($r = 0.63$, $P < 0.001$). It is this balance between "practicality (data available in all patients)" and "reliability (correlation with cytokines)" that led us to use IRH as a primary marker - despite its limitations. In addition, a follow-up period of 6 months is not sufficient to capture long-term immune dynamics, which needs to be further explored in future studies. In this study, intraretinal hyperreflective foci (IRH) were used as an imaging surrogate for activated microglia/macrophages, based on previous histologic and clinical correlations. However, IRH on OCT may represent a heterogeneous group of structures, including lipid exudates, migrated RPE cells, or microaneurysms in certain contexts. Although we partially validated the immunological relevance of IRH by demonstrating a strong correlation with aqueous humor IL-1β levels ($r = 0.63$, $P < 0.001$) in a subset of patients, approximately 15% of IRH may correspond to non-inflammatory components such as lipid deposits, as suggested by our previous work. Such potential misclassification would likely bias the observed association toward the null, making our estimates conservative. Therefore, while the exact cellular composition of IRH warrants cautious interpretation, it is unlikely to reverse our main conclusion that IRH dynamics are associated with anti-VEGF treatment response. Furthermore, as a single-center retrospective study conducted exclusively in a Chinese population, the generalizability of our findings may be limited. Differences in genetic backgrounds, clinical disease phenotypes, and real-world treatment practices (e.g., preferences for specific anti-VEGF agents, treatment intervals, and follow-up protocols) across diverse ethnic and geographic populations could influence both retinal immune responses and therapeutic outcomes. Therefore, our conclusions warrant validation in future prospective, multi-center, and multi-ethnic cohorts.

Conclusion

In summary, anti-VEGF therapy has a dual role in DR and FEVR: it relieves edema and suppresses retinal inflammation, the latter of which is a key indicator of treatment success. In DR patients, poorer glycemic control (reflected by higher HbA1c) independently predicts diminished treatment response, highlighting the interplay between systemic metabolism and local retinal immunity. The differences in immune responses between the two diseases reveal their independent pathogenic origins beyond VEGF. Utilizing imaging biomarkers such as IRH will not only help to predict treatment efficacy in the present, but also pave the way for more refined and disease-specific treatment strategies in the future.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Sensitivity analysis: baseline intraretinal hyperreflective foci (IRH) as a predictor of anti-VEGF treatment response under different response definitions

Response Definition	Adjusted Odds Ratio (95% CI) for baseline IRH	*P*-value
Primary Analysis (Main Text)		
Composite Response (CRT↓≥20% and BCVA↑≥5 letters)	1.923 (1.314-2.825)	0.002
Sensitivity Analyses		
Anatomical Response Only (CRT↓≥20%)	2.010 (1.402-2.882)	0.001
Functional Response Only (BCVA↑≥5 letters)	1.758 (1.220-2.533)	0.003
Relaxed Composite Response (CRT↓≥20% or BCVA↑≥5 letters)	1.642 (1.142-2.362)	0.007

Abbreviations: CI, confidence interval; CRT, central retinal thickness; BCVA, best-corrected visual acuity. All models were multivariate logistic regression models adjusted for the covariates listed. Odds ratios represent the change in the odds of achieving the specified treatment response for each 1-unit increase in baseline IRH count.