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ORIGINAL RESEARCH

Dynamic Complement Protein Changes in Aqueous Humor and Plasma of Patients With Retinal Vein Occlusion During Ranibizumab Treatment

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Purpose: To assess dynamic changes of complement protein in aqueous humor (AH) and plasma of retinal vein occlusion (RVO) patients during ranibizumab treatment, and to explore the differential expression of complement proteins in branch retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO).

Patients and Methods: This prospective, consecutive case series study collected AH and plasma samples from 27 RVO patients at baseline, 1 and 2 months after ranibizumab treatment, including 19 BRVO and 8 CRVO patients. The concentrations of 13 complement proteins and vascular endothelial growth factor A (VEGF-A) were measured using Luminex® × MAP® technology.

Results: During ranibizumab treatment, a reduction in the levels of C1q ($p < 0.001$), C2 ($p = 0.030$), C4 ($p = 0.001$), C4b ($p = 0.026$), C3b/iC3b ($p < 0.001$), C5 ($p = 0.007$), C5a ($p = 0.005$), CFD ($p = 0.022$), CFH ($p < 0.001$), and CFI ($p < 0.001$) in AH was observed. No significant changes were observed in the plasma levels of all measured factors. At baseline, CRVO had higher levels of C4 ($p = 0.003$), C4b ($p < 0.001$), C3b/iC3b ($p < 0.001$), C5 ($p = 0.020$), C5a ($p = 0.007$), CFD ($p = 0.002$), CFH ($p < 0.001$), and CFI ($p < 0.001$) in AH compared to BRVO.

Conclusion: Ranibizumab treatment reduced the intraocular but not circulating activation of classical and alternative complement pathways in RVO patients. Differences in intraocular complement proteins were observed between BRVO and CRVO patients, which may reflect different pathogenesis.

Keywords: retinal vein occlusion, complement protein, aqueous humor, plasma

Introduction

Retinal vein occlusion (RVO), the second most common cause of retinal vascular disorders, can be classified into central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO) depending on the occluded vessel.[1] CRVO arises from thrombosis of the central retinal vein as it traverses the lamina cribrosa. BRVO occurs due to venous thrombosis at arteriovenous crossings where the artery and vein are encased within a shared vascular sheath.[2] The most frequent complication in RVO patients is Macular Edema (ME), which can lead to significant vision loss and potentially blindness.[3] Ranibizumab, a recombinant humanized monoclonal antibody fragment, is recognized as a first-line treatment for RVO.[4] It functions by binding to and inhibiting all human forms of VEGF, which are key contributors to the

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pathogenesis of RVO-ME.[5] The drug has demonstrated effectiveness in improving vision and reducing macular edema in RVO patients.[6]

The complement system, a complex network comprising over 30 soluble proteins, membrane-bound proteins, and complement receptors, is crucial to the innate immune system.[7] Under physiological conditions, the majority of complement components are found in an inactive proenzyme state and are activated through a series of cascading enzymatic reactions triggered by various stimuli. This activation is essential for maintaining tissue homeostasis and immune surveillance against pathogens, thereby providing a critical protective mechanism for the human body.[8] The activation pathways of the complement system are primarily categorized into three: the classical pathway (CP), the alternative pathway (AP), and the mannan-binding lectin (MBL).[9] Excessive or deregulated activation of the complement system has been associated with a range of disorders.[10] Moreover, there is a strong association between the complement system and ocular diseases, such as uveitis, diabetic retinopathy (DR), and age-related macular degeneration (AMD).[11–13] Abnormal expression of complement proteins has also been noted in RVO patients. Reich et al conducted a proteomic analysis of vitreous samples from RVO patients and controls, identifying C3 as significantly statistically relevant, suggesting its potential as a biomarker for RVO.[14] Elevated levels of C3, C5, and complement factor H (CFH) has been detected in BRVO patients, correlating with central retinal thickness (CRT).[15,16] Similarly, in CRVO patients, an upregulation of complement proteins including C5, C6, C7, C9, complement factor B (CFB), and CFH has been observed.[17] Additionally, our previous research found that the levels of C1q, C2, C4, C4b, C3b/iC3b, C5, C5a, CFB, CFH, and MBL in the aqueous humor, as well as the levels of C4, C4b, C3b/iC3b, CFB, and CFH in the plasma of RVO patients, were significantly higher than control group.[18]

Although the role of anti-VEGF therapy in treating RVO has been widely recognized, there is currently a lack of studies that have detailed the impact of this treatment on the levels of complement proteins in the aqueous humor (AH) and systemic circulation of RVO patients. The complement system may play a role in the pathological process of RVO. Therefore, understanding the effects of anti-VEGF therapy on the complement system is essential for a deeper comprehension of the immunopathological mechanisms of the disease. The purpose of this study was to assess the dynamic changes in complement protein levels in AH and plasma of RVO patients undergoing intravitreal ranibizumab injection (IRI) treatment. Additionally, this study analyzed the differences in complement proteins in AH of BRVO and CRVO patients before and after ranibizumab injection treatment, to provide more precise guidance and reference for clinical decisions.

Materials And Methods

Patients

This study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants, and the study was approved by the Institutional Review Board (IRB) at the Changsha Aier Eye Hospital [Ethical approval number: (2024) KYPJ009]. To estimate the required number of participants, we utilized the online sample size calculator G*Power, a commonly used tool in statistical power analysis. The sample size was calculated to achieve 80% power with a significance level of 0.05 to detect a medium effect size (followed by Cohen's guidelines). Based on these calculations, 27 participants would be necessary for this study. Twenty-seven patients (27 eyes) with RVO were recruited to the study at Changsha Aier Eye Hospital from January 2021 to December 2023.

Inclusion criteria: (1) patients aged 18 years or older; (2) RVO confirmed by fundus stereoscopy, optical coherence tomography (OCT), and fluorescein fundus angiography (FFA); (3) CRT exceeding 250μm with the best corrected visual acuity (BCVA) ≤ 0.5 and require medical attention (ie, intravitreal anti-VEGF injection, ranibizumab); (4) no previous treatment for RVO including intravitreal anti-VEGF injections or corticosteroids, focal/grid macular photocoagulation, pan-retinal photocoagulation, or vitreoretinal surgery. Exclusion criteria: (1) presence of other retinal pathologies including glaucoma, DR, and retinal neovascularization from other etiologies; (2) current or history of intraocular or systemic inflammatory or autoimmune diseases; (3) history of intraocular surgery or laser treatments.

Clinical characteristics, including gender, age, body mass index (BMI), RVO duration, and blood test results were collected from the electronic medical record system. Before each treatment, all patients received comprehensive

ophthalmologic examinations including BCVA (reported as the log of the minimum angle of resolution (logMAR)), slit-lamp examination, intraocular pressure (IOP), FFA, and OCT. All participants received an intravitreal ranibizumab 0.5-mL injection 3.5mm behind the corneal limbus at baseline, month 1 and month 2. Patients were followed up for 3 months. The CRT change was calculated by subtracting the CRT thickness after three ranibizumab injections from the CRT thickness at baseline.

Sample Collection and Cytokine Analysis

Immediately before the intravitreal injection of ranibizumab 50–60μL of AH were collected under a surgical microscope in an aseptic operating room. 5 mL of blood samples were collected into purple sterile vacuum blood collection tube before each treatment. AH and plasma samples were stored in sterile Eppendorf tubes at −80°C until laboratory measurements.

The levels of complement proteins C1q, C2, C3, C3b/iC3b, C4, C4b, C5, C5a; complement factors B, D, H, I (CFB, CFD, CFH, CFI); and mannose-binding lectin (MBL); and Vascular Endothelial Growth Factor A (VEGF-A) in the AH and plasma samples were measured using the Luminex® × MAP® technology following manufacturer's instructions. Each individual microsphere is identified and the result of its bioassay is quantified based on fluorescent reporter signals. We combined the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of the new MILLIPLEX®Analyst 5.1, integrating data acquisition and analysis seamlessly with all Luminex® instruments. A total of 25μL of aqueous humor (1:3 dilution) and plasma (1:1,000 dilution or 1:40,000 dilution) were collected from each sample and were used to measure the above complement proteins in the study, respectively. Ratios of C3b/C3, C4b/C4 and C5a/C5 were also determined. Standard curves and a four-parameter curve fit were employed to calculate the concentrations in nanograms per milliliter (ng/mL).

Statistical Analysis

Data were analyzed using the SPSS 26.0 software and plots were generated with GraphPad Prism V.9.0 and Origin 2024. Continuous variables were presented as mean ± SD. The Kolmogorov–Smirnov test was employed to assess the normality of the distribution. Independent sample t-tests and the $\chi 2$ test were utilized to compare the continuous and categorical variables between the BRVO and CRVO groups. A generalized estimating equation (GEE) model and Bonferroni post-hoc tests were employed to assess differences in the levels of complement proteins and ratios of C3b/C3, C4b/C4 and C5a/C5 in RVO patients during ranibizumab treatment. Pearson correlation analysis was used to evaluate the correlation between measurements. $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

We obtained 76 AH samples and 71 plasma samples from 27 RVO patients. The baseline characteristics of the participants are summarized in Table 1. Within the RVO patients, the average age was 57.22 years, the mean of BCVA (logMAR) was 0.63, and the mean of CRT was 520.78μm. There was no significant difference in age, sex distribution, history of hypertension, history of diabetes, RVO duration, BMI and the BCVA (logMAR), IOP, AL, PT, TT, APTT, FIB, TG, TC, HDL-C, LDL-C between BRVO and CRVO patients. However, CRVO had significantly higher CRT (678.25μm ± 245.31μm) than BRVO patients (454.47μm ± 132.46μm) at baseline (Table 1).

The Levels of Complement Proteins and VEGF-A in AH and Plasma

The concentrations of complements and VEGF-A in AH at baseline, month 1, and month 2 are detailed in Table 2. 11 out of 14 measurements demonstrated significant statistical differences during the treatment process. C3 plays a crucial role in all three activation pathways of the complement system. Activation of C3 occurs through proteolytic cleavage by C3 convertases associated with either the AP (C3bBb) or the CP and MBL (C4b2a). C3 cleavage releases C3a and C3b, which can be further processed to generate iC3b, an inactive version of C3b.[19] Our results indicated that, compared to the baseline and month 1, there was a significant reduction in the levels of C3b/iC3b in month 2. Nevertheless, there was no

Table 1 Baseline Characteristics of the Study Populations

	RVO (n = 27)	BRVO (n = 19)	CRVO (n = 8)	P value
Age (mean ± SD, years)	57.22 ± 14.38	58.37 ± 12.16	54.50 ±19.37	0.613[a]
Male (n, %)	8 (29.6)	6 (31.6)	2 (25.0)	1.000[b]
Hypertension (n, %)	13 (48.1)	10 (52.6)	3 (37.5)	0.678[b]
Diabetes (n, %)	2 (7.4)	1 (5.3)	1 (12.5)	0.513[b]
Duration of RVO (n, %)				0.921[b]
≤ 1-month	12 (44.4)	8 (42.1)	4 (50.0)	/
>1, <3-month	8 (29.6)	6 (31.6)	2 (25.0)	/
≥3-month	7 (25.9)	5 (26.3)	2 (25.0)	/
BMI (mean ± SD, kg/m^2)	24.48 ± 3.48	24.57 ± 3.86	24.27 ± 2.59	0.843[a]
BCVA logMAR (mean ± SD)	0.63 ± 0.27	0.55 ± 0.18	0.83 ± 0.38	0.103[a]
IOP (mean ± SD, mmHg)	13.74 ± 2.20	14.04 ± 1.99	13.03 ± 2.64	0.283[a]
AL (mean ± SD, mm)	23.57 ± 1.70	23.39 ± 1.55	24.00 ± 2.05	0.406[a]
PT (mean ± SD, s)	11.16 ± 0.58	11.11 ± 0.60	11.29 ± 0.52	0.477[a]
TT (mean ± SD, s)	11.47 ± 0.56	11.46 ± 0.62	11.50 ± 0.43	0.864[a]
APTT (mean ± SD, s)	29.95 ± 2.80	29.35 ± 2.34	31.36 ± 3.43	0.088[a]
FIB (mean ± SD, g/L)	3.14 ± 0.68	3.09 ± 0.62	3.27 ± 0.84	0.559[a]
TG (mean ± SD, mmol/L)	1.33 ± 0.59	1.31 ± 0.50	1.39 ± 0.80	0.768[a]
TC (mean ± SD, mmol/L)	4.55 ± 1.13	4.72 ± 1.22	4.16 ± 0.83	0.243[a]
HDL-C (mean ± SD, mmol/L)	1.34 ± 0.42	1.40 ± 0.47	1.19 ± 0.25	0.133[a]
LDL-C (mean ± SD, mmol/L)	2.67 ± 1.00	2.74 ± 1.11	2.50 ± 0.69	0.577[a]
CRT (mean ± SD, μm)	520.78 ± 197.97	454.47 ± 132.46	678.25 ± 245.31	**0.005[a]**

Notes: Values are given as mean ± SD. Bold $p < 0.05$. [a] independent-sample *t*-test. [b] Chi-square test.
Abbreviations: RVO, retinal vein occlusion; BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; BMI, body mass index; BCVA, best corrected visual acuity; IOP, intraocular pressure; AL, axial length; PT, prothrombin time; TT, thrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; TG, Triglycerides; TC, total Cholesterol; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; CRT, central retinal thickness.

significant change in C3b/C3 ratio. C5a is produced by the cleavage of C5 during the activation of the complement cascade.[20] C5a is a potent pro-inflammatory anaphylatoxin that can stimulate RPE cells to produce VEGF and induce local inflammation through ICAM-1. We observed a significant decrease in the levels of C5 ($p = 0.006$) and C5a ($p = 0.004$) at month 1. Regarding ratio measurements, the C5a/C5 ratio exhibited statistical differences between baseline and month 1 ($p = 0.002$). (Table 2).

Based on the levels of proteins such as C1q, C2, C4, and C4b, the CP of complement activation was assessed. The results showed that there was a statistically significant difference between baseline and month 2 for C1q ($p = 0.020$), C2 ($p = 0.039$), and C4 ($p = 0.001$). Additionally, these factors also demonstrated statistical significance between month 1 and month 2. Compared to the baseline, C4b ($p = 0.026$) significantly decreased at month 1. However, no significant changes were observed in the C4b/C4 ratio. Factor D is crucial for catalyzing the creation of the C3 convertase and for the downstream activation and functioning of the AP.[21] At month 2, the level of CFD was statistically significantly reduced compared to the baseline levels ($p = 0.018$) (Table 2). CFH and CFI, which serve negative regulator functions in the AP, have also shown a significant decrease in concentration during the treatment process. Specifically, a statistically significant reduction in VEGF-A ($p = 0.001$) was observed at month 1 after ranibizumab treatment (Table 2).

No significant changes in the plasma concentration of all the cytokines measurements were observed, as well as in the ratios of C3b/C3, C4b/C4 and C5a/C5 (Table 3). The results suggest that ranibizumab treatment significantly impacted complement proteins and VEGF-A levels in the RVO eyes, but not the blood circulation. Additionally, we observed that the plasma concentrations of C4 in RVO patients are correlated with disease duration. Nonetheless, no significant differences were noted in AH complement proteins between disease duration > 1 month and ≤ 1 month (Supplementary Table S1).

Table 2 Aqueous Humor Levels of Complement Proteins and VEGF-A at Baseline and During the Follow-up in RVO Patients

Variables (unit)	Baseline	Month 1	Month 2	P value	P value	P value	P value
	(n=27) Mean (SE)	(n=27) Mean (SE)	(n=22) Mean (SE)	BL vs 1 vs 2	BL vs 1	BL vs 2	1 vs 2
C1q (ng/mL)	63.26 (12.10)	41.85 (6.37)	28.03 (5.86)	**<0.001**	0.245	**0.020**	**0.001**
C2 (ng/mL)	467.27 (136.54)	236.43 (61.64)	142.78 (35.41)	**0.030**	0.082	**0.039**	**0.040**
C4 (ng/mL)	413.06 (36.27)	364.09 (29.52)	279.02 (33.18)	**0.001**	0.208	**0.001**	**0.005**
C4b (ng/mL)	100.28 (17.03)	71.02 (13.67)	57.02 (18.40)	**0.026**	**0.026**	0.085	1.000
C3 (ng/mL)	393.11 (23.10)	472.67 (33.38)	393.44 (34.20)	0.105	0.139	1.000	0.265
C3b/iC3b (ng/mL)	3532.79 (559.81)	2543.42 (582.99)	1427.35 (401.81)	**<0.001**	0.221	**<0.001**	**0.034**
C5 (ng/mL)	428.65 (83.01)	238.94 (50.37)	225.44 (61.93)	**0.007**	**0.006**	0.062	1.000
C5a (ng/mL)	0.38 (0.07)	0.23 (0.04)	0.25 (0.07)	**0.005**	**0.004**	0.340	1.000
CFB (ng/mL)	247.03 (16.15)	243.74 (16.81)	195.22 (21.52)	0.044	1.000	0.086	0.051
CFD (ng/mL)	58.63 (6.48)	49.65 (5.59)	43.16 (6.64)	**0.022**	0.179	**0.018**	0.234
CFH (ng/mL)	358.54 (42.30)	247.83 (33.23)	149.03 (25.95)	**<0.001**	**0.004**	**<0.001**	**<0.001**
CFI (ng/mL)	543.89 (76.55)	466.88 (69.47)	318.35 (56.13)	**<0.001**	0.774	**0.005**	**0.001**
MBL (ng/mL)	0.51 (0.11)	0.29 (0.03)	0.28 (0.09)	0.125	0.129	0.254	1.000
VEGF-A (ng/mL)	0.35(0.09)	0.01 (0.01)	0.01 (0.00)	**0.001**	**0.001**	**0.001**	1.000
C3b/C3	10.89 (2.95)	6.60 (1.75)	4.65 (1.28)	0.055	0.453	0.133	0.196
C4b/C4	0.30 (0.10)	0.16 (0.02)	2.08 (1.81)	0.211	0.504	0.976	0.864
C5a/C5	0.0009 (0.00)	0.001 (0.00)	0.001 (0.00)	**<0.001**	**0.002**	0.176	1.000

Notes: Values are presented as mean (SE) deviation. Bold $p < 0.05$. p-values calculated by generalized estimating equations (GEE) and Bonferroni post-hoc tests.
Abbreviations: Month 1, after the first intravitreal injection; Month 2, after the second intravitreal injection. BL, baseline; RVO, retinal vein occlusion; CFB, complement factor B; CFD, complement factor D; CFH, complement factor H; CFI, complement factor I; MBL, mannose-binding lectin.

Table 3 Plasma Levels of Complement Proteins and VEGF-A at Baseline and During the Follow-up in RVO Patients

Variables (unit)	Baseline	Month 1	Month 2	P value	P value	P value	P value
	(n=26) Mean (SE)	(n=24) Mean (SE)	(n=21) Mean (SE)	BL vs 1 vs 2	BL vs 1	BL vs 2	1 vs 2
C1q (ng/mL)	156829.23 (5106.79)	153029.19 (5041.55)	155987.74 (7731.94)	0.813	1.000	1.000	1.000
C2 (ng/mL)	32964.30 (2761.64)	34286.65 (3661.85)	28801.93 (2110.18)	0.348	1.000	0.624	0.541
C4 (ng/mL)	1106533.85 (61,626.57)	1044124.09 (51,023.96)	1027239.57 (57,193.54)	0.090	0.108	0.498	1.000
C4b (ng/mL)	71540.85 (4220.02)	69836.89 (4063.01)	65172.06 (4405.07)	0.097	1.000	0.116	0.141
C3 (ng/mL)	1334007.69 (244,868.04)	1081854.72 (192,478.12)	1089710.25 (241,249.94)	0.069	0.075	1.000	1.000
C3b/iC3b (ng/mL)	114876.92 (20,969.00)	95390.83 (13,658.21)	88241.08 (16,656.00)	0.132	0.140	0.733	1.000
C5 (ng/mL)	32453.97 (2104.70)	30625.56 (1914.74)	28760.82 (12,760.67)	0.320	0.553	1.000	1.000
C5a (ng/mL)	9.47 (0.57)	9.72 (0.74)	8.40 (0.67)	0.071	1.000	0.123	0.122
CFB (ng/mL)	337263.08 (17,025.95)	313700.04 (12,520.43)	311340.33 (18,893.78)	0.191	0.249	0.684	1.000
CFD (ng/mL)	6250.47 (1357.78)	5207.06 (331.30)	4729.89 (279.97)	0.070	1.000	0.677	0.067
CFH (ng/mL)	516144.62 (19,741.69)	495898.28 (15,919.35)	507747.02 (30,130.75)	0.441	0.641	1.000	1.000
CFI (ng/mL)	91973.12 (6424.19)	88602.33 (6248.70)	83937.99 (6381.46)	0.103	0.935	0.101	0.516
MBL (ng/mL)	4594.85 (902.67)	3833.26 (617.71)	3873.43 (621.38)	0.221	0.338	0.255	1.000
VEGF-A (ng/mL)	0.05 (0.02)	0.04 (0.01)	0.07 (0.02)	0.397	1.000	1.000	0.602
C3b/C3	0.09 (0.00)	0.09 (0.00)	0.09 (0.00)	0.274	0.366	1.000	1.000
C4b/C4	0.07 (0.00)	0.07 (0.00)	0.06 (0.00)	0.361	0.829	1.000	0.785
C5a/C5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.113	0.205	1.000	0.383

Notes: Values are presented as mean (SE) deviation. p-values calculated by generalized estimating equations (GEE) and Bonferroni post-hoc tests.
Abbreviations: Month 1, after the first intravitreal injection; Month 2, after the second intravitreal injection. BL, baseline; RVO, retinal vein occlusion; CFB, complement factor B; CFD, complement factor D; CFH, complement factor H; CFI, complement factor I; MBL, mannose-binding lectin.

The Comparison of Complement Protein and VEGF-A Levels in AH of BRVO and CRVO Groups at Baseline and During Follow-up

We further analyzed the differences in complement protein levels between the BRVO and CRVO groups. At baseline, the levels of C3b/iC3b ($p < 0.001$), C5 ($p = 0.020$), and C5a ($p = 0.007$) were significantly lower in the BRVO group

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compared to the CRVO group. C4 ($p = 0.003$) and C4b ($p < 0.001$) showed significant statistical differences between the BRVO and CRVO groups at baseline. Several complement proteins that play a role in the AP, including CFD ($p = 0.002$), CFH ($p < 0.001$), and CFI ($p < 0.001$), were found to be at higher levels in the CRVO group than in the BRVO group at baseline. In contrast, the levels of C1q, C2, C3, CFB, MBL, and VEGF-A showed no significant differences between the two groups at baseline (Table 4).

There remained a statistically significant difference in C3b/iC3b ($p = 0.015$) between BRVO and CRVO groups at month 1. The levels of C1q ($p = 0.024$), C4 ($p = 0.001$) and C4b ($p < 0.001$) were significantly lower in the BRVO group compared to the CRVO group. Additionally, CFH ($p = 0.003$) and CFI ($p = 0.027$) were lower in the BRVO group than in the CRVO group. At month 2, only C4 ($p = 0.023$), C3b/iC3b ($p = 0.034$), and CFH ($p = 0.026$) showed differences between the BRVO and CRVO groups (Table 4).

Changes in the Mean CRT and the Mean logMAR of BCVA Before and After Intravitreal Injection of Ranibizumab for RVO Patients

The mean CRT at baseline for RVO patients was 520.78μm, which decreased by 197.34μm after the first month of treatment, and the average value was 290.24μm at month 2 (Supplement Figure 1). Following ranibizumab treatment, both the BRVO and CRVO groups exhibited a significant reduction in CRT. Compared to the baseline, the BCVA of RVO patients showed a notable improvement post-treatment. In the BRVO group, there was a marked enhancement in BCVA after treatment when compared to baseline (Supplement Figure 1). For the CRVO group, a significant statistical difference was observed between baseline and month 1 (Supplement Figure 1). However, no correlation was observed between the levels of complement proteins and the BCVA logMAR in AH (Supplementary Table S2).

Correlation Analysis Between Complement Proteins in AH at Baseline and Change in CRT After Three Intravitreal Ranibizumab Treatments in RVO Patients

After three intravitreal injections of ranibizumab in RVO patients, CRT was recorded in 10 patients. The correlation between AH cytokine levels at baseline and changes in CRT from baseline to after three treatments was analyzed. There was a strong positive correlation between the changes in CRT and baseline levels of C5a ($r = 0.651$, $p = 0.041$), CFB ($r = 0.764$, $p = 0.010$), CFH ($r = 0.672$, $p = 0.033$), and CFI ($r = 0.706$, $p = 0.023$) in AH (Figure 1). However, no correlation was found between the changes in CRT and the other cytokines.

Discussion

During the ranibizumab treatment, we observed a reduction not only in VEGF-A levels but also in several complement proteins within the AH. The study showed significant decreases in the levels of C3b/iC3b, C5, and C5a during treatment. Additionally, it was found that the levels of C1q, C2, C4, and C4b, which participate in the CP, as well as CFD, CFH, and CFI, which are involved in the AP, were reduced. However, no significant changes were observed in any complement factors in the plasma. At baseline, CRVO patients exhibited higher levels of C4, C4b, C3b/iC3b, C5, C5a, CFD, CFH, and CFI compared to BRVO patients in AH. At month 2, differences remained only in the levels of C4, C3b/iC3b, and CFI between BRVO and CRVO groups. In the 10 patients followed up after three injections, a significant positive correlation was found between the change in CRT and the levels of C5a, CFB, CFH, and CFI at baseline.

We observed a significant decrease in VEGF-A levels in AH from baseline to month 1, which is accordance with those of previous studies.[22,23] VEGF-A enhances vascular permeability and angiogenesis and plays a significant role in the pathophysiological processes associated with RVO, being highly correlated with the development of ME and the impairment of vision.[24] Ranibizumab rapidly reduces VEGF-A levels in AH of RVO patients and provides excellent long-term results in controlling ME.[25] C3, as a central element of the complement system, serves as the hub for all activation pathways, while C5 is a crucial protein in the terminal pathway of the complement system.[19] Our study indicated that C3b/iC3b, C5, and C5a have decreased compared to baseline. Interestingly, Nozaki et al found that C3a and C5a can induce retinal pigment epithelial cells to produce VEGF. In a laser-induced AMD model, the absence of C3a (C3aR) or C5a (C5aR1) receptors leads to a reduction in VEGF expression.[26] However, the level of C5a in AH was very

Table 4 Aqueous Humor Levels of Complement Proteins and VEGF-A at Baseline and During the Follow-up in BRVO and CRVO Patients

Variables (unit)	Baseline			Month 1			Month 2		
	BRVO (n=19)	CRVO (n=8)	P	BRVO (n=19)	CRVO (n=8)	P	BRVO (n=14)	CRVO (n=8)	P
C1q (ng/mL)	44.95 ± 39.82	106.75 ± 89.94	0.098	32.54 ± 26.14	63.97 ± 40.95	**0.024**	20.80 ± 19.33	44.92 ± 41.34	0.075
C2 (ng/mL)	214.62 ± 171.87	1067.33 ± 1131.72	0.071	136.11 ± 114.12	474.70 ± 519.64	0.109	111.05 ± 117.77	253.09 ± 245.85	0.080
C4 (ng/mL)	345.62 ± 168.21	573.22 ± 150.38	**0.003**	305.89 ± 126.09	502.31 ± 137.29	**0.001**	233.98 ± 144.56	401.10 ± 167.61	**0.023**
C4b (ng/mL)	63.36 ± 66.87	187.97 ± 78.95	**<0.001**	42.03 ± 39.37	139.87 ± 88.21	**<0.001**	51.21 ± 97.39	86.92 ± 85.76	0.399
C3 (ng/mL)	394.82 ± 125.53	389.05 ± 122.55	0.913	467.44 ± 174.19	485.10 ± 194.26	0.818	376.96 ± 201.80	427.62 ± 59.47	0.394
C3b/iC3b (ng/mL)	2242.05 ± 2121.06	6598.32 ± 2409.74	**<0.001**	1634.15 ± 2310.98	4702.93 ± 3753.56	**0.015**	755.05 ± 1160.64	3229.55 ± 2634.28	**0.034**
C5 (ng/mL)	251.04 ± 208.26	850.47 ± 563.07	**0.020**	166.34 ± 139.62	411.37 ± 407.15	0.137	183.22 ± 213.92	359.27 ± 419.47	0.204
C5a (ng/mL)	0.22 ± 0.18	0.74 ± 0.39	**0.007**	0.16 ± 0.13	0.38 ± 0.35	0.120	0.24 ± 0.33	0.32 ± 0.35	0.599
CFB (ng/mL)	228.81 ± 87.15	290.28 ± 67.84	0.088	229.82 ± 85.53	276.79 ± 94.02	0.217	171.79 ± 109.05	238.55 ± 89.57	0.158
CFD (ng/mL)	45.98 ± 30.69	88.69 ± 21.85	**0.002**	42.63 ± 27.47	66.32 ± 29.38	0.056	40.72 ± 33.93	59.38 ± 37.98	0.248
CFH (ng/mL)	268.48 ± 162.89	572.44 ± 209.31	**<0.001**	185.80 ± 133.32	395.14 ± 184.58	**0.003**	106.76 ± 90.33	262.74 ± 154.58	**0.026**
CFI (ng/mL)	360.59 ± 265.46	979.20 ± 348.24	**<0.001**	367.25 ± 342.11	703.49 ± 332.84	**0.027**	279.57 ± 281.31	496.70 ± 316.30	0.111
MBL (ng/mL)	0.47 ± 0.68	0.60 ± 0.30	0.589	0.26 ± 0.17	0.37 ± 0.16	0.124	0.31 ± 0.52	0.26 ± 0.12	0.782
VEGF-A (ng/mL)	252.96 ± 273.72	564.95 ± 761.22	0.124	7.61 ± 12.79	24.56 ± 49.48	0.369	10.93 ± 23.72	13.36 ± 24.26	0.821

Notes: Values are given as mean ± SD. Bold $p < 0.05$. p-values calculated by independent-sample *t*-test.

Abbreviations: Month 1, after the first intravitreal injection; Month 2, after the second intravitreal injection. BL, baseline; RVO, retinal vein occlusion; BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; CFB, complement factor B; CFD, complement factor D; CFH, complement factor H; CFI, complement factor I; MBL, mannose-binding lectin.

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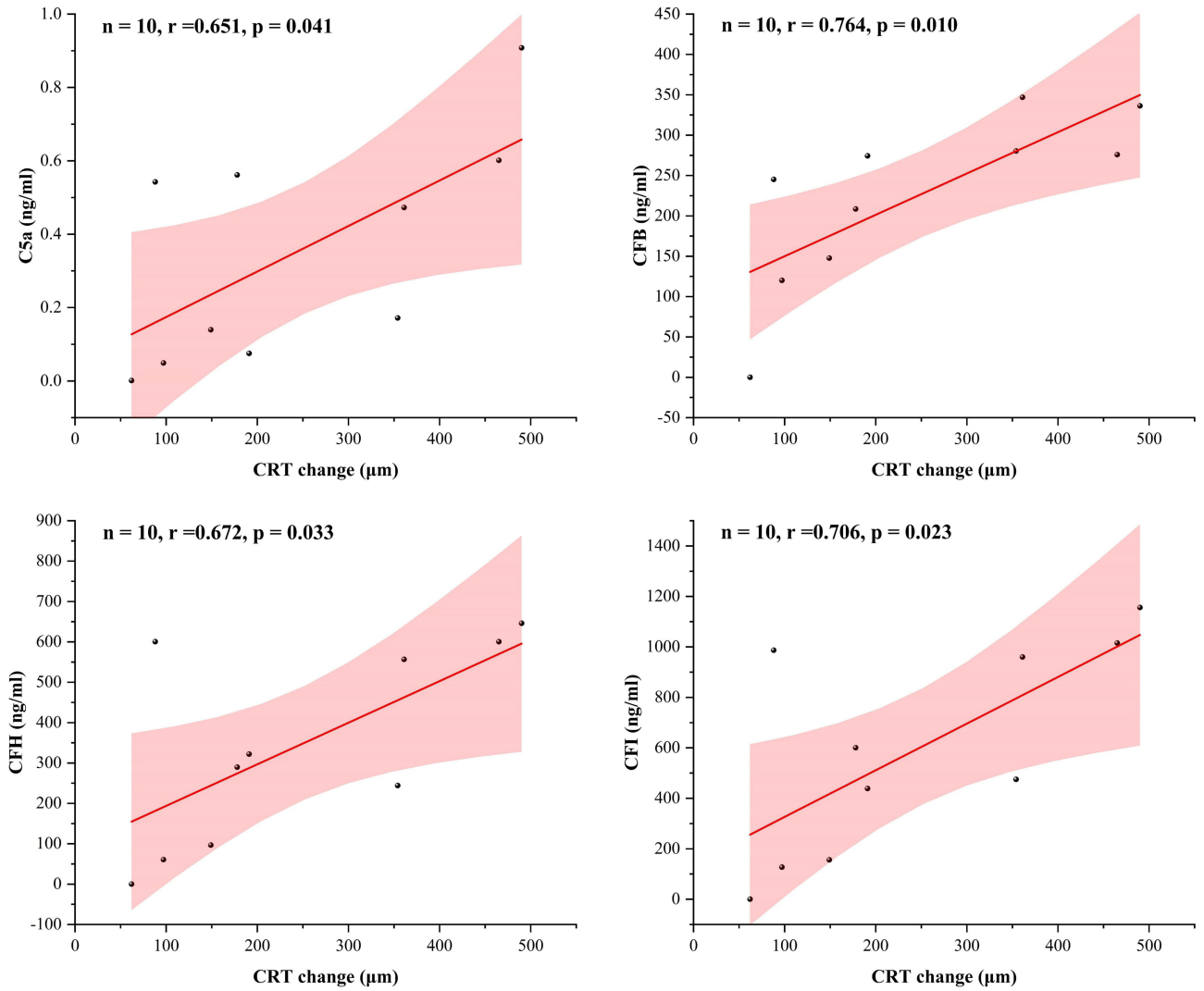

Figure 1 The correlation between baseline cytokine levels in the aqueous humor and the changes in CRT after three IVR treatments. The pink area represents the 95% confidence interval. n represents the sample size. Pearson's correlation analysis was used.

low. Although the ratio of C5a/C5 showed statistical differences between baseline and month 1, the clinical significance of this finding should be interpreted with caution.

Our results suggested that C1q, C2, C4, and C4b, which are involved in the CP, significantly decreased after treatment. C1q is the first component of the CP in the complement cascade. A study indicated that C1q might promote vascular remodeling after a stroke through the LAIR1-HIF1α-VEGF pathway, suggesting that C1q has a potential role in angiogenesis and may function by influencing the expression and secretion of VEGF.[27] However, Keir et al reported that C3a was elevated in AH 48h after intravitreal bevacizumab injections in 10 nAMD patients.[28] Another study measuring C3a and C4a in AH during aflibercept injections for CNV showed a significant increase after one month.[29] This discrepancy may be attributed to differences in the pathogenesis of RVO compared to AMD and CNV, as well as variations in the anti-VEGF drugs employed. CFD, CFH, and CFI play critical regulatory roles in the AP, and this study also showed that the levels of these complement proteins decrease during ranibizumab treatment.

The systemic elimination half-life of ranibizumab is estimated to be approximately 2 hours.[30] The intravitreal injection of ranibizumab was eliminated in the vitreous before reaching the systemic circulation, resulting in minimal systemic exposure. Therefore, we observed no changes in complement proteins during the treatment in the plasma, as well as in the ratios of C3b/C3, C4b/C4 and C5a/C5.

Retinal circulation obstruction can lead to RVO from the nonischemic type into the more severe ischemic type. Anti-VEGF treatment is more effective for nonischemic RVO than for ischemic RVO. Therefore, early and adequate anti-VEGF can improve the vision of RVO patients.[31,32] However, we only found that the concentrations of C4 in plasma correlated with RVO duration. Future studies with larger sample sizes will be needed to explore the relationship between disease duration and complement factors.

In this study, the CRT in CRVO patients was higher than in BRVO patients at baseline, suggesting more severe macular oedema in CRVO patients in general. Compared to BRVO, CRVO patients exhibit a more extensive area of embolism as well as more severe retinal ischemia and hypoxia.[33] Another interesting finding in our study was that complement proteins involved in the CP and AP show differences in BRVO and CRVO subgroup analyses, such as C4, C4b, C3b/iC3b, C5, C5a, CFD, CFH, and CFI. Therefore, we speculated that the CP and AP are not only related to the occurrence of RVO but also affect the severity of RVO. After ranibizumab treatment, some complement factors in the CRVO group remained higher than in the BRVO group, which may indicate that CRVO patients need more treatment. However, the small number of patients we included may limit further interpretation. In a study on AMD patients, no significant correlation was found between the increase in C3a levels after anti-VEGF treatment and changes in logMAR.[29] Similarly, We did not find a correlation between complement levels and the BCVA logMAR changes. These undesirable responses may not reflect clinical outcomes since visual acuity data were obtained before each treatment session.

Furthermore, our study revealed a significant positive correlation between baseline levels of C5a, CFB, CFH, and CFI, and changes in CRT. C5a is an anaphylatoxin that promotes inflammation by attracting immune cells to sites of injury or infection. CFB is a component of the C3 convertase, which is crucial for the activation of the AP, leading to the formation of C3b and initiation of the membrane attack complex. CFH is a key inhibitor of the AP, while CFI works in conjunction with CFH.[19] The study indicated that these complement proteins as potential biomarkers for predicting the response to anti-VEGF treatment in RVO patients. Furthermore, the association between complement factor levels and the resolution of macular edema highlights the intricate interplay between the complement system and vascular permeability in the pathophysiology of RVO.

This study has several limitations. Firstly, the sample size was relatively small, with only eight CRVO patients in the subgroup analysis. Secondly, while previous studies have indicated significant differences in complement cytokine concentrations between RVO patients and the control group, our study focused on the changes in complement levels during ranibizumab treatment and did not include a control group without RVO, which may limit further interpretation.

Conclusion

In conclusion, our findings demonstrate that ranibizumab treatment reduced the intraocular but not circulating activation of classical and alternative complement pathways in RVO patients. Differences in intraocular complement proteins were observed between BRVO and CRVO patients, which may reflect different pathogenesis. These findings may have some significance for ophthalmologists in predicting the outcomes of ranibizumab treatment for RVO patients. Additionally, it may be possible to discover targets for personalized therapy for RVO.

Ethics

Consent was obtained directly from patient(s), and the study was approved by the Institutional Review Board (IRB) at the Changsha Aier Eye Hospital [Ethical approval number: (2024) KYPJ009].

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Scott IU, Campochiaro PA, Newman NJ, Biousse V. Retinal vascular occlusions. *The Lancet*. 2020;396(10266):1927–1940. doi:10.1016/S0140-6736(20)31559-2
2. Nicholson L, Talks SJ, Amoaku W, Talks K, Sivaprasad S. Retinal vein occlusion (RVO) guideline: executive summary. *Eye*. 2022;36(5):909–912. doi:10.1038/s41433-022-02007-4
3. Hayreh SS. Photocoagulation for retinal vein occlusion. *Progress in Retinal and Eye Research*. 2021;85:100964. doi:10.1016/j.preteyeres.2021.100964
4. Campa C, Alivernini G, Bolletta E, Parodi MB, Perri P. Anti-VEGF therapy for retinal vein occlusions. *Curr Drug Targets*. 2016;17(3):328–336. doi:10.2174/1573399811666150615151324
5. Ferrara N, Damico L, Shams N, Lowman H, Kim R. Development of ranibizumab, an anti–vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. *Retina*. 2006;26(8):859. doi:10.1097/01.iae.0000242842.14624.e7
6. Hogg HJ, Di Simplicio S, Pearce MS. Ranibizumab and aflibercept intravitreal injection for treatment naïve and refractory macular oedema in branch retinal vein occlusion. *European Journal of Ophthalmology*. 2021;31(2):548–555. doi:10.1177/1120672120904669
7. Akhtar-Schäfer I, Wang L, Krohne TU, Xu H, Langmann T. Modulation of three key innate immune pathways for the most common retinal degenerative diseases. *EMBO Molecular Medicine*. 2018;10(10):e8259. doi:10.15252/emmm.201708259
8. Pouw RB, Ricklin D. Tipping the balance: intricate roles of the complement system in disease and therapy. *Semin Immunopathol*. 2021;43(6):757–771. doi:10.1007/s00281-021-00892-7
9. Reis ES, Mastellos DC, Hajishengallis G, Lambris JD. New Insights into the Immune Functions of Complement. *Nat Rev Immunol*. 2019;19(8):503–516. doi:10.1038/s41577-019-0168-x
10. Mastellos DC, Ricklin D, Lambris JD. Clinical promise of next-generation complement therapeutics. *Nat Rev Drug Discov*. 2019;18(9):707–729. doi:10.1038/s41573-019-0031-6
11. Clark SJ, Bishop PN. The eye as a complement dysregulation hotspot. *Semin Immunopathol*. 2018;40(1):65–74. doi:10.1007/s00281-017-0649-6
12. Xu H, Chen M. Targeting the complement system for the management of retinal inflammatory and degenerative diseases. *Eur J Pharmacol*. 2016;787:94–104. doi:10.1016/j.ejphar.2016.03.001
13. Armento A, Ueffing M, Clark SJ. The complement system in age-related macular degeneration. *Cell mol Life Sci*. 2021;78(10):4487–4505. doi:10.1007/s00018-021-03796-9
14. Reich M, Dacheva I, Nobl M, et al. Proteomic analysis of vitreous humor in retinal vein occlusion. *PLoS One*. 2016;11(6):e0158001. doi:10.1371/journal.pone.0158001
15. Cehofski LJ, Kojima K, Terao N, et al. Aqueous fibronectin correlates with severity of macular edema and visual acuity in patients with branch retinal vein occlusion: a proteome study. *Invest Ophthalmol Vis Sci*. 2020;61(14):6. doi:10.1167/iovs.61.14.6
16. Dacheva I, Reich M, Nobl M, et al. Proteome analysis of undiluted vitreous humor in patients with branch retinal vein occlusion. *Ophthalmologe*. 2018;115(3):203–215. doi:10.1007/s00347-017-0469-z
17. Cehofski LJ, Kojima K, Kusada N, et al. Macular edema in central retinal vein occlusion correlates with aqueous fibrinogen alpha chain. *Invest Ophthalmol Vis Sci*. 2023;64(2):23. doi:10.1167/iovs.64.2.23
18. Liu H, Zhou Y, Qi J, et al. Intraocular complement activation is independent of systemic complement activation and is related to macular vascular remodelling in retinal vein occlusion. Biochemistry. 2023. doi:10.21203/rs.3.rs-3239512/v1
19. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement system part i – molecular mechanisms of activation and regulation. *Front Immunol*. 2015;6. doi:10.3389/fimmu.2015.00262
20. Kim BJ, Mastellos DC, Li Y, Dunaief JL, Lambris JD. Targeting complement components C3 and C5 for the retina: key concepts and lingering questions. *Prog Retin Eye Res*. 2021;83:100936. doi:10.1016/j.preteyeres.2020.100936
21. Lesavre PH, Müller-Eberhard HJ. Mechanism of action of factor D of the alternative complement pathway. *J Exp Med*. 1978;148(6):1498–1509. doi:10.1084/jem.148.6.1498
22. Yong H, Qi H, Yan H, Wu Q, Zuo L. The correlation between cytokine levels in the aqueous humor and the prognostic value of anti-vascular endothelial growth factor therapy for treating macular edema resulting from retinal vein occlusion. *Graefes Arch Clin Exp Ophthalmol*. 2021;259(11):3243–3250. doi:10.1007/s00417-021-05211-2

23. Cui W, Sun XY, Sun LP, Li J, Liu ZL, Zhang H. Comparison of the effect of intravitreal conbercept and ranibizumab on aqueous humor cytokines in central retinal vein occlusion-related macular edema. *Journal of Ocular Pharmacology and Therapeutics*. 2021;37(1):52–59. doi:10.1089/jop.2020.0035
24. Apte RS, Chen DS, Ferrara N. VEGF in signaling and disease: beyond discovery and development. *Cell*. 2019;176(6):1248–1264. doi:10.1016/j.cell.2019.01.021
25. Campochiaro PA, Sophie R, Pearlman J, et al. Long-term outcomes in patients with retinal vein occlusion treated with ranibizumab: the RETAIN study - ScienceDirect. *Ophthalmology*. 2014;121(1):209–219. doi:10.1016/j.ophtha.2013.08.038
26. Nozaki M, Raisler BJ, Sakurai E, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proceedings of the National Academy of Sciences*. 2006;103(7):2328–2333. doi:10.1073/pnas.0408835103
27. Fan G, Li Q, Qian J. C1q contributes to post-stroke angiogenesis via LAIR1-HIF1α-VEGF pathway. *FBL*. 2019;24(6):1050–1059. doi:10.2741/4767
28. Keir LS, Firth R, Aponik L, et al. VEGF regulates local inhibitory complement proteins in the eye and kidney. *J Clin Invest*. 2017;127(1):199–214. doi:10.1172/JCI86418
29. Tanaka K, Oguchi Y, Omori T, et al. Changes in complement activation products after anti-VEGF injection for choroidal neovascularization in age-related macular degeneration and pachychoroid disease. *Sci Rep*. 2021;11(1):8464. doi:10.1038/s41598-021-87340-6
30. Zhang Y, Yao Z, Kaila N, et al. Pharmacokinetics of ranibizumab after intravitreal administration in patients with retinal vein occlusion or diabetic macular edema. *Ophthalmology*. 2014;121(11):2237–2246. doi:10.1016/j.ophtha.2014.05.012
31. Noma H, Yasuda K, Shimura M. Cytokines and pathogenesis of central retinal vein occlusion. *Journal of Clinical Medicine*. 2020;9(11):3457. doi:10.3390/jcm9113457
32. Noma H, Yasuda K, Shimura M. Cytokines and the pathogenesis of macular edema in branch retinal vein occlusion. *Journal of Ophthalmology*. 2019;2019(1):5185128. doi:10.1155/2019/5185128
33. Sivaprasad S, Amoaku WM, Hykin P. The Royal College of Ophthalmologists Guidelines on retinal vein occlusions: executive summary. *Eye*. 2015;29(12):1633–1638. doi:10.1038/eye.2015.164