• Clinical Research •

Increased serum levels of soluble CD146 and vascular endothelial growth factor receptor 2 in patients with exudative age-related macular degeneration

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Abstract

• AIM: To investigate serum levels of soluble CD146 (sCD146) and vascular endothelial growth factor receptor 2 (VEGFR2) in patients with age-related macular degeneration (AMD).

• METHODS: Eighty-eight patients with exudative AMD and 45 sex- and age-matched healthy controls were enrolled in this study conducted in China. Serum samples was obtained from the patients with exudative AMD and from the controls. Serum sCD146 and VEGFR2 protein levels were measured using an enzyme-linked immunosorbent assay.

• RESULTS: We found that serum sCD146 and VEGFR2 protein levels were significantly higher in the patients with exudative AMD group than in the controls (t=3.859, P<0.001 and t=3.829, P<0.001, respectively). Serum sCD146 levels were significantly higher in patients with classic choroidal neovascularization (CNV) than in those with occult CNV (t=9.899, P<0.001). There was a significant difference in the trend for exudative AMD in the highest versus lowest quartile of circulating sCD146 levels (χ^2=10.29, P=0.001). The receiver operating characteristic curve analysis showed that the area under the curve was 0.696 for sCD146 (95%CI: 0.601-0.791) with an optimum diagnostic cut-off value of 157.16 ng/mL, a sensitivity of 55.7%, and a specificity of 82.2%.

• CONCLUSION: The serum sCD146 level increases and may be a biomarker for exudative AMD.

• KEYWORDS: age-related macular degeneration; soluble CD146; vascular endothelial growth factor receptor 2; serum

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INTRODUCTION

Age-related macular degeneration (AMD) is a chronic progressive disease that causes irreversible loss of central vision in older populations[1]. There are two types of AMD, *i.e.* atrophic and exudative. The clinical features of atrophic AMD are the presence of drusen and geographic atrophy of the retina, while those of exudative AMD are invasion of abnormal choroidal membranes by newly formed blood vessels with leaking of fluid under the retina. Exudative AMD accounts for more than 90% of severe vision loss in patients with the disease[2]. There are many risk factors for AMD, including age, heredity, and smoking. However, the pathogenesis of exudative AMD is poorly understood, and there is an urgent need to find novel biomarkers for more precise prediction of the prognosis of the disease.

CD146 is a cell adhesion molecule that plays an important role in the integrity of vessels and angiogenesis and is a potential endothelial biomarker[3]. Soluble CD146 (sCD146) is generated by loss of surface CD146 and reflects an impaired endothelial monolayer[4-5]. Evaluation of circulating sCD146 levels is helpful in studies of diseases associated with impairment of vascular endothelial cells, such as heart failure, carotid atherosclerosis, and systemic sclerosis[6-8]. Recent in-depth preclinical and clinical research on the pathogenesis of exudative AMD has found that VEGFR2 is strongly linked with progression of the disease. VEGFR2 is an immunoglobulin-like structure that has been used in anti-VEGF agents, such as aflibercept and conbercept, to treat patients with exudative AMD[9-11]. CD146 is a coreceptor for VEGFR2 and is involved in nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase downstream signaling and participates in VEGF-induced signal transduction and formation of the microvasculature[12].

A recent study found that sCD146 enhanced secretion of VEGFR2 and VEGF from endogenous endothelial cells and that sCD146 and VEGFR2 participated synergistically in the recruitment, proliferation, and formation of vascular-like structures in endothelial progenitor cells[13]. However, there has no research on sCD146 and VEGFR2 in the various subtypes of exudative AMD. In this study, we explored the relationships between serum sCD146 and VEGFR2 levels and the various clinical manifestations of exudative AMD, such as severity of exudative AMD and subtype of choroidal neovascularization (CNV), to determine the significance of serum sCD146 level as a sensitive biomarker for this disease.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. Signed consent forms were obtained from all subjects and all of the experiments performed followed the guidelines of the Declaration of Helsinki.

Patients and Samples This study involved 88 Chinese patients with exudative AMD and 45 healthy age- and sex-matched controls were enrolled in the study. All subjects were recruited from the First Affiliated Hospital of Chongqing Medical University from June 2015 to April 2018. All study participants underwent a detailed ophthalmic examination, including slit-lamp biomicroscopy, dilated binocular ophthalmoscopy, fundus photography, optical coherence tomography, indocyanine green angiography, and fundus fluorescein angiography. The patients were classified as having occult or classic CNV using the Wisconsin Age-related Maculopathy Grading system[14]. These two subtypes of CNV were distinguished based on their appearance on fundus fluorescein and indocyanine green angiography[15]. Patients with fundus disease, such as polypoidal choroidal vasculopathy, diabetic retinopathy, or retinal inflammatory disease, were excluded. Data were collected on patient sex and age, smoking history, history of diabetes, hypertension, and coronary heart disease, and whether the exudative AMD was unilateral or bilateral. Circulating triglyceride, total cholesterol, high-density and low-density lipoprotein cholesterol, and apolipoprotein A1 and B levels were also recorded.

Enzyme-linked Immunosorbent Assay Peripheral venous blood samples were obtained from the antecubital vein and stored in vacuum blood collection tubes. All samples were centrifuged at 2000× g for 10min within 4h of collection and then separated and frozen at -80℃ until assayed in a standardized manner. The serum sCD146 and VEGFR2 levels were measured using commercial enzyme-linked immunosorbent assay kits following the manufacturer's protocol (CY-QUANT ELISA sCD146; Biocytex, Marseille, France; R&D Systems, Minneapolis, MN, USA).

Table 1 Demographics and clinical characteristics in the study groups at baseline *n* (%)

Variable	Healthy controls (*n*=45)	Patients with exudative AMD (*n*=88)
Sex		
Female	20 (44.4)	35 (39.8)
Male	25 (55.6)	53 (60.2)
Age, mean±SD (y)	73.62±8.29	72.45±9.93
Smoking status		
Never	29 (64.5)	52 (59.1)
Former	6 (13.3)	8 (9.1)
Current	10 (22.2)	28 (31.8)
Systemic disease		
Diabetes	7 (15.6)	17 (19.3)
Hypertension	14 (31.1)	33 (37.5)
Cardiovascular disease	2 (4.4)	7 (7.9)
Lipid profile (mmol/L), mean±SD		
Triglycerides	5.24±2.20	4.91±1.73
Total cholesterol	1.86±1.60	1.75±1.28
High-density lipoprotein	1.49±0.44	1.49±0.37
Low-density lipoprotein	2.81±1.11	2.84±1.00
Apolipoprotein A1	1.47±0.29	1.47±0.28
Apolipoprotein B	1.11±0.42	1.04±0.36
Subtype of CNV, *n*		
Classic		66
Occult		22
Laterality of exudative AMD, *n*		
Unilateral		44
Bilateral		44

There was no significant difference in sex, age, smoking status, or presence of systemic disease between the study groups (all $P>0.05$, Student's *t*-test). AMD: Age-related macular degeneration; CNV: Choroidal neovascularization.

Statistical Analysis Data are shown as the mean±standard deviation (SD) and as the interquartile range. Student's *t*-test was used for the independent samples. Trends of serum sCD146 levels in exudative AMD samples were analyzed by the Chi-square test. Receiver-operating characteristic (ROC) curve analysis was used to determine the ability of the serum sCD146 level to discriminate cases and controls. Specificity and sensitivity were evaluated by the area under the curve (AUC). Statistical analyses were performed using SPSS version 21 software (IBM Corp., Armonk, NY, USA). A *P*-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics The demographics and clinical characteristics of the exudative AMD group (*n*=88) and control group (*n*=45) at baseline are shown in Table 1. Patients in the exudative AMD group were categorized according to whether or not they had classic CNV (*n*=66) or occult CNV (*n*=22) and

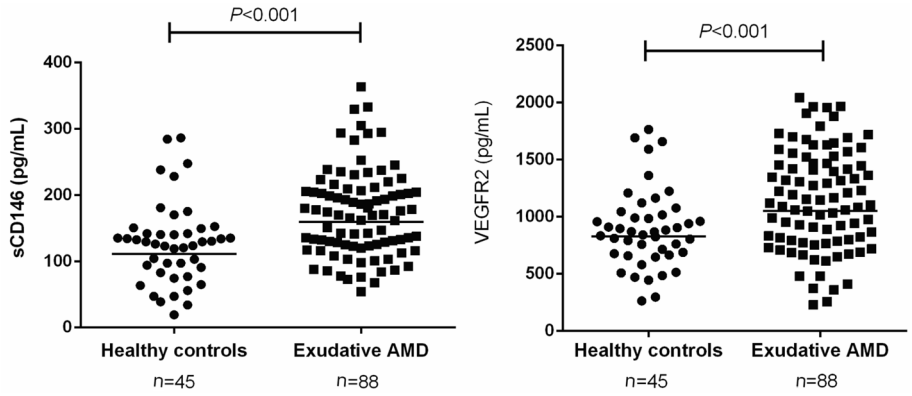

Figure 1 Serum sCD146 and VEGFR2 levels in the exudative AMD and the control groups Each square or spot represents the value for an individual subject. The bars represent the mean values.

Table 2 Distribution of sCD146 levels in the exudative AMD and control groups

Group	*n*	sCD146 level (ng/mL)			Test for normal distribution	
		Mean±SD	Min	Max	*Z*	*P*
Control	45	125.52±60.99	54.10	363.40	0.882	0.418
Exudative AMD						
Total	88	171.88±65.67	19.18	286.40	1.049	0.221
Unilateral CNV	44	165.69±68.43	67.74	333.20	0.967	0.307
Bilateral CNV	44	178.06±62.96	54.10	363.40	0.633	0.818
Occult CNV	22	103.09±27.53	54.10	161.66	0.559	0.914
Classic CNV	66	194.81±58.24	100.60	363.40	0.825	0.503

P>0.05 indicates a normal distribution (one-sample Kolmogorov-Smirnov test). AMD: Age-related macular degeneration; CNV: Choroidal neovascularization; sCD146: Soluble CD146; SD: Standard deviation.

Table 3 Distribution of VEGFR2 levels in the exudative AMD group and the control group

Group	*n*	VEGFR2 level (pg/mL)			Test for normal distribution	
		Mean±SD	Min	Max	*Z*	*P*
Control	45	892.64±342.86	264.00	1765.00	0.810	0.528
Exudative AMD						
Total	88	1155.34±429.39	257.20	2041.70	0.652	0.789
Unilateral CNV	44	1107.46±397.15	229.90	1963.90	0.406	0.996
Bilateral CNV	44	1191.85±476.77	257.20	2041.70	0.654	0.786
Occult CNV	22	1199.20±467.15	229.90	1967.00	0.389	0.998
Classic CNV	66	1133.14±430.71	257.20	2041.70	0.669	0.762

P>0.05 indicates a normal distribution (one-sample Kolmogorov-Smirnov test). AMD: Age-related macular degeneration; CNV: Choroidal neovascularization; sCD146: Soluble CD146; SD: Standard deviation; VEGFR2: Vascular endothelial growth factor receptor 2.

whether or not the exudative AMD was unilateral (*n*=44) or bilateral (*n*=44).

Serum sCD146 and VEGFR2 Levels in Patients with Exudative AMD *vs* Controls The distributions of serum sCD146 and VEGFR2 levels in the exudative AMD group (including for laterality and CNV subtype) and the control group were confirmed to be normal using the one-sample Kolmogorov-Smirnov test and compared using the Student's *t*-test (Tables 2 and 3). The serum levels of sCD146 and VEGFR2 were significantly higher in the exudative AMD group than in the control group (*t*=3.859, *P*<0.001 and *t*=3.829, *P*<0.001, respectively; Figure 1).

Relationship Between Serum sCD146 and VEGFR2 Levels and Clinical Manifestations and Type of CNV We then examined the relationship between serum sCD146 and VEGFR2 levels and the progressive nature of exudative AMD according to laterality and subtype of CNV (Figure 2). The distributions of sCD146 and VEGFR2 levels in the subgroups were confirmed to be normal using the one-sample Kolmogorov-Smirnov test and compared using the Student's *t*-test (Tables 2 and 3). There were no significant differences in the mean sCD146 and VEGFR2 level according to whether or not the exudative AMD was unilateral or bilateral (*t*=0.882, *P*=0.380 and *t*=-0.902, *P*=0.370, respectively). However, the

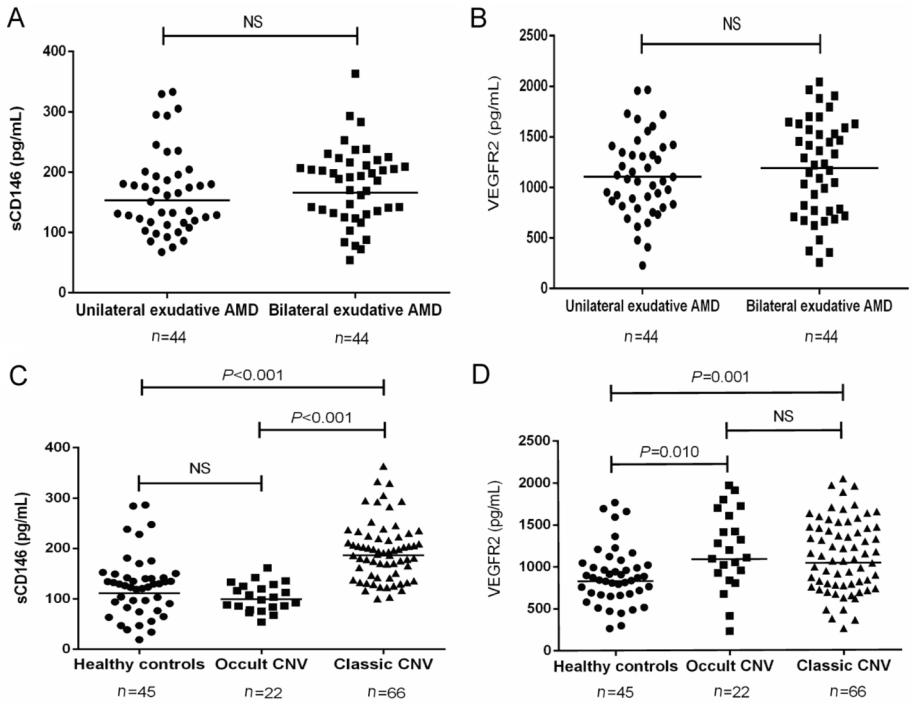

Figure 2 Relationship between serum sCD146 and VEGFR2 levels and the severity of exudative AMD A: Comparison of the mean sCD146 level between patients with bilateral and unilateral involvement; B: Comparison of VEGFR2 levels between patients with unilateral and bilateral exudative AMD; C: Compared the serum concentrations of sCD146 in patients with classic CNV or occult CNV and healthy controls; D: Compared the serum concentrations of VEGFR2 in patients with classic CNV or occult CNV and healthy controls. Each square, spot or triangle represents one participant. Bars represent the mean.

sCD146 level was significantly higher in the classic CNV subgroup than in the occult CNV subgroup (t=9.899, P<0.001). The mean serum sCD146 level was found to be significantly higher in the subgroup with classic CNV than in the control group (t=5.950, P<0.001) but not in the subgroup with occult CNV (t=-2.165, P=0.054). There was no significant difference in the mean VEGFR2 level between the occult and classic CNV subgroups (t=0.610, P=0.543). However, the mean VEGFR2 levels were significantly higher in the classic and occult CNV subgroups than in the control group (t=3.266, P=0.001 and t=2.739, P=0.010, respectively).

Relationship Between Serum sCD146 Protein Level and High-Risk of Exudative AMD The serum sCD146 levels in subjects in both study groups were divided into quartiles and assessed for their potential clinical relevance. There were significant between-group differences in the proportions of subjects in the highest quartile (Q4) versus lowest quartile (Q1) and in the second highest quartile (Q3) versus Q1 (χ^2=10.29, P=0.001 and χ^2=5.22, P=0.022, respectively, Chi-square test; Table 4).

Feasibility of Using Serum sCD146 Level as a Biomarker of Exudative AMD ROC curve analysis was used to test the ability of the serum sCD146 level to predict exudative AMD. The ROC curve was created by plotting the true-positive rate against the false-positive rate at different threshold settings. The AUC represents the probability of a randomly selected patient having AMD when compared with a randomly selected control subject. Youden index (J) was used to confirm the optimal cut-off value for sCD146. As shown in Figure 3, the ROC curve for sCD146 revealed a strong distribution for an AUC of 0.696 (95%CI 0.601-0.791). The optimum diagnostic cut-off value for sCD146 was 157.16 ng/mL. with a sensitivity and specificity of 55.7% and 88.2%, respectively.

Table 4 Classification of subjects in the study groups according to quartile for serum sCD146 level *n* (%)

sCD146 quartile	Control group[a]	Exudative AMD group[a]
Q1	17 (37.78)	16 (18.18)
Q2	15 (33.33)	18 (20.45)
Q3	8 (17.78)	25 (28.41)
Q4	5 (11.11)	29 (32.96)
Total, *n*	45	88

Q1: <112.7 ng/mL; Q2: 112.8-140.44 ng/mL; Q3: 140.45-198.32 ng/mL; Q4: >198.33 ng/mL. [a]Percentage of total number of subjects in quartile.

DISCUSSION

AMD is a progressive disease and a leading cause of irreversible blindness in the aging population. In recent years, anti-VEGF therapy has become the first-line treatment for exudative AMD. However, this treatment method cannot maintain sufficiently high drug concentrations for an adequate period of time, so requires frequent intraocular injections[16-17]. A number of studies have investigated anti-VEGF treatment and attempted to predict disease outcomes but their results

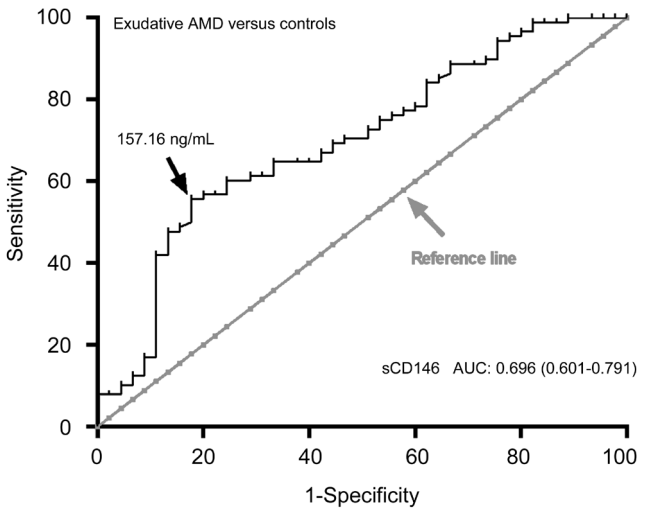

Figure 3 Serum concentrations of sCD146 as a biomarker for exudative AMD The ROC curve was formed by the information of 44 healthy controls and 88 AMD patients. The AUC for sCD146 was 0.696 (95%CI 0.601-0.791). The optimal cut-off value for sCD146 was 157.16 ng/mL.

have not been satisfactory. Nowadays, in the clinical setting, a low-cost, highly sensitive and specific blood biomarker that can be measured in a minimally invasive way would the first choice for detection of exudative AMD. However, no biomarker that detects or characterizes the development of exudative AMD alone has been found. In this study, circulating levels of sCD146 and VEGFR2 were found to be increased in patients with exudative AMD. We found a clear relationship between an increased serum sCD146 level in particular and a greater likelihood of exudative AMD, suggesting that serum sCD146 is a candidate biomarker for the disease.

CD146 plays a crucial role in angiogenesis in the retina. One research group reported that downregulation of CD146 expression in the vitreous body attenuated abnormal neovascularization in a murine model of oxygen-induced retinopathy[18]. Furthermore, growth of chorioretinal angiogenesis was reported to be significantly decreased by intraocular injection of anti-CD146 antibody in rats[19]. To the best of our knowledge, ours is the first report to demonstrate an association between circulating levels of sCD146 in patients and the progression and severity of exudative AMD. Several studies have demonstrated altered serum sCD146 protein levels in heart failure, carotid atherosclerosis, and systemic sclerosis, all of which are diseases in which VEGF/NF-κB signaling is upregulated[6-8]. Moreover, recent research indicates that sCD146 increases expression of VEGFR2 and secretion of VEGF, which in turn increases migration, proliferation, and angiogenic activity of endothelial progenitor cells[13]. CD146, together with VEGFR2 as a co-receptor for the VEGF ligand on endothelial cells, can augment the VEGF/NF-κB signaling pathway and promote formation of microvessels[12]. We have demonstrated that circulating levels of both sCD146 and VEGFR2 are higher in patients with exudative AMD than in controls, which suggests that these factors are involved in exudative AMD. Therefore, we hypothesize that sCD146 and VEGFR2 are involved in the pathophysiology of exudative AMD by activating the VEGF/NF-κB signaling pathways.

The NF-κB signal pathway is involved in many pathologic processes and is known to be upregulated in a number of diseases, including cancer-related angiogenesis and several inflammatory and neurodegenerative diseases[20-24]. It has been reported that hypoxic stress-induced changes can upregulate NF-κB signaling, which increases the secretion of inflammatory cytokines, complement factors, and metalloproteinases[25]. Furthermore, activation of the NF-κB signaling pathway can increase expression of VEGF in human retinal pigment epithelial cells and multiple growth factors and inflammation-associated cytokines can exacerbate exudative AMD[26-27]. CD146 plays an important role in regulation of NF-κB signaling, and CD146 knockout has been confirmed to reverse VEGF-induced microvascularization in endothelial cells[28]. Moreover, animal experiments have demonstrated that the anti-CD146 antibody, knockout of CD146, and anti-VEGF treatment have additive inhibitory effects on the NF-κB signaling pathway associated with angiogenesis in cancer[29-30].

Several other potential biomarkers of exudative AMD have been identified, including interferon-gamma-inducible protein 10[31], eotaxin[32], soluble FMS-like tyrosine kinase[33], cholesterol[34], C-reactive protein[35], and VEGF[36], which have helped to shed light on the pathogenesis and risk of AMD, but none alone can accurately predict the outcome of exudative AMD[37]. We identified a strong association between circulating sCD146 levels and exudative AMD, especially in patients with classic CNV. We also tested our hypothesis using ROC curve analysis and determined from the AUC that sCD146 had a 69.6% probability of correctly distinguishing between patients with exudative AMD and controls with a sensitivity of 55.7% and specificity of 82.2% at a cut-off value of 157.16 ng/mL. Therefore, sCD146 holds promise as a serum biomarker that could be added to the biomarkers already identified to increase the efficiency of diagnosis of exudative AMD.

A strength of our study is that we grouped the patients according to whether they had unilateral or bilateral exudative AMD and according to whether they had occult or classic CNV and then measured the circulating sCD146 protein levels in the different subtypes. However, the study also has several limitations. Firstly, we measured the circulating levels of sCD146 and VEGFR2 but did not measure these levels in the ocular tissues, such as vitreous fluid and the choroidal neovascular membrane. Secondly, we limited our exclusion criteria to diseases of the fundus and did not exclude patients

with systemic diseases, such as diabetes, hypertension, and coronary heart disease. Not excluding these patients may have influenced the serum sCD146 and VEGFR2 measurements obtained.

In conclusion, we have investigated circulating sCD146 and VEGFR2 levels in patients with exudative AMD for the first time. Our findings demonstrate a relationship between increased sCD146 and VEGFR2 levels and the presence and progression of exudative AMD. Increased circulating levels of sCD146 in particular may contribute to activation of the VEGF/NF-κB pathway, which is known to participate in the angiogenesis and inflammation that occurs in exudative AMD. Our study may have clinical significance in terms of not only improving our understanding of the pathogenesis of exudative AMD but also providing a basis for development of a novel intervention targeting this potential biomarker of the disease.

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