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The reduction of serum soluble Flt-1 in patients with neovascular age-related macular degeneration

Hironori Uehara, PhD¹, Christina Mamalis, BS¹, Molly McFadden, PhD², Michael Taggart, BS¹, Brian Stagg, MD¹, Samuel Passi, BS¹, Phillip Earle, PhD³, Usha Chakravarthy, MD, PhD³, Ruth E. Hogg, PhD³, and Balamurali K. Ambati, MD, PhD^{1,*}

¹John A Moran Eye Center, University of Utah, 65 Mario Capecchi Drive, Salt Lake City, UT 84132, USA

²Division of Epidemiology, University of Utah, 295 Chipeta Way, Salt Lake City, UT 84108 , USA

³Centre for Experimental Medicine, Queen's University Belfast, Grosvenor Road, Belfast, Co. Antrim. Northern Ireland U.K. BT126BA

Abstract

Purpose—To evaluate serum soluble Flt-1 (sFlt-1) in age-related degeneration (AMD) patients.

Design—Case control study.

Methods—Fifty-six non-AMD participants, fifty-three early AMD patients and ninety-seven neovascular AMD patients from Belfast in Northern Ireland. Serum samples were collected from each patient. Serum sFlt-1 was measured by human sVEGFR1/sFlt-1 ELISA kit. The results were analyzed by Excel and SPSS.

Results—Serum sFlt-1 concentration of non-AMD, early AMD, and neovascular AMD were 90.8±2.9 pg/mL (±SEM), 88.2±2.6 pg/mL and 79.9±2.2 pg/mL. sFlt-1 from neovascular AMD

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*corresponding author Address for reprints: Balamurali K. Ambati, MD, PhD, University of Utah, 65 Mario Capecchi Dr., Salt Lake City, UT 84132 USA, Bala.ambati@utah.edu.

Contributions to Authors

Design (H.U, U.C, R.E.H, B.K.A), Sample collection (P.E, U.C, R.E.H), Measurement (H.U, C.M), Analysis (H.U, M.M, R.E.H, B.K.A), Interpretation of the data (H.U, M.M, U.C, R.E.H, B.K.A), Manuscript preparation (H.U, M.T, B.S, B.K.A), Revision of paper (H.U, M.M, R.E.H, S.P, B.K.A).

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patients was significantly decreased compared to non-AMD and early AMD patients (ANOVA, $p<0.01$). For each 10 point increase in sFlt-1, the odds for having neovascular AMD compared with non-AMD and neovascular AMD decreases by 27.8% OR=0.722 (95% CI: 0.588-0.888, $p=0.002$) and 27.0% OR=0.730 (95% CI: 0.594–0.898, $p=0.003$), respectively. In patients over 73 years of age, serum sFlt-1 <80 pg/mL was associated with a >6-fold higher risk of neovascular AMD.

Conclusions—Reduced serum sFlt-1 differentiates those patients with neovascular AMD from both early AMD and non-AMD participants. In those aged over 73, serum sFlt <80 pg/mL seems to indicate a particularly high risk of neovascular AMD. Our results indicate serum sFlt-1 could be a biomarker for development of neovascular AMD.

Introduction

Age-related Macular Degeneration (AMD) is the leading cause of irreversible visual blindness over the age of 50 in the western world ^{1,2}. Prior to the development of visual loss, degenerative changes manifest initially as the accumulation of extracellular protein and lipid which occur at the Bruch's membrane, retinal pigment epithelium (RPE) and photoreceptor interfaces and characteristically manifest as drusen. These features are not associated with overt sight loss and are termed early AMD. Approximately 1 in 2 persons with extensive macular drusen will progress within 5 years to sight threatening geographic atrophy (GA) and/or neovascularization (neovascular AMD)³, which are late stage manifestations of the condition. In a large long-term epidemiologic study, roughly a quarter of patients over the age of 75 were found to have some features of AMD, about 80% of whom have dry AMD and are at risk of converting to exudative AMD ⁴. The onset of neovascular AMD generally results in sudden acute vision loss, and if left untreated, can cause permanent loss of sight. Neovascular AMD accounts for 90 percent of the blindness due to AMD ².

While age is the strongest demographic risk factor associated with AMD, there is also a significant genetic component with at least 19 loci reported to be associated with AMD⁵. The most significant associations are found with a tyrosine-histidine change at amino acid 402 of Complement factor H (CFH), which is involved in innate immunity^{6–8} and with the HTRA1/ARMS2 locus, which is thought to affect matrix stability^{9,10}. Compared with genetic risk factors, serum markers are less well characterized. Although various candidates have been proposed as risk factors for AMD, currently the most studied serum biomarker is elevated C-reactive protein (CRP) ¹¹. CRP is a systemic marker of inflammation and is associated with a variety of different diseases, including heart disease ¹², collagen-vascular disorders ¹³, and temporal arteritis¹⁴, a recent meta-analysis has confirmed that that high serum levels (>3 mg/L) of CRP are associated with a two-fold likelihood of late AMD ¹⁵.

Vascular endothelial growth factor (VEGF) plays an important role in the pathogenesis and treatment of neovascular AMD. Soluble fms-like tyrosine kinase-1(sFlt-1 or VEGFR1) is an anti-angiogenic receptor for VEGF that binds and sequesters VEGF, reducing its angiogenic activity¹⁶. Recently, we demonstrated that the decoy VEGF receptor, sFlt-1 in RPE is mechanistically required for subretinal vascular demarcation ¹⁷. Moreover, patients with neovascular AMD expressed less sFlt-1 in the RPE and conditional knockout of sFlt-1 in the

RPE showed spontaneous choroidal neovascularization. As sFlt-1 is also found in serum, we hypothesized that serum sFlt-1 might be reduced in patients with neovascular AMD, either as a byproduct or a cause of their condition. To further explore this potential link, we examined sFlt-1 in sera from non-AMD (controls), early AMD and neovascular AMD patients.

Methods

The study adhered to the tenets of the Declaration of Helsinki on research into human volunteers, and the design was approved by the Research Ethics Committee of Queen's University of Belfast. After informed consent, serum samples were collected in Northern Ireland (50 mile radius around Belfast) and then analyzed in a de-identified manner. To examine sFlt-1 level in patient's serum, we used Human sVEGF R1/Flt-1 Quantikine ELISA Kit (R&D, USA) following manufacturer's instructions.

Sample Collection

Controls—These were obtained as part of population based study cohort collected in Belfast according to the methods outlined previously¹⁸. In brief adults men and women 65 years or older were randomly identified from 2 general practice registers. The study participants' general practitioner excluded those with severe physical disability, dementia, or other debilitating disease that would preclude a visit to the study clinic. From the 205 who participated we randomly selected 52 with no evidence of early or late AMD in either eye with the classification based on systematic grading of their color fundus photographs.

Cases—These were obtained from the Belfast participants in the CARMA study whose recruitment and data collection methods are outlined elsewhere¹⁹. A random sample of those with bilateral drusen $\geq 63\mu\text{m}$ and unilateral neovascular AMD who had serum samples and gradable fundus photographs were obtained.

Cigarette Smoking—Participants were categorized as nonsmokers, or ever smokers.

Age-Related Macular Degeneration Grade—Fundus photographs were graded in the photographic reading center at Queen's University of Belfast by trained graders using the definitions of the Wisconsin Age-Related Maculopathy Grading System²⁰. In brief, the presence and type of drusen and pigmentary irregularities were evaluated within segments of the 3000 μm -radius grid, which was placed with its center on the fovea. A graded categorical approach was used to obtain an estimate of the number of drusen within the grid. Geographic atrophy (GA) was defined as areas of atrophy in excess of 175 μm with well-delineated margins within which choroidal vessels could be observed. Choroidal neovascularization was said to be present if stereoscopic examination showed subretinal fluid, exudate, hemorrhage, and/or scar tissue in the macular region of the fundus. When both GA and choroidal neovascularization were present in the same eye, the person was classified as having neovascular AMD. Participants were categorized based on the features observed in the more severely affected eye. In the absence of drusen $>63\mu\text{m}$ or pigmentary irregularities in either eye, participants were designated as non-AMD (controls). Participants

with drusen $\geq 63 \mu\text{m}$ or pigmentary irregularities in at least one eye were classified as early AMD. The remaining participants had neovascular AMD.

Statistical Analysis

Student's t-test (two-tail), χ^2 test and ANOVA were performed using Microsoft Excel. Logistic regressions were performed using SAS software version 9.3. A logistic regression model was used to determine the effects of sFlt-1 level, age, gender and smoking history on the stage of macular degeneration (AMD stage). When considering the levels of non-AMD, early AMD and neovascular AMD in a polytomous (multilevel) logistic regression, the results of the score test indicated that the proportional odds assumption was not met. Therefore outcomes were considered in pairs of non-AMD vs. early AMD, early AMD vs. neovascular AMD and non-AMD vs. neovascular AMD.

Results

Demographics

Table 1 displays the age, gender and smoking status of those participants from whom the samples were derived. Participants with neovascular AMD were older and more likely to have been smokers compared to those with early AMD or non AMD.

Serum sFlt-1 is lower in neovascular age-related macular degeneration patients

Serum sFlt-1 concentrations from non-AMD, early AMD and neovascular AMD cohorts were $90.8 \pm 2.9 \text{ pg/mL}$ ($\pm\text{SEM}$), $88.2 \pm 2.6 \text{ pg/mL}$ and $79.9 \pm 2.2 \text{ pg/mL}$, respectively (Table 1). sFlt-1 serum levels in neovascular AMD patients was significantly lower than in sera from non-AMD and early AMD patients (Figure 1, $p=0.0033$ and 0.019 , respectively).

We also analyzed serum sFlt-1 levels of all participants by age, gender and smoking history (Table 1). When the participants were divided into two groups by age 73 (the median age of our population sample), serum sFlt-1 levels were $84.7 \pm 2.1 \text{ pg/mL}$ ($<73 \text{ yrs}$) and $84.8 \pm 2.1 \text{ pg/mL}$ ($\geq 73 \text{ yrs}$), respectively. Serum sFlt-1 level in female ($81.7 \pm 2.0 \text{ pg/mL}$) was lower than that in male ($88.3 \pm 2.2 \text{ pg/mL}$), significantly ($p=0.027$). Serum sFlt-1 was not significantly different between non-smoker ($87.4 \pm 2.2 \text{ pg/mL}$), past smoker ($83.7 \pm 2.6 \text{ pg/mL}$) and current smoker ($79.8 \pm 3.0 \text{ pg/mL}$).

Reduced serum sFlt-1 was associated with neovascular age-related macular degeneration in patients over 73 years of age

Since participants with nAMD were older than those with early AMD and or non AMD, we compared serum sFlt-1 concentration in patients age $<73\text{yrs}$ or $\geq 73\text{yrs}$ (Table 2). In those aged $<73\text{yrs}$, serum sFlt-1 concentrations from non-AMD, early AMD and neovascular AMD were $87.9 \pm 3.8 \text{ pg/mL}$, $85.3 \pm 3.3 \text{ pg/mL}$ and $79.7 \pm 3.3 \text{ pg/mL}$, respectively. This was not statistically significant (ANOVA $p=0.31$). In subjects with age $\geq 73\text{yrs}$, serum sFlt-1 concentrations from non-AMD, early AMD and neovascular AMD were $93.9 \pm 4.1 \text{ pg/mL}$, $93.5 \pm 4.0 \text{ pg/mL}$ and $80.0 \pm 2.6 \text{ pg/mL}$, respectively. Serum sFlt-1 in neovascular AMD subjects was significantly decreased compared with non-AMD and early AMD subjects ($p=0.014$ and 0.016 , respectively).

Gender and Smoking Status did not affect serum sFlt-1

To determine if gender impacted the relationship between AMD stage and sFlt-1 concentration, we stratified the data by gender (Table 3). In females, serum sFlt-1 levels of non-AMD, early AMD and neovascular AMD were 88.4 ± 3.8 pg/mL ($\pm$ SEM), 83.2 ± 3.6 pg/mL and 76.5 ± 2.9 pg/mL, respectively. In males, serum sFlt-1 levels of non-AMD, early AMD and neovascular AMD were 91.8 ± 4.4 pg/mL, 95.9 ± 2.9 pg/mL and 83.2 ± 3.3 pg/mL, respectively. In both males and females, sFlt-1 was lower in neovascular AMD subjects than in non-AMD and early AMD subjects. Male serum sFlt-1 levels were relatively higher than those in females in early AMD ($p=0.0148$).

To examine whether smoking affect serum sFlt-1 in our data set, we compared serum sFlt-1 level across AMD groups stratified by smoking state (Table 4). In non-smokers, serum sFlt-1 levels of non-AMD, early AMD and neovascular AMD were 95.2 ± 4.4 pg/mL, 87.5 ± 3.8 pg/mL and 82.0 ± 3.4 pg/mL, respectively. In past smokers, serum sFlt-1 levels of non-AMD, early AMD and neovascular AMD were 93.0 ± 8.8 pg/mL, 88.0 ± 4.7 pg/mL and 80.2 ± 3.4 pg/mL, respectively. In current smokers, serum sFlt-1 levels of non-AMD, early AMD and neovascular AMD were 80.9 ± 3.9 pg/mL, 92.5 ± 9.3 pg/mL and 72.0 ± 8.5 pg/mL, respectively. We found that the sFlt-1 level of neovascular AMD patients is lowest in all group. When comparing serum sFlt-1 between nonsmokers, past smokers and current smokers, we could not find any significant differences among non-AMD, early AMD and neovascular AMD groups.

Logistic Regression Model

A logistic regression model was used to investigate the relationship between sFlt-1 concentration and AMD group, corrected for age, gender and smoking (Table 5).

When non-AMD and early AMD groups were compared, none of the effects were significant. Next, non-AMD and neovascular AMD were compared. Controlling for gender, smoking, history and age, for each 10 point increase in serum sFlt-1 the odds for having neovascular AMD decreased by 27.8% (OR=0.722 (95% CI: 0.588-0.888, $p=0.002$). Controlling for gender, sFlt-1 level and smoking history, for each increase in year of age the odds for having neovascular AMD increased by 8.3% OR=1.083 (95% CI: 1.019–1.152, $p=0.011$). Gender and smoking history were not significant. Knowledge of the serum sFlt-1 improved the prediction of neovascular AMD vs non-AMD subjects

Finally, we compared early AMD and neovascular AMD. Controlling for gender, smoking history and age, for each 10 point increase in sFlt-1 the odds for having neovascular AMD decreases by 27% (OR=0.730 (95% CI: 0.594–0.898, $p=0.003$)). Controlling for gender, smoking history and serum sFlt-1 level, for each increase in year of age the odds for having neovascular AMD increased by 12.8% OR=1.128 (95% CI: 1.066–1.193, $p<0.001$). Gender and smoking history were not significant. To estimate whether knowledge of the serum sFlt-1 level improves the prediction of neovascular AMD, we determined receiver operating curves and compared the area under the curve in >73 patients in a model accounting for gender and smoking status (Table 6). Adding sFlt to the model increased the AUC for

prediction of neovascular AMD, but this difference did not meet statistical significance ($p=0.097$).

The predicted probabilities analysis for neovascular AMD versus early AMD from the above logistic regression, are graphically represented in figure 2. As shown, rising age and declining serum sFlt-1 concentration are associated with neovascular AMD.

Serum sFlt-1 <80 pg/mL is highly associated with neovascular age-related macular degeneration in subjects over 73 years of age

To clarify the potential clinical relevance, we categorized the data by patient age greater or lesser than 73 yrs and patient serum sFlt-1 greater than or less than 80pg/mL (Table 7). From our analysis, serum sFlt <80 pg/mL has a very high association with neovascular AMD in the over-73 population. From table 7, we also calculated a more than 6-fold odds ratio for neovascular AMD in this subpopulation, relative to both no AMD and early AMD (Table 8). This would seem to indicate a synergistic interaction between declining sFlt in the serum and rising age in raising the risk of neovascular AMD. Stratification by decade of life did not affect the statistically significant association between declining serum sFlt-1 and risk of neovascular AMD (Supplemental Table).

Discussion

We found that neovascular AMD patients had lower serum sFlt-1 than non-AMD or early AMD patients, especially in those aged 73 years (the median age of our population) or older. Coupled with our previous results, these findings suggest that reductions in sFlt-1 that are observed in neovascular AMD occur both locally at the level of the RPE and are also reflected systemically¹⁷. Although this study does not address the question of cause or effect, these results indicate that reduced sFlt-1 may have a role as a biomarker for development of neovascular AMD.

It is well established that VEGF-A plays an important role in the development of neovascular AMD. Our results demonstrating reduced serum sFlt-1 levels in patients with neovascular AMD have a clear pathophysiologic link. Flt-1 is the highest-affinity receptor for VEGF-A. The Flt-1 gene produces two kinds of major isoform by alternative splicing; membrane bound Flt-1 (mbFlt-1) and soluble Flt-1 (sFlt-1)²¹. MbFlt-1 consists of seven IgG-like domains, a transmembrane domain, and tyrosine kinase domains. On the other hand, sFlt-1 has only six extracellular IgG-like domains, no transmembrane domain and no tyrosine kinase domains. As it is soluble, sFlt-1 binds to and sequesters VEGF-A and therefore suppresses angiogenesis¹⁶. Thus, reduced sFlt-1 in the RPE is associated with the onset of choroidal neovascularization¹⁷.

Intravitreal anti-VEGF therapy is the mainstay of treatment for neovascular AMD. Interestingly, multiple previous studies have shown that serum VEGF-A levels are increased in wet AMD patients^{22,23}. Our finding of decreased serum sFlt in neovascular AMD patients suggests a possible cause for the increased serum VEGF-A levels. This may, in part, explain some of the pathophysiology behind the development of neovascular AMD.

Cigarette smoking is a strong environmental risk factor for neovascular AMD, and it is responsible for a two- to three- fold risk increase of developing AMD²⁴. Smoking is associated with both early and neovascular AMD, but there is a stronger correlation between smoking and the development of neovascular AMD²⁴. Previous studies have indicated that serum sFlt-1 is reduced in smokers^{25,26}. However, we did not find a significant difference of serum sFlt-1 levels between current/past smokers and non-smokers. Future studies that are larger and more longitudinal will likely better define the relationship between smoking and serum sFlt-1.

Many genetic loci have been identified that correlate with risk for development of AMD. A recent large, population-based study identified 19 loci associated with AMD at a high probability⁵. The genes associated with AMD are involved in the regulation of complement activity, lipid metabolism, extracellular matrix remodeling, and angiogenesis⁵⁻⁸. Specifically, a recent systematic review and meta-analysis suggested that two VEGF-A polymorphisms (rs1413711 and rs833061) might contribute to AMD susceptibility²⁷. An important linkage variant is the HTRA1/ARMS2 locus, which presumably affects matrix stability and is associated with neovascular AMD^{9,10}. Previous reports suggest that there are polymorphisms in the VEGFR2 (KDR) gene that may correlate with increased risk of AMD, though this relationship is unclear^{28,29}. In future, we would like to explore the relationship between serum sFlt-1 and known risk factors including CFH and ARMS2.

There are no reported genetic variations in Flt that are associated with increased risk for AMD, though several papers report that there are specific SNPs in Flt gene that are associated with either increased or decreased response to anti-VEGF therapy in patients with wet AMD^{30,31}. As genetic analysis has not revealed Flt variations associated with AMD or exudative AMD development, we speculate that the sFlt-1 reduction seen in our study may arise from some environmental factors rather than genetic factors, though genetic factors may also contribute. Future research could explore the interaction between sFlt-1 serum levels, SNPs in the Flt-1 gene, and development of neovascular AMD.

Compared with the genetic risk factors, serum markers are less well characterized. Many studies have attempted to associate various biomarkers in the pathogenesis of AMD. Serologic markers that have been associated with AMD development include elevated inducible costimulator, elevated MDA, elevated superoxide dismutase, elevated C-reactive protein, and elevated LDL^{11,32-36}. CRP is the most well understood and studied of these biomarkers. A large meta-analysis by Hong et al found a significant association between high serum and plasma CRP levels and late AMD (OR= 1.69 95%CI= 1.28-2.23, p=0.001)¹⁵. Serologic markers reported elevated in neovascular AMD include eotaxin-2 and homocysteine. Sharma et al found that eotaxin-2 expression was significantly elevated in AMD patients compared with controls (ANOVA, n= 47, p = 0.0001)³⁷. Similarly, homocysteine was elevated in patients with neovascular AMD when compared with controls (p= 0.0186), although this study included a relatively smaller sample size of 32 cases³⁸. Another study reported elevated serum VEGF-receptor 2 in patients with AMD (OR= 2.13, CI=1.011-4.489, p=0.047) though this relationship is not well studied and is of unclear significance²⁹, especially as soluble VEGF-receptor-2 (unlike sFlt-1) is monomeric and hence has very low affinity for VEGF-A⁵⁵.

The present study shows sFlt-1 from patients with neovascular AMD is significantly decreased compared to early AMD and normal controls (ANOVA, $p < 0.01$). For each 10 point increase in sFlt-1 the odds for having neovascular AMD decreases by 27% (OR=0.730 95% CI: 0.594–0.898, $p = 0.003$). Further, in patients over 73, sFlt-1 levels under 80 pg/ml were associated with over a 6-fold higher risk of neovascular AMD. While we do not feel that 73 years can be interpreted as a “cut-off” as age is a continuous variable, further studies should elucidate whether the early 70s represent an inflection point where declining serum sFlt-1 synergizes with age to produce a profoundly higher risk of neovascular AMD. This finding is in keeping with recent work reporting the reduced effect of the major AMD risk alleles (ARMS2 and CFH) in nonagenarians³⁹, in that factors other than those mediated via these genes must be underlying the risk of AMD in older age-groups. Reduced sFlt-1 may such a contributing factor.

Comparatively, this study has a similar or stronger effect and statistical significance than the aforementioned serologic biomarker studies. This study also indicates a clinically relevant stepwise correlation between sFlt-1 levels and the risk of developing neovascular AMD, which has not been shown in with the other serum biomarkers. The interplay of serum sFlt-1 with these various serologic markers is currently unclear and should be an item of further research. Future development of serologic panels that include biomarkers such as CRP, eotaxin-2, homocysteine, and possibly sFlt-1 could be important clinical tools in risk stratification, early diagnosis & intervention, and prevention of vision loss in neovascular AMD.

Future prospective investigations should explore whether declining serum sFlt-1 levels herald the onset of neovascular AMD, why sFlt-1 levels decline in these patients, and if interventions that restore normal systemic serum sFlt-1 levels ameliorate the disease and/or improve therapeutic outcomes. We must emphasize that our current finding cannot determine causality because it is uncertain whether sFlt-1 reduction precedes or is a consequence of neovascular AMD. However, it would seem that serum sFlt-1 < 80 pg/mL in patients over 73 indicates a dramatically higher risk of neovascular AMD. To determine a precise relationship, a longitudinal, prospective, controlled cohort study is necessary. If declining sFlt-1 is confirmed as a predictive biomarker for progression of early to neovascular AMD, this would enable earlier intervention, potentially preventing vision loss from the onset of neovascular AMD. In future, we will examine whether age and serum sFlt-1 are correlated in each subset of patients also.

In summary, sFlt-1 in neovascular AMD patient sera was reduced compared to that in non-AMD and early AMD patients. In conjunction with our previous report, which showed that sFlt-1 was decreased in RPE of AMD patients, sFlt-1 in the serum is likely associated with the development of neovascular AMD. Determining the clinical relevance of serum sFlt-1 in individual patients will require further study. Predictive diagnostics which enable targeted home monitoring⁴⁰ or more frequent clinical observation may result in earlier detection of choroidal neovascularization which may allow earlier intervention and better visual preservation for patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biography

Hironori Uehara is a post-doctoral fellow in Moran Eye center, University of Utah. He graduated from Tokyo Institute of Technology, and completed Ph.D in the same institute. He is interested in RNA splicing and polyadenylation mechanism as a drug target.

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Figure 1.

Serum sFlt-1 concentraion in Non age-related macular degeneration, Early and Neovascular age-related macular degeneration. A line in the box graph indicated the average of serum sFlt-1 concentraion. The box indicates the standard deviation. Error bars indicate the range. p-values are calculated from a two-tail student's t-test.

Figure 2.
Graphic of the estimated probability for the risk of neovascular age-related macular degeneration from early age-related macular degeneration. sFlt-1 concentration is pg/mL, and age is year.

Table 1

Serum sFlt-1 concentration by age related macular degeneration grade, age, gender and smoking state

	All participant (n=206)	AMD grade				Age			Gender			Smoking				Missing
		Non-AMD D (n=56)	Early AMD (n=53)	Neovascular AMD(n=97)	p-Value	<73 (n=96)	73 (n=109)	p-Value	Female (n=108)	Male (n=92)	p-Value	Non (n=87)	Past (n=78)	Current (n=35)	p-V alue	
Age (yrs. ±S.D)	73.0 ± 6.9	71.9±4.7	70.4± 7.4	75.1±7.0	<0.001	67.0± 4.4	78.3 ± 3.6		73.3 ± 6.7	72.6±7.1	0.47	73.9± 6.7	73.3± 7.1	70.0± 6.5	0.017	1
Gender F/M	110/95	30/25	32/21	48/49	0.44	51/45	59/50	0.89	-	-		64/23	26/52	18/17	<0.001	1
Non/past/current smoker	87/78/35	24/9/17	27/20/ 6	36/49/12	<0.001	38/33/ 23	49/45/ 12	0.050	67/26/18	23/52/17	<0.001	-	-	-		6
Non-AMD(%)	27.2	-	-	-		37.5	17.4	<0.001	27.3	26.3	0.20	27.6	11.5	48.6	<0.001	
Early AMD (%)	25.7	-	-	-		35.4	17.4		29.1	22.1		31.0	25.6	17.1		
Neovascular AMD(%)	47.1	-	-	-		27.1	65.1		43.6	51.6		41.4	62.8	34.3		
Serum sFlt-1 concentration (pg/mL, ± S.E.M)	85.0 ± 1.5	90.8±2.9	88.2± 2.6	79.9±2.2	0.0043	84.7± 2.1	84.8 ± 2.1	0.99	81.7 ± 2.0	88.3±2.2	0.027	87.4± 2.2	83.7± 2.6	79.8± 3.0	0.19	-

P-value was calculated using student's t-test for the average between two groups, χ^2 test for categories and ANOVA for the average between three groups

AMD: age related macular degeneration

Table 2

Serum sFlt-1 concentration by age related macular degeneration grade and Age 73yrs.

	Age<73yrs (n=96)				Age 73 (n=109)				Missing	
	Non-AMD (n=36)	Early AMD (n=34)	Neovascular AMD (n=26)	P-value	Non-AMD (n=19)	Early AMD (n=19)	Neovascular AMD (n=71)	P-value		
Gender F/M	20/16	20/14	11/15	0.42	10/9	12/7	37/34	0.68	5	
Non/Past/Current smoker	18/4/12	13/16/5	7/13/6	0.008	6/5/5	14/4/1	29/36/6	0.007		
Serum sFlt-1 concentration (pg/mL, ±S.E.M)	87.9±3.8	85.3±3.3	79.7±3.9	0.31	93.9 ±4.1 (0.32)	93.5 ±4.0 (0.13)	80.0±2.6 (0.95)	0.007		

AMD: age related macular degeneration

Table 3

Serum sFlt-1 concentration by age related macular degeneration stage and gender

Female				
	Non-AMD (n=30)	Early AMD (n=32)	Neovascular AMD (n=48)	P-value
Age($\pm$ S.D)	71.7 $\pm$ 4.7	70.5 $\pm$ 7.6	76.2 $\pm$ 6.0	<0.001(ANOVA)
Non-smoker/Ever smoker	17/4/7	21/6/5	26/16/6	0.26 (χ^2 test)
Serum sFlt-1 concentration (pg/mL. $\pm$ S.E.M)	88.4 $\pm$ 3.8	83.2 $\pm$ 3.6	76.5 $\pm$ 2.9	0.041 (ANOVA)
Male				
	Non-AMD (n=25)	Early AMD (n=21)	Neovascular AMD (n=49)	P-value
Age($\pm$ S.D)	72.2 $\pm$ 4.8	70.2 $\pm$ 7.6	73.9 $\pm$ 7.8	0.127(ANOVA)
Non-smoker/Ever smoker	7/5/10	6/14/1	10/33/6	<0.001 (χ^2 test)
Serum sFlt-1 concentration (pg/mL. $\pm$ S.E.M)	91.8 $\pm$ 4.4	95.9 $\pm$ 2.9	83.2 $\pm$ 3.3	0.045 (ANOVA)

Table 4

Serum sFlt-1 concentration by age related macular degeneration stage and smoking state

Non-smoker				
	Non-AMD (n=24)	Early AMD (n=27)	Neovascular AMD (n=36)	P-value of Row
Age(±S.D)	71.1±4.1	72.2±7.7	76.9±6.1	<0.001 (ANOVA)
Gender F/M	17/7	21/6	26/10	0.83 (χ^2 test)
Serum sFlt-1 concentration (pg/mL. ±S.E.M)	95.2±4.4	87.5±3.6	82.0±3.4	0.055(ANOVA)
Past smoker				
	Non-AMD (n=9)	Early AMD (n=20)	Neovascular AMD (n=49)	P-value
Age(±S.D)	73.9±5.4	68.7±6.9	75.0±6.7	0.0028(ANOVA)
Gender F/M	4/5	6/14	16/33	0.74 (χ^2 test)
Serum sFlt-1 concentration (pg/mL. ±S.E.M)	93.0±8.8	88.0±4.7	80.2±3.4	0.19 (ANOVA)
Current smoker				
	Non-AMD (n=17)	Early AMD (n=6)	Neovascular AMD (n=12)	P-value
Age(±S.D)	71.2±5.1	67.5±5.9	69.6±8.5	0.49(ANOVA)
Gender F/M	7/10	5/1	6/6	0.20 (χ^2 test)
Serum sFlt-1 concentration (pg/mL. ±S.E.M)	80.9±3.9	92.5±9.3	72.0±8.5	0.066 (ANOVA)
P-value of Column				
Age	0.29 (ANOVA)	0.16 (ANOVA)	0.006 (ANOVA)	
Gender	0.13 (χ^2 test)	0.002 (χ^2 test)	0.002 (χ^2 test)	
Serum sFlt-1 concentration	0.10 (ANOVA)	0.85 (ANOVA)	0.38 (ANOVA)	

AMD: age related macular degeneration

Table 5
Multivariate Logistic Regression Model: Risk factors for age related macular degenerations

Effect	non-AMD vs early AMD		non-AMD vs neovascular AMD		early AMD vs neovascular AMD	
	Odds Ratio[95%CI]	P value	Odds Ratio[95%CI]	P value	Odds Ratio[95%CI]	P value
Age units=1	0.959[0.897–1.026]	0.222	1.083[1.019–1.152]	0.011	1.128[1.066–1.193]	<0.001
sFlt-1 units=10	0.972[0.787–1.200]	0.789	0.722[0.588–0.888]	0.002	0.730[0.594–0.898]	0.003
Gender F vs M	1.572[0.643–3.843]	0.321	0.759[0.335–1.720]	0.509	0.470[0.201–1.099]	0.082
Ever smoker vs non smoker	0.365[0.120–1.112]	0.076	0.496[0.183–1.344]	0.168	2.577[0.733–9.058]	0.140
Past smoker vs. Never	2.688[0.955–7.566]	0.061	4.225[1.624–10.996]	0.003	1.884[0.792–4.481]	0.152

AMD: age related macular degeneration

Table 6

Receiver Operating Curves for Impact of sFlt on Association with Neovascular vs Early age related macular degeneration

	AUC	SE	95% CL for AUC	P value *
Full Model: Gender + Smoking Hx. + sFLT level for Neovascular vs. Early AMD where age = 73	0.76	0.05	0.65–0.87	
Full Model without sFLT	0.68	0.06	0.55–0.81	0.097

* difference in AUC for Full Model vs. Full Model without sFLT

Table 7

Patient number categorized by Age 73yrs, Serum sFlt-1 80pg/mL and age related macular degeneration stage

		Age 73yrs				Age <73yrs				P value	
		non-AMD	early AMD	neovascular AMD	P value	non-AMD	early AMD	neovascular AMD			
Serum sFlt-1 concentration	<80pg/mL	3	3	39	<0.001	17	13	16	0.196		
	80pg/mL	15	16	32		20	21	10			

Table 8

Stratification by age and serum sFlt-1 demonstrates high risk of neovascular AMD in patients over 73 with low serum sFlt-1 (<80pg/mL).

Effect	Category	non-AMD vs neovascular AMD		early AMD vs neovascular AMD		non-AMD vs early AMD	
		Odds Ratio[95%CI]	P value	Odds Ratio[95%CI]	P value	Odds Ratio[95%CI]	P value
Serum sFlt-1 <80pg/mL vs 80pg/mL	In Age 73yrs	6.094[1.620–22.922]	0.004	6.500[1.738–24.303]	0.002	0.938[0.163–5.387]	0.942
	In Age <73yrs	1.882[0.678–5.225]	0.222	2.585[0.904–7.387]	0.073	0.728[0.283–1.877]	0.511

AMD: age related macular degeneration