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Effect of intravitreal injection of aflibercept on blood coagulation parameters in patients with age-related macular degeneration

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Abstract

Purpose: Treatment with intravitreal injections of anti-vascular endothelial growth factor agents has been associated with an increased risk of arterial thromboembolic events. The aim of the present pilot study was to assess the effect of a single intravitreal injection of aflibercept on coagulation.

Methods: Treatment-naïve patients with age-related macular degeneration ($n = 47$), who were scheduled to undergo treatment with intravitreal injections of aflibercept, were enrolled. None of the included patients received any anticoagulation therapy or had a history of a recent arterial thromboembolic event. Blood samples were collected before the first intravitreal injection, and at 7 and 30 days after aflibercept administration. We evaluated coagulation parameters, such as platelet count and plasma fibrinogen and D-dimer levels; functional clotting parameters, such as prothrombin time, international normalized ratio, and activated partial thromboplastin time; and anticoagulant parameters, such as the levels of Proteins S and C.

Results: The levels of all of the evaluated biomarkers were within the normal range at baseline and at both the time points throughout the study. No statistically significant changes were observed in any of the measured parameters at 1 week and 1 month after aflibercept administration.

Conclusion: A single intravitreal injection of aflibercept in treatment-naïve patients with exudative age-related macular degeneration has no statistically significant effect on blood coagulation parameters for up to 1 month after aflibercept administration. Our results also provide an explorative statistical data, and further studies are required to evaluate any significant clinical effects of aflibercept on blood coagulation parameters.

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Keywords: activated partial thromboplastin time, aflibercept, age-related macular degeneration, arterial thromboembolic events, fibrinogen, international normalized ratio, plasma D-dimer, Protein C, Protein S, prothrombin time

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Introduction

Aflibercept is a recombinant fusion protein that binds to and disables all forms of vascular endothelial growth factor-A (VEGF-A); it is also placental growth factor. Intravitreal injections of aflibercept (IVA) has been approved for the treatment of neovascular age-related macular degeneration (AMD), diabetic macular edema, macular edema due to retinal vein occlusion, and for myopic choroidal neovascularization.[1] Pharmacokinetic studies have shown that after a single IVA (2.0 mg), aflibercept is transferred quickly into the bloodstream, it potently suppresses free plasma VEGF, resulting in early reduction of the plasma VEGF concentration that remains below the lower limit of quantitation for at least 7 days post-IVA.[2]

VEGF serves as a survival factor for endothelial cells and its suppression causes vascular endothelial cell dysfunction and death. This triggers a dysregulation in coagulation homeostasis, resulting in a predisposition to thrombosis.[3,4] Consequently, there is an increased concern regarding systemic safety, and more precisely, regarding the potential risk of arterial thromboembolic (ATE) events, including stroke and myocardial infarction in patients undergoing treatment with intravitreal injections of anti-VEGF agents, such as aflibercept. This concern predominantly applies to the elderly population that suffers from AMD and requires prolonged treatment with anti-VEGF agents.

Hypercoagulability, endothelial damage, and circulatory stasis have been implicated in thromboembolic events.[5] Several biomarkers have been evaluated to study coagulation alterations that result in hypercoagulability, and therefore, potentially increased thromboembolic risk in patients under systemic chemotherapy or under treatment with intravitreal injections of ranibizumab or bevacizumab.[6–11]

The aim of this study was to examine changes in coagulation parameters, such as platelet count and plasma fibrinogen and D-dimer levels; functional clotting parameters, such as prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT); and anticoagulant parameters, such as the levels of Proteins S and C, in treatment-naïve patients with AMD after the first intravitreal injection of aflibercept.

Patients and methods

This was a prospective, nonrandomized, one-arm, uncontrolled pilot study that received approval from the Institutional Review Board of the University Hospital of Patras (516/26 July 2017) and was conducted in accordance with the principles of the Declaration of Helsinki. The study was carried out at the Departments of Ophthalmology and Hematology at the University Hospital of Patras in Greece. Treatment-naïve patients with neovascular AMD scheduled to undergo treatment with IVA in one eye were eligible for enrollment. All the patients provided written informed consent before enrollment in the study.

Patients were excluded if they were undergoing systemic treatment with anti-VEGF agents, if they had received treatment with anti-VEGF agents for the other eye, if they had received any treatment with anti-VEGF agents in the study eye in the past, or if they were unwilling to return 1 week and 1 month after the first IVA. Patients undergoing dialysis, suffering from chronic liver disease or malignancy, who had undergone treatment with nonsteroidal anti-inflammatory drugs systemically, and those undergoing anticoagulation therapy were also excluded. Finally, patients with a history of any ATE event during the last 6 months were also excluded.

Fasting blood samples were collected 10–15 min before the first IVA, and at 7 and 30 days postinjection using a 19-gauge needle under minimum stasis.

Blood sample analysis

Venous blood samples were collected in vacutainers containing K3EDTA (IMPROVACUTER® EDTA; Guangzhou Improve Medical Instruments Co., Ltd, Guangzhou, China) for platelet count analysis. For the evaluation of the other parameters, blood samples were collected in citrate tubes (IMPROVACUTER® Citrate Tube, Guangzhou Improve Medical Instruments Co., Ltd), centrifuged for 15 min at 2000–2500*g* at 18°C, and the plasma samples were transferred into new plastic tubes. Plasma samples were tested undiluted. All laboratory analyses were conducted on the same day. Platelet count was evaluated using the Sysmex XE 2100 instrument (Sysmex, Kobe, Japan). Plasma fibrinogen and D-dimer levels, PT, INR, APTT, and Proteins S and C levels were measured using the STA Compact analyzer (Diagnostica Stago, Saint-Ouen-l'Aumône, France) using the appropriate kits (Diagnostica Stago S.A.S., Asnières-sur-Seine, France) at a routine research laboratory.

Fibrinogen level was determined using the clotting method of Clauss with the STA-Liquid Fib kit (Diagnostica Stago, Asnieres, France), according to the instructions. D-dimer levels were evaluated using an immuno-turbidimetric assay and more specifically the STA-Liatest D-Di PLUS kit (Diagnostica Stago, Asnieres, France). The STA-Neoplastine R kit was used for the determination of PT, and the STA-Cephascreen kits was used for the determination of APTT. The levels of Protein S were evaluated using the STA-Staclot Protein S kit, where the quantitative measurement of the functional Protein S levels was based on

Table 1. Values of the platelet count, plasma fibrinogen levels, plasma D-dimer levels, PT, INR, APTT, Protein S, and Protein C at baseline, before the intravitreal injection of aflibercept, and at 1 week and 1 month after aflibercept administration as well as the reference values of these parameters.

	Baseline	1 week	1 month	*p* value	Reference values
Platelets ($10^3/\mu L$)	245.05 ± 49.72	240.50 ± 51.34	247.95 ± 43.75	0.267	150–400
PT (s)	12.8791 ± 0.28218	12.9875 ± 0.41434	12.826 ± 0.42467	0.114	Ref. Time 13
INR	0.985 ± 0.027	0.992 ± 0.029	0.989 ± 0.024	0.494	
APTT (s)	30.00 ± 2.45	29.86 ± 2.58	31.10 ± 4.17	0.160	24–36
Fibrinogen (mg/dL)	345.92 ± 36.80	349.36 ± 37.07	347.04 ± 36.42	0.156	200–400
D-dimer (μg/dL)	0.38 ± 0.13	0.37 ± 0.11	0.35 ± 0.11	0.276	0.0–0.5
Protein S (%)	114.70 ± 22.45	117.96 ± 22.66	112.74 ± 24.68	0.298	65–140
Protein C (%)	106.97 ± 17.54	106.34 ± 17.11	106.77 ± 17.30	0.111	70–130

APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time.

the principle of factor Va inhibition. Finally, the STA-Stachrom Protein C kit (Diagnostica Stago, Asnieres, France) was used to determine functional Protein C levels in the plasma by the synthetic chromogenic substrate method. The test is based on the activation of Protein C by a specific activator derived from the venom of *Agkistrodon contortrix*, and the quantity of the enzyme that was formed was measured based on its amidasic activity toward the synthetic chromogenic substrate CBS 42.46. The intensity of the color produced was directly proportional to the level of Protein C initially present in the test plasma.

Statistical analysis

All statistical analyses were performed with SPSS 23.0 software (SPSS, Inc., Chicago, IL, USA). All variables were tested for normality using the Kolmogorov–Smirnov test. Repeated measures analysis of variance (ANOVA) was used to compare parametric values with Bonferroni post hoc test for comparisons between values within subjects; $p < 0.05$ indicated statistical significance.

Results

All the analyzed data were normally distributed. A total of 47 patients (28 male, 19 female) were included in the study. The mean age of the patients was 77.7 ± 8.04 years. All the enrolled patients completed the study. Treatment with IVA was well tolerated, as no complications or side effects were observed during the study period. Table 1 shows the values for platelet count, plasma fibrinogen levels, plasma D-dimer levels, PT, INR, APTT, and Protein S and C levels at baseline, before the IVA, and at 1 week and 1 month after aflibercept administration, along with the reference values of these parameters. According to our results, the levels of all evaluated biomarkers were within the normal range at baseline and at 1 week and 1 month after aflibercept. We observed that one IVA in treatment-naïve patients with AMD did not result in any statistically significant changes in any of the evaluated parameters for up to 1 month after the administration of aflibercept.

Discussion

Intravitreal administration of anti-VEGF agents has become the mainstay of treatment for AMD. One of the main concerns regarding treatment with anti-VEGF agents is their safety profile and more specifically the potential risk of ATE events. A majority of the clinical trials have demonstrated a good safety profile for ranibizumab and aflibercept, as these trials failed to show any definite increase in the risk for ATE events.[3,12–14] However, the design of the clinical trials excludes patients who are at increased risk of ATE events. This may not apply to the patients treated in routine clinical practice. Interestingly, the summary of product characteristics of aflibercept and ranibizumab does not overlook the potential dangers of ATE events and suggests caution when these agents are administered to patients with a history

of stroke, transient ischemic attacks, or myocardial infarction.[1,15]

Virchow's triad is a key conception of the pathophysiologic basis of all thromboembolic events. The three elements of this crucial triad are endothelial injury, hypercoagulability, and abnormal blood flow. These are interrelated factors that contribute to thrombosis and thromboembolic events.[5] The importance of the endothelium and the role of the blood constituents, namely of the platelets and of the soluble coagulation factors, has been recently highlighted.[5]

VEGF suppression by anti-VEGF agents results in endothelial cell dysfunction, causes defects in the endothelial layer with exposition of the underlying matrix, and thus predisposes to thrombosis.[3,4] Furthermore, endothelial cells, as aforementioned, play a pivotal role in the pathogenesis of thrombosis through the production of several regulators of coagulation and fibrinolysis.[3,5]

Platelets play a fundamental role in the coagulation cascade and are involved in primary hemostatic mechanisms. Following damage to the vascular endothelium, the platelets adhere to the site of injury and are activated by releasing mediators that promote platelet aggregation and the formation of the primary platelet plug.[16] Consequently, the platelets are implicated in the initial development of the blood clot in the injured vascular wall. It has been found that high platelet count in patients with cancer under chemotherapy is associated with increased risk of symptomatic venous thromboembolic events.[17] *In vitro* studies have shown that incubation of platelets with ranibizumab or low concentrations of bevacizumab did not influence their activation or aggregation. Nevertheless, platelet exposure to higher bevacizumab concentrations resulted in inhibition of platelet activation.[10] Another study demonstrated different results. More precisely, platelet exposure to a mixture of bevacizumab and VEGF-A or to a mixture of aflibercept and VEGF-B or placental growth factor resulted in their activation, while ranibizumab had no effect on the platelets.[18] Our study did not demonstrate any statistically significant effect on platelet count at 1 week and 1 month after the first IVA in AMD patients. Differences in the agents studied, study settings, and underlying diseases could explain these results.

The secondary hemostasis includes the activation of the coagulation cascade.[17] This is a complex cascade that involves the conversion of prothrombin to thrombin, which triggers the proteolysis of fibrinogen to fibrin monomers. Fibrin monomers are then polymerized to fibrin polymers, which interact with the platelets and other plasma components to eventually form a stable clot at the site of vascular injury.[17] An increase in the plasma fibrinogen levels is associated with increased risk of cardiovascular disease.[5,19] Yi and colleagues[8] found that plasma fibrinogen levels did not change significantly for up to 1 month after the first intravitreal administration of ranibizumab and 1 month after the second injection of ranibizumab in patients with AMD. Similarly, in our trial, plasma fibrinogen levels were within normal limits throughout the study and did not change significantly, as compared with the baseline at 1 week and 1 month after the first IVA.

D-dimer is produced from fibrinolysis of blood clot. An increase in plasma D-dimer levels is associated with increased risk of ATE events.[20] Hee and colleagues[9] measured serum levels of D-dimer in order to evaluate the risk of ATE events after intravitreal injection of bevacizumab and ranibizumab in patients with AMD. The authors found that the levels of D-dimer did not change significantly during 3 months of monthly intravitreal injections of both anti-VEGF agents. Nevertheless, bevacizumab-administered patients who were at risk of thromboembolic events showed significant increase in serum D-dimer levels at 1 day and 1 week after its administration.[9] In our study, the levels of D-dimer were not affected significantly by aflibercept for up to 1 month after the first intravitreal injection.

Routine tests of coagulation are the evaluation of PT, INR, and APTT in order to assess the overall blood clotting function. The PT test measures the duration of time for the blood to clot. INR is calculated in order to standardize PT and it takes into account the sensitivity of the reagent used by each laboratory to calculate PT. More precisely, INR is based on the ratio of each patient's PT and the laboratory's normal mean PT, thus, providing a value which is comparable between different laboratories. The APTT is used in conjunction with PT in order to evaluate coagulation. Decreased values obtained from the above-mentioned tests imply a relative hypercoagulable state, while increased values reveal a predisposition to bleeding.[21] According to our results, PT, INR, and APTT were not affected by the administration of aflibercept. However, different results have been

reported after treatment with intravitreal injections of bevacizumab. More precisely, Li and colleagues[22] reported that the regular dose of bevacizumab administered intravitreally resulted in significant decrease in PT 2 h after the injection, while no effect was observed on APTT. Furthermore, a short-term transient reduction in APTT has been reported 1 week after the first intravitreal injection of ranibizumab in AMD patients.[8]

Hemostatic balance involves the action of several anticoagulant agents, including Protein C and Protein S. When Protein C is activated by thrombin, it exerts antithrombotic actions, while it also has anti-inflammatory and cytoprotective activities. Protein S is a vitamin K–dependent glycoprotein that enhances the anticoagulant activity of Protein C, whereas it can also directly inhibit coagulation reactions.[23] Deficiency of Protein C and Protein S predisposes to recurrent venous thromboembolism.[24] According to our results, the levels of Protein S and Protein C were within normal limits in our patients and were not affected by one IVA.

The results of our study refer to treatment-naïve AMD patients without any anticoagulation therapy or a recent history of ATE event. Consequently, our results could not completely refer to patients with an underlying cardiovascular disease and other indications for treatment with aflibercept, such as diabetic macular edema or macular edema secondary to retinal vein occlusion. Someone could recognize certain limitations of our study. We could evaluate any possible effect of aflibercept on endothelium directly, using plasma markers of endothelial dysfunction and injury, such as the von Willebrand factor and soluble thrombomodulin.[5] Furthermore, due to the short duration of the study, potential long-term effects of aflibercept in the evaluated biomarkers could not been estimated. In addition, our cohort cannot correspond to the typical group of AMD patients as we excluded patients under anticoagulation therapy or with a history of a recent ATE event. It is worth emphasizing that this is a pilot study, without a hypothesis, where power analysis was not conducted. We also have to point out that our results are based on a statistical analysis and we might have overlooked the clinical significance of the changes of the exact values of the evaluated parameters. We could have enrolled a larger group of patients that would have resulted in statistically significant outcomes and a control group with patients receiving no treatment in order to provide further significance to our analysis and be more conclusive.

We designed this pilot study to assess how coagulation parameters, such as platelet count and fibrinogen and D-dimer levels; functional clotting parameters, such as PT, INR, and APTT; and anticoagulant parameters, such as Protein S and C levels might be altered after one IVA in treatment-naïve AMD patients. According to our results, aflibercept seems to have no statistically significant effect on the evaluated parameters. However, our study provides an explorative statistical analysis without proving any clinical relevance. Further studies with a larger sample size are required to evaluate the long-term systemic safety of aflibercept treatment with respect to ATE events.

Conflict of interest statement

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