Acta Medica Alanya

RESEARCH ARTICLE

ARAŞTIRMA

Acta Medica Alanya

2021;5(1):66-74

DOI:10.30565/medalanya.828026

Relationship between Whole Blood Viscosity and Lower Extremity Peripheral Artery Disease Anatomical Complexity and Symptom Severity

Tam Kan Viskozitesi ile Alt Ekstremite Periferik Arter Hastalığının Anatomik Kompleksliği ve Semptom Şiddeti Arasındaki İlişki

Mustafa Yenerçağ[1], Uğur Arslan[2*], Metin Çoksevim[3], Seçkin Dereli[1], Mustafa Doğduş[4], Güney Erdoğan[2]

1.Deparment of Cardiology, Ordu University Faculty of Medicine, Ordu, Turkey
2.Department of Cardiology, University of Health Sciences Samsun Training and Research Hospital, Samsun, Turkey
3.Deparment of Cardiology, Ondokuz Mayis University Faculty of Medicine, Samsun, Turkey
4.Department of Cardiology, University of Usak Training and Research Hospital, Usak, Turkey

ABSTRACT

Aim: Increased blood viscosity (BV) has good correlaton with lower extremity peripheral artery disease (LEAD). However, the relationship between BV and peripheral arterial disease (PAD) anatomical complexity and symptom severity have not been studied adequately so far. The aim of the present study was to assess the relationship between whole blood viscosity (WBV) and LEAD anatomical complexity and symptom severity.

Methods: The study included 240 consecutive patients with suspected PAD who had lower extremity peripheral angiography between March 2016 and March 2020. A Transatlantic İntersociety Consensus II (TASC II) A-B lesion was defined as anatomical simple LEAD, and a TASC II C-D lesion was defined as anatomical complex LEAD. Symptom severity of all patients were categorized from 0 to 6 according to Rutherford classification. WBV was assessed using a validated calculation formula derived from hematocrit and total plasma protein levels, both at low (LSR) and high (HSR) shear rate.

Results: TASC II C-D group presented significantly higher WBV values both at LSR (40.2 ± 9.5 vs. 46.5 ± 13.2; p < 0.001) and HSR (15.9 ± 0.5 vs. 16.5 ± 0,7; p < 0.001). In ROC analysis, a cut-off value of 16.1 WBV at HSR had 74.5% sensitivity and 68% specificity for predicting TASC II C-D (AUC: 76.2%, p < 0.001) and a cut-off value of 42.9 WBV at LSR had 73.4% sensitivity and 66.6% specificity for predicting TASC II C-D (AUC: 74.2%, p < 0.001). In multivariate analysis, both high WBV at LSR (OR: 2.121, 95% CI: 1.079 – 3.164, p < 0.001) and high WBV at HSR (OR: 2.737, 95% CI: 1.671 – 4.483, p < 0.001) were independent predictors for TASC II C-D. There was a significant positive correlation between WBV at LSR and Rutherford symptom category (0-6) (r = 0.412, p <0.001) and WBV at HSR and Rutherford symptom category (0-6) (r = 0.402, p <0.001).

Conclusion: Our data suggests that; increased WBV values may be associated with TASC II C-D lesions, which indicated more anatomically complex LEAD. Also WBV values positively correlated with Rutherford symptom severity.

Keywords: Blood viscosity, lower extremity, peripheral arterial disease

ÖZ

Amaç: Artmış kan viskozitesinin (KV), alt ekstremite periferik arter hastalığı (AEPAH) ile iyi korelasyonu vardır. Ancak, KV ve peripherik arter hastalığının (PAH) anatomik kompleksiliği ve semptom şiddeti arasındaki ilişki şu ana kadar yeterince çalışılmamıştır. Bu çalışmanın amacı, tam kan viskozitesi ve alt ekstremite periferik arter hastalığının anatomik kompleksliği ve semptom şiddeti arasındaki ilişkiyi değerlendirmektir.

Yöntemler: Çalışmaya Mart 2016 ile Mart 2020 tarihleri arasında alt ekstremite periferik anjiyografisi yapılan ve PAH şüphesi olan 240 ardışık hasta dahil edildi. Transatlantic Intersociety Consensus II (TASC II) A-B lezyonu anatomik olarak basit AEPAH olarak, TASC II C-D lezyonu anatomik olarak kompleks AEPAH olarak tanımlandı. Tüm hastaların semptom şiddeti Rutherford sınıflandırmasına göre 0 ile 6 arasında kategorize edildi. Tam kan viskositesi (TKV), hem düşük (DKH) hem de yüksek kayma hızında (YKH) hematokrit ve toplam plazma protein seviyelerinden türetilen doğrulanmış bir hesaplama formülü kullanılarak değerlendirildi.

Bulgular: TASC II C-D grubu hem DKH'de (40.2 ± 9.5'e karşı 46.5 ± 13.2; p <0.001) hem de YKH'de (15.9 ± 0.5'e karşı 16.5 ± 0,7; p <0.001) anlamlı olarak daha yüksek TKV değerleri gösterdi. ROC analizinde, YKH'deki 16,1'lik TKV kesme değeri TASC II C-D'yi tahmin etmek için % 74,5 duyarlılık ve % 68 özgüllük (p <0,001) gösterdi. DKH'deki 42,9'luk TKV kesme değeri TASC II C-D'yi tahmin etmek için %73,4 duyarlılık ve %66.6 özgüllük (p <0.001) gösterdi. Çok değişkenli analizde, hem DKH'de yüksek TKV (OR: 2.121, % 95 CI: 1.079 - 3.164, p <0.001) hem de YKH'de yüksek TKV (OR: 2.737, % 95 CI: 1.671 - 4.483, p <0.001) TASC II C-D için bağımsız predictor olarak saptandı. DKH'deki TKV ile YKH'deki TKV değerleri Rutherford semptom kategorisi ile (0-6) anlamlı pozitif korelasyon gösterdi (r = 0.412, p <0.001; r = 0.402, p <0.001, sırasıyla).

Sonuç: Verilerimiz gösteriyor ki; artmış TKV değerleri, daha anatomik olarak kompleks AEPAH'ı gösteren TASC II C-D lezyonları ile ilişkili olabilir. Ayrıca TKV değerleri, Rutherford semptom şiddeti ile pozitif korelasyon gösterdi.

Anahtar Kelimeler: Kan viskozitesi, alt ekstremite, periferal arteriyel hastalık

Received: 20.10.2020 Accepted: 23.12.2020 Published (Online): 23.04.2021

*Coresponding Author: Uğur Arslan, University of Health Sciences Samsun Training and Research Hospital, Department of Cardiology, Samsun, Türkiye. +905326039983, ugurarslan5@yahoo.com

ORCİD: 0000-0001-8572-3571

To cited: Yenerçağ M, Arslan U, Çoksevim M, Dereli S, Doğduş M, Erdoğan G. Relationship Between Whole Blood Viscosity and Lower Extremity Peripheral Artery Disease Anatomical Complexity and Symptom Severity Acta Med. Alanya 2021;5(1):66-74. doi:10.30565/medalanya.828026

INTRODUCTION

From mild plaque formation to chronic total occlusion lower extremity peripheral arterial disease (PAD) is a spectrum of atherosclerotic disease. There can be asymptomatic patients in the peak of the disease, however, symptoms may occur such as pain in the leg in any form (resting or active), gangrene and intermittent claudication within the process of the disease progression. Clinical representation of the disease is often draw parallel with the austerity of atherosclerosis [1]. TASC II sorting is the unity of ideas that is utilized for the evaluation of lower extremity peripheral arterial disease (LEAD) in consonance with the anatomic distribution and number and nature of lesions (stenosis, occlusion). The suggested classification for grading the symptom severity of PAD is the Rutherford classification [2]. TASC II classification, indicates the anatomical complexity of LEAD. Rutherford classification, indicates the symptom severity of LEAD.

A common cardiovascular disease that occur by the inflammation and endothelial dysfunction of which result in occlusive plaque formation within the arterial wall is atherosclerosis. Thus, endotelial shear stress (ESS) and haematological factors have a crucial role in the pathophysiology of the atherosclerotic process. The most crucial predictive factor of ESS is blood viscosity (BV) [3] whose major predictive factor is hematocrit, is a primary property of blood correlated to its internal erosion that causes blood to withstand flow. In consonance with the data, whole blood viscosity (WBV) has been proven as the predictor in several cardiovascular diseases. Furthermore, up-to-date studies have indicated that BV was in correlation with the increased prevelence of PAD [4,5]. Relationship between WBV and severity of LEAD has not been taken into consideration yet. In this study, we aim to evaluate the relationship between WBV and LEAD and anatomical complexity and sypmtom severity

MATERIAL AND METHODS

Patient selection

This cross-sectional retrospective study enrolled 240 patients with LEAD who underwent peripheral lower extremity angiography at Samsun Training and Research Hospital cardiology and cardiovascular surgery clinics due to suspected PAD in noninvasive tests between March 2016 and March 2020. Demographical data, baseline cardiovascular risk factors, medical history, antiagregan and anticoagulan drug used, echocardiographic data, laboratory values, ankle-brachial index (ABI) and peripheral angiography recordings were obtained using the hospital's medical database. Patiens who had former peripheral revascularization, anticoagulant drug utilization for each and every reason, acute coronary syndromes, non-atherosclerotic stenosis, patients with baseline anemia, history of blood transfusion within the last three months, active infection, chronic inflammatory diseases, malignancy, decompensated heart failure and missing data were not included in the study. The research procedures were revised and approved by the local hospital's ethics committee, according to the ethical considerations stipulated in the Helsinki Declaration.

Evaluation of LEAD complexity and symptom severity

Peripheral angiography was executed with a 6-French pigtail catheter by the utilization of an automatic pump injector. In order to do the assessment of lower extremities, the tip of the catheter was placed on top of the aorto iliac bifurcation. LEAD anatomical complexity was evaluated with TASC II classification. The Trans-Atlantic Inter Society Consensus (TASC) II score analysis (Table 1) was performed on bilateral aorto-iliac and femoro-popliteal arterial segments [6]. The patients' angiographic data were taken into consideration from the records of catheter laboratory by two interventional cardiologists and the TASC II grade was noted for each patient. Interobserver variations for measurements were less than 5%. A TASC II A-B lesion was defined as simple LEAD, and a TASC II C-D lesion was defined as complex LEAD.

Anamnesis, physical examination and ABI of all patients were recorded from the hospital database. The severity of their symptoms was categorized according to Rutherford's classification (symptom category, 0-6 score) [7] (Table 2).

Echocardiography

Table 1. TASC II classification for the assessment of anatomical complexity of peripheral arterial diseases [6]

	AORTO-ILIAC LESIONS	FEMORO-POPLITEAL LESIONS
TASC II-A	Single stenosis (<3 cm in length) in the CIA or EIA (unilateral / bilateral)	Single stenosis (<3 cm in length) in the superficial femoral artery or popliteal artery
TASC II-B	1. Single stenosis (3–10 cm in length) not extending into the CFA 2. Heavily calcified stenosis up to 3 cm in length 3. Unilateral CIA occlusion	1. Single stenosis (3–10 cm in length) not involving distal popliteal artery 2. Heavily calcified stenosis up to 3 cm in length 3. Multiple lesions, each <3 cm in length (stenoses or occlusions) 4. Single or multiple lesions in the absence of continuous tibial runo_ to improve inflow for distal surgical bypass
TASC II-C	1. Bilateral stenosis (5–10 cm in length) in the CIA and / or EIA, not extending into the CFA 2. Multiple stenoses or occlusions (each 3–5 cm in length) 2. Unilateral EIA occlusion not extending into the CFA with or without heavy calcification 3. Unilateral EIA stenosis extending into the CFA 4. Bilateral CIA occlusion	1. Single stenosis or occlusion >5 cm in length 2. Unilateral EIA occlusion not extending into the CFA with or without heavy calcification
TASC II-D	1. Diffuse, multiple unilateral stenosis involving the CIA, EIA, and CFA (usually >10 cm in length) 2. Unilateral occlusion involving both the CIA and EIA 3. Bilateral EIA occlusions 4. Di_use disease involving the aorta and both iliac arteries 5. Iliac stenosis in a patient with abdominal aortic aneurysm or other lesions requiring aortic or iliac surgery	Complete CFA or superficial femoral artery occlusion or complete popliteal and proximal trifurcation occlusions

TASC-II: Trans-Atlantic Inter-Society Consensus-2; CIA: Common iliac artery; CFA: Common femoral artery; EIA: External iliac artery

Table 2. Rutherford classification in patients with lower extremity artery disease [7]

Grade	Category	Symptoms
0	0	Asymptomatic.
I	1	Mild claudication
I	2	Moderate claudication
I	3	Severe claudication.
II	4	Ischemic rest pain
III	5	Ischaemic ulceration not exceeding ulcers of the digits of the foot (Minor tissue loss)
III	6	Severe ischaemic ulcers or frank gangrene (Major tissue loss)

Echocardiographic assessment was executed by the utilization of a VIVID 9-dimensional cardiovascular ultrasound system (Vingmed-General Electric, Horten, Norway) with a 3.5 MHz transducer. Echocardiographic examination were executed in left lateral decubitus position. Parasternal long and short axis views and apical views were used as standard imaging windows. The left ventricular ejection fraction (LVEF) was determined from the apical window by the utilization of the modified Simpson method.

Blood sampling

Biochemistry tubes without anticoagulants were used for biochemical tests and EDTA tubes for hematological tests.

WBV was calculated from hematocrit (HCT) and plasma total protein (TP) at a low shear rate (LSR; 0.5 s-1) and a high shear rate (HSR; 208 s-1) by the previously validated formula of de Simone et al. [8] :

For HSR, the WBV (208/sec-1) formula: (0.12 × HCT) + 0.17 (TP − 2.07)

For LSR, the WBV (0.5/sec-1) formula: (1.89 ×

HCT) + 3.76 (TP − 78.42).

The two of the WBV values were determined by taking into consideration Nwose and Richards' suggestions in the series on 'Whole Blood Viscosity Assessment Issues' that extrapolation of WBV from HCT in % and TP in g/L is the most applicable approach, specifically for estimating WBV in LSR [9,10]. Overall data were measured up between the TASC II A-B (simple LEAD) and TASC II C-D (complex LEAD) groups. Two different logistic regression models were established for WBV at a LSR and WBV at a HSR (models 1 and 2) to determine the risk factors affecting LEAD anatomical complexity. Separate risk elements of which are affecting LEAD austerity were measured up between each other by using these models.

Statistical Analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences 22.0 (SPSS Inc., Chicago, IL, USA) program. In order to test normality of distribution Kolmogorov–Smirnov test was used. Quantitative variables with a normal distribution were specified as the mean ± standard deviation. Categorical variables were shown as number and percentage values. The independent samples t-test was used to compare normally distributed quantitative data and the Mann–Whitney U-test was used to analyze data that did not follow a normal distribution. Categorical data were analyzed using the chi-square test. The individual effects of all variables were examined in a univariate binary logistic regression analysis. Two different logistic regression models were established separately for LSR and HSR (Model 1 and 2); independent risk factors affecting outcome were calculated using multivariate logistic regression analysis, and the comparative results are presented. To determine the best predictive WBV for both shear rate for LEAD anatomical complexity, we used ROC curve analysis, and area under the curve, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy values corresponding to each cut-off value are presented. Odds ratios and 95% confidence intervals were calculated, and sensitivity and specificity values were generated for outcome classification. The correlation of WBV values at low shear rates with WBV values at high shear rates was assessed with Spearman's correlation coefficient. In addition, spearman correlation analysis was performed to examine the relationship between WBV at HSR, WBV at LSR and Rutherford symptom severity category (0-6). A p value of <0.05 was accepted as statistically significant.

RESULTS

Demographic, clinic and laboratory data of the study groups are summarized in Table 3. A total of 135 patients (44.1%) were male, and the mean age of the study population was 70.4 ± 10.5 years old. There were no significant differences between the two groups in terms of baseline demographic characteristics, medical history and medical treatment. Smoking was significantly higher in the TASC II C-D group. Hemoglobin (13.3 ± 1.2 g/dl vs. 13.9 ± 1.0 g/dl; p = 0.002), hematocrit (40.0% ± 3.6 vs. 42.3% ± 3.5; p < 0.001), total protein (6.8 ± 2.5 mg/dl vs. 7.3 ± 2.7 mg/dl; p = 0.012), low density lipoprotein cholesterol (97.9 ± 23 mg/dl vs. 112.8 ± 240 mg/dl; p = 0.005) levels, ABI (0.63 ± 0.12 vs 0.45 ± 0.22) and WBV values were significantly higher both for HSR (15.9 ± 0.5 vs. 16.5 ± 0,7; p < 0.001) and for LSR (40.2 ± 9.5 vs. 46.5 ± 13.2; p < 0.001) were significantly higher in the TASC II C-D group than the TASC II A-B group. The ROC curve analysis explored the discriminatory capability of WBV values at both shear rates for complex PAD. Using a cut-off value 42.9 of WBV at LSR (area under the curve = 0.742, p<0.001) had a 73.4% sensitivity and 66.6% specificity, and a cut-off value of 16.1 WBV at HSR (area under the curve = 0.762, p < 0.001) had a 74.5% sensitivity and 68.0% specificity (Figure 1). The associations of possible risk factors with anatomically complex LEAD were evaluated in univariate and multivariate logistic regression analysis. Univariate regression analyses showed that smoking, age, hypertension, diabetes mellitus, WBV at HSR and WBV at LSR were significantly associated with the occurrence of anatomically complex LEAD (for all, p < 0.05). We constituted two different models to further analyze the predictiveness of WBV levels for each shear rate at multivariate analysis (Model 1 and 2). In multivariate models adjusted with smoking, age, hypertension, diabetes mellitus and low density lipoprotein cholesterol; having a high WBV at LSR (OR: 2.121, 95% CI: 1.079 – 3.164,

p < 0.001) and having a high WBV at HSR (OR: 2.737, 95% CI: 1.671 – 4.483, p < 0.001) were found as independent predictors of anatomically complex LEAD (Table 4). In spearmen correlation analysis, WBV at HSR was significantly correlated with WBV at LSR (rs = 0.906; p < 0.001). There was a significant positive correlation between LSR at WBV and Rutherford categories (0-6) (r = 0.412, p < 0.001) and HSR at WBV and Rutherford categories (0-6) (r = 0.402, p < 0.001) (Figure 2A-B, respectively).

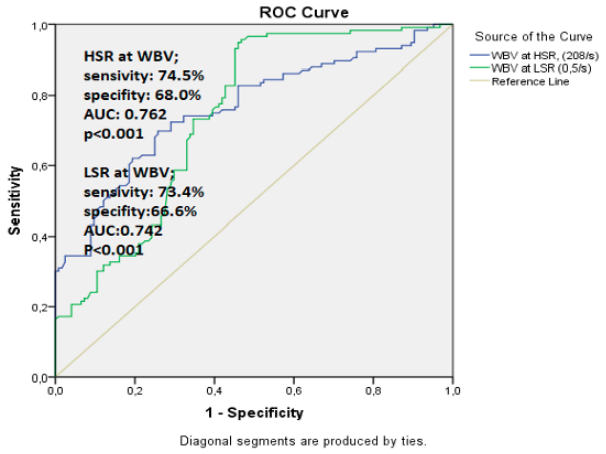

Figure 1. ROC curve analysis showing the predictive cut-off value of WBV at a high shear rate (blue line) and low shear rate (green line) for anatomically complex LEAD. LEAD, lower extremity peripheral artery disease; AUC, area under the curve; HSR, high shear rate; LSR, low shear rate; ROC, receiver operating characteristic; WBV, whole blood viscosity.

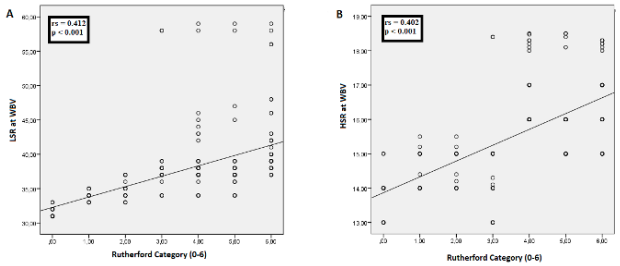

Figure 2. (A) Correlation between WBV at LSR and Rutherford categories, (B) Correlation between WBV at HSR and Rutherford categories.

DISCUSSION

This study demonstrates for the first time in the literature that the increased WBV is may be independent risk factor for the anatomical complexity of LEAD. Increased WBV levels may be associated with TASC II C-D lesions, which indicated a more anatomically complex LEAD. Besides, increased WBV values in LSR and HSR correlated positively with symptom severity assessed by Rutherford categorization.

Blood viscosity is the intrinsic resistance of blood flow in vessels. Its major determinants are: red blood cells, plasma viscosity, red cell deformation and aggregation. It has been hypothesized that increasing levels of blood viscosity within the general population may promote cardiovascular events through its potential theoretical effects on atherogenesis, thrombogenesis, or ischaemia [5]. Recent epidemiological studies demonstrated that association between blood viscosity and the conventional risk factors such as male sex, smoking, blood pressure and plasma lipids. But, in routine clinical practice, evaluation of WBV is limited. As a major component of Virchow's triad, WBV was rarely studied because of the requirement for various parameters during its evaluation. In their study De Simone et al. evaluated WBV with a simple, costless equation using total protein and HCT levels at different shear rates [8]. The prognostic value of estimated WBV calculated by this formula has been demonstrated in many studies for cardiovascular diseases [11-15]. In our study, we also preferred to use this formula to evaluate WBV.

Atherosclerosis is a leading cause of vascular disease around the worldwide. Its major clinical manifestations include ischemic heart disease, ischemic stroke, and PAD. The process of atherosclerosis is complex and multifactorial. Regular vascular physiology includes prevention of pathological conditions such as inflammation, proliferation, thrombosis and atherosclerosis. Endothelium is the most important structure that provides this protective effect. Shear stress created by blood flow on the endothelial surface plays an important role in the atherosclerotic process. Endothelial shear stress (ESS) is the most important component of blood viscosity [16]). Changes in blood viscosity affect the atherosclerotic process by direct and indirect mechanisms. Systemic vascular factors such as smoking, hyperlipidemia, hyperglycemia and hypertension, with increased blood viscosity causes endothelial dysfunction and promotes chronic fibro-inflammatory response. This response is associated with a disarming of the

Table 3. Demographic, procedural and clinical data for the study group.

Variables	TASC II A-B	TASC II C-D	p-value
	(n=124)	(n=116)	
Age, years	69.1 ± 10.9	71.7± 11.1	0.070
Gender, male n,(%)	67 (54)	68 (58.6)	0.471
Hypertension n,(%)	92 (74.1)	95 (81.8)	0.102
Diabetes Mellitus n,(%)	44 (35.4)	46 (39.7)	0.073
Hyperlipidemia n,(%)	46 (37.1)	42 (36.2)	0.880
Coronary Artery Disease n,(%)	40 (32.2)	47 (31.9)	0.090
Smoking n,(%)	49 (39.5)	61 (52.6)	0.042
eGFR, ml/min/1.73 m2	14.5 ± 0.2	15 ± 0.3	0.850
LVEF, %	53.6 ± 8.0	53.2 ± 8.7	0.740
Ankle-brachial index	0.63 ± 0.12	0.45 ± 0.22	0.001
Hemoglobin, g/Dl	13.3 ± 1.2	13.9 ± 1.0	0.002
Hematocrit, %	40.0 ± 3.6	42.3 ± 3.5	<0.001
White Blood Cell, 10^3/µl	10.2 ± 2.8	10.3 ± 2.9	0.690
Platelet, 10^3/µl	241 ± 73	244 ± 71	0.570
Total Protein, g/L	68.8 ± 2.5	73.4 ± 2.7	0.012
Albumin, g/Dl	3.6 ± 0.3	3.9 ± 0.4	0.071
Glucose, mg/dl	105 ± 17	107 ± 14	0.120
Total cholesterol, mg/dL	172 ± 35	169 ± 37	0.770
LDL cholesterol, mg/dl	97.9 ± 23	112.8 ± 240	0.005
HDL cholesterol, mg/dl	39 ± 10	36 ± 11	0.880
Triglycerides, mg/dl	118 ± 51	123 ± 56	0.470
Antidiabetic n,(%)	44 (35.4)	46 (39.6)	0.262
Statin n,(%)	50 (40.3)	54 (46.5)	0.183
ACEi/ARB, n (%)	75 (60.4)	78 (67.2)	0.691
CCB n,(%)	23 (18.5)	20 (17.2)	0.380
Beta blocker n,(%)	50 (81)	47 (78)	0.320
Pentoxifylline n,(%)	60 (48.3)	63 (50.8)	0.260
Cilastazol n,(%)	50 (40.3)	53 (42.7)	0.165
Acetylsalicylic acid n,(%)	40 (32.2)	44 (37.9)	0.196
Clopidogrel n,(%)	19 (15.3)	20 (17.2)	0.234
WBV at LSR (0,5/s-1)	40.2 ± 9.5	46.5 ± 13.2	<0.001
WBV at HSR (208/s-1)	15.9 ± 0.5	16.5 ± 0.7	<0.001

Quantitative variables with a normal distribution were specified as the mean ± standard deviation. Categorical variables were shown as number and percentage values . LVEF, Left ventricular ejection fraction; eGFR, estimated Glomerular filtration rate; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; ACE-i/ARB, Angiotensin converting enzyme inhibitor/angiotensin receptor blocker; CCB, Calcium channel blocker; WBV at HSR, Whole blood viscosity at high shear rate; WBV at LSR, Whole blood viscosity at low shear rate.

Table 4. The effects of variables on anatomically complex LEAD in univariate and multivariate logistic regression analysis

Variables	Odds Ratio (OR)	95% CI	p-value
Univariate logistic regression analysis			
Age	1.022	(0.998– 1.047)	0.030
Hypertension	1.869	(0.879– 3.975)	0.021
Diabetes Mellitus	1.739	(1.011 – 2.992)	0.011
eGFR	0.962	(0.562 – 1.628)	0.885
Coronary artery disease	1.347	(0.769 – 2.359)	0.292
Smoking	1.698	(1.017– 2.833)	0.045
LDL cholesterol, mg/dL	1.008	(0.998– 1.019)	0.021
Triglycerides, mg/dl	1.002	(0.997 – 1006)	0.470
White Blood Cell (10^3 µl)	1.017	(0.934 – 1.108)	0.690
Platelet (10^3 µl)	1.001	(0.997– 1.004)	0.681
WBV at LSR (0,5/s-1)	1.047	(1.022 – 1.074)	<0.001
WBV at HSR (208/s-1)	2.547	(1.541 – 3.917)	<0.001
Multivariate logistic regression analysis			
MODEL-1			
LDL cholesterol, mg/dL	1.010	(0.997– 1.023)	0.128
Age	0.531	(0.157 – 1.797)	0.309
Hypertension	1.939	(0.798– 4.712)	0.055
Diabetes Mellitus	1.254	(1.209 – 1.303)	0.078
Smoking	1.911	(1.108– 3.652)	0.052
WBV at LSR (0,5/s-1)	2.121	(1.079 – 3.164)	<0.001
MODEL-2			
LDL cholesterol, mg/dL	1.008	(0.997 – 1.020)	0.172
Age	1.023	(0.998 – 1.050)	0.079
Hypertension	1.914	(0.849 – 4.314)	0.067
Diabetes Mellitus	1.290	(1.112 – 1.562)	0.062
Smoking	1.914	(1.100 – 3.032)	0.058
WBV at HSR (208/s-1)	2.737	(1.671 – 4.483)	<0.001

WBV at HSR: Whole blood viscosity at high shear rate, WBV at LSR: Whole blood viscosity at low shear rate. LDL, low-density lipoprotein. eGFR, estimated Glomerular filtration rate

atheroprotective defenses encouraged by laminar blood flow. The consequences of this dynamic interaction between shear stress and systemic risk factors not only promotes atherosclerosis but also influences the disease progression and clinical outcomes. [17]. Recent studies have shown that WBV is associated with carotid thickening, coronary artery disease and PAD suggesting that rheologic factors are involved in the subclinical phase of atherosclerosis [18]. Futhermore, several large prospective studies have shown the important link between WBV and the risk of cardiovascular events [19,20]. In our study, we have found that WBV, LDL cholesterol

were higher and smoking was more prevalent in patients with anatomical complex LEAD (TASC II C-D) when compared to patients with anatomical simple LEAD (TASC II A-B). Accordingly, we have thought that these parameters act concordantly in the pathogenesis of atherosclerosis progression and may be closely related with the anatomical complexity of LEAD.

PAD is an atherosclerotic disease and can vary from small plaque formation to chronic totally occlusion of the arteries. Therefore, mild forms of the disease may be asymptomatic, and critical leg ischemia may occur as the condition progresses. The clinical presentation correlates with the involved artery segment. Numerous schemes have been developed to classify patients for clinical or prognostic features. Classification schemes are based on the symptoms of the patients, the anatomical distribution of the disease, or a combination of clinical factors such as the presence of limb ischemia and/or infection. Two systems are most commonly used to classify PAD; Rutherford classification [7] and TASC II classification [2]. LEAD symptom severity can be evaluated with the Rutherford classification. TASC II classification reflects the anatomical complexity of LEAD. TASC II C and TASC II D groups indicate a more complex and more diffused atherosclerotic involvement. Interventional treatment of the TASC II C-D lesions are also challenged when compared to TASC II A-B [6]. Therefore, in our study, we evaluated the patients as a simple (TASC II A-B) and complex (TASC II C-D) group in order to obtain more accurate statistical results.

Blood rheological factors are associated with peripheral arterial disese in the general population. Any change in hemorheological factors affects atherosclerotic process and microvascular circulation. The relationship of many hematological parameters with PAD has been evaluated [21,22]. WBV among hematological parameters plays an important role in peripheral artery disease. Increased WBV can contribute to the progression of atherosclerosis by changing ESS. Also, increased stasis, thrombosis and increased peripheral vascular resistance are thought to cause exacerbation of peripheral artery disease. Edinburgh Artery Study, involving 1581 adults assessed for peripheral artery disease, showed that blood viscosity independently associated with peripheral arterial occlusion and severity of the disease [23]. In the West of Scotland Coronary Prevention Study that enrolled 6595 patients, it was shown that WBV were associated with an peripheral atherosclerosis [4]. In our study, higher WBV values were found in the TASC II C-D group, in which the anatomical complexity of PAD was evaluated and accepted as an indicator of atherosclerotic burden, compared to the TASC II A-B group. In addition, WBV has been shown to be independently associated with the anatomical complexity of LEAD. These results support the close relationship between anatomical complexity of LEAD and WBV.

Blood rheological factors elevations may further contribute to the aggravation of symptoms by further reducing blood flow in patients with PAD. In Edinburgh study, the relationship between increased blood viscosity and symptom severity of PAD was shown [23]. Dormandy et al. found that there was a significant relationship between the worsening of peripheral circulatory disorders and baseline blood viscosity levels [24]. A second study by the same group reported that blood viscosity was higher in PAD patients with resting pain than in those with intermittent pain [25]. A third study by Dormandy et al., compared intermittent claudication with controls gruops and found blood viscosity was significantly higher in claudication group [26]. The researchers suggested that hyperviscosity may be the determining cause of claudication in LEAD patients. The Rutherford classification is used to evaluate lower extremity arterial ischemia according to the presence of ischemic signs and symptoms. Requirement of perfusion is determined according to the Rutherford category. In our study, we found that the symptom severity of LEAD evaluated by the Rutherford category was positive correlated with WBV. This result demonstrated that the positive correlation of increased WBV with increased symptom severity in LEAD. These results suggest that blood viscosity plays a critical role in the pathogenesis of lower-limb ischemia in the general population.

Smoking and blood viscosity are important risk factors of atherosclerosis. The close relationship between smoking and blood viscosity has been

also shown in many studies. Shimada et al. found that high blood viscosity was closely related to smoking and significantly decreased when smoking was quitted [27]. Increased hematocrit levels in smokers may also increase the blood viscosity which is calculated according to the formula used in our study. We have found that both smoking history and whole blood viscosity were significantly higher in anatomically complex LEAD when compared to simple LEAD. However, in our study, we have also shown that there is an independent relationship between whole blood viscosity and anatomical complexity of LEAD after the multivariate analysis which includes smoking as a separate parameter.

Limitations: Our study has several limitations. The other clinical classifications representing the severity of LEAD was not performed. The comments were done according to angiographic TASC II classification. The modest sample size, retrospective, non-randomized and single-center study are important methodological shortcomings. WBV was not validated by accurate measurement of viscosity using a viscometer. The extrapolation formula that we used in our study has been validated and utilized in several other studies but direct comparison of estimated and directly measured WBV in this patient population may strengthen our results and serve precision. In addition, other hemorheological factors that may affect blood viscosity such as platelet and erythrocyte aggregability and rigidity were not evaluated.

Conclusıon: To the best of our knowledge, this is the first study to evaluate the relationship between WBV and LEAD anatomical complexity and symptom severity. The results of this study demonstrate that the increased WBV values at LSR and HSR were an independent risk factor for TASC II C-D lesions, which indicated a more anatomically complex LEAD. Besides, WBV values in LSR and HSR positively correlated with symptom severity. Multicentre and prospective studies are needed in large populations to better characterize the relationship between anatomical complexity and symptom severity of LEAD and WBV.

Conflict of Interest: No conflict of interest was declared by the authors.

Funding sources: The authors declared that this study received no financial support.

Ethics Committee Approval: Samsun Training and Research Hospital non-interventional clinical research ethics committee (aprroval date-number:05.06.2020-8)

Peer-review: Externally and internally peer-reviewed.

REFERENCES

1. Campia U, Gerhard-Herman M, Piazza G, Goldhaber SZ. Peripheral Artery Disease: Past, Present, and Future. Am J Med. 2019;132(10):1133-41. doi:10.1016/j.amjmed.2019.04.043.
2. Hardman RL, Jazaeri O, Yi J, Smith M, Gupta R. Overview of classification systems in peripheral artery disease. Semin Intervent Radiol. 2014;31(4):378-88. doi:10.1055/s-0034-1393976.
3. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J Am Coll Cardiol. 2007;49(25):2379-93. doi: 10.1016/j.jacc.2007.02.059.
4. Lowe G, Rumley A, Norrie J, Ford I, Shepherd J, Cobbe S, et al. Blood rheology, cardiovascular risk factors, and cardiovascular disease: the West of Scotland Coronary Prevention Study Thromb Haemost. 2000;84(4):553-8. PMID: 11057849.
5. Lowe GD, Lee AJ, Rumley A, Price JF, Fowkes FG. Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. Br J Haematol. 1997;96(1):168-73. doi: 10.1046/j.1365-2141.1997.8532481.x.
6. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). J Vasc Surg. 2007;45 Suppl S:S5-67. doi: 10.1016/j.jvs.2006.12.037.
7. Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, Ahn S, et al. Recommended standards for reports dealing with lower extremity ischemia: revised version. J Vasc Surg. 1997;26(3):517-38. doi:10.1016/s0741-5214(97)70045-4.
8. de Simone G, Devereux RB, Chien S, Alderman MH, Atlas SA, Laragh JH. Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults. Circulation. 1990;81(1):107-17. doi: 10.1161/01.cir.81.1.107.
9. Nwose EU, Richards RS. Whole blood viscosity extrapolation formula: Note on appropriateness of units. N Am J Med Sci. 2011;3(8):384-86. doi:10.4297/najms.2011.3384.
10. Daniel J. Cho. Blood Viscosity Abnormalities in Large and Small Vessel Diseases: Future Directions for Plasma Medicine. 2012;2: 221–235. doi: 10.1615/PlasmaMed.2014010786.
11. Cetin EH, Cetin MS, Canpolat U, Aydin S, Aras D, Topaloglu S, et al. Prognostic significance of whole blood viscosity estimated by de Simone's formula in ST-elevation myocardial infarction. Biomark Med. 2016;10(5):495–511. doi: 10.2217/bmm.16.10.
12. Çekici Y, Kılıç S, Saraçoğlu E, Çetin M, Veysel Düzen İ, Yılmaz M. The Relationship between Blood Viscosity and Isolated Coronary Artery Ectasia. Acta Cardiol Sin. 2019;35(1):20-6. doi:10.6515/ACS.201901_35(1).20180701A.
13. Erdogan G, Yenercag M, Arslan U.The Relationship between Blood Viscosity and Acute Arterial Occlusion. Journal of Cardiovascular Emergencies. 2020;6:7-12. doi: 10.2478/jce-2020-0002.
14. Ozcan Cetin EH, Cetin MS, Çağlı K, Temizhan A, Özbay MB, Ediboglu E, et al. The association of estimated whole blood viscosity with hemodynamic parameters and prognosis in patients with heart failure. Biomark Med. 2019;13(2):69-82. doi: 10.2217/bmm-2018-0309.
15. K Dolu A, Korkmaz A, Kundi H, Guray U. Whole blood viscosity predicts nondipping circadian pattern in essential hypertension. Biomark Med. 2020;14(14):1307-16. doi: 10.2217/bmm-2020-0249.
16. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. Lab Invest. 2005;85(1):9-23. doi: 10.1038/labinvest.3700215.
17. Celik T, Balta S, Ozturk C, Iyisoy A. Whole Blood Viscosity and Cardiovascular Diseases: A Forgotten Old Player of the Game. Med Princ Pract. 2016;25(5):499-500. doi: 10.1159/000446916.
18. Celik T, Yilmaz MI, Balta S, Ozturk C, Unal HU, Aparci M, et al. The Relationship Between Plasma Whole Blood Viscosity and Cardiovascular Events in Patients With Chronic Kidney Disease. Clin Appl Thromb Hemost. 2017;23(6):663-70. doi:

10.1177/1076029616634888.

19. Stone PH, Saito S, Takahashi S, Makita Y, Nakamura S, Kawasaki T, et al. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. Circulation. 2012;126(2):172-81. doi: 10.1161/CIRCULATIONAHA.112.096438.
20. Lee AJ, Mowbray PI, Lowe GD, Rumley A, Fowkes FG, Allan PL. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh Artery Study. Circulation. 1998;97(15):1467-73. doi: 10.1161/01.cir.97.15.1467.
21. Smith FB, Lowe GD, Lee AJ, Rumley A, Leng GC, Fowkes FG. Smoking, hemorheologic factors, and progression of peripheral arterial disease in patients with claudication. J Vasc Surg. 1998;28(1):129-35. doi: 10.1016/s0741-5214(98)70208-3.
22. Cho YI, Cho DJ, Rosenson RS. Endothelial shear stress and blood viscosity in peripheral arterial disease. Curr Atheroscler Rep. 2014;16(4):404. doi: 10.1007/s11883-014-0404-6.
23. Lowe GD, Fowkes FG, Dawes J, Donnan PT, Lennie SE, Housley E, et al. Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study. Circulation. 1993;87(6):1915-20. doi: 10.1161/01.cir.87.6.1915.
24. Dormandy JA, Hoare E, Khattab AH, Arrowsmith DE, Dormandy TL, et al. Prognostic significance of rheological and biochemical findings in patients with intermittent claudication. Br Med J. 1973;4(5892):581-3. doi: 10.1136/bmj.4.5892.581.
25. Dormandy JA, Hoare E, Colley J, Arrowsmith DE, Dormandy TL, et al. Clinical, haemodynamic, rheological, and biochemical findings in 126 patients with intermittent claudication. Br Med J. 1973;4(5892):576-81. doi: 10.1136/bmj.4.5892.576.
26. Dormandy JA, Hoare E, Postlethwaite J. Importance of blood viscosity. Rheological claudication. Proc R Soc Med. 1974;67(6 Pt 1):446-7. PMID: 4852367.
27. Shimada S, Hasegawa K, Wada H, Terashima S, Satoh-Asahara N, Yamakage H, Kitaoka S, Akao M, Shimatsu A, Takahashi Y. High blood viscosity is closely associated with cigarette smoking and markedly reduced by smoking cessation. Circ J. 2011;75(1):185-9. doi: 10.1253/circj.cj-10-0335

Author / ORCID	Authorship Contrubition
Mustafa Yenerçağ 0000-0002-0933-7852	Consept and/or Design, Materials and/ orPractices, Data collection and/or Processing, Manuscript Writing, Final approval.
Uğur Arslan 0000-0001-8572-3571	Consept and/or Design, Süpervision and/or Critical Review, Materials and/ or Practices, Manuscript Writing,Final approval.
Metin Çoksevim 0000-0001-6907-6941	Literatüre Search, Data collection and/or Processing, Analysis and/ or Interpretation:, Materials and/or Practices.
Seçkin Dereli 0000-0003-0090-3835	Analysis and/or Interpretation, Literatüre Search, Materials and/or Practices.
Mustafa Doğduş 0000-0002-3895-1923	Analysis and/or Interpretation, Critical Review, Materials and/or Practices, Data collection and/or Processing.
Güney Erdoğan 0000-0001-5205-1326	Data collection and/or Processing, Analysis and/or Interpretation, Supervision and/or Critical Review, Materials and/or Practices.