Tissue Factor and Vascular Endothelial Growth Factor in Detecting Thromboembolic Complications in Diabetic Atherosclerotic Patients

Tijen Alkan Bozkaya[1,2,3] (ID), Şanser Ateş[1], Ünsal Veli Üstündağ[4], Çağrı Çakıcı[4], İlknur Keskin[5], Pakize Yiğit[6], Ahmet Yiğitbaşı[3] and Nesrin Emekli[4]

[1]Department of Cardiovascular Surgery, Koç University Hospital, Istanbul, Turkey. [2]Department of Cardiovascular Surgery, Yeditepe University Hospital, Istanbul, Turkey. [3]Biochemistry Program, Institute of Health Sciences, İstanbul Medipol University, Istanbul, Turkey. [4]Departmant of Biochemistry, School of Medicine, Istanbul Medipol University, Istanbul, Turkey. [5]Department of Histology and Embryology, School of Medicine, Istanbul Medipol University, Istanbul, Turkey. [6]Department of Biostatistics and Medical Informatics, School of Medicine, Istanbul Medipol University, Istanbul, Turkey.

ABSTRACT

BACKGROUND: Atherosclerosis, which is one of the leading causes of death all over the world, can create major or minor thromboembolic complications with the exponentially increasing diabetic status. Despite all the studies, the mechanism by which endothelial damage in atherosclerosis is triggered in diabetic setting is still not fully understood.

METHODS: In this study, tissue factor (TF), which is thought to act together in the formation of vasular endothelial growth factor (VEGF-A) and coagulopathy in diabetic atherosclerotic patients, may be an important indicator in this regard, a total of 100 cases who were undergone off-pump coronary artery bypass (OPCAB) which were at same risk group examined by dividing into diabetic status. Early postoperative process and biochemical parameters analyzed in terms of TF and VEGF-A levels measured before and after the operation.

RESULTS: TF and VEGF-A expression of the T1DM group were statistically high compared to non-diabetics. Significantly longer hospital stays with changes in TF and VEGF-A were found in patients in the diabetic group compared to pre- and postoperatively, respectively; TF (95% CI: 0.879–0.992; p = 0.025), VEGF-A (95% CI: 0.964–0.991; $p = 0.001$) and hospital stay (95% CI: 1.96–7.49; $p = 0.0001$). Preoperatively measured carotid intima-media thickness (CT) was higher in diabetics and was significantly associated with atrial fibrillation (AF), (r = 0.873). Surgical team and protocols were same and OPCAB procedures were routinely applied to all patients in our clinic. No minor or major events were observed in any of the cases.

CONCLUSION: TF and VEGF-A values in patients with diabetic atherosclerosis may be important in the early detection of thromboembolic complications.

KEYWORDS: atherosclerosis, diabetes, tissue factor, vascular endothelial growth factor, atrial fibrillation

Background

Diabetic status is one of the leading causes of death worldwide due to its vascular effects, and its association with atherosclerosis increases the risk of cardiovascular disease.[1] Endothelial cells, which also take part in vascularization, function dynamically via synthesize many activators as well as on the surface between the blood and vessel wall, and inhibitor molecules for establish an optimal balance.[2]

In this endothelial dynamic balance, it has been reported in many studies that tissue factor (TF) has multidimensional biological effects affecting hemostasis, thrombosis, angiogenesis, and inflammation, and thus is effective in micro and macrovascular complications that occur when this delicate balance is disrupted.[3] Since high TF values, which are the main triggers of coagulation, are seen in atherosclerotic plaque, plaque rupture,

and thrombus formation usually follow each other.[4] TF has also been shown to be essential for normal embryonic development. When the TF gene is silenced, embryonic death is encountered.[5] TF induces migration of vascular SMCs, angiogenesis, activation of protease-activated receptors, and an inflammatory response.[6,7] It was observed that TF values of atherectomy samples taken from patients with acute coronary syndrome were higher than patients with stable angina pectoris.[8,9]

Angiogenesis, like hyperglycemia, is one of the important clinical manifestations of the diabetic status.[10] About a quarter of a century ago vascular endothelial growth factor (VEGF-A) a new multifunctional protein isolated and involved in angiogenesis, was found to be collateralized in a wide spectrum of ischemic and inflammatory tissue from tumor

growth, wound healing, age-related macular degeneration, rheumatoid arthritis, and diabetic retinopathy.[10,11] In diabetic-atherosclerotic patients especially type II diabetics, the chronicity of the process and angiogenesis, which is a more prominent finding in the prolonged inflammatory process, may play a key role in the formation of micro and macrovascular complications.[12] As a result of endothelial proliferation created by VEGF-A, which ensures the migration of endothelial cells, vascular permeability increases and it regulates the thrombogenic surface and process in the endothelium.[13,14]

The aim of our study is to investigate the possible prognostic features of TF and VEGF-A in terms of looking at the early postoperative outcomes of diabetes, especially from a thromboembolic perspective, in a randomized and homogenized series that underwent off-pump coronary artery bypass (OPCAB)- thus free from possible pump effects - in advanced atherosclerotic patients.

Patients and Methods
Patient Selection

100 consecutive elective patients who were going to undergo coronary artery bypass surgery for the first time with the OPCAB technique due to angiographically severe coronary artery atherosclerotic lesions were randomized to those in the moderate risk category according to the EuroScore risk classification and included in the study. The system, which enables preoperative preparation and risk assessment according to the risks carried by the patients who will be taken into cardiac surgery, was prepared 24 years ago based on the European clinical database data, and since it is the risk scoring system, EuroSCORE (The European System for Cardiac Operative Risk) Evaluation) is named. This scoring system was updated as EuroScoreII in 2012, with the data being expanded to the whole world. It shows the risk category of the post-operative death risk according to the patient's own risk score before heart surgery and thus provides standardization.[15]

The ages of the cases are between 40 and 60 years old and 1/ 3 of whom were women, were divided into two groups in equal numbers according to whether they were diabetic or not. The analysis parameters of the patients in the low-intermediate risk group with a mean Euroscore II[15] of 4.31 ± 0.65 were compared with the serum samples taken from 15 volunteer patients in the same age range as the non-aerosclerotic and non-diabetic patient group. In our series, 12 of 35 female cases were in the menopausal period, and this factor was also investigated in the relevant biochemical analyzes.

Surgical Techniques

In our study, off-pump coronary bypass surgery, which is routinely performed in our clinic, was applied to all cases. Under general anesthesia, after appropriate heparinization with a midline sternotomy approach, full-arterial revascularization

was achieved by performing sequential artery anastomosis to the ITA (internal thoracic artery) with composite grafts created with the radial artery. A heart-lung machine was not used in any of the cases. Myocardial stabilization was achieved with special suspenders-technique[16] (Figure 1). Preoperative rate control was performed with beta-blocker in all cases. The operation was performed normothermic without partial clamping of the aorta. The carotid intima-media thicknesses (CT) were determined by preoperative carotid doppler ultrasound in all cases.

Blood and Tissue Sample Collection and Analysis

After ethics committee approvals and the consent forms obtained from each patient and volunteer, pre- and postoperative blood samples from the patients in the study group on the morning of the day before surgery and 72 h after the surgery, and blood samples taken from healthy volunteers on an empty stomach in the morning (8 ml), vacuum gel red taken to capped tubes. Blood samples were centrifuged (3000 g/10 min) using the NUVE (NF-800R) brand centrifuge, and serum samples were separated. The serum samples obtained were placed in Eppendorf tubes and stored at −80°C.

TF and VEGF-A Measurements

To determine protein levels of TF (Cat. No. E-EL-H0040) and VEGF-A (Cat. No. E-EL-H0111), (Elabscience Biotechnology Co., Ltd, Wuhan, China) in blood samples from patients method was used. Hemolyzed plasma samples were excluded from the study as it would affect the results. To determine the TF and VEGF-A protein levels, the protocol
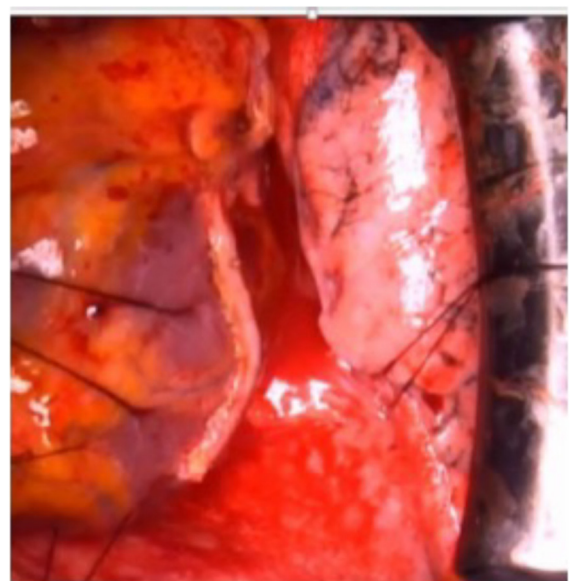

Figure 1. Myocardial stabilization was achieved with special suspenders stitches for off-pump coronary bypass surgery.

will be followed and the ready-made ELISA kit was used. Samples were placed in microwells of ELISA 96 well plates. Horseradish peroxidase (HRP) conjugate, substrate reagent, and stop solution were added to each well, respectively. Washing with washing solution was carried out at each step. Protein levels were determined with a microplate reader (Synergy HTX Multimode reader) set to 450 nm.

HbA1c and hsCRP Measurements

The turbidimetric inhibition method and the particle surface expanded immunoturbidimetric test were used to measure HbA1c and hsCRP levels, respectively. (Roche Cobas 6000 device, Roche, Germany).

Western Blot Analysis

Tissue samples of 5 mg were lysed in cold RIPA lysis buffer with a Qiagen homogenizer. It was centrifuged at 10,000 g for 20 min and the supernatant was transferred to Eppendorf tubes. Protein concentration was determined using the Thermo Fischer Nanodrop instrument and conducted in gel electrophoresis (SDS-PAGE). Then, the proteins were transferred electrophoretically onto the polyvinylidene fluoride (PVDF) membrane using the Bio-Rad Trans-Blot system. They were incubated with TF and VEGF-A primary antibodies (dilution ratios of 1/1000) at +4°C. β-actin primary antibody (dilution ratio 1/3000) was used as a control. Primary antibodies for the parameters to be examined were purchased as immunohistochemistry (IHC) compatible primary antibodies.[17]

Immunohistochemistry (IHC)

Vascular tissues embedded in paraffin were kept in an oven at 37°C overnight, using a microtome, with 5 μm thick sections. It was microwaved in 10% citrate buffer to remove deparaffinization, hydration, and antigen masking, respectively. Hydrogen peroxide blockade and protein blockade were performed. The primary antibody (Anti-TF or Anti-VEGF-A) whose concentration was determined was dropped and kept at +4°C overnight. Excess of primary antibody was removed and

secondary antibody was administered. After washing with phosphate buffer, 3,3-diaminobenzidine chromogen was applied. Core staining was done with Mayer Hematoxylin. After dehydration, entellan was dripped onto the sections and closed with the help of forceps. It was kept at room temperature until the image was taken with a microscope (Sterio Discovery V16, Carl Zeiss).[9]

Statistical Analysis

Data were analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Oneway Anova and Kruskall-Wallis Test were used to examine differences between groups for continuous variables. Kolmogorov-Smirnov test was used to test the normality. Chi-square test of independence was used to test group differences for categorical variables. Partial spearman rank correlation analysis was used to examine associations between variables. Binary analysis was used to determine the confidence interval. $P < 0.05$ was considered significant, $P < 0.001$ was considered statistically significant.

A logistic regression was performed to ascertain the effects of age, gender, smoking, hospital time, and difference of pre- and post-measurements of TF, VEGF, CRP on the likelihood that participants have diabetes.

Results

Results of HbA1c and hsCRP Analysis

HbA1c levels were significantly higher in the T1DM and T2DM groups compared to nondiabetics. Although hsCRP values were higher in TIDM compared to nondiabetics preoperatively but there is no difference between the groups (Table 1A,1B).

Results of ELISA Analysis of TF and VEGF-A in Blood

Δ TF (post-pre) levels in blood decreased significantly in the T2DM group compared to the NonDM ($p < 0.001$) and T1DM ($p < 0.001$) groups. Similarly, Δ VEGF-A (post-pre) levels in blood decreased in the T1DM and T2DM groups compared to the NonDM group ($p < 0.001$) (Figure 2).

Table 1. HbA1c (A) and hsCRP(B) levels in groups.

A	Non DM ($n = 50$)	T1DM ($n = 25$)	T2DM ($n = 25$)	Statistical Analysis	P value	s/ns
HbA1c (%)[a]	4.08 ± 0.22	8.02 ± 1.72*	5.60 ± 0.55*▲	$X^2 = 86.824$	0001	s
B	**Control**		**NonDM (n = 50)**	**T1DM (n = 25)**	**T2DM (n = 25)**	
C-reactive protein (mg/L)	Ref. value < 0.5	**preOP**	1.36 ± 0.91	3.52 ± 3.75*	1.82 ± 1.98	
		postOP	2.62 ± 1.45	3.71 ± 2.80	2.35 ± 1.41	
		Δpost-pre	1.26 ± 1.44	0.19 ± 3.82	0.81 ± 2.27	

Data are expressed as mean ± standard deviation. *$p < 0.001$: Significantly different from the NonDM group, ▲$p < 0.001$: Significantly different from the T1DM group. ns = non-significant mean comparison, n = significant mean comparison.
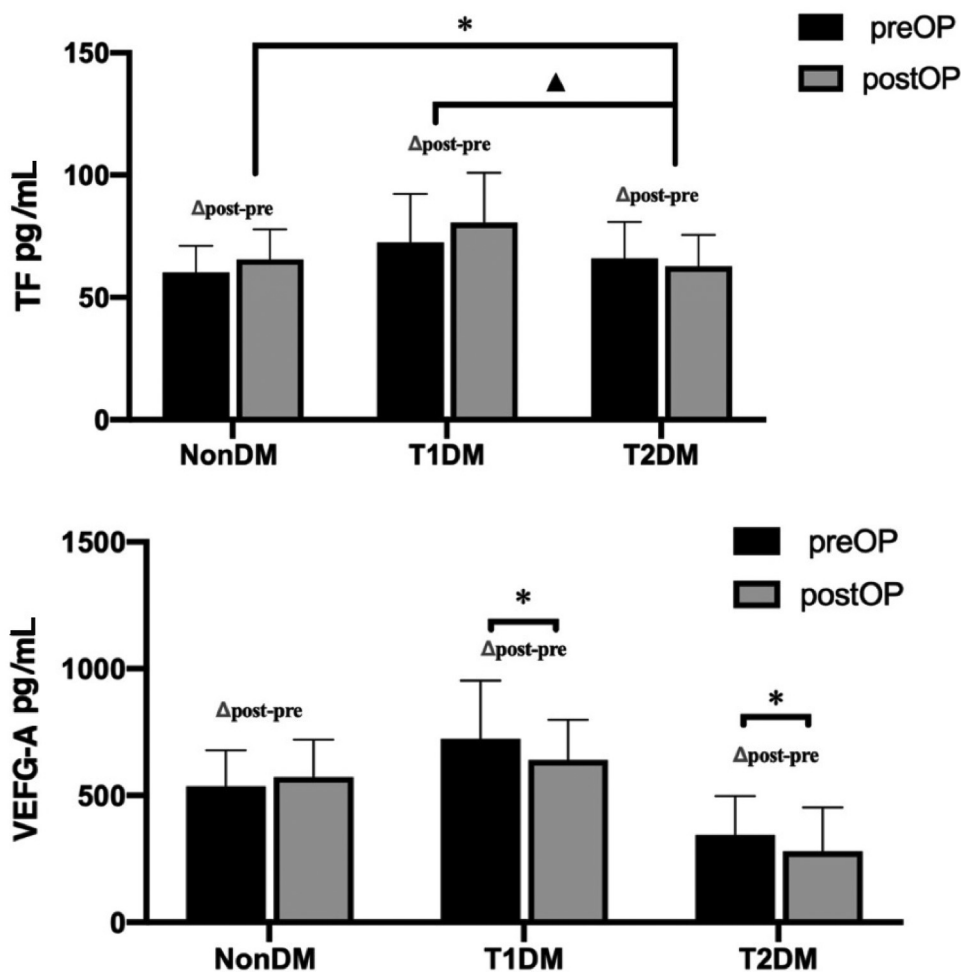
Figure 2. TF and VEGF-A levels in diabetic and non-diabetic patients with atherosclerosis. Data are expressed as mean ± standard deviation. *$p < 0.001$: Significantly different from the NonDM group, ▲$p < 0.001$: Significantly different from the T1DM group.

Results of Western Blot Analysis of TF and VEGF-A

T1DM was significantly higher in the TF and VEGF-A values compared to nondiabetics. ($p < 0.05$) (Figure 3).

Results of Immunohistochemical (IHC) Analysis of TF and VEGF-A

When TF and VEGF-A expression was compared observationally by immunohistochemical methods, it was observed that TF expression was slightly higher in the tunica media in the T1DM group compared to the other groups. When VEGF-A expression was compared observationally between groups, it was found to be more expressed in T1DM group compared to the non-diabetic group. Intensity analysis showed that TF expression increased significantly in T1DM group when compared to non-diabetic group (*$p < 0.05$). Also, VEGF-A TF expression increased significantly in T1DM group when compared to non-diabetic group (**$p < 0.001$) (Figure 4).

TF, VEGF-A, hsCRP, AF, and Carotid Intima-Media Thicknesses Correlations

Correlations between HbA1c and hsCRP levels with TF and VEGF-A levels of total diabetic patients are given in Table 2. TF and VEGF-A levels were correlated with HbA1c levels, and a more significant correlation was found between VEGF-A and hsCRP.

Correlations according to AF, hsCRP, TF, VEGF-A, and carotid intima-media thicknesses (CT) in the non-diabetic group are given in Table 3. Preoperatively measured carotid intima-media thickness measurements were significantly correlated in diabetic patients who developed atrial fibrillation (AF) after coronary bypass. In the non-diabetic-atherosclerotic group; while AF developed in 6 of 50 cases, in the diabetic-atherosclerotic group in 15 of 50 cases, in the T1DM and T2DM subgroups, respectively; AF was seen in 7 and 8 cases.

The correlation between AF patients in the non-diabetic group and CT measurements was $r = 0.550$; In the diabetic group, AF-CT correlation was significantly correlated in the T1DM sub-group, $r = 0.725$, and in the T2DM sub-group, $r = 0.873$.
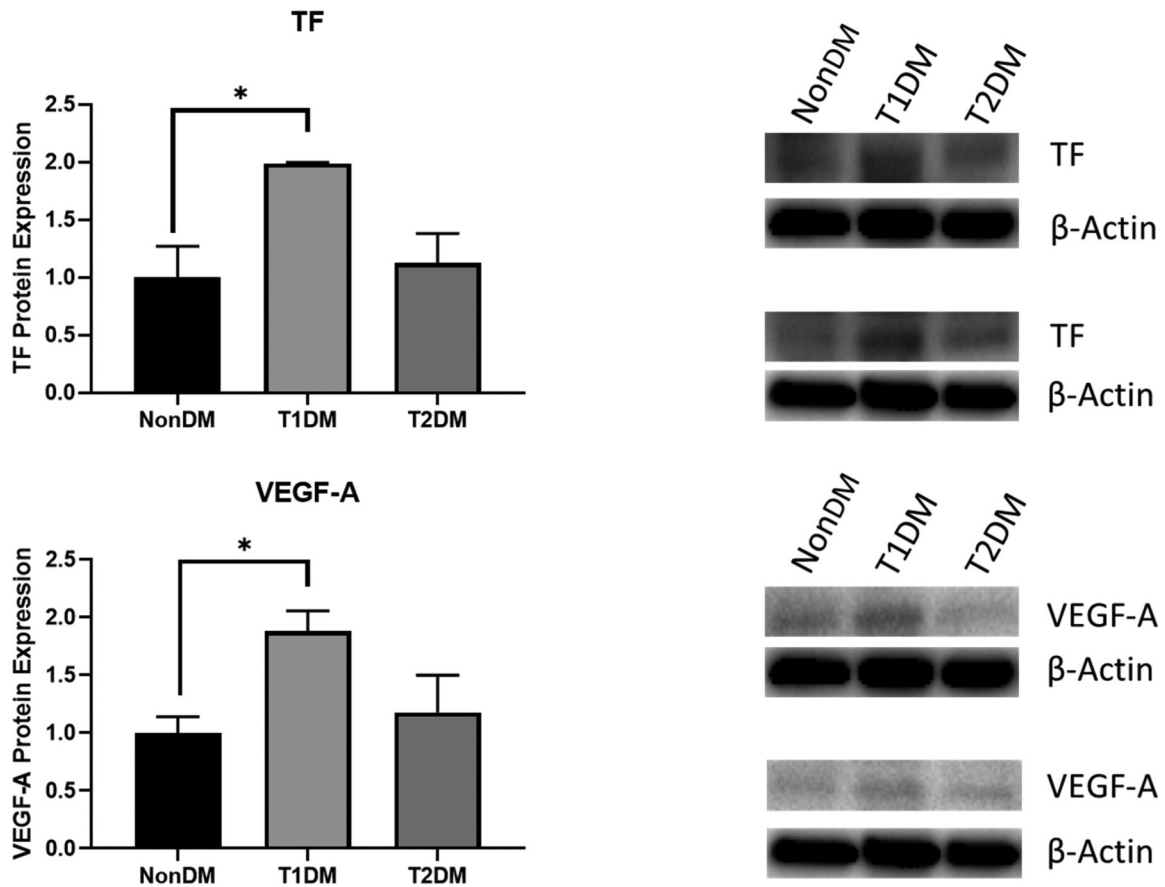
TF

VEGF-A

Figure 3. Western blot results of TF and VEGF-A proteins. * p < 0.05 denotes significance.

However, logistic regression analysis could not be performed because the relative number was low in subgroups. It was planned to reevaluate the series in larger cases (Table 3 and 4).

AF transition was generally in the first 48 h and did not persist in any of the cases at discharge from the hospital, and no anticoagulant treatment was required at discharge. No major or minor neurological events and mortality were observed.

Logistic Regression Test Results

The logistic regression model was statistically significant, $\chi2(7) = 87.722$, $p < .0001$. The model explained 77.9% (Nagelkerke $R2$) of the variance in heart disease and correctly classified 89% of cases. Diabetics length of stay was 3.83 (95%CI: 1.96; 7.49) times higher than non-diabetic patients. Increasing difference between TF and VEGF-A was associated with a reduction in the likelihood of exhibiting diabetics ($p < 0.05$) (Table 4).

Discussion

Our primary goal is to detect the initiator/trigger factors of atherosclerosis in terms of public health early. The thrombotic process, which started with vascular endothelial damage, blood flow, and abnormalities in blood's elements, which is

the classical triad defined by Virchow about two centuries ago, still maintains its validity even though it has been renewed with the discovery of new biochemical formations and it is aimed to predict before complications develop with advanced biochemical analyzes.[1,18–20]

Gleissner et al showed that microvascular diabetic complications can be reduced by providing normoglycemia. As a result, they emphasized the importance of early detection of inflammatory and thrombogenic factors that may be effective in addition to providing normoglycemia in diabetic patients.[21]

In another study showing the role of oxidative stress in diabetic and morbid obese patients, there was no difference between TF and Interleukin-33 (IL-33) values when compared to the healthy group, while significantly higher in the diabetic morbid-obese groups and they found also a positive correlation was found between HbA1c and TF.[6,7] In our study, preoperative hsCRP values were significantly higher in the diabetic-atherosclerotic group, which we defined as uncontrolled diabetes and whose HbA1c level was 7 and above, and both TF and VEGF values were higher in these cases also.

Tissue factor, which is one of the basic building blocks of the cell membrane and in the glycoprotein structure, combines with the active form of Factor VII and triggers the coagulation cascade. TF plays an active role in both vascular angiogenesis
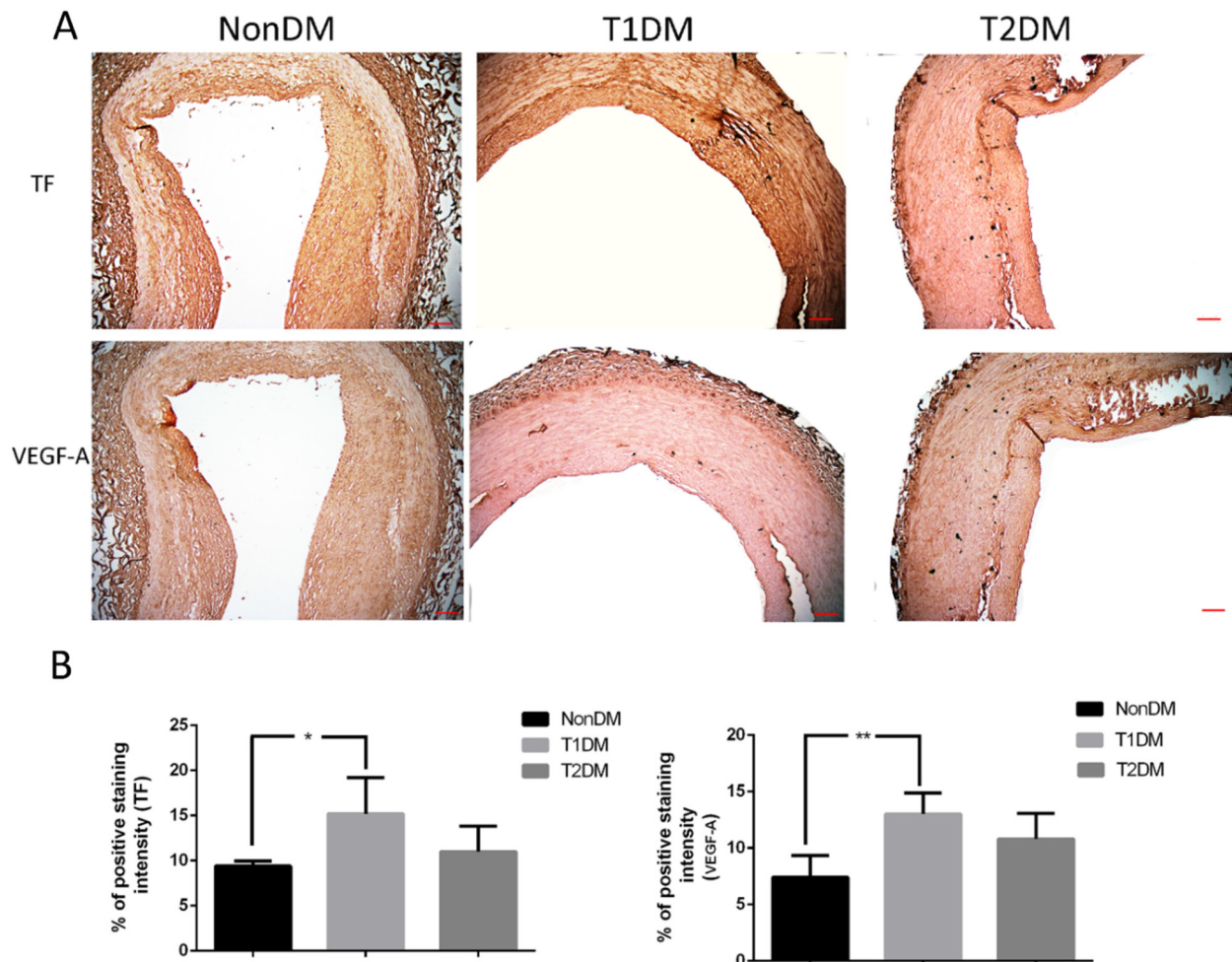
Figure 4. A) Immunohistochemical examination of TF and VEGF-A expression in type I diabetic, type ii diabetic and non-diabetic patients with atherosclerosis. B) Graphics showing intensity of TF and VEGF-A expression in vessels. Values expressed in arbitrary unit (*$p < 0.05$, **$p < 0.001$). (TF; tissue factor, VEGF-A; vascular endothelial growth factor, NonDM; Non-Diabetic, T1DM; Type1 Diabetes, T2DM; Type2 Diabetes. Bar: 100 µm).

and tumor formation and metastatic spread. The interaction with VEGF in its role in angiogenesis is emphasized. The TF-aFVII complex has been shown to initiate VEGF synthesis by lung-fibroblasts through proteolytic activity.[22] While endothelial cells are not thrombogenic under normal conditions, the pathological angiogenesis process in which VEGF takes an active role is triggered by the effect of endothelial damage developing in chronic inflammatory processes accompanied by atherosclerosis and diabetes or both, or tumor formation processes reported that both in the form of abnormal blood vessels in the glomeruli of diabetics.[3,4,22,23]

VEGF production may give important clues about the severity or extent of atherosclerosis in diabetic atherosclerotic patients. VEGF is a glucoprotein that has a protective effect by providing angiogenesis to ischemic tissues during the formation of atherosclerosis. Studies have indicated the vital role of VEGF in abnormal angiogenesis, especially in chronic atherosclerotic or carcinogenic processes. In this series, it is known that TF is effective in micro and macrovascular complications

with its versatile biological effects affecting hemostasis, thrombosis, angiogenesis, and inflammation. Although the cause of microvascular complications of diabetes is not fully explained, various hypotheses state that the cause of microvascular complications is related to angiogenesis. It has been reported that VEGF inhibitors reduce macular degeneration and macular edema. In patients with diabetic atherosclerosis, it has been reported that the VEGF receptor signal is impaired in the myocardium with the increase in VEGF expression. Mahdy et al in which they examined micro and macrovascular complications in type 2 diabetic patients and they showed the VEGF levels and vascular complications were related in diabetics. We found that TF and VEGF were significantly associated with length of hospital stay and especially in diabetics.[12,24,25]

Inoue M. et al, in their study, showed that VEGF is an important angiogenic factor that induces migration and proliferation of endothelial cells, increases vascular permeability and modulates thrombogenicity. It has been shown that VEGF expression in cultures of different phases of atherosclerotic

Table 2. Correlations between HbA1c, hsCRP levels with Tissue Factor (TF) Levels (A) and Vascular Endothelial Growth Factor (VEGF-A) levels (B) of total diabetic patients (NonDM, T1DM, T2DM) *p < 0,05, ** p < 0.001

| | TF | | | |
| | pre-op | | post-op | |
A	r	p	r	p
HbA1c	0.336**	$p < 0.01$	0.127	$p > 0.05$
hsCRP pre-op	0.100	$p > 0.05$	0.008	$p > 0.05$
hsCRP post-op	0.214*	$p < 0.05$	0.065	$p > 0.05$
VEGF-A pre-oop	0.170	$p > 0.05$	0.435**	$p < 0.01$
VEGF-A post-op	0.162	$p > 0.05$	0.462**	$p < 0.01$
B	VEGF-A			
	pre-op		post-op	
	r	P	r	P
HbA1c	0.153	$p > 0.05$	0.072	$p > 0.05$
hsCRP pre-op	0.200*	$p < 0.05$	0.149	$p > 0.05$
hsCRP post-op	0.178	$p > 0.05$	0.186	$p > 0.05$

Table 3. Correlations according to AF, hsCRP, TF, VEGF-A, and carotid intima-media thicknesses in the nondiabetic group (NonDM) (A) and diabetic group (T1DM, T2DM) (B) *p < 0.05, ** p < 0.01.

| | Nondiabetics | | |
A	AF	CT(int-med)	hsCRP
AF	1	0550**	0.221
CT (int-med/mm)	0.55	1	0181
hsCRP mg/L	0221	0181	1
TF pg/mL	−0025	−0191	−0058
VEGF-A pg/mL	−0011	−0,4	0318*
	Diabetics		
B	AF	CT(int-med)	hsCRP mg/L
AF	1	0720**	0157
CT(int-med/mm)	0.72	1	−0193
hsCRP mg/L	0157	0193	1
TF pg/mL	−0143	−0086	0005
VEGF-A pg/mL	−0066	0208	0234

plaque formation from the initial stage of atherosclerotic plaque formation to the advanced stage, which narrows the entire vessel lumen, is associated with intraplaque microvascular increase, unlike in normal arteries.[14] VEGF, which has been shown to have multipotent effects in experimental studies, may be a determining factor in atherosclerosis as well as a clinically helpful factor in staging.[26–28]

In a study pointing out that VEGF may be a precursor factor in the early detection of atherosclerosis; Between the ages of 30–75, 400 people who come to the normal annual check-up, approximately 10% have hypertension and hyperlipidemia in 5% and they reported that there is a significant correlation between VEGF and atherosclerotic process, especially in smoking men, who have diabetics. In our study, all of cases were in the same risk category and objective critical (≥70%) atherosclerotic stenosis in the coronary arteries was detected angiographically in all 100 cases, and half of the cases consisted of diabetic cases requiring treatment. In this respect, we think that the atherosclerotic burden of our series is stronger and more specific in this regard. Off-pump coronary bypass surgery, which is routinely performed in our clinic, was performed in all cases, and carotid intima-media thicknesses were determined by pre-operative carotid doppler evaluation of all cases. We found that TF expression in the T1DM group was statistically high compared to the non-diabetics (p < 0.05) but in T2DM group showed a slight increase compared to the non-diabetic group. VEGF protein expression in the T1DM group was statistically high also compared to non-diabetics (p < 0.05). According to our results, the correlation between VEGF-A and CT values is especially evident in uncontrolled diabetic patients with an increase in carotid intima-media thickness, while this increase can be thought to be caused by microangiogenesis induced by VEGF. We also agree with the researchers in terms of analyzing VEGF-A values in serum for possible leaks.[29,30]

Although new-onset atrial fibrillation is reported as the most common arrhythmia after coronary bypass surgeries in 1/3–1/5 of cases, many publications have not shown a difference between on-pump or off-pump coronary bypass techniques. Considering the effects of atrial fibrillation on long hospital stays due to possible thromboembolic co-morbidities and prolonged medical treatment processes, its importance in terms of postoperative course is clear. Although recent studies have reported that critical atherosclerosis and diabetic status trigger postoperative atrial fibrillation in patients, the underlying mechanism and related factors have not been clearly identified. In our study, we compared transient atrial fibrillation attacks, which we see more frequently in diabetic-atherosclerotic cases (30% vs 12%, $p = 0.0272$), and preoperative CT in all cases; Although there was a significant correlation between AF and CT ($r = 0.720$) in diabetic patients but not significant in the T1DM and T2DM subgroup analysis, since it was less in number. In our series, 35 of 100 cases were women, and since only 12 of them were in the menopausal period, no significant correlation was observed with the transition to AF. Therefore, it was planned to reevaluate the group in a larger series.[31–33]

Table 4. **Logistic regression test results:** difTF: difference between before and after measurement of TF; difVEGF: difference between before and after measurement of VEGF-A; difCRP: difference between before and after measurement of CRP

Logistic Regression Table							
						95% C.I. for OR	
		Estimate	Standard error	Sig.	Odds Ratio (OR)	Lower	Upper
Step 1[a]	DifTF	−0.071	0.033	0.032	0.931	0.872	0.994
	DifVEGF-A	−0.020	0.006	0.001	0.980	0.968	0.992
	Age	0.087	0.069	0.211	1.091	0.952	1.250
	Gender(1)	0.489	0.859	0.570	1.630	0.303	8.781
	Cigarettes(1)	−0.417	0.849	0.623	0.659	0.125	3.479
	Hospital time (days)	1.343	0.342	0.000	3.829	1.958	7.488
	DifhsCRP	−.452	0.365	0.216	0.636	0.311	1.301
	Constant	−10.999	4.303	0.011	0.000		

Conclusion

The coexistence of diabetes and atherosclerosis is an important cause of morbidity and mortality. Especially in diabetic atherosclerotic patients, VEGF and TF, which is thought to act together in the formation of coagulopathy, may be precursors before thromboembolic complications develop and also they can be useful tool as a marker for after bypass surgery graft patency rate. Significantly longer hospital stays with changes in TF and VEGF-A were found in patients in the diabetic group. Preoperatively measured CT (carotid intima-media-media/mm) was higher in diabetics and was significantly performed with AF and TF – VEGF-A values were found to be correlated. This indicates that TF and VEGF-A can be clinically important guides in reducing morbidity and mortality rates, especially in diabetic-atherosclerotic patients.

Author contribution(s)
İlknur Keskin: Investigation; Validation.
Nesrin Emekli: Conceptualization; Investigation; Methodology; Supervision; Writing – original draft; Writing – review & editing.

Ethics Approval and Consent to Participate
The study was initiated after the consent forms of the cases and the Ethics Committee decisions, and the Research Fund approvals for the Biochemistry Doctoral Thesis; this thesis was supported by the Istanbul Medipol University Scientific Research Projects Commission with the project number 2019/16.

All experimental protocols were approved by Istanbul Medipol University Ethics Committee, number and date: 10840098-604.01.01- E.47329/25.10.18 and Koç University Hospital Ethics Committee, number and date: EMA/hd /54/ 2019/07.01.2019 Informed consent was obtained from each patient and voluntary participant.

All methods were performed according to the guidelines and regulations of the Declaration of Helsinki.

Actual study start date: 20/01/2019
Actual primary completion date: 01/01/2020
Actual completion date: 01/05/2020

Availability of Data and Materials
Data is available as an additional file (Data.xlsx)

ORCID iD
Tijen Alkan Bozkaya https://orcid.org/0000-0002-4728-1079

REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, et al. American Heart association council on epidemiology and prevention statistics committee and stroke statistics subcommittee (2022). Heart disease and stroke statistics — 2022 update: A report from the American Heart Association. *Circulation.* 2022;145(8):e153-e639.
2. Krychtiuk KA, Astl SP, Speidl WS, et al. Inflammation and coagulation in atherosclerosis. *Haemostaseologie.* 2013;33(4):269-282.
3. Grover SP, Mackman N. Tissue factor in atherosclerosis and atherothrombosis. *Atherosclerosis.* 2020;307:80-86. doi:10.1016/j.atherosclerosis.2020.06.003
4. Steffel J, Luscher TF, Tanner FC. Tissue factor in cardiovascular disease: Molecular mechanisms and clinical implications. *Circulation.* 2006;113(5):722-731.
5. Bugge TH, Xiao Q, Kombrinck KW, et al. Fatal embrynonic bleeding events in mice lacking tissue factor, the cell-associated initiator of blood coagulation. *Proc Natl Acad Sci USA.* 1996;93(13):6258-6263.
6. Stojkovic S, Kaun C, Basilio J, et al. Tissue factor is induced by interleukin-33 in human endothelial cells: A new link between coagulation and inflammation. *Sci Rep.* 2016;6(1):1-11.
7. Çakıcı Ç, Yigitbası T, Alagoz MH, et al. Relationship between interleukin 33 and tissue factor in non-diabetic and diabetic obese patients. *Acta Pharmaceutica Sciencia.* 2021;59(4):559-573.

8. Wilcox JN, Smith KM, Schwartz SM, et al. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaques. *Proc Natl Acad Sci USA*. 1989;86(8):2839-2843.

9. Ustundag UV, Sahin S, Ak K, et al. The effects of tacrolimus on the activity and expression of tissue factor in the rat ovary with ischemia– reperfusion induced injury. *Reprod Biol*. 2015;15(3):139-145.

10. Ferrara N, Bunting S. Vascular endothelial growth factor, a specific regulator of angiogenesis. *Curr Opin Nephrol Hypertens*. 1996;5(1):35-44.

11. Dvorak HV, Brown LF, Detmar M, et al. Vascular permeabiliy factor vascular endothelial growth factor, microvascular hypermemeabilitiy and angiogenesis. *Am J Pathol*. 1995;146(5):1029-1039.

12. Mahdy RA, Nada WM, Hadhoud KM, et al. The role of vascular endothelial growth factor in the progression of diabetic vascular complications. *Eye(Lond)*. 2010;24(10):1576-1584.

13. O'Brien ER, Garvin MR, Dev R, et al. Angiogenesis in human coronary atherosclerotic plaques. *Am J Pathol*. 1994;145(4):883-894.

14. Inoue M, Itoh H, Ueda M, et al. Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions. Possible pathophysiological significance of VEGF in progression of atherosclerosis. *Circulation*. 1998;98(20):2108-2116.

15. Nashefa SAM, Roquesb F, Sharplesc LD, et al. EuroSCORE II. *Eur J Cardiothorac Surg*. 2012;41(4):734-745. doi:10.1093/ejcts/ezs043

16. Gönenç A, Hacevki A, Bakkaloğlu B, et al. Oxidative stress is decreased in off-pump versus on-pump coronary artery surgery. *J Biochem Mol Biol*. 2006;39(4):377-832.

17. Bass JJ, Wilkinson DJ, Rankin D, et al. An overview of technical considerations for western blotting applications to physiological research. *Scandinavian J Med*. 2017;27(1):4-25.

18. Emekli-Alturfan E, Başar I, Malali E, et al. Plasma tissue factor levels and salivary tissue factor activities of periodontitis patients with and without cardiovascular disease. *Pathophysiol Haemost Thromb*. 2010;37(2-4):77-81.

19. Chung I, Lip GYH. Virchow's triad revisited blood constituents. *Pathophysiol Haemost Thromb*. 2003/2004;33(5-6):449-454.

20. Posma JJ, Grover SP, Hisada Y, et al. Roles of coagulation proteases and protease-activated receptors in mouse models of inflammatory diseases. *Arterioscler.Thromb. Vasc. Biol*. 2019;39(1):13-24. doi: 10.1161/ATVBAHA.118. 311655

21. Gleissner CA, Galkina E, Nadler J, et al. Mechanisms by which diabetes increases cardiovascular disease. *Drug Discov Today Dis Mech*. 2007;4(3):131-140.

22. Ollivier V, Bentolila S, Chabbat J, et al. Tissue factor-dependent vascular endothelial growth factor production by human fibroblasts in response to activated factor VII. *Blood*. 1998;91(8):2698-2703.

23. Osterby R, Nyberg G. New vessel formation in the renal corpusles in advanced diabetic glomerulopathy. *J Diabet Compliacations*. 1987;1(4):122-127.

24. Costa PZ, Soares R. Neovascularization in diabetes and its complications. Unraveling the angiogenic paradox. *Life Sci*. 2013;92(22):1037-1045.

25. Chait A, Bornfeldt KE. Diabetes and atherosclerosis: Is there a role for hyperglycemia? *J.Lipid Res*. 2009;50(Suppl):S335–S339.

26. Shimizu Y, Arima K, Noguchi Y, et al. Vascular endothelial growth factor (VEGF) polymorphism rs3025039 and atherosclerosis among older with hypertension. *Sci Rep*. 2022;12(1):1-6.

27. Harper SJ, Bates DO. VEGF A splicing: The key to anti-angiogenic therapeutics? *Nat Rev Cancer*. 2008;8(11):880-887.

28. Celletti FL, Waugh JM, Amabile PG, et al. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med*. 2011;7(4):425-429.

29. Kimura K, Hashiguchi T, Deguchi T, et al. Serum VEGF-as a prognostic factor of atherosclerosis. *Atherosclerosis*. 2007;194(1):182-188.

30. Wang F, Li G, Guan X, et al. Overexpression of eNOS decrease tissue factor (TF) level in CD34+ cells exhibit increased antithrombogenic property in small caliber vascular graft. *J Cardiovasc Surg (Torino)*. 2019;60(1):136-143. doi: 10.23736/S0021-509.18.08951-6. Epub 2015 Sep 3.

31. Junior MSB, Matkovski PD, Di Giovanni FJ, et al. Incidence of postoperative atrial fibrillation in patients undergoing on-pump and off-pump coronary artery bypass grafting. *Braz J Cardiovasc Surg*. 2015;30(3):316-324.

32. Alkan-Bozkaya T, Akçevin A, Türkoğlu H, et al. Impact of pulsatile perfusion on clinical outcomes of neonates and infants with complex pathologies undergoing cardiopulmonary bypass procedures. *Artif Organs*. 2013;37(1):82-86.

33. Wang A, Green JB, Halperin JI, et al. Atrial fibrillation and diabetes mellitus. *JACC Review Topic of the Week, JACC*. 2019;14(8):1107-1115.