# Tissue-type plasminogen activator and plasminogen activator inhibitor type 1 as compared to lower extremity artery disease.

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# Abstract

Background Chronic lower extremity artery disease (LEAD) involves progressive arterial narrowing manifested by intermittent claudication (IC). LEAD entails endothelial dysfunction and fibrinolytic disorders. The main objective of the present study was to analyze selected parameters of the fibrinolytic system in the blood of patients with symptomatic LEAD depending on clinical parameters. Methods The test group consisted of 80 patients with LEAD (27F/53M) with an average age of 63.5±9 years. The control group included 30 healthy, non-smoking volunteers (10F/20M), with their median age of 56±6 years. The research material comprised venous blood to determine concentrations of tissue-type plasminogen activator (t-PA Ag), plasminogen activator inhibitor type 1 (PAI-1 Ag) and D-dimer, fibrinogen and platelet count (PLT). Results Elevated concentrations of t-PA Ag and PAI-1 Ag as well as D-dimer and fibrinogen were found in the plasma of subjects with symptomatic LEAD. Various stages of the Fontaine classification demonstrated a gradual, statistically significant increase in the concentrations of fibrinogen and PLT count as the disease progressed. The subgroup of LEAD patients aged ≥ 65 years was reported to have significantly higher levels of D-dimer than the group of younger subjects. Besides, the LEAD group demonstrated: negative correlations between IC distance and fibrinogen concentrations, and PLT count, negative correlations between ABI at rest and concentrations of D-dimer and PLT count, and positive correlations between age and D-dimer levels. Conclusion High t-PA Ag concentrations in LEAD patients reflect damaged endothelium which comprises the main source of this factor. With high PAI-1 Ag levels, inactive fibrinolytic t-PA-PAI-1 complexes are formed. Increasing fibrinogen concentrations at the subsequent stages in accordance with the Fontaine classification, suggest a growing inflammatory condition. Elevated values of D-dimer reflect the aggregation of secondary fibrinolysis activation as the patient ages and along with impaired extremity vascularization manifested by the decreasing ABI.


# Background

It is estimated that chronic lower extremity artery disease (LEAD) affects more than 200 million people all over the world [1,2,3]. LEAD involves progressive arterial narrowing manifested mainly by intermittent claudication (IC). One of clinical classifications for LEAD is the Fontaine classification according to which stage I means asymptomatic, IIa describes intermittent claudication after more than 200 meters of walking, IIb means intermittent claudication after less than 200 meters, stage III means rest pain and IV stands for ischemic ulcers [4]. One of key diagnostic tools is the non-invasive Ankle-Brachial Index (ABI) measurement. This method uses a blood pressure monitor and a Doppler device. The ABI is the ratio of blood pressure at the ankle and blood pressure measured at the upper arm. The value of ≤ 0.9 is considered to be a hemodynamic definition of LEAD and in patients with symptomatic LEAD, it has shown 95% sensitivity in detecting the disease when compared to arteriography and nearly a 100% specificity for identifying healthy individuals [5].

Chronic ischemia depends on the presence of atherosclerotic plaque in the artery and is associated with damaged endothelium, which may lead to synthesis disorder and release numerous factors to blood which, in turn, are important for maintaining vessel hemostasis, including tissue-type plasminogen

activator (t-PA). The main role of t-PA is to transform the inactive proenzyme of plasminogen into plasmin which dissolves blood microclots caused by endothelial dysfunction. Increased release of t-PA from endothelium and its intensive activity enhance fibrinolysis [6]. Due to the influence of plasmin on stable fibrin networks, there occur fibrinolytic degradation products (FDP) including D-dimer. D-dimer is a cross-linked fibrin fragment between monomeric chains and measures fibrinolytic activity and, indirectly, indicates the level of stable fibrin formed in vessels as a result of coagulation activation [7,8].

Plasminogen activator inhibitor type 1 (PAI-1 Ag) inactivates plasma t-PA with which it forms inactive fibrinolytic t-PA-PAI-1 complexes, which prevents plasmin formation. The main source of PAI-1 are endothelial cells, platelets and vascular smooth muscle cells [9].

Recent studies indicate the significance of endothelial inflammation in the development of atherosclerosis [10]. The role of fibrinogen as an acute-phase protein and an inflammatory risk factor, is incredibly important for the pathogenesis of atherosclerosis. Fibrinogen contributes to cholesterol esterification due to monocytes / macrophages and to the formation of foam cells. This may be explained by atherosclerosis progression in patients with hyperfibrinogenemia even with normal cholesterol levels [11].

A number of publications concerning cardiovascular diseases have analyzed such platelet parameters as increased thrombopoiesis, morphology (including the platelet volume) of platelets, markers of platelet activation, i.e. substances produced by granules (e.g. β-thromboglobulin, platelet factor 4, sP-selectin) and receptors engaged in adhesion and aggregation (e.g. glycoproteins GPI and GPIIb-IIIa). These research studies have provided reasons for the implementation of antiplatelet therapy (initially aspirin which is commonly used these days) in atherosclerotic diseases [12,13]. Undeniably fewer studies have analyzed the absolute value of PLT in the blood of patients with atherosclerosis including LEAD, which is an easily accessible laboratory parameter and it seems to be underestimated in everyday clinical practice.

The main objective of the present study was to analyze the fibrinolytic system based on selected parameters such as concentrations of t-PA Ag, PAI-1 Ag, D-dimer, fibrinogen and platelet count (PLT) in the blood of patients with symptomatic LEAD in comparison to healthy individuals. The studies also included value of fibrinolysis factors depending on the severity of disease in accordance with the Fontaine classification, age and sex, IC distance and the ABI value.

# Methods

### Patients and controls

The study group included 80 patients (27 females and 53 males) with an average age of 40 – 88 years (63.5 ± 9 on average) with symptomatic LEAD. These were patients admitted to hospital to confirm an outpatient diagnosis (by general practitioners) of LEAD, determine the stage of severity and then to implement a relevant strategy for further specialized treatment.

The diagnosis was preceded by a clinical history with particular focus on questions about typical ailments, i.e. intermittent claudication and distance, possible rest pain in lower limbs, ischemic ulcers as well as previous pharmacotherapy (acetylsalicylic acid, statin).

During the physical examination, special attention was paid to skin appearance (changes in hair, color, ulcerations) and bilateral palpation of the pulse, especially at the level of femoral popliteal, dorsal and posterior tibial arteries. Intermittent claudication distance was objectively confirmed using the walk test on a treadmill in standard conditions (treadmill speed of 3.2 km/h, angle of 10O). The ABI test was performed on patients at rest.

The exclusion criteria included the lack of consent to participate in the research, the history of cancer, a surgical operation during last month, venous thromboembolism, oral administration of vitamin K antimetabolites (acenocoumarol, warfarin) or the so-called new oral anticoagulants (dabigatran, rivaroxaban, apixaban), diabetic retinopathy, nephrotic syndrome, history of systemic connective tissue diseases, asthma, chronic obstructive pulmonary disease, nonspecific inflammatory bowel disease, pregnant women.

The control group included 30 healthy, non-smoking, sex-matched volunteers (10 females / 20 males) with their median age of 56±6 years.

The studies were authorized by the local Bioethics Commission of Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, no. KB 509/2011 and were carried out in accordance with the Declaration of Helsinki.

Obtaining blood samples of 15 ml was preceded by giving informed consent - participation in the research was voluntary. Blood samples were collected from patients before the implementation of further treatment.

The collected material was used to measure the concentrations of t-PA Ag (*Diagnostica Stago®, France*) and PAI-1 Ag (*Sekisui Diagnostics®, USA*) by means of the immunoenzymatic method (ELISA), the concentrations of D-dimer and fibrinogen (*Bio-ksel®, Poland*) using CC-3003 apparatus and PLT count by means of XT-4000i apparatus (*Symex®, Japan*).

### Statistics

The statistical analysis was carried out by means of Statistica 12.0 software (*StatSoft®, Poland*). The compatibility of analyzed parameters distribution with the standard normal distribution was assessed by the Shapiro-Wilk test. In case of variables whose distribution was not normal, nonparametric tests were used (U Mann-Whitney test, Kruskal-Wallis test). For normal distributions, though, parametric tests were applied (t – Student test, Tukey HSD test). If variables in test groups were not characterized by normal distribution, the values of the median (Me) and quartiles: lower (Q25) and upper (Q75) were given, whereas for variables with normal distribution, the values of mean (X) and standard deviations (SD) were provided. To study the correlations of the parameters whose distribution was not normal, the Spearman

correlation coefficient (*r*) was determined. For variables with normal distribution, the Pearson correlation coefficient (*r*) was calculated to measure the correlation. The significance level was set at $p < 0.05$.

# Results

The characteristics of the study group is presented in Table 1.

Table 1. General characteristics of the study group (LEAD, *n = 80*).

A clear majority of people from the test group were administered acetylsalicylic acid and statin.

Table 2 presents the values of parameters in the study group against the control group.

**Table 2. Values of studied parameters in the test group and control group.**

The concentrations of t-PA Ag, PAI-1 Ag and D-dimer were 2.5-times higher in the test group as compared to the control group and the differences were statistically significant. Fibrinogen levels were found significantly higher in patients against healthy subjects.

Table 3 compares the values of analyzed parameters with division to LEAD patients in accordance with the Fontaine classification and in comparison to the control group.

Table 3. Values of studied parameters in the test group in the subgroups of patients in accordance with the Fontaine classification of disease severity in comparison to the control group.

Significant differences were found between the analyzed parameters, i.e. the concentrations of t-PA Ag, PAI-1 Ag, D-dimer, fibrinogen and PLT count in patients in particular classes in accordance with the Fontaine classification and the values of these parameters in the blood of healthy individuals. Statistically significant increasing levels of fibrinogen were observed in subsequent disease categories in accordance with the Fontaine classification, and an insignificant trend of increasing concentrations of D-dimer and significant differences in terms of PLT count were reported in the studied subgroups. It was noted that an elevated PLT count in class IV was significantly higher in comparison to healthy subjects.

Table 4 illustrates the values of investigated parameters in the test group and the control group disaggregated by age (< 65 years of age and ≥ 65 years of age) – the age of 65 was considered the old age.

Table 4. Values of studied parameters in the test group and the control group disaggregated by age (< 65 years of age and ≥ 65 years of age).

LEAD patients < 65 years of age were found significantly lower values of D-dimer ($p = 0.03$) in comparison to patients ≥ 65 years of age. Significantly higher PLT levels were reported in LEAD subjects < 65 years of age against healthy subjects from the same age range ($p = 0.04$).

Table 5 displays the values of analyzed parameters in the group of patients with LEAD against the control group with sex-based division.

Table 5. Values of studied parameters in the test group and the control group with sex-based division.

No distinctive differences were found in the values of studied parameters between the subgroups of females and males in the LEAD group and between these subgroups in the control group.

Table 6 summarizes the analysis results concerning correlations of studied parameters (concentrations of t-PA Ag, PAI-1 Ag, D-dimer, fibrinogen, PLT count) and selected demographic parameters (age) and clinical parameters (intermittent claudication distance, ABI value) in the test group.

**Table 6. Correlations of studied parameters.**

The analysis of studied biochemical parameters and selected clinical factors conducted in the LEAD group revealed the following: a positive correlation of age and D-dimer concentrations, negative correlations of intermittent claudication, fibrinogen levels and platelet count as well as negative correlations between the values of the ABI at rest, D-dimer concentrations and platelet count.

An additional analysis of correlations between the studied parameters did not show any interdependencies in the LEAD group, however, the control group was found to have a significant positive correlation between fibrinogen concentrations and platelet count ($r = 0.47, p = 0.01$).

# Discussion

The present study revealed significantly higher concentrations of investigated parameters (t-PA Ag, PAI-1 Ag, D-dimer, fibrinogen) and insignificantly elevated PLT count in LEAD patients when contrasted with healthy subjects. Moreover, the observations indicated significantly increasing values of fibrinogen and PLT, and an insignificant (due to the number of subjects) but clear trend of increasing concentrations of D-dimer at the successive stages of LEAD (in accordance with the Fontaine classification). This was also reflected in the obtained negative correlation of IC distance, fibrinogen concentrations and PLT count. Besides, the ABI negatively correlated with D-dimer concentrations and PLT count. No influence of gender on the concentrations of the studied parameters was found, yet, the age of patients with LEAD positively correlated with D-dimer concentrations and the subgroup of patients ≥ 65 years of age revealed significantly elevated values of this parameter when compared to the subjects in the younger age group.

The world literature refers to LEAD patients as PAD or PAOD. Zalewska-Rydzkowska *et al*. measured t-PA Ag and PAI-Ag levels in 40 patients with PAOD classified as stage II of Fontaine's classification. They found significantly elevated concentrations of t-PA Ag and PAI-1 Ag in the blood patients against healthy subjects [14]. Similar results – high values of t-PA Ag and PAI-1 Ag – in the study of 103 patients (also stage II of Fontaine's classification) were obtained by Strano *et al*. [15]. A comparative analysis conducted by Killewich *et al*. on two groups of patients: with PAD and mild ischemia (stage IIa of

Fontaine's classification, *n = 18*) and subjects with PAD and severe ischemia (stage IIb, *n = 51*) found significantly higher values of t-PA Ag in patients at stage IIb of Fontaine's classification, in comparison to IIa subjects and the control group, as well as a negative correlation between t-PA Ag levels and the claudication-free distance [16]. The studies carried out by Rość *et al*. on patients at stage II (*n = 13*), III (*n = 10*) and IV (*n = 2*) of Fontaine's classification reported increasing values of t-PA Ag with the disease progress [17]. Elevated concentrations of PAI-1 in patients with lower extremity artery disease against healthy subjects were also observed by Trifiletti *et al*. [18]. No distinctive differences were obtained in this research in terms of concentrations of t-PA Ag and PAI-1 Ag in patients with division to LEAD patients.

One of significant cardiovascular risk factors is the reduced fibrinolytic activity of plasma depending on t-PA synthesis and/or the increased activity of PAI-1 [19,20]. A considerable part of plasma t-PA circulates as complexes with PAI-1, therefore, the concentration of t-PA is believed to be primarily dependent on PAI-1 levels [21]. t-PA-PAI-1 complexes do not demonstrate any catalytic activity to plasminogen and their active form is free t-PA. The observations made in the present study, just like the examples provided by the scientific literature about high concentrations of t-PA in patients with LEAD/PAD indirectly indicate that patients with atherosclerosis have damaged endothelium which is the main source of t-PA. However, given the high concentration of PAI-1 Ag in PAD patients, t-PA forms inactive fibrinolytic complexes with this inhibitor.

The research studies conducted by van der Bom *et al*. reported elevated D-dimer levels in the blood of patients with PAD [22]. Similar, significantly higher values of D-dimer were observed by Trifiletti *et al*. in patients with lower extremity artery disease in comparison to healthy subjects [18]. Likewise, significantly higher D-dimer levels were obtained by Strano et *al*. in the blood of 103 patients with PAD (stage II of Fontaine's classification) in comparison to the control group [15]. High FDP levels were reported by Zalewska-Rydzkowska *et al*. in 40 patients at stage II of Fontaine's classification [14].

Our studies on D-dimer are consistent with the findings of the authors referred to above. They indicate increased dissolution of stabilized fibrin that is excessively formed during blood coagulation activated by the tissue factor (TF). The research of our center presented in another publication demonstrates increased thrombogenesis and excessive formation of TF in the blood of PAD patients. The present study, despite the observed tendency to increasing D-dimer values at successive Fontaine stages (IIa < IIb < III < IV), did not find statistical significance which may have been associated with a relatively small number of subjects, especially in the group of stage III patients.

This research study observed a significant increase in the concentrations of fibrinogen in the course of successive disease stages in accordance with the Fontaine classification (IIa, IIb, III and IV) in the test group, and the values in patients classified as IIb, III and IV were significantly higher when compared to healthy subjects. The above-mentioned study by Zalewska-Rydzkowska *et al*. found significantly higher fibrinogen levels in the blood of patients with PAOD classified as II against healthy subjects [14]. Increased fibrinogen concentrations are an independent thrombosis factor in patients with

atherosclerosis. A high fibrinogen concentration changes the rheological properties of the blood through increased aggregation of platelets and erythrocytes and intense adhesion of granulocytes. It also contributes to increase the content of fibrinogen in thrombus and to change the thrombus structure.

Available publications do not analyze the absolute number of PLT at subsequent Fontaine stages. Various platelet parameters including platelet count in patients with PAD were investigated by Zeiger *et al*. who did not find any significant differences between patients and healthy subjects in terms of PLT count. Yet, they demonstrated a negative correlation between platelet count and volume [23]. The role of platelet count, in addition to other platelet parameters, was also mentioned by Bigalke *et al*. [24]. Still, Braun *et al*. in their pilot study reported elevated PLT count in patients with PAD against the control group [25]. The study by Demirtas *et al*. analyzed 82 PAD patients divided in accordance with the Fontaine classification and determined mean platelet volume (MPV) obtaining the increasing values of MPV respectively in class I, II and III of Fontaine's classification [26]. The importance of MPV as a significant PAD risk factor was highlighted by Berger *et al*. in the *NHANES* (*National Health and Nutrition Examination Survey*) that included as many as 6354 subjects [27]. The analysis of subgroups of PAD patients classified as IIa, IIb and IV of Fontaine's classification – in the present publication – found a significant increase in PLT count at successive stages of disease. In the present study, lack of statistical significance between these subgroups of patients and class III of Fontaine's classification was probably affected by a small sample size of patients in this class (*n = 4*). It should be also added that the values in class IIa, IIb and III were not significantly higher in comparison to the control group, whereas a significant difference was observed between class IV and healthy subjects. Regardless of the aforementioned limitations, it appears that determination of PLT count, due to the fact that this test is readily available in everyday medical practice, should be more appreciated. The findings require further studies on a larger number of LEAD patients.

The ABI is an indispensable parameter in the diagnostics and assessment of LEAD/PAD stage. Van der Bom *et al*. analyzed t-PA and PAI-1 concentrations depending on the severity of disease classified in accordance with the decreasing value of ABI in 325 patients with PAD selected from a large cohort study group - *Rotterdam Study* (including nearly 8 thousand people). They demonstrated higher values of t-PA Ag and PAI-1 Ag in patients against healthy subjects, and a significant increase in t-PA Ag with the decreasing ABI [22]. The publication of Mota *et al*. indicated elevated levels of PAI-1 in PAD patients as compared to healthy subjects and a negative correlation between PAI-1 concentrations and ABI values [28]. However, in the study conducted by McDermott *at al*. on a large number of patients with PAD (*n = 370*), significant correlations were not observed between t-PA Ag and ABI concentrations and the levels of PAI-1 Ag and ABI [29]. This study also did not report any statistically significant correlations between t-PA Ag, PAI-1 Ag and IC distance or rest ABI.

Findings reported by Mota *et al*. highlighted high concentrations of D-dimer in patients with PAD which insignificantly increased with the decreasing ABI [28]. In the above-mentioned research, however, McDermott *et al*. obtained a significant negative correlation of D-dimer concentrations and the ABI [29]. The severity of lower extremity artery disease assessed as the decreasing ABI, correlated – in the study by

Reich *et al*. – with the increasing values of D-dimer [30], which is consistent with the statistically significant negative correlations between D-dimer levels and the ABI observed in our patients. In their publication, McDermott *et al*. also described a significant negative correlation between fibrinogen concentrations and the ABI [29]. A similar relationship between increasing values of fibrinogen with the decreasing ankle-brachial index was evidenced by Reich *et al*. [30]. Such an interdependence was not reported in the present study, but a significant negative correlation was observed between the values of fibrinogen levels and IC distance in the walk test.

Important observations were made in this study in terms of statistically significant positive correlations of D-dimer levels and age in the LEAD group, in addition to significantly higher concentrations of D-dimer in the subgroups of patients over the age of 65 in comparison to LEAD patients under 65. It is evident that the process of blood coagulation increases with age due to atherosclerosis progression and endothelial dysfunction [31]. Since the concentration of D-dimer reflects secondary-to-coagulation fibrinolysis, high D-dimer levels in patients over 65 years of age manifest intensified coagulation and an increase in secondary fibrinolysis. Even though the present study did not find a significant difference in PLT count between LEAD patients and healthy subjects, the comparison of patients and healthy subjects under the age of 65, allowed to determine significantly higher PLT count in patients as compared to healthy subjects from the same age group. Elevated PLT count in the blood of patients with atherosclerosis always increases the risk of thrombosis.

## Limitations of the study

Based on the study results it is hard to explain the apparent contradiction of high concentrations of t-PA Ag, PAI-1 Ag and D-dimer in PAD patients. A considerable limitation of the present study was the use of methods for quantitative detection of antigen proteins only (t-PA Ag, PAI-1 Ag) without measuring the activity of t-PA and PAI-1 at the same time. The measurement of t-PA activity allows to determine the size of t-PA active fraction responsible for transforming plasminogen into plasmin within the entire antigen range of t-PA protein. The research methodology of the activity demonstrated by these parameters is complicated and incredibly hard to conduct considering the approved research protocol of the present study. Another limitation of the present study is also a small number ($n = 4$) of LEAD patients classified as III of Fontaine's classification.

## Conclusion

1. High t-PA Ag concentrations in LEAD patients reflect damaged endothelium which comprises the main source of this factor. With high PAI-1 Ag levels, inactive fibrinolytic t-PA-PAI-1 complexes are formed.

2. Increasing concentrations of fibrinogen and rising PLT count at the successive stages of Fontaine's classification, confirmed by negative correlations with IC distance (and with the ABI value in case of PLT count) suggest increasing ischemia (hypoxia) and inflammatory condition which is associated with progression of atherosclerosis.

3. Elevated values of D-dimer reflect the aggregation of secondary fibrinolysis activation as the LEAD patient ages and along with impaired extremity vascularization manifested by the decreasing ABI at rest.

# Abbreviations

LEAD: lower extremity artery disease

IC: intermitten claudication

CLI: critical limb ischemia

t-PA Ag: tissue-type plasminogen activator

PAI-1 Ag: plasminogen activator inhibitor type 1

PLT: platelet count

ABI: ankle-brachial index

BMI: body-mass index

# Declarations

**Acknowledgements:** not applicable.

**Authors' contributions:** RW, AMW and DR made the concept and design, acquisition of data, analysis, interpretations of data, drafted the article, and approved the final version.

Fundind: not applicable.

**Ethics approval and consent to participate:** The studies were authorized by the local Bioethics Commission of Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, no. KB 509/2011 and were carried out in accordance with the Declaration of Helsinki. Obtaining blood samples was preceded by giving informed consent.

**Consent for publication:** not applicable.

**Competing interest:** none declare.

# Tables

Table 1. General characteristics of the study group (LEAD, *n = 80*).

| Parameter | Unit | Value |
|---|---|---|
| Sex (females / males) | n (%) / n (%) | 27 (34%) / 53 (66%) |
| Average age ± SD | years | 63.5 ± 9 |
| Number of patients classified as F IIa stage | n (%) | 11 (14%) |
| Number of patients classified as F IIb stage | n (%) | 54 (67%) |
| Number of patients classified as F III stage | n (%) | 4 (5%) |
| Number of patients classified as F IV stage | n (%) | 11 (14%) |
| Medium IC distance ± SD | m | 100 ± 87 |
| Average ABI ± SD | [ ] | 0.5 ± 0.25 |
| Number of patients taking ASA | n (%) | 80 (100%) |
| Number of patients taking statin | n (%) | 80 (100%) |

**Table 2. Values of studied parameters in the test group and control group.**

| Analyzed parameter and unit | Assumed value | Test group (LEAD, *n = 80*) | Control group (C, *n = 30*) | *p* |
|---|---|---|---|---|
| **t-PA Ag** | Me (Q25;Q75) | 12.22 | 4.79 | ***< 0.001*** |
| [ng/mL] | | (9.01; 16.39) | (2.62; 5.77) | |
| **PAI-1 Ag** | X | 48.5 | 17.79 | ***< 0.001*** |
| [ng/mL] | (± SD) | (± 14.03) | (± 6.91) | |
| **D-dimer** | X | 828.49 | 312.58 | ***< 0.001*** |
| [ng/mL] | (± SD) | (± 274.96) | (± 93.25) | |
| **fibrinogen** | Me (Q25;Q75) | 4.51 | 3.36 | ***< 0.001*** |
| [g/L] | | (3.66; 5.17) | (2.8; 3.7) | |
| **PLT** | Me (Q25;Q75) | 246.5 | 223 | *NS* |
| [G/L] | | (203.5; 290) | (182; 282) | |

Me = median, Q25 = lower quartile, Q75 = upper quartile, X = mean, SD = standard deviation

Table 3. Values of studied parameters in the test group in the subgroups of patients in accordance with the Fontaine classification of disease severity in comparison to the control group.

| Analyzed parameter and unit | Assumed value | Test group (LEAD, *n = 80*) | | | | Control group (C, *n = 30*) | *p* |
|---|---|---|---|---|---|---|---|
| | | IIa (*n = 11*) *a* | IIb (*n = 54*) *b* | III (*n = 4*) *c* | IV (*n = 11*) *d* | *e* | |
| **t-PA Ag** [ng/mL] | Me (Q25;Q75) | 11.14 (7.31;13.41) | 13.16 (8.69;16.57) | 13.07 (11.14;15.6) | 11.38 (8.69;16.89) | 4.79 (2.62;5.77) | *a vs. b vs. c vs. d NS*<br>*a vs. e = 0.006*<br>*b vs. e < 0.,001*<br>*c vs. e = 0.01*<br>*d vs. e < 0.001* |
| **PAI-1 Ag** [ng/mL] | Me (Q25;Q75) | 50.85 (43.85;54.61) | 50.75 (42.83;56.58) | 55.75 (41.86;71.3) | 42.35 (28.1;58.34) | 16.81 (14.02;22) | *a vs. b vs. c vs. d NS*<br>*a vs. e = 0.002*<br>*b vs. e < 0.001*<br>*c vs. e = 0.002*<br>*d vs. e < 0.001* |
| **D-dimer** [ng/mL] | X (± SD) | 762.28 (± 323.49) | 797.92 (± 277.31) | 963.54 (± 283.61) | 967.48 (± 194.577) | 312.58 (± 93.25) | *a vs. b vs. c vs. d NS*<br>*a vs. e < 0.001*<br>*b vs. e < 0.001*<br>*c vs. e < 0.001*<br>*d vs. e < 0.001* |
| **fibrinogen** [g/L] | Me (Q25;Q75) | 3.81 (3.5;4.22) | 4.25 (3.39:5.12) | 4.85 (4.74;5.67) | 5.38 (4.64;6.16) | 3.36 (2.8;3.7) | ***a vs. b vs. c vs. d = 0.02***<br>*a vs. e NS*<br>*b vs. e < 0.001*<br>*c vs. e = 0.008*<br>*d vs. e < 0.001* |
| **PLT** | Me (Q25;Q75) | 208 | 243 | 190 | 350 | 223 | ***a vs. b vs. c vs. d < 0.001*** |

| | | | | | | |
|---|---|---|---|---|---|---|
| [G/L] | | (198;297) | (211;270) | (175.5;199) | (268;398) | (182;282) | *a vs. e NS*<br>*b vs. e NS*<br>*c vs. e NS*<br>***d vs. e < 0.001*** |

Me = median, Q25 = lower quartile, Q75 = upper quartile, X = mean, SD = standard deviation

Table 4. Values of studied parameters in the test group and the control group disaggregated by age (< 65 years of age and ≥ 65 years of age).

| Analyzed parameter and unit | Assumed value | Test group (LEAD, *n = 80*) | | Control group (C, *n = 30*) | | *p* |
|---|---|---|---|---|---|---|
| | | < 65 years (*n = 48*) | ≥ 65 years (*n = 32*) | < 65 years (*n = 26*) | ≥ 65 years (*n = 4*) | |
| | | *a* | *b* | *c* | *d* | |
| **t-PA Ag** [ng/mL] | Me (Q25;Q75) | 11.95 (9.29;15.95) | 13.16 (9.01;16.66) | 4.37 (2.62;5.6) | 6.02 (3.52;7.17) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d = 0.01* |
| **PAI-1 Ag** [ng/mL] | X (± SD) | 47.55 (± 13.9) | 49.98 (± 14.37) | 17.64 (± 7.09) | 18.78 (± 6.42) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d < 0.001* |
| **D-dimer** [ng/mL] | X (± SD) | 761.87 (± 260.47) | 929.87 (± 270.61) | 314.12 (± 96.65) | 302.6 (± 77.8) | ***a vs. b = 0.03***<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d < 0.001* |
| **fibrinogen** [g/L] | X (± SD) | 4.5 (± 1.28) | 4.36 (± 1,09) | 3.08 (± 0.59) | 3.83 (± 0.02) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d NS* |
| **PLT** [G/L] | Me (Q25;Q75) | 246.5 (209.5;296.5) | 246 (200.5;277) | 221 (182;264) | 297.5 (238;320) | *a vs. b NS*<br>*c vs. d NS*<br>***a vs. c = 0.04***<br>*b vs. d NS* |

Me = median, Q25 = lower quartile, Q75 = upper quartile, X = mean, SD = standard deviation

Table 5. Values of studied parameters in the test group and the control group with sex-based division.

| Analyzed parameter and unit | Assumed value | Test group (LEAD, *n = 80*) | | Control group (C, *n = 30*) | | *p* |
|---|---|---|---|---|---|---|
| | | women (*n = 27*) | men (*n = 53*) | women (*n = 10*) | men (*n = 20*) | |
| | | *a* | *b* | *c* | *d* | |
| **t-PA Ag** [ng/mL] | Me (Q25;Q75) | 12.04 (8.69;15.65) | 12.4 (9.84;17.12) | 3.84 (2.62;5.59) | 5.04 (2.49;6.23) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d < 0.001* |
| **PAI-1 Ag** [ng/mL] | X (± SD) | 47.86 (± 15.22) | 48.82 (± 13.57) | 15.03 (± 7.56) | 19.17 (± 6.31) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d < 0.001* |
| **D-dimer** [ng/mL] | X (± SD) | 835.82 (± 253.31) | 824.02 (± 290.82) | 243.82 (± 73.04) | 346.96 (± 83.74) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d < 0.001* |
| **fibrinogen** [g/L] | Me (Q25;Q75) | 4.61 (3.81;5.38) | 4.22 (3.54;5.17) | 3.13 (2.38;3.57) | 3.39 (2.84;3.81) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c < 0.001*<br>*b vs. d < 0.001* |
| **PLT** [G/L] | Me (Q25;Q75) | 273 (203;321) | 234 (204;267) | 234.5 (182;286) | 222 (184;275) | *a vs. b NS*<br>*c vs. d NS*<br>*a vs. c NS*<br>*b vs. d NS* |

Me = median, Q25 = lower quartile, Q75 = upper quartile, X = mean, SD = standard deviation

**Table 6. Correlations of studied parameters.**

| LEAD (*n = 80*) | | Age | IC distance | ABI |
|---|---|---|---|---|
| | unit | years | m | [ ] |
| **t-PA Ag** | ng/mL | *NS* | *NS* | *NS* |
| **PAI-1 Ag** | ng/mL | *NS* | *NS* | *NS* |
| **D-dimer** | ng/mL | *r = 0.4*<br>*p = 0.002* | *NS* | *r = -0.32*<br>*p = 0.027* |
| **fibrinogen** | g/L | *NS* | *r = -0.32*<br>*p = 0.027* | *NS* |
| **PLT** | G/L | *NS* | *r = -0.33*<br>*p = 0.02* | *r = -0.42*<br>*p = 0.003* |