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Article

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Abstract: Intermittent claudication is a frequent complaint in lower extremity artery disease, but approximately two thirds of patients are asymptomatic, mostly in diabetic population. Non-invasive angiological and microrheological tests among diabetic patients with and without intermittent claudication were studied. 98 diabetic patients were included and divided into two groups: 20 patients (63.5±8.8 yrs, 55% men, 45% women) had intermittent claudication, 78 patients (65.5±9.3 yrs, 61.5% men, 38.5% women) were asymptomatic. Hand-held Doppler, transcutaneous tissue partial oxygen pressure ($tcpO_2$) measurement, tuning fork test and 6-minute walk test were performed and erythrocyte aggregation was investigated. Ankle/brachial index ($p<0.02$), and $tcpO_2$ measured during provocational tests ($p<0.003$), 6-minute walk test ($p<0.0001$) were significantly deteriorated in the symptomatic group. Higher erythrocyte aggregation index and faster aggregate formation could be observed among claudicant patients ($p<0.02$). In spite of the statistically better results of the asymptomatic group, 13% of these patients had severe limb ischemia based on the result of $tcpO_2$ measurement. Claudication could be associated with worse hemodynamical and hemorheological status in diabetics; nevertheless, severe ischemia can develop even in asymptomatic subjects which can be revealed by noninvasive vascular tests, which highlights the importance of the early instrumental screening of the lower limbs.

Keywords: diabetes mellitus; lower extremity artery disease; red blood cell aggregation; transcutaneous partial tissue oxygen pressure

1. Introduction

Based on the data of the International Diabetes Federation, 425 million people suffered from diabetes mellitus in 2017, and this number is increasing rapidly [1,2]. Diabetes can cause multiple complications, both at the macro- and microvascular levels. Even in the case of newly discovered diabetes, early diagnosis and the detection of pre-existing complications (e.g. retinopathy, nephropathy, neuropathy, peripheral artery disease) are important [3]. Microvascular complications may draw attention to possible involvement of other vascular areas (e.g. coronary vessels, carotid or lower limb arteries) [4]. LEAD is 2-4 times more frequent in diabetic patients than in the general population [5,6]. The 5-year mortality of LEAD patients is very high (30%), this is not only due to the deterioration of the limb, but also the life-threatening condition caused by major cardiovascular events (e.g. atherosclerosis of the coronary or cerebral vessels) [7]. It is known that the survival of LEAD patients, even in the asymptomatic stage, is worse compared to non-vascular patients [8]. 20-30% of LEAD patients also have diabetes. The severity and duration of diabetes affect the development of the disease and its progression, and it has also been proven that there is a much greater opportunity of critical limb ischemia among diabetic patients and the major amputation rate of the lower limb is 7-15 times higher than in the non-diabetic population [9]. Diabetes is one of the main causes of lower limb amputation due to nontraumatic reason; moreover, patients can suffer another major or minor amputation within 3 years of the first amputation [10]. In patients with T2DM,

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involvement of the infrapopliteal arteries is more common with advanced calcification, multisegmental appearance, and poor distal outflow tract. LEAD can appear 10 years earlier compared to the non-diabetic population. In a late phase, critical limb ischemia (CLI) may develop with high risk for major adverse limb and/or cardiac events (MALE, MACE) [11]. The diagnosis of CLI is confirmed by non-invasive vascular procedures: ABI<0.4, ankle pressure <50 mmHg, toe pressure <30 mmHg or $tcPO_2$ <30 mmHg. International guidelines recommend WIFI classification which gives a risk stratification for amputation based on the severity of the wound, ischemia, and foot infection [12,13]. Due to the possibility of asymptomatic (or silent) lower extremity artery disease in diabetes, it is important to perform vascular screening. Following palpation of arterial pulse, the ankle-brachial index is recommended as the first non-invasive diagnostic tool based on several guidelines with evidence of I/A [14,15]. Sensitivity and specificity of this test are high in non-diabetic subjects, however, because of media sclerosis in diabetes, renal failure or severe calcification in the elderly, arteries become incompressible resulting in false negative test [16]. In this case, toe blood pressure or transcutaneous tissue oxygen pressure measurement could be good alternative [17]. In addition to the vascular examination, the calibrated tuning fork test should be essential in the diabetic population and could be easily performed as a screening method. Sensory neuropathy leads to loss of different types of sensitivity: beside vibration, the sensation of pain is also impaired, which can mask tissue ischemia and the classic claudication symptom does not develop while walking, therefore LEAD remains undiscovered [18]. In addition to macrovascular damage, microvascular involvement also plays a significant role, e.g. causing retinopathy, nephropathy. In diabetic microvascular damages, hemorheological alterations also play a role beside hemodynamic and endothelial factors. Several studies have proven that hemorheological characteristics, such as erythrocyte deformability and aggregation are significantly worse in the diabetic population compared to the non-diabetics. [19,20].

2. Materials and Methods

2.1. Focus and aim of the study

The aim of our study was to find out whether there is a significant difference of the angiological and the hemorheological results between claudicant and non-claudicant diabetic patients. We studied whether the ankle-brachial index in a diabetic population can reveal lower limb ischemia appropriately, or an additional non-invasive angiological method can identify more patients having peripheral artery disease.

2.2. Participants

98 diabetic patients with previously known diabetic retinopathy were included in our study. These patients were followed regularly for their retinopathy at the Department of Ophthalmology. The participants were divided into two groups: 20 patients (63.5±8.8 yrs, 50% men, 50% women) suffered from intermittent claudication, 78 patients (65.5±9.3 yrs, 61.5% men, 38.5% women) were asymptomatic. Comorbidities, risk factors, concomitant medication and physical status {including palpation of peripheral arteries, e.g. dorsal pedal artery (DPA), posterior tibial artery (PTA), popliteal, femoral artery} were recorded. Additionally to the routine examination, hand-held Doppler, transcutaneous tissue partial oxygen pressure measurement, tuning fork and 6-minute walk test were performed and red blood cell aggregation was also investigated.

2.3. Non-invasive arterial diagnostic procedures

2.3.1. Hand-held Doppler measurement, ankle/brachial index calculation

Hand-held Doppler ultrasound (MultiDoppy, 8 MHz, Medicad Ltd., Hungary, serial number: 141203) and manual sphygmomanometer were used to measure the systolic ankle pressure in both legs and arms; posterior tibial, dorsal pedal and brachial arteries were checked. To calculate the ankle-brachial index (ABI), the higher systolic blood pressure was used as a denominator while the higher

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pressure from the posterior tibial and dorsal pedal arteries at each ankle was considered as the numerator [13].

2.3.2. Transcutaneous tissue oxygen pressure

Transcutaneous partial tissue oxygen pressure was measured after 15 minutes acclimatization at room temperature of the lying subject using a two-channel oximeter (Tina TCM 4000, Radiometer, Copenhagen, Denmark). For the reference measurement, the first electrode was placed on the right side of the chest in the subclavicular region; then the electrode was repositioned to the lateral part of the leg, another electrode was fixed on the dorsal part of the foot in the first intrametatarsal space. The measurement was carried out at 44°C locally causing a maximum vasodilation [21]. The measurement at rest was followed by a test performed while elevating and hanging the leg.

2.3.3. Calibrated tuning fork test

To check diabetic polyneuropathy, Rydel-Seiffer calibrated 128 Hz tuning fork test was performed on the radius and big toe on both sides [22,23].

2.3.4. 6-minute walk test

Supervised 6-minute walk test (6MWT) was completed by each patient. During the test, the patients' maximum walking distance was recorded [24].

2.4. Blood sampling, sample preparation, red blood cell aggregation measurement

Blood samples were processed within 2 hours after vein puncture preceded by overnight fasting. For red blood cell (RBC) aggregation measurement LORCA (Laser-assisted Optical Rotational Cell Analyzer; R&R Mechatronics, Hoorn, The Netherlands) aggregometer was used. Red blood cell aggregation index (AI), RBC disaggregation threshold shear rate (γ) and $t_{1/2}$ characterizing the time that elapses to reach the half of maximal aggregation amplitude were measured [25].

2.5. Statistical analysis

IBM® SPSS® Statistics version 23.0 and one-way repeated ANOVA statistical test and Tamhane post-hoc tests were used to evaluate differences between the groups after using Kolmogorov-Smirnov test to check normality of the data distribution. Multinominal linear regression and stepwise analyses of the data were performed to predict the presence of claudication from RBC aggregation data (AI, $t_{1/2}$) considering the principle of multicollinearity. Data are shown as means ± standard deviation (SD). Results were considered significant at $p < 0.05$.

3. Results

Patients with intermittent claudication had a slightly poorer glycemic control, but the difference was not significant (p=0.239), and smoking habits were more common than in asymptomatic patients. The characteristics of the study population are summarized in Table 1.

Table 1. Characteristics of the study population.

	Symptomatic (%) (n=20)	**Asymptomatic (%) (n=78)**
Male	55	61.5
Female	45	38.5
Smoking habits	30	7.7
Mean duration of DM (yrs)	15	15.9
Mean HbA1c±SD	8.16±1.69	7.44±1.5
Chronic coronary syndrome (CCS)	35	29.4

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Cerebrovascular disease (CVD)	5	7.7
CCS+CVD	10	--
PAD (previously diagnosed)	30	15.4
Dyslipidemia	75	57.7
Hypertension	100	78.2
Ulcer/gangrene	10	11.53
Minor/major amputation	20	3.85

In 20% of the claudication patients very low ABI (<0.4) was found, which indicates severe limb ischemia; in 25% of the patients, moderately deteriorated ABI could be measured (0.4-0.7). Claudicant poatients had significantly lower mean ABI compared to asymptomatic ones (0.79±0.38 vs. 1.01±0.34, p<0.02). Despite the statistically better results of the symptom-free patients, 16% had absolute values characteristic of severe or moderate limb ischemia, and every fifth patient had non-compressible peripheral arteries, which implies non-informative measurements regarding intraluminal pressure potentially masking severe ischemia. The distribution of the ankle-brachial index in various ABI ranges is shown in Table 2.

Table 2. Distribution of ankle-brachial index in the study population.

ABI range	Symptomatic patients(%) n=20	Asymptomatic patients(%) n=78
<0.4	20% (n=4)	6.41% (n=5)
0.4-0.7	25% (n=5)	10.26% (n=8)
0.7-0.9	10% (n=2)	24.35% (n=19)
0.9-1.0	25% (n=5)	17.95% (n=14)
1.0-1.4	15% (n=3)	20.51% (n=16)
>1.4	5% (n=1)	20.51% (n=16)

In the claudication group, severe ischemia was confirmed at 40% of the patients based on $tcpO_2$ measurement (<30 mmHg), in half of these patients critically low value was revealed (<10 mmHg). In the asymptomatic group good tissue oxygenation (>50 mmHg) were measured only in 19 patients (24.6%), while 12 patients (15.4%) had severely low tissue oxygen pressure (<30 mmHg) (Table 3).

Table 3. Transcutaneous partial tissue oxygen pressure values of claudicant and non-claudicant patients.

$tcpO_2$ values	Symptomatic patients(%)	Asymptomatic patients(%)
>50 mmHg	15	24.6
30-50 mmHg	45	60
10-30 mmHg	20	15.4
<10 mmHg	20	-

We examined the ankle-brachial index indicating severe ischemia by low tissue oxygen tension (<30 mmHg) values in both claudication and non-claudication groups. In the claudicant group, the patients with severely (<0.4) or moderately decreased (<0.7) ABI, 7 out of 9 people were identified with severe ischemia with the $tcpO_2$ measurement. In the asymptomatic group the patients with severe (<0.4) or moderate decreased (<0.7) ABI, 3 out of 13 people were certified with severe ischemia.

60% of the asymptomatic patients with borderline and/or normal ABI (0.9-1.4) had low tissue oxygen tension values, precisely 7 patients out of 30 were justified with severe ischemia. 2 patients in both groups were found with non-compressible arteries (ABI >1.4), and severe tissue ischemia could be confirmed by the $tcpO_2$ measurement at them.

These results are summarized in Table 4.

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Table 4. Distribution of ankle-brachial indices with severe limb ischemia based on partial tissue oxygen pressure values (<30 mmHg) in the study population.

ABI range	Symptomatic patients (%) n=8	Asymptomatic patients (%) n=12
<0.4	4 (50%)	1 (8.33%)
0.4-0.7	2 (25%)	2 (16.67%)
0.7-0.9	-	-
0.9-1.0	-	5 (41.67%)
1.0-1.4	-	2 (16.67%)
>1.4	2 (25%)	2 (16.67%)

The absolute $tcpO_2$ value on the leg and the foot in a resting position was more deteriorated in the symptomatic than in the asymptomatic group. Foot elevation as a provocation test could worsen ischemia further (Table 5).

Table 5. Results of $tcpO_2$ measurement.

Position of the electrode	Symptomatic patients	Asymptomatic patients	p values
Chest	51.64±19.09	52.47±12.64	p=0.831
Leg at rest	41.11±17.05	49.50±11.45	p=0.018
Leg at elevation	38.08±16.54	44.26±11.46	p=0.119
Leg at stasis	57.5±14.64	58.58±12.83	p=0.795
Foot at rest	31.70±17.47	43.33±11.53	p=0.001
Foot at elevation	26.00±18.41	39.78±14.56	p=0.003
Foot at stasis	45.71±19.99	53.00±14.75	p=0.122

The tuning fork values were below the normal range (6-8) in both groups. We obtained worse results on the hallux of the diabetic claudicants (Table 6).

Table 6. Results of the calibrated tuning fork test.

Localization	Symptomatic patients	Asymptomatic patients	p values
Right hallux	3.36±2.06	4.45±1.92	p=0.045
Left hallux	3.28±2.05	4.54±1.99	p=0.038
Proc. styl.radii	5.85±1.02	6.41±1.31	p=0.149

The symptomatic patients had significantly lower maximal walking distance during the 6-minute walk test (192.33 ±120 m for claudicants vs. 310.31 ± 97.07 m for non-claudicants, ($p < 0.0001$).

The red blood cell aggregation index was significantly higher in symptomatic patients, the aggregate formation was faster and disaggregation shear rate was higher in this group. The nominal regression analyses regarding the prognostic power of RBC aggregation parameters for the presence of claudication revealed that the variables AI and $t_{1/2}$ statistically significantly predicted the dependent variable, $F(1,1) = 85$, $p < 0.002$. Results of these micro-hemorheological parameters are summarized in Table 7.

Table 7. Results of red blood cell aggregation.

Aggregation parameter	Symptomatic patients	Asymptomatic patients	p values
AI	71.94±5.93	65.78±6.78	p<0.001
$t_{1/2}$(sec)	1.36±0.54	1.94±0.71	p=0.002

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γ	197.11±80.29	138.12±41.58	p<0.001

4. Discussion

Diabetes mellitus is associated with higher risk for peripheral artery disease which can develop earlier, progresses more rapidly than in the nondiabetic patients and causes multisegmental appearance on both lower extremities. Early detection of LEAD among diabetics is of crucial importance because of the high rate of life-threatening cardio-, cerebro- and peripheral vascular complications. Due to incompressible arteries and peripheral neuropathy, LEAD can be hidden, and diagnostic procedures may be misleading. Diabetic retinopathy as a microvascular complication of diabetes was proved to be an independent risk factor for LEAD. [26,27].

In our study we aimed to assess the prevalence of LEAD among diabetics with already confirmed microvascular complication, i.e. diabetic retinopathy, and to reveal the differences of symptomatic and asymptomatic diabetic subjects by the means of a routine and an alternative non-invasive vascular test complemented by red blood cell aggregation parameters which can alter microvascular circulation.

Since diabetes is nearly asymptomatic in the early stages, approximately 50% of the patients are diagnosed late, so the development and progression of complications may go unnoticed [28]. In a large prospective study (UKPDS 59) among other findings the possible interaction between HbA1c and retinopathy were present. They found that hyperglycemia represents a higher risk of LEAD in patients with retinopathy, which may be a marker for vascular dysfunction and can draw attention to the disease even before the diagnosis of diabetes [29]. In our study the prevalence of LEAD based on the ABI was equally high, 60% both in the claudicant and the non-claudicant group. The role of peripheral neuropathy and LEAD in the development of diabetic foot and lower extremity ulcers has long been known; neuropathy can hide (mask) lower limb ischemia. The screening for neuropathy could be carried out easily by the calibrated tuning fork or monofilament test. Approximately 1/3 of diabetic patients develop diabetic neuropathy, which creates the basis for diabetic foot syndrome and causes neuropathic complaints [30,31]. Polyneuropathy is responsible for more than half of all limb amputations, and it has high economic and quality-of-life costs. In many cases the patient does not have complaints, such as intermittent claudication, due to neuropathy. Therefore, screening for neuropathy should be essential for diagnosis, patient education, provision of further impetus for optimizing glycemic control, and improving foot care for the reduction of lower-extremity complications. The diabetic neuropathy is symmetrical and has a distal appearance, the lower limbs are most frequently affected [32]. By the calibrated tuning fork test, we found abnormal values indicating neuropathy in more than half of the examined patients. This prevalence is in accordance with the literature, half of the diabetic patients develop neurological damage causing symptoms during their lifetime, most often distal symmetric polyneuropathy [33]. We found significant difference between the results of the tuning fork test in the two groups. Symptomatic patients in general could be in a more advanced stage of LEAD. Although neuropathy may reduce the patient's sensation of ischemia, the vasomotor damage caused by autonomic neuropathy may enhance microcirculatory disturbances, which can worsen tissue oxygenation further provoking ischemic pain upon exertion (e.g., walking).

LEAD may have histological implications as well, suggested in a study describing lower extremity small vessels of diabetic individuals with known peripheral artery disease [34]. They found that microvascular involvement may magnify peripheral resistance and intensify arterial atherosclerosis. Low ABI (below 0.9) indicating LEAD is an independent predictor of cardiovascular diseases and is a risk factor for cardiovascular morbidity and mortality [35]. In a Hungarian nationwide study hypertensive patients between 50 and 75 years of age were examined. Based on the ABI measurement, 14.4% were diagnosed with PAD [36]. In another clinical trial the LEAD prevalence was even higher and may exceed 20% when its definition was based on abnormal ankle–brachial index (ABI) [6,37].

Among the multiple complications of diabetes, the diabetic foot takes an extremely important place, due to its severity and often unfavorable outcome [38]. LEAD is one of the major components

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of diabetic foot: impaired circulation, superinfections, and neuropathy can lead to the development of non-healing ulcers and amputation. LEAD was present in 49% of patients with diabetic foot in the EURODIALE study, and one-third of the study population had both LEAD and infection [39]. In the ADVANCE study, the prevalence of LEAD determined on a very strict definition (peripheral revascularization procedure-either surgical or endovascular, at least one finger amputation of the lower limb, chronic leg ulcer due to arterial stenosis) was estimated in 4.6% [40]. In the Hungarian nationwide large retrospective study (HUNVASCDATA project) Kolossvary et al. found that the lifetime risk of ulcer formation in the diabetic population is estimated in 25%. The amputation was preceded by the development of an ulcer in 85% of all cases, and 50.4% of the patients underwent amputation were diabetic [9]. In our study, the proportion of patients living with ulcers was similar, 10% among claudication patients and 11.5% in the asymptomatic population. Larger portion of the claudication group had previously undergone amputation comparing to the non-claudicants.

The prevalence of LEAD increases with the duration of diabetes as shown in the UK Prospective Diabetes Study (UKPDS): at the time of diabetes diagnosis, it was 1.2%, while 18 years later the prevalence of peripheral artery disease was 12.5% [29]. The study of Marso et al described 1 in 3 people with diabetes over the age of 50 suffers from LEAD [41]. In our study the mean duration of T2DM was 15-15.9 years and LEAD was already present in 30% of claudicant patients, and 15.4% in the asymptomatic population. The high incidence of the peripheral artery disease can be attributed to the fact that our patients already had microvascular complications.

The gold standard for diagnosis of LEAD is invasive angiography. The guidelines recommend hand-held Doppler examination as a first non-invasive vascular diagnostic method performed on four limbs. The measurement is a commonly accepted tool to investigate macrocirculation, to define ABI and to presume LEAD [13,42]. It has a great value in classifying non-diabetic LEAD, but it can give unreliable results in diabetes. In a review, Xu et al. found the sensitivity of Doppler method to be 15-79% for detecting at least 50% lower extremity stenosis. The sensitivity was lower in diabetic and elderly patients. Doppler's specificity is very high, ranging from 83.3 to 99% in various studies [43]. This screening test is fundamental, it has a high importance from an epidemiological point of view, since the survival of LEAD patients, even in the asymptomatic stage, is significantly worse compared to non-vascular patients. [8]. The test can indicate peripheral artery disease even in an asymptomatic state (usually with a value between 0.75-0.9). If the ABI already indicates an abnormal value, the background is advanced atherosclerosis, and usually 50-70% lower limb artery stenosis can be verified [14].

Based on AHA/ACC 2016 recommendation, in patients with normal (1.00–1.40) or borderline (0.91–0.99) ABI in the setting of nonhealing wounds or gangrene, it is reasonable to diagnose CLI by using toe-brachial index (TBI) with waveforms, $tcpO_2$, or skin perfusion pressure (SPP) measurement. In PAD with an abnormal ABI (≤0.90) or with noncompressible arteries (ABI >1.40 and TBI ≤0.70) in the setting of nonhealing wounds or gangrene, TBI with waveforms, $tcpO_2$, or SPP can be useful to evaluate local perfusion [44]. Measurement of $tcpO_2$ is a non-invasive method detecting microcirculation and tissue oxygen supply. It can give information on skin perfusion and ischemia, which can be used to define the degree of lower extremity arterial disease [45]. Our results of the $tcpO_2$ measurements showed significantly lower $tcpO_2$ value measured on the leg at rest and on the foot at rest and at elevation in symptomatic patients compared to the asymptomatic group. This result supports the assumption of the more severe macro- and microcirculatory disorders in the diabetic claudicants; cramps as a sign of ischemia can be objectified with $tcpO_2$ measurement. Our study showed that the hand-held Doppler and $tcpO_2$ measurements clearly identify PAD patients in both groups; moreover, in the borderline (0.9-1) and normal (1-1.4) ABI ranges, high portion of the asymptomatic patients (41.67% and 16.67%, respectively) had severe limb ischemia (<30 mmHg). A part of the patients with noncompressible arteries (ABI > 1.4) also had severe ischemia (25% in the claudication, 16.67% in the asymptomatic group). Combining the two methods, we were able to identify 13 new patients with previously hidden severe limb ischemia, which accounts for 13% of the entire study population.

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In addition to severe hemodynamic and neuropathic abnormalities, hemorheological parameters also play a significant role in diabetic patients due to the poor distal outflow. In the study Dupuy-Fons et al. showed that diabetic patients who needed major amputation (above or below knee) presented significantly deteriorated hemorheological parameters (blood viscosity, RBC aggregation, RBC rigidity index, hematocrit/viscosity ratio, fibrinogen level) compared to diabetic patients without major amputation needs (no amputation or only toes' amputation) [46]. In a previous study, our team confirmed that patients suffering from diabetic retinopathy had more increased RBC aggregation compared either to an age-matched non-diabetic population or a young, healthy control group [19]. In the present study, an increased red blood cell aggregation and faster aggregation formation was proved in patients with claudication compared to asymptomatic patients. Furthermore, we could prove, that RBC aggregation parameters can predict the presence of claudication as well. These findings may suggest, that hemorheological factors have a strong influence on blood flow in LEAD patients. Ernst and his coworkers described the relevance of rheological factors in the maintenance of pain free walking distance in LEAD patients. The researchers found that physical activity had a beneficial effect on hemorheological parameters, and even seemingly minor changes in the flow properties of blood may increase nutritive flow in the hypoxic vascular bed [47]. Our result supports these previous findings; the higher RBC aggregation is, the more probable claudication occurs. This could be resulted from the diminished oxygen delivery to distal tissues in case of hemodynamic and hemorheological disturbances and may have prognostic significance in chronic peripheral occlusive arterial disease in advanced cases.

5. Conclusions

There were less patients in the claudicant group, the sensitivity of the study could have been increased if an equal number of patients had been included in both groups. Toe pressure was not measured due to unavailability at the beginning of this study.

6. Summary

Because of lower limb neuropathy ischemic symptoms are not recognized neither by the patient in time nor even by physicians taking ankle pressures, because of false negative results. Hence LEAD may remain undiscovered, supplementary non-invasive diagnostic tools, e.g., transcutaneous partial oxygen pressure and/or toe pressure measurements could be needed as an alternative diagnostic step to clarify the patient's condition especially in patients with neuropathy [21]. Limb loss is a severe complication of LEAD, and could seriously impair quality of life, therefore an early recognition of ischemia by non-invasive vascular tests (screening) could play a key role for preventing diabetic patients from the development of ischemic ulcer, amputation, and years of life lost. Our results suggest that in case of diabetes the Doppler measurements should be routinely complemented by tissue oxygenation measurements to improve diagnostic power. Based on our data, even diabetic patients without claudication should be screened for LEAD, as neuropathy could obscure the complaints, but together with the deteriorated rheological status could promote the progression of the disease. If claudication is present, our investigation evolved higher and faster red blood cell aggregation, more severe neuropathy and lower tissue oxygenation, which all should refer to a seriously impaired macro-, and microcirculation. These facts should evoke attention to early and multitool screening procedures for diabetes patients with but even without claudication.

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Informed Consent Statement: In accordance with institutional guidelines written informed consent was obtained from all subjects before their participation.

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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