Diploma Thesis

Cardiac biomarkers and their importance for the prognosis in peripheral arterial disease patients

submitted by

Aryan Aliabadi

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under the supervision of

Assoz. Prof. Priv.-Doz. Dr. Thomas Gary

Univ. FA Priv.-Doz. Dr. med. Gabor Toth-Gayor, PhD.

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Statutory Declaration

I hereby declare that I have authored this diploma thesis fully on my own, that I have not used any other than the declared sources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 04.02.2022 *Aryan Aliabadi eh.*

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List of abbreviations

ABI	Ankle-brachial index
ACC	American College of Cardiology
ACE	Angiotensin-converting enzyme
AHA	American Heart Association
AMI	Acute myocardial infarction
ANP	Atrial natriuretic peptide
AST	Aspartate transaminase
BNP	Brain natriuretic peptide
CHD	Coronary heart disease
CK	Creatine kinase
CLI	Critical limb ischemia
CNP	C-type natriuretic peptide
COX-1	Cyclooxygenase-1
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular diseases
ESC	European Society of Cardiology
ESH	European Society of Hypertension
HDL	High-density lipoprotein
HF	Heart failure
IC	Intermittent claudication
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein

LMR	Lymphocyte-to-Monocyte ratio
MI	Myocardial infarction
MRA	Magnetic resonance angiography
mRNA	Messenger ribonucleic acid
NLR	Neutrophil-to-Lymphocyte ratio
NSTEMI	Non-ST-segment-elevation myocardial infarction
NT-proBNP	N-terminal pro brain natriuretic peptide
PAD	Peripheral arterial disease
PAES	Popliteal artery entrapment syndrome
PLR	Platelet-to-Lymphocyte Ratio
PTA	Percutaneous transluminal angioplasty
PWV	Pulse wave velocity
TnC	Troponin C
TnI	Troponin I
TnT	Troponin T

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Zusammenfassung

Einleitung: Die periphere arterielle Verschlusskrankheit (pAVK) ist eine Manifestation der Atherosklerose, die zu arteriellen Stenosen und Okklusionen führt und häufiger die unteren Extremitäten betrifft. In der Folge kommt es zu einer verminderten Perfusion, was sich klinisch als Claudicatio intermittens äußern kann und im Weiteren zu einer kritischen Extremitätenischämie (CLI) mit Ruheschmerzen und Nekrosen fortschreiten kann.

Troponin und brain natriuretic peptide (BNP) sind kardiale Biomarker, welche eine wichtige Rolle bei Herzerkrankungen wie Myokardinfarkt und Herzinsuffizienz besitzen. Diese Arbeit soll die Relevanz dieser Biomarker bei Patient*innen mit pAVK untersuchen.

Methoden: In dieser retrospektiven Studie wurden 1743 Patient*innen, die an der Klinischen Abteilung für Angiologie der Universitätsklinik Graz behandelt wurden, eingeschlossen. Dazu wurden die Parameter Alter, Geschlecht, kardiovaskuläre Risikofaktoren wie arterielle Hypertonie und Diabetes mellitus und die Einnahme von Thrombozytenaggregationshemmer wie Acetylsalicylsäure und Clopidogrel erhoben. Zusätzlich wurden die Biomarker C-reaktives Protein (CRP), Troponin T und BNP gemessen. Im Folgenden wurde der Zusammenhang zwischen kardialen Biomarkern und das Auftreten von vaskulären Endpunkten einschließlich CLI, Myokardinfarkt und Schlaganfall mittels univariater und multivariater Regression analysiert.

Resultate: Die Studienpopulation hatte ein mittleres Alter von 69,2 ± 11,9 Jahren. 1046 Männer (60,0%) und 697 Frauen (40,0%) wurden in die Studie eingeschlossen, arterielle Hypertonie war in 81,2%, Diabetes mellitus in 33,2% als Risikofaktor vorhanden. Die Studienpopulation für BNP war kleiner und inkludierte 332 Patient*innen. Regressionsanalysen zeigten statistische Signifikanz für CLI (univariate Regression: Troponin T $p < 0,001$, BNP $p < 0,001$; multivariate Regression: Troponin T $p < 0,001$). Troponin T > 0,01 ng/ml war assoziiert mit einer OR von 2,439 (1,827 – 3,255) für CLI.

Schlussfolgerung: Erhöhtes Troponin T ist signifikant assoziiert mit Patient*innen mit CLI. Troponin T ist ein häufig eingesetzter Test im Zusammenhang mit Myokardischämie, jedoch scheint er auch mit einem aggressiveren Verlauf einer pAVK assoziiert zu sein.

Abstract

Introduction: Peripheral arterial disease (PAD) is a manifestation of atherosclerosis that results in arterial stenoses and occlusions, predominantly in the lower extremities. Consequently, the perfusion is impaired, which manifests clinically as intermittent claudication and can progress to critical limb ischemia (CLI) with ischemic rest pain and tissue loss.

Troponin and brain natriuretic peptide (BNP) are primarily cardiac biomarkers which play a crucial role in heart diseases such as myocardial infarction and heart failure, respectively. The subject of this diploma thesis is to evaluate the significance of these biomarkers for PAD.

Methods: A retrospective study was conducted including 1743 patients who were treated at the Division of Angiology, Medical University of Graz. Collected data included age, sex, cardiovascular risk factors such as arterial hypertension and diabetes, and intake of antiplatelet drugs such as aspirin and clopidogrel. Additionally, several biomarkers were measured including C-reactive protein (CRP), troponin T, and BNP. In the following, the correlation between cardiac biomarkers and the occurrence of vascular endpoints including CLI, myocardial infarction (MCI), and stroke were analyzed by means of univariate and multivariate regression.

Results: The study population was characterized by a mean age of 69.2 ± 11.9 years. 1046 men (60.0%) and 697 women (40.0%) were included in the study, arterial hypertension was present in 81.2% and diabetes in 33.2%. The study population for BNP was smaller and included 332 patients. Regression analyses showed statistical significance for CLI (univariate regression: troponin T $p < 0.001$, BNP $p < 0.001$; multivariate regression: troponin T $p < 0.001$). Troponin T > 0.01 ng/ml was associated with an OR of 2.439 (1.827 – 3.255) for CLI.

Conclusion: Increased troponin T is significantly associated with patients with CLI. Troponin T measurement is an often-used test in the context of myocardial ischemia, however, it also appears to be associated with a more aggressive course of PAD.

1. Introduction

1.1. Peripheral arterial disease

1.1.1. Definition

Peripheral arterial disease (PAD) is a common manifestation of arteriosclerosis, along with coronary heart disease and cerebrovascular disease. In the context of arteriosclerosis, a progressive narrowing of the arteries takes place, which leads to stenoses and occlusions and furthermore to perfusion deficits and ischemia. These pathological processes predominantly occur in the arteries of the lower limbs, however, the arteries of the upper limbs can also be affected (Shanmugasundaram *et al.*, 2011).

Peripheral arterial disease is often underdiagnosed and undertreated, which could be explained by the lack of screening measures. However, the diagnosis at an early stage and adequate therapy are of utmost importance, as patients with peripheral arterial disease have a high probability of suffering from other cardiovascular diseases such as coronary heart disease, myocardial infarction, and stroke (Shanmugasundaram *et al.*, 2011).

1.1.2. Epidemiology

Estimates reveal that there are approximately 40 million people with PAD in Europe, which equals a prevalence of 5.3%. Interestingly, the numbers across Europe differ strongly, for example, the prevalence in Greece is about 28%, while it is about 7% in Belgium (Olinic *et al.*, 2018). This could be explained by different lifestyle customs such as physical exercise and smoking (Cimminiello *et al.*, 2011).

Worldwide there are more than 200 million people suffering from PAD and the prevalence has increased significantly over the last decades (Firnhaber and Powell, 2019). What is important in this regard is the difference between high-income developed countries and low-income developing countries, the latter showing lower PAD rates (Criqui and Aboyans, 2015).

The prevalence of PAD is affected by several factors such as age, gender, ethnic origin, and social status. It is known that with age the prevalence surges, in Germany, for instance, the prevalence rises from 3% in men between 45 and 49 years to 18.2% in men between 70 and

75 years. The influence of the factor gender is not completely clear since there are several studies that conclude with differing results concerning this matter. In contrast, the influence of ethnic origin and social status has been well analyzed, and there is an acknowledged correlation between the prevalence of PAD and these factors. Studies from the United States show that ethnicity itself can increase the risk of PAD, African-Americans, for instance, have a higher risk compared to the other ethnicities, and this risk is not ascribable to traditional risk factors. Finally, social status affects the risk of PAD considerably, including factors such as marital status, education, employment, and socioeconomic status. Marital difficulties, lower education and unemployment contribute to a higher risk (Olinic *et al.*, 2018).

1.1.3. Risk factors

Smoking

In the context of PAD, smoking is often considered the most significant risk factor, and there is a direct correlation between smoking exposure and severity of PAD, rates of lower limb amputation and mortality (Diehm *et al.*, 2011). This applies not only to PAD but to all cardiovascular diseases, smoking is accountable for nearly one third of all coronary heart diseases and leads to a doubling of the risk of ischemic stroke (Planas *et al.*, 2002).

Tobacco consumption is directly proportional to the degree of PAD. Affected patients who continue smoking have to undergo procedures such as invasive interventions and amputations more often. Additionally, the success rates of leg revascularization are lower when patients continue to smoke. Conversely, patients who successfully quit smoking have many beneficial effects such as higher exercise tolerance and less fatal vascular complications, which can be reached within few years (Gottsäter, 2006).

Arterial hypertension

Arterial hypertension is strongly associated with an elevated risk for PAD. Many studies have indeed shown moderate elevations in relative risks, due to its high prevalence, though it is considered the second most contributive risk factor after smoking (Criqui and Aboyans, 2015).

The higher the degree of arterial hypertension, the more severe the degree of PAD, which is why blood pressure therapy is an important part of the general therapeutic strategy of PAD (Eraso *et al.*, 2014).

Diabetes mellitus

Diabetes mellitus is considered a major risk factor for PAD. In this connection, it is important to emphasize that a strong association especially exists between longstanding or more severe diabetes mellitus and PAD, whereas newly diagnosed diabetes mellitus and impaired glucose tolerance show no or hardly any association with PAD. Important parameters in the context of diabetes mellitus are its duration, the use of insulin and the degree of glycemic control which can be measured by the HbA_{1c} level (Criqui and Aboyans, 2015).

Diabetes mellitus contributes to atherosclerosis as it enhances platelet activity. Additionally, augmentations of coagulation factors and inflammatory biomarkers occur, which altogether lead to an environment that favors inflammation and thrombosis and therefore advancement of arteriosclerosis (Vrsalovic *et al.*, 2017).

PAD is a serious condition in patients with diabetes, since it leads to a higher risk of cardiovascular diseases and death, limb loss, disabilities and thus socioeconomic impairments (Nativel *et al.*, 2018). This circumstance and the relatively high commonness of PAD among diabetic patients necessitate regular screening for the purpose of an optimal risk management strategy (Norman *et al.*, 2006).

Dyslipidemia

Risk factors in the context of dyslipidemia are elevated concentrations of total cholesterol, low-density lipoprotein (LDL), triglyceride and lipoprotein (a) (Diehm *et al.*, 2011). In contrast, high-density lipoprotein (HDL) has a protective effect against PAD (Criqui and Aboyans, 2015). When compared to coronary heart disease dyslipidemia seems to have a less significant role and other risk factors such as smoking and arterial hypertension are placed ahead, still dyslipidemia has an adverse impact on the process of PAD and its reduction has non-neglectable consequences. As many studies have shown, by reducing the

lipids, improvements of symptoms, lower complication rates after revascularization and lower mortality can be achieved (Gottsäter, 2006).

Obesity

Obesity is an underestimated risk factor for PAD. As part of the so-called metabolic syndrome, it is often accompanied by hypertension, hyperglycemia, and dyslipidemia and therefore poses a major risk factor in the process of atherosclerosis. Intra-abdominal fat is involved in several metabolic processes and can raise the risk for metabolic disorders through different mechanisms. One of them is the dysregulation of the adipocytes, which leads to increased secretion of tumor necrosis factor α, free fatty acids, and various interleukins while at the same time the secretion of adiponectin is decreased. Altogether, obesity, which can be measured by an increased waist circumference, contributes to the genesis and progress of atherosclerosis and by this means to PAD (Brouwer *et al.*, 2007).

1.1.4. Classification

PAD varies notably in its presentation and patients can either suffer from acute or chronic symptoms or no symptoms at all. For clinical purposes, a pragmatic classification is one that considers the symptoms in the first place since those are the basis for categorizing the disease and determining the needed therapy. Nevertheless, aside from the classification scheme based on the patient's presentation, there are also several other schemes that focus on other factors such as anatomical aspects and special clinical factors such as the presence of wounds and infections (Hardman *et al.*, 2014).

The first classification was the Fontaine classification, which was published in 1954 and was grounded on symptomatology. In 1984, a new symptomatic classification was introduced which resembled Fontaine but added hemodynamic parameters to increase objectivity. This classification is known as the Rutherford classification (table 1) and is up to this day broadly applied in clinical settings (Hardman *et al.*, 2014; Teraa *et al.*, 2016).

The Bollinger and Graziani classification schemes are anatomical classifications that are based on angiographic findings. The WIfI classification (wound, ischemia, and foot infection) combines existing schemes with foot ulcer schemes and is therefore a very useful tool in the context of diabetic mellitus (Hardman *et al.*, 2014).

Grade	Category	Symptoms
0	0	Asymptomatic
	1	Mild claudication
I	2	Moderate claudication
	3	Severe claudication
II	4	Ischemic rest pain
III	5	Minor tissue loss
	6	Major tissue loss

Table 1: Rutherford classification adapted from (Hardman *et al.*, 2014)

1.1.5. Clinical presentation

PAD can present itself in many various ways, the classic presentation with intermittent claudication (IC) as a cardinal symptom is less frequent. This symptom is defined as pain in different parts of the lower limbs, depending on the localization of the stenoses, usually in the calves, which is triggered by exercise and can be relieved by rest. Normally, patients can walk for a certain distance until on grounds of ischemia IC occurs and forces them to halt. However, most patients have no symptoms at all or symptoms that are not as distinct as IC (Aboyans *et al.*, 2018a; Firnhaber and Powell, 2019).

1.1.6. Physical examination

Every patient who has a higher risk for PAD based on his or her risk factors or a suggestive clinical presentation should be assessed by means of a thorough physical examination. Part

of that is a general inspection of the body, especially of the limbs and feet which need to be exposed in a proper manner. At this point, general signs of cardiovascular disease such as scars from previous cardiovascular surgery, amputations, and xanthelasma related to hyperlipidemia should be the focus of examination. While examining the limbs trophic skin lesion, livedo reticularis, ulceration, and necrosis are to be searched. The next step after the inspection is the palpation in which the pulses of the femoral, popliteal, dorsalis pedis, and posterior tibial arteries should be evaluated, compared bilaterally, and categorized as present, weak, or absent. Furthermore, skin temperature and capillary refill time should also be checked. Finally, auscultation of the heart, abdomen, and the above-mentioned arteries should be conducted to assess possible pathologies such as valvular pathologies, aortic aneurysm, and arterial bruits (Bailey, Griffin and Julian Scott, 2014; Tummala and Scherbel, 2018).

1.1.7. Diagnosis

Medical history and physical examination

The first step in diagnosing PAD is to gather a complete medical history, which should contain all cardiovascular risk factors and comorbidities. In fact, a thorough medical history is considered a key step in the diagnostic process (table 2). For this purpose, the personal and family medical history should be enquired (Lawall *et al.*, 2016; Aboyans *et al.*, 2018a).

Family history of CVD (coronary artery disease, cerebrovascular disease, aortic aneurysm, lower extremity artery disease) and premature CVD (fatal or non-fatal CVD event or/and established diagnosis of CVD in first degree male relatives before 55 years or female relatives before 65 years).
Personal history of: • Hypertension • Diabetes • Dyslipidemia • Smoking (present and/or past), passive smoking exposure • Prior CVD • Chronic kidney disease

• Sedentary life • Dietary habits • History of cancer radiation therapy • Psycho-social factors
Transient or permanent neurological symptoms
Arm exertion pain, particularly if associated with dizziness or vertigo
Symptoms suggesting angina, dyspnea
Abdominal pain, particularly if related to eating and associated with weight loss
Walking impairment/claudication • Type: fatigue, aching, cramping, discomfort, burning • Location: buttock, thigh, calf, or foot • Timing: triggered by exercise, uphill rather than downhill, quickly relieved with rest; chronic • Distance
Lower limb pain (including foot) at rest, and evolution at upright or recumbent position
Poorly healing wounds of the extremities
Physical activity assessment: • Functional capacity and causes of impairment
Erectile dysfunction

Table 2: Medical history for assessment of PAD from (Aboyans *et al.*, 2018b)

By taking a detailed medical history, patients at increased risk can be identified, namely those older than 65 years, those between 50 to 64 years with additional risk factors of arteriosclerosis or family history of peripheral arterial disease, those younger than 50 years

with diabetes mellitus and one other risk factor, and those who already suffer from another atherosclerotic disease (Firnhaber and Powell, 2019).

Physical examination alone may not lead to the diagnosis due to poor sensitivity, still it should always be conducted as part of the overall view. Besides, findings in the physical examination do not only play a part in the diagnostic process but are also valuable in terms of prognostics. For example, a femoral bruit is considered an independent indicator for ischemic cardiac events (Aboyans *et al.*, 2018a).

Laboratory testing

There are routine laboratory tests that should be performed in every patient, while others are required as additional tests to specific findings from medical history, clinical examination, and routine laboratory tests. The routine tests include blood count, uric acid, fasting plasma glucose, fasting serum lipid profile, serum creatinine, creatinine clearance, and urine analysis with urinary protein and microalbuminuria. Additional tests such as lipoprotein (a) or glycated hemoglobin are required if there is a relevant cardiovascular family history or fasting plasma glucose is elevated (Aboyans *et al.*, 2018b).

Ankle-brachial index

The ankle-brachial index (ABI) is a very useful and effective tool in the diagnostic process of PAD. It is performed by measuring the highest systolic pressure in the dorsalis pedis or posterior tibial artery in each leg and in the brachial artery and putting these values in ratio to each other. The blood pressure is determined by using a Doppler probe and hence making the ABI a non-invasive measurement. That is, among other things, why the ABI is considered an excellent tool. Besides, the quick implementation and the high sensitivity and specificity pose major advantages (Bendermacher *et al.*, 2012; Firnhaber and Powell, 2019).

The ABI should be conducted in every patient with a suspected PAD on grounds of typical clinical presentation in form of intermittent claudication or wound in the lower extremities which do not heal. In addition, due to its simplicity, it should be performed as a screening measurement in primary care. In this way elderly patients with missing or poor symptoms can easily be identified and the progression of PAD can possibly be stopped (Aboyans *et al.*, 2018a).

Ratios between 0.9 to 1.3 are considered normal for adults, a ratio below 0.9 indicates a possible PAD, below 0.5 a CLI (table 3). In addition to the importance of ABI in terms of diagnosing PAD, it should be stressed that ABI in general is a strong marker for atherosclerosis and cardiovascular risk and that a decreased ratio is accompanied by higher rates of cardiovascular events and mortality. There are also other measurements such as coronary calcium score and carotid intima-media thickness, which can deliver similar information, but are much harder to obtain when compared to ABI (Crawford *et al.*, 2016; Aboyans *et al.*, 2018a).

ABI	Rating
1.0 to 1.3	Normal
0.9 to 1.0	Borderline
0.7 to 0.9	Mild
0.4 to 0.7	Moderate
< 0.4	Severe

Table 3: Diagnostic criteria for PAD on ankle-brachial-testing adapted from (Firnhaber and Powell, 2019)

Duplex ultrasound

While the ABI is used to determine whether PAD is present or not, the next step in the diagnostic process is usually medical imaging for the purpose of localizing stenoses and further measurements. The duplex ultrasound combines the different modes in ultrasound technology and can produce a B-mode image that is underlaid with Doppler modes. Being a non-invasive and hence safe method, which still provides lots of information, the duplex ultrasound is often considered as the gold standard when it comes to vascular imaging methods. Within the context of diagnosing PAD the purpose of the duplex ultrasound is to

localize the stenoses and determine their degree, which can be accomplished with both sensitivity and specificity above 90% in stenoses > 50% (Mathew and Kramer, 2018; Shabani Varaki *et al.*, 2018).

The disadvantages are the strong dependence on the operating person, who must be familiar with this special form of imaging, and the natural limitation of ultrasound. For instance, the iliac vessels can be hard to evaluate due to bowel gas, also swelling, excessive fat tissue and calcifications can make the assessment difficult (Mathew and Kramer, 2018; Shabani Varaki *et al.*, 2018).

Computed tomography

Arterial imaging by computed tomography (CT) is usually applied when the diagnosis is still uncertain after performing vascular ultrasound or in the context of revascularization (Kinlay, 2014). Non-invasively stenoses can be detected and plaques characterized, however, this is hampered by artefacts which are the result of calcifications (Tanaka *et al.*, 2019).

When compared to MRA and duplex sonography, both CTA and MRA show higher sensitivities, but require radiation or contrast agents and are not as easily accessible. The biggest disadvantage with CTA is certainly the usage of ionizing radiation that is required for this technology (Met *et al.*, 2009).

Magnetic resonance angiography

Another imaging modality used in the diagnosis of PAD is magnetic resonance angiography (MRA), which is used for determining the localization and severity of the stenoses. There are different types of MRA techniques with certain advantages and disadvantages which is why the adequate technique must be chosen wisely in order to obtain the best results (Mathew and Kramer, 2018). For instance, the MRA with gadolinium contrast is superior to non-contrast techniques in terms of resolution though in case of a chronic kidney disease, the latter is the preferred one (Aboyans *et al.*, 2018a).

Pulse wave velocity

Although the pulse wave velocity (PWV) is a rather old technique, it has recently assumed a relevant position in the diagnostics of PAD and in general cardiovascular diseases. Basically, PWV is a measurement of arterial stiffness by measuring the speed of the arterial pulse wave in the arteries. In PAD, the PWV is higher and hence the arterial stiffness is more distinct when compared to healthy individuals, though an elevated PWV can also be caused by hypertension and diabetes, for instance (Shabani Varaki *et al.*, 2018).

1.1.8. Differential diagnosis

When a patient is admitted to the hospital with intermittent claudication, the first and most important suspected diagnosis is PAD, since the most frequent cause for vascular insufficiency is an impaired perfusion due to atherosclerosis. However, there are several other etiologies, both vascular and nonvascular, which should be included in the list of differential diagnoses for IC and similar lower extremity pain. Vascular diseases that can mimic PAD are summarized under the term nonatherosclerotic vascular disorders (table 4). Although the prevalence of these diseases is rather low, they should always be taken into consideration, especially if the diagnosis of PAD does not stand to reason or if the affected patients are young and show no risk factors (Lyden and Joseph, 2006; Korngold and Jaff, 2009; Mintz and Weinberg, 2015).

Mural abnormity	Fibromuscular dysplasia
	Cystic adventitial disease
	Connective tissue disorders
	Idiopathic midaortic syndrome
Inflammatory disease	Thromboangiitis obliterans
	Takayasu arteritis

	Giant-cell arteritis
	Behçet disease
	Radiation-induced arteritis
Artery subject to abnormal forces	Iliac artery endofibrosis
	Popliteal artery entrapment syndrome
	Thoracic outlet syndrome
Abnormal coagulation	Inherited and acquired arterial thrombophilias
Abnormal veins	Venous claudication and gangrene

Table 4: Nonatherosclerotic vascular pathologies adapted from (Sharma, Norton and Zhu, 2014)

Popliteal artery entrapment syndrome

Popliteal artery entrapment syndrome (PAES) can feature intermittent claudication because of reduced perfusion through the popliteal artery due to compression. This compression can be caused by muscles and ligaments situated around the popliteal artery. PAES is subdivided into six types, which differ from each other by which anatomical structure or variation is responsible for the compression. It is noteworthy that PAES occurs predominantly in young patients on the one hand and male patients on the other hand (Sharma, Norton and Zhu, 2014; Mintz and Weinberg, 2015).

Cystic adventitial disease

Another nonatherosclerotic vascular disorder that can cause similar symptoms to PAD is cystic adventitial disease. The reason behind that is the compression and eventual occlusion of the vessels caused by cysts in the adventitial layer. The underlying etiology is not clear yet and there are different hypotheses, according to one of them certain mesenchymal cells in the adventitia seem to be the origin of the pathological process. These cells produce and

secrete mucin, which clusters in the form of cysts and exerts pressure on the inner layers of the vessels (Korngold and Jaff, 2009; Sharma, Norton and Zhu, 2014).

Vasculitides

As already mentioned, other reasons for intermittent claudication are rather rare. They also include inflammatory diseases such as vasculitides including giant-cell arteritis, Takayasu's arteritis, Buerger's disease, which is also known as thromboangiitis obliterans, polyarteritis nodosa and Behçet disease. Buerger's disease, for instance, can be a difficult and important differential diagnosis to PAD since both share the risk factor of smoking and can be very alike when it comes to the clinical presentation. A distinctive feature is that almost exclusively smokers are affected by this disease. Furthermore, men have a higher incidence than women. An important factor that should be taken into consideration for differential diagnosis is age, as Buerger's disease normally occurs in the fifth decade of life and consequently much earlier compared to PAD (Celecova *et al.*, 2013; Mintz and Weinberg, 2015).

Venous claudication

Venous hypertension based on a chronic venous insufficiency causes venous claudication with exercise as a trigger factor. The differentiation from PAD can be difficult, however, there are differences such as the regression time of the symptoms or the presence of edema in chronic venous insufficiency (Lyden and Joseph, 2006; Sharma, Norton and Zhu, 2014).

Nonvascular differential diagnoses

Besides nonatherosclerotic vascular disorders, there are a few important nonvascular differential diagnoses that should always be considered. Neurological diseases such as spinal cord compression or neuropathy can produce symptoms similar to those of PAD. The former is characterized by pain, weakness, and neurological symptoms such as paresthesia in the hip and thigh region. The symptoms can be triggered by simple upright standing and walking and can be eased by sitting. In contrast, neuropathy is not depending so gravely on the position as the associated pain can also occur at rest. The causes for neuropathy can be

various, frequent ones are nerve root compression and diabetic neuropathy (Lyden and Joseph, 2006).

Furthermore, several disorders affecting the musculoskeletal system can cause PAD-like symptoms. Hence, osteoarthritis of the hips and knees should always be considered a differential diagnosis, especially because the prevalence of this disease is high. The pain in osteoarthritis can occur at rest or at the beginning of a movement and can be influenced by different factors such as daily condition and weather. Contrary to osteoarthritis, chronic compartment syndrome occurs in young people, in particular young athletes who tend to develop muscular hypertrophy and thickened fascia. In these cases, the pathologically raised compartment pressure leads to reduced perfusion and leg pain which is triggered by exercise (Lyden and Joseph, 2006).

1.1.9. Therapy

Patients with PAD have a higher risk of mortality and cardiovascular events in opposition to people without PAD and that is the case regardless of the presence of symptoms. Furthermore, patients with IC have reduced rates of physical activity, increased rates of mobility loss, and worse quality of life, while most of the PAD patients never progress to the stage where the limb is threatened by ischemia. Based on this knowledge the main treatment goals are reducing the cardiovascular risk, alleviating claudication symptoms, and avoiding mobility loss. These goals can be accomplished by medical management and revascularization (Pereira, 2018).

Medical therapy

The medical treatment of PAD consists of risk factor modification, medications, and exercise. It has two goals, namely the reduction of cardiovascular morbidity and mortality and an improved quality of life (Ratchford, 2017).

One of the most important points in the treatment of PAD is smoking cessation since smoking is a crucial risk factor in the emergence of arteriosclerosis and hence PAD. Quitting in early stages results in reduced progression of PAD and increased walking distance, however, it is also highly recommendable in later stages, even after a bypass procedure as studies show that smoking increases the risk of graft failure. Furthermore, cessation reduces

the risks of death, myocardial infarction, and amputation. Therefore, it is important to advise every smoking patient to quit at any stage. Physicians can use the five "A's" approach consisting of the steps ask, advise, assess, assist, and arrange for that purpose. Many patients may benefit from medications such as varenicline which is a partial agonist of nicotinic acetylcholine receptors and thus this approach should also be taken into consideration (Ratchford, 2017).

Another relevant risk factor that needs to be modified in the treatment of PAD is hypertension, which should always follow the latest guidelines. There can be slight differences internationally when it comes to the actual recommendations. For instance, the definition of hypertension by the 2018 European Society of Cardiology (ESC)/European Society of Hypertension (ESH) guidelines is > 140/90 mm Hg and the target blood pressure is below this value, whereas the 2017 American College of Cardiology (ACC)/American Heart Association (AHA) guidelines define hypertension as a blood pressure > 130/90 mm Hg and hence have a lower target blood pressure. Though this seems to be the major disagreement, as there are a lot of agreements and similarities in the guidelines (Bakris, Ali and Parati, 2019). The best nonpharmacological measures for the prevention and treatment of arterial hypertension are physical activity, healthy nutrition, reduction of weight, decreased intake of dietary sodium, increased intake of dietary potassium, and moderation in alcohol intake. Frequently, these measures are not sufficient and pharmacological interventions are necessary. Angiotensin-converting enzyme (ACE) inhibitors are often used in the treatment of hypertension in PAD and can further decrease the cardiovascular risk in addition to reducing the blood pressure (Ratchford, 2017; Bakris, Ali and Parati, 2019).

Diabetes is a notable risk factor for cardiovascular disease and PAD, and therefore a suitable therapy should take place. The multidisciplinary approach should be performed by specialists of primary care, podiatry, endocrinology, and ophthalmology and should include nutrition advice, weight management and pharmacological measures for glycemic control. It is important to design an individualized therapy plan with proper glycemic goals for every patient, however, a $HbA_{1c} < 7\%$ could be a goal for patients who do not show a high risk for hypoglycemia. A relevant component of the therapy is regular and meticulous foot care, which should be monitored each visit by removing shoes and socks and thoroughly inspecting the feet. Pathological conditions can hereby be detected at early stages and treated appropriately, so that more severe complications such as maceration and ulceration can be prevented (Ratchford, 2017; Parvar *et al.*, 2018).

Treatment of dyslipidemia is another component of the medical treatment, as all patients with PAD should take a statin regardless of their cholesterol levels. According to the ESC guidelines, it is recommended to reduce the LDL-C to < 70 mg/dL or by at least 50% if the original level was between 70 and 135 mg/dL. This can be achieved by potent statins such as rosuvastatin and atorvastatin to reduce the risk of cardiovascular events. Moreover, according to studies statins can also have positive effects on PAD symptoms such as claudication. In terms of adverse effects, it should be stressed that the positive impacts clearly outweigh possible side effects, which are mostly mild and reversible (Gandhi *et al.*, 2011; Ratchford, 2017; Aboyans *et al.*, 2018a).

Platelets play a decisive role in the pathophysiology of PAD and in general atherosclerosis. They adhere to locations of arterial injury which is based on the interaction between subendothelial proteins and platelet receptors and thus contribute on the one hand to the chronic atherosclerotic process, on the other hand to acute ischemic complications. To reduce the risks of these complications such as myocardial infarction and stroke, antiplatelet drugs are put to use and are recommended for patients with established PAD (Foley, Waldo and Armstrong, 2016).

Usually, the first line antiplatelet treatment consists of monotherapy with aspirin or clopidogrel, which is slightly more effective. Aspirin irreversibly inhibits cyclooxygenase-1 (COX-1), which results in reduced production of thromboxane A2 and thus reduced platelet aggregation. Clopidogrel, however, inhibits ADP, which normally stimulates platelet adhesion and aggregation. Therefore, the administration of aspirin 81 mg/d or clopidogrel 75 mg/d is recommended for all patients with symptomatic PAD, while anticoagulation drugs are unsuitable for the therapy of PAD. Previous studies have shown no additional benefit to combining anticoagulation and antiplatelet therapy compared to antiplatelet therapy alone, while the risk of dangerous bleeding is increased (Foley, Waldo and Armstrong, 2016; Ratchford, 2017; Melfi and Ricottini, 2018). However, recent studies recommend the administration of rivaroxaban 2.5 mg together with low-dose aspirin if the patient suffers from chronic symptomatic PAD and has no high risk of bleeding. While this combination leads to increased risks such as major bleedings, they are outweighed by the benefits, especially in patients with diabetes, renal dysfunction, polyvascular disease, and heart failure (Aboyans *et al.*, 2021).

Exercise is of paramount importance to the therapy of PAD based on several effects. On the one hand, supervised exercise programs can improve claudication symptoms, the patients

can eventually extend their pain-free and maximal walking distance. On the other hand, regular physical activity improves general health with positive effects on endothelial function, metabolic parameters, and systemic cardiovascular health. Thus, guidelines recommend daily exercise for 30 minutes in which the patients should walk until they feel pain, pause for alleviation, and then restart the exercise. Over time they should push their limits by elevating the pace and altering the downhill gradient. These exercise programs have several positive effects on the symptoms of PAD such as claudication, among them improved muscular oxygen extraction (Ratchford, 2017; Parvar *et al.*, 2018).

Question	Measure
Is the patient on antiplatelet therapy?	Consider antiplatelet therapy.
Is the patient on a statin?	Consider statin therapy for instance with atorvastatin.
Is the patient currently smoking?	Recommend smoking cessation.
Is the blood pressure above goal?	Adjust antihypertensive therapy.
Is the hemoglobin $A_{1c} > 7\%$?	Refer to diabetes counselling.
Is BMI > 25 kg/m^2?	Set appropriate weight loss goals.

Table 5: Assessment for medical therapy adapted from (Ratchford, 2017)

Revascularization therapy

Another important part of the treatment of PAD is revascularization therapy, which can be divided into open surgery and endovascular therapy, though it should be stressed that revascularization poses no alternative to medical therapy but rather serves as a subsequent one. In former times, open surgery was the gold standard, but over the years endovascular therapy has surpassed it regarding different aspects and is nowadays often seen as the first-

line revascularization therapy in many cases. This development is due to technological advances and new devices in this field (Lazar and Morrissey, 2020).

The decision if a patient should undergo revascularization therapy depends on different factors, in particular symptoms and stage of the disease. Patients with PAD who receive comprehensive medical therapy that does not alleviate their IC and thus are limited in their lifestyle and daily activities are candidates for revascularization. Furthermore, when a certain stage of the disease is reached, namely critical limb ischemia, such therapy should also be considered (Thukkani and Kinlay, 2015).

Endovascular therapy can be performed by means of several techniques which include percutaneous transluminal angioplasty, endovascular stents and atherectomy. Percutaneous transluminal angioplasty (PTA) is a procedure in which a wire is introduced in the vessel, led to the site of the lesion, and an attached balloon is inflated there with a certain pressure in order to expand the artery. Advanced technologies have improved the standard balloons and in addition introduced several new balloons, which include among others cutting balloons, cryoplasty balloons and drug-coated balloons. Stent techniques, however, are based on specially designed endoprostheses that are placed at the site of the lesion and remain there in order to keep the vessel open. Stents can be divided into bare-metal stents and drug-eluting stents, which are coated with chemotherapeutic drugs such as paclitaxel. These drugs prevent the intimal layer from proliferation and thus these stents are less vulnerable to stent-thrombosis and in-stent restenosis and are in these aspects superior to bare-metal stents. Lastly, atherectomy is another choice in endovascular therapy and is especially used in interventions of the common femoral artery and popliteal artery since at certain locations the other techniques are less feasible. For this procedure, custom-built blades and drills are needed with which plaques can be ablated and subsequently removed (Lazar and Morrissey, 2020).

The second option in revascularization therapy is open surgery, in which the stenotic part of the artery is bypassed for which autogenous vein grafts, homografts, and synthetic grafts can be used. In contrast to endovascular interventions, bypass procedures have advantages regarding clinical durability and anatomical patency, though endovascular therapy features lower rates of morbidity and mortality and quicker times of recovery due to its minimally invasive nature. However, both options share the same therapeutic goals and in case of success they ensure that IC is alleviated, ulcers can heal, amputations are prevented and ultimately the quality of life is improved. There are many key factors that have an influence

on the revascularization strategy, among others surgical risk, life expectancy, and anatomical circumstances, therefore the decision should always be made individually taking into accounts all relevant factors (Friedell *et al.*, 2014; Antoniou *et al.*, 2017).

Lower extremity amputation is exclusively used in far advanced stages of PAD and is applied when all other therapy options are exploited since it is associated with increased rates of morbidity, mortality, and causes a high social and economic burden for the patients. Impossible or failed revascularization attempts, and considerable tissue loss can necessitate this treatment option, however, it should be stressed that averting amputation is of paramount importance. Lower extremity amputations can be divided into two groups, namely minor and major amputations. Those performed below ankle level are termed as minor amputations, whereas those at and above the ankle are called major amputations, these again are divided into below knee and above the knee amputations (Swaminathan *et al.*, 2014; Hussain *et al.*, 2019).

1.1.10. Prognosis

Cardiovascular diseases (CVD) are the leading cause of death in the Western World with mortality rates of 120-238/100,000 for men and 76-180/100,000 for women in European, North American, and Australian countries. The main cause of death from cardiovascular diseases is coronary heart disease (CHD) with approximately a fifth of overall deaths in men and women, followed by stroke which causes 10% male mortality and 15% female mortality. PAD and other CVD account for another 10% and 15% mortality in men and women, respectively (Olinic *et al.*, 2018).

Patients with PAD are faced with premature cardiovascular events and death, which is not only referable to PAD itself but rather the fact that those patients frequently suffer additionally from other cardiovascular diseases like coronary heart disease and cerebrovascular disease since they share the same risk factors and are all based on the process of atherosclerosis. Among patients with PAD, there are differences regarding their prognosis which hinge on the severity and the stage of the disease. Quality of life in patients who suffer from claudication is inferior when compared to healthy people, yet it is even worse in patients with CLI who face higher risks of complications like amputations and higher mortality. Progression to CLI can take place in patients with claudication, though the risk of this amounts to 20% over a period of five years. Moreover, patients with PAD are more

likely to develop depressive symptoms, which in turn results in a higher overall and cardiovascular mortality. Concerning risk assessment, the number of arterial lesions could be a useful indicator since adverse prognosis correlates with a higher number of such lesions (Olinic *et al.*, 2018; Agnelli *et al.*, 2020).

1.1.11. Biomarkers

There are several biomarkers that are important for the prognosis of PAD and CLI patients. The Neutrophil-to-Lymphocyte ratio (NLR) is an easy test that can be obtained by performing a white blood cell count. Neutrophils are an important part of the inflammation response and release various metabolites and factors to support this process. Thus, the neutrophils in this ratio show the inflammatory response, whereas a low lymphocyte count is considered to show the stress response which is triggered by cortisol. There is not only an association between NLR > 3.95 and high risk for CLI in patients with PAD but also associations with myocardial infarction and stroke (Gary, Pichler, Belaj, Hafner, Gerger, Froehlich, Eller, Pilger, *et al.*, 2013).

Another similar biomarker is Platelet-to-Lymphocyte ratio (PLR), which can be easily obtained by performing a blood test. Platelets are an important factor for atherosclerosis and atherothrombosis because of their interaction with other involved cells such as endothelial cells and leukocytes and their release of inflammatory substances. Analogue to NLR there is an association between PLR > 150 and increased risk for CLI. Furthermore, there are also associations with other vascular endpoints (Gary, Pichler, Belaj, Hafner, Gerger, Froehlich, Eller, Rief, *et al.*, 2013).

The Lymphocyte-to-Monocyte ratio (LMR) which can be obtained by a white blood cell count is another relevant biomarker. Monocytes enter the subendothelial space as macrophages and compile lipids until they turn into foam cells. Thus, monocytes have an important part in the process of atherosclerosis. There is an association between LMR < 3.1 and high risk for CLI as well as other vascular endpoints (Gary *et al.*, 2014).

1.2. Troponin

1.2.1. Structure

Muscle contraction is a complex process and hence requires a specially built cytoskeleton of participating cells. Several proteins are involved, and they undertake important functions regarding the structure and regulation. One of the participating proteins is troponin, which is considered a key regulator alongside tropomyosin (Henderson *et al.*, 2017).

Troponin is subdivided into three subunits: troponin C (TnC), troponin I (TnI), and troponin T (TnT). Every subunit has its own function and role in the process of muscular contraction. Together they form the so-called troponin complex which interacts with the actin helix along with tropomyosin (Katrukha, 2013).

There are three different isoforms of TnT: slow skeletal, fast skeletal, and cardiac. The main function of TnT is to fix the troponin complex in its entirety on the actin filament and establish a connection to tropomyosin. Additionally, it participates in the organization of the complex and regulation of muscle contraction as well. In the presence of calcium, TnT changes its conformation and consequently interacts with TnI (Katrukha, 2013; Henderson *et al.*, 2017).

The expression of TnI follows TnT, and the same isoforms are expressed. The binding of myosin to actin is inhibited by the troponin complex and TnI serves as a directly conducting part. High flexibility is a feature of TnI and is required to adapt the shape when interacting with different partners such as TnT, TnC, and actin (Katrukha, 2013; Cheng and Regnier, 2016).

Lastly, TnC is a protein in form of a dumbbell and serves as a calcium binding subunit of the troponin complex. It contains sections where calcium and magnesium bind with high affinity, the quantity of these sites depends on the isoform. The role of TnC is to interact with calcium and initiate the process of muscle contraction (Cheng and Regnier, 2016).

1.2.2. Role at muscle contraction

In the process of muscle contraction and relaxation, many participants are involved, among them different proteins like troponin, tropomyosin, and actin, but also non-protein participants such as calcium. In fact, calcium is considered the key element in the process of

muscle contraction as it is responsible for electromechanical coupling. Calcium is involved both in the process of membrane depolarization and binding to myofilaments and hence couples electrical potentials to mechanical responses (Pfeiffer *et al.*, 2014).

Concerning the heart, there are two different distinguished phases, namely systole and diastole, which are the different contraction phases of the heart in which the hearts contracts and pumps blood into the vessels and then relaxes and fills again with blood. These phases show different features, among them the concentration of calcium in the cytosol. In diastole, there is a low concentration which results in the blocking of actin and myosin. This is accomplished by the presence of TnI, which forces tropomyosin into a blocking position so that the interaction between actin and myosin is not possible. However, in systole, membranes of cardiomyocytes are depolarized, which ultimately leads to the release of intracellular calcium which is deposited in the sarcoplasmic reticulum. Subsequently, the concentration of calcium within the cell rises, and the interaction of calcium with TnC triggers a series of further events affecting the other subunits of troponin, tropomyosin, actin, and myosin. The interaction with calcium leads to an altered structure of troponin, which ultimately results in the resolution of the blocking effects of TnI and tropomyosin and hence the binding sites on actin are accessible for myosin. Finally, the interaction between actin and myosin is enabled and the cross-bridge cycling can take place (Cheng and Regnier, 2016; Marston and Zamora, 2020).

1.2.3. Clinical significance

Nowadays, cardiac troponins are an inherent part of the diagnostics of acute coronary syndrome expressing injury of cardiomyocytes, however, this was not the case in the past. The first biomarkers were established in the 1960s and 1970s, namely aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK). At that time, they were used for the diagnostics of acute myocardial infarction, but all of them had one common weakness which was the lack of specificity for cardiac muscle. Novelties were the application of myoglobin and cardio-specific iso-enzymes such as CK-MB and LDH 1 and 2, which posed an improvement in the diagnostics since the level of isoenzymes depends on the tissue and the level of CK-MB, for instance, is much higher in cardiac muscle than in skeletal muscle. However, the search for a more reliable biomarker continued, and eventually troponin was discovered which proved to be a cornerstone in the diagnostics of myocardial injury which

is due to the high sensitivity and specificity of TnI and TnT to cardiomyocytes (Garg *et al.*, 2017).

Eventually, troponin superseded all other biomarkers, and the troponin standard was established. This revolutionized the diagnosis of acute myocardial infarction (AMI) and also has its part in the definition of AMI, which requires a dynamic change of troponin (Park *et al.*, 2017). In the case of non-ST-segment-elevation myocardial infarction (NSTEMI), the measurement of TnT or TnI is needed in most patients since clinical presentation and electrocardiogram are insufficient to establish the diagnosis. Therefore, these blood tests that measure the concentration of troponin in the blood come into operation, and over the years there has been substantial progress in the technology of these tests which has resulted not only in more accurate and more reliable testing but also in recent strategies which, based on troponin measurement, can early rule-out or early rule-in a myocardial infarction (MI). For this purpose, troponin tests with higher sensitivity have been developed, which are called sensitive and high-sensitive troponin assays and can detect significantly lower levels. However, these tests come with an important disadvantage as cardiac troponin is organ-specific but not disease-specific, which means that an elevation of troponin indeed indicates injury of cardiomyocytes, however, it gives no information about the underlying cause. Apart from MI, there are several other diseases such as heart failure, hypertensive heart disease, and valvular heart disease (table 6) that can result in an elevation of troponin (Twerenbold *et al.*, 2017).

Reasons for troponin elevation
Myocardial infarction
Tachyarrhythmia
Heart failure
Hypertensive emergency
Critical illness such as shock or sepsis

Myocarditis
Takotsubo cardiomyopathy
Structural heart diseases such as aortic stenosis
Aortic dissection
Pulmonary embolism, pulmonary hypertension
Renal dysfunction and associated cardiac disease
Coronary spasm
Acute neurological events such as stroke
Cardiac contusion or cardiac procedure such as CABG and PCI
Hypothyroidism and Hyperthyroidism
Infiltrative diseases such as amyloidosis and sarcoidosis
Myocardial drug toxicity and poisoning
Extreme endurance efforts
Rhabdomyolysis

Table 6: Reasons for troponin elevations adapted from (Twerenbold *et al.*, 2017)

1.3. BNP

1.3.1. Structure and physiology

Brain natriuretic peptide (BNP) is counted among the natriuretic peptide family along with atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP). While the production of ANP and BNP mainly occurs in the heart, CNP is produced in the central nervous system and peripheral tissue. Precursors of BNP are preproBNP, which consists of 134 amino acids, and proBNP, which is formed from preproBNP by the removal of the signal peptide. Further processing of proBNP provides biologically active BNP, which consists of 32 amino acids, and NT-proBNP, which consists of 76 amino acids, and is biologically inactive. BNP and its mRNA can be found both in the atria and in the ventricles and although the tissue concentration is higher in the atria, the content of BNP and mRNA in the ventricles surpasses the atrial one due to the considerably larger size of the ventricles. ANP, however, can be indeed found in the atria and the ventricles though the content is significantly higher in the atria (Nakagawa, Nishikimi and Kuwahara, 2019).

Once BNP is secreted in the blood flow it develops its physiological effects that ultimately lead to diminution of pre-load and after-load. Both ANP and BNP interfere as cardiac hormones with blood pressure and fluid regulation by increasing diuresis and natriuresis. Specifically, they antagonize the renin-angiotensin-aldosterone system and influence the excretion of water and electrolytes. Additionally, a shift of fluid into the interstitial space which altogether results in a reduction of pre-load can be observed. Reducing the post-load is accomplished by relaxing vascular smooth muscle cells in acute situations and by preventing these cells from further growing and proliferating. Lastly, also the heart itself is affected by natriuretic peptides, as ANP and BNP prevent hypertrophy and fibrosis (Maries and Manitiu, 2013; Nakagawa, Nishikimi and Kuwahara, 2019).

1.3.2. Clinical significance

BNP and NT-proBNP have high clinical relevance, especially for heart failure (HF) and cardiac dysfunction, but also for acute diseases such as acute coronary syndrome and acute pulmonary embolism. These two biomarkers are considered as the most important ones in the diagnosis of HF, however, their application field also comprises the classification, therapy and prognosis of heart diseases (Cao, Jia and Zhu, 2019).

In clinical practice, it is recommended to measure natriuretic peptides in every case in which new or worsening HF is suspected since it contributes to the diagnosis or early exclusion. In this respect, higher levels of natriuretic peptides make the diagnosis more probable, while lower levels have a high negative predictive value. However, natriuretic peptides should always be interpreted in synopsis with medical history and clinical presentation, and can thus contribute importantly in several areas such as primary care (Mueller *et al.*, 2019).

2. Material and Methods

This retrospective data analysis reviews the data of 1743 patients who were treated at the Division of Angiology, Medical University of Graz. There were no exclusion criteria for this study.

Diagnosis of peripheral arterial disease was made by means of medical history, clinical examination, determination of ankle-brachial index, and duplex ultrasound following the TASC II consensus. The classification of PAD was made by means of the Fontaine classification, which defines critical limb ischemia as the advanced stage of PAD with ischemic rest pain or tissue loss in the form of ulceration or gangrene. Myocardial infarction and stroke were defined according to the latest guidelines.

The inquiry of data involved comprehensive medical history, concomitant diseases, and cardiovascular risk factors. Additionally, thorough clinical examinations and laboratory tests were conducted, in which among others CRP, troponin T, and BNP were investigated.

Statistical analysis was performed with IBM SPSS Statistics. Qualitative variables are described with frequency, while quantitative variables are described with mean and standard deviation or median and interquartile range. CRP, troponin T, and BNP were analyzed by means of univariate and multivariate regression analyses, and their relation to the endpoints CLI, myocardial infarction, and stroke was investigated. P-value of under 5% is considered significant and is used alongside odds ratio (OR) with 95% confidence intervals for the results.

3. Results

3.1. Characterization of the study population

1743 patients were included in this part of the study, 1046 which equals 60.0% of them were men and 697 which equals 40.0% were women. The average age of the study population was 69.2 ± 11.9 years.

Further characterization of the study population (table 7) follows the presence of cardiovascular risk factors and the intake of antiplatelet drugs. Arterial hypertension was present in 1416 patients (81.2%), diabetes mellitus in 578 patients (33.2%). 1056 patients which equals 60.6% were treated with aspirin, whereas 687 patients which equals 39.4% were not. 786 patients which equals 45.1% were treated with clopidogrel, whereas 957 which equals 54.9% were not.

Additionally, laboratory tests were conducted, and the patients were characterized by the measured levels of troponin T and CRP. 308 patients which equals 17.7% had troponin higher than 0.01 ng/ml, whereas 1435 patients which equals 82.3% had troponin less than or equal to 0.01 ng/ml. Median CRP was 4.8 mg/l with an interquartile range from 2.0 to 13.1 mg/l.

Characterization	**Mean ± SD Median (IQR) n (%)**
Age (years)	69.2 ± 11.9
Male gender	1046 (60.0%)
Female gender	697 (40.0%)
Arterial hypertension	1416 (81.2%)
Diabetes mellitus	578 (33.2%)

Aspirin	1056 (60.6%)
Clopidogrel	786 (45.1%)
CRP (mg/l)	4.8 (2.0 – 13.1)
Troponin T > 0.01 ng/ml	308 (17.7%)

Table 7: Characterization of the study population for troponin T

For the other part of the study there is fewer data available since BNP was only measured in some patients, namely 332 patients (table 8). 198 which equals 59.6% of them were men and 134 which equals 40.4% women. The average age of the study population was 69.8 ± 12.1 years.

Arterial hypertension was present in 275 patients (82.8%) and diabetes mellitus in 112 patients (33.7%). 214 patients were treated with aspirin which equals 64.5%, whereas 118 which equals 35.5% were not. 179 patients which equals 53.9% were treated with clopidogrel, whereas 153 which equals 46.1% were not.

In addition, laboratory tests were conducted in which BNP was measured with the median value of 384 pg/ml and an interquartile range from 143 to 1472 pg/ml. Median CRP amounted to 4.2 mg/l with an interquartile range from 1.6 to 12.4 mg/l.

Characterization	**Mean ± SD Median (IQR) n (%)**
Age (years)	69.8 ± 12.1
Male gender	198 (59.6%)
Female gender	134 (40.4%)

Arterial hypertension	275 (82.8%)
Diabetes mellitus	112 (33.7%)
Aspirin	214 (64.5%)
Clopidogrel	179 (53.9%)
CRP (mg/l)	4.2 (1.6 – 12.4)
BNP (pg/ml)	384 (143 – 1472)

Table 8: Characterization of the study population for BNP

In the following, statistical computing was conducted with the larger patient sample except from BNP, where the smaller sample was used.

3.2. Critical limb ischemia

First, the correlation between the ascertained factors and critical limb ischemia (CLI) was examined by means of a univariate regression analysis (table 9). This analysis showed a significant correlation between CLI and age ($p < 0.001$), male sex ($p = 0.001$), diabetes mellitus ($p < 0.001$), CRP ($p < 0.001$), troponin T ($p < 0.001$), and BNP ($p < 0.001$). There was no correlation between CLI and arterial hypertension ($p = 0.861$).

	Sign.	**OR (95% CI)**
Age	< 0.001	1.055 (1.045 – 1.066)
Male sex	0.001	0.700 (0.570 – 0.860)

Diabetes mellitus	< 0.001	2.65 (2.15 – 3.28)
CRP	< 0.001	1.015 (1.012 – 1.019)
Troponin T	< 0.001	4.03 (3.12 – 5.20)
BNP	< 0.001	2.801 (1.914 – 4.100)
Arterial hypertension	0.861	

Table 9: Univariate regression analysis CLI

For further evaluation, a multivariate regression analysis was conducted in which the significant factors age, diabetes mellitus, CRP, and troponin were considered (table 10). The multivariate regression analysis showed statistical significance for age ($p < 0.001$), diabetes mellitus ($p < 0.001$), CRP ($p < 0.001$) and troponin T ($p < 0.001$).

	Sign.	**OR (95% CI)**
Age	< 0.001	1.045 (1.034 – 1.057)
Diabetes mellitus	< 0.001	2.095 (1.654 – 2.653)
CRP	< 0.001	1.010 (1.007 – 1.014)
Troponin T	< 0.001	2.439 (1.827 – 3.255)

Table 10: Multivariate regression analysis CLI

3.3. Myocardial infarction

The univariate regression analysis of myocardial infarction (table 11) showed statistical significance regarding age ($p = 0.002$), diabetes mellitus ($p = 0.014$), CRP ($p = 0.005$), troponin T ($p < 0.001$), and BNP ($p < 0.001$). There was no correlation between myocardial infarction and male sex ($p = 0.771$) and arterial hypertension ($p = 0.690$).

	Sign.	**OR (95% CI)**
Age	0.002	1.036 (1.014 – 1.060)
Diabetes mellitus	0.014	1.804 (1.127 – 2.890)
CRP	0.005	1.006 (1.002 – 1.010)
Troponin T	< 0.001	6.7 (4.2 – 10.9)
BNP	< 0.001	3.368 (1.710 – 6.632)
Clopidogrel	0.091	
Male sex	0.771	
Arterial hypertension	0.690	

Table 11: Univariate regression analysis myocardial infarction

The multivariate regression analysis was conducted for further evaluation (table 12), which showed statistical significance for intake of clopidogrel ($p = 0.01$) and troponin T ($p < 0.001$).

	Sign.	OR (95% CI)
Clopidogrel	0.010	1.907 (1.164 – 3.124)
Troponin T	< 0.001	5.700 (3.353 – 9.691)

Table 12: Multivariate regression analysis myocardial infarction

3.4. Stroke

The univariate regression analysis of stroke (table 13) showed statistical significance regarding intake of aspirin (p = 0.004), intake of clopidogrel (p = 0.036), CRP (p = 0.001), and troponin T (p = 0.007). However, there was no correlation between stroke and age (p = 0.085), male sex (p = 0.692), arterial hypertension (p = 0.055), diabetes mellitus (p = 0.865), and BNP (p = 0.166).

	Sign.	OR (95% CI)
Aspirin	0.004	0.606 (0.430 – 0.855)
Clopidogrel	0.036	1.45 (1.02 – 2.04)
CRP	0.001	1.006 (1.002 – 1.009)
Troponin T	0.007	1.73 (1.16 – 2.57)
Age	0.085	
Male sex	0.692	

Arterial hypertension	0.055	
Diabetes mellitus	0.865	
BNP	0.166	

Table 13: Univariate regression analysis stroke

Further evaluation with a multivariate regression analysis (table 14) showed statistical significance regarding intake of aspirin (p = 0.043), intake of clopidogrel (p = 0.022), and CRP (p = 0.001).

	Sign.	**OR (95% CI)**
Aspirin	0.043	0.693 (0.486 – 0.989)
Clopidogrel	0.022	1.519 (1.063 – 2.171)
CRP	0.001	1.006 (1.002 – 1.009)

Table 14: Multivariate regression analysis stroke

4. Discussion

PAD is a global disease that affects more than 200 million people worldwide with an increasing tendency, the prevalence has steadily risen over the last decades (Firnhaber and Powell, 2019). This disease is often underdiagnosed and in further consequence many patients do not receive appropriate therapy, or they receive it with delay (Shanmugasundaram *et al.*, 2011).

The correlation between various biomarkers and PAD has been the subject of several studies in order to find reliable biomarkers for this disease. Since PAD is essentially a manifestation of atherosclerosis, some authors have focused on biomarkers that indicate inflammation and vessel remodeling in the context of atherosclerosis, including acute-phase proteins such as C-reactive protein (CRP) and different interleukins. In this regard, CRP occupies a special position since its role has been studied for many years. Previous studies suggest that CRP is a marker of early PAD and major cardiovascular events such as myocardial infarction and stroke with worse outcomes. Furthermore, PAD patients with elevated CRP apparently have a more severe course of their disease. It is still uncertain to what extent and in which context CRP could be used, though there is evidence that its application regarding diagnosis and prognosis of PAD is possible (Saenz-Pipaon *et al.*, 2021). Besides cardiac biomarkers the patients' CRP values were measured, and statistical significance for the vascular endpoints, namely CLI and stroke could be found. This supports evidence that CRP is an important biomarker for cardiovascular diseases such as PAD, however, its strength and significance could not be determined, and further studies are needed to shed light on this issue.

Troponin and in particular troponin T are the most important biomarkers for myocardial infarction and reliably indicate cardiomyocyte stress and injury. Troponin has been established in the diagnostics of myocardial infarction, especially as a high-sensitive assay (Twerenbold *et al.*, 2017; Saenz-Pipaon *et al.*, 2021). There are several studies that have investigated the connection between PAD and this biomarker, and there have been indications that it could be linked to the evolution and prognosis of PAD. It is known that CHD and PAD share common risk factors and thus often occur concomitantly, however, it is not yet completely clear in which relation release of troponin stands in this context (Saenz-Pipaon *et al.*, 2021).

In a recent study published by Matsushita et al., the authors found associations between hs-cTnT and NT-proBNP with future risk of PAD. These associations did neither depend on

each other nor on established cardiovascular risk factors. Furthermore, these associations were even more significant with CLI. A possible explanation for these associations could be the link of these biomarkers with heart failure and atrial fibrillation. Patients with these diseases suffer from a higher risk of systemic embolism, which in turn can affect the perfusion of the legs and thus lead to leg symptoms. As a result, those patients would more often seek for medical advice, which would increase PAD hospitalizations and revascularizations (Matsushita *et al.*, 2018). Pohlhammer et al. found an association of detectable hs-cTnT with an 84% higher probability for symptomatic PAD in a study with male patients with IC (Pohlhammer *et al.*, 2014).

Besides the diagnostic value in patients with PAD, cardiac biomarkers have also proven to be useful to prognosis. Mueller et al. showed that there is an association between high concentrations of NT-proBNP and increased risk of 5-year all-cause mortality, and NT-proBNP was considered valuable regarding stratifying risk of PAD patients. The authors stated that this could be an advance in the treatment of patients with PAD since detecting patients with concomitant undiagnosed heart failure early could lead to better general outcomes (Mueller *et al.*, 2009).

This study demonstrates that elevations of troponin T are associated with a high risk for CLI in patients with PAD and consequently confirms the results of previous studies that investigated the correlation between cardiac biomarkers and PAD. This leads to the conclusion that cardiac biomarkers such as troponin T and BNP could be useful tools in the diagnostics and prognosis of PAD and CLI, however, further studies are required to determine the strength and significance of these biomarkers. A possible explanation for the association between BNP and CLI could be attributable to the pathophysiology of heart failure. It is known that heart failure results in forward failure with a reduction of cardiac output per minute. This results, among other things, in reduced renal perfusion, which together with the activation of the renin-angiotensin-aldosterone system and antidiuretic hormone leads to typical edema. However, this reduced perfusion would also explain that the legs are supplied worse, and an existing PAD could deteriorate to the more severe form of CLI.

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