Neuropeptide Y in patients presenting with acute myocardial infarction

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NEUROPEPTIDE Y IN PATIENTS PRESENTING WITH ACUTE MYOCARDIAL INFARCTION

DIPLOMA THESIS

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TABLE OF CONTENTS

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This work is the cumulative and finalizing effort of the last 6 years and dedicated to my family and close friends.

Additionally, I would like to thank my mentor and friend Assoc. Prof. Josko Bozic, MD, PhD, for always supporting me.

LIST OF ABBREVIATIONS

CVD – Cardiovascular disease

CAD – Coronary Artery disease

CHD – Coronary heart disease

IHD – Ischemic heart disease

ACS – Acute Coronary Syndrome

STEMI – ST-Elevation Myocardial Infarction

NPY – Neuropeptide Y

GIT – Gastrointestinal Tract

UA – Unstable Angina

NSTEMI – Non-ST Segment Elevation Myocardial Infarction

MI – Myocardial Infarction

EC – Endothelial Cell

SMC – Smooth muscle cell

ECM – Extracellular Matrix

t-PA – Tissue-type plasminogen activator

u-PA – Urokinase-type plasminogen activator

NADH/NADPH – nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate

1. INTRODUCTION

1.1 Terminology

CVD (cardiovascular disease) is a joined category of diseases that affects the heart and blood vessels. Although frequently used interchangeably, CAD (coronary artery disease) in which atherosclerotic plaques are formed within the coronary vessels of the heart is a condition that will lead to CHD (coronary heart disease), also known as IHD (ischemic heart disease). On the other hand, ACS (acute coronary syndrome) describes the acute onset clinical symptomatology that goes mostly with either occlusion of coronary arteries due to CAD or lowered perfusion, which is precipitated by other cardiac conditions such as severe aortic stenosis or apical hypertrophic cardiomyopathy (1-4).

1.2 Coronary artery disease

The most common underlying cause of CAD and peripheral arterial disease is atherosclerosis, a multifactorial inflammatory process. The plaque formation by itself is rarely deadly; rather it is the formation of thrombi that are either superimposed on previously ruptured or eroded plaques that results in ACS and strokes, which can be fatal (5). The formation and progression of atherosclerosis is based on environmental and genetic factors. The process of plaque formation can be affected by genetic predisposition either directly or via cardiovascular risk factors (Figure 1). While the formation of atherosclerotic plaque is a process that is already beginning during fetal development, the clinically evident ischemic cardiovascular incidents start appearing in men and women, in their 40s and 50s, respectively (6, 7).

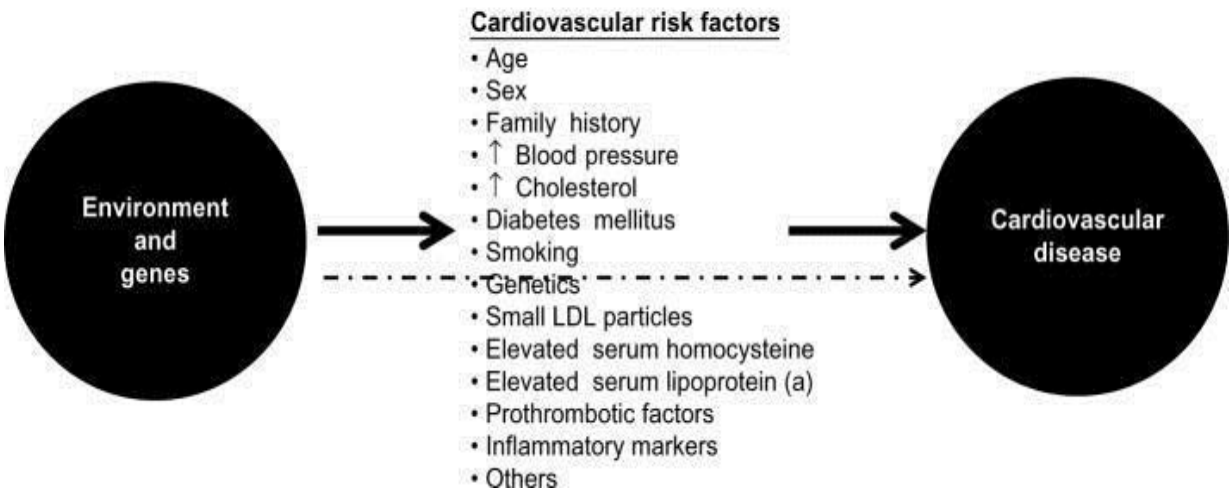

Figure 1. Direct and indirect factors affecting cardiovascular disease. Source: Sayols-Baixeras S, Lluís-Ganella C, Lucas G, Elosua R. Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. Appl Clin Genet. 2014;7:15-32.

1.2.1. Cell types of the normal artery

The material that lines the lumen of arteries consists partially of EC (endothelial cells), whose presence allow blood to maintain a liquid state while constantly being in contact. This blood compatibility is possible due to ECs remarkable antithrombotic properties, enabled through the expression of heparan sulfate, thrombomodulin, t-PA and u-PA (Tissue- and Urokinase-type plasminogen activator). While heparan sulfate acts as a cofactor for antithrombin III, thrombomodulin activates protein C and S. The plasminogen activators enable plasmin to dissolve clots with its fibrinolytic action. Another important constituent of the arterial wall are smooth muscle cells, which regulate blood flow via contraction and synthesize the complex arterial ECM (extracellular matrix) that plays a major role in the pathogenesis of intimal hyperplastic lesions and thus atherosclerosis. Loss of arterial SMCs (smooth muscle cells) may also destabilize atherosclerotic plaques, which could give rise to ACS and ischemic heart disease (8).

1.2.2 Arterial layer anatomy

The innermost layer of the artery is known as the tunica intima and undergoes significant changes during our lifetime. While the younger intima consists mostly of ECs residing upon a basement membrane with nonfibrillar collagen types, arterial SMC and its produced fibrillar

interstitial collagen modify the tunica intima as the human ages. This frequently gives rise to a characteristic pattern, which is often referred to as “diffuse intimal thickening” by pathologists. That intimal thickening process can form regardless of lipid accumulation and without any atheromas. The tunica intima is finally limited by its internal elastic membrane, which separates the intima from the tunica media (8, 9).

The tunica media is mainly comprised of concentrically arranged SMCs and is interwoven with an elastin rich ECM, which is surrounded by the external elastic lamina (9). In a healthy artery, mitosis and cell death rates of SMCs are low, thus ECM synthesis and degradation cancel out (8).

Lastly, the adventitial layer contains more loosely arranged collagen fibrils and harbors the vasa vasorum, vascular nerve endings, fibroblasts and mast cells (8).

1.2.3 Pathogenesis of atherosclerotic plaques

1.2.3.1 Initiation of atherosclerosis

Upon exposure to an atherogenic diet that contains large amounts of cholesterol and saturated fats, small lipoprotein particles attach to proteoglycans of the tunica intima and aggregate (*Figure 2: 1, 2*). While aggregated, the lipoproteins are more susceptible to chemical alterations, such as oxidation, due to alterations in the nascent atheroma that include, the reduced production of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidases by vascular cells, lipoxygenases from migrating leukocytes and enzymes such as myeloperoxidase (8).

Usually, ECs are resistant to leukocyte adhesion, but during hypercholesterolemia monocytes tend to adhere to the endothelium more readily, migrate between the junctions of neighboring ECs and take up lipids to become foam cells (10). This increased adherence is due to the expression of several adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), which interacts with very late antigen 4 (VLA-4), an integrin that is present on monocytes and T cells, thus favoring their accumulation in nascent atheromas. Other leukocyte adhesion molecules of interest are the selectins. Although, E-selectin does not appear to be involved in atherogenesis despite its abundance on endothelial cells, P-selectin is expressed by ECs overlying atheromas, thereby facilitating adherence by enabling a saltatory locomotion of leukocytes along the ECs (*Figure 2: 3*). After adhesion, various chemokines emitted due to

local oxidative stress are believed to promote penetration. One particular chemokine involved in attracting leukocytes is the monocyte chemoattractant protein 1 (MCP-1). Once migrated, leukocytes seem to be retained within the intimal lesion due to retention factors such as netrin-1, hindering the macrophages exit (11, 12).

After lipid uptake, the monocyte becomes a lipid-laden macrophage known as foam cell. This uptake is enabled by so called scavenger receptors (*Figure 2: 4, 5*) that show affinity towards chemically altered lipoproteins and apoptotic cells, further complexing the foam cell's role during various stages of atherosclerosis (13). Studies in mice have shown that monocytes tend to be primarily derived from blood in early lesions, local foam cell replication seems to account for their abundance in older lesions (14). Hematopoietic growth factors such as macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and Interleukin-3 seem to promote this macrophage cell division within the atherosclerotic plaque, making its appearance dominated by lipid-laden macrophages in this early stage of atherosclerosis, giving them the characteristic name "fatty streaks" (8).

1.2.3.2 Evolving atherosclerotic lesions

Within the lesion, Lipid-laden macrophages produce various cytokines, eicosanoids. lipid mediators and radical oxygen species that together promote inflammation and further progress the atherosclerotic plaque. In addition to this innate immunity response, adaptive immunity contributes to this progression as well. Dendritic cells can present various lesion antigens such as lipoproteins, heat shock proteins and beta$_2$-glycoprotein 1b and recruit Th1 (T-helper 1) cells, which in turn secrete interferon-y, lymphotoxin and TNF-α (tumor-necrosis-factor α). This cytokine response could then lead to plaque desquamation and thrombogenesis. Also attracted CD8 (Cytolytic T Cells) can trigger cell death of SMCs, ECs, and macrophages via Fas ligand expression and other cytotoxic factors, furthering plaque progression and giving rise to complications (15). On the contrary, stimulation of Th2 (T-helper 2) cells and Treg (T regulatory) could inhibit inflammation through secretion of interleukin 10. Tregs can also produce TGF-β (transforming growth factor β), constituting another inhibitory adaptive immunity cell response (8, 16, 17).

According to Knorr *et al*. (18), there is a strong interaction between NK cells and monocytes in ACS. Macrophages can activate NK cells by secreting IL-12 or IL-18 or by direct cell-to-cell contact, whereas NK cells use IFN- to induce monocytes to become macrophages and/or DCs. IL-12 and IL-18 are also produced by macrophages and DCs, which combine to

induce NK cells to release IFN-, resulting in a positive feedback loop (19, 20). The Renin-Angiotensin axis appears to regulate this interaction, and certain elements of reciprocal activation in mice arterial hypertension models have been shown (21). ATII and aldosterone have a regulatory function by promoting or inhibiting the production of chemokines and cytokines by monocytes and/or NK cells (22, 23). The data on NK cell function impairment in terms of ACS seems to be consistent, in contrast to NK cell number. Yan *et al*. looked at mRNA expression in both activating and inhibitory receptors on NK cells and discovered that both types of receptors were considerably lower in MI patients than in SA and healthy controls (24). Furthermore, although no functional impairment was found, patients with SA had a lower number of NK cells than those with MI (22, 25). Strassheim *et al.* looked at how NK cells can help with myocardial remodeling and heart failure in general (26). NK cells appear to protect against the development of cardiac fibrosis by lowering the concentration of certain inflammatory populations in the heart and directly lowering collagen formation in cardiac fibroblasts (27). Ormiston et al. and Tamosiuniene et al. used monocrotaline-induced pulmonary artery hypertension (PAH) animal models to show that NK cells reduce right ventricular hypertrophy and right ventricular systolic pressure growth (28, 29).

As time progresses, SMCs replicate within the intima in a burst like fashion *(Figure 2: 7*) due to bouts of mitogen exposure during plaque disruptions, thrombosis and consequent healing. This replication is countered by apoptosis, which is mediated by inflammatory cytokines, ultimately resulting in a back-and-forth shift between cell replication and death (*Figure 2: 8*) (8, 30).

Further contributing to plaque growth are the ECM molecules produced by said SMCs, catalyzed by PDGF (platelet derived growth factor) and TGF-β. In similar fashion of SMCs life cycles within the lesion, ECM also gets degraded by MMPs (matrix metalloproteases), additionally facilitating SMC migration from the tunica media to the tunica intima and remodeling the arterial wall. While initial plaque growth is mediated outward, this process leads to compensatory thickening of the entire artery caliber. After approximately 40% of the cross-sectional area of the arterial wall consists of atherosclerotic plaque, luminal stenosis is going to become evident (31).

Angiogenic peptides within the atherosclerotic lesion such as PlGF (placental growth factor) and oncostatin-M promote rich neovascularization, which then provides additional entry and exit to leukocytes through increased expression of VCAM-1 on the newly formed ECs.

Similarily to angiogenesis in malignancy, new vessels nourish and enable further plaque growth. Hemorrhage and/or thrombosis of neovasculature could in turn stimulate further SMC proliferation and thus ECM production locally (31, 32).

Lastly, SMC subpopulations can calcify via activation of the RANKL (receptor activator of NF-κB) mediated bone morphogenetic protein 4-dependent pathway. Furthermore, another way of mineral formation is enabled through Runx-2, a transcription factor that initiates the AKT pathway (8).

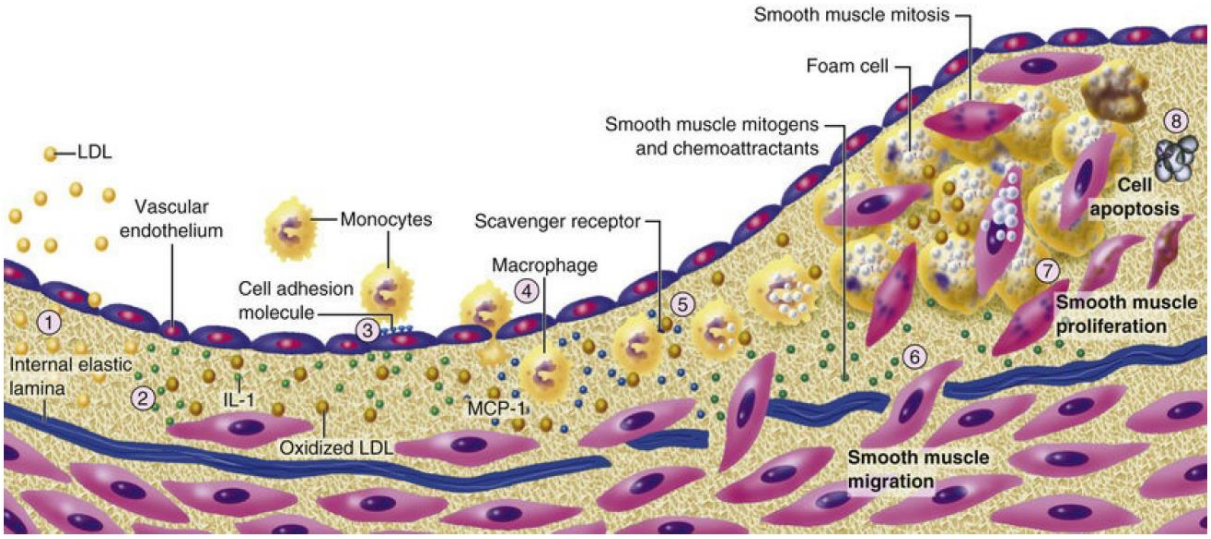

Figure 2. Initiation and progression of atherosclerotic lesion. Source: Zipes D. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 11th ed. Philadelphia: Elsevier; 2019. p. 2230

1.2.4 Complications of atherosclerosis

As previously discussed, atherosclerotic lesions progress in a discontinuous manner, which can be characterized as quiescent periods interpolated with rapid growth spurts. Most lesions are asymptomatic because it takes more than 60% of luminal occlusion in order to disturb flow under high demand conditions, giving rise to the clinical picture of stable angina. Acute MI on the other hand is most often caused by a thrombus that is formed on top of a disrupted plaque that was previously not symptomatic on its own. Although this mechanism predominates in frequency of occurrence, highly stenotic lesions are more likely to cause MI individually (33). The integrity of the fibrous cap is due to SMCs collagen synthesis, which is affected by a lot of different factors. Inhibition by T cells Interferon (IFN)-γ and degradation

by MMPs are countered by collagen-synthesis stimulating factors like TGF-β and PDGF. Depending on which direction of balance is favored, the thrombosis can take different forms (34).

Roughly 66% of all MI are believed to be caused by fibrous cap fractures of atherosclerotic lesions, while superficial erosions constitute the remainder. Superficial erosions possess intact fibrous caps and depend on collagen exposure and the subsequent attraction of neutrophils. This form of thrombosis mostly presents itself as NSTEMI. Thrombosis due to cap rupture on the other hand is triggered by exposed tissue factor and occur mostly after large amounts of expansive remodeling have occurred leading to STEMIs, rather than NSTEMIs (33, 34).

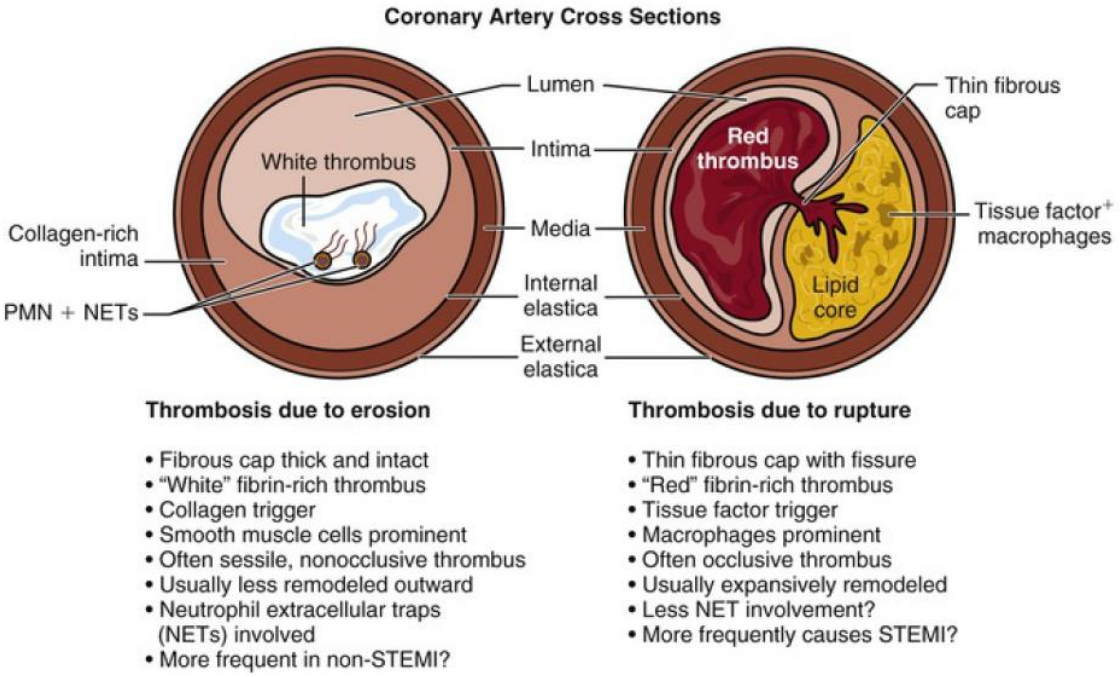

Figure 3. Comparison of thrombotic mechanisms

Source: Zipes D. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 11th ed. Philadelphia: Elsevier; 2019. p. 2242

1.3 Global Burden of Ischemic heart disease

IHD affects about 126 million people worldwide (1,655 per 100,000), or around 1.72 percent of the world's population and is rising in prevalence (*Figure 4*). IHD claimed the lives of nine million people worldwide, generally affecting more men than women. The current

prevalence rate of 1,655 per 100,000 people is projected to rise to 1,845 by 2030, with the highest prevalence being seen in Eastern European countries (*Figure 5*) (35).

Even though the general mortality of CHD (coronary heart disease) has been declining in western countries for the past decades (*Figure 6*), it is still the cause of death in one third of deaths worldwide. Additionally, it is important to mention that mortality due to CHD in developing countries is projected to further increase, which warrants more and thorough investigations regarding diagnoses and treatment guidelines (2).

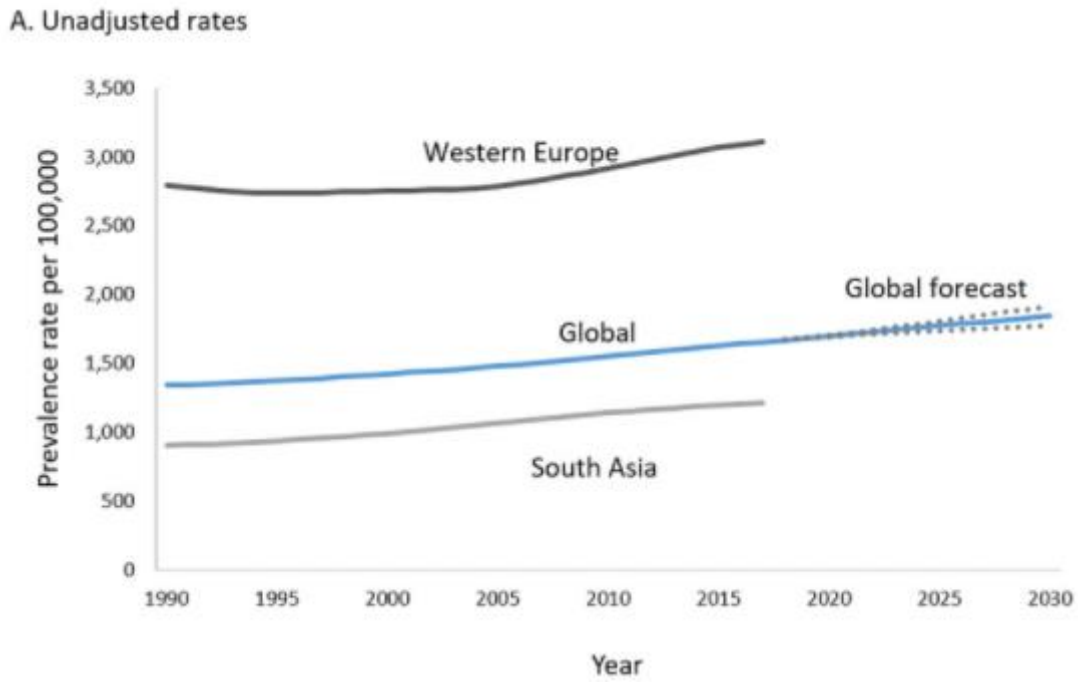

Figure 4. Unadjusted rates of IHD prevalences in Western Europe, South Asia, and the world. Source: Khan MA, Hashim MJ, Mustafa H, Baniyas MY, Al Suwaidi SKBM, Al Katheeri R, Alblooshi FMK, et. al. Global Epidemiology of Ischemic Heart Disease: Results from the Global Burden of Disease Study. Cureus. 2020;12:9349.

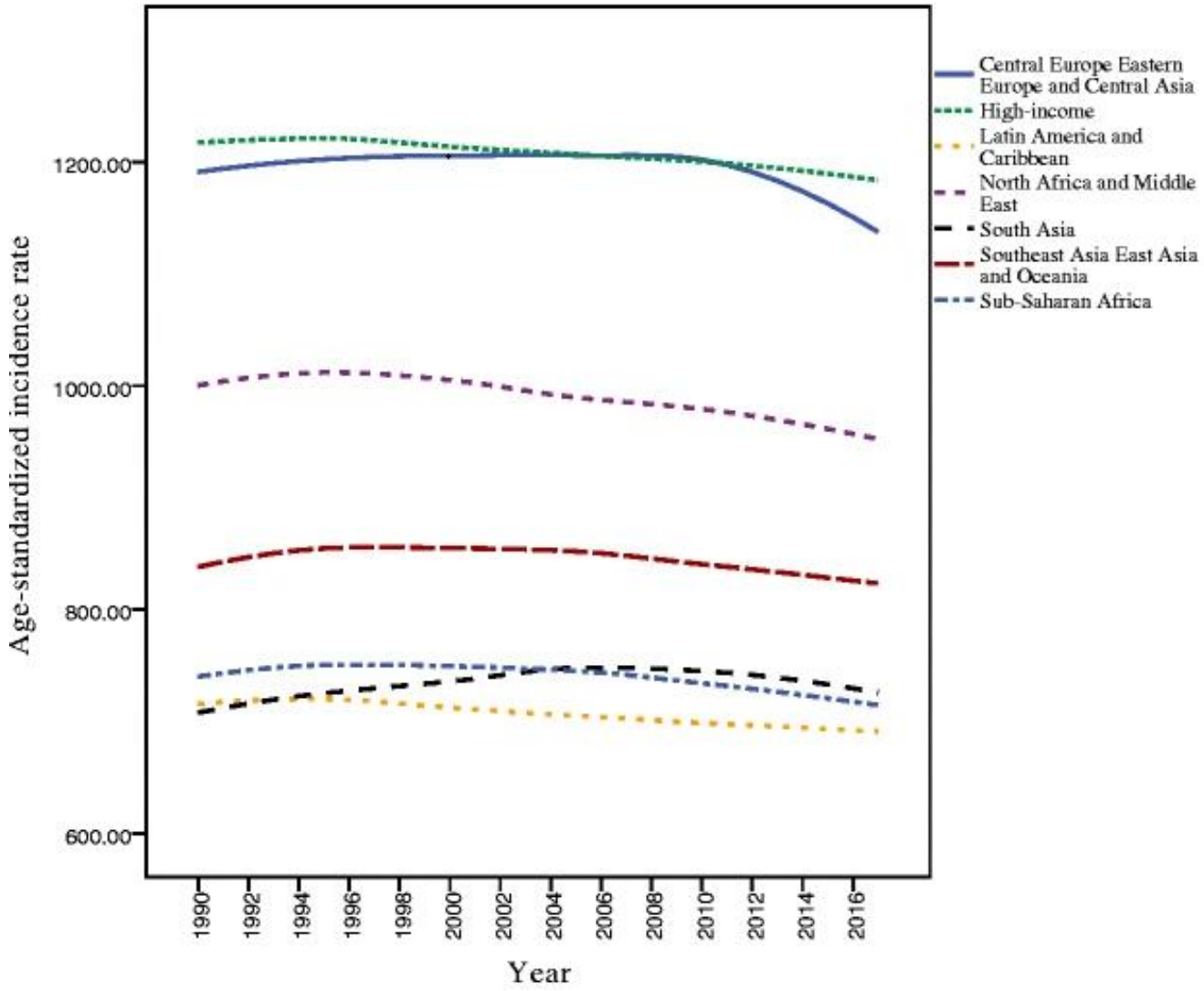

Figure 5. Age-Standardized incidence rate from 1990-2017 in different areas of the world. Source: Amini, M., Zayeri, F. & Salehi, M. Trend analysis of cardiovascular disease mortality, incidence, and mortality-to-incidence ratio: results from global burden of disease study. BMC Public Health. 2017;21:401.

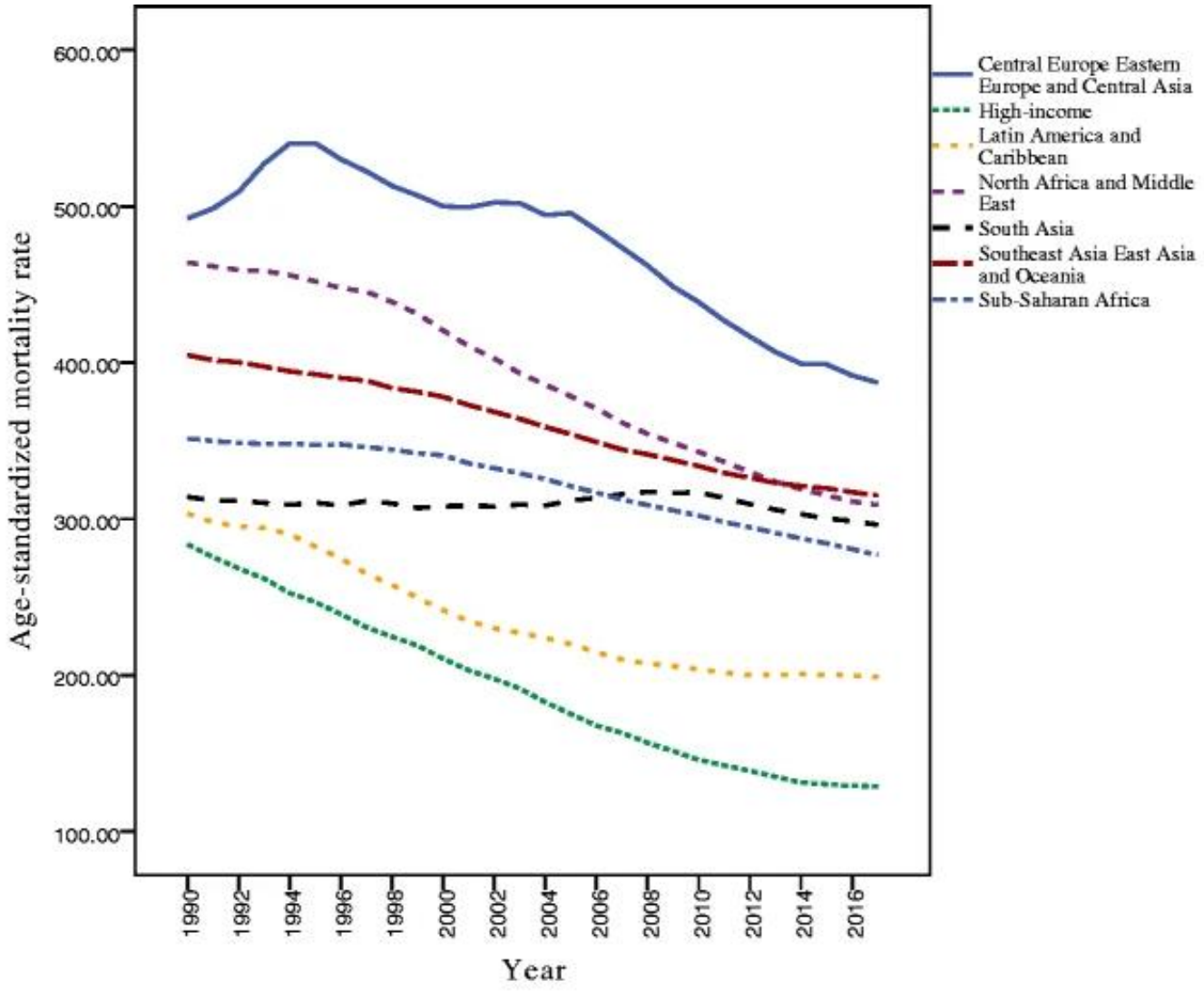

Figure 6. Age-Standardized mortality rate from 1990-2017. Source: Amini, M., Zayeri, F. & Salehi, M. Trend analysis of cardiovascular disease mortality, incidence, and mortality-to-incidence ratio: results from global burden of disease study. BMC Public Health. 2017;21:401.

1.4 Pathophysiology of acute coronary syndrome

ACS encompasses a group of symptoms as a result of myocardial ischemia and can present as unstable angina, non-ST-segment elevation myocardial infarction and lastly as ST-segment elevation myocardial infarction (36). Reduced blood supply to portion of the heart muscle is the underlying pathophysiology in ACS, which is generally caused by plaque rupture and thrombus development. ACS can sometimes be caused by vasospasm, which can occur with or without underlying atherosclerosis. As a result, blood supply to a segment of the heart musculature is reduced, leading to ischemia and subsequently infarction of said segment (37).

Afferent sympathetic nerve activity, which is primarily stimulated by adenosine, appears to be responsible for the majority of pain signaling from the heart to the spinal cord and brain in the event of cardiac pain. The vagus nerve appears to play a modest function in the transmission of afferent pain (38).

Various biomarkers (Table 1) are being frequently used in order to further aid precise diagnosis. Cardiac enzymes, particularly troponin and the CK-MB/CK ratio, are useful in determining the difference between NSTEMI and myocardial ischemia without tissue loss (37). Cardiac troponins have surpassed creatine kinase-MB as the leading biomarker for myocardial infarction diagnosis (MI). Even low-level increases of cardiac troponin T or I are associated with a greater mortality risk and recurrent ischemic episodes in patients with an acute coronary syndrome (ACS) as compared to those with troponin levels below the cut-off. Antithrombin and antiplatelet drugs, as well as an early invasive care plan, are most beneficial to individuals with increased troponin levels. While cardiac troponins are highly selective for myocardial necrosis, they do not distinguish between ischemia and non-ischemic myocardial damage etiologies (39). Another interesting marker is dephosphorylated-uncarboxylated Matrix Gla-Protein (dp-ucMGP). Dp-ucMGP levels were substantially greater in NSTEMI patients than in STEMI patients. Furthermore, as compared to non-high-risk patients, those with a high risk of in-hospital death had considerably greater levels of dp-ucMGP. Furthermore, greater dp-ucMGP levels in ACS patients are likely to indicate increased calcification and may assist in the identification of NSTEMI patients who are at an elevated risk of in-hospital death (40).

1.4.1 Stable angina

The presence of chest pain is responsible for around 50% of all incidents and is often precipitated by exertion or emotional distress. The pain is usually characterized as tight, squeezing, analogous to a weight on the chest, or as indigestion and begins gradually, reaching its maximum after a few minutes; however, as with any visceral pain, the location is ambiguous, and there is significant individual variation. Adenosine, lactate, and H+ are believed to stimulate nerve endings near the endocardium, resulting in cardiac ischaemic pain that may radiate from the retrosternal area to the arms, epigastrium or the jaw. The pain can also be accompanied or replaced by dyspnea during the angina episode, known also as “anginal equivalent”. The severity of angina and dyspnea can be measured using the NYHA or Canadian Cardiovascular Society scales, which range from I (very mild) to IV (symptoms at rest or on minimal exertion) (41, 8). While NSTEMI and STEMI are primarily occlusive in nature, Stable

angina, on the other hand, is caused by a mismatch between myocardial oxygen delivery and demand in the presence of stable coronary stenosis (42).

1.4.2 Unstable angina

Unstable angina can be found in a variety of forms, such as angina at rest, nocturnal angina, variant angina in which the electrocardiogram indicates temporary ST-segment elevation due to coronary vasospasms and non-Q-wave MI and post-myocardial infarction angina after 24 hours (43). New onset angina that is CCSC class III or above within 2 months of presentation or angina advancing to CCSC III or IV can point towards UA (44).

1.4.3 Non-ST segment elevation myocardial infarction

NSTEMI can be caused by disruption of atherosclerotic plaque, coronary vasoconstriction, progressing atherosclerosis or restenosis after stenting and lastly oxygen supply-demand mismatch. Although the clinical presentation is very similar to stable angina, NSTEMIs tend to last longer and are more intense. Subsequently, the discomfort may be accompanied by symptoms such as diaphoresis, fatigue, stomach pain, dyspnea, and syncope. Other anginal equivalents such as dyspnea without chest pressure, isolated epigastrium pain localization, or indigestion can occasionally occur. Women, elderly adults, and patients with diabetes mellitus (DM), CKD, or dementia are more likely to have these atypical results, which can contribute to delayed recognition, undertreatment, and poor outcomes. These clinical signs can arise unexpectedly, with extreme, new-onset symptoms arising at rest (CCSC IV) or during reduced exertion (CCSC III), accelerating reoccurrence, pain or duration of angina or angina occurring soon after MI (8).

1.4.4 ST-Elevation myocardial infarction

A precipitating cause or prodromal signs are present in up to one-third of STEMI cases. The most common causes are unusually vigorous exercise (especially in fatigued or inactive patients), emotional stress, and acute illness. There is a tendency for STEMI to occur in the morning. Clinically, the patient might experience a prodrome that consists of classic anginal symptoms, yet it tends to occur at less activity than usual or even at rest. Other signs that precede STEMI often include a sense of general malaise or outright fatigue. If there is no reperfusion, anginal pain can often last for a minimum of 30 minutes up to several hours. Coagulation, necrosis and contraction band necrosis are common findings, with patchy regions of myocytolysis at the infarct's periphery. Myocytes die in the infarct zone during the acute process

of MI, which is followed by inflammation, necrotic debris clearance, reconstruction, recovery and eventual scar formation (45, 8).

Table 1. Overview of acute coronary syndrome subtypes (41-50)

	SA	UA	NSTEMI	STEMI
Duration	<5min	>15min	>10min	≥30min
Degree of luminal obstruction	≥50 %	≥50%	<50-70%	≥70%
Laboratory Markers			TnI TnT CK-MB	TnI, TnT, CK-MB, BNP, NT-proBNP
Features		Occurs frequently at night	Likely due to fibrous cap erosion	Likely due to fibrous cap rupture

SA – stable angina, UA – unstable angina, NSTEMI – non-ST elevation myocardial infarction, STEMI – ST elevation myocardial infarction, Tn – troponin, NT-proBNP – N-terminal pro brain natriuretic peptide, CK-MB – creatin kinase muscle/brain isoform.

1.5 Neuropeptide Y

1.5.1 Structure

Tatemoto & Mutt discovered neuropeptide Y (NPY) in a porcine hypothalamus in 1982 (51). It is a 36-amino-acid peptide that belongs to the class of pancreatic polypeptides. Since it includes several tyrosine residues, including an amidated C-terminal tyrosine residue, its name is derived from the single-letter code (Y) for the amino acid tyrosine (51).

1.5.2 Functions relative to location

The widespread dissemination of NPY is linked to a variety of biological effects, including cardiovascular control, seizures and cognition, stress, neuroendocrine system stimulation, and appetite regulation (52).

1.5.2.1 Brain

NPY is found in the cortex, hippocampus, hindbrain, and hypothalamus of the brain, being abundantly synthesized in the latter region by neurons of the arcuate (ARC) nucleus (52). Neuropeptide Y is a neurotransmitter that is synthesized in GABAergic neurons which functions as a neurotransmitter. The majority of NPY's effects are mediated by G-protein coupled receptor proteins, primarily Y1, Y2, Y4, and Y6.While both receptors are involved in post-synaptic communication, the Y2 receptor has also been found to be involved in pre-synaptic production. The G protein-coupled receptor that NPY targets belongs to the rhodopsin-like 7-transmembrane GPCR family. In animals, there are five subtypes of the NPY receptor, four of which are functional in humans (53, 54). Subtypes Y1 and Y5 are believed to play a role in appetite activation, while Y2 and Y4 appear to play a role in appetite inhibition (55). NPY increases cell proliferation by binding on and activating Y1 receptors on progenitor cell membranes (56). Higher levels of the Y1 and Y5 receptors in the amygdala have been linked to lower anxiety levels. The Y1 receptor has also been related to anxiolytic effects in the forebrain, while the Y2 receptor has been linked to the pons (53). Consequently, higher levels of NPY have been linked to rehabilitation from posttraumatic stress disorder. as well as dampening the anxiety reflex, helping people to respond better under pressure (57). Additionally, NPY inhibits glutamate release and seizure activity in the rodent hippocampus (58).

1.5.2.2 Immune System

The discovery that sympathetic neurons innervating lymphoid organs contain NPY and that NPY is co-released with noradrenaline upon stimulation provided the first proof that NPY may have immunological implications. Since immune cells can produce and release NPY, nerve-derived NPY has a direct effect on immune cells. NPY also serves as a paracrine and autocrine immune mediator. NPY can either stimulate or inhibit the immune system, depending on a variety of factors such as the Y receptors activated and the cell types involved (59).

1.5.2.3 Cardiovascular System

NPY is involved in several physiological processes, including cardiovascular activity, vasomotion, angiogenesis, and heart remodeling. NPY is co-released with catecholamines (mostly NE) and galanin from cardiac sympathetic nerve terminals in the cardiovascular system (60). It is also the heart's most common neuropeptide, found in post-ganglionic sympathetic neurons that supply the vasculature, endocardium, and cardiomyocytes, as well as intracardiac ganglia and parasympathetic neurons. It is actively involved in the pathophysiology of a variety

of cardiovascular disease pathways, in addition to its essential role in natural physiological regulation mechanisms. Cardiac-related NPY receptors Y1R, Y2R, and Y5R, have been implicated in the pathogenesis of cardiovascular disorders such as hypertension, atherosclerosis, myocardial ischemia/infarction, stress, hypertrophic cardiomyopathies, and heart failure (61, 62, 63).

Along with NE, NPY is highly expressed in sympathetic nerve endings around the vasculature and acts as a potent vasoconstrictor (64). As the sympathetic nervous system is activated, such as during exercise or exposure to the cold, concentrations increase. In hypertensive patients and rodents, plasma levels of NPY and NE are also higher, indicating elevated sympathetic function (65, 66, 67, 68). To initiate vasoconstriction, NPY can promote Ca2+/CaM-mediated MLCK phosphorylation. The actions of several vasodilators, including substance P, acetylcholine, and vasoactive intestinal peptide, are impeded via NPYs inhibition on adenylyl cyclase (64).

In patients with arterial disease, studies have found a connection between atherosclerotic burden, susceptibility and increased NPY activation. Endothelial cells, SMCs, macrophages, and platelets all have receptors for NPY (61). Elevations in sympathetic glial-derived and platelet NPY and its receptors have been observed in mammalian experiments under inflammatory conditions such as vascular endothelium injury. The stimulation of Y1Rs and Y5Rs in endothelial cells and SMCs may also cause cellular proliferation, which leads to intima thickening, platelet aggregation, and adhesion during thrombosis (69, 70).

Although its function in this sense is still unknown, animal studies indicate that cardiac NPY is released from sympathetic nerves during experimentally induced myocardial infarction. In patients with STEMI, NPY and galanin had a direct capacity to influence the vagus nerve to release acetylcholine and regulate heart rate, whereas NPY plasma levels exhibited a high link with coronary microvascular performance (60, 71). NPY's pro-angiogenic effects have been shown to be beneficial in ischemic conditions such as hindlimb ischemia and chronic myocardial ischemia in several trials (72, 73). Despite these potentially beneficial effects, other findings have shown that NPY has negative effects on myocardial ischemia. In dogs, the vasoconstrictive effects of NPY in the coronary arteries trigger ST-T wave changes, as well as a decrease in intra-myocardial pH and left ventricle ejection fraction, resulting in myocardial ischemia (74). Recently, it was discovered that NPY levels in the peripheral venous system are

significantly elevated for at least 48 hours in patients undergoing primary percutaneous surgery for ST-elevation myocardial infarction (75).

Given the complexity of NPYs actions, determining if it plays a primarily cardio-protective or pathogenic role is exceedingly difficult. Indeed, its ultimate effect could be dose dependent, with beneficial effects at low doses and pathogenic effects at higher doses, according to some theories. Understanding the disease's regulatory function can therefore depend on the precise time point in the disease phase. The way NPY levels are tested can confuse studies even more, as plasma and local tissue concentrations are likely to vary. The high prevalence of cardiovascular disease, combined with NPY's pleiotropic impact, emphasizes the importance of more research into NPY's function in cardiovascular disease (63).

2. OBJECTIVES

The main aim of this study was to compare serum concentrations of NPY between patients presenting with STEMI and patients presenting with NSTEMI. In addition, we sought to compare NPY concentrations with various anthropometric, clinical and laboratory data.

Hypotheses:

1. NPY concentrations will be higher in patients with NSTEMI in comparison to patients with STEMI
2. NPY concentrations will be higher in hypertensive patients in comparison to non-hypertensive patients
3. NPY concentrations will positively correlate with diastolic blood pressure in patients
4. NPY concentrations will be higher in patients with DM in comparison to patients without DM
5. NPY concentrations will correlate with left ventricular ejection fraction (LVEF) of patients
6. NPY concentrations will correlate with The Global Registry of Acute Coronary Events (GRACE) score
7. NPY concentrations will correlate with markers of kidney function (urea, creatinine, eGFR)

3. SUBJECTS AND METHODS

3.1. Subjects and ethical considerations

In this cross-sectional study we enrolled a total of 60 patients with acute MI, 30 of which were diagnosed with STEMI, and 30 with NSTEMI. All of them received treatment according to European Society of Cardiology (ESC) latest guidelines (1). Patients were excluded from the study if they were in circulatory shock, had an active malignant disease, had a disease or were on therapy that altered bone metabolism.

This study was conducted in the period between February and July of 2019 at the University Hospital of Split. All ethical guidelines of the Declaration of Helsinki were fulfilled, and every included patient was informed about performed measurements in the study, after which an informed consent was obtained.

3.2. Clinical and laboratory measurements

Anthropometric measurements of height and weight were obtained using a calibrated scale (Seca, Birmingham, UK), from which BMI was calculated. Furthermore, using tape measure, waist and hip circumferences were obtained following standard procedures, while waist-to-hip ratio (WHR) was calculated by dividing the two measurements. Detailed physical examination and medical history assessment were performed.

Furthermore, every subject underwent a transthoracic echocardiography (TTE) at rest and left lateral position, in the time of 24 hours of admission. Ejection fraction of left ventricle (LVEF) was measured using modified Simpson rule, while Vivid 9 ultrasound system (GE Medical Systems, Milwaukee, WI, USA) was used for image acquisition. Killip classification was used for categorization of severity of heart failure, and GRACE score was used for calculation of in-hospital mortality (2,3).

Collected blood samples were centrifuged and placed in -80 °C containers for further analyses by a blinded medical biochemistry specialist. Serum NPY levels were determined by an enzyme-linked immunosorbent assay (ELISA) (Cat. no. EK-049-03, EIA kit, Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). Furthermore, chemiluminescent microparticle immunoassay (CMIA) of ARCHITECT STAT High Sensitive Troponin-I assay (Abbott Laboratories, Illinois, United States) was used for determination of Hs-cTnI concentrations. Finally, all other biochemical parameters were determined with standard laboratory procedures.

3.3. Statistical analysis

For the present study we used SPSS Statistics for Windows® (version 26.0, IBM, Armonk, NY, USA) and Prism 6 for Windows® (version 6.01, GraphPad, La Jolla, CA, USA) for data analysis. Categorical data were shown as numbers (N) and percentages (%), whereas continuous data were shown as mean ± standard deviation (SD) or median (interquartile range). We used the Kolmogorov–Smirnov test to assess normality of data. Differences between two groups (STEMI vs. NSTEMI) were assessed using independent samples t-test and Mann–Whitney U test for continuous variables and Chi-squared test for categorical variables or Fisher's exact test, where appropriate. Spearman's correlation was performed to assess correlation between NPY levels and multiple clinical and laboratory parameters. Statistical significance was set at $P<0.05$.

4. RESULTS

In comparison of baseline characteristics between NSTEMI and STEMI group of our study population, the patients statistically differed only with respect to tobacco use. Namely, patients with STEMI were smokers more often than patients with NSTEMI (18 (60%) vs. 10 (33.3%, P=0.040). The rest of the baseline characteristics were presented in **Table 2**.

Table 2. Baseline characteristics of patients

Parameter	STEMI (N=30)	NSTEMI (N=30)	P-value
Age, *years*	66.4 ± 6.4	69.6 ± 7.9	0.090†
Male sex	21 (70%)	24 (80%)	0.375*
Body mass index, kg/m^2	27.65 ± 2.70	26.53 ± 1.87	0.068†
Waist-to-hip ratio	1.03 ± 0.08	1.04 ± 0.07	0.669†
Diabetes mellitus	3 (10%)	7 (23.3%)	0.169*
Arterial hypertension	19 (63.3%)	20 (66.7%)	0.788*
Smoking	18 (60%)	10 (33.3%)	0.040*
Dyslipidemia	4 (13.3%)	8 (26.7%)	0.201*
Beta-blocker use	9 (30%)	11 (36.7%)	0.587*
ACE inhibitor or ARB use	14 (46.7%)	15 (50%)	0.798*
Calcium channel blocker use	7 (23.3%)	6 (20%)	0.756*
Statin use	3 (10%)	8 (26.7%)	0.098*
Diuretic use	3 (10%)	8 (26.7%)	0.098*

Data are presented as mean ± standard deviation or n (%)
*Chi-squared test.
†t-test for independent samples
Abbreviations: ACE – angiotensin converting enzyme, ARB – angiotensin receptor blocker, STEMI – ST elevation myocardial infarction, NSTEMI – non ST elevation myocardial infarction.

Upon admission, patients with STEMI did not differ from patients with NSTEMI in clinical characteristics except with regard to leukocyte count which was greater in the STEMI group (10.53 ± 2.59 vs. 9.16 ± 2.35 x 10^9/L, P=0.037), and creatinine levels (84.0 ± 14.7 vs. 104.5 ± 51.3 μmol/L, P=0.041), which were higher in the NSTEMI group. The rest of the clinical characteristics upon hospital admission were presented in **Table 3**.

Table 3. Clinical characteristics of patients upon admission

Parameter	STEMI (N=30)	NSTEMI (N=30)	P-value
Systolic blood pressure at admission, *mmHg*	133.5 ± 22.0	138.9 ± 20.5	0.329*
Diastolic blood pressure at admission, *mmHg*	79.2 ± 9.5	79.3 ± 13.4	0.973*
Heart rate at admission, *bpm*	71.9 ± 14.3	73.0 ± 13.2	0.773*
Left ventricular ejection fraction, *%*	52.2 ± 7.65	53.0 ± 10.6	0.730*
Erythrocytes, $x10^{12}$/L	4.57 ± 0.59	4.64 ± 0.48	0.634*
Leukocytes, $x10^{9}$/L	10.53 ± 2.59	9.16 ± 2.35	0.037*
Thrombocytes, $x10^{9}$/L	239.96 ± 54.60	231.96 ± 72.98	0.632*
Glucose	7.62 ± 2.70	8.48 ± 4.62	0.382*
Prothrombin time – INR	1.08 ± 0.30	1.15 ± 0.60	0.567*
Activated partial thromboplastin time, *s*	24.32 ± 4.90	24.91 ± 4.39	0.621*
C-reactive protein, *mg/L*	20.9 ± 4.6	15.0 ± 2.8	0.540*
High-sensitivity cardiac troponin I at admission, *ng/L*	391.69 ± 66.8	393.17 ± 46.4	0.992*
Potassium, *mmol/L*	3.94 ± 0.39	4.04 ± 0.43	0.383*
Urea, *mmol/L*	6.92 ± 1.88	8.32 ± 4.36	0.110*
Creatinine, *μmol/L*	84.0 ± 14.7	104.5 ± 51.3	0.041*
AST, *mmol/L*	77.3 ± 10.5	46.00 ± 28.39	0.120*
GRACE score, *points*	116.8 ± 13.5	123.2 ± 15.8	0.098*
CRUSADE score, points	25.1 ± 9.1	300 ± 13.6	0.108*

Data are presented as mean ± standard deviation or n (%)

* t-test for independent samples

Abbreviations: INR – international normalized ratio, AST – aspartat aminotrasferase, GRACE – The Global Registry of Acute Coronary Events, CRUSADE – Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines.

We observed that NPY levels were significantly higher in patients with NSTEMI compared to patients with STEMI (0.590 (0.550 – 0.650) vs. 0.735 (0.630 – 0.820) ng/mL, P < 0.001) (**Figure 7**).

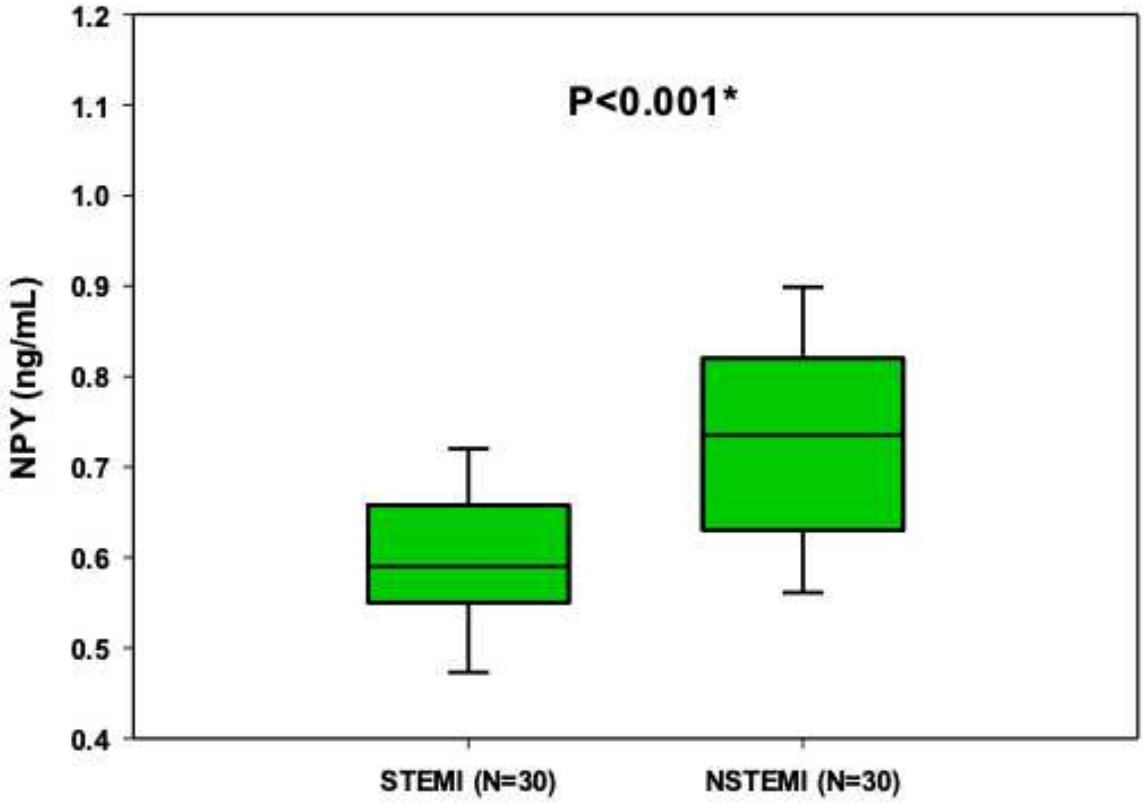

Figure 7. Comparison of NPY serum concentrations with respect to different types of acute coronary syndrome (STEMI vs. NSTEMI). Abbreviations: NPY – neuropeptide Y; STEMI – ST elevation myocardial infarction, NSTEMI – non ST elevation myocardial infarction. *Mann Whitney U test

Patients with DM had significantly higher NPY serum concentrations (0.640 (0.550 – 0.650) vs. 0.740 (0.720 – 0.754), P=0.046) than patients without DM. The Spearman's correlation showed that there was no correlation between NPY serum concentrations and neither LVEF (r = 0.073, P=0.609) nor diastolic blood pressure (r = -0.089, P=0.499), respectively, but NPY serum concentrations positively correlated with GRACE score (r = 0.293; P=0.023) (**Figure 8**). Correlation between NPY and various clinical and laboratory parameters are presented in **Table 4**.

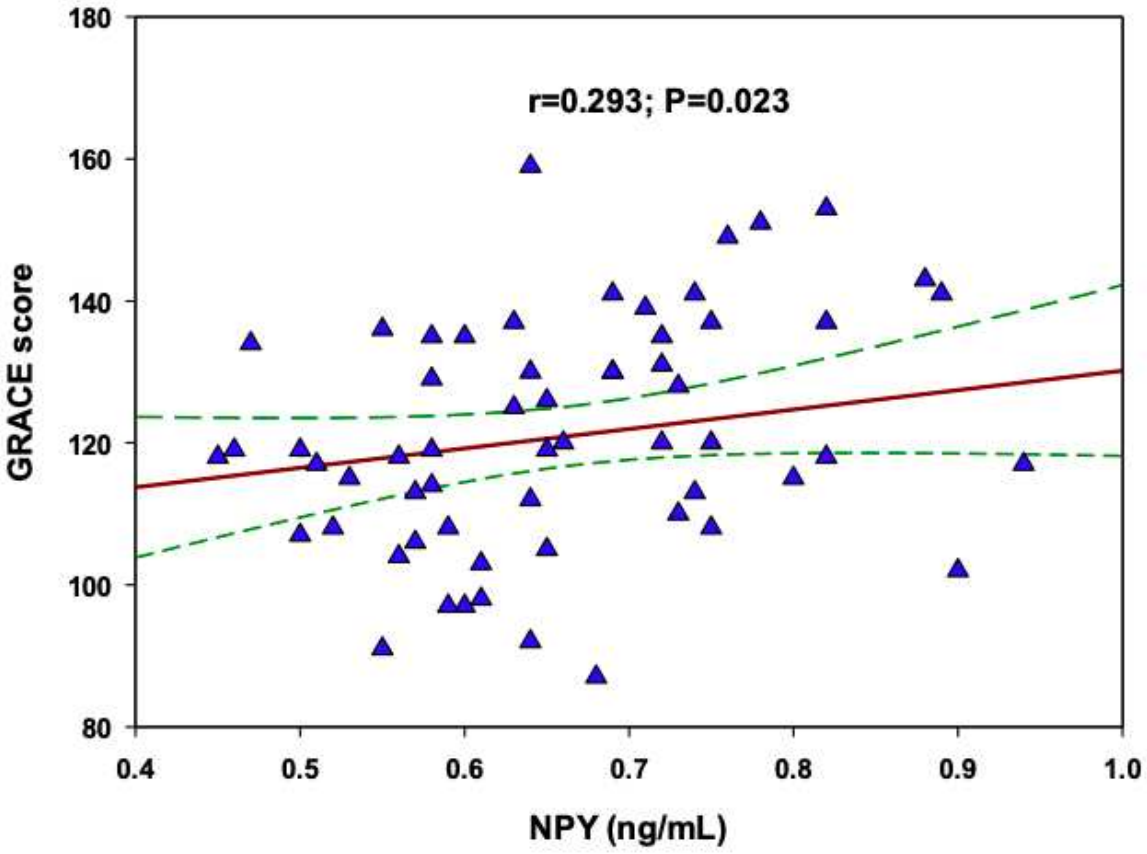

Figure 8. Spearman's correlation of NPY and GRACE score in patients with acute coronary syndrome. Abbreviations: NPY – neuropeptide Y; GRACE – The Global Registry of Acute Coronary Events.

Table 4. Spearman's correlation between neuropeptide Y serum concentrations and various clinical and laboratory parameters

Parameter	r-correlation coefficient	P-value
Activated partial thromboplastin time, s	-0.062	0.640
Urea, mmol/L	0.318	0.013
Creatinine, mmol/L	0.298	0.020
eGFR, mL/min/1.73m^2	-0.374	0.003
Heart rate, /min	0.006	0.966
Leukocytes, x10^9/L	-0.132	0.315
INR	-0.048	0.717
Troponin, ng/L	0.035	0.791
Waist-to-hip ratio	-0.196	0.134

Abbreviations: INR – international normalized ratio; eGFR – estimated glomerular filtration rate

5. DISCUSSION

Despite immense improvement in invasive cardiology in the recent years, myocardial infarction (MI) and concomitant repercussions, still represent a major global health burden. This addresses the need for further investigation of the complex pathophysiological network underlying this clinical entity. Hence, in the present study we aimed to bring further data to the field by inspecting the role of NPY in the setting of acute MI.

In this study we demonstrated that NPY concentrations are significantly higher in patients with NSTEMI in comparison to patients with STEMI. Furthermore, we observed higher levels of NPY in patients with DM in contrast to patients without DM, whereas there was no difference between hypertensive and non-hypertensive patients in NPY. In line with this, there was no correlation between diastolic blood pressure and NPY serum concentrations in patients with MI. Finally, according to our observations, NPY concentrations correlated with risk score in patients with MI, assessed by GRACE score, and renal function parameters, whereas there was no correlation between NPY concentrations and LVEF in patients presenting with acute MI.

Even though animal studies suggest that cardiac NPY is released from sympathetic nerves during experimentally induced MI, its role in this context still remains elusive (76). Multiple studies underlined the benefits of the pro-angiogenic properties of NPY in ischemic environments including chronic myocardial ischemia and limb ischemia (77-80). However, unlike the observed pro-angiogenic effects that may be beneficial, various studies have suggested detrimental actions of NPY in the ischemic *milieu* (81). In a study by Maturi et al. the authors demonstrated that intra-coronary administration of low dose NPY reduced blood flow in the coronary artery of dogs by 39% (82). Similar effects of NPY-induced myocardial ischemia was observed in patients with microvascular angina in a recent study by Rosano et al. (83). Clinical studies have shown that peripheral venous NPY activity is elevated during MI and left ventricular failure correlating with 1-year mortality (84, 85). These observations are in line with our results, which imply correlation between GRACE score, a well-established risk score model in MI, and NPY serum concentrations. Our main observation that NSTEMI patients exhibit higher serum concentrations than STEMI counterpart is rather hard to interpret. However, as NPY has been shown to alter microcirculation in the ischemic myocardium, it is plausible that in certain patients presenting with NSTEMI impairment of coronary circulations is at least in part owing to high levels of local NPY (86, 87). On the other hand, respecting the differences between STEMI and NSTEMI plaque pathobiology, and bearing in mind the complex interaction of NPY with multiple parts of the atherosclerotic network, it is also

possible that higher NPY concentrations, observed in NSTEMI, reflect the differences in plaque pathophysiology between the two entities. Overall, NPY seems to play a dual-role in worsening ischemia in the short term, beneficially by promoting angiogenesis in the longer term. Explaining the receptor signaling pathways underlying these responses remain to be elucidated in further studies.

The relationship between DM and NPY is rather complex. Namely, even though plasma levels of NPY were shown to be higher in patients with chronic type 2 DM, Ejaz et al. argue that the observed difference may be owing to compensatory increase in extra-neuronal NPY, whereas atrial NPY mRNA expression is actually lower in patients with DM in comparison to patients without DM (80, 88). Results from the present study are thus in line with the available data.

Unlike multiple studies that reported higher NPY serum concentrations among hypertensive patients, especially in preeclampsia, we found no significant difference between NPY in hypertensive vs non-hypertensive patients presenting with acute MI (89, 90, 91). Namely, by being expressed in sympathetic nerve endings around the blood vessels, NPY acts as a potent vasoconstrictor (92, 93). Furthermore, NPY levels are very low in healthy individuals, yet they abruptly rise during conditions of sympathetic activation which we observe in stressful situations such as hypoxia, cold weather and strenuous exercise (66, 67) (94). Finally, the observed discrepancy between the present and other studies could arise from the differences in mechanisms of hypertension, as NPY-mediated hypertension is related to increased sympathetic activity, which may not be the most common underlying mechanism of hypertension in our studied population.

It has so far been well established that heart failure (HF) is associated with increased sympathetic nervous system activity (95). Early studies have showed that baseline levels of NPY in HF patients are high (96, 97). Moreover, Liu et al. demonstrated that severity of HF may be correlated to NPY plasma levels (98). Nonetheless, these observations are not in concordance with results from the present study. Yet, we merely failed to establish a correlation between NPY and EF, which does not exclude correlation of NPY with HF. For instance, our results would be more indicative if we compared NPY to BNP blood levels. Various mechanisms that could mediate the association of NPY signaling and HF have been suggested (99, 100). Overall, it seems that NPY has both protective and detrimental effects in this setting,

hence, it remains to be elucidated in large longitudinal studies which of these effects prevail in HF.

Studies suggest that NPY is also implicated in renal pathology. Results from the present study, but other studies as well, indicate a firm and negative correlation between kidney function and NPY serum concentrations (101, 102). In fact, it has been demonstrated that NPY represents an independent, robust predictor of CV events in both predialysis and dialysed patients with chronic kidney disease (103, 104). Zoccali et al. even suggest the risk for such events is age-dependent, maximal being in youngest patients (103). The above-noted observations are in line with the emerging experimental evidence that suggests that high sympathetic activity plays a role in CKD progression (104).

This study bears several notable limitations. Firstly, the present study is of cross-sectional design which prevents us of making any causal inferences. Secondly, sample size in our study was relatively low. Finally, peripheral venous sampling tends to be of poor accuracy in determining NPY release, as hepato-mesenteric release of NPY also contributes significantly to its circulating levels.

6. CONCLUSIONS

1. NPY concentrations are significantly higher in patients with NSTEMI in comparison to patients with STEMI.
2. There is no difference in NPY concentrations between hypertensive and non-hypertensive patients with MI.
3. There is no correlation between NPY concentrations and diastolic blood pressure in patients with MI.
4. Among patients presenting with ACS, NPY concentrations are significantly higher in patients with DM in comparison to patients without DM.
5. NPY concentrations do not correlate with left ventricular ejection fraction (LVEF) in patient presenting with ACS.
6. NPY concentrations positively correlate with The Global Registry of Acute Coronary Events (GRACE) score.
7. NPY concentrations negatively correlate with glomerular filtration rate in patients with MI.

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8. SUMMARY

Objectives: The main aim of this study was to compare serum concentrations of NPY between patients presenting with ST elevation myocardial infarction (STEMI) and patients presenting with non ST elevation myocardial infarction (NSTEMI). In addition, we sought to compare NPY concentrations with various anthropometric, clinical and laboratory data.

Subjects and methods: In this cross-sectional study we enrolled a total of 60 patients with acute myocardial infarction (MI), 30 of which were diagnosed with STEMI, and 30 with NSTEMI. Patients were submitted to thorough clinical examination where anthropometric data was obtained. Subsequently, peripheral blood sample was drawn, and serum Neuropeptide Y (NPY) levels were determined.

Results: NPY levels were significantly higher in patients with NSTEMI compared to patients with STEMI (0.590 (0.550 – 0.650) vs. 0.735 (0.630 – 0.820) ng/mL, $P < 0.001$). Furthermore, patients with DM had significantly higher NPY serum concentrations (0.640 (0.550 – 0.650) vs. 0.740 (0.720 – 0.754) ng/mL, P=0.046) than patients without DM. The Spearman's correlation showed that there was no correlation between NPY serum concentrations and neither LVEF (r = 0.073, P=0.609) nor diastolic blood pressure (r = -0.089, P=0.499), respectively, however NPY serum concentrations positively correlated with GRACE score (r = 0.293; P=0.023). Finally, NPY serum concentrations positively correlated with urea (r = 0.318, P=0.013) and creatinine (r = 0.298, P=0.020) levels, and negatively with estimated glomerular filtration rate (r = -0.374, P=0.003).

Conclusions: We concluded that NPY concentrations are significantly higher in patients with NSTEMI in comparison to patients with STEMI, and that NPY concentrations in patients with acute MI are significantly higher among patients with diabetes but with no difference between hypertensive and non-hypertensive patients. In addition, NPY concentrations correlate with the risk of mortality and renal function parameters in patients with MI.

9. CROATIAN SUMMARY

Naslov: Neuropeptid Y u bolesnika s akutnim infarktom miokarda

Cilj: Glavni cilj ovog istraživanja bio je usporediti serumske koncentracije neuropeptida Y (NPY) među bolesnicima s infarktom miokarda sa ST elevacijom (engl. *ST elevation myocardial infarction,* STEMI) i infarkta miokarda bez ST elevacije (engl. *non ST elevation myocardial infarction,* NSTEMI). Također, usporedili smo razine NPY s raznim kliničkim i laboratorijskim parametrima.

Materijali i metode:. U ovu presječnu studiju uključili smo 30 bolesnika sa STEMI-jem i 30 s NSTEMI-jem. Bolesnici su podvrgnuti detaljnom kliničkom pregledu gdje su uzeti i antropometrijski podaci. Konačno iz uzoraka krvi smo analizirali razne laboratorijske parametre uključujući NPY.

Rezultati: Serumske razine NPY-a su bile značajno veće u bolesnika s NSTEMI-jem u usporedbi s bolesnicima s STEMI-jem (0,590 (0,550 – 0,650) vs. 0,735 (0,630 – 0,820) ng/mL, P < 0,001). Nadalje, bolesnici koji boluju od šećerne bolesti imali značajno veće koncentracije NPY-a od ispitanika koji ne boluju od iste (0,640 (0,550 – 0,650) vs. 0,740 (0,720 – 0,754) ng/mL, P=0,046). Spearmanova korelacijska analiza pokazala je da serumske koncentracije NPY-a ne koreliraju s ejekcijskom frakcijom (r = 0,073, P=0,609) ni s vrijednostima dijastoličkog tlaka (r = -0,089, P=0,499), ali koreliraju s GRACE zbirom (r = 0,293; P=0,023). Konačno, serumske koncentracije NPY-a pozitivno su korelirale s urejom (r = 0,318, P=0,013) i kreatininom (r = 0,298, P=0,020), a negativno s procijenjenom glomerularnom filtracijom (r = -0,374, P=0,003).

Zaključci: Utvrdili smo značajno veće koncentracije NPY u bolesnika s NSTEMI-jem u odnosu na bolesnike sa STEMI-jem. Uz to, demonstrirali smo da su koncentracije značajno veće u dijabetičara u odnosu na ne-dijabetičare, te da nema značajne razlike između hipertoničara i ispitanika koji nisu bolovali od hipertenzije. Konačno, pronašli smo značajnu korelaciju razina NPY-a s GRACE zbirom i markerima bubrežne funkcije.

10. CURRICULUM VITAE

PERSONAL INFORMATION:

NAME AND SURNAME: Christian Libers

DATE AND PLACE OF BIRTH: Bad Kissingen, Germany February 15, 1997

NATIONALITY: German

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EDUCATION:

2015-2021 University of Split, School of Medicine, MD / Degree of Medical Doctor.

2007-2015 E.T.A Hoffmann-Gymnasium Bamberg, Germany

AWARDS:

Dean's Award for highest Academic GPA in 2016/2017 school year

RELEVANT EXPERIENCE:

Clinical Rotations in Gynaecology, Cardiology and General Surgery Regiomed Kliniken, Coburg, December 2020 - February 2021

Able to advance and fortify my theoretical knowledge, daily work and repetition of many procedures steadied my skills and improved my confidence greatly; Being able to perform under everyday clinical pressure was an integral aspect.

Nurse Internships 3 Months, 30 days each, 2017 - 2018 Hospital Ebern, Hassberge Kliniken (Germany), Hospital Krizine (Split, Croatia)

Gave me the opportunity to fully grasp the concept of working in the hospital environment as a combined entity of many individuals. Taught the ways of caring for patients professionally on a medical and social level, heavily influencing my work ethic.

OTHER

Languages: German, Russian, English, Norwegian, Croatian, Spanish