Aus der Klinik für Kardiologie und Angiologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Midregional Pro-A-Type Natriuretic Peptide as Part of a Dual Biomarker Strategy for the Early Rule Out of non-ST Segment Elevation Acute Coronary Syndrome

Mittregionales Pro-Atriales Natriuretisches Peptid als Teil einer dualen Biomarker-Strategie für den früheren Ausschluss des akuten Koronarsyndroms ohne ST-Strecken-Hebung

zur Erlangung des akademischen Grades
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von

Miloš Tajsić

aus Belgrad, Serbien

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Inhalt

I Abkürzungsverzeichnis

ANP	Atrial Natriuretic Peptide
NPR_A	natriuretic peptide receptor-A
NPR_B	natriuretic peptide receptor-B
NPR_c	natriuretic peptide clearance receptor-C
ACS	Acute Coronary Syndrome
ACEI	angiotensin converting enzyme inhibitor
ARNI	angiotensin receptor neprilysin inhibitor
AUROC	area under the receiver operating characteristics curve
BNP	brain natriuretic peptide
BMI	body mass index
BP	blood pressure
CNP	C-type natriuretic peptide
CABG	coronary artery bypass grafting
CA	coronary angiography
CAD	coronary artery disease
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CK-MB	creatine kinase-muscle/brain
CI	confidence Interval
ER	Emergency Room
ECG	electrocardiogram
eCRF	electronic case report form
GRACE Score	Global Registry of Acute Coronary Events Score
IQR	interquartile ranges
hs-cTn	high sensitive cardiac troponin assay
KDIGO	Kidney Disease: Improving Global Outcomes

LoD	limit of detection
LoQ	limit of quantification
Mr-proANP	mid-regional pro a-type natriuretic peptide
MI	myocardial infarction
NPS	Natriuretic peptide system
NCC	sodium-chloride symporter
NT-proANP	N-terminal portion of proANP
NEP	enzyme-neutral endopeptidase
NSTE-ACS	non ST elevation myocardial infarction
NPV	negative predictive value
PAD	peripheral arterial disease
PCI	percutaneous coronary intervention
RAAS	renin-angiotensin-aldosterone system
RSS	Renal sympathetic system
STE-ACS	ST elevation myocardial infarction
s-cTnI	sensitive cardiac troponin I assay
SOT	symptom onset time
TIA	transitory ischemic attack
UA	unstable angina
URL	upper reference limit

II Abstract

II.1 Abstract (englisch)

Background:

Mr-proANP is a biomarker produced in atrial and left ventricular myocardium. We investigated the effect of combined measurement of mr-proANP and high-sensitive cardiac Troponin I assay of the penultimate generation (s-cTnI) for an early type-1 and type-2 NSTE-ACS rule-out with emphasis on the very early presenters' subgroup with symptom onset time (SOT) ≤ 2 h.

Methods:

This was a prospective cohort study of 311 consecutive patients admitted to ER with symptoms suggestive of an acute coronary syndrome (ACS). All patients had baseline mr-proANP and s-cTnI measurements.

Results:

Of the total cohort, 17.6% (n = 55) had final diagnosis of NSTE-ACS: 9.6% (n = 30) had an angiographically-confirmed type-1 infarction and 8.0% (n = 25) had type-2 infarction.

In the subgroup of very early presenters (SOT ≤ 2 h) the negative predictive value (NPV) of s-cTnI for type-1 NSTEACS was 96.7% (95%-CI: 87.5–99.4) and the NPV of mr-proANP was 100% (95%-CI: 87.1–100). The dual biomarker strategy yielded an NPV of 100% (95%-CI: 86.7–100). In the same time-related subgroup, the NPV of s-cTnI alone for type-2 was 98.3% (95%-CI: 89.8–99.9) and the NPV of mr-proANP was 97.0% (95%-CI: 82.5–100). The combination of biomarker increased the NPV to 100% (95%-CI: 86.7–100).

Conclusions:

Our study demonstrated an immediate release pattern of mr-proANP in NSTE-ACS that may bridge the silent troponin time phenomenon when highest-sensitivity cardiac troponin assays are not used. This concept performed best in the very early presenters' subgroup with an excellent NPV of 100% and might result in an early rule-out of NSTE-ACS thus accelerating the diagnostic work-up.

II.1 Abstract (deutsch)

Hintergrund:

Mr-proANP ist ein Biomarker, der im atrialen und linksventrikulären Myokard produziert wird. Wir haben den Effekt der kombinierten Messung von mr-proANP und hochsensitivem kardialen Troponin I der vorletzten Generation (s-cTnI) für einen früheren Ausschluss vom NSTE-ACS Typ-1 und Typ- 2 untersucht mit Akzentsetzung auf jene Patienten, die sich sehr früh nach Symptombeginn (≤ 2 h) in der Notaufnahmen vorstellten.

Methoden:

Es handelte sich um eine prospektive Kohortenstudie von 311 hintereinander kommenden Patienten, die sich in der Notaufnahme mit Symptomen, die auf ein akutes Koronarsyndrom (ACS) hindeuteten, vorstellten. Bei allen Patienten fand die Bestimmung von mr-proANP- und s-cTnI-Spiegel unmittelbar nach dem Eintreffen in der Notaufnahme statt.

Ergebnisse:

Von der Gesamtkohorte hatten 55 (17,6%) Patienten die endgültige Diagnose eines NSTE-ACS, 30 (9,6%) Patienten hatten einen angiographisch bestätigten Typ-1-Infarkt und 25 (8,0%) Patienten hatten einen Typ-2-Infarkt.

In der Subgruppe der Patienten, die sich sehr früh nach Symptombeginn (SOT ≤ 2 h) vorstellten, betrug der negative prädiktive Wert (NPW) von s-cTnI für Typ-1-NSTE-ACS 96,7% (95% -CI: 87,5–99,4) und der NPW von mr-proANP 100% (95% -CI: 87,1–100). Die duale Biomarker-Strategie erbrachte einen NPW von 100% (95% -CI: 86,7–100). In derselben Subgruppe betrug der NPW von s-cTnI allein für Typ-2 98,3% (95% -CI: 89,8–99,9), der NPW von mr-proANP betrug 97,0% (95% -CI: 82,5– 100). Die Kombination zweier Biomarker erhöhte den NPW auf 100% (95% -CI: 86,7–100).

Schlussfolgerungen:

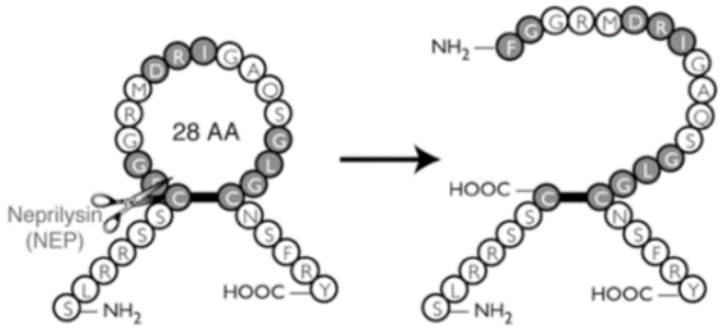
Unsere Studie zeigte ein sofortiges Freisetzungsmuster von mr-proANP in NSTE-ACS, was möglicherweise das Phänomen der „silent troponin time“ überbrücken könnte, wenn keine Herz-Troponin-Assays mit höchster Empfindlichkeit verwendet werden. Dieses Konzept erzielte in der Gruppe der Patienten, die sich sehr früh nach Symptombeginn vorstellten, einen NPW von 100%. Somit könnte ein früherer Ausschluss von NSTE-ACS erzielt werden, wodurch die gesamte diagnostische Aufarbeitung beschleunigt werden könnte.

III Dissertation

1. Current State of Research

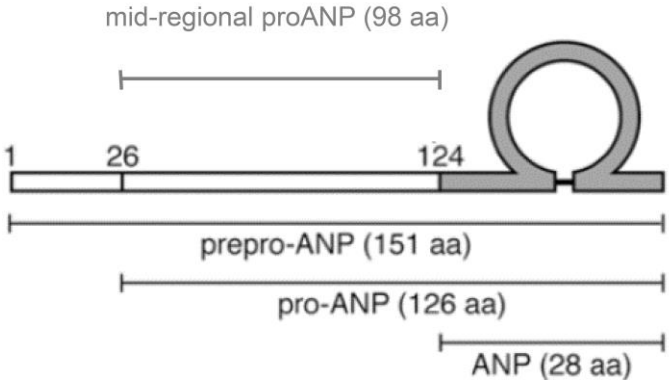
1.1 Atrial Natriuretic Peptide and the Natriuretic Hormones Family

Atrial Natriuretic Peptide (ANP) is a cardiac regulatory hormone, synthetized mainly by the myocytes of the atria. ANP belongs to the family of natriuretic hormones: the group of nine structurally similar hormones synthetized in the heart atria, intestinal, renal, and adrenal tissue. ANP is a 28-amino acid peptide with a 17-amino acid ring in the center of the molecule, which consists of a disulfide bond between two residues cysteine (at positions 7 and 23). (Graph 1) (1)
The other members of this hormone cluster are BNP (brain natriuretic peptide) and CNP (C-type natriuretic peptide), which all share a similar amino acid ring structure. (2, 3)



Graph 1: Structure of ANP (Potter et al.)

Prepro-ANP is the prohormone and the precursor of ANP and it is released in atria. This polypeptide consists of 151 amino acids and is in its nature susceptible to fragmentation. (Graph 2) The mature atrial natriuretic peptide (ANP), a 28-amino acids polypeptide, represents a smaller part of the whole prepro-ANP molecule, and has a short half-life. (1) On the other hand, the N-terminal portion of prepro-ANP called mid-regional proANP, comprises the larger portion of proANP with its 98 amino acids, with a much longer half-life than mature ANP and therefore poses as more reliable analyte for clinical routine measurement (4).



Graph 2: Structure of the precursor hormone for ANP (Potter et al.)

As mentioned, ANP is mainly synthetized and stored by the myocytes of the atria. Its release from the atrial granules is primarily stimulated by the:

(1) Stretch of the atrial wall induced by an increased intravascular volume and the

(2) Pressor hormones (norepinephrine, epinephrine, vasopressin, dopamine, dobutamine, etc.). (5-7)

After secretion of the ANP-precursor (prepro-ANP) and its cleavage into the mature peptide (Graph 1), ANP enters the coronary sinus and reaches its receptors in target organs via the bloodstream. ANP binds to a specific set of receptors called natriuretic peptide receptors (NPR receptors). There are three main types of the NPR-receptors: natriuretic peptide receptor-A (NPR_A), natriuretic peptide receptor-B (NPR_B), natriuretic peptide clearance receptor (NPR_C).

The NPR_A and NPR_B receptors have a single segment that spans through the membrane. An extracellular domain binds the ligand and an intracellular domain maintains two of the catalytic domains for guanylyl cyclase activity. ANP_C is a receptor that binds all natriuretic peptides and functions as a clearance receptor by attaching and sequestering ANP from the bloodstream. (1)

The plasma levels of ANP in healthy individuals were shown to be low at 11 ± 0.9 fmol/ml (11 pmol/L) and demonstrated a significant increase with maximal median values of 71 ± 9.9 fmol/ml (71 pmol/L) ($p<0.01$) when measured in patients with chronic heart failure. (8)

The levels of the mr-proANP measured using the sandwich immunoassay (B.R.A.H.M.S SERISTRA®, B.R.A.H.M.S AG) developed by Morgenthaler et al, demonstrated aged and gender-related differences in levels of mr-proANP in healthy population. (9) The highest levels were measured in the oldest age group with median values of 68.6 pmol/L. Furthermore, there was a clear correlation

between the mr-proANP levels and increasing age, with significant gender differences in the 26-35 years group in favor of women and in 46-55 years group in favor of men. (Graph 3.)

On the other hand, the Leicester Acute Myocardial Infarction Peptide (LAMP) Study demonstrated significantly elevated plasma levels of mr-proANP in patients with AHF. The patients who died due to AHF had median mr-proANP median values of 310 pmol/L (IQR 48 to 1150 pmol/L). The median value of the mr-proANP in patients with AHF that survived was measured at 108 (IQR 4.9 to 1,210 pmol/l).

	Age group									
	18–25 years		26–35 years		36–45 years		46–55 years		56–65 years	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
n	19	25	33	35	54	45	41	27	25	21
Median, pmol/L	35.0	43.3	31.7	45.1	44.0	41.1	57.4	41.4	64.8	68.6
Range, pmol/L	19.8–121.0	11.3–123.0	12.5–116.0	9.6–181.0	18.9–119.0	13.6–120.0	17.4–241.0	23.1–144.0	31.2–183.0	26.6–313.0
P, Mann–Whitney test	0.28		0.02		0.65		0.05		0.74	

Graph 3: Midregional proANP in healthy donors stratified by age and gender (Morgenthaler et al.)

The half-life of ANP in human plasma is approximately 2 min, before being degraded (10, 11) by an enzyme-neutral endopeptidase (NEP) also called neprilysin (Graph 1) (12, 13) or through binding to the natriuretic peptide clearance receptor (NPR_C). Identical pathway of degradation and neutralization applies for BNP.

ANP_C is a cell surface receptor lacking guanylyl cyclase activity thereby neutralizing the concentrations of natriuretic peptides via receptor-mediated internalization and degradation. The inhibition of neprilysin prolongs the half-life of ANP both in vitro (12) and in vivo (14), suggesting that neprilysin activity contributes to the rapid clearance of ANP, although the degradation of ANP is a synergetic effect of both neprilysin activation and ANP_C binding.

ANP together with its matching receptors (NPR_A, NPR_B, and NPR_C) and neprolisyn/enzyme-neutral endopeptidase (NEP) play a pivotal role as a part of three major systems that regulate the extracecullar fluid volume:

(1) Electrolyte retention systems i.e. renin-angiotensin-aldosterone system (RAAS)

(2) Renal sympathetic system (RSS)

(3) Salt (Natriuretic Peptide) hormone elimination system i.e. natriuretic peptide system.

These systems are working in an antagonistic manner. The activation of the RASS and RSS leads to reduction of the extracellular space and vasoconstriction thereby increasing the periphery vascular resistance. In contrast, the activation of the natriuretic peptide system leads to the expansion of both atria of the heart.

1.1.2. The physiological effects of the ANP-receptor-agonist binding

The ANP-receptors are widely present in the body, where the binding of the receptor-agonists causes the increase in renal sodium excretion, which results in a decreased extracellular fluid and blood volume thereby reducing the systematic blood pressure and cardiac afterload. This leads to an improvement in cardiac ejection fraction.

Renal physiological effects

Binding of the ANP to its receptors in the kidney, increases the sodium and water elimination (natriuresis) through several physiological mechanisms (15, 16):

- ANP increases glomerular filtration rate and glomerular permeability. By binding to NPR_A and NPR_B receptors, ANP directly facilitates the dilatation of the afferent arteriole and prevents the vasoconstriction of the vessel caused by norepinephrine. Furthermore, ANP relaxes the glomerular mesangial cells by directly inhibiting Angiotensin II and increases the diameter and number of glomerular pores. As a consequence, the glomerular permeability increases and leads to a greater filtration turnover of sodium and water. (17, 18)

- On the other hand, in an interaction with sodium-chloride symporter (NCC) and cortical collecting duct of the nephron, stimulation of the NPR_A and NPR_B receptors reduces the sodium reabsorption in the distal convoluted tubule; ANP also inhibits the Sodium Potassium ATPase pump resulting in less sodium re-absorption and more sodium excretion. (17, 18)

- Furthermore, ANP inhibits the renal sympathetic nervous system by blocking the renin secretion and consequently the renin-angiotensin-aldosterone axis thereby inhibiting the production of angiotensin and aldosterone and ultimately the vasopressin secretion. This emphasizes the antagonizing effect that ANP has regarding aldosterone: aldosterone increases renal sodium retention and ANP increases renal sodium loss (19).

Blood pressure homeostasis

The major signaling pathway for the regulation of the blood pressure homeostasis is the binding of ANP to NPR_A receptors (20-23). The activation of the NPR_A decreases blood pressure through several mechanisms: (1) vasorelaxation, (2) natriuresis and diuresis, (3) increased endothelium permeability and (4) by antagonizing of the renin-angiotensin system. The experiments demonstrated that volume injection in the heart atrium leads to a prompt release of ANP as well as activation of NPR_A with subsequent triggering of the abovementioned pathway mechanisms (24). Furthermore, mice with genetically engineered reduction in NPR_A-expression in the vascular endothelium demonstrated impairment on the blood pressure regulation with increased volume expansion, reduced albumin clearance and subsequent hypertension (25).

Effects of Natriuretic Peptides on Cardiac Hypertrophy and Fibrosis/Remodeling

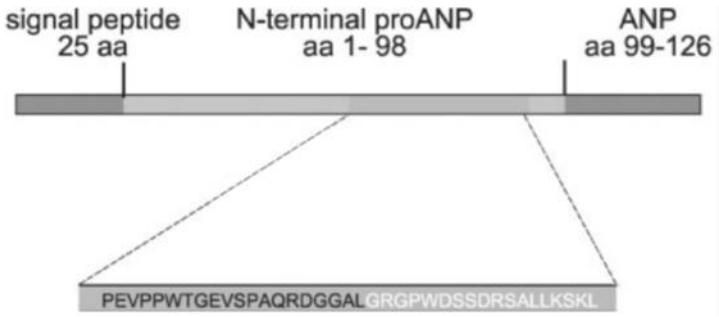
An additional effect of ANP, beside the regulation of blood pressure, is the inhibition of the cardiac hypertrophy as well as the antagonization of the cardiac fibrosis and remodeling. Hypertrophy is regulated along the ANP/BNP-NPR_A receptors pathway, whereas remodeling is regulated by both the ANP/BNP-NPR_A and the ANP/BNP-NPR_B pathways.

Cardiac hypertrophy is caused by calcium influx facilitated by norepinephrine. By binding to NPR_A receptors, ANP inhibits this influx and therefore prevents the hypertrophy. (18) Although prolonged hypertension alone induces the cardiac hypertrophy, experiments demonstrated that in NPR_A deficient mice, the level of hypertrophy was significantly greater than that observed in other genetic models without the NPR_A knockout genotype. These findings suggest that NPR_A generates a local growth inhibitory signal in the heart. Furthermore, the data showed that NPR_A knockout mice had myocardial hypertrophy, resistant to the treatment with antihypertensive drugs, even when treated from birth (26), whereas mice with overexpressing ANP/NPR_A genotype had smaller hearts. (27, 28)

The pathophysiology of cardiac fibrosis and remodeling is determined by prolonged inflammation followed by the migration of the fibroblasts into the myocardium and their replication. The activation of both NPR_A and NPR_B receptors through ANP and BNP, respectively, controls the inflammation process and inhibits the fibroblast-migration and replication. (28)

1.2. Midregional pro-A-type natriuretic peptide

The original form of the ANP released in atria is a prohormone (proANP). This polypeptide consists of 126 amino acids and is in its nature susceptible to fragmentation. The mature atrial natriuretic peptide (ANP), a 28-amino acids polypeptide, represents a smaller part of the whole proANP molecule, and has a short half-life (2 to 5 minutes). On the other hand, the N-terminal portion of proANP (NT-proANP), comprises the larger portion of proANP with its 98 amino acids, with a much longer half-life than mature ANP (40 to 60 minutes) and therefore poses as more reliable analyte for clinical routine measurement (4). (Graph 4)



Graph 4: Structure of the proANP with the location of the mid-regional section (Morgenthaler et al. 2004)

The sandwich immunoassays developed for proANP employ an antibody against the N-terminal region of proANP (1–52 amino acids) combined with a second antibody against the mid-region of pro ANP (amino acids 53-90). (29, 30) However, under certain conditions, the N-terminal region provides an impaired antibody binding (31, 32), despite its long half-life. This could be due to its innate proneness to fragmentation. (33, 34) However, the mid-region of pro ANP (known as mid-regional proANP), a polypeptide with 38 amino acids (53-90) proved to provide a stable antibody binding, allowing accurate immunoassay measurements.

Utilizing this property of mid-regional proANP (mr-proANP), a sandwich immunoassay (B.R.A.H.M.S SERISTRA®, B.R.A.H.M.S AG) was developed, targeting only the mr-proANP for an antibody biding. Therefore, the mr-proANP was established as biomarker in the clinical routine-providing the stabile and reliable determination of the ANP plasma levels. (9)

1.3 Acute Coronary Syndrome

Acute coronary syndrome (ACS) is a set of signs and symptoms occurring due to an impaired perfusion of the coronary arteries, subsequently leading to the dysfunction or the necrosis of the heart muscle (myocardium). (35) The cardinal symptom of critically impaired myocardium perfusion is chest pain, experienced as tightness around or over the chest, occasionally radiating to the left arm and the left angle of the jaw, sometimes accompanied with vegetative symptomatic: nausea, sweating (diaphoresis) and shortness of breath (dyspnoe). In a large portion of patients, the symptoms present as "atypical", with different qualitative and quantitative onset of pain, or pain being completely absent, with dyspnea being the leading symptom. This atypical symptomatology is more likely to occur in female and older patient as well as the patient with diabetes mellitus. (36)

Chest pain can also be the common leading symptom in brady- or tachycardia, hypertensive crisis, severe aortic valve stenosis, pulmonary artery hypertension, severe anemia and multiple other conditions.

Clinical Classification of Acute Coronary Syndrome

Regarding the acute treatment concept i.e. reperfusion therapy, the diagnostic golden standard is the allocation of the patients with ACS to three clinical entities: ST elevation myocardial infarction (STE-ACS), non-ST elevation myocardial infarction (NSTE-ACS) or unstable angina (UA). (37)

Patients with chest discomfort or other ischemic symptoms, who develop new ST-segment elevations in two contiguous leads or new bundle branch blocks with ischemic repolarization patterns, are designated as an STE-ACS.

Patients without ST-segment elevation at presentation, yet with the rise of circulating troponin levels above the 99th percentile are usually designated as NSTE-ACS.

Patients with stabile angina experiencing sudden new-onset of angina pectoris symptoms, often at rest or with minimal exertion, or at lesser degrees of exertion than the individual's previous angina ("crescendo angina") without ST-segment elevation at presentation and without the rise of circulating troponin levels above the 99th percentile are diagnosed as UA. UA should be distinguished from stable angina, which develops during physical activity or stress and resolves at rest.

The categories of patients with STE-ACS and NSTE-ACS are regularly included in the concept of Myocardial Infarction (MI). Furthermore, the categories of patients with STE-ACS, NSTE-ACS, or unstable angina are customarily included in the concept of ACS. (37)

Types of myocardial Infarction according to the Fourth Universal Definition of myocardial infarction

In addition to the clinical classification of ACS/MI, another classification based on etiology, pathogenesis, clinical, and prognostic differences, along with different treatment strategies allocates the ACS/MI into various types. (37)

Type 1 MI is caused by atherothrombotic coronary artery disease (CAD) and commonly facilitated by the rupture or erosion of the atherosclerotic plaque in the coronary artery. The lesion is labeled as "culprit" and could have varying degrees of atherosclerosis and thrombosis burden with thrombotic component leading to distal coronary embolization resulting in myocardial necrosis. (38, 39)

Typ 2 MI is determined by the pathophysiological mechanism leading to ischemic myocardial injury due to a mismatch between oxygen supply and oxygen demand in the myocardial tissue. (40, 41). By definition, acute atherothrombotic plaque disruption is not the cause of type 2 MI. In patients with stable CAD, an acute impairment factor such as an acute gastrointestinal bleeding, sustained tachyarrhythmia, and hypertensive crisis may result in myocardial injury and a type 2 MI. This occurs due to dysfunctional myocardial perfusion failing to compensate the increased myocardial oxygen demand. The occurrence of the impaired demand/supply ratio with subsequent myocardial injury varies among the patients with CAD and depends on the properties of the impairment factor, presence of non-cardiac comorbidities, CAD severity scale and cardiac structural abnormalities.

Type 3 MI is defined as typical presentation of myocardial ischemia/infarction symptoms, including ischemic ECG changes or ventricular fibrillation, with death occurring before it is possible to obtain blood for cardiac biomarker determination; or the patient dies promptly after the onset of symptoms before an elevation of biomarker values has occurred i.e. the suspicion of an acute myocardial ischemic event is high, even when cardiac biomarker evidence of MI is lacking. (40, 41)

Type 4 MI is the percutaneous coronary intervention (PCI) related MI that occurs during a PCI procedure or is related to in-stent restenosis, or restenosis following balloon angioplasty in the infarct territory i.e. the PCI is the only plausible explanation for the MI since no other culprit lesion or thrombus can be identified. (37)

Type 5 MI is Coronary artery bypass grafting (CABG) related MI with numerous factors that may lead to procedural myocardial injury during a CABG. These factors are related to the details of the cardiac preservation, the extent of the direct traumatic injury to the myocardium, as well as any potential ischemic injury. (37)

1.4 Role of mr-proANP/ANP in diagnosis and treatment of acute and chronic heart failure

Current European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of acute and chronic heart failure indicate that the plasma concentration of natriuretic peptides, mr-proANP and BNP/NT-proBNP, are to be used as an initial diagnostic test when echocardiography is not immediately available (Class I, Level A Recommendation). (42) Upon presentation of the patients with acute dyspnoe and suspected AHF a measurement of plasma natriuretic peptide level (BNP, NT-proBNP or MR-proANP) is recommended to help in the differentiation of AHF from non-cardiac causes of acute dyspnoe. Elevated NPs facilitate an initial working diagnosis, identifying those who are in need of further cardiac investigation. Furthermore, patients with natriuretic peptide levels below the cutoff for the exclusion of important cardiac dysfunction do not require echocardiography. The cutoff for a diagnosis of acute HF for mr-proANP is recommended at 120 pmol/L. The upper limit of normal in the acute setting for B-type natriuretic peptide (BNP) is 100 pg/mL and for N-terminal pro-BNP (NT-proBNP) it is 300 pg/mL. On the other hand, patients with normal plasma NP levels are unlikely to have heart failure. (42)

Diagnostic values of both mr-proANP as well as BNP/NT-proBNP, apply similarly to HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF). Furthermore, their median values are lower for HFpEF than for HFrEF. (43, 44) At the above mentioned recommended thresholds of 120 pmol/L, mr-proANP has a sensitivity ranging from 0.95 (range 0.90-0.98) to 0.97 (0.95-0.98) and a negative predictive value ranging from 0.90 (0.80-0.96) to 0.97 (0.96-0.98). (43, 45-50) At the recommended threshold of 100 ng/L for BNP and 300 ng/L for NT-proBNP, the natriuretic peptides have sensitivities of 0.95 (95% CI, 0.93 to 0.96) and 0.99 (95% CI, 0.97 to 1.00) and negative predictive values of 0.94 (0.90 to 0.96) and 0.98 (0.89 to 1.0), respectively, for a diagnosis of acute heart failure. Therefore, the use of NPs is recommended for ruling-out HF, but not to establish the diagnosis i.e. for the rule-in diagnostic algorhytmus of HF.

There are numerous cardiovascular and non-cardiovascular causes of elevated NPs that may weaken their diagnostic utility in HF. Among them atrial fibrillation, age and renal failure are the most important factors impairing the interpretation of NP measurements. (44) On the other hand, NP levels may be disproportionally low in obese patients. (51)

Neprilysin inhibitor-Sacubitril-as part of ARNI

As mentioned neprilysin, also called neutral endopeptidase (NEP), is an enzyme responsible for degrading the ANP and BNP molecule, resulting in its rapid clearance. This yields a short half-life of ANP in human plasma (2 min).

A new therapeutic class of agents acting on the RAAS and the neprilysin has been developed. They are called angiotensin receptor/neprilysin inhibitor (ARNI).

The first medication in class is LCZ696 agent (52), registered since 2015 as Entresto® (EPAR summary for the public; Entresto EMA/782147/2015; European Medicines Agency, 2015). Entresto® is a molecule that combines the portions of valsartan (angiotensin-1(AT1)-receptor inhibitor) and sacubitril (neprilysin inhibitor) in a single substance. By inhibiting neprilysin, the degradation of NPs, bradykinin and other peptides is hindered.

High circulating levels of ANP and BNP exert physiologic effects through binding to NP receptors and the augmented generation of cGMP, thereby enhancing diuresis, natriuresis and myocardial relaxation and anti-remodeling. ANP and BNP also inhibit renin and aldosterone secretion. Selective AT1-receptor blockade reduces vasoconstriction, sodium and water retention and myocardial hypertrophy. (52, 53, 54)

PARADIGM-HF trial investigated the long-term effects of sacubitril/valsartan compared with an ACE inhibitor (enalapril) on morbidity and mortality in patients with ambulatory, symptomatic HFrEF with LVEF ≤35%, elevated plasma NP levels (BNP ≥150 pg/mL or NT-proBNP ≥600 pg/mL or, if they had been hospitalized for HF within the previous 12 months, BNP ≥100 pg/mL or NT-proBNP ≥400 pg/mL), and an estimated GFR (eGFR) ≥30 mL/min/1.73 m^2 of body surface area, who were able to tolerate separate treatments periods with enalapril (10 mg b.i.d.) and sacubitril/valsartan (97/103 mg b.i.d.) during a run-in period. (52)

In this population, sacubitril/valsartan (97/103 mg b.i.d.) was superior to ACEI (enalapril 10mg b.i.d.) in reducing hospitalizations for worsening HF, cardiovascular mortality and overall mortality. (52)

1.5 Clinical importance of mr-proANP/ANP as biomarker

As above described, the secretion of ANP occurs in response to atrial distension, hypervolemia, increased peripheral vascular resistance and raising levels of Angiotensin-II and other vasoconstrictors. (55) However, ANP is released in the ventricles as well, in response to hemodynamic stress induced by increased afterload (aortic stenosis, hypertension) or myocardial injury (myocardial infarction). ACS leads to impairment of myocardial relaxation and contractility, to myocardial wall stress, further to changes in systolic and diastolic function, thereby triggering the immediate secretion of natriuretic peptides (both ANP and BNP). The ANP secretion arises as an instant response to transient myocardial ischemia, even before myocardial necrosis in ACS occurs, and reaches its peek 3.7±0.4 hours after onset of symptoms. (56)

Data from previous studies demonstrated that plasma levels of ANP in patients with congestive heart failure and acute myocardial infarction are strong predictors of mortality, regardless of troponin levels, extent of kidney dysfunction or left ventricular performance. (57-63)

Data from the Leicester Acute Myocardial Infarction Peptide (LAMP) Study suggested that plasma mr-proANP concentrations, measured 3-5 days after the onset of ischemic symptoms, have strong prognostic potential comparable to that of plasma N-terminal proBNP (NT-proBNP). (60) Furthermore, a subgroup analysis of the APACE study demonstrated strong prediction value of mr-proANP for 360-day mortality/AMI, linearly correlating with mr-proANP quartiles (61). In addition, this study put forward the idea of utilization of mr-proANP together with hs-cTn in early rule-out of AMI. Although, the study claimed no significant diagnostic merit of mr-proANP regarding AMI diagnostics, it had several limitations and failed to demonstrate the diagnostic yield of mr-proANP for rapid exclusion of NSTE-ACS.

1.6 Clinical importance of cardiac troponin as biomarker

Cardiac troponin I (cTnI) and T (cTnT) compose the contractile structure of the myocardial cells and can be found almost exclusively in myocardium. (64-66) The rise of cTnI levels in blood are unknown to happen following the injury of non-cardiac tissues. Increase in the values of cTnT rarely occurs in skeletal muscle injury. Due to its fairly high specificity for myocardial cells and favorable sensitivity for the myocardial injury, cTnI and cTnT are the preferred biomarkers and the golden standard for the evaluation (44, 65-67) and diagnostic of MI. In terms of diagnosing a MI, other biomarkers like (CK-MB) provide both, less sensitivity and specificity. (68)

Myocardial injury is defined as being present when blood levels of cTn are increased above the 99th percentile upper reference limit (URL). (44, 65-67) The injury may be acute, as evidenced by the dynamic rising and/or falling pattern of cTn values above the 99th percentile URL, or chronic, in the setting of persistently elevated cTn levels.

Although elevated cTn values reflect injury to myocardial cells, they do not indicate the underlying pathophysiological mechanisms of the injury and can be detected in the normal heart after a preload-induced mechanical stretch or physiological stress. (69-71) However, regardless of the mechanism, acute myocardial injury, when associated with a rising and/or falling pattern of cTn values with at least one above the 99th percentile URL and caused by myocardial ischemia, is designated as an acute MI. (44, 65-67)

The myocardial injury with myocyte death occurs also in clinical conditions associated with non-ischemic mechanisms of myocardial injury. These conditions can be cardiac: heart failure, myocarditis, cardiomyopathy, Takotsubo syndrome, catheter ablation, defibrillator shocks, and cardiac contusion and systematic: sepsis, infectious disease, chronic kidney disease, stroke, subarachnoid hemorrhage, pulmonary embolism, pulmonary hypertension, infiltrative diseases (amyloidosis, sarcoidosis, etc.), chemotherapeutic agents, critically ill patients.

Major shortcoming of cardiac troponin is its incapability to generally detect an Acute Coronary Syndrome (ACS) in the early hours after symptom onset, due to the delayed increase of its circulating levels after the actual ACS occurrence (‘silent Troponin time’ phenomenon). (72)

1.7 Current Biomarker Algorithms in Diagnosis of NSTE-ACS

The ESC guidelines (73, 74) as well as the Biomarker Study Group of the ECS (75-77) suggested the new algorithms for NSTE-ACS diagnosis, recommending high-sensitivity assays over less sensitive ones based on the ratio of the higher sensitivity and diagnostic accuracy of these assays for the detection of acute MI at presentation. Hence, the time interval to the second cardiac troponin assessment in patients with baseline values under the cutoff or under LoD can be shortened when using these assays. This seems to substantially reduce the delay to diagnosis, translating into shorter stays in the emergency department and lower costs. (78-81) Two rule-out algorithms are suggested, depending of the assay being used. It is recommended to use the 0 h/1 h algorithm (best option, blood draw at 0 h and 1 h) or the 0 h/2 h algorithm (second best option, blood draw at 0 h and 2 h). These have been derived and well-validated in large multi-center diagnostic studies using central adjudication of the final diagnosis for all currently available hs-cTn assays. (82-87)

They are based on the results of several large validation cohorts (75, 76, 88-96), which demonstrated NPV for NSTE-ACS that exceeded 98%. Optimal thresholds for rule-in were selected to allow for a minimal positive predictive value (PPV) of 70%. According to ESC guidelines, as an alternative, 0 h/3 h algorithm should be considered. (97) Furthermore, in daily clinical routine the algorithm with additional 6h-retesting (0h/3h/6h), recommended when conventional troponin assays are used, is being widely utilized. (75)

Aside from abovementioned caveats in utilization of assays of the highest sensitivity ('early presenters' and 'late raisers') these new algorithms are assay-specific, as well as their proposed cut-offs and delta values.

1.8 The Dual Biomarker Concept

Thus far, copeptin is the only biomarker proven to be suitable for routine clinical utilization as a part of a dual-biomarker strategy in NSTE-ACS rule-out. Similar to mr-proANP, copeptin is an unspecific, high-sensitive biomarker of a low-specificity that unlike mr-pro-ANP arises in the brain instead the heart. (98) The dual biomarker strategy using either copeptin (99) or, as in our case, mr-proANP in addition to cardiac troponin may enable a timely NSTE-ACS rule-out and simultaneously limit itself to only one blood draw at admission. It is, however, not clear to date whether copeptin or mr-pro-ANP perform better in association with cTn in order to rule-out an underlying NSTE-ACS at the earliest convenience. Further studies directly comparing the diagnostic yield of both biomarkers in combination with cardiac troponin are required.

2. Methods

2.1. Study population

Patients who presented to the emergency room (ER) with symptoms suggestive of an ACS were recruited in the study. The study was single-center and was conducted in Wilhelminen Hospital of the City of Vienna. These symptoms were classified as: chest pain, dyspnea and other atypical or mixed symptoms that might be caused by an ACS. Atypical symptoms were: epigastric pain, nausea, palpitations, inner anxiety and cold sweat. Mixed symptoms were defined as combination of symptoms were chest pain or dyspnea were associated with atypical symptoms whereby a clear leading symptom couldn't have been differentiated.

Patients with a life expectancy below six months or presenting in cardiogenic shock were not eligible for participation in this study. Furthermore, patients admitted with STE-ACS were excluded from the analysis, as the primary diagnosis of these patients is based on the findings of the electrocardiogram (ECG) instead of biomarker assessments.

The emergency room of Wilhelminen Hospital has approximately 80,000 cases each year which makes this ER a high-volume one.

2.2. Study procedures

Patients were recruited early after admission and blood was drawn at initial presentation to the ER. Blood samples were processed timely and processing steps and times were documented in a standardized protocol. Blood was processed on site and biomarker measurements were performed immediately. After measurement, the blood was stored in a blood bank by processing on ice and then frozen in aliquots at -80°C. The recruitment of the patients in the study occurred from January till November 2013.

2.3. Biomarker testing

Mr-proANP was measured directly in EDTA plasma using the mr-proANP sandwich immunoassay (B.R.A.H.M.S. SERISTRA®) on the Kryptor device (Thermofisher Scientific, Berlin, Germany). This assay has a limit of detection (LOD) of 2.1 pmol/L. The median mr-proANP value of a normal reference population is 46.1 pmol/L and the 97.5th percentile is 85.2 pmol/L according to manufacturer's information. The functional assay sensitivity (20% coefficient of variation) is below 10 pmol/L and the limit of quantification (LOQ) is 4.5 pmol/L. Troponin I was measured using the high-sensitive troponin

I assay of the penultimate generation (Siemens Dimension EXL) with the 99th percentile of a normal reference population at 56 ng/L, 10% coefficient of variation and the LOQ at 17 ng/L.
As the troponin I assays of the highest sensitivity i.e. ultimate generation (e.g. hs-cTnI Abbott Architect or hs-cTnI Siemens Dimension Vista) already exist, in purpose of differentiating the assay generations, we referred to the assay used in this study as the sensitive cardiac troponin I assay (s-cTnI). The assay used in our study was approved by the US Food and Drug Administration in 2013 (Clearance Number K130276).
Although the sensitive and high-sensitive assays are to great extent established as the standard in Europe, outside Europe and particularly in USA, conventional troponin assays are still being widely utilized in clinical practice. Furthermore, nearly no point-of-care cTn assays meet the criteria for s-cTn or hs-cTn, respectively. (95)

2.4. Endpoints

The primary endpoint of this analysis was the rule-out of NSTE-ACS, both type-1 and type-2, in a cohort of patient presenting with symptoms suggestive of an ACS in the ER. The diagnosis of type-1 and type-2 infarction was based on the Third and Fourth Definition of Myocardial Infarction. (37, 41) In addition, the diagnosis of type-1 infarction was based on the gold standard diagnostic procedure i.e. coronary angiography. Patients without coronary-related myocardial necrosis i.e. with type-2 infarction were differentially diagnosed by standard clinical evaluation for other, none-coronary, reasons that led to s-cTnI increase.

2.5. Statistics

Categorical variables are shown as relative (%) and absolute (n) frequencies, numeric variables as median and inter quartile ranges (IQR). Statistical significance of categorical variables was computed by Chi-Square test, for numeric variables non-parametric Mann-Whitney test was used for comparison of two groups and Kruskal-Wallis test for more than two groups due to skewed distribution. A p-value of below 0.05 was considered statistically significant. The overall discriminatory ability of biomarkers was assessed by the area under the receiver operating characteristics curve (AUROC).
Sensitivity and specificity were calculated at predefined cut-off values: for mr-proANP, the 97.5th percentile (85.2 pmol/L) and for the troponin assay the 99th percentile of a healthy reference population (56.0 ng/L for s-cTnI) was used. As a measure of uncertainty, 95% confidence Intervals (CI) were calculated when appropriate. All computations were performed in SPSS (Statistical Package for Social Science; IBM).

2.6. Data processing

All data were stored and processed electronically. To verify accuracy of the data, it underwent the range, validity and consistency checks. Implausible or missing data were corrected or added after consulting the hospital documents and attending physicians. Documentation for these corrections was stored with the CRFs. All validated data were stored in the eCRF. After termination of the study and after completion of all entries, the database was closed (frozen) for further entries. This process has been documented.

All patient data were saved under a pseudonym. The study software automatically generated a pseudonym for every new patient. The pseudonym for new patients could be typed into the study software. A document regarding a precise patient allocation was printed and kept in a safe place. The author had the main role in recruiting of the patients. Every patient signed a consent form document.

2.7. Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethic Committee of the City of Vienna (EK 13-161-VK).

2.8. Scientific hypothesis

In our prospective, single-center cohort study, we hypothesized the beneficiary effect of a dual biomarker concept: mr-proANP, a biomarker with raising levels within the scope of myocardial ischemia and cTnI, a well-established biomarker of myocardial necrosis, diagnostically less conclusive in patients presenting to the emergency room early after onset of symptoms. We intended to utilize this combined measurement for early exclusion of NSTE-ACS, i.e. timely, prompt, reliable rule-out of NSTE-ACS.

3. Results

3.1. Patients' characteristics

In total, 311 consecutive patients were included in the study. The median age of the study population was 63 years (IQR: 24.9). The majority of patients was female (n=171, 54.9%). Women had higher median value of mr-proANP 98.2 pmol/l (IQR: 130.5) than men with mr-proANP median value of 78.11 pmol/l (IQR: 99.6). Nevertheless, there was no significant difference between the gender (p=0.91). (Figure 1)

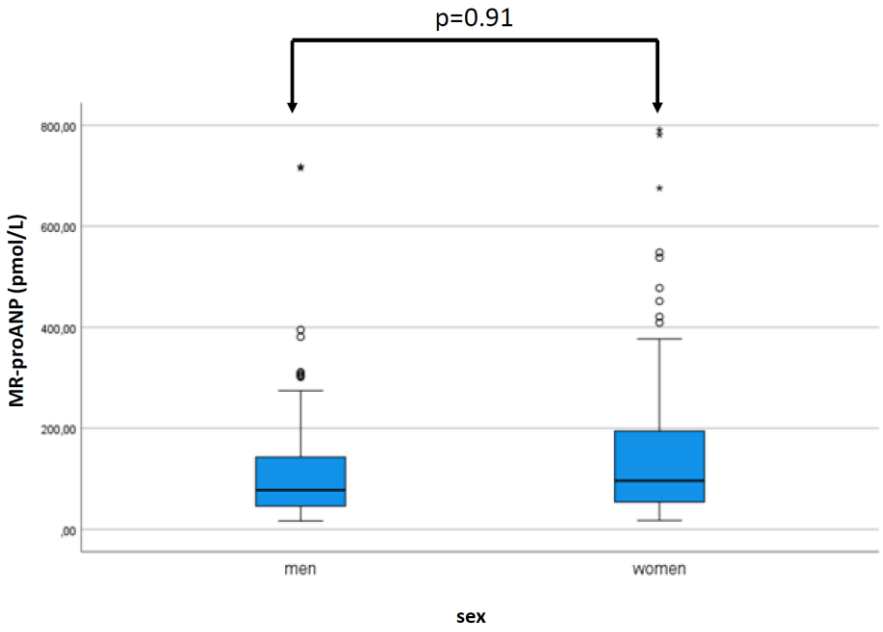


Figure 1: Boxplot representing the mr-proANP values of patients according to sex

There was a strong and significant correlation between the increasing age of the patients and increasing mr-proANP levels. The coefficient of correlation (Spearman) between mr-proANP and age was significant at 0.637 (p<0.0001). Nevertheless, the stratification of the mr-proANP levels depending on sex and age demonstrated no significant difference within the age/sex groups. (Table 1)

	Age group							
	< 40 years		**41-60 years**		**61-80 years**		**> 80 years**	
sex	male	female	male	female	male	female	male	female
n	12	23	43	75	48	56	37	17
Median (pmol/L)	41.9	49.2	58.8	48.9	96.1	112.9	231.3	157.1
IQR (pmol/L)	53.8	38.5	74.4	95.8	156.9	100.8	182.5	155.0
p value	0.85		0.92		0.77		0.47	

Table 1: Midregional proANP in study patients stratified by age and gender

For detailed information on patient characteristics allocated according to mr-proANP quartiles, see Table 2.

	All patients n=311	**mr-proANP 1st Quartile n=79**	**mr-proANP 2nd Quartile n=77**	**mr-proANP 3rd Quartile n=78**	**mr-proANP 4th Quartile n=77**	**p value**
Male sex, n (%)	140 (45.1%)	28 (35.4%)	37 (48.5%)	33 (42.3%)	42 (54.5%)	0.154
Age, years (median, IQR)	63.0 (24.9)	48.6 (62.9)	55.9 (42.0)	68.1 (64.8)	77.5 (69.1)	<0.0001
Risk factors						
Diabetes mellitus, n (%)	76 (24.4%)	11 (13.9%)	16 (20.8%)	21 (26.9%)	28 (36.4%)	0.062
Hypertension, n (%)	221 (71.1%)	37 (46.8%)	49 (63.6%)	69 (88.5%)	66 (85.7%)	<0.001

Hyperlipidemia, n (%)	137 (44.1%)	21 (26.6%)	35 (45.4%)	39 (50.0%)	42 (54.5%)	0.836
Family history of CAD, n (%)	43 (13.8%)	17 (21.5%)	17 (22.1%)	6 (7.7%)	3 (3.9%)	<0.001
Smoking, n (%)	113 (36.3%)	32 (40.5%)	35 (45.4%)	27 (34.6%)	19 (24.7%)	0.106
Vital signs						
BMI, kg/m^2 (median, IQR)	27.7 (7)	29.2 (22.0)	27.3 (20.9)	26.3 (21.1)	27.7 (19.1)	0.042
systolic BP, in mmHg	146 (32)	148 (67)	137.5 (95)	150 (113)	152 (145)	0.29
diastolic BP, in mmHg	78 (20)	83 (39)	76 (40)	76 (56)	78 (78)	0.65
Heart rate, bpm	74 (23)	75 (40)	72 (41)	68 (52)	83 (105)	<0.001
Medical History						
Heart failure, n (%)	37 (11.9%)	3 (3.8%)	5 (6.5%)	11 (14.1%)	18 (23.4%)	<0.001
PAD, n (%)	26 (8.4%)	5 (6.3%)	4 (5.2%)	7 (8.9%)	10 (12.9%)	0.195
TIA/Stroke, n (%)	22 (7.1%)	3 (3.8%)	3 (3.9%)	8 (10.3%)	8 (10.4%)	0.132
Renal disease, n (%)	23 (7.4%)	1 (1.3%)	2 (2.6%)	5 (6.4%)	15 (19.5%)	<0.001
CAD, n (%)	107 (34.4%)	16 (20.2%)	20 (25.9%)	31 (39.7%)	40 (51.9%)	<0.001
Prior MI, n (%)	63 (20.2%)	8 (10.1%)	11 (14.3%)	19 (24.3%)	25 (32.4%)	0.002
GRACE score (median, IQR)	95 (54)	70 (47)	82 (30)	108 (32)	131 (51)	<0.001
Procedures						
prior CABG, n (%)	13 (4.2%)	1 (1.3%)	3 (3.9%)	2 (2.6%)	7 (9.1%)	0.309
prior PCI, n (%)	79 (25.4%)	12 (15.2%)	13 (16.9%)	27 (34.6%)	27 (35.1%)	0.005

Table 2: Patients' characteristics in subgroups based on the initial mr-proANP values (quartiles)

Regarding the differences in mr-proANP levels depending on the medication that patients had as permanent home treatment there was no significance between the quartile-subgroups of the mr-proANP. For detailed information on patients' medication at time of presention to ER allocated according to mr-proANP quartiles, see Table 3.

Medication at presention	**Frequency (n/%)**	**mr-proANP 1st Quartile**	**mr-proANP 2st Quartile**	**mr-proANP 3st Quartile**	**mr-proANP 4st Quartile**	**p value**
Aspirin, n (%)	107 (34.4%)	32 (29.9%)	20 (18.7%)	26 (24.3%)	29 (27.1%)	0.47
$P2Y_{12}$-inhibitors, n (%)	32 (10.3%)	11 (34.4%)	6 (18.8%)	5 (15.6%)	10 (31.2%)	0.34
Beta-blocker, n (%)	125 (40.2%)	32 (25.6%)	29 (23.2%)	32 (25.6%)	32 (25.6%)	0.96
ACE inhibitors/AT_1-antagonists, n (%)	148 (47.6%)	35 (23.6%)	38 (25.7%)	39 (26.3%)	36 (24.4%)	0.97
Aldosteron-antagonists, n (%)	7 (2.3%)	3 (42.8%)	0	1 (14.4%)	3 (42.8%)	0.44
Calcium channel blockers, n (%)	71 (22.8%)	20 (28.2%)	15 (21.1%)	21 (29.6%)	15 (21.1%)	0.71
Amiodaron, n (%)	5 (1.6%)	0	1 (20.0%)	1 (20.0%)	3 (60%)	0.43
Loop diuretics, n (%)	34 (10.9%)	12 (35.3%)	4 (11.7%)	9 (26.5%)	9 (26.5%)	0.24
Thiazide, n (%)	66 (21.2%)	22 (33.3%)	12 (18.3%)	16 (24.2%)	16 (24.2%)	0.42
Oral anticoagulants, n (%)	20 (6.4%)	5 (25.0%)	3 (15.0%)	4 (20.0%)	8 (40.0%)	0.76
Oral antidiabetics, n (%)	51 (16.4%)	17 (33.3%)	9 (17.7%)	17 (33.3%)	8 (15.7%)	0.21

Table 3: Diagnoses of patients' medication at time of presention allocated according to mr-proANP values (quartiles)

Of the total patient cohort, n=55/311 (17.6%) had increased levels of s-cTnI either already at presentation (n = 42) or 3 h after presentation (n = 13). Thirty patients (9.6% of the cohort) had an angiographically confirmed diagnosis of type-1 infarction (all of them received PCI and stent implantation) and n=25 (8.0%) had type-2 infarction as confirmed by coronary angiography (n = 21) or clinical evaluation only (n=4). Causes for type-2 NSTE-ACS were acute heart failure (n=9), arrhythmias (n = 4), pulmonary embolism (n = 2), myocarditis (n = 2), hypertensive crisis (n = 1), perimyocarditis (n = 1) and other cardiac conditions (n = 6), respectively.

	All patients **n=311**	**mr-proANP** **< 97.5th percentile** **n=160**	**mr-proANP** **≥ 97.5th percentile** **n=151**	**p value**
Chronic CAD, n (%)	107 (34.4%)	36 (22.5%)	71 (47.0%)	<0.0001
NSTE-ACS type-1, n (%)	30 (9.6%)	10 (6.2%)	20 (13.2%)	0.068
NSTE-ACS type-2, n (%)	25 (8%)	6 (3.7%)	19 (12.6%)	0.09
Acute heart failure, (n) %	18 (5.8%)	3 (1.8%)	15 (9.9%)	<0.0001
Arrhythmia, n (%)	43 (13.8%)	14 (8.7%)	29 (19.2%)	<0.001
Hypertensive crisis, n (%)	30 (9.6%)	15 (9.4%)	15 (9.4%)	*ns*
COPD/asthma, n (%)	26 (8.4%)	11 (6.8%)	15 (9.9%)	*ns*
Non-cardiac chest pain, n (%)	118 (37.9%)	84 (52.5%)	34(22.5%)	<0.0001
Non-cardiac disease, n (%)	214 (68.8%)	126 (78.7%)	88 (58.3%)	<0.001

Table 4: Diagnoses of all patients stratified by mr-proANP value. The cut-off for positive and negative classification is the 97.5th percentile of the mr-proANP assay (85.2 pmol/l; BRAHMS Thermofisher Scientific).

The most frequent diagnoses in the total study cohort were non-cardiac chest pain (n=118, 37.9%), arrhythmia (n=43, 13.8%), hypertensive crisis (n=30, 9.6%), COPD/asthma (n=26, 8.4%), type-1 NSTE-ACS (n=30, 9.6%), and type-2 NSTE-ACS (n=25, 8.0%), respectively.

In the entire patient cohort, 259 (83.3%) patients presented to the ER with characteristic chest pain, 20 (6.4%) had dyspnea only and 9 (2.9%) had atypical symptoms and 23 (7.4%) mixed symptoms. Median value for symptom onset time (SOT) until blood collection was 151 min (IQR: 336.3). The very early presenters' group i.e. patients presenting within 2 h after symptom onset represented (n=68/311) 21.9% of the overall study population; (n=243/311) 78.1% presented ≥ 2 h after symptom onset, of which (n=44/243) 18.1% presented between 3 and 4 h, (n=19/243) 7.8% between 5 and 6 h, and (n=180/243) 74.1% between 7 and 24 h after symptom onset.

3.2. Biomarkers

In this study population the median mr-proANP-value was 82.1 pmol/l (IQR: 113.8). Patients with type-1 NSTE-ACS had a median mr-proANP value of 174.1 pmol/l (IQR: 240.8) and for patients with type-2 NSTE-ACS median mr-proANP value was at 226.2 pmol/L (IQR: 349). Mr-proANP levels in both NSTE-ACS types were higher compared to patients with other diagnosis (73.6 pmol/l; IQR: 91 pmol/l; p=0.02 and p<0.001; **Figure 2**). There was no significant difference between the mr-proANP levels in patients with NSTE-ACS type-1 and type 2 (p=0.28). Half of the patient cohort (n=151, 48.6%) had initially elevated mr-proANP-values above the 97.5th percentile of a normal reference population. Of these patients, 20/151 (13.2%) had a final diagnosis of type-1 infarction and 19/151 (12.6%) the final diagnosis of type-2. The frequency of other diagnoses in all patients and stratified by the initial mr-proANP test result are illustrated in **Table 4**.

The initial troponin I value was above the 99th percentile of a healthy reference population in 42/311 (13.5%) patients. After the second Troponin measurement performed 3 hours after the initial, additional 13 patient had hs-cTnI levels above the 99th percentile.

The proportion of patients with initial s-cTnI levels above the cutoff was comparable in patients with type-1 NSTE-ACS (n=22; 73.3%) and type-2 NSTE-ACS (n=20; 80%) (p=0.508). Furthermore, the portion of the patients with mr-proANP levels above cutoff was significantly higher in type-1 NSTE-ACS (n=20; 66.7% vs. n=112; 43.8%, p=0.04) and type-2 NSTE-ACS patients (n=19; 76% vs. n=112; 43.8%, p=0.037) than in patient with other diagnoses (n=112; 43.8%). The frequency of other diagnoses stratified by the initial Troponin I and mr-proANP values are illustrated in **Table 5.**

In patients with initial s-cTnI values below the 99th percentile of a healthy reference population, the prevalence of type-1 NSTE-ACS was 8/256 (3.1%) and 5/256 (1.9%) for type-2 NSTE-ACS. In this subgroup of initially s-cTnI negative patients with type-1 NSTE-ACS, 7/8 (87.5%) patients had elevated mr-proANP values at admission and only one patient with type-1 infarction was tested false negative for both biomarkers. In the type-2 NSTE-ACS subgroup 3/5 (60%) patients had elevated mr-proANP levels and 2/5 (40%) patients were false negative for the biomarker combination.

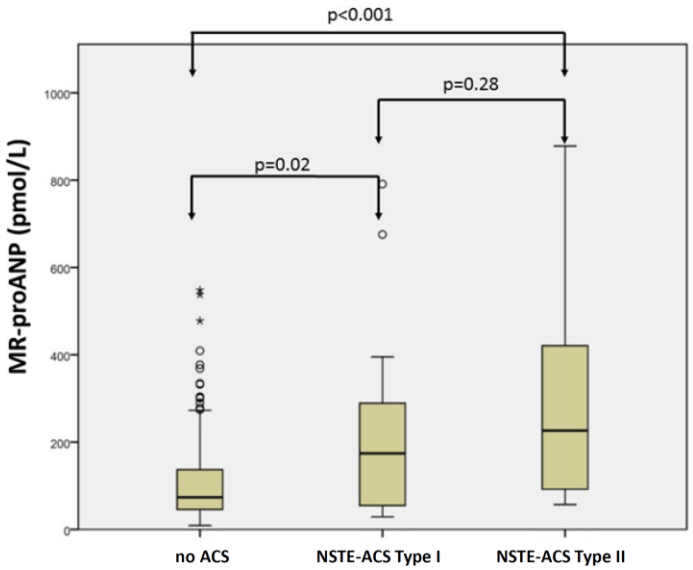


Figure 2: Boxplot representing the mr-proANP values of patients with type-1 and type-2 NSTE-ACS as compared to patients with other diagnoses in the study population.

	All patients n=311	**mr-proANP < 97.5th percentile & Troponin < 99th n=147**	**mr-proANP < 97.5th percentile & Troponin > 99th n=13**	**mr-proANP > 97.5th percentile & Troponin < 99th n=122**	**mr-proANP > 97.5th percentile & Troponin >99th n=29**
NSTE-ACS type-1, n (%)	30 (9.6%)	1 (0.7%)	9 (69.2%)	7 (5.7%)	13 (44.8%)
NSTE-ACS type-2, n (%)	25 (8%)	2 (1.4%)	4 (30.1%)	3 (2.4%)	16 (55.2%)
Acute heart failure, n (%)	9 (2.9%)	2 (1.4%)	0	7 (5.7%)	0
Arrhythmia, n (%)	43 (13.8%)	14 (9.5%)	0	27 (22.1%)	2 (6.9%)
Hypertensive crisis, n (%)	30 (9.6%)	15 (10.2%)	0	15 (12.3%)	0
COPD/asthma, n (%)	26 (8.4%)	11 (7.5%)	0	15 (12.3%)	0
Non-cardiac chest pain, n (%)	118 (37.9%)	84 (57.1%)	0	34 (27.9%)	0
Non-cardiac disease, n (%)	214 (68.8%)	126 (85.7%)	0	88 (72.1%)	0

Table 5: Diagnoses of all patients stratified by mr-proANP and troponin I values. The cut-off for positive and negative classification is the 97.5th percentile of the mr-proANP assay (85.2 pmol/l; BRAHMS Thermofisher Scientific) and 99th percentile of troponin I assay (56ng/l; Siemens Dimension EXL).

The median GRACE score for the study population was 95 (IQR: 53), for type-1 NSTE-ACS 109 (IQR: 46) and for type-2 NSTE-ACS 140 (IQR: 85). Mr-proANP levels correlated with an increasing GRACE score: patients in the 4th quartile of mr-proANP (>161 pmol/L) exhibited a median GRACE score of 129 (IQR: 46) and were at an intermediate to high-risk for in-hospital death and 6-month death post-discharge. The coefficient of correlation (Spearman) between mr-proANP and the GRACE score was significant at 0.626 (p<0.001). (**Figure 3**)

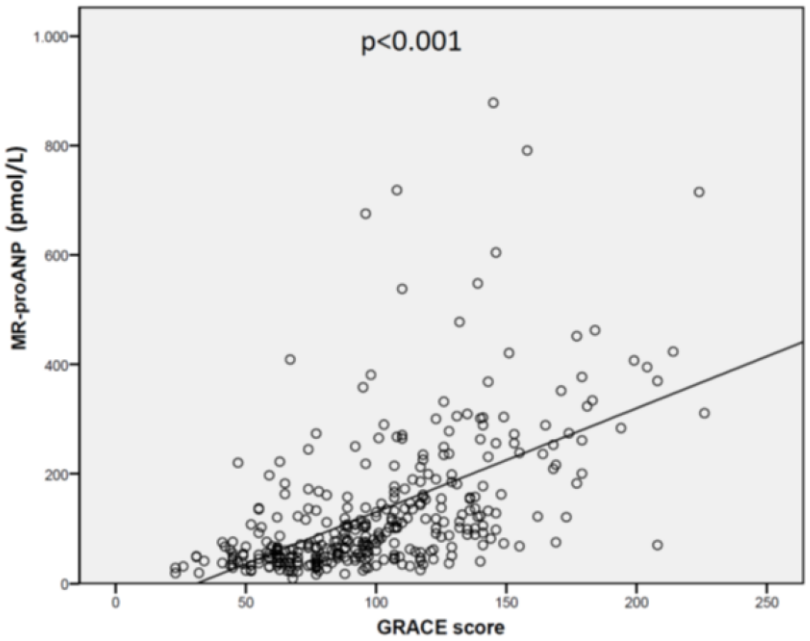


Figure 3: Dot-plot demonstrating the correlation between mr-proANP levels and GRACE Score in overall study population

In relation to renal function, mr-proANP levels demonstrated strong correlation with increased levels of creatinine i.e. the increased impairment of the renal function was closely associated with higher mr-proANP levels. This interrelation was also confirmed throughout the stages of the renal dysfunction according to KDIGO (Kidney Disease: Improving Global Outcomes (KDIGO)) classification. (**Figure 4**). There were no patients in the study cohort that had terminally impaired renal function i.e. GFR<15 ml/min, therefore there were no stage 5 (KDIGO) patients. Furthermore, the coefficient of correlation (Spearman) between mr-proANP and creatinine levels was significant at 0.261 (p<0.0001).

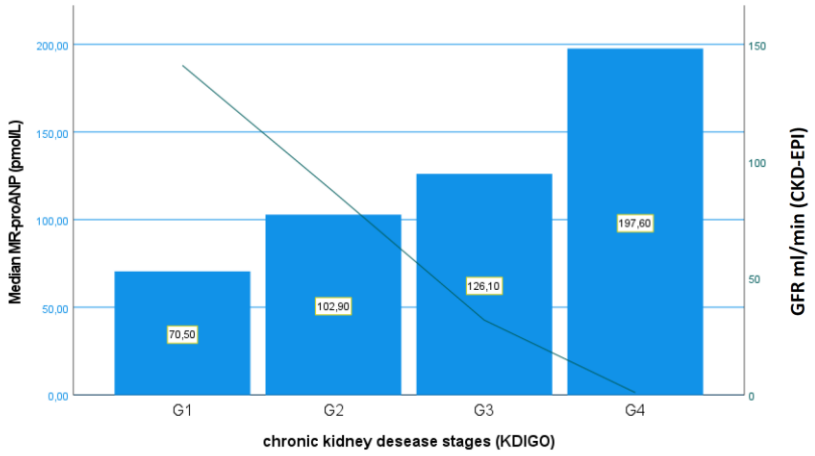


Figure 4: Mr-proANP median levels stratified according to KDIGO stages of renal dysfunction

In relation to SOT, release kinetic of mr-proANP in type-1 NSTE-ACS patients showed its immediate increase of >3.5-fold above cutoff within 2 h after symptom onset with a median value of 289.2 pmol/L (IQR: 66.8) and reached its peak at 5-6 h after symptom onset with a median of 369.7 pmol/L (IQR: 324.5). For patients with type-2 NSTE-ACS, mr-proANP demonstrated an immediate increase of over 3-fold above cutoff within 2 h SOT (median at 236.5; IQR: 277.6) and reached its peak at 3-4 h SOT with median at 392.6 pmol/L (IQR: 770.6). Patients without NSTE-ACS showed stable mr-proANP values at 73.6 pmol/L (IQR: 91.1) (**Figure 5**).

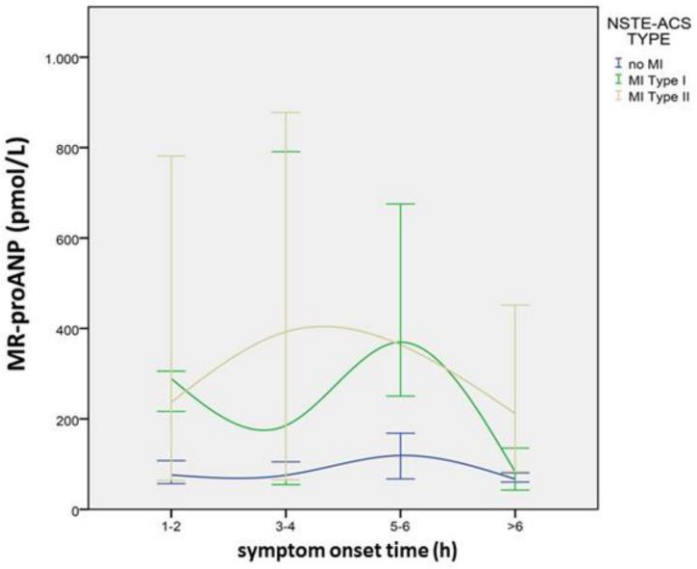


Figure 5: mr-proANP-levels in relation to symptom onset time stratified by patients with and without type-1 and type-2 NSTE-ACS

The area under the receiver operating characteristic (AUROC) curve of s-cTnI for the diagnosis of type-1 NSTE-ACS was 0.903 (95%-CI: 0.836–0.970) and 0.642 (95%-CI: 0.525–0.760) for mr-proANP. The combination of both, s-cTnI and mr-proANP, resulted in a lower c-statistic with an AUROC of 0.827 (95%-CI: 0.744–0.911) (**Figure 6**).

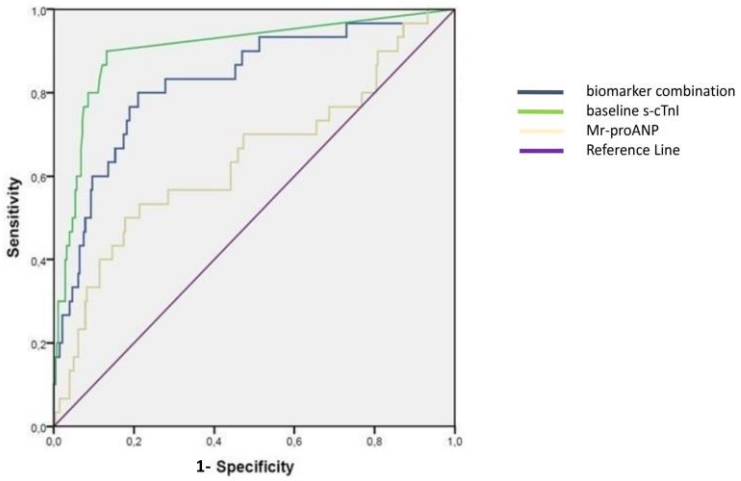


Figure 6: ROC-curves representing the overall discriminatory abilities of mr-proANP, sensitive-cardiac troponin I and the combination of both biomarkers for the identification of patients with type-1 NSTE-ACS

C-statistic for type-2 NSTE-ACS was comparable to type-1 NSTE-ACS and exhibited an AUROC for s-cTnI at 0.903 (95%-CI: 0.836–0.970) and for mr-proANP at 0.642 (95%-CI: 0.525–0.760), while the combination of both biomarkers resulted in an AUROC of 0.772 (95%-CI: 0.676–0.869) for the type-2 NSTE-ACS (**Figure 7**).

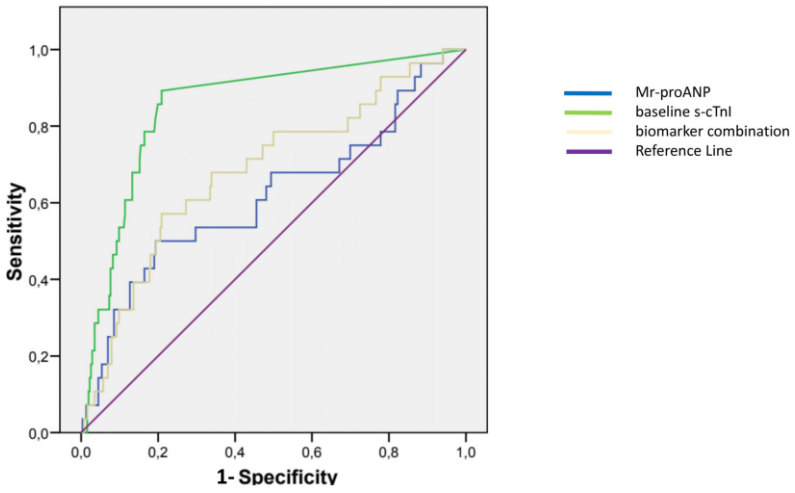
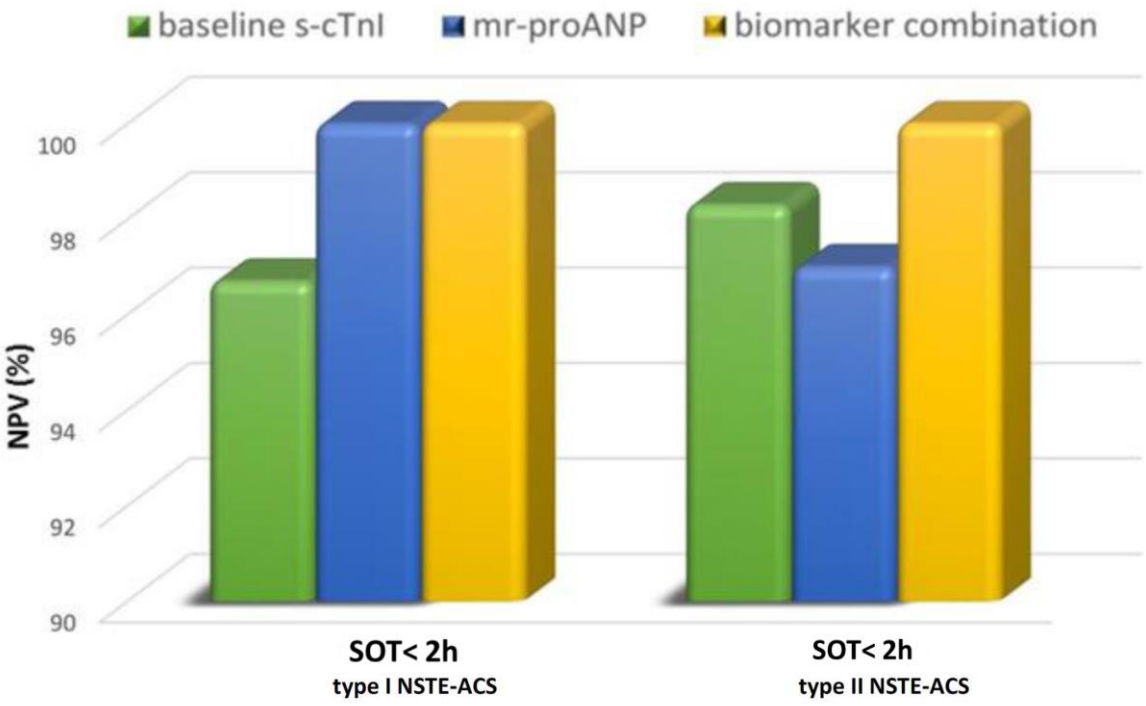


Figure 7: ROC-curves representing the overall discriminatory abilities of mr-proANP, sensitive-cardiac troponin I and the combination of both biomarkers for the identification of patients with type-2 NSTE-ACS

Regarding the diagnostic performance in the total study cohort for an early rule-out of type-1 NSTE-ACS, sensitivity and the negative predictive value (NPV) of s-cTnI increased in combination with mr-proANP. The NPV was 97.5% (95%-CI: 93.7–98.4) for s-cTnI and 94.4% (95%-CI: 93.7–98.4) for mr-proANP, respectively. The combination of both biomarkers resulted in NPV of 99.5% (95%-CI: 95.8–99.9) and only one patient with the final diagnosis of type-1 NSTE-ACS was false negative. Sensitivity of mr-proANP for type-1 NSTE-ACS was 82.9% with low specificity (39.3%), while sensitivity and specificity of the s-cTnI assay used in the analysis were high (86.3% and 83.9%, respectively). By combining both biomarkers, further increase of sensitivity up to 98.9% was achieved, thereby reflecting the different pathophysiological properties of both markers. Due to the low specificity of mr-proANP, the combined effect failed to yield a better diagnostic performance with a specificity for biomarker combination of only 37.5%. With respect to the diagnostic performance in the total study cohort for the early rule-out of type-2 NSTE-ACS, sensitivity and NPV increased slightly when the dual marker strategy was employed. The NPV for type-2 NSTE-ACS was at 98.1% (95%-CI: 95.5–99.3) for s-cTnI and 96.3% (95%-CI: 91.7– 98.5) for mr-proANP. The combination of both biomarkers led to a NPV of 98.6% (95-%: 94.6–99.8) with only two false negative-patients with final diagnosis of type-2 NSTE-ACS.

In the subgroup of very early presenters (SOT ≤ 2 h), the NPV of s-cTnI for type-1 NSTE-ACS was 96.7% (95%-CI: 87.5–99.4) and the NPV of mr-proANP was 100% (95%-CI: 87.1–100). The dual biomarker strategy yielded an NPV of 100% (95%-CI: 86.7–100) (**Figure 8, left panel**). Comparable to type-1 NSTE-ACS, the NPV of s-cTnI in patients presenting within 2 h from symptom onset was 98.3% (95%-CI: 89.8–99.9) for type-2 NSTE-ACS while the NPV of mr-proANP was 97.0% (95%-CI: 82.5–100). The combination of biomarker increased the NPV to 100% (95%-CI: 86.7–100) (**Figure 8, right panel**).



s-cTnI	96.7 (87.5-99.4)	98.3 (89.8-99.9)
mr-proANP	100 (87.1-100)	97 (82.5-99.8)
dual-biomarker	100 (86.7-100)	100 (86.7-100)

Figure 8: Negative predictive value (NPV) of the respective biomarkers for the diagnosis of type-1 and type-2 NSTE-ACS in the *very early presenters'* subgroup (SOT ≤ 2h)

As expected, the diagnostic accuracy for the rule out of type-1 NSTE-ACS increased in patients presenting <2h after symptom onset. NPV for the combination of the biomarker reached 100% in this subgroup, thereby increasing the NPV of s-cTnI for type-1 NSTE-ACS from 97.5% (95%-CI: 94.7–98.7). In contrast, the dual biomarker strategy did not significantly increase the rule-out efficacy for type-2 NSTE-ACS patients presenting longer than 2 h after onset of symptoms.

4. Scientific Yield and Clinical Applications

4.1 Scientific Yield

This prospective cohort study of 311 patients, presenting to the ER with symptoms suggestive of ACS, investigated the release pattern and clinical efficacy of mr-proANP, as part of a dual biomarker strategy, in addition to s-cTnI for the diagnosis of type-1 and type-2 NSTE-ACS. This concept increased the NPV of s-cTnI for type-1 and type-2 NSTE-ACS in very early presenters (SOT<2 h) to 100%. The dual biomarker strategy with mr-proANP and s-cTnI results in a very early rule-out of NSTE-ACS, regardless of the infarction type and thus helps accelerating the diagnostic procedure itself.

The study population was unselected, included patients with chest pain and/or dyspnea or atypical and mixed symptoms and was comparable to other ER populations. (36, 100)

In the analysis of the mr-proANP values in context of gender, this biomarker demonstrated no sex-related differences in its levels. (Figure 1) In relation to the mr-proANP levels and age, the study demonstrates a clear dependency between the high levels of mr-proANP and advanced age. This property of the biomarker is attributed to the comorbidities associated with advanced age with emphasis on chronic heart and renal failure as part of the complex cardiorenal syndrome. Furthermore, the study confirmed previous findings regarding the relation of mr-proANP levels and renal function. The strong correlation between increased levels of mr-proANP and increased impairment of the renal function was confirmed throughout all stages of the renal dysfunction according to KDIGO classification. (Figure 4)

Despite significant correlation between higher mr-proANP levels and age, this correlation weakened when compared in relation to sex-subgroups. The general property of mr-proANP to demonstrate no sex-related differences proved further on solid when tested through various age groups. (Table 1)

As shown in previous studies, the triggers for mr-proANP release include: atrial distension, increased preload/afterload and vasoconstriction. In NSTE-ACS, the acute ventricular dysfunction might cause hemodynamic impairment on atrial level thus provoking an immediate mr-proANP release that serves as an actual compensatory mechanism in order to improve ventricular performance by reducing pre- and afterload of the heart. (56)

As previously demonstrated, this mr-proANP-response occurs immediately (2 to 5 minutes) (4) after myocardial ischemia onset and reaches its peak 3.7±0.4 h after onset of symptoms (56) with a half-life of 40 to 60 minutes (4). This is of importance for patients with both type-1 and type-2 NSTE-ACS

presenting within 2 h after onset of symptoms when troponin levels measured by commercial, non-highest sensitivity assays still remain below cutoff (Figure 5). Such immediate increase of mr-proANP appears to be of special importance for the rule-out of an underlying NSTE-ACS and might as well help solving important caveats in the diagnostic performance of high sensitivity troponin assays of the ultimate generation: the issues of ”early presenters” or “late risers”. (73, 75, 76, 101)
Furthermore, there are currently no mr-proANP-assays available for point of care testing (POCT). Our study demonstrated that the properties of mr-proANP as biomarker are suitable for potential development and utilization of both POCT-assay and device.
The first study investigating the use of dual biomarker strategy in NSTE-ACS diagnostic with mr-proANP and troponin T (both sensitive and high-sensitive assays) claimed no diagnostic improvement when using mr-proANP (61). However, the investigators of this study did not address the rule-out strategy for NSTE-ACS in general or for the type-1 or type-2 NSTE-ACS, as they exclusively focused on the rule-in strategy by validating only the diagnostic accuracy for combination of mr-proANP and sensitive cTnT assays using c-statics.
It has been shown in previous studies that copeptin, in combination with cardiac troponin, is a biomarker suitable for clinical utilization as part of a dual biomarker strategy in NSTE-ACS rule-out. Similar to mr-proANP, copeptin is another biomarker of high sensitivity but low specificity that unlike mr-proANP arises in the brain instead the heart. (98) Our findings suggest that the combined use of mr-proANP and s-cTnI might be even superior to a dual biomarker strategy including copeptin for a quick rule-out in very early presenters.
Although, the high-sensitivity troponin I assay of the penultimate generation and not the highest-sensitivity assays of the ultimate generation was used, there is a rationale for testing the dual biomarker strategy using these generations of troponin assays: 1) the conventional and high-sensitive assays of penultimate generation are still widely used in Europe and almost exclusively in USA and 2) inability of hs-cTn assays of the ultimate generation to detect the subgroups of ‘early presenters’ and ‘late risers’.
Albeit the concept of employing the mr-proANP for a rule-out of NSTE-ACS in early presenters proved plausible, the study was conducted in a relatively small cohort of patients and therefore should be put to the test in further studies.

4.2 Clinical Applications

The current ESC guidelines (74), suggest several new algorithms for NSTE-ACS diagnosis, trying to overcome the shortcomings of the assays of the highest sensitivity ('early presenters' and 'late raisers'). Furthermore, these new algorithms and their cut-offs are specific for each of the hs-cTn assays. Regardless which of the assessment concepts is used it demands serial blood testing, therefore presenting the challenge for both patients and medical staff: additional consumption of personnel, resources, prolonged length of stay for the patients and unfavorable economic effects in already congested ERs.

Our prospective cohort analysis demonstrated a favorable immediate release pattern of mr-proANP in NSTE-ACS that might help to bridge the `silent troponin time` phenomenon when highest-sensitivity cardiac troponin assays are not used. The high sensitivity of mr-proANP for NSTE-ACS was reflected in a high NPV, which was even further improved by employing the dual biomarker strategy. This concept performed best in the subgroup of patients presenting very early, within 2 h of symptom onset (21.9% of the study population), with an excellent NPV = 100% for both type-1 and type-2 NSTE-ACS. Accordingly, this strategy using mr-proANP in addition to cardiac troponin might result in an early, rapid and reliable rule-out of NSTE-ACS thus accelerating the diagnostic work-up and shortening the length of stay for patients presenting in ER with symptoms attributable to ACS.

4.3 Limitations

There are several limitations of this study that need to be taken into consideration.

Primarily, this is a non-randomized, prospective, single-center cohort study and a potential bias regarding the patient selection cannot be excluded. Although our study cohort was unselected, in terms of patients' characteristics, it included the population with symptoms comparable to other published ER population studies.

By definition, cohort studies differ from clinical trials in that no intervention, treatment, or exposure is administered to participants in a cohort design and no control group is defined. In this study population, there were in total 68 patients with SOT under <2 hours and they were identified as 'early presenters'. Although the concept of employing the mr-proANP for a rule-out of NSTE-ACS in early presenters proved plausible, the study was conducted in a relatively small cohort and subgroups of patients and therefore should be put to the test in further, larger studies.

Furthermore, it was a single-center study with its issues embedded in the nature of this particular study design. On the other hand, the advantage of a single-center approach was reflected in a reduced variability regarding conduct of the study and data collection.

Importantly, we used the high-sensitivity troponin I assay of the penultimate generation and not the highest-sensitivity assays of the ultimate generation. Nevertheless, considering that conventional and high-sensitive assays of penultimate generation are still widely used in Europe and primarily in USA and regarding the hs-cTn assays' caveats of the ultimate generation in the ‘early presenters’ and ‘late risers’ subgroups, there is a rationale for testing the dual biomarker strategy using these generations of troponin assays.

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IV Eidesstattliche Versicherung

„Ich, Miloš Tajsić, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema:

Midregional Pro-A-Type Natriuretic Peptide as Part of a Dual Biomarker Strategy for the Early Rule Out of non-ST Segment Elevation Acute Coronary Syndrome

=

Mittregionales Pro-Atriales Natriuretisches Peptid als Teil einer dualen Biomarker-Strategie für den früheren Ausschluss des akuten Koronarsyndroms ohne ST-Strecken-Hebung

selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Betreuer, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Berlin, 29.05.2019 Unterschrift

Anteilserklärung an der erfolgten Publikation

Miloš Tajsić hatte folgenden Anteil an der folgenden Publikation:

Tajsic M, Járai R, Koch J, Stangl K, Wojta J, Dreger H, Huber K.
Midregional pro-A-type natriuretic peptide as part of a dual biomarker strategy for the early rule out of non-ST segment elevation acute coronary syndrome - The WilCop study.
Int J Cardiol. 2018

Beitrag im Einzelnen: Der Anteil von Miloš Tajsić an der Publikation bestand in der selbstständigen Erfassung der Daten sowie in der komplette statistische Auswertung der Ergebnisse und deren kritischen Analyse innerhalb der Diskussion mit PD Dr. med. Dreger und Prof. Dr. Huber. Darüber hinaus Miloš Tajsić verfasste, unter der Betreuung von PD Dr. Dreger sowie Prof. Dr. Huber, den kompletten Text der Publikation.

Berlin, 29.05.2019

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Unterschrift der promovierenden Person

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Unterschrift Erstbetreuer

V Auszug aus der Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI Selected Categories: **"CARDIAC and CARDIOVASCULAR SYSTEMS"** Selected Category Scheme: WoS

Gesamtanzahl: 128 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
24	Cardiovascular Diabetology	4,796	5.235	0.011190
25	EUROPACE	9,232	5.231	0.026860
26	REVISTA ESPANOLA DE CARDIOLOGIA	3,338	5.166	0.004720
27	Circulation-Cardiovascular Quality and Outcomes	4,337	5.036	0.016830
28	JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY	27,492	4.880	0.042650
29	HEART RHYTHM	11,723	4.743	0.033520
30	Circulation-Arrhythmia and Electrophysiology	6,026	4.712	0.021390
31	TRENDS IN CARDIOVASCULAR MEDICINE	2,588	4.598	0.004040
32	European Journal of Preventive Cardiology	3,478	4.542	0.013060
33	CANADIAN JOURNAL OF CARDIOLOGY	6,035	4.524	0.017810
34	ATHEROSCLEROSIS	23,013	4.467	0.039120
35	Clinical Research in Cardiology	2,789	4.455	0.007260
36	Journal of the American Heart Association	9,057	4.450	0.047030
37	EuroIntervention	5,742	4.417	0.019300
38	CURRENT PROBLEMS IN CARDIOLOGY	569	4.190	0.001040
39	AMERICAN HEART JOURNAL	21,762	4.171	0.035520
40	HEART FAILURE REVIEWS	2,288	4.104	0.005280
41	INTERNATIONAL JOURNAL OF CARDIOLOGY	27,788	4.034	0.077900
42	JOURNAL OF CARDIAC FAILURE	5,177	3.942	0.010430
43	JOURNAL OF NUCLEAR CARDIOLOGY	3,508	3.847	0.004120
44	ANNALS OF THORACIC SURGERY	34,006	3.779	0.043550
45	AMERICAN JOURNAL OF PHYSIOLOGY-HEART AND CIRCULATORY PHYSIOLOGY	28,039	3.569	0.027570
46	EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY	15,001	3.504	0.026110

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VI Publikation

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VII Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

VIII Publikationsliste

1. **Tajsic M**, Járai R, Koch J, Stangl K, Wojta J, Dreger H, Huber K.
Midregional pro-A-type natriuretic peptide as part of a dual biomarker strategy for the early rule out of non-ST segment elevation acute coronary syndrome - The WilCop study.
Int J Cardiol. 2018 Dec 15; 273:243-248.
doi: 10.1016/j.ijcard.2018.09.084. Epub 2018 Sep 26. PubMed PMID: 30282602.

2. Koehler F, Koehler K, Deckwart O, Prescher S, Wegscheider K, Kirwan BA, Winkler S, Vettorazzi E, Bruch L, Oeff M, Zugck C, Doerr G, Naegele H, Störk S, Butter C, Sechtem U, Angermann C, Gola G, Prondzinsky R, Edelmann F, Spethmann S, Schellong SM, Schulze PC, Bauersachs J, Wellge B, Schoebel C, **Tajsic M**, Dreger H, Anker SD, Stangl K.
Efficacy of telemedical interventional management in patients with heart failure (TIM-HF2): a randomised, controlled, parallel-group, unmasked trial.
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