Charles University in Prague
Third Faculty of Medicine

Doctoral dissertation

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The role of apoptosis in patients with coronary artery disease

Role apoptózy u pacientů s ischemickou chorobou srdeční

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Prague, 2018

Declaration:

Hereby I declare that this doctoral dissertation was written by me and all mentioned sources and literature were cited properly. This work was not used to obtain any other or same degree.

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Key words: apoptosis; coronary artery disease; acute myocardial infarction; heart failure

Klíčová slova: apoptóza, ischemická choroba srdeční, akutní infarkt myokardu, srdeční selhání

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List of abbreviations

ACS	acute coronary syndrome
AIF	apoptosis inducing factor
AMI	acute myocardial infarction
Apaf-1	apoptotic protein activating factor 1
BNP	B-type natriuretic peptide
CAD	coronary artery disease
CFR	coronary flow reserve
CMR	cardiac magnetic resonance imaging
DISC	death-inducing signaling complex
EDRF	endothelium-derived relaxing factor
EndoG	endonuclease G
Fas	apoptosis stimulating fragment
FasL	apoptosis stimulating fragment ligand
FFR	fractional flow reserve
HF	heart failure
hs-cTnT	high-sensitive cardiac troponin T
I/R	ischemia/reperfusion
IAPs	inhibitor of apoptosis proteins
LV	left ventricular
LVEF	left ventricular ejection fraction
MACE	major advert cardiovascular events
MMPs	matrix metalloproteinases
MI	myocardial infarction

MVO	microvascular obstruction
NO	nitrous oxide
NSTEMI	non-ST-elevation myocardial infarction
PCI	percutaneous coronary intervention
pPCI	primary percutaneous coronary intervention
ROS	reactive oxygen species
sFas	soluble apoptosis stimulating fragment
STEMI	ST-elevation myocardial infarction
TNF-α	tumor necrosis factor alpha
TNFR1	tumor necrosis factor receptor 1
TNFR2	tumor necrosis factor receptor 2
TRAIL	tumor necrosis factor-related apoptosis stimulating ligand
TRAIL-R1	tumor necrosis factor -related apoptosis stimulating ligand receptor 1
TRAIL-R2	tumor necrosis factor -related apoptosis stimulating ligand receptor 2
UA	unstable angina

Content

1. Introduction

Coronary artery disease (CAD) is a leading cause of death worldwide. Acute myocardial infarction (AMI) is the most common first manifestation of CAD and represents a major cause of morbidity and mortality [1]. In the past decades, treatment of patients with AMI has significantly improved. However, more patients subsequently suffer from left ventricular (LV) dysfunction and heart failure [2]. Post-AMI heart failure represents a high-risk condition with a poor long-term prognosis. Although necrosis was thought to be the sole cause of death in myocardial infarction for a long time, recent studies provide growing evidence that apoptosis plays an important role in the process of myocyte loss after AMI, as well as in the process of LV remodelling and development of heart failure [3, 4]. Recognizing a sensitive apoptotic marker that would help define high-risk patients after AMI and offer new therapeutic strategies is thus of a great importance. Inhibition of apoptosis through anti-apoptotic therapy could represent a new way forward to improve poor prognosis of these patients.

The aim of this dissertation is to discuss the role of known apoptotic markers in coronary artery disease and highlight their potential benefit in clinical practice and, in the second part, to clarify serum kinetics of two well-known apoptotic markers TRAIL and sFas and their relation to left ventricular ejection fraction and two-year prognosis in patients with ST-elevation myocardial infarction.

1.1. Definition and epidemiology of coronary artery disease

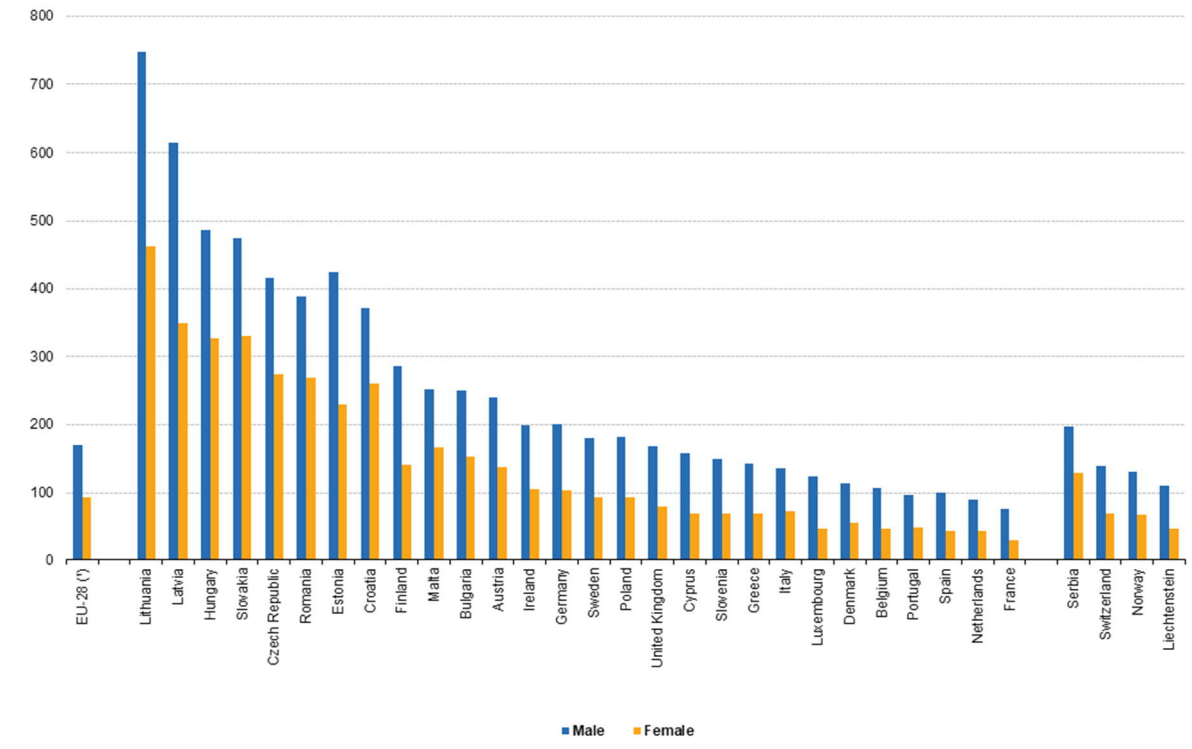
Coronary artery disease represents a group of clinical syndromes that are characterised by myocardial ischemia - an imbalance between myocardial oxygen supply and demand. The most common cause of CAD is coronary atherosclerosis. The manifestation of CAD depends on duration, severity and acuity of the ischemic periods. Acute forms of CAD include unstable angina, acute myocardial infarction and sudden cardiac death. Acute coronary syndrome (ACS) is a term used to describe acute myocardial infarction presenting with ST-elevation (STEMI) and ACS without ST-elevation: unstable angina (UA) and non-ST-elevation myocardial infarction (NSTEMI) [5]. Stable forms of CAD can be divided according to their clinical presentation to effort-induced angina, vasospastic angina, asymptomatic forms of CAD (silent ischemia and asymptomatic phases with history of ACS), and ischemic cardiomyopathy [6]. The underlying mechanisms are listed in Table 1.

Table 1. Stable forms of coronary artery disease – their clinical presentation and cause [6]

Stable forms of coronary artery disease	
Clinical presentation	**Cause**
Effort-induced angina	• Epicardial stenosis • Microvascular dysfunction • Vasoconstriction at the site of dynamic stenosis
Vasospastic angina	• Coronary vasospasm focal / diffuse
Asymptomatic	• Silent ischemia • Medical history of ACS
Ischemic cardiomyopathy	• Prior myocardial infarction • Hibernation

Clinical syndromes of coronary artery disease cause higher mortality, morbidity and also financial burden in developed countries than any other group of diseases. In 2015, CAD affected 110 million of people worldwide and resulted in 8.9 million deaths, what represents 15.9% of all deaths in 2015 [7]. CAD thus remains the leading cause of death globally. In European countries, CAD accounted for 19% of all deaths in 2015 [8]. In Czech Republic, mortality rates are above European average: 333 deaths from CAD per 100 000 inhabitants in Czech Republic while 126 deaths per 100 000 inhabitants in Europe (Figure 1) [9].

Figure 1. **Deaths from coronary artery disease in European countries** – standardized death rate (per 100 000 inhabitants) in 2014 [9]



Note: the figure is ranked on the average of male and female.
(*) For the age standardisation, among older people, the age group aged 85 and over was used rather than separate age groups for 85-89, 90-94 and 95 and over.
Source: Eurostat (online data code: hlth_cd_asdr2)

The relative incidence of STEMI is decreasing and the incidence of NSTEMI is increasing [10]. The incidence rate of STEMI in European countries ranges from 44 to 142 per 100 000 inhabitants per year [11]. In Czech Republic, estimated incidence of acute myocardial infarction is 168 per 100 000 inhabitants a year, while estimated incidence of STEMI is 66 per 100 000 a year

[11, 12]. According to National Register of Cardiovascular Interventions in Czech Republic, total number of 21 415 percutaneous coronary interventions were performed in 2012, of which 26.3% were due to non-ST elevation myocardial infarction, 25.6% due to ST-elevation myocardial infarction and 31.3% due to stable forms of CAD [13].

Despite progressive decline in death rates following ACS due to improved treatment strategies and preventive measures, mortality remains substantial. The in-hospital mortality of STEMI patients in EU varies between 4 to 12% [1]. In Czech Republic, where majority of STEMI patients are treated with primary percutaneous coronary intervention (pPCI), the in-hospital mortality for STEMI is 2.5% [13]. 30-day mortality in patients with STEMI is 8.2% and one-year mortality 12,9%. 30-day and 1-year mortality for both STEMI and NSTEMI divided according to age are shown in Table 2.

Table 2. 30-day and 1-year mortality of patients with STEMI and NSTEMI in Czech Republic according to age [13]

30-day mortality			
	< 75 years	≥ 75 years	Total
NSTEMI	2.0%	5.2%	2.9%
STEMI	5.9%	17.2%	8.2%
1-year mortality			
	< 75 years	≥ 75 years	Total
NSTEMI	5.0%	16.2%	8.2%
STEMI	9.0%	28.1%	12.9%

1.2. Pathophysiologic mechanisms of coronary artery disease

1.2.1. Impairment of coronary reserve

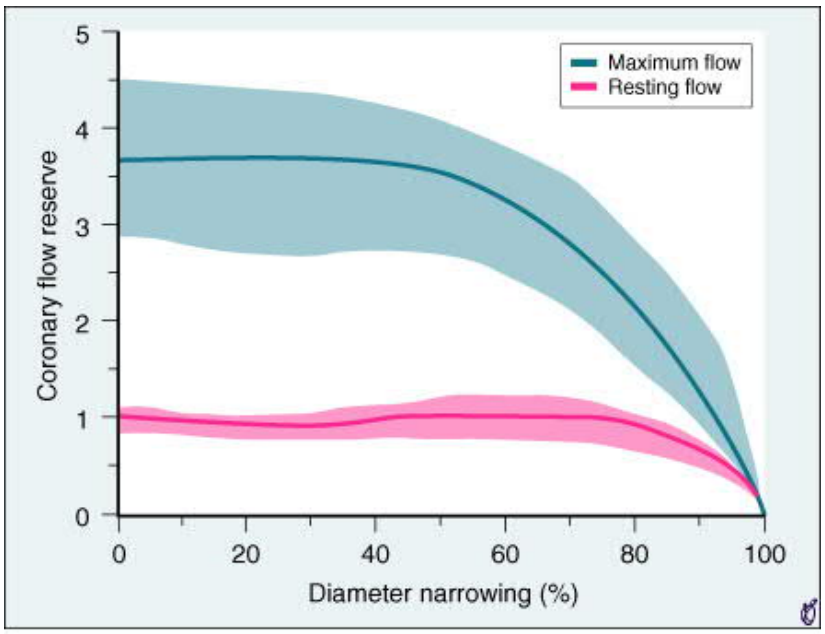
Coronary circulation provides blood supply to myocardium according to its current demands. Main components of coronary circulation are epicardial arteries (left and right coronary artery and their branches) that give rise to pre-arterioles, further dividing to arterioles. Arterioles are connected to capillary network and together with venules form microvascular circulation. Oxygen extraction in coronary circulation reaches a very high level already upon rest conditions. Thus the main mechanism to increase myocardial oxygen supply upon increased oxygen demand is through increased coronary blood flow. Given stable blood pressure, coronary vasodilatation represents a key mechanism to increase blood flow. Vasodilatation in coronary arterioles is able to increase coronary blood flow 4 to 5-times compared to resting blood flow, what represents so-called coronary reserve. Main determinants of myocardial oxygen demand are heart rate, systolic blood pressure, myocardial contractility, left ventricular wall tension and myocardial mass [14].

Regulation of coronary blood flow is secured by mechanical, neural and mainly humoral mechanisms. The most important is endothelial regulation through vasoactive substances [15]. The main vasodilator is endothelium-derived relaxing factor (EDRF), chemically nitrous oxide (NO). EDRF production is induced by shear stress, pulsatility and indirectly acting vasodilators (e.g. serotonin, ADP). After its production in endothelium, EDRF penetrates to smooth muscle cells of media, activates guanynyl cyclase and causes accumulation of cGMP, which reduces concentration of intracellular Ca^{2+} and leads to vasodilatation [16]. Some endothelial vasodilators act directly on vascular smooth muscle, e.g. adenosine and prostacyclin. Adenosine directly reduces input of Ca^{2+} into smooth muscle cells of media and thus causes vasodilatation [17]. Permanent basal production of NO is inevitable to adjust tonus of epicardial parts of coronary arteries and arterioles.

Endothelial dysfunction, which characterizes initial stages of coronary atherosclerosis, results in impaired vasodilatation with prevailing vasoconstriction mechanisms [18]. Impairment of coronary vasodilatation causes reduced coronary reserve and therefore may lead to myocardial ischemia. Endothelial dysfunction occurs predominantly in patients with risk factors for atherosclerosis (metabolic syndrome, dyslipidaemia, diabetes mellitus, hypertension, smoking and positive family history of cardiovascular disease).

Progression of coronary atherosclerosis leads to further reduction of coronary reserve. Vasodilatation of arterioles is able to secure adequate coronary blood flow until a certain degree of epicardial stenosis. Resting coronary blood flow is sufficiently provided until a coronary stenosis exceeds 80-90% diameter stenosis. The maximal coronary blood flow and coronary reserve is reduced when the coronary stenosis exceeds 50% diameter stenosis (Figure 2) [19, 20]. Functional significance of epicardial coronary stenosis can be determined by fractional flow reserve (FFR) and coronary flow reserve (CFR) – methods, which facilitate clinical decision making in patients with equivocal coronary stenosis.

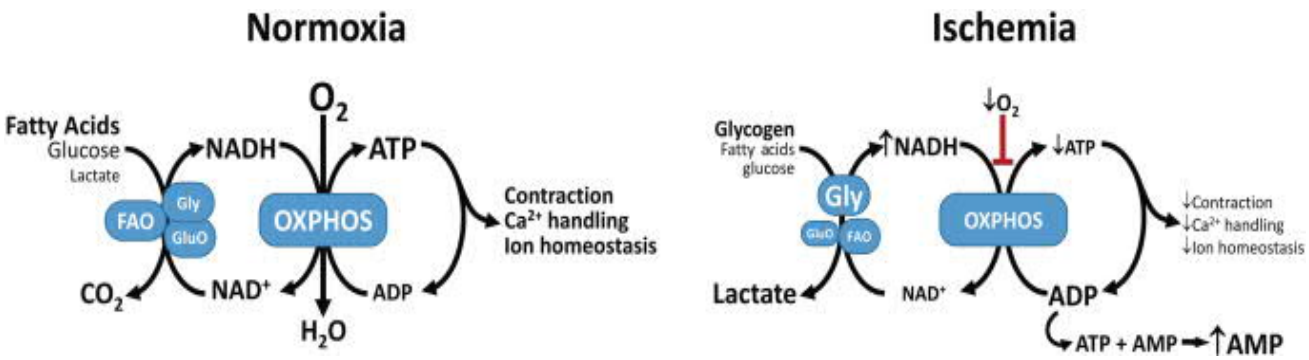
Figure 2. Relationship between coronary flow reserve and the severity of coronary artery stenosis [19] Resting coronary blood flow is sufficiently provided until a coronary stenosis exceeds 80-90% diameter stenosis. The maximal coronary blood flow and coronary reserve is reduced when the coronary stenosis exceeds 50% diameter stenosis.



1.2.2. Myocardial ischemia

Myocardial energy metabolism is highly dependant on ATP production through oxidative phosphorylation since its anaerobic capacity is very limited. Under aerobic conditions, the majority of cardiac ATP is generated by oxidation of fatty acids. The ATP pool in myocardium is relatively small and can be quickly exhausted. Impairment of ATP production thus rapidly induces contractile dysfunction. Under anaerobic conditions, glucose utilization becomes the main mechanism for ATP generation (Figure 3) [21, 22].

Figure 3. Myocardial energy metabolism under normal and ischemic conditions [21] (OXPHOS = oxidative phosphorylation; FAO = fatty acids oxidation; Gly = glycolysis; GluO = glucose oxidation)



Anaerobic glycolysis represents a significantly less effective mechanism of ATP production, however, is able to provide sufficient amount of ATP for myocyte survival (with substantial restriction of mechanical activity) even during prolonged ischemia. Nonetheless, decrease in ATP level below 10-20% of its normal cell content results in irreversible cell damage [23]. Myocardial ischemia with significant ATP depletion results in the cascade of ischemic events, which usually occur in the following sequence: *metabolic abnormalities* – including loss of intracellular K^+ and accumulation of lactate, *LV diastolic dysfunction* – with reduced ventricular compliance and elevated ventricular filling pressure, followed by *LV systolic dysfunction* -

resulting in decreased ejection fraction, reduced stroke volume and increased LV end-diastolic pressure, *ECG changes* and lastly development of *angina pectoris* – due to accumulation of lactate, serotonin and adenosine, which activate peripheral pain receptors (Table 3) [24].

Table 3. The time sequence of ischemic cascade

The ischemic cascade
Local ischemia
Metabolic abnormalities
LV diastolic dysfunction
LV systolic dysfunction
ECG abnormalities
Angina pectoris

1.2.3. Consequences of ischemia on myocardium

The fate of myocardium is ultimately determined by the severity and duration of the ischemic periods. The spectrum of consequences ranges from rapid and complete recovery of myocardial function (e.g. after short episodes of effort-induced angina), to prolonged contractile dysfunction with potential recovery of normal function (stunned and hibernating myocardium), till complete myocardial necrosis (myocardial infarction).

Ischemic preconditioning

Ischemic preconditioning represents an adaptation process where repeated short episodes of myocardial ischemia cause protection to myocardium against subsequent ischemic insult. Ischemic preconditioning is able to reduce infarct size and, in addition, protect myocardium against post-AMI arrhythmias and LV dysfunction [25-27]. Ischemic preconditioning has two windows of protection – early, which lasts a few hours, and late preconditioning, covering a few days. The exact molecular mechanism behind this phenomenon has not yet been fully understood. Early phase is apparently related to substances released by ischemic cells - adenosine, opiates and bradykinin, while late phase is associated with increased synthesis of protective proteins [28].

Stunned myocardium

Stunned myocardium represents a prolonged post-ischemic dysfunction that persists despite the absence of irreversible damage and despite restoration of normal or near-normal coronary blood flow [29]. Stunned myocardium is completely reversible and occurs usually after an acute ischemic attack. Spontaneous functional recovery is reached within days to weeks, depending on the size of the risk area, duration of the ischemic insult and collateral circulation. Stunning is supposedly a multifactorial process that includes interaction of several

pathophysiological mechanisms. The theory of oxygen-derived free radicals and the calcium theory are two most often listed theories behind this phenomenon [29, 30]. Free-radicals theory assumes stunning is cased by oxidative stress, while calcium hypothesis considers stunning as a result of calcium overload.

Hibernating myocardium

Myocardial hibernation represents a chronic myocardial dysfunction, which is reversible upon reperfusion [31]. The underlying pathophysiological mechanism has not yet been fully understood. It was thought that hibernation is cause by reduced coronary blood flow. However, more recent studies, based on quantitative evaluation of coronary blood flow by 13N-NH3 positron emission tomography, disprove the former theory. Quantitative evaluation indicated that coronary blood flow to hibernating myocardium remains normal at rest, while coronary flow reserve is decreased [32, 33]. Thus exercise or stress cause repetitive periods of myocardial ischemia, what results in hibernating myocardium. Morphological alterations of hibernating cardiomyocytes include reduction in contractile proteins, disorganisation of sarcoplasmic reticulum, increased intracellular glycogen content and alterations of cell nucleus [29]. Up to a certain degree of severity, these alterations are reversible upon reperfusion. Longer the hibernation process lasts, more severe alterations to myocardium occur and less significant function recovery is achieved by reperfusion [31, 34]. Early detection of hibernating myocardium thus provides important information from a long-term perspective and substantially influences further therapeutic approach in patients with chronic forms of CAD with LV dysfunction.

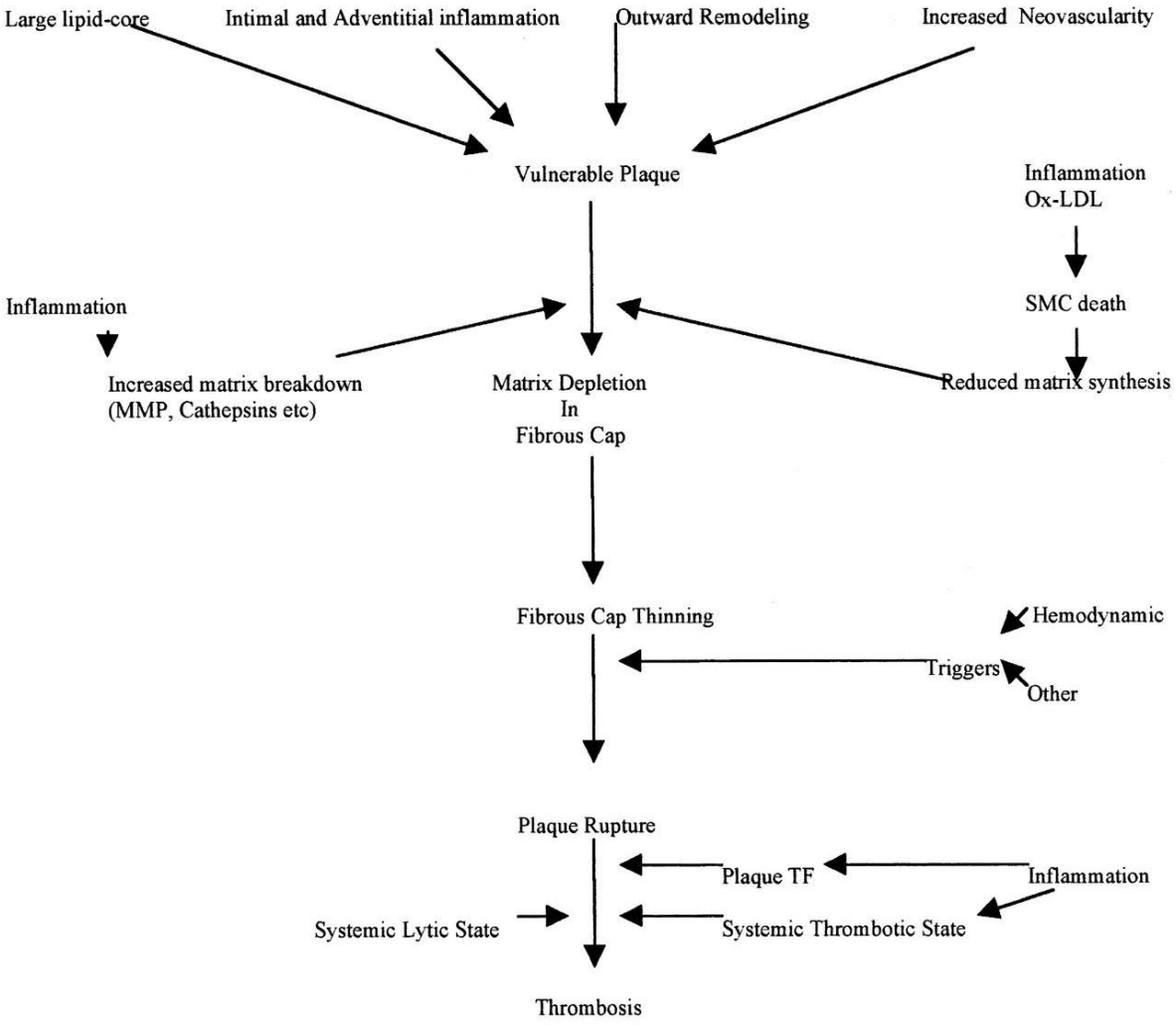
Detection methods of viable myocardium, both stunned and hibernating, include dobutamin echocardiography (evaluating coronary reserve), myocardial contrast echocardiography (evaluating preserved myocardial perfusion), single-photon emission computed tomography with ^{201}Tl or ^{99m}Tc-sestamibi and positron emission tomography with ^{18}F-FDG (evaluating combination

of preserved perfusion, membrane integrity and metabolic activity), and late gadolinium-enhancement cardiac magnetic resonance – LGE-MR (evaluating size of extracellular space). Nowadays, dobutamin echocardiography together with scintigraphic techniques and LGE-MR represent the most commonly used methods evaluating myocardial viability in clinical practise.

Myocardial infarction

Acute myocardial infarction involves irreversible myocardial damage caused by myocyte necrosis and apoptosis due to prolonged ischemia. Complete process of myocardial cell death requires at least 2-4 hours of ischemia, depending on the presence of collateral circulation to the ischemic region, persistent or intermittent coronary occlusion, the sensitivity of myocytes to ischemia, pre-conditioning, and individual demands on oxygen and nutrients [35]. Common initiating pathophysiological mechanism involves disruption of an atherosclerotic plaque with subsequent thrombus formation. Unstable (vulnerable) plaques are rich in macrophages and foam cells and the fibrous cup is thin and prone to rupture, what leads to exposure of thrombogenic material into circulation and thrombus formation (Figure 4) [36, 37]. The intraluminal thrombus can be completely occlusive or suboclusive. Complete occlusion of epicardial part of a coronary artery results in acute myocardial infarction presenting with ST-elevation, while sub-total or intermittent occlusion leads to acute myocardial infarction without ST-elevation [38, 39].

Figure 4. The potential pathophysiologic mechanisms of plaque vulnerability, rupture, and thrombosis [37] (Ox-LDL = oxidized low-density lipoprotein; MMP = matrix degrading metalloproteinases; SMC = smooth muscle cells; TF = tissue factor)



1.2.4. Reperfusion injury

After the onset of acute myocardial ischemia, especially in the setting of ST-elevation myocardial infarction, prompt myocardial reperfusion therapy is essential to safe viable myocardium, limit infarct size, preserve LV function and prevent the onset of heart failure and other possible complications of AMI. On the other hand, sudden reperfusion of acutely ischemic myocardium can independently lead to further myocardial injury [40, 41]. The underlying pathophysiological mechanisms of reperfusion injury include oxidative stress, ionic imbalance and inflammation [42, 43]. There are four recognized forms of reperfusion injury: myocardial stunning, reperfusion-induced arrhythmias, microvascular obstruction and lethal myocardial reperfusion injury (Figure 5).

Pathophysiological mechanisms of reperfusion injury

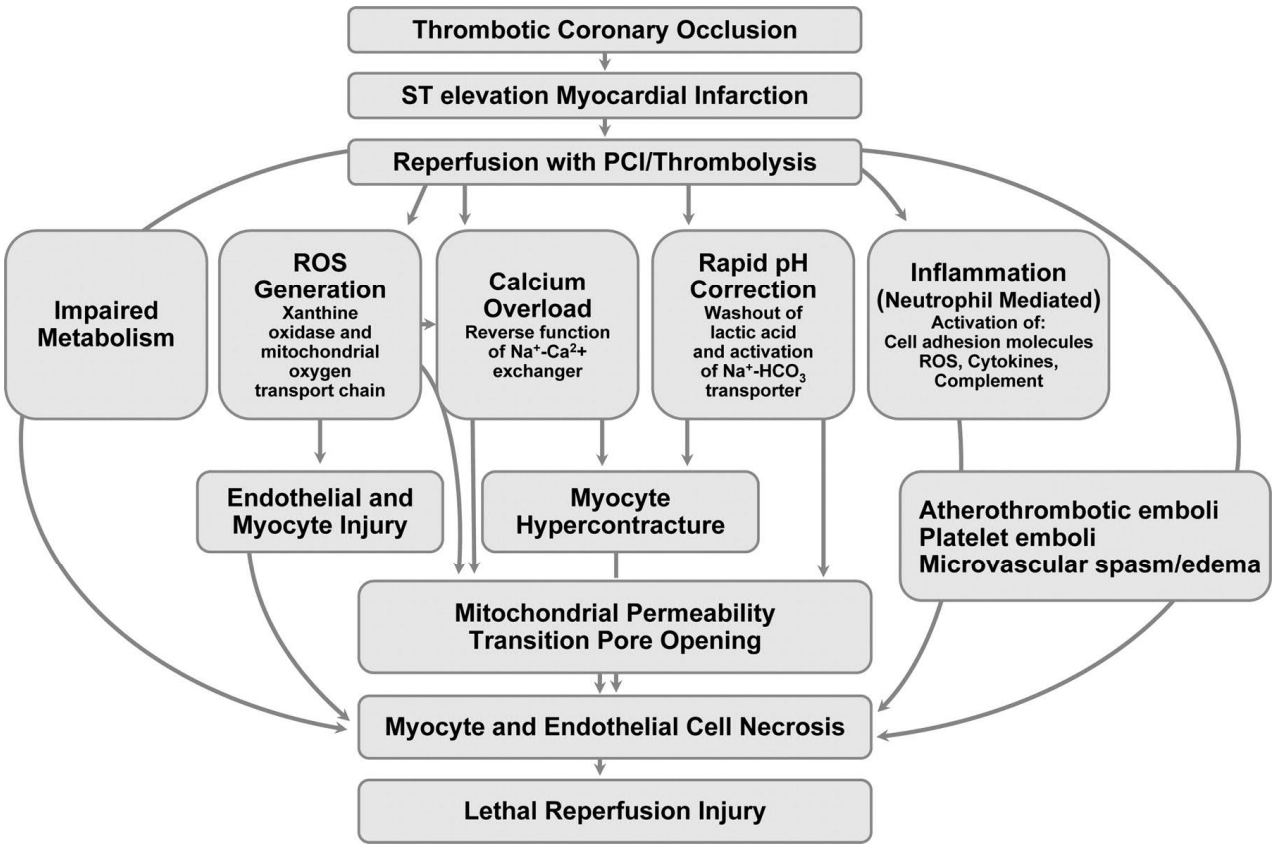
Oxidative stress represents a key mechanism of reperfusion injury [42]. It is characterized by a significantly increased production of reactive oxygen species (ROS, such as superoxide anion radical, hydroxyl radical and peroxynitrite anion) and/or altered cellular mechanisms of protection against ROS [43, 44]. Under physiological conditions, ROS are formed in a small amount as a natural byproduct of oxygen metabolism and their optimum level is guarded by a complex system of antioxidants [45]. However, during myocardial reperfusion injury, the fragile equilibrium is impaired. The production of ROS is increased while the defence mechanisms of myocytes are altered. Sudden re-admission of oxygen during reperfusion in previously ischemic myocardium causes rapid increase in ROS production and results in worsening of the tissue damage by causing cellular dysfunction, apoptosis and necrosis [42].

Ionic imbalances and intracellular pH-changes represent another important contributing factor to reperfusion injury. Intracellular Na^+ and Ca^{2+} overload begins during the ischemic phase

and is exacerbated at reperfusion due to damage caused to plasma and intracellular membranes [46]. Moreover, ischemia-induced intracellular acidosis and subsequent rapid restoration of physiological pH during reperfusion (by washing out lactate and activation of Na^+-H^+ exchanger and Na^+-HCO^- symporter) results in further cell injury [47].

Inflammation accompanying myocardial infarction also contributes to the pathogenesis of reperfusion injury. The ischemic insult together with overproduction of ROS triggers the release of pro-inflammatory cytokines (such as TNF-α, IL-11β, IL-6) and expression of adhesive molecules, what results in activation of inflammatory cells, enhanced thrombocyte activity, microvascular thrombus formation and mircovascular dysfunction. Oxidative stress together with inflammation also participates in the initiation of apoptosis and in the process of myocardial remodelling after myocardial infarction [48, 49].

Figure 5. Pathophysiological mechanisms of ischemia-reperfusion injury [50]



Consequences of reperfusion injury

Stunned myocardium and *reperfusion-induced arrhythmias* represent two reversible forms of ischemia-reperfusion injury. Stunned myocardium results from negative effects of oxidative stress and intracellular Ca^{2+} overload on the contractile apparatus of cardiomyocytes [51]. Reperfusion-induced arrhythmias are often represented by potentially malignant ventricular arrhythmias, caused by ionic imbalances [52].

Microvascular obstruction (MVO) is characterized by dysfunction of myocardial microvascular circulation with no–reflow phenomenon despite successful restoration of blood flow of an infarct-related artery through reperfusion therapy [53, 54]. The major contributing factors include: microembolization of the atherosclerotic plaque material causing direct microvascular obstruction, formation of platelet micro-thrombi due to enhanced inflammatory reaction, vasoconstriction induced by soluble vasoconstrictors released from the infarcted region, direct capillary damage with impaired vasodilatation, and external compression of microvascular circulation due to edema of the surrounding myocardium [55]. Microvascular obstruction is associated with lager infarct size, reduced LV ejection fraction, adverse LV remodelling, and worse prognosis in AMI patients [56, 57]. MVO can be detected during coronary angiography through assessment of the antegrade coronary blood flow beyond the coronary occlusion. This so-called TIMI Grade Flow represents a scoring system where TIMI equal or less than 2 is considered “no reflow”. In AMI patients, TIMI 0 and 1 after PCI occurs in 2-3%. [58]. MVO is more frequent in pure STEMI patients, while in patients with cardiogenic shock the incidence of no reflow even reaches 20% [59, 60]. However, TIMI system evaluates the microvascular circulation only indirectly. A more precise diagnostic method is Myocardial blush grade, where the status of microcirculation is assessed by the density of contrast in the myocardial area irrigated by the culprit artery [61]. Noninvasive methods include myocardial contrast echocardiography and magnetic resonance imaging.

Lethal myocardial reperfusion injury is defined as an irreversible reperfusion-induced damage to myocardium that was viable at the end of the ischemic phase. Experimental studies suggest that lethal myocardial reperfusion injury may be responsible for up to 50% of the final infarct size, however, majority of human studies failed to prove significant cardioprotective benefit of therapies aimed at limiting reperfusion injury [62, 63]. Some promising results brought a recent meta-analysis of randomized trials with adjunctive manual thrombectomy. In STEMI patients undergoing pPCI, the use of adjunctive manual thrombectomy devices improved myocardial perfusion, decreased distal embolization and led to reduced mortality [64].

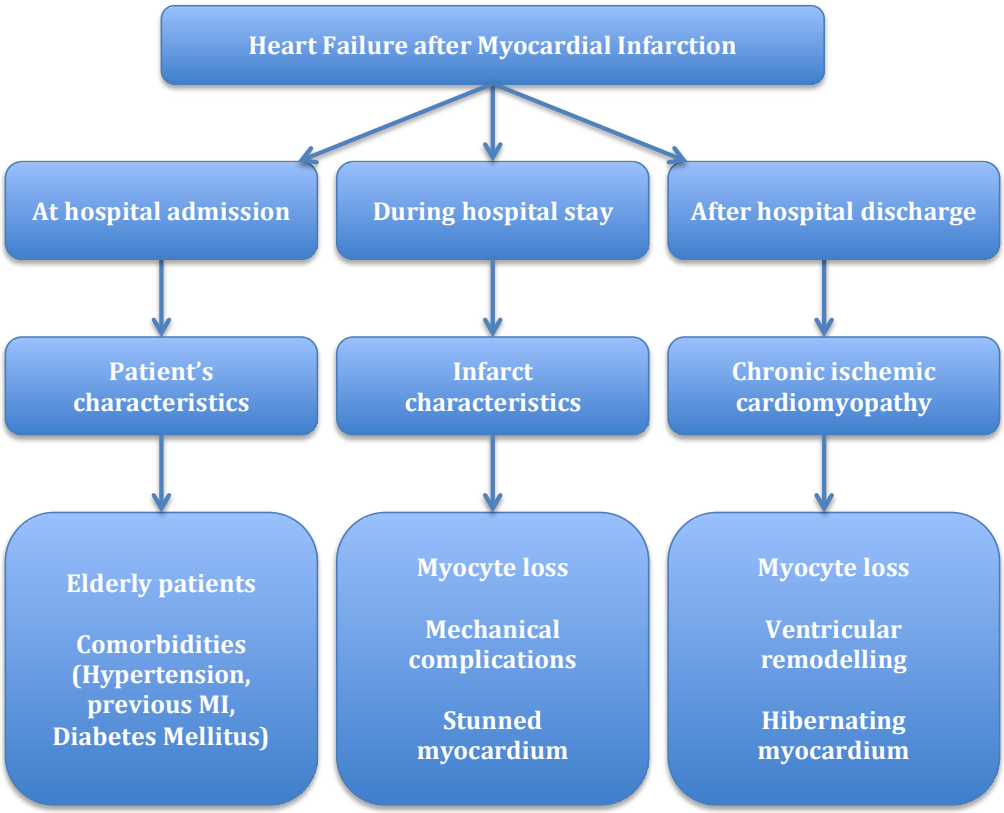
1.2.5. Ventricular remodelling and development of heart failure

Acute myocardial infarction remains the most common cause of heart failure (HF) worldwide [65]. At the same time, heart failure represents a major cause of late morbidity and mortality after AMI [66]. Significant improvement in the treatment of acute forms of CAD, especially introduction of percutaneous coronary intervention to routine clinical use, revolutionised the outcomes of AMI patients [67-69]. Successful reperfusion treatment led to a rapid reduction in the incidence of post-AMI heart failure and improved long-term survival rates [70]. However, despite the success, the incidence of heart failure after AMI remains substantial. About 25% of patients develop features of heart failure following AMI. The exact incidence of post-AMI heart failure according to the recent epidemiological studies varies from 13-39% [71-83], depending on the study definition of heat failure and AMI, the time of the diagnosis ascertainment and the duration of follow-up. Post-AMI heart failure represents an important adverse prognostic feature, with 1-year mortality 5 fold higher in those with HF [66, 84]. Thus early prediction of high-risk patients and novel therapeutic strategies remain a key requirement in modern medicine.

Heart failure is defined as a clinical syndrome with typical symptoms (breathlessness, ankle swelling, fatigue etc.) caused by structural or functional cardiac abnormality that results in impaired ability of the ventricle to fill or eject blood [85]. First clinical diagnostic criteria such as the Killip and New York Heart Association classifications were later extended by the development of echocardiography and other imaging methods, which allowed objective measurements of ejection fraction and ventricular volumes [86]. As a result, three types of heart failure have been defined: heart failure with reduced ejection fraction (HFrEF, with LVEF < 40%), HF with mild-range EF (HFmrEF, with LVEF 40-49%) and HF with preserved EF (HFpEF, with LVEF $\geq$ 50%) [85]. Concerning post-AMI heart failure, it is important to distinguish three key time periods of heart failure development: HF present at the hospital admission, HF occurring during the hospital stay or HF developed after the hospital discharge. The time of HF onset following AMI is

reflected by the presence of certain contributing factors (Figure 6) [87]. Moreover, the time of heart failure development has a prognostic significance, with higher mortality rates in patients with delayed onset of post-AMI heart failure compared to HF with early onset [75]. Unfortunately, timing of heart failure development is often not well defined in the research studies making comparisons more complicated.

Figure 6. Timing of heart failure development after acute myocardial infarction – characterised by the presence of certain contributing factors [87]

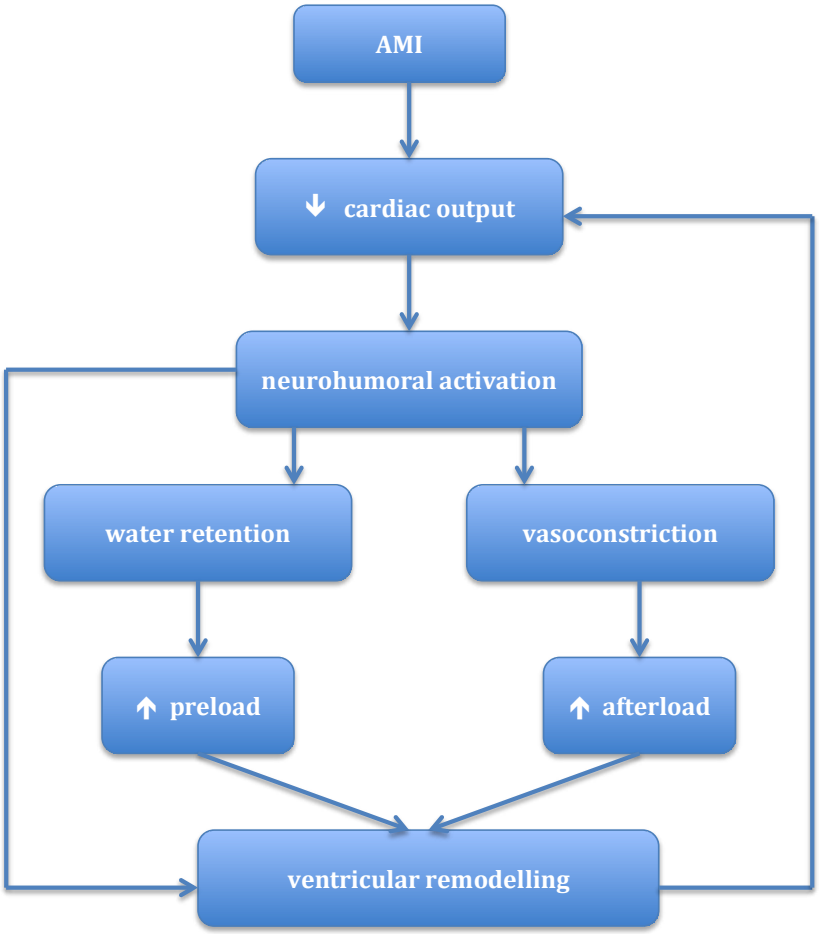


Pathophysiologic mechanism of heart failure development after AMI

Development of heart failure after acute myocardial infarction depends on several factors, such as time from the onset of ischemic symptoms to reperfusion therapy, result of culprit-lesion recanalization, restoration of myocardial perfusion, stunned myocardium, extent of LV remodelling, and further ischemic changes to non-infarcted myocardium in case of concomitant coronary stenoses in other locations [88, 89]. Contractile dysfunction after AMI with decreased cardiac output leads to neurohumoral activation, including activation of sympathoadrenal system,

renin-angiotensin-aldosterone system, release of antidiuretic hormone and several cytokines. Neurohumoral activation results in systemic vasoconstriction and retention of water and solutes as a compensatory mechanism to maintain perfusion of the vital organs. At the same time, cardiac hemodynamic compensatory mechanisms are activated. The neurohumoral activation results in increased preload and afterload and initiates complex of structural changes in the heart, together identified as ventricular remodelling [90]. The alteration of ventricular function leads to further neurohumoral activation and results in further ventricular remodelling – completing the vicious circle (Figure 7).

Figure 7. Pathophysiologic mechanism of LV remodelling and development of heart failure



The process of LV remodelling is defined as changes at molecular, cellular and interstitial level that clinically manifest as changes in size, shape and function of the heart after the insult [91]. The process begins within the first few hours after AMI and progressively continues [92, 93]. The early phase involves thinning and dilatation of the infarcted region, resulting in the infarct expansion, which can lead to early ventricular rupture or formation of aneurysm. The later phase involves architectural rearrangements of the whole left ventricle and leads to mural hypertrophy, reshaping (from elliptical to spherical), dilatation and finally wall thickening [88]. With the progression of the LV dilatation, the end-systolic volume index increases and ejection fraction decreases. These were identified as important predictors of mortality [94, 95]. Processes that occur within LV remodelling are summarized in Table 4 [91, 96-100].

Table 4. Processes occurring within ventricular remodelling after myocardial infarction

LV remodelling after AMI
Myocyte hypertrophy due to pressure overload
Myocyte lengthening due to volume overload
Ongoing myocyte loss via apoptosis and necrosis
Inflammation and reabsorption of necrotic tissue
Infarcted area expansion, scar formation
Alterations in extracellular matrix, including interstitial fibrosis
Dilatation and reshaping of the left ventricle
Ventricular wall thinning

LV remodelling is driven by a complex of contributing factors, such as ongoing cardiomyocyte death, altered energy metabolism, oxidative stress, inflammation, alterations in extracellular matrix, changes in structure of contractile proteins, calcium transport alterations, changes in LV geometry and further neurohumoral activation. Main changes caused by these factors and following consequences are listed in Table 5 [101].

Table 5. Pathophysiologic mechanisms involved in ventricular remodelling [101]

Mechanism	Main changes	Consequence
Cell death	↑ apoptosis, ↑necrosis ↓ autophagy	Progressive myocyte loss
Energy metabolism	β oxidation Triglyceride accumulation ↑ glycolysis Mitochondrial dysfunction Mitochondrial atrophy	Lipotoxicity ↓ energy ↑ oxidative stress
Oxidative stress	↑ NADPH oxidase ↑ catecholamine degradation ↑ xanthine oxidaseMitochondrial dysfunction ↓ antioxidant systems	Lipid peroxidation Protein oxidation DNA damage Cell dysfunction Fibroblast proliferation Metalloproteinase activation ↑ apoptosis ↑ signaling pathways to hypertrophy
Inflammation	innate response Adaptive response dysfunction	↑ inflammatory cytokines Macrophage, T cell and B cell dysfunction
Collagen	Fibroblast proliferation ↑ metalloproteinases	Degradation of normal collagen Fibrosis
Contractile proteins	β-myosin ↓ α-myosin ↑ troponin T type 2 ↓ troponin I phosphorylation	↓ contractility
Calcium transport	↓ L-type calcium channels ↓ ryanodine ↓ calsequestrin ↓ calmodulin ↓Phospholamban phosphorylation ↓ SERCA 2a	↓ Calcium in systole ↑ Calcium in diastole
Geometry	LV cavity ↓ wall thickness	↑ parietal stress of the LV

Risk stratification of heart failure development after AMI

Precise and early risk stratification of post-AMI heart failure remains challenging. Several clinical, angiographic, imaging and biomarker approaches have been identified in AMI patients; however, their applicability in clinical practise remains limited. Therefore, a search for an accurate and early predictor of high-risk patients that could be easily introduced to everyday clinical use is of a great importance.

Clinical evaluation

Several predictors of heart failure development have been recognized in AMI patients, including age, diabetes, renal insufficiency, ejection fraction post-AMI, and Killip class ≥ 2 [102, 103]. A scoring system integrating these prognostic parameters could represent a valuable stratification tool. Recently, GRACE score, originally aimed at stratification of mortality risk after ACS, has shown to have the ability to predict development of heart failure after AMI [104].

Angiographic evaluation

Coronary angiography provides prognostic markers such as presence of multivessel disease, post-procedural TIMI flow ≤ 2 and absent myocardial blush indicating microvascular obstruction [105-107]. Patients with no-reflow present with higher incidence of arrhythmias, LV remodelling, heart failure and mortality [108].

Imaging methods

Echocardiographic examination after acute myocardial infarction provides important information on several prognostic parameters, including ejection fraction, chamber volumes, wall motion score index, E/E' ratio and right ventricular function [109]. Recently, also longitudinal and circumferential strain rate have been reported as predictors of mortality and hospitalization for heart failure after AMI [110].

Cardiac magnetic resonance imaging (CMR) provides prognostic information through determination of infarct size, myocardial salvage index and extent of microvastular obstruction [111-115]. A recent study using combined imaging by ^{18}F-FDG PET-MRI (^{18}F-fluorodeoxyglucose positron emission tomography/magnetic resonance imaging) in AMI patients has also reported its potential prognostic significance: the intensity of ^{18}F-FDG uptake in the myocardium after AMI correlated with the infarct size and predicted cardiac function at 6-month follow up [116].

Biomarkers

Clinically well-established biomarkers Troponin and B-type natriuretic peptide (BNP) has become a core use for the diagnosis of acute myocardial infarction and heart failure, providing also prognostic significance in long-term outcomes [117]. As recently reported, also combined assessment of traditional biomarkers (including N-terminal pro-BNP, hs-cTnT, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and high-sensitivity C-reactive protein) can provide prognostic information on LV remodeling after AMI [118].

Besides that, several new potential biomarkers of post-AMI heart failure have been identified, that could provide improvement in the risk stratification of AMI patients, including markers of apoptosis, inflammation and cardiac extracellular matrix remodelling.

Significance of apoptotic markers in the prognostic stratification of AMI patients will be discussed in detail in the following chapter.

Markers of inflammation with promising prognostic value include galectin-3 and ST2. According to PROVE IT-TIMI 22 study, galectin-3, a biomarker of inflammation and cardiac fibrosis, represents a strong predictor of heart failure development after AMI, even after adjustment for clinical risk factors in the multivariate analysis [119]. However, galectin-3 level failed to prove an association with parameters of LV remodelling after AMI in another study [120]. Serum level of the interleukin-1 receptor family member ST2 has been reported as an

important predictor of mortality and heart failure development after AMI [121, 122]. Moreover, ST2 showed high sensitivity and specificity relative to heart failure diagnosis in patients presenting with chest pain to the emergency department [122].

Markers of cardiac extracellular matrix remodelling with prognostic potential in AMI patients include tenascin-C and several matrix metalloproteinases (MMPs). Tenascin-C, an extracellular matrix glycoprotein, which serum level is increased after AMI, represents an independent predictor of LV remodelling, heart failure and MACE [123, 124]. Levels of matrix metalloproteinases are also increased after AMI, since they are involved in both the healing process as well as in adverse remodelling [125, 126]. MMP-2 assessed in AMI patients has been reported as an independent predictor of mortality during two-year follow-up [127]. Another study demonstrated MMP-3 level association with LV dysfunction, adverse LV remodelling and prognosis after AMI [128]. However, due to diverse roles of different MMPs at different time-points after AMI and their complex interactions, determining the best prognostic marker among MMPs represents a rather complicated task.

Another emerging biomarker - copeptin (C-terminal part of pro-vasopressin), has been reported a strong prognostic predictor of post-AMI heart failure according to the OPTIMAAL study [129, 130]. Looking ahead, further studies are needed to validate prognostic power of the above-mentioned markers and, if confirmed, adjust the treatment approach accordingly.

1.3. Apoptosis in coronary artery disease

1.3.1. Definition and role of apoptosis

Apoptosis is a highly regulated and energy-requiring process by which activation of specific signalling cascades leads to a cell death. Apoptosis is characterized by distinct morphological characteristics including cell shrinkage, plasma membrane blebbing, nuclear condensation, and fragmentation of the DNA and nucleus. This is followed by cell fragmentation into apoptotic bodies that are quickly removed by phagocytosis, thereby preventing an inflammatory process [131].

In contrast to apoptosis, necrosis represents an energy-independent form of cell death, characterized by a rapid cell swelling, early plasma membrane rupture, and disruption of cellular organelles. As a result of the membrane rupture and subsequent leakage of cellular material into the surrounding tissue, an inflammatory response is induced [132].

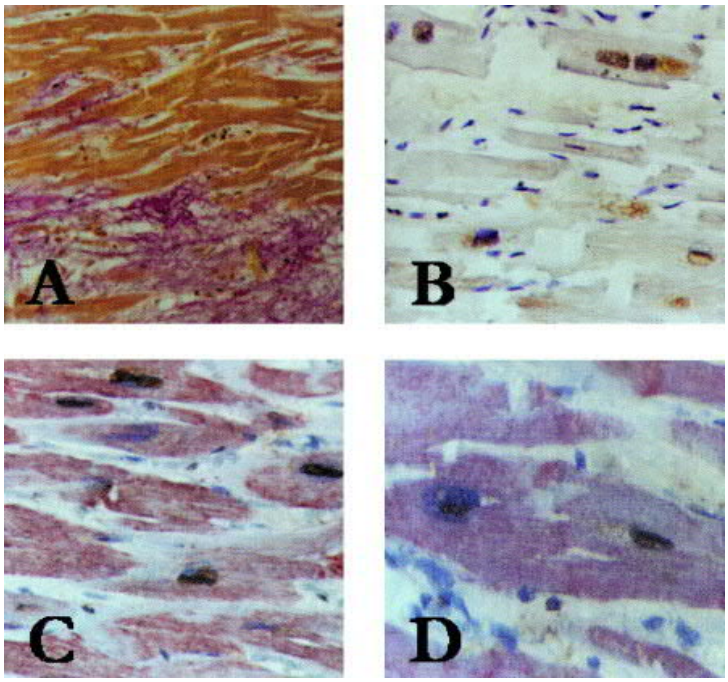
Although apoptosis and necrosis differ in the mechanism and the morphologies, there are no sharp boundaries between the two processes. Apoptosis and necrosis can occur simultaneously and predominant type of a cell death depends on the specific type of stimuli, the degree of the insult, the intracellular ATP concentration and the availability of caspases [133, 134].

Apoptosis is primarily beneficial since it plays an important role in various biological processes including embryogenesis, normal tissue homeostasis and aging [135-137]. However, excessive or insufficient apoptosis results in many diseases, including cancer, infectious and autoimmune diseases, some neurological illnesses (Parkinson's disease, Alzheimer's disease, Huntington's disease) and depression [138-145]. Moreover, growing evidence confirms an important role of apoptosis in coronary artery disease and development of heart failure [4].

The role of apoptosis in coronary artery disease

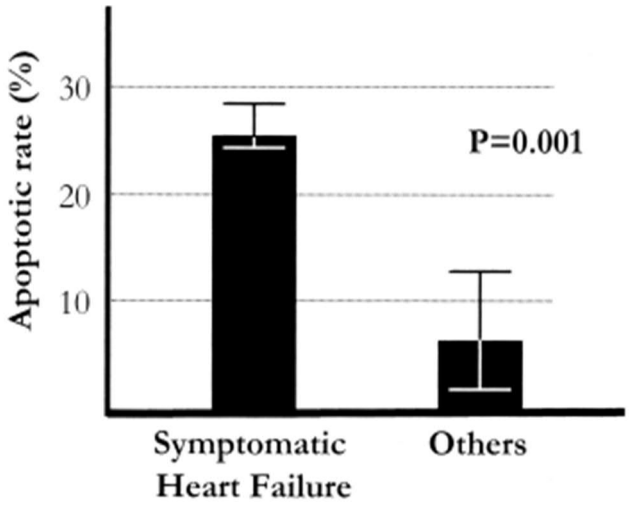
Various experimental studies have extensively investigated cell death in coronary artery disease. Besides necrosis, recent studies demonstrate an important role of apoptosis in the process of myocyte loss after myocardial infarction, as well as in the subsequent process of LV remodelling and development of heart failure [146-149]. Quantitative measurement of apoptotic versus necrotic myocardial cells in an *in vivo* rat model with constant myocardial ischemia showed even higher levels of apoptosis than necrosis in the ischemic region. Unlike necrosis, apoptosis was present also in the bordering zone and in the remote myocardium from the ischemic region, suggesting its role in determining the infarct size and in the process of LV remodelling after AMI [146]. Lower myocardial apoptosis occurred in the ischemic area when ischemia was followed by reperfusion, however, reperfusion accelerated apoptosis in the non-infarcted regions [147]. Apoptosis was detected up to 3 months in the ischemic area and in its border zone and 1 month in the remote myocardium after AMI. Cardiomyocyte apoptosis also correlated with the increased diastolic diameter 4 weeks after AMI, supporting its role in LV remodelling [148].

Figure 8. Apoptotic cardiomyocytes at the site of infarction [4] (A) Hematoxylin/van Gieson stain. (B) In situ end labelling of deoxyribonucleic acid (TUNEL) staining - brown nuclei. (C) Double staining: nuclear staining for TUNEL (brown) and cytoplasmic staining for muscle actin. (D) Double staining: nuclear staining for TUNEL and cytoplasmic staining for caspase-3.



Ex vivo human studies in patients who died of AMI support the concept of apoptosis as a determinant of infarct size and a contributor to LV remodelling after myocardial infarction. In myocardial samples obtained from 8 patients who died of AMI, apoptosis was observed particularly in the border zone to the infarcted area [3]. Another larger study in 20 patients, who died shortly after AMI, presented significant number of apoptotic cardiomyocytes in the border zone as well as in the remote regions [150]. High grade of ongoing apoptosis was detected also in later phases after AMI (20-30days) in the infarcted region [151]. A similar study confirmed significantly higher apoptotic rates in the infarcted region in later phases post AMI (up to 62 days) and demonstrated a correlation between apoptotic rates and LV longitudinal and transverse diameters [152]. Abbate et al. showed that patients who developed heart failure shortly after AMI were associated with significantly increased apoptotic rate at the site of infarction (Figure 9) [4]. Moreover, apoptotic rates in the infarcted area as well as in the non-infarcted regions significantly correlated with parameters of progressive LV remodelling. Thus apoptosis is shown to play an important role in LV remodelling and development of early symptomatic heart failure after AMI.

Figure 9. Apoptotic rate in patients with early post-infarction heart failure [4] Apoptotic rate at the site of infarction was increased nearly fourfold in patients who developed heart failure shortly after AMI compared to remaining patients.



Since apoptosis represents an intracellular process with minimal reaction to extracellular space, quantitative measurement of apoptosis in clinical practise remains a challenge. In *ex vivo* human studies, the rate of apoptotic cardiomyocytes ranges from 0.8% to 44% of total cardiomyocytes in the infarct-related area, depending mainly on the chosen detection method [3, 150-152]. In clinical studies, defining the significance of the apoptotic process is even more complicated, since detection of apoptosis is possible only indirectly – through soluble markers of apoptosis. Apoptotic markers include either apoptotic ligands, extracellular parts of apoptotic receptors or intracellular apoptotic mediators that manage to escape to circulation. The importance of several apoptotic markers has been confirmed by their proven association with parameters of the infarct size, the extent of LV remodelling or the ability to predict prognosis after AMI. Individual apoptotic markers and their role in CAD will be introduced in the following chapter.

1.3.2. Mechanism of apoptosis

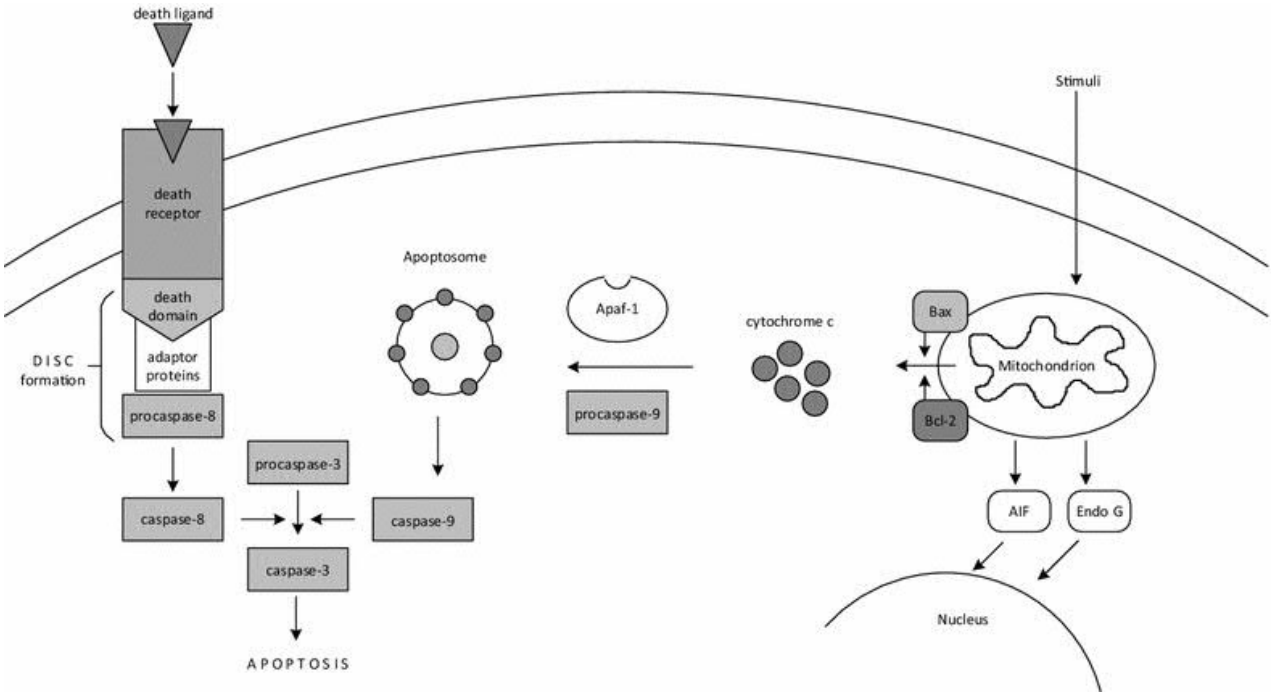
There are two well-characterized apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. These two pathways have distinct modes of activation, however, molecules that act in one pathway can influence the other [153]. Both pathways converge on the same terminal or execution pathway.

Extrinsic or Death receptor pathway

Activation of the extrinsic pathway is initiated through death receptors localized at the cell membrane. Death receptors contain a cytoplasmic domain called "death domain", which plays an important role in transmitting the death signal from the cell surface into the intracellular space [154]. After an apoptotic ligand binds to its death receptor, death-inducing signaling complex (DISC) is formed. DISC contains multiple proteins including adaptor proteins and results in procaspase-8 activation. Subsequently, caspase-8 activates effector caspases 3 and 7 and thus triggers the terminal phase of the apoptotic cascade (Figure 10).

Best-characterized apoptotic ligands and their receptors include apoptosis stimulating fragment – Fas ligand and its Fas receptor, tumor necrosis factor alpha - TNF-α and its receptors TNFR1 and TNFR2, and TNF-related apoptosis stimulating ligand TRAIL and its receptors TRAIL-R1 and TRAIL-R2 [154-158].

Figure 10. Schematic diagram of apoptotic signalling pathway: Apoptosis can be induced by the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. The extrinsic pathway is initiated after an apoptotic ligand (e.g. FasL, TNF-α, TRAIL) binds to its death receptor. Subsequently, death-inducing signalling complex is formed resulting in caspase-8 activation. Caspase-8 activates effector caspases and triggers the terminal phase of the apoptotic cascade. The intrinsic pathway is initiated through wide range of none-receptor mediated stimuli (e.g. deprivation of growth factors, hypoxia, oxidative stress), resulting in changes of the mitochondrial membrane permeability. After its release from mitochondria, cytochrome c together with Apaf-1 and procaspases-9 forms an apoptosome, resulting in caspase-9 activation. Caspase-9 then activates effector caspases, such as caspase-3. AIF and EndoG are also released from mitochondria and translocate to cell nucleus where they cause DNA fragmentation, independently of caspase activation. Both extrinsic and intrinsic pathways converge on the same terminal pathway.



Extrinsic pathway in coronary artery disease

Several studies have demonstrated that death receptor pathway plays an important role in inducing myocardial apoptosis after ischemia/reperfusion (I/R) injury.

Fas-mediated apoptosis

In experimental studies, Fas-mediated apoptosis is described as a critical pathway of cardiomyocyte death during I/R injury. A study using neonatal cardiomyocytes *in vitro* and adult rat cardiomyocytes *in vivo* showed that activation of Fas-receptor pathway through adenoviral overexpression of Fas ligand results in higher levels of apoptosis. Moreover, transgenic mice lacking functional Fas exhibit significantly reduced infarct sizes compared to wild type mice after I/R injury [159].

However, levels of soluble Fas receptor (sFas) and Fas ligand (FasL) measured in patients with different forms of CAD provided rather controversial results. Levels of sFas were significantly higher in patients with AMI compared to healthy controls or patients with chronic coronary artery disease but failed to correlate with the infarct size. Concerning FasL, no difference was found in its serum levels among patients with AMI, chronic forms of CAD or healthy controls [160].

In the study by Fertin et al., levels of FasL were measured in patients 1 month after AMI and several echocardiographic studies were performed up to 1-year post AMI to evaluate LV volumes. LV remodelling was documented by a significant increase of LV volumes; however, changes in LV volumes were not associated with FasL levels [161].

Similar finding were documented by Nilsson et al. who measured levels of both sFas and FasL in patients with STEMI prior to PCI and 24 hours after the procedure. Cardiac MRI was used

to evaluate infarct size and LV dysfunction 5 days and 4 months after STEMI. No correlation was found between sFas or FasL and infarct size or LV dysfunction [162].

In line with this, serum levels of sFas measured in patients with acute coronary syndromes were not associated with patient prognosis during 6-month follow-up [163].

In conclusion, sFas levels were significantly higher in patients with acute myocardial infarction but failed to correlate with the infarct size, LV dysfunction, or prognosis. Thus the role of Fas-mediated apoptosis in CAD is yet unclear.

However, sFas levels seem to be helpful as a prognostic marker in patients with various forms of heart failure. Prognostic value of sFas was demonstrated in patients with dilated cardiomyopathy, decompensated heart failure, as well as in patients with compensated heart failure [164-166]. Higher sFas concentrations were associated with higher risk of mortality or hospitalization for heart failure.

TNF-α mediated apoptosis

Experimental studies also demonstrate an important role of TNF-α –mediated apoptosis in coronary artery disease. TNF-α is a proinflammatory cytokine with various biological functions. After binding to its receptor, TNF-α can induce apoptosis in cardiomyocytes, as shown in an *in vitro* rat study [167]. In a myocardial infarction model in mice, TNF-α demonstrated its diverse effects. Elevated levels of TNF-α contributed to acute myocardial rupture and chronic left ventricle dysfunction by inducing myocardial apoptosis, local inflammatory response, increased matrix metalloproteinase activity, and matrix and collagen degradation [168].

TNF-α shows dual effect in the heart depending on its concentration and on the type of its receptor. Low dose of TNF-α improves myocardial function while high dose of TNF-α increases myocardial injury following ischemia and reperfusion [169]. In an *in vivo* murine model of

myocardial infarction, TNF-α showed its cardiotoxic effect through receptor TNFR1 and cardioprotective effect through TNFR2. Mice lacking TNFR2 demonstrated worse post-IM survival, more severe ventricular dysfunction, and exacerbated myocyte hypertrophy and interstitial fibrosis in non-infarct myocardium - compared to TNFR1-knockout mice [170].

TNF-α is suggested to play its role also in progression of heart failure. Studies in patients with heart failure demonstrate that high levels of TNF-α are expressed within failing human myocardium [171, 172]. However, anti-TNF-α clinical trials using TNF-α antagonist or TNF-α antibody showed no benefit to patients with heart failure [173, 174]. These disappointing results support the presumption of ambivalent effects of TNF-α in the cardiovascular system.

Levels of TNF-α and its receptors are elevated also in patients with acute myocardial infarction and can predict infarct size, LV dysfunction and prognosis. A study by Kehmeier et al. showed that TNF-α levels measured in STEMI patients after pPCI can predict infarct size [175]. Nilsson et al. measured soluble TNF-receptors in STEMI patients prior to and 24 hours after PCI and showed that concentrations of TNFR1 and TNFR2 are associated with infarct size and LV dysfunction [162].

TNFR1 has proven to have a prognostic value in patients after acute myocardial infarction. Valgimigli et al. show that plasma levels of soluble TNFR1 are a predictor of mortality and new onset of heart failure in patients with AMI [176]. Ueland et al. demonstrate that levels of sTNFR1 can predict all-cause mortality and cardiovascular death in patients who developed heart failure after AMI [177].

Other studies suggest that also TNF-α has a prognostic value in patients with AMI [178-180]. However, a clinical trial with TNF-α antagonist in patients with acute myocardial infarction provided no evidence of its beneficial effect for these patients [181].

These findings suggest that involvement of TNF-α in coronary artery disease is very complex and includes wide range of biological processes – both harmful and beneficial. Further

research is needed to clarify the exact molecular mechanism of TNF-α in CAD and find possible ways to inhibit only the negative TNF-α effects.

Since TNF-α is involved in CAD pathological pathway, TNF-α gene polymorphisms and their association with the risk of myocardial infarction and coronary artery disease have been extensively studied. A meta-analysis investigating relationship between TNF-α gene polymorphisms and CAD risk, however, showed no association [182].

TRAIL mediated apoptosis

TRAIL is a member of TNF superfamily that can induce apoptosis. After binding to its receptors TRAIL-R1 and TRAIL-R2, TRAIL initiates intracellular signalling cascade resulting in the apoptotic cell death [156, 157]. In an I/R model with isolated rat and mouse hearts, TRAIL was released from the postischemic hearts early after the onset of reperfusion [183]. However, the exact molecular mechanism of TRAIL has not yet been completely understood. Some experimental data suggest that TRAIL-R1 and TRAIL-R2 can also mediate cell type-dependent prosurvival and proliferation signals [184]. In experimental studies, administration of soluble recombinant TRAIL showed protective activity. In a diabetic mouse model, direct administration of TRAIL reduced development of cardiomyopathy [185]. Another similar study demonstrated that systemic TRAIL delivery showed anti-atherosclerotic activity in diabetic mice [186].

Several clinical studies report that levels of TRAIL are decreased in patients with acute myocardial infarction [163, 187, 188]. Moreover, TRAIL is reported to be a potential marker of severity of coronary artery disease and predictor of prognosis in patients after acute myocardial infarction. Mori et al. measured serum TRAIL levels in patients undergoing coronary angiography. TRAIL levels were significantly lower in patients with coronary artery disease compared to those without. Moreover, TRAIL levels were inversely associated with the severity of CAD, suggesting potential use of TRAIL as a marker of CAD severity [187].

Secchierro et al. measured TRAIL in patients with acute myocardial infarction in serial serum samples during hospitalization and in a 12 month-follow-up. Serum levels of TRAIL were significantly decreased in AMI patients at baseline (compared to healthy controls) and low TRAIL levels at patient discharge were associated with increased incidence of cardiac death and heart failure at the 12-month follow-up, even after adjustment for demographic and clinical risk parameters. Thus low TRAIL levels represent a potential predictor of cardiovascular events following acute myocardial infarction [188].

A study by Osmancik et al. provided similar findings. TRAIL levels were measured in acute coronary syndrome patients, who were then followed for 6 months. Low serum TRAIL concentrations were the strongest significant and independent predictor of the composite endpoint of death and hospitalization for heart failure [163].

Low TRAIL levels can predict worse prognosis also in patients with chronic heart failure and in older patients with cardiovascular disease [165, 189].

In conclusion, serum TRAIL levels seem to represent an important predictor of prognosis in patients with acute myocardial infarction. Low TRAIL levels are associated with worse prognosis of AMI patients while higher TRAIL levels seem to be protective. It is unclear whether decreased TRAIL levels reflect reduced production or increased consumption. Metalloproteinase 2, which level is elevated in patients with acute coronary syndromes, can cleave TRAIL, as shown in an *in vitro* study [190]. This could be a potential explanation of decreased TRAIL levels in patients with acute myocardial infarction. Better understanding of the exact molecular mechanism of TRAIL might provide new therapeutic strategies in patients with acute myocardial infarction.

Intrinsic or Mitochondrial pathway

Activation of intrinsic pathway is initiated through wide range of non-receptor mediated stimuli, such as deprivation of growth factors, hypoxia or oxidative stress. These stimuli produce intracellular signals that cause changes in permeability of mitochondrial membrane, resulting in the release of two groups of pro-apoptotic proteins into the cytosol. One group initiates caspase-dependent apoptosis while the second one initiates caspase-independent apoptosis [191] (Figure 10).

Caspase-dependent mitochondrial pathway

Cytochrome C together with other pro-apoptotic proteins triggers caspase-dependent mitochondrial pathway. After its release from mitochondria, cytochrome c binds to Apaf-1 (apoptotic protein activating factor 1) as well as to pro-caspase 9, forming an activation complex called apoptosome. Apoptosome formation leads to caspase-9 activation, which then activates effector caspases, such as caspase 3 [192, 193].

Inhibitors of apoptosis proteins (IAPs) represent a regulatory mechanism of caspase-mediated apoptosis. IAPs usually bind directly to caspases and thus block their function. Additional level of regulation is represented by inhibitors of IAPs, which promote apoptosis by inhibiting IAPs. These regulators are released from mitochondria together with cytochrome c [194-196].

Caspase-independent mitochondrial pathway

Upon apoptotic stimulation, a group of pro-apoptotic proteins is released from mitochondria that initiate caspase-independent apoptotic pathway, such as apoptosis inducing factor (AIF) and endonuclease G (EndoG). AIF and EndoG translocate to cell nucleus and cause DNA fragmentation, independently of caspase activation [197-199].

Regulatory mechanism of mitochondrial pathway

The release of apoptotic proteins from mitochondria is controlled and regulated by Bcl-2 family of proteins. Bcl-2 family of proteins contains both anti-apoptotic (e.g. Bcl-2) and pro-apoptotic proteins (e.g. Bax) [200].

Regulatory Bcl-2 family of proteins in coronary artery disease

Experimental studies show that cardiac specific overexpression of Bcl-2, an inhibitor of apoptosis, significantly reduces infarct size after I/R injury. This reduction of I/R injury correlates with the reduction of cardiomyocyte apoptosis [201, 202].

In an *in vivo* dog model, I/R injury resulted in myocyte apoptosis accompanied by a significant reduction of Bcl-2 expression and increase in Bax expression in the ischemic area [149]. Bcl-2 to Bax ratio is suggested to play an important role in determining whether a cell commits an apoptotic suicide or not.

Expression of anti-apoptotic Bcl-2 and pro-apoptotic Bax was studied also in the hearts of patients who died either of AMI (up to 20 days after the onset of AMI), more then a month after AMI and in normal control hearts. Bcl-2 expression was found in the area surrounding the infarcted area in the hearts with AMI, while Bax overexpression was the most evident in the hearts with older MI, predominantly again in the border zone to infarcted myocardium. Balance between Bcl-2 and Bax expression may be related to occurrence of myocyte apoptosis in these areas [203].

No clinical study with AMI patients was performed to examine serum levels of either Bcl-2 or Bax. Bcl-2 serum levels were measured in some studies with e.g. cancer patients, COPD patients or β-Thalassemia Minor patients [204-206]. Evaluation of Bcl-2 serum levels and its tissue expression, however, showed correlation bordering on statistical significance [206].

Terminal or Execution pathway

Terminal apoptotic pathway is common for both extrinsic and intrinsic pathway. Activation of execution caspases begins this last phase of apoptosis. Execution caspases, such as caspase-3, caspase-6 and caspase-7, activate cytoplasmic endonuclease, which degrades nuclear material, and proteases, that degrade the nuclear and cytoskeletal proteins [207]. This is followed by fragmentation into apoptotic bodies that are quickly removed by phagocytes. Cellular constituents are not released into the surrounding tissue and thus no inflammatory process is induced [208].

Caspase-3 in coronary artery disease

The most important caspase of the terminal apoptotic pathway is caspase-3. Increasing number of experimental studies demonstrate its role in the apoptotic cell death after I/R injury. Overexpression of cardiac specific caspase-3 in transgenic mice showed increased infarct size and pronounced susceptibility to die after I/R injury [209]. Vice-versa, downregulation of caspase-3 decreased the infarct size, lowered the apoptotic index of myocytes and improved the heart function in an experimental model with myocardial infarction [210].

The cleaved caspase-3 p17 peptide was shown to escape from apoptotic cancer cells and was detectable in extracellular medium after induction of apoptosis, suggesting the concept that p17 peptide can escape also into circulation from apoptotic cells in patients. This presumption was used in a clinical study with 27 STEMI patients undergoing PCI [211]. p17 peptide was measured within initial 24 hours after STEMI and the second measurement was performed 3 months after STEMI (88± 29days). Compared to healthy subjects, peak p17 levels were nearly 4-fold higher in the acute phase of STEMI and stayed significantly higher also in the late post-STEMI samples. However, the p17 peptide kinetics and its correlation with the myocyte apoptosis after myocardial infarction have not yet been sufficiently examined.

1.2.3. Inhibition of apoptosis

Since apoptosis is significantly involved in myocardial injury after AMI, inhibition of apoptosis represents an appealing target for a therapeutic intervention.

TNF-α inhibition therapy

TNF-α inhibition therapy was examined in large clinical trials with heart failure patients, using TNF-α antagonist infliximab or TNF-α antibody etanercept. Anti-TNF-α therapy, however, provided no evidence of clinical benefit to heart failure patients.

Two trials RECOVER and RENAISSANCE evaluated a TNF-α antagonist etanercept in 2000 patients with chronic heart failure. Both trials were terminated prematurely because etanercept failed to show clinically relevant benefit on the patient clinical status, the rate of death or hospitalization due to chronic heart failure [173].

ATTACH trial evaluated a TNF-α antibody infliximab in 150 patients with moderate-to-severe heart failure. Results showed that short-term inhibition of TNF-α with infliximab did not improve and high dose adversely affected the clinical condition of examined patients [174].

In patients with acute myocardial infarction, TNF-α antagonist etanercept was used in one trial with 26 patients. Etanercept reduced systemic inflammation markers but increased platelet activation. TNF-α antagonism thus provided no evidence of immediate beneficial effect for AMI patients [181]. Effect of anti-TNF-α therapy on adverse outcomes in AMI patients was not evaluated.

These findings suggest that involvement of TNF-α in the pathogenesis of coronary artery disease and development of heart failure includes various biological processes and simple inhibition of all TNF-α effects does not provide clinical benefit to cardiac patients.

On the other hand, TNF-α inhibition therapy brought a revolution in the treatment of several autoimmune diseases, such as rheumatoid arthritis, psoriasis and non-specific inflammatory bowel diseases. Interestingly, clinical trials indicate that anti-TNF-α therapy in patients with psoriasis is associated with significantly decreased risk of myocardial infarction, compared to treatment with topical agents [212, 213]. The risk of myocardial infarction is markedly reduced also in patients with rheumatoid arthritis who respond to anti-TNF-α therapy [214, 215].

TRAIL-targeted therapy

No clinical trials in coronary artery disease have been done using TRAIL as a therapeutic target yet. However, TRAIL therapy using agonistic antibodies against TRAIL receptors has been assessed within several clinical trials for cancer treatment [216]. Proapoptotic receptor agonists, including the recombinant human TRAIL ligand (dulanermin) that targets both TRAIL receptors or agonistic antibodies targeting against one of TRAIL death receptors (e.g. mapatumumab, lexatumumab, conatumumab), showed virtually no negative side effects, while indicating a strong therapeutic potential to a subset of cancer patients, who respond to TRAIL therapy. Further research is needed to evaluate possibility of exploiting TRAIL-based treatment also in cardiovascular diseases.

1.2.4. Conclusion

Apoptotic cell death represents a significant contributor to myocardial damage in patients with acute myocardial infarction and participates in the process of subsequent LV remodelling and development of heart failure. Finding a sensitive marker of apoptosis that would help predict prognosis of AMI patients is of a great importance. Among intracellular apoptotic mediators, the only one evaluated in serum of patients with AMI is a fragment of caspase-3 - p17 peptide. Serum levels of p17 peptide brought some hopeful results in one study with STEMI patients; however, further research is needed to prove p17 peptide as a substantial marker of apoptosis. Among extracellular markers of apoptosis, several soluble forms of receptors and their ligands were evaluated in clinical studies with AMI patients. The most promising apoptotic marker seems to be TRAIL. Serum TRAIL levels represent an important predictor of prognosis in AMI patients. Low TRAIL levels are associated with worse prognosis and high TRAIL levels seem to be beneficial. So far, it is unclear whether decreased TRAIL levels represent a reduced production or an increased consumption. Degradation of TRAIL by metalloproteinase 2, which level is elevated in AMI patients, might be a potential explanation for low TRAIL levels. Deeper understanding of the exact molecular mechanism of TRAIL is essential before its use in clinical practice.

2. Original research

Relationship between TRAIL and left ventricular ejection fraction in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention

2.1. Aims of the study

The aim of the present study was to assess levels of two apoptotic markers TRAIL and sFas in a homogenous group of patients with ST-elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI) in series of serum samples obtained during hospitalization and at one-month follow-up, to clarify the kinetics of serum levels of the two above-mentioned apoptotic markers after STEMI. Since apoptosis represents an important contributor to cardiomyocyte loss after AMI (initiated during the ischemic insult, subsequent reperfusion injury as well as within the process of ventricular remodelling) [4, 42, 146, 150, 152], we aimed to test the correlation between levels of apoptotic markers and LV ejection fraction (LVEF) after STEMI. Furthermore, we aimed to determine whether levels of TRAIL and sFas relate to LVEF change during one-month follow-up. Lastly, we aimed to validate their prognostic significance during 2-year clinical follow-up.

2.2. Methods

Study population and follow-up

Study participants were prospectively enrolled in the Cardiocenter at the University Hospital Kralovske Vinohrady, Prague, from December 2012 till June 2014. The inclusion criterion was STEMI treated using primary percutaneous coronary intervention (pPCI). Diagnosis

was made based on typical ischaemic symptoms and changes in electrocardiogram (ECG) according to the guidelines of the European Society of Cardiology for the management of STEMI [217]. The exclusion criteria were: 1) no revascularisation possible, 2) life expectancy less than one year due to non-cardiac reasons, 3) reluctance to cooperate in a long-term project. Echocardiographic examination was performed in all patients on the first day of hospitalization for STEMI. The study complies with the Declaration of Helsinki and was approved by the local Ethics Committee. Each patient signed written informed consent.

Follow-up visits including echocardiographic examination were arranged one month after the index procedure at the outpatient department. Patients were further followed for two years for mortality and morbidity endpoints either by clinical controls or telephonically.

Blood sampling and laboratory analysis

Apoptotic markers were analysed from venous blood samples obtained from each patient at four different time points: prior to PCI (day 0), 24 hours +/- 6hours after PCI (day 1), two days after PCI (day 2), and at 30-day control. After centrifugation (3500 rpm, 15 min), serum was stored at -70°C. Commercially available Enzyme-Linked Immuno-Sorbent Assays (ELISA) were used to measure serum concentrations of the reported apoptotic markers (sFas and TRAIL - R&D Systems, Minneapolis, MN, USA). Intra- and inter-assay coefficients were 4.60% and 6.70% for sFas and 5.60% and 7.40% for TRAIL. The lowest concentration detectable was 20pg/mL for sFas and 7.87pg/ml for TRAIL. All measurements were performed by staff unaware of the clinical data.

High-sensitive cardiac troponin T (hs-cTnT) was measured by Roche assay at admission and one and two days after PCI – at the same time points as meassurement of levels of apoptotic markers. Blood samples for biochemistry and haematology tests were taken at admission.

Clinical and echocardiographic evaluation

Echocardiographic examination was performed in all patients on the first day of hospitalization and at one-month clinical follow-up. A standard echocardiographic imaging protocol was used with the apical 4- and 2-chamber views and long and short parasternal axis views. The left ventricular ejection fraction (LVEF) was determined by using the biplane modified Simpson rule. To limit the variation, final LVEF was determined as a result of two examiners consensus. All echocardiographic examinations were analysed at the Echocardiographic laboratory of the Cardiocenter at the University Hospital in Kralovske Vinohrady, Prague.

Patients were followed for two years after the index event and major advert cardiovascular events (MACE - i.e. death, re-IM, hospitalisation for heart failure, and stroke) were analysed.

Statistical analysis

Continuous data were tested for distribution using the Kolmogorov-Smirnov test. Continuous data with normal distribution are presented as mean $\pm$ SD, with non-Gaussian distribution as median (inter-quartile range). Statistical comparison of change in apoptotic markers within individual patients was done using Friedman test and Kendall's W, post hoc analysis was performed using Wilcoxon signed-rank tests with Bonferroni correction. Relation between continuous values was described using Pearson's correlation coefficient and its significance (both crude and adjusted for confounding factors). Potential confounding factors which were taken in consideration: age, gender, BMI, presence of diabetes mellitus, arterial hypertension, Killip class, and infarct-related artery. Predictive power of analysed markers for the improvement of LVEF was analysed using ROC analysis and described by its AUC and specificity and sensitivity at cut-off. The ability of TRAIL and sFas to predict 2-year MACE was analysed using logistic regression. Two tailed p-value of less than 0.05 was considered to be significant; statistical analysis was computed using SPSS 22.0.0.1 (IBM Corporation, 2014).

2.3. Results

Baseline characteristics

A total of one hundred and fifteen patients were enrolled in the study. One-month follow-up was achieved in one hundred and one patients (87.8%). Baseline characteristics of the study population are summarized in Table 6.

Table 6. Baseline characteristics of the study population including medical history, index event and angiography characteristics and medication at discharge (n = 101)

Baseline characteristics	
Age, years (mean, SD)	59.36 ± 10.00
Male gender (n, %)	75 (74.3)
BMI (mean, SD)	28.00 ± 4.07
DM (n, %)	17 (16.8)
Hypertension (n, %)	53 (52.5)
Smoking status (n, %)	82 (81.2)
History of MI (n, %)	10 (9.9)
Index event and angiography characteristics	
Time-to-PCI, minutes (median, 25th, 75th percentile)	180 (120, 370)
Killip class	
Killip class I-II	100 (99.0)
Killip class III	1 (1.0)
CAD severity (mean, SD)	1.85 ± 0.79
Infarct related artery	
LAD (n, %)	41 (40.6)
LCx (n, %)	16 (15.8)
RCA (n, %)	44 (43.6)
Type of stent	
BMS (n, %)	16 (15.8)
DES (n, %)	32 (31.7)
Absorb (n, %)	49 (48.5)
TIMI flow 3 after PCI (n, %)	98 (97)
Complete revascularization (n, %)	60 (60.0)
Medication at discharge	
Beta-blocker (n, %)	92 (91.1)
ACE inhibitor (n, %)	90 (89.1)
Aspirin (n, %)	96 (95.1)
Statin (n, %)	99 (98.0)
Clopidogrel (n, %)	18 (17.8)
Prasugrel (n, %)	48 (47.5)
Ticagrelor (n, %)	35 (34.7)

BMI – body mass index, DM – the presence of diabetes mellitus, smoking status – smoking before admission, MI – myocardial infarction, time-to-pPCI – time from the onset of symptoms to primary percutaneous coronary intervention, LAD – left anterior descending artery, LCx – left circumflex artery, RCA – right coronary artery, BMS – bare metal stent, DES – drug eluting stent, Absorb – bioresorbable stent, TIMI flow – "thrombolysis in myocardial infarction" grade flow, complete revascularization – the absence of any stenosis of 60% or more in at least one coronary artery at discharge.

Dynamic changes in serum levels of TRAIL and sFas after STEMI

Serum levels of TRAIL and sFas measured in STEMI patients during hospitalization and at 1-month follow-up are summarized in Table 7.

Concerning TRAIL, its level decreased one day after pPCI compared to admission level (day 0). TRAIL subsequently increased on day 2 and reached its highest level measured in our study at 1-month (Table 7).

On contrary, sFas level increased one day after pPCI compared to admission. sFas subsequently decreased on day 2 and second rise of sFas was marked at 1-month (Table 7).

All changes of sFas and TRAIL levels within individual patients were statistically significant.

Table 7. Serum concentrations of soluble TRAIL and sFas

	Day 0	Day 1	Day 2	1 month	p-value[1]	p-value[2]
TRAIL (pg/mL)	56.7 (41.6; 68.1)	50.5 (34.3; 62.1)	58.8 (42.5; 77.1)	70.3 (59.0; 84.8)	**p<0.001**	W=0.209 **p<0.001**
p-value[3]		p=0.058	**p<0.001**	**p<0.001**		
sFas (pg/mL)	5073 (3716; 6415)	6370 (5032; 7867)	5548 (4322; 7070)	7024 (5898; 8875)	**p<0.001**	W=0.422 **p<0.001**
p-value[3]		**p<0.001**	**p=0.001**	**p<0.001**		

Values are given as median (25th, 75th percentile). P-values are shown for [1]Friedman test and [2]significance of Kendall's W (coefficient of concordance). In post hoc analysis, [3]Wilcoxon tests with Bonferroni corrections were used. TRAIL - TNF-related apoptosis-stimulating ligand, sFas – soluble apoptosis-stimulating fragment

Correlation between markers of apoptosis and necrosis

Statistical analysis showed a significant negative correlation between levels of TRAIL and troponin on day 1 and 2 after pPCI. sFas levels correlated with troponin only on day 2 after pPCI and the correlation was bordering on the statistical significance. Results are summarized in Table 8. Relationships between troponin and apoptotic markers TRAIL and Fas are visualised in Figure 11 and 12.

Table 8. The correlation between markers of apoptosis and troponin

	r	p-value	r (adj.)	p-value
Correlation between TRAIL and hs-cTnT				
Day 0	-0.106	0.299	-0.062	0.561
Day 1	**-0.387**	**<0.001**	**-0.379**	**<0.001**
Day 2	**-0.486**	**<0.001**	**-0.510**	**<0.001**
Correlation between sFas and hs-cTnT				
Day 0	0.127	0.216	0.152	0.154
Day 1	-0.049	0.639	-0.019	0.861
Day 2	-0.225	0.054	**-0.249**	**0.042**

Correlation is described using Pearson's correlation coefficient and its significance (both crude and adjusted for confounding factors). TRAIL - TNF-related apoptosis-stimulating ligand, hs-cTnT – high sensitive cardiac troponin T

Figure 11. Relationship between serum level of TRAIL and hs-cTnT

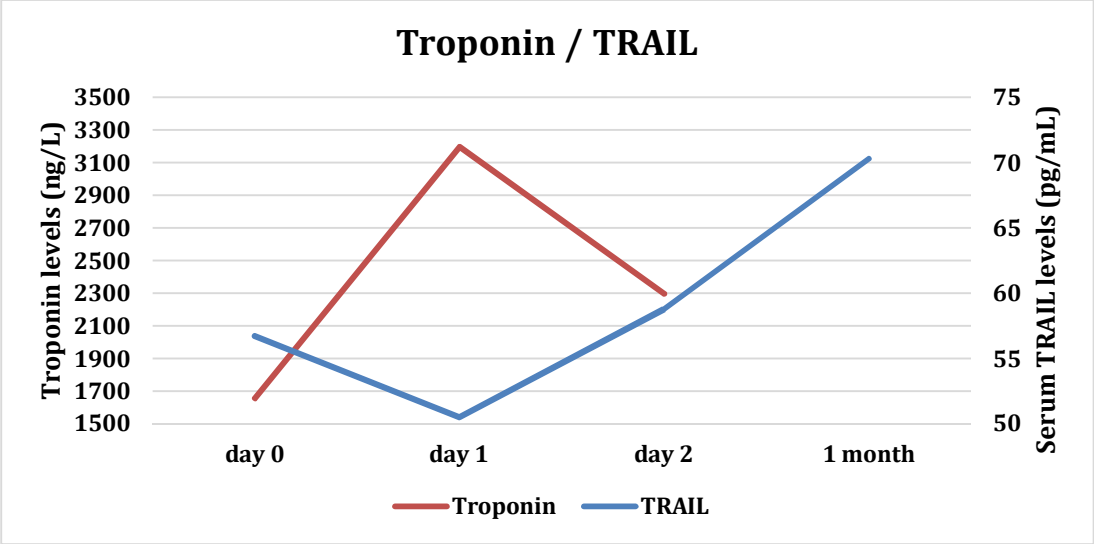
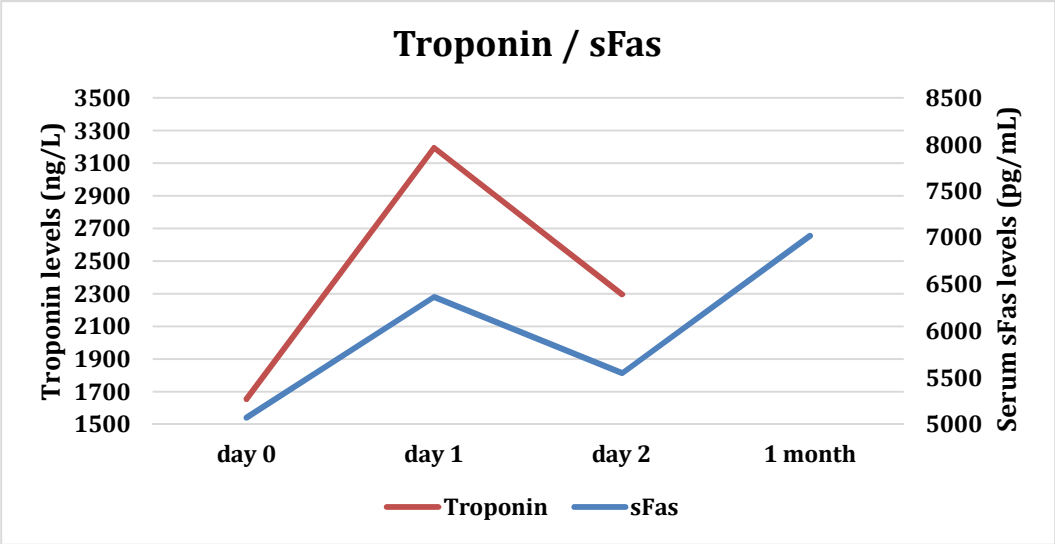


Figure 12. Relationship between serum level of sFas and hs-cTnT



Correlation between markers of apoptosis and time to pPCI

There was a negative correlation between time from the onset of symptoms to pPCI and level of TRAIL at admission (day 0: r= - 0.33, p=0.002, day 1: r= -0.19, p=0.08). No correlation was found between time to pPCI and Fas levels.

Correlation between markers of apoptosis and LVEF

Among 101 patients who completed 1-month follow-up, echocardiographic examination was available in 94 patients. Mean LVEF at baseline was 47.25% $\pm$ 8.82. One month after STEMI, mean LVEF improved to 55.78% $\pm$ 8.96, what represents an average improvement of 8.62% $\pm$ 8.16. One month after STEMI, improvement of LVEF $\geq$ 10% was present in 51 patients.

Statistical analysis showed a positive correlation between levels of TRAIL and LVEF at baseline – results are summarized in Table 4. Moreover, TRAIL levels correlated positively also with LVEF at 1-month (Table 9). There was no correlation found between Fas levels and LVEF at baseline or at 1-month.

Apoptotic markers were further tested for their ability to predict improvement of LVEF. However, receiver-operating characteristic curve analysis showed that neither TRAIL nor Fas were able to predict improvement of LVEF $\geq$10% one-month after STEMI. Similarly to apoptotic markers, also troponin failed to predict improvement of LVEF one month after STEMI. Results are shown in Tables 10 and 11.

Table 9. The correlation between TRAIL and LVEF at baseline and at 1-month (described using Pearson's correlation coefficient and its significance – crude and adjusted for confounding factors)

	r	p-value	r (adj.)	p-value
Correlation between TRAIL and LVEF at baseline				
Day 0	**0.252**	**0.013**	0.168	0.113
Day 1	**0.301**	**0.003**	**0.320**	**0.002**
Day 2	**0.455**	**<0.001**	**0.554**	**<0.001**
Correlation between TRAIL and LVEF at 1-month				
Day 0	0.158	0.136	0.076	0.495
Day 1	**0.368**	**<0.001**	**0.302**	**0.006**
Day 2	**0.505**	**<0.001**	**0.398**	**0.001**

Table 10. The correlation between troponin and LVEF at baseline and at 1-month (described using Pearson's correlation coefficient and its significance – crude and adjusted for confounding factors)

	r	p-value	r (adj.)	p-value
Correlation between hs-cTnT and LVEF at baseline				
Day 0	**-0.284**	**0.005**	**-0.287**	**0.007**
Day 1	**-0.550**	**<0.001**	**-0.542**	**<0.001**
Day 2	**-0.613**	**<0.001**	**-0.612**	**<0.001**
Correlation between hs-cTnT and LVEF at 1-month				
Day 0	**-0.460**	**<0.001**	**-0.448**	**<0.001**
Day 1	**-0.513**	**<0.001**	**-0.520**	**<0.001**
Day 2	**-0.656**	**<0.001**	**-0.690**	**<0.001**

Table 11. Ability of apoptotic markers to predict improvement of LVEF ≥10%

	AUC (95% CI)	p-value
TRAIL		
Day 0	55.0 (42.7; 67.2)	0.421
Day 1	50.2 (37.7; 62.8)	0.970
Day 2	57.9 (45.0; 70.8)	0.220
sFas		
Day 0	53.2 (41.1; 65.3)	0.606
Day 1	54.6 (42.5; 66.7)	0.455
Day 2	55.9 (43.4; 68.4)	0.362
hs-cTnT		
Day 0	54.0 (34.1; 57.8)	0.502
Day 1	53.8 (41.6; 66.0)	0.533
Day 2	50.0 (37.0; 63.0)	0.999
Peak hs-cTnT	52.9 (40.7; 65.0)	0.633

(Predictive power of analysed markers for the improvement of LVEF was analysed using ROC analysis and described by its AUC).

Two-year follow-up

Two-year follow-up was achieved in 91 patients (90%). Major advert cardiovascular events were present in 3 patients, what represents 3.3%. One patient had died, two patients had had re-MI, no one had been hospitalised for heart failure, and no one had experienced stroke.

2.4. Discussion

In our study, we demonstrated how serum levels of soluble TRAIL and Fas evolve after STEMI treated with pPCI. TRAIL decreased one day after pPCI compared to admission, then progressively increased on day 2 and reached its highest level measured in our study at one-month. Our findings confirm and extend recently published studies, which have demonstrated that TRAIL level is significantly decreased in AMI patients [163, 188]. Our results provide a detailed description of how TRAIL serum level ranges in the acute phase of STEMI as well as one month after STEMI.

TRAIL represents a promising marker of prognosis in AIM patients and is considered a protective mediator in post-AMI injury. Lower TRAIL level is associated with worse patient prognosis while higher TRAIL level seems to be protective [163, 188]. Secchiero et al. measured TRAIL in a population of 60 AMI patients and demonstrated that TRAIL levels were significantly lower at admission for AMI compared to healthy controls, increased at discharge and normalized at 6-12months [188]. In our study, more detailed examination of the first three days of STEMI showed that TRAIL reached its minimum one day after pPCI and then progressively increased. Decrease in TRAIL level 24 hours after PCI could be related to reperfusion injury. Reperfusion injury with enhanced inflammatory reaction is associated with increased level of many cytokines and proteolytic enzymes, such as matrix metalloproteinases [218]. Metalloproteinase 2 was shown to have the ability to cleave recombinant TRAIL in vitro [190]. Thus degradation of TRAIL by proteolytic enzymes released at reperfusion, such as metalloproteinase 2, could represent one of potential explanations for decreased TRAIL level after PCI. The exact molecular mechanism of TRAIL's function, however, has not yet been completely understood. In tumor cell lines, TRAIL binds to its receptors (TRAIL receptor 1 and 2) and initiates intracellular signaling cascade resulting in the apoptotic cell death [155, 156]. The effect of TRAIL on normal cells is yet unclear. Some authors reported that TRAIL-induced apoptosis could be specific to cancer cells, sparing the normal cells [219] while others described that TRAIL can induce apoptosis also in

normal human hepatocytes and endothelial cells [220, 221]. TRAIL has also been referred to as a modulator of inflammatory response [222] and some experimental data suggest that TRAIL receptors 1 and 2 can also mediate cell type-dependent prosurvival and proliferation signals [184]. In a diabetic mouse model, direct administration of TRAIL reduced development of cardiomyopathy [185] and another similar study in diabetic mice demonstrated that systemic TRAIL delivery exhibited anti-atherosclerotic activity [186]. Despite undetermined function of TRAIL at the molecular level, in clinical studies, lower TRAIL levels have been associated with worse patient prognosis while higher levels of TRAIL seem to be protective. Thus inhibition of TRAIL degradation or/and an enhancement of TRAIL availability could represent an interesting field of investigation and a new target of therapeutic intervention.

Our study also showed that TRAIL level at admission correlated inversely with the time from the onset of symptoms to PCI. The trend also continued on the 1st day after PCI. Longer the ischemic insult, lower was the level of TRAIL and higher the level of troponin. Osmancik et al. examined TRAIL level in 295 acute coronary syndrome patients and followed them for 6 months. Low TRAIL level was the strongest significant and independent predictor of death and hospitalization for heart failure [163]. In line with Osmancik results, TRAIL in our study correlated significantly with important prognostic markers: inversely with concentration of troponin and positively with LVEF. Moreover, our study showed a significant positive correlation between TRAIL and LVEF one-month after STEMI. These findings support TRAIL as a protective mediator in post-AMI injury. However, TRAIL failed to have the ability to predict improvement of LVEF at one month. We assume this failure could be explained by the size and the spectrum of our study group. Small sample size and a selected group of patients according to their willingness to cooperate in a long-term project could have influenced the results. TRAIL levels in our study group were generally higher compared to Osmancik's study [163]. There, TRAIL concentration of 44.6pg/mL at admission was identified as a cut-off value for prediction of poor prognosis. TRAIL level at admission in our study group was 56,7pg/mL. These results also correspond with a small number of end-points during our two-year follow-up. Similarly to

TRAIL, also troponin failed to predict improvement of LVEF one month after STEMI in our study, even though some recent studies reported troponin as an important predictor of LVEF after STEMI [223, 224]. Thus larger study group with consecutive patients could have provided a better understanding of TRAIL's role in LVEF recovery after STEMI. Additionally, evolution of post-AMI LVEF represents a multifactorial process influenced by several other co-factors besides cardiomyocyte loss (such as the extend of stunned myocardium, function of myocardial microvascular circulation, level of oxidative stress, inflammatory response, extracellular matrix alterations etc.). As a result, simple assessment of markers of apoptosis might not reach the ability to predict improvement of LVEF after AMI.

Concerning sFas levels, previous studies demonstrated elevated sFas levels in patients with acute myocardial infarction [160, 225]. However, sFas levels failed to correlate with infarct size [160], measures of LV remodelling [225], or patient prognosis [163]. These findings were confirmed also in a study with pure STEMI population - Nilsson et al. measured sFas levels in 48 STEMI patients prior to PCI and 24 hours after the procedure and used cardiac MRI to assess infarct size and parameters of LV dysfunction and remodelling at 5 days and 4 months after STEMI [162]. sFas levels did not show any consistent correlation with any of the measured parameters. Interestingly, level of sFas at 24 hours after PCI was significantly higher then sFas measured at admission. In concordance with Nilsson's results, sFas levels measured in our study behaved similarly. sFas level increased significantly one day after PCI compared to baseline. Serum samples obtained at later time points revealed that sFas level significantly decreased two days after PCI and increased again at one-month. However, sFas showed no correlation with LVEF at baseline or 1-month after STEMI. Increase in the serum level of sFas after AIM is a result of release from myocardial tissue [225], however, the role of Fas-mediated apoptosis in post-AMI injury remains yet undetermined. Studies with heart failure patients demonstrated association of increased sFas levels with worse patient prognosis [164-166]. In our STEMI group, significantly highest sFas levels were measured 1-month after STEMI, but still sFas levels were dramatically lower compared to sFas levels reported in high-risk heart failure patients [165]. Thus

measuring sFas levels in the acute phase of AIM that are significantly lower and without prognostic value, seems to be inefficient.

In conclusion, our results demonstrate how serum levels of TRAIL and sFas evolve after STEMI treated with pPCI. TRAIL decreases one day after pPCI compared to admission, then increases on day 2 and reaches its highest level measured in our study one month after STEMI. TRAIL levels show significant inverse correlation with troponin levels and with time-to-PCI interval. TRAIL correlates positively with LVEF at baseline as well as with LVEF one month after STEMI. Low TRAIL levels are associated with worse LVEF after STEMI. Thus TRAIL seems to be a protective mediator of post-AMI injury. On contrary, sFas level increased one day after PCI compared to admission, then decreased on day 2 and increased again one month after STEMI. sFas failed to correlate with LVEF at baseline or at one month. The role of sFas in post-AMI injury is yet uncertain.

Study limitations

Our study limitations are related mainly to sample size, patient selection, follow-up completion and absence of a control group. Patients participating in the study were not enrolled consecutively but selected, due to better coordination with the project from a long-term prospective. Also, one-month follow-up was not achieved in 12.2% of patients, of who 2-year follow-up was not completed in 10%. These limitations could have influenced our results.

3. Conclusion

Coronary artery disease remains a leading cause of death worldwide, accounting for about 16% of all deaths. Clinical syndromes of coronary artery disease cause higher mortality, morbidity and also financial burden in developed countries than any other group of diseases. Despite significant improvement in the treatment of CAD in the past decades, many patients subsequently suffer from left ventricular dysfunction and heart failure. Post-AMI heart failure represents a high-risk condition with a poor long-term prognosis. Necrosis was thought to be the sole cause of death in myocardial infarction for a long time. However, recent studies provide growing evidence that also apoptosis represents a significant contributor to myocardial damage after AMI and participates also in the process of subsequent LV remodelling and development of heart failure. Thus recognizing a sensitive apoptotic marker that would help define high-risk patients after AMI and offer new therapeutic strategies is of a great importance. Inhibition of apoptosis through anti-apoptotic therapy could represent a new way forward to improve poor prognosis of these patients.

Based on the literature research, several apoptotic markers have been evaluated in serum of patients with coronary artery disease. Among intracellular apoptotic mediators, serum levels of caspase-3 fragment - p17 peptide - brought some hopeful results in one study with STEMI patients; however, further research is needed to prove it as a substantial marker of apoptosis. Among extracellular markers, several soluble forms of receptors and their ligands were evaluated in clinical studies with AMI patients. The most promising apoptotic marker seems to be TRAIL, since its serum level represents an important predictor of prognosis in AMI patients. Therefore, we decided to arrange a clinical study with TRAIL in an effort to better understand its role in the pathophysiological mechanisms occurring after AMI.

The results of our study demonstrate how serum levels of TRAIL evolve in patients with STEMI treated with pPCI. TRAIL decreases one day after pPCI compared to admission, then

increases on day 2 and reaches its maximum one month after STEMI. TRAIL levels show significant inverse correlation with troponin and with time-to-PCI interval. At the same time, TRAIL correlates positively with LV ejection fraction at baseline as well as with LVEF one month after STEMI. Low TRAIL levels are associated with worse LVEF after STEMI. The results of our study provide further evidence that TRAIL represents a protective mediator of post-AMI injury. We believe that these results brought us a step forward to better understanding of TRAIL's role in post-AMI injury and provide important information for building up next research projects.

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5. List of publications related to dissertation topic

1. **Teringova E,** Kozel M, Knot J, Kocka V, Benesova K, Tousek P. Relationship between TRAIL and left ventricular ejection fraction in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. Biomed Res Int. 2018. Currently in press.

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2. **Teringova E,** Tousek P. Apoptosis in ischemic heart disease**.** J Transl Med. 2017 May;15(1):87. doi: 10.1186/s12967-017-1191-y.

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3. Osmancik P, **Teringova E**, Tousek P, Paulu P, Widimsky P. Prognostic value of TNF-related apoptosis inducing ligand (TRAIL) in acute coronary syndrome patients. PLoS ONE. 2013;8(2):e53860. doi: 10.1371/journal.pone.0053860.

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