Università degli Studi di Ferrara

DOTTORATO DI RICERCA IN
"Medicina Molecolare e Farmacologia"

CICLO XXX

COORDINATORE Prof. Di Virgilio Francesco

Factor XIIIA in myocardial infarction: a dual role as specific prognostic biomarker and proposed reparative molecule in left ventricular remodeling.

Settore Scientifico Disciplinare MED/03

Dottorando
Dott. Vigliano Marco

Tutore
Prof. Gemmati Donato

Anni 2015/2017

INDEX

1. INTRODUCTION

1.1 CARDIOVASCULAR DISEASE, EPIDEMIOLOGY

Cardiovascular diseases (CVD) are the first cause of death in the world. 17.7 million people die every year from cardiovascular disease, resulting in 31% of all global deaths [1]. In Europe as a whole, diseases related to myocardium and to the circulatory system take the lives of 3.9 million people every year, 45% of all deaths (40% and 49% of all deaths respectively in men and in women). CVD, mainly culprits the heart [ischemic heart disease (IHD) and coronary artery disease (CAD)], the brain (cerebrovascular disease, e.g. stroke) and the blood vessels (aorta and other main arteries). In Europe, among CVDs ischaemic heart disease and stroke are the first and the second single causes of mortality respectively. By comparing with cancer, the second most common cause of death, CVD causes 2 millions of deaths for year in Europe (23% of all deaths). These data provide a generalized indication of cardiovascular diseases incidence. However, there are variations, sometimes remarkable, within specific macro geographic areas and among different nations. Accordingly, women CVD mortality ranges from 25% in Denmark to 70% in Bulgaria, while in men, ranges from 23% in France to 60% in Bulgaria. Among developed nations, death rates from CVD are higher in the Eastern Europe, intermediate in the United States and Western Europe, and lower in Japan.

Over the past 30 years, mortality rates form CVD have been declining in most European countries, mainly due to downward trends in some impactful CVD risk factors like smoking, alcohol consumption, circulating cholesterol and thanks to the improvement of acute patients care technique and technology. Conversely, survivor patients from infarction have in turn unavoidable increasing in hospitalization rate with significant cost for society. Only in EU, the cost due to CVD is around € 210 billion year, considering health care, productivity losses and informal care costs of people with cardiovascular diseases [2].

1.2 MYOCARDIAL INFARCTION: DEFINITION

The acute myocardial infarction (AMI) is the death of heart muscle caused by prolonged and severe ischemia, characterized by irreversible alteration of myocardial anatomy, unlike what happens in angina where the alteration is reversible. It may be the first manifestation of Coronary Artery Disease (CAD) or a minor event in a chronic Ischemic Heart Disease (IHD) [3].

AMI is the ultimate result of a complex and articulated series of pathological events that progressively evolve during the life, often starting several years before acute accident.

The main underlying disease in blood vessels that results in AMI is known as atherosclerosis. It is responsible for a large proportion of CVDs and in particular, it is a complex pathological and inflammatory process affecting the wall of blood vessels. The process starts with the exposition of the endothelium to increased levels of low density lipoprotein (LDL) and other substances leading to an intense change in the regular properties and in the permeability of endothelium to lymphocytes and monocytes, which migrate in the deep layers of the vessels dramatically altering the wall steady state. Over the years, this leads to consequent reactions, propagating and cumulating in fatty material and cholesterol deposition. These deposits, are the main composition of the plaques, and considerably modify the inner surface of vessels, which become irregular and less pliable, and lumen become narrow, making it harder for blood to flow through[4]. In most case of AMI the initial events is a sudden modification of this atheromatic plaque like breakage, creaking, ulceration or erosion. With the rupture of the plaque, lipid fragments and cellular debris are released into the vessel lumen and the exposure to sub-endothelial collagen and the content of necrotic plaque induce platelet activation and adhesion with thrombus formation. Thrombus, within minutes, grows until the vessel is completely closed. The arrest of blood circulation rapidly drops the pressure to zero in the coronary branches following the blockage. The downstream area of the obstruction can only be sprayed if it is reached by other vessels, otherwise tissue necrosis will inevitably occur. Necrosis begins after 20-40 minutes from occlusion, after that it becomes irreversible, even if the blood flow is restored. For this reason, this time window defines the so-called "no-return point"[5] (Figure 1).

With less frequency (5-10%), AMI is caused by other mechanisms different to atherosclerotic plaque rupture and subsequent thrombosis, such us vasospasm, which can reduce coronary blood flow without obstructive coronary atherosclerosis[6].

Clinical score and morphological characteristics of a myocardial infarction depend on:

-localization and severity of coronary occlusion;

-area extension of the occluded vessels;

-number of vessel involved;

-duration of the occlusion;

-presence/absence of collateral circles.

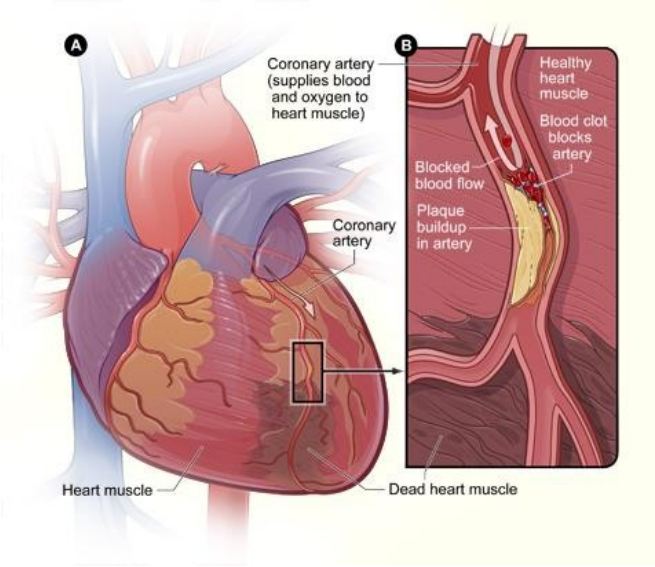

Figure 1. Acute myocardial infarction with consequent necrosis of a cardiac district due to the occlusion of the left coronary artery by a thrombus resulting from atheromatous plaque rupture.

In the recent past, the World Health Organization (WHO) had defined MI as the presence of at least two of these three characteristics[7]:

- Typical symptoms such as chest pain and discomfort;
- Detection of rise of circulating cardiac enzyme (CK-MB, Cardiac Troponin);
- Presence of the characteristic electrocardiogram (ECG) pattern with Q-waves development.

However, tanks to the development of even more sensitive and tissue specific cardiac biomarkers and ever more sensitive imaging techniques, that allows the detection of very small amount of injury and damage in the myocardium, the definition has become more detailed. Following the guideline of the Third Universal Definition of Myocardial Infarction the term MI should be used when there is a clinical setting consistent with acute myocardial ischemia and evidence of myocardial necrosis. Under this condition any one of the following criteria meets the diagnosis for AMI:

-Detection of rise and/or fall of cardiac biomarker values (preferably cardiac Troponin, cTn) with at least one value above 99th percentile upper reference limit (ULR) and with at least one of the following:

-Symptoms of ischemia.

-New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB).

-Development of pathological Q waves in ECG.

-Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

-Identification of an intracoronary thrombus by angiography or autopsy.[3]

1.3 ELECTROCARDIOGRAPHIC DETECTION OF MI

An ECG is a painless test that detects and records heart's electrical activity using electrodes placed on the skin. The electrodes detect the tiny electrical changes that arise from the heart muscle's electro-physiologic pattern of depolarizing and repolarizing during each heartbeat. The conventional 12-lead ECG measure the overall magnitude of the heart's electrical potential from 12 different angles (leads) and is recorded over a period of time (10 seconds) generating a graph of voltage versus time, the electrocardiogram (Figure 2). In healthy Population, the heart pulse is formed in the Sinus-node and propagates through the atrium that contract. This electrical activity generates the so-called P wave. After that, the impulse propagates to the atrioventricular node and the excited ventricles contracts. This event is represented by the QRS wave complex. The last stage consists in the repolarization of the cardiac muscle that consequently generates the T wave. ECG, has a key role in the diagnosis of AMI, so is fundamental to perform it promptly after clinical presentation (within 10 min)[8]. In particular, the ST segment is an indicator of blood flow, myocardial oxygen level and coronary assessment.

Based on ECG, AMI can be classified as:

- ST elevation MI (STEMI)
- Non-ST elevation MI (NSTEMI).

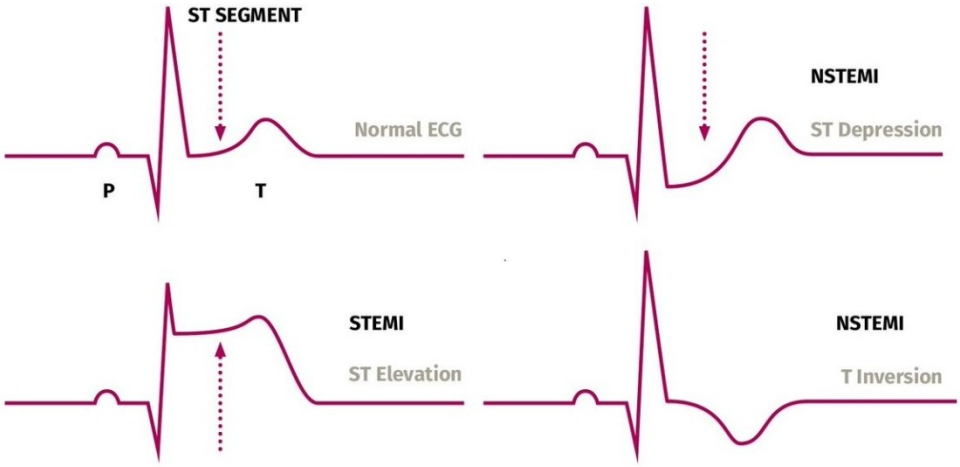

Figure 2. Main differences in the electrocardiographic profile: Normal, STEMI, NSTEMI

During the initial stage of the acute phase of MI, total occlusion of an epicardial artery produces a ST segment elevation (STEMI). A large amount of patients initially presenting with ST elevation, evolve in Q-waves on the ECG and are diagnosed as having sustained a Q-wave MI. Otherwise, when the obstructing thrombus is not totally occlusive, the obstruction is transient or a collateral network is present, no ST elevation is seen. Such patients are initially considered to be experiencing unstable angina or NSTEMI.

In case of necrosis, both of these clinical frameworks may further evolve. One of these evolutions is represented by the development of abnormal Q wave (after 8-12 h), that usually reflects the presence of necrotic tissue. Acute changes in the ST segment or evolution of the Q-waves are useful for dating the event, hypothesizing which coronary is occluded and estimating the extent of damaged myocardium.

Nevertheless, the ECG is not enough to completely and correctly perform a MI diagnosis: in fact, alteration in the ST-segment can also be observed in other pathological conditions such as ventricular hypertrophy, left branch block or pericarditis.

1.4 BIOMARKERS FOR CARDIAC INJURY DETECTION

Over the last few decades several myocardial damage and necrosis biomarkers have been evaluated, but only a few have been widely used in the diagnosis and prognosis of CVDs. An ideal cardiac biomarker should be specific to the heart muscle (and therefore absent in the smooth muscle), should be released quickly and only after the onset of the damage, and should be quantifiable and informative about the extent of the damaged area[9].

Until a few years ago, creatine kinase (CK) was the most commonly used cardiac biomarker in the laboratory routine. CK is an enzyme expressed in various tissues and cell types that catalyses the conversion of creatine to phosphocreatine (PCr), an energy reservoir in cells and tissue that consumes ATP rapidly. In cells, the CK enzymes consist of two subunits, which can be either B (brain type) or M (muscle type), that give origin to three different isoenzymes: CK-MM, CK-BB and CK-MB. The last one, CK-MB, has the advantage that is not present in significant concentrations in not-cardiac tissues and is considerably more specific. After an MI, serological level of CK-MB rises within 4-8 hours, reaches a peak within 24 hours, and returns to normal levels after 48-72 hours. Another indicator related to CK-MB is the CK ratio. Two serological forms of MB, MB1 and MB2 are known, usually in a ratio 1:1 (MB2/MB1). At the onset of MI, the tissue form MB2 is released in small amount, not responsible for total CK-MB elevation, but enough to increase the ratio MB2/MB1 >1.5 becoming informative for early MI diagnosis.

Another biomarker is Myoglobin (Mb), an iron- and oxygen-binding protein found in the muscle tissue that can be found in bloodstream only after muscle injury. Although it is not specifically released by heart muscle, is an early marker of muscle damage because it is rapidly detected in the bloodstream (within 1-2 hours) originating from the infracted myocardium and it rapidly returns within normal ranges after 24 hours. Finally, normal circulating Myoglobin levels detected between the 4^{th} and 18^{th} hour after the onset of symptoms may exclude myocardial infarction [10].

In recent years, CK (-MB), Mb, and other necrosis indicators such as the enzymes lactate dehydrogenase (LDH) and the transaminases GOT (glutamic oxalacetic transaminase) and GPT (glutamic pyruvic transaminase) have been increasingly replaced by cardiac troponins in laboratory practice. Cardiac troponins are specific to the myocardium and are considered the ideal cardiovascular biomarkers for their high specificity and sensitivity. Cardiac Troponin (cTn) is composed by: Troponin C (TnC) which binds Calcium ions (Ca^{2+}); Troponin I (cTnI) which binds actin and inhibits the actin-myosin interaction; and Troponin T (cTnT) which binds tropomyosin and facilitates contraction. In the heart muscle the cTnT and cTnI possesses amino acid sequences (epitopes) different from those of the skeletal muscle form. These differences permitted the development of quantitative assays using highly specific monoclonal antibodies. After heart damage caused by an MI, cardiac troponins are slowly released in the blood flow and in plasma levels they rise rapidly after 3-6 hours and may remain elevated for 7-10 days, in relation to the extension of the damage. Due to this delay, a single negative screening of cTns is not sufficient to

definitely exclude MI, so a second blood withdrawal six hours after the onset of symptoms has to be repeated[11].

1.5 TREATMENT THERAPIES

Based on ECG and biomarkers results findings, the patient is given the most suitable therapy under his condition. The main objective of STEMI patient treatment is the rapid reperfusion of ischemic myocardium. Over the years, two main reperfusion strategies have been developed: the pharmacological one, defined as thrombolysis, and the mechanical one, the "percutaneous transluminal coronary angioplasty (PTCA).

1.5.1 Thrombolysis

Thrombolytic therapy consists in the intravenous administration of drugs that can activate endogenous plasminogen (zymogen) to plasmin (active enzyme), which breaks down fibrin meshwork and dissolves coronary thrombus, restoring the flow through the occluded coronary artery[12]. Guidelines suggest use of several approved thrombolytic agents: Streptokinase (SK), Urokinase (UK), recombinant tissue-type plasminogen activator (rtPA), anisoylated plasminogen streptokinase activator (APSCA).
The effectiveness of thrombolytic therapy is strongly time dependent. The amount of myocardium that can be saved by necrosis depends on how quickly the thrombus is removed from the vessel and blood flow restored. The efficacy of this approach is highest in patients hospitalized within the first-third hour after the onset of symptoms and decrease progressively over time. Different aspects concerning patient anamnesis and comorbidity influence the decision whether or not to administer thrombolytic agents. However, fibrinolytic therapy is associated with significant (0.9-1%) increase in stroke episodes during the first day of treatment mainly caused by cerebral hemorrhage and thrombotic episodes in selected subset of patients. Significant predictors of intracranial hemorrhage are: elder age, low body weight, female gender, previous cerebrovascular disease, and diastolic or systolic hypertension at the time admission[13].

1.5.2 Angioplasty

Nowadays, primary PTCA (or angioplasty) can be considered the elite reperfusion strategy in STEMI patients. In fact, it is demonstrated that primary angioplasty in STEMI significantly lowers the rates of death, stroke, recurrent ischemia and reinfarction compared with fibrinolytic therapy [14]. PTCA is performed after diagnostic angiography with the help of X-ray based imaging and contrast agent injected into the blood vessel so any obstructed segments can be highlighted. The procedure is somewhat articulated: starting from a peripheral artery (usually the femoral artery), the occluded coronary artery is reached by the introduction of a guide wire. At this point, a catheter with the balloon is inserted into the blockage site. The balloon is inflated, also several times, to compress the blockage and widen the passage. This treatment may be repeated at other blocked or narrowed sites. Depending on the clinical and/or anatomical criteria, can be applied one or more coronary stents that helps prevent arteries from becoming narrow or blocked again in the months or years after PTCA[15,16] (Figure 3). To get the best benefit, PTCA should be performed within two hours of first medical contact[17]. Absolute contraindications to PTCA are: absence of hemodinamically significant stenosis (< 50% of vessel lumen), allergy to the contrast agent, impossibility of obtaining arterial access.

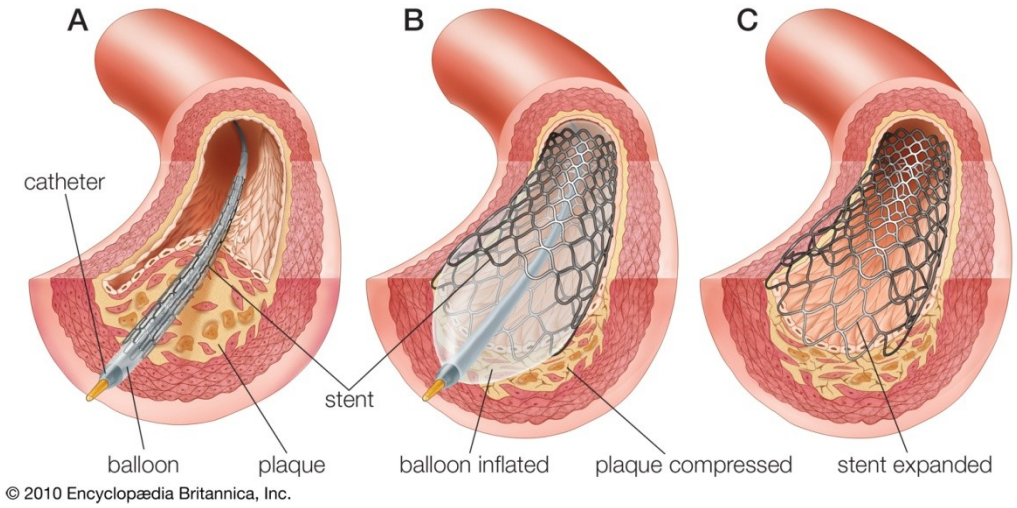

Figure 3. Representation of balloon angioplasty with stent application.

1.5.3 Drugs and Secondary Prevention

Patients who survive a STEMI represent a high risk group of new events and premature deaths. 8-10% of these patients undergo a re-infarction within 1 year of discharge[18].

Lifestyle changes and precise secondary prevention therapies are crucial to reduce this risk. Smoking stoppage, tight blood pressure control, proper diet and body weight control, moderate aerobic exercise and stress management represent the main risk factors that can be modified. Among the secondary prevention therapies it is worth quoting at least the antiplatelets drugs, mainly aspirin is essential, both in acute and lifelong therapy[15]. In fact, a low dosage for long-term of acetylsalicylates (ASA), irreversibly blocks the formation of thromboxane A2 in platelets resulting in an inhibitory effect on platelet aggregation, resulting in blood fluidification. Frequently, aspirin is combined with an ADP receptor inhibitor, such as Clopidogrel, Prasugrel, or Ticagrelor to prevent blood clots. This is called double antiplatelets therapy (DAPT). United States and European Union guidelines disagree somewhat about how long, and for what indications this combined therapy should be continued after surgery. U.S. guidelines recommend DAPT for at least 12 months, while EU guidelines recommend DAPT for 6–12 months after a drug-eluting stent placement[19]. However, they agree that aspirin should be continued indefinitely after DAPT is complete.

1.6 PROGNOSIS

The mortality rate for STEMI patients is influenced by several concomitant factors, primarily age, intervention time, Killip class, prior infarction, diabetes mellitus, renal failure, and treatment strategies. In the last years, short-term mortality and in-hospital mortality have been greatly reduced by modern coronary revascularization techniques and secondary prevention therapies. However, the long-term prognosis is conditioned by not so rare post-MI complication such as the ventricular remodeling and the impaired ventricular function, resulting in a high incidence of adverse post-MI events such as heart failure, malignant ventricular arrhythmias and cardiac death.

1.7 LEFT VENTRICULAR REMODELLING

Left ventricular remodelling (LVR) is a clinically characterized process of alterations in the size, shape and function of the ventricle following a cardiac injury like an MI. Three crucial mechanisms dived in two chronological steps contribute to LVR:
-The early step (within the first 72 hours) of early infarct expansion;

-The late steps (beyond 72 hours) composed by infarct extension into non infracted myocardium;

-The late hypertrophy in the remote LV.

To better understand this process, is necessary to explain the phases that led to the creation of a mature scar out of infracted myocardium. The initial inflammatory phase after reperfusion is characterized by cytokine/chemokine signalling and the infiltration of neutrophils that begins to clean the infarct of necrotic cardiomyocytes and cellular debris. This is followed by the beginning of the proliferative phase during which monocytes invade the infarct and differentiate into macrophages. During the proliferative phase, neovessels are formed to support the proliferation of novel myofibroblasts in the infracted area. The myofibroblasts elaborate extracellular matrix proteins including the collagen necessary for a properly elastic and extensible scar formation. As the neutrophils complete the process of clearing the necrotic cells from the infarct area, they enter apoptosis phase and are phagocytised by macrophages. This induces the macrophages to synthesize and release TGF-β which down regulates inflammation promotes the transition from the proliferative phase to the maturation phase. During this final phase, fibroblasts undergo apoptosis, neovessels regress from the infarct and the collagen-based matrix matures by condensation into mature scar[20].

The expansion of the infarct comes from an unrestrained degradation of collagen bonds by serine proteases and the activation of matrix metalloproteinases (MMPs) in particular MMP9 released by neutrophils[21]. Collagen network associates and align adjacent cardiomyocytes through intracellular myofilaments links, optimizing strength development and uniformly distributing tension on the ventricular wall, these complex and well tuned action properly prevent unwanted sarcomer deformation. Collagen degradation and myofibrils necrosis causes the loss of this support tissue, and consequently, makes this region more sensitive to deformation. Therefore, in the necrotic area we can assist to sliding and realigning of cardiomyocytes causing in the affected area wall thinning and distension is defined as infarction expansion. As a result, affected area thinning and cavity dilation are observed. That acute ventricular dilation characterized by infarcted wall thinning and distension is defined as infarction expansion [22]. The second key point is the expansion of the infarction in an adjacent non-ischemic region. There, cardiomyocytes juxtaposed between the infarct scar, consisting of non-contractile tissue, and viable contracting myocardium, are submitted to a detrimental and anomalous mechanical stress. Biologically, mechanical stress induces oxidative stress (iNOS) and active pro-inflammatory pathways (TNFalfa)[23,24]. This combination leads to apoptosis of

cardiomyocytes in this border region and their replacement with fibrous tissue and consequent and further extension of infarct scar. This phenomenon compromises the contractile functionality of the whole ventricle. It suggests that the later, yet unavoidable progression, of heart failure in patients presenting large MI is partially driven by this insidious mechanism[25]. The last aspect concerns the remote areas of the ventricle, vital non-ischemic tissue beyond the region adjacent to the infarcted area. This tissue is made up of adult cardiomyocytes, differentiated cells that cannot proliferate but respond to stress with hypertrophic growth. An overload condition, especially of volume, due to an extensive heart attack causes eccentric hypertrophy of the cardiomyocytes in the remote region. A gene expression pattern that has remarkable similarities with that of fetal development is activated[26]. It must be taken into account that in normal hearts both systolic and diastolic tension are at maximal value at midventricle, intermediate level at the base, and lowest level at the apex. As a result of post-infarction expansion, the ventricle loses its elliptical form and takes up spherical configuration. Under this new format, apical parietal tension is significantly increased to reach midventricle level, with midventricle level also being increased. In addition to that redistribution, diastolic parietal tension is shown to be significantly higher than systolic tension.

LVR, at least at beginning, can be described as a positive adaptive process. In fact it allows the heart to maintain its function despite the overload of volume and pressure in the acute phase of cardiac damage, and a fibrotic scar with significant tensile strength serves to contain and prevent rupture. However, this remodelling of the LV continues progressively in response to increases in wall stress, provoking cardiomyocytes hypertrophy in the infarct border zone, wall thinning and chamber dilatation. This global adverse remodelling response leads to increases in both end-diastolic and end-systolic volume and a reduced ejection fraction responsible in turn of severe heart failure[27].

1.7.1 Drugs therapy to counteract LVR

The early reperfusion of the occluded artery, responsible for infarction, is the key therapeutic strategy to limit the extent of infarct and maintain ventricular function. ACE inhibitors and β-blockers constitute the therapeutic standard in the post-infarct phase. The effects of ACE inhibitors and beta blockers appear to be complementary. They act by modifying the remodeling process, reducing morbidity and mortality related to heart failure. Administration of ACE inhibitors causes resolution of symptoms and significantly

improves survival of patients with heart failure, positively influencing post-infarct cardiac remodeling, significantly reducing the left ventricular end-diastolic and left-ventricular volume indexes and increases the ejection fraction. Mechanisms are related in part to peripheral vasodilatation and the attenuation of ventricular dilatation. In addition, ACE inhibitors have a direct effect on the myocardial tissue, preventing the inappropriate growth and hypertrophy of myocytes, stimulated by angiotensin II and other growth factors[28]. Several clinical trials have shown the benefits of β-blockers in the treatment of heart failure, thanks to the improvement of the function of the left ventricle. B-blocking agents when associated with ACE inhibitors have been shown to reduce the mortality of patients with heart failure.

Novel therapeutic strategies should be focused to limit remodeling by controlled orchestration of the molecular and cellular factor involved in tissue repair, including hypertrophy, fibrosis, and the capillary microcirculation. For instance, the use of novel IIb/IIIa platelet inhibitors, by reducing platelet aggregation, could preserve capillary microcirculation; further improving cardiomyocytes salvage and limit remodeling. Furthermore, contrasting the breakdown of extracellular collagen scaffold, e.g. with MMP inhibitors, could stiffen the infarct zone, arrest infarct expansion and ventricular dilatation reducing the increased wall stress that initiates the intracellular signalling for enzymatic degradation of collagen[29]. Despite the currently available therapies, post-infarct ventricular remodeling and progression to heart failure significantly affect patient survival.

1.8 COAGULATION FACTOR XIII

1.8.1 FXIII, structure and main functions

Coagulation factor XIII (FXIII) is a circulating transglutaminase that works in the final steps of blood coagulation cascade and it plays a pivotal role in maintaining the mechanical and functional integrity of fibrin clots.

FXIII is a tetrameric molecule composed of 2 A-subunits of 83.2 kDa and 2 B-subunits of 79.7 kDa that are held together non covalently in a heterologous tetramer of 325 kDa [30]. FXIII is present in plasma either as hetero tetramer bounded to the B subunits (FXIIIA2B2) or alone as intracellular homodimer (FXIIIA2). The A-subunit, the active subunit, is mainly synthesized by hepatocytes, monocytes, megakaryocytes as well as by their early precursors, and platelets that express about 3% of the total FXIII-A subunits[31].

The B-subunit serves as a carrier for the catalytic A-subunit in plasma, is synthesized by the liver, and is secreted as a monomer that binds circulating A-subunit. The gene for A-subunit (locus 6p24-p26) encodes for a protein of 731 amino acids and the gene for B-subunit (locus 1q31-q32.1) encodes for a mature protein of 641 amino acids[32,33]. The A-subunit contains the cleavage site for the activation peptide consisting of 37 amino acids (AP; R37-G38), the active site (-Tyr-Gly-Gln-Cys-Trp), and the substrate-recognition regions. The activation of the FXIII is the result of a multi-step process that ends with the exposure of the active site. FXIII is proteolytically activated by Thrombin (FIIa), which hydrolyzes the activation peptide and, in the presence of Ca^{2+} ions, the plasma FXIII-A dissociates from the B subunits and assumes the same active enzyme conformation of the cellular component (FXIIIA2). FXIII-A can be also activated without proteolytic cleavage: in non-physiological conditions, with high levels of Ca^{2+} (> 50 mM) released from the activated platelets during vessel injury, the tetrameric form spontaneously dissociates into the active form A2 (Figure4)[34].

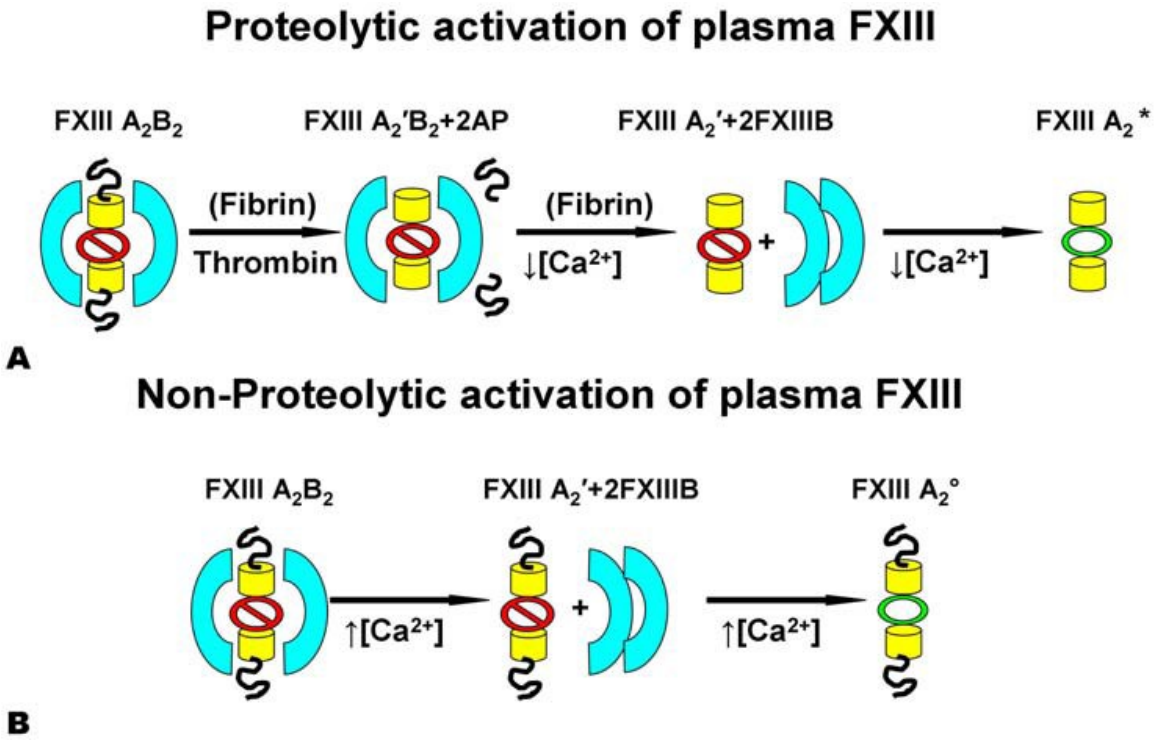

Figure 4. Mechanisms of FXIII activation. A: Proteolytic activation of FXIIIA2B2 tetramer in presence of thrombin; B: non-proteolytic activation of FXIIIA2B2 tetramer in presence of high concentration of Ca^{2+} ions.

Fibrin polymers are an important cofactor to factor XIII activation, in fact Thrombin hydrolysis of plasma FXIII is accelerated in the presence of fibrin-I, originated from Fibrinogen[35].

Fibrinogen is a dimer of three chains (Aα, Bβ and γ) held together by disulfides bonds. This homodimer (AαBβγ)2 is rod-like and composed of two globular D-regions joined to a centrally located E-region by coiled coils of Aα, Bβ, and γ chains. The serine protease thrombin cleaves fibrinopeptides A and B (FpA and FpB, respectively) from the N-termini of the Aα and Bβ chains, thus converting fibrinogen into fibrin. Loss of FpA results in fibrin-I; the additional loss of FpB yields fibrin-II. The resultant A and B knobs within the E-region of a fibrin monomer can align with complementary a and b holes in the D-regions of other fibrin monomers to produce a soft clot[36].

Activated FXIII later catalyzes the formation of γ-glutamyl-ε-lysyl covalent crosslink in the fibrin network and in fibrin-enzyme complexes leading to formation of a mechanically stable and degradation resistant hard clot. In fact, in addition to the fibrin crosslink function, the FXIII possesses numerous other substrates showing an active role in different physiological mechanisms.

In the haemostatic process, the FXIII also interacts with the FVa, whose presence contributes to its enzymatic activity, with the von Willebrand factor and with the Thrombospondin-1, a platelet glycoprotein that plays a role in the platelet aggregation. On the other hand, FXIII plays a important role in the fibrinolytic system interacting with α2-antiplasmin (glycoprotein which inhibits the activity of Plasmin, preventing fibrin degradation), TAFI (Thrombin Activable Fibrinolysis Inhibitor), Vitronectin (involved in regulation of coagulation, fibrinolysis and cell adhesion)[37].

Not less relevant is the role of FXIII in angiogenesis. Indeed, FXIII is involved in the down-regulation of Thrombospondin-1(important anti-angiogenic factor) and in over-expression of Vascular Endothelial Growth Factor Receptor 2 (VEGF2)[38]. There are also numerous interactions with other macromolecules of the extracellular matrix (ECM) such as fibronectin, vitronectin, collagen, laminin; such interactions modulate cellular functions and the formation/stability of the extracellular matrix[39]. Factor XIII interact with various components with active role in haemostasis, fibrinolysis and angiogenesis showing a generalized function throughout the entire “wound healing” process (Figure 5).

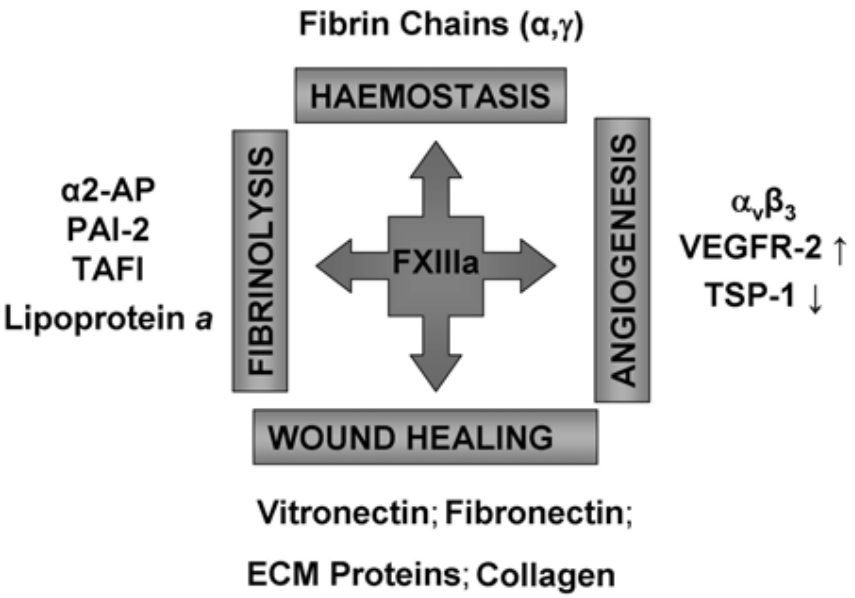

Figure 5. Schematic representation of the multitask role of FXIIIa and the relatives different substrates

1.8.2. FXIII and Cardiovascular Disease

The interactions of FXIII with the above mentioned systems are reflected within the pathophysiology of complex diseases like chronic venous lesion [40], and cardiovascular disease.

Regarding cardiovascular diseases and in particular post-myocardial infarction healing, one of the first important evidences that directly demonstrate the essential role of FXIII in the stability of infarct scar has been obtained from an experimental animal model with genetically reduced FXIII-A levels[41]. In this study, 100% of mice with genetically reduced FXIIIA levels died within five days after induced MI. Left ventricular rupture were the cause of death, and no mice died due to severe bleeding or internal haemorrhage. Accordingly, intravenous FXIII treatment gave back a survival rate comparable to that of wild-type mice, though the cardiac magnetic resonance revealed anomalous left ventricular remodelling responsible for poor heart performances. The role of FXIII in supporting the post-MI healing was further confirmed and demonstrated by additional studies suggesting and supporting the use of FXIII as supplementary therapy to avoid anomalous left ventricular remodelling and loss of heart functions[42,43]. In addition, FXIII-based advanced treatments have been recently proposed to counteract the negative post-MI effects suggesting even intramyocardial injection of FXIII-modifiable biomaterial[44]. Finally, recent papers dealt with the role of platelet rich plasma (PRP) in MI healing.

Intra-myocardial injections of autologous PRP have been successfully utilized during MI to accelerate and optimize local healing and contrast ROS-generation in the ischemic/reperfused heart[45,46]. Altogether these data support the hypothesis that appropriate levels of FXIII-A at the injury site or of its derived by-products is essential requisite for optimal myocardial healing particularly in the earliest phases. Some studies at the end of the seventies had already examined in detail the changes in the levels of FXIII and fibrinogen after ischemic events[47]. More recently, the interest towards FXIII fluctuations during the acute venous or arterial accidents or comparing the presence/absence of the ischaemic event has reawakened the interest in making attempts at ascribing to this phenomenon diagnostic/prognostic information[48,49].

Recently, we have also published a study on the dynamics of factor XIII, founding a relation with MI prognosis[50]. A Population of 350 MI patients (STEMI and not STEMI) was enrolled in this study, measuring FXIII-A circulating levels during the first six days adding a control after 30 days. A one year follow-up was performed for all patients. The primary endpoint was a composite of major adverse cardiac events (MACE) consisting of cardiovascular death and heart failure (HF) at 30-days and one year. The experimental data collected showed an acute and transient fall in FXIII-A levels occurs in the whole cohort (Figure 6).

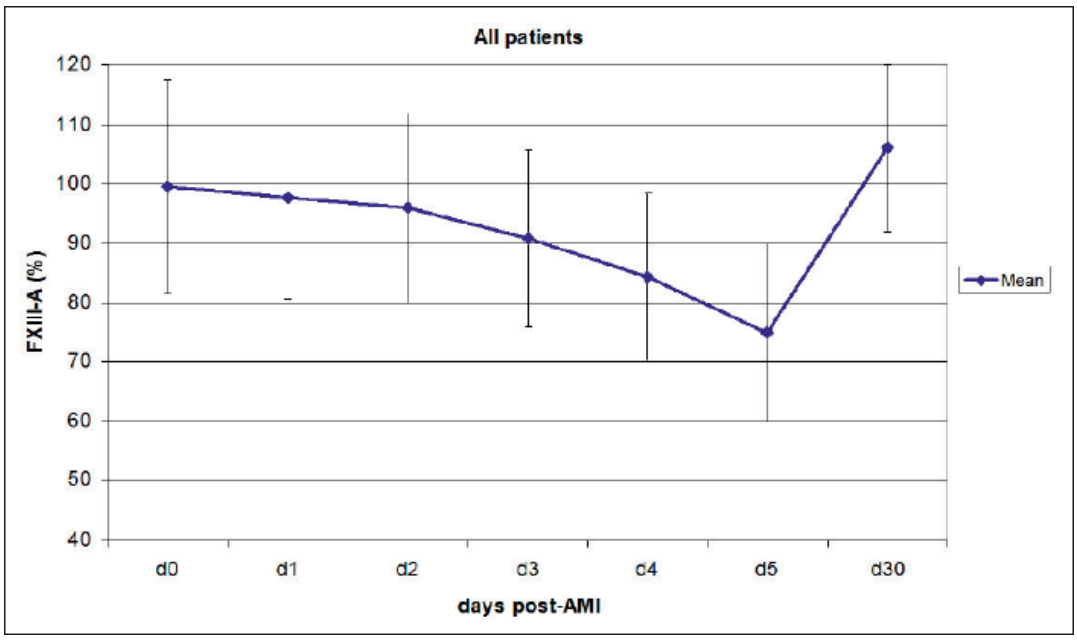

Figure 6. Dynamics of FXIII-A (mean and SD) at scheduled times in the whole cohort of MI patients.

Furthermore, we discovered patients undergoing excessive FXIII-A consumption at the time of MI were more prone to die or to develop HF. Finally, we focused on the survival

analysis looking at the clinical outcome (i.e. risk of death or HF) in patients stratified by FXIII-A levels at day 4 which revealed an inverse relationship between the risk and the FXIII-A levels; with low FXIII-A level, higher was the risk. Accordingly, the worst prognosis was reserved to those cases with FXIII-A below the 25th percentile with an increased risk of about four-fold to reach the combined endpoint at both 30-days and one-year follow-up. Moreover, since there was no correlation between FXIII variation and common cardiac damage markers (TnT and CKMB) we proposed FXIII to be used as a novel prognostic biomarker.

1.8.3 Main Polymorphisms of FXIII

Several polymorphisms are known in the genes coding for factor XIII and fibrinogen chains. Some of these variants are related to different pathologies including cardiovascular disease and the onset of myocardial infarction.

A common polymorphism in the FXIII A gene, the Val34Leu (rs5985) is one of the most important functional polymorphisms described in literature. Codon 34 is located at three amino acids away from the site for activation by thrombin (Arg37-Gly38). The presence of the Leucine in position 34 results in an increased catalytic activity which in turn increases the speed of stabilization of the clot and alters the three-dimensional structure of the fibrin mesh which appears less permeable and with finer fibrils[51] (Figure 7). Studies have reported the prevalence of the Leu encoding allele is lower in patients with myocardial infarction[52], deep venous thrombosis[53] and cerebral infarction when compared with matched control groups. These clinical studies suggest this polymorphism may be a risk determinant of thrombosis in both the arterial and venous systems. However, other studies and meta-analyses do not agree with these results. Specifically, one study didn't found relationship between Val34Leu polymorphism and the risk of early-onset of myocardial infarction, but has found instead a relationship with a not well characterized polymorphism of FXIII-A gene in Intron 13 (variant T/C rs17141831)[54].

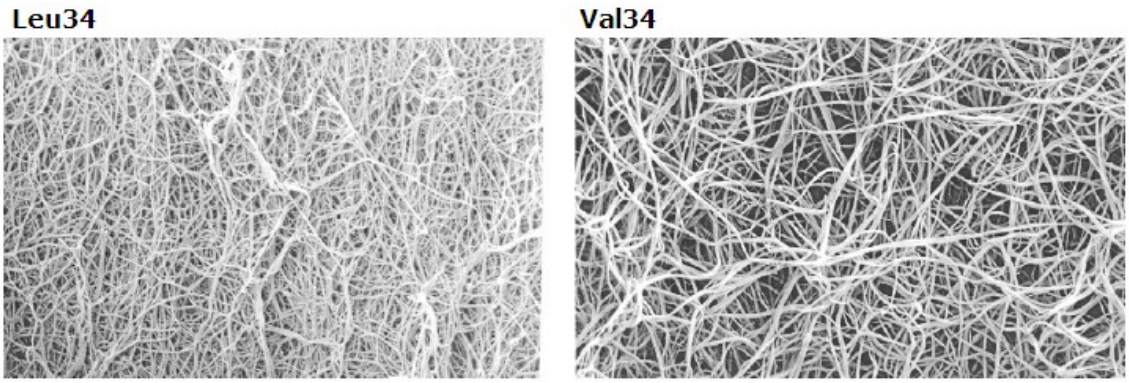

Figure 7. SEM of a fibrin clot prepared with purified fibrinogen, respectively in the presence of FXIIIA Leu34 (left) and Val34 (right) (from Lim BCB et al, Lancet, 2003).

Pro564Leu (rs5982) variant constitutes a C-to-T transition at +1694 in exon 12 and alters proline 564 to leucine in barrel 1 with an observed increase in the activity. Recently, this polymorphism has been associated to a decrease in circulating FXIII levels[55]. Case-control studies conducted on a population of women under the age of 45 years have highlighted its involvement in increasing the risk of on fatal hemorrhagic attack, especially when associated with another polymorphism: Tyr204Phe. Leu564 alters the domain surface that allow the interaction between subunits A and B. Proline and leucine are amino acids with very different properties, therefore the subunit A structure is sufficiently altered in its efficiency to interacts with subunit B[56]. It has been hypothesized that Leu564 variant prevents efficient binding between A and B subunits. This less stable complex would result in lower plasma FXIII levels[57]. For these reasons, the Leu564 variant could be important in several clinical events where lower FXIII plasma levels are present, such as Crohn 's disease, ulcerative colitis and in both benign and malignant gynaecological tumors[58].

The Tyr204Phe (rs3024477) variant is characterized by a transversion in exon 5 G→T. The presence of Phe in position 204 is associated, besides recurrent miscarriages, also to a reduction in enzyme activity which can cause a decrease in the haemostatic plug stability, resulting in a high risk condition of intracranial bleeding and ischemic attacks, in particularly in young women constitutionally presenting low FXIII levels[59].

The His95Arg (rs6003) variant of the B subunit, due to the A8259G transition in the second sushi domain in exon 3, is responsible of an increase in the dissociation rate of the B subunits from the A subunits and therefore with significant effects on FXIIIA activation kinetics[60]. The Arg95 allele co-inherited to the Leu34 allele is associated with increased post-MI survival and reduction of the risk of AMI or reinfarction in menopausal women on estrogenic therapy (the risk is reduced by 70% compared to non-estrogenic women but

carriers of allelic variants)[61]. This result was explained by the coexistence of both alleles with cooperative effects on the activation kinetics of FXIII[62].

Regarding fibrinogen gene, a polymorphism of the α-fibrinogen gene has been identified that consist in a threonine-to-alanine amino acid substitution at position 312 (Thr312Ala, rs6050).This polymorphism lies close to the FXIIIa cross-linking site at position Aα328. A significant interaction between Thr312Ala and atrial fibrillation was identified in relation to post-stroke mortality[63].

Regarding the β-fibrinogen gene, a common polymorphism in the promoter region is the G -455→A (rs1800790). This polymorphism is an independent predictor of plasma fibrinogen due to his association with elevated plasma fibrinogen levels. However, several studies have shown that this is not related to the incidence of ischemic disease[64,65].

2 AIM OF THE STUDY

Advances in therapeutic and pharmacological approaches have considerably reduced the death rate after acute myocardial infarction. However, the hospitalization rate and long-term mortality contextually increased mainly due to heart failure. Heart failure remains the first cause of long-term mortality of these patients. After MI, complex and not fully characterized healing mechanisms and damage compensation are activated. Structural and left ventricular capacity changes are likely to be the basis of the disease.

The rationale of this thesis is the natural progression of the results achieved in our previous published studies. As described previously, the main result of our previous works was the evidence that patients undergoing excessive FXIII-A consuming at the time of MI were more prone to die or to develop heart failure. In addition, the residual circulating FXIII levels were independent from the increasing of classical cardiac biomarker (TnT and CKMB) ascribing to FXIII as a possible role as novel independent prognostic biomarker.

These findings prompted us to move forward to gain more insights on the prognostic role of FXIII also thanks to the long-lasting collaboration with the Cardiology Unit of the University-Hospital of Ferrara. In this study we focused on the wall remodeling of heart (Left Ventricular Remodeling, LVR), one of the most life-threatening adverse effects after AMI. Since FXIII is involved in tissue healing, it could have a key role in tuning the complex mechanisms taking place to repair the damaged heart after AMI. We focused our interests and research to STEMI patients receiving elective primary percutaneous coronary intervention within a definite and short period of time after AMI and therefore representing not only a more homogeneous cohort of patients, compared to the one addressed in our previous studies, but also the perfect *in vivo* model to monitor FXIII consuming in a narrow window of time.

Contextually, we decided to investigate key single nucleotide polymorphisms (SNPs) in the gene of FXIII and fibrinogen to evaluate also if they play a role in the prognosis after AMI by influencing myocardium repair after AMI.

In fact, though FXIII and Fibrinogen belong to the coagulation cascade, and therefore mainly involved in coronary occlusion via thrombosis, numerous evidences and experimental data consider fibrinogen as the main determinant of 3D-biscaffold organization with role in regenerative medicine and healing processes. In this line, we strongly believed that together with FXIII the two genes might have similar crucial role in heart healing steps after AMI.

Overall, the aim of the present study is to investigate and evaluate the role of circulating levels of FXIII as active biomarker responsible for optimal repairing of the damaged heath after AMI and the genetic contribution of the two key genes considered as molecular biomarkers useful to predict the proper organization of the reparative structure at the myocardium injury site.

The results obtained from these studies will help to select in advance those patients at risk of poor prognosis with the aim of a personalized approach in regenerative medicine.

3. MATERIALS AND METHODS

3.1 PATIENTS

From January 2015 to October 2017 we progressively enrolled 235 patients with a diagnosis of STEMI (mean age 65.75 ± 12.25 years, 75% men). The patients have been hospitalized at Cardiac Intense Care Unit of the University-Hospital of Ferrara for acute myocardial infarction with persistent elevation of ST segment and treated with primary percutaneous coronary angioplasty (P-PCI) within 90 minutes of hospital admission.

The diagnosis of STEMI was performed in accordance with the criteria established by The Joint ESC/ACCF/AHA WHF Task Force for the Universal Definition of Myocardial Infarction, in the presence of symptoms of ischemia and electrocardiographic alterations such as persistent elevation of ST segment in two or more contiguous branches, left branch block (LBBB) of new onset or persistent sub stimulation of ST in the preclinical V1-V3 derivations with ST elevated in V7-V9; followed by elevation of myocardial cell biomarkers (Troponin I, CK-MB mass) and / or development of Q waves in the electrocardiogram. All hospitalized patients have received standard medical therapies according to European Society of Cardiology unless contraindication, for the treatment of acute MI including aspirin, platelets inhibitors (Clopidogrel, Ticagrelor, Prasugrel) low molecular weight heparin, β-blockers, ACE inhibitors, statins, nitrates, inhibitors of glycoproteins IIb / IIIa (Tirofiban, Abciximab), renin and angiotensin blockers.

The baseline demographic, clinical, echocardiographic, and angiographic test results were collected in all patients. All subjects enrolled in the study came from Northern Italy, had the same ethnic background and signed an informed consent approved by the local Ethics Committee.

3.2 BLOOD SAMPLE

Blood was collected in Trisodium Citrate Coagulation tubes (BD Vacutrainer Na Citrate3.8%, 2.7mL) at admission (D0) and at fourth and fifth day from the acute confirmed MI event. Additional blood samples (extended time) were not available for the patients under study. Platelet poor plasma (PPP) was obtained by blood centrifugation (2,500 g x 10 minutes), and different aliquots were stored at –80 °C.

3.3 FACTOR XIII-A LEVEL MEASUREMENT

The quantitative determination of FXIII in each sample was measured with the use of a commercial kit (HemosIL Factor XIII Antigen IL, Instrumentation Laboratories, Werfen Group, Milan). The method is based on a latex immuno-turbidimetric assay. The latex reagent is a suspension of homogeneous polystyrene particles coated over the entire surface with rabbit polyclonal antibodies highly specific for sub-units A of FXIII. When the platelet poor plasma of a patient containing the active sub-units A of FXIII is mixed with the latex reagent and buffer (TRIS buffer added with BSA bovine serum albumin and others stabilizers and preservatives) the recognition Antigen:Antibody occurs and the particles agglutinate. The degree of agglutination is directly proportional to the FXIII antigen concentration in the sample and is determined by measuring the decrease in transmitted light caused by the aggregates. The antigenic quantity of FXIII is returned as percentage respect to the normal ranges. All measurements were performed using the ACL TOP 500 instrument (IL, Instrumentation Laboratories, Werfen Group, Milan). According to the manufacturers' instruction, the machine was calibrated using a Calibration Plasma before each session of work (IL, Instrumentation Laboratories, Werfen Group, Milan FXIII-A concentration= 100%). As references, additional normal control plasma (Normal plasma Control, FXIII-A concentration= 75.5%) and pathological plasma (Special test Control Level 2, FXIII-A concentration = 37.7%) were used instead.

3.4 GENOTYPE ANALYISIS

Peripheral blood samples were collected at admission in order to avoid loss of cases. Genomic DNA was extracted using a commercial kit (QIAamp Dna Blood Mini kit, QIAGEN) on Robotic workstation for automated purification of DNA (QIACube, QIAGEN). All the samples of DNA extracted were measured at the spectrophotometer and diluted at the concentration of 80 ng/µL. Seven different single nucleotide polymorphisms (SNPs) were investigated: FXIIIA-V34L, FXIIIA -P564L, FXIIIA-Y204P, FXIII-A intronic variant T/C, FXIIIB-H95R, FGA T312A, FGB -455 G/A (Table A). Genotyping was performed by PCR-amplification followed by Pyrosequencing[66]. All PCR cycles were performed using Sure Cycler 8800 (Agilent Technologies) using Taq DNA Polymerase (Roche). Pyrosequencing technology is based on the sequencing by synthesis principle. After successful incorporation of a nucleotide by a polymerase using a single-stranded PCR (or RT-PCR) fragment as template, the release of inorganic

pyrophosphate (PPi) allows the conversion of a substrate into light by an enzymatic cascade: ATP sulfurylase converts PPi to ATP in the presence of adenosine 5' phosphosulfate (APS). This ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin that generates visible light, which is detected by CCD sensors and seen as a peak in the raw data output. Apyrase continuously degrades unincorporated nucleotides and ATP. The height of each peak (light signal) is proportional to the number of nucleotides incorporated. Sequential addition of nucleotides allows quantitative decoding of the sequence to analyze. Two primers for target DNA amplification and one primer for sequencing were designed by PyroMark Assay Design 2.0 (Biotage). The 5' ending base of the Forward primer was labelled with a biotin. PCR amplification reactions contained 5 µl 10x Buffer, 4 µl dNTP (200mM), 0.25 U Taq DNA Polymerase (Roche), 1 µl genomic DNA, and 40 pmol of amplification primers in a reaction volume of 50 µl. Following PCR, the fragments were checked by 2% agarose gel electrophoresis. The single-stranded PCR product labelled with a biotin was tested on a PyroMark ID (Biotage) according to the manufacturer's recommendations. Confirmation of genotypes was carried out by regenotyping a random selection of samples for each polymorphism investigated, there were no discrepancies between genotypes determined in duplicate.

Gene	dbSNP	Functional Variation	Primer	Sequence (5'-3')
FXIIIA1	Rs5985	Val34Leu	Forward	AATGCAGCGGAAGATGACC
			Reverse	GCTCATACCTTGCAGGTTGAC (biot)
			Sequencing	CACAGTGGAGTCTCAG
	Rs3024477	Tyr204Phe	Forward	TGGTGTGAAGATGATGCTGTGTA
			Reverse	TCCATAAAAAATTACCCCGAT (biot)
			Sequencing	TGAGAAAGAAAGAGAAGAGT
	Rs5982	Pro564Leu	Forward	CACAACCGTTACACCATCACA (biot)
			Reverse	GCGTCACGTCGAACGTCT
			Sequencing	CCTTCTTGAATTCTGCC
	Rs17141831	intron 13 variant T/C	Forward	AGGCTATGTGGAGGGAGTCTACAA
			Reverse	AGAAAAAGCGGCTCCGACTTG (biot)
			Sequencing	AATTTTGGATGAGAGCTT
FXIIIB	Rs6003	His95Arg	Forward	AAAATGCACTAAGCCTGACCTGA (biot)
			Reverse	TCCTTCCCTCCAGTGGTTTTGTAC
			Sequencing	TGAAGCGCAACCATA
FGA	Rs6050	Thr311Ala	Forward	TGGGACTGGAGGGACTGC (biot)
			Reverse	TTCCGGTACTACCAGGTCTAGG
			Sequencing	CCCAGAGTTCCAGCT
FGB	Rs1800790	-455G /A	Forward	CAAAAAAAGGGTCTTTCTGATGTG (biot)
			Reverse	CAAGGCAACCACTAAAATCGTGAC
			Sequencing	CTATTTCAAAAGGGGC

Table A. List of primers used by Pyrosequencing.

3.5 CONVENTIONAL ECHOCARDIOGRAPHY

The patients enrolled underwent to conventional two-dimensional transthoracic echocardiography (2D-EC) after three days (D3) and three months (M3) from the STEMI. Patients were examined in left lateral decubitus using a 3.5 MHz probe, using a Vivid E9 echocardiograph (GE Vingmed, Horten, Norway), in conventional projections: parasternal (long axis and basal short axis, medioventricular and apical) and apical (two -, three -, four - rooms). Images and cine-loop sequences were recorded by an expert operator and analyzed offline independently by two experienced operators. All measurements used for statistical analysis were obtained from an average of three consecutive cycles. Volumes of the left ventricle and ejection fraction were calculated using the modified Simpson biplane method. Left ventricular remodeling was defined as

an increase of ≥ 20% of the tele-diastolic volume (LVEDV) and / or ≥ 15% of the tele-systolic volume (LVESV) of the left ventricle in the three-month assessment compared to three-day[67].

3.6 FOLLOW-UP AND END-POINT DESCRIPTION

The study provides a one year follow- after discharge, with the following scheduled controls:

- Conventional transthoracic echocardiography after three-month;
- Telephone survey at one year to monitor the state of health.

The primary endpoint is the incidence of left ventricular remodeling.

Secondary endpoints include the incidence of the following major cardiac events: cardiovascular death, myocardial re-infarction, heart failure, and need for myocardial revascularization.

Cardiovascular death includes death resulting from an acute myocardial infarction, sudden cardiac death, death due to HF, death due to stroke, death due to cardiovascular procedures, death due to cardiovascular haemorrhage, and death due to other cardiovascular causes. Cardiovascular origin of death was established clinically or at autopsy. An HF event is defined as hospitalisation or an urgent unscheduled outpatient visit for HF, with documented new or worsening symptoms due to HF, objective evidence of new or worsening HF at physical examination and/or laboratory tests, prompting the initiation or intensification of treatment specifically for HF. The cardiovascular events were defined according to the ACC/AHA and ESC guidelines for the management of patients with STEMI, NSTE-ACS and HF and the Standardized Definitions[68].

3.7 STATISTICAL ANALYSIS

The continuous parameters were described as mean ± standard deviation (SD) and the statistical significance of the difference between the means was evaluated by t-test. The categorical variables were represented in terms of number and percentage and the statistical significance of the differences was calculated with the chi-square test. The Fisher exact-test and the Yates correction were applied when appropriate. Statistical analyses were performed with the use of the program MedCalc 17.9.7. version.

4. RESULTS

4.1 Clinical characteristic of enrolled patients.

From January 2015 to October 2017 we progressively enrolled 235 patients with a diagnosis of STEMI. 147 patients (63%) completed the three-month follow-up (FU). 3 patients died early after the STEMI event before they complete the 3 months FU. Among these, one patient died before the first discharge for intractable ventricular arrhythmia, one patient died after one month of STEMI event for postoperative septic complications after myocardial revascularization, and one patient died two months after STEMI event because of ischemic stroke complications. In addition, six further patients refused the three month echocardiography provided by the FU. In the three-month echocardiographic FU, 22 (15%) patients showed establishment of left ventricular remodeling (LVR+).
The table 1R shows the main basic characteristics of the study population such as the mean age and SD, the male gender number and percentage, in the whole cohort and in the LVR+ and LVR– subgroups. The mean age is not significantly different between the two subgroups (LVR+ *vs* LVR-), while the frequency of females was significantly higher within the LVR+ subgroup (LVR+ 12, 54.54% *vs* LVR–23, 18.4%; P=0.0007) males in the LVR– group is higher than that of LVR +, in fact have remodelled much more women (45%) than men (12%) (P = 0.0007).

Table 1R. The main basic characteristics of enrolled patients. . In bold the statistically significant p values.

	WHOLE (n=147)	**LVR+ (n=22)**	**LVR– (n=125)**	**P**
Age (y, SD)	63.67 ± 11,18	64.86 ± 11,28	63.46 ± 11,21	NS
Gender (n female, %)	35 (23.8%)	12 (54.54%)	23 (18.4%)	**0.0007**

Table 2R shows the main classical cardiovascular risk factors considered as: hypertension, diabetes mellitus, smoking, dyslipidaemia, Body Mass Index (BMI), and familiarity for cardiovascular disease. No significant difference between the studied subgroups was observed except for diabetes mellitus, more frequently found in the LVR+ subgroup (LVR+ 49% vs LVR– 15%, P=0.0112).

Table 2R. The main cardiovascular disease risk factor considered. In bold the statistically significant p values. BMI=Body Mass Index.

RISK FACTORS	WHOLE (n=147)	LVR+ (n=22)	LVR– (n=125)	P
Hypertension (n, %)	91 (61%)	16 (73%)	75 (60%)	NS
Diabetes (n, %)	28 (19%)	9 (41%)	19 (15%)	**0,0112**
Smoking (n, %)	79 (53%)	12 (55%)	67 (54%)	NS
Dyslipidaemia (n, %)	69 (46%)	9 (41%)	60 (48%)	NS
Familiarity (n, %)	39 (26%)	9 (41%)	30 (24%)	NS
B.M.I. (index, SD)	27.81 ± 5,49	27.99 ± 5,89	27.30 ± 4,38	NS

Evaluating the cardiological profile (Table 3R) at the time of admission, previous infarct and location of the infarct in the anterior wall did not show a significant difference in the two subgroups. As far as the Killip class is concerned, a class greater than 1 (>1) was more frequently observed in the LVR+ subgroup than in the LVR– subgroup (27% *vs* 12%; P=0.0086). With regard to the common cardiac damage biomarkers, the peak reaching times and the peak value recorded for Troponin and CK-MB were evaluated. Patients in the LVR+ subgroup reached cTnI peak after an average time of 7.64 ± 5.20 hours with a mean peak value of 69.01 ± 23.12 %, conversely, patients in the LVR– group reached the peak in a longer time (10.79 ± 6,26 hours) with an average peak value of 43.54 ± 28.58 % (P=0.0001). A similar trend was also found for the CK-MB enzyme. Accordingly, LVR+ patients reached the enzymatic peak significatively earlier than LVR– patients (7.64 ± 5.20 hours *vs* 10.79 ± 6.26; P=0.0001) and reaching higher mean values (244 ± 97.78 % vs 168.2 ± 95.62 %; P=0.0001).

Table 3R. Main cardiological characteristics of the patients considered and in the two subgroups LVR+ and LVR–. In bold the statistically significant p values.

CARDIOLOGICAL PROFILE	**WHOLE (n=147)**	**LVR + (n=22)**	**LVR - (n=125)**	**P**
Killip Class 1 (n, %)	133 (91%)	16 (73%)	117 (94%)	**0.0086**
Killip Class 2 (n, %)	9 (6%)	4 (18%)	5 (4%)	
Killip Class 4 (n, %)	5 (3%)	2 (9%)	3 (2%)	
Anterior STEMI (n, %)	55 (37%)	11 (50%)	44 (35%)	NS
First ischemic event (n, %)	126 (85%)	16 (73%)	110 (88%)	NS
Troponin Peak (n, %)	47.12 ± 29.2	69.01 ± 23.12	43.54 ± 28.58	**0.0001**
peak time Troponin (n, %)	10.31 ± 6.22	7.64 ± 5.20	10.79 ± 6.26	**0.0176**
CK-MB Peak (n, %)	179.61 ± 99.45	244 ± 97.78	168.2 ± 95.62	**0.0008**
peak time CK-MB (n, %)	7.73 ± 4.99	5.41 ± 3.79	8.14 ± 5.06	**0.0187**

Among the pharmacological therapies (Table 4R), angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARBs) were mainly administered to those belonging to the LVR– subgroup than to those in the LVR+ subgroup (90% vs 59%; P=0.0015). No statistically significant differences between the two subgroups was found for the administration of anti-platelet drugs (Aspirin or Clopidogrel, Prasugrel and Ticagrelor) oral anticoagulants (Coumadin or Novel Oral Anticoagulants), ACEI/ARBs, β-Blockers, anti-Aldosterone, Statins and Proton Pump Inhibitors.

Table 4R. The main pharmacological therapies administered as a result of myocardial infarction. ACE I= Angiotensin Converting Enzyme Inhibitors; ARBs= Angiotensin Receptor Blockers; PPIs= Proton Pump Inhibitors. O.A.* Coumadin or Novel Oral Anticoagulants. In bold the statistically significant p values.

DRUGS THERAPIES	WHOLE (n=147)	LVR + (n=22)	LVR - (n=125)	P
Aspirin (n, %)	142 (97%)	21 (95%)	121 (97%)	NS
Clopidogrel (n, %)	25 (17%)	4 (18%)	21 (16%)	NS
Ticagrelor (n, %)	87 (59%)	16 (73%)	71 (57%)	NS
Prasugrel (n, %)	35 (24%)	2 (9%)	33 (26%)	NS
ACEI / ARBs (n, %)	126 (86%)	13 (59%)	113 (90%)	**0.0015**
β- Blockers (n, %)	130 (88%)	21 (95%)	109 (87%)	NS
anti Aldosterone (n, %)	15 (10%)	5 (23%)	10 (8%)	NS
Statins (n, %)	145 (98%)	22 (100%)	123 (98%)	NS
PPIs (n, %)	105 (71%)	18 (82%)	87 (70%)	NS
O. A.* (n, %)	13 (9%)	2 (9%)	11 (9%)	NS

4.2 Clinical Follow-up and endpoint.

After one year follow-up (365 days from STEMI event) we had data on 122 (52%) patients among those examined in our study. The major adverse cardiovascular events (MACEs) considered were: death due to cardiovascular causes, heart failure, reinfarction and stroke. After one year, a total of 22 adverse events occurred in 18 patients (5.5%).
During the three months FU, globally six patients died of cardiovascular causes: one of these had LVR in the three-month window; further three patients died before the three months' time window; one deceased patient refused evaluation at three months and died due to stroke, another one patient, negative for LVR evaluation, died due to stroke.
During the one year FU, globally eight patients experienced ~~a~~ Heart Failure (HF). Three of these had LVR+; one patient died before the three months ~~for~~ LVR evaluation; and four patients were considered LVR–.

Six patients suffered from a re-infarction (Re-I): one Re-I patients was LVR+, five patients were LVR–, and among these latter one also experienced HF.
Three patients had stroke, and none of these was defined LVR+; two of these latter died during the extended one-year follow-up.
By defining remodeling as an adverse event, we recreated a new study group by considering those patients who developed at least one among these MACEs: heart failure, reinfarction, stroke, cardiovascular death and LVR. This new combined group has been defined MACE+/- and now account for 122 patients, 36 patients (30%) in the MACE+ subgroup and 86 patients (70%) in the MACE– subgroup.

4.3.1 FXIII-A levels after STEMI, LVR group.

Table 5R shows the mean and median values of FXIII-A obtained from the entire cohort of patients and in the LVR+ and LVR- subgroups. The analysis of the factor XIII-A levels measured in the plasma of the entire cohort of patients who underwent to LVR monitoring both at admission (D0, mean 95.26 ± 25.57) and the lowest value found between the fourth and fifth day post-STEMI event (D4/D5, mean 86.59 ± 26.8), gave us the confirmation of a physiological consumption of Factor XIII-A after infarction (Δ_{mean} of consumption for paired data 11.63, 95% CI 8.181 to 15.07). Statistical significance was assessed comparing $D0_{mean}$ *versus* $D4/D5_{mean}$, $P<0.0001$ (Figure 1R).

Table 5R. main statistically relevant values in the description of FXIII-A trends on the days considered for LVR group.

	ALL LVR (n=145)		LVR+ (n=22)		LVR– (n=123)	
Day(s) after STEMI	**D0**	**D4/D5**	**D0**	**D4/D5**	**D0**	**D4/D5**
Mean	95.26	86.59	91.69	86.73	96.19	86.56
Std. Deviation	25.57	26.8	24.37	25.84	26.51	27.09
Std. Error	2.102	2.298	5.744	5.509	2.461	2.537
25% Percentile	77.83	67.5	74.88	75.63	81.45	66.6
Median	94.4	84.35	87.4	90.4	95.1	83.95
75% Percentile	107.2	101.8	104	108.6	110.1	101.3
Minimum	45	26.6	50	26.6	45	34.6
Maximum	185.5	175.3	143.5	129	185.5	175.3
Lower 95% CI of mean	91.1	82.04	79.57	75.27	91.31	81.53
Upper 95% CI of mean	99.41	91.13	103.8	98.18	101.1	91.59

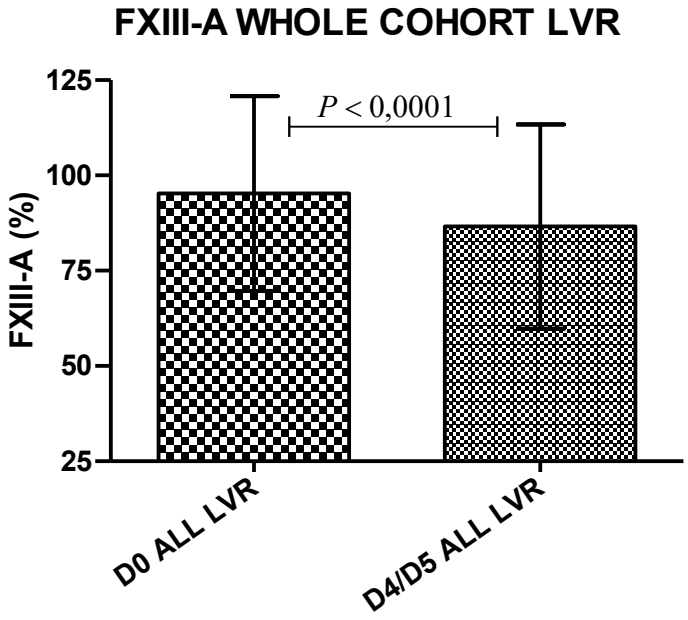

Figure 1R. The figure shows the mean and standard deviation of FXIII-A levels for all LVR patients on the day of STEMI (D0) and the lowest recorded value between the fourth and the fifth day (D4/D5).

The consumption trend, in term of reduction levels, is also confirmed by analyzing separately the two distinct LVR subgroups (Figure 2R). Although, the consumption of FXIII-A is on average higher among the LVR– subgroup (Δ_{mean} of consumption for paired data 11.45%, 95%CI 7.66-15.23; P<0.0001) than among the LVR+ subgroup (Δ_{mean} of consumption for paired data 9.96%, 95%CI 1.41-18.42; P=0.0158), LVR+ subgroup presented in D0 with lower mean levels (91.69 ± 24.37) than those found in LVR– subgroup (96.19 ± 26.51) (P=N.S.). Both groups reached similar average levels in D4/D5 (LVR–, 86.73±25.84; LVR+ 86.56±27.09; P=NS), suggesting that the earlier FXIII-A levels at the myocardium injury site is crucial at the earliest acute moments of infarction.

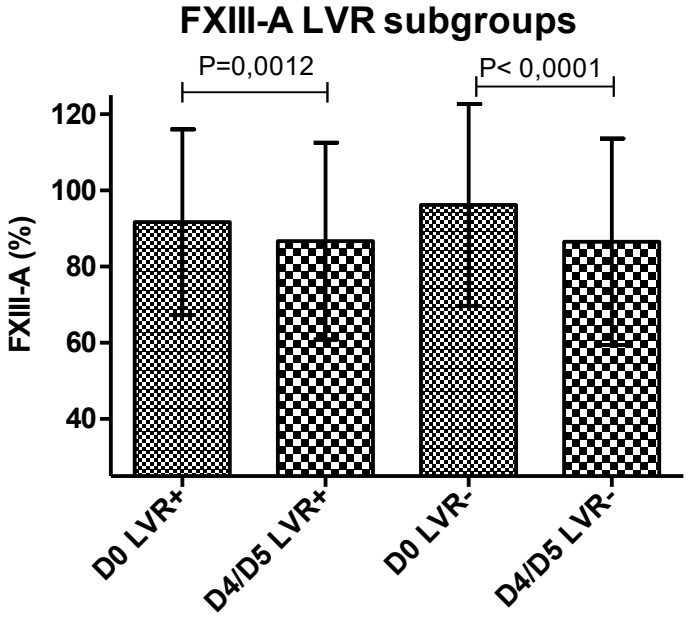

Figure 2R. The figure shows the mean and standard deviation of FXIII–A levels for all (n=122) patients on the day of STEMI (D0) and the lowest recorded value between the fourth and the fifth day (D4/D5) for the subgroups LVR+ and LVR– . The P value is referred to that obtained by t-test for paired data comparing D0 values with D4/D5 values in each subgroup.

4.3.2 FXIII-A levels after STEMI, MACE group.

The same considerations made for the LVR group were made for the combined MACE subgroup. The statistically relevant data in the MACE group and in the MACE + and MACE– subgroups were shown in table 6R.

In figure 3R are shown the mean values and standard deviations obtained in D0 (95.18 ± 28.64) and D4/D5 (86.34 ± 28.13) obtained evaluating the entire cohort of MACE patients (Δ_{mean} of consumption for paired data 10.07, 95% CI 5.99 to 14.14). Statistical significance was assessed comparing $D0_{mean}$ *versus* $D4/D5_{mean}$, P<0.0001. These data confirm a consumption of factor XIII also in the MACE group.

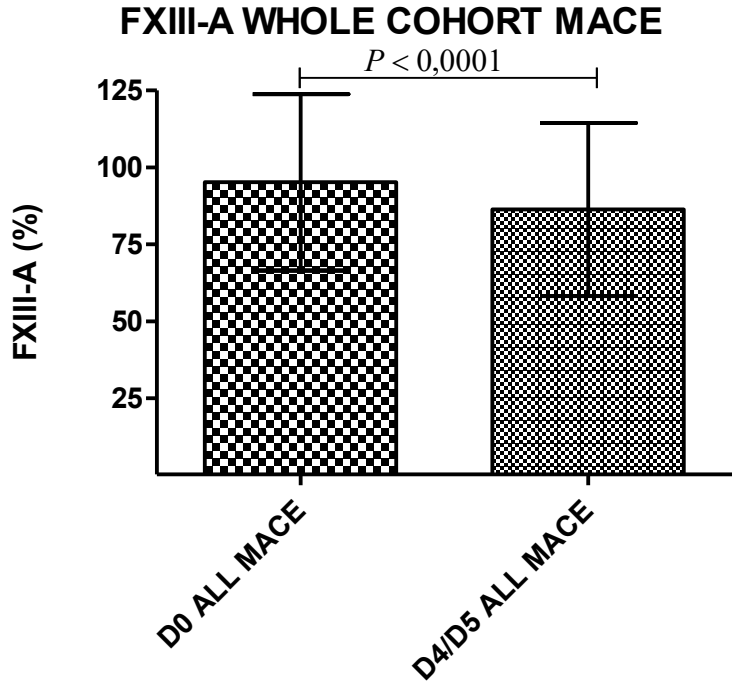

Figure 3R. The figure shows the mean and standard deviation of FXIII-A levels for all LVR patients on the day of STEMI (D0) and the lowest recorded value between the fourth and the fifth day (D4/D5).

The consumption trend is also confirmed within the two subgroups MACE+ and MACE– (Figure 4R) which show very similar consumption average values (MACE+ Δ_{mean} of consumption for paired data 10.0, 95% CI 1.92 to 18.09; MACE– Δ_{mean} of consumption for paired data 10.09 , 95% CI 5.25 to 14.92, P=N.S.). However, the MACE+ subgroup presented in D0 with significantly (P=0.047) lower mean levels (86.30 ± 24.12) than those found in MACE– subgroup (98.33 ± 29.58). Significantly different are also the comparison of the mean values of FXIII-A reached in D4/D5, the MACE+ subgroup reaches lower mean levels then MACE– subgroup (76.93 ± 24.29 *versus* 89.47 ± 28.76; P=0.048)(Figure 4R).

Table 6R. main statistically relevant values in the description of FXIII-A trends on the days considered for MACE group.

	ALL MACE (n=122)		MACE+ (n=36)		MACE - (n=86)	
Day(s) after STEMI	**D0**	**D4/D5**	**D0**	**D4/D5**	**D0**	**D4/D5**
Mean	95.18	86.34	86.3	76.93	98.33	89.47
Std. Deviation	28.64	28.13	24.12	24.29	29.58	28.76
Std. Error	2.822	2.758	4.641	4.763	3.393	3.257
25% Percentile	72.7	67.83	70.6	57.78	75.35	68.13
Median	93.3	83.95	86.5	78.2	98.1	85.45
75% Percentile	111.1	101.2	97.6	93.58	114.5	102.1
Maximum	185.5	175.3	143.5	129	185.5	175.3
Minimum	45	26.6	45	26.6	50.1	38.4
Lower 95% CI of mean	89.58	80.87	76.76	67.12	91.57	82.99
Upper 95% CI of mean	100.8	91.81	95.84	86.74	105.1	95.96

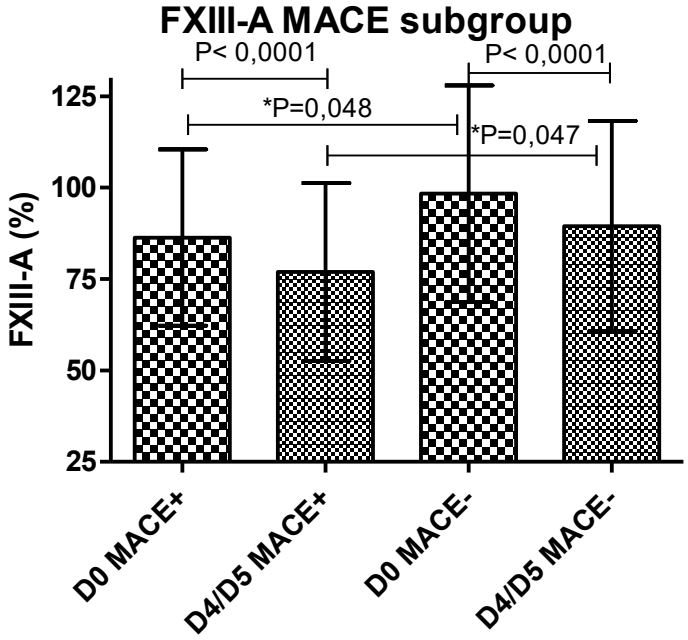

Figure 4R. The figure shows the mean and standard deviation of FXIII–A levels for all patients on the day of STEMI (D0) and the lowest recorded value between the fourth and the fifth day (D4/D5) for the subgroups MACE+ and MACE–. The P value is referred to that obtained by t-test comparing D0 values with D4/D5 values in each subgroup. The *P value is referred to that obtained by t-test comparing D0 values in MACE+ and MACE- subgroups and comparing D4/D5 values in MACE+ and MACE- subgroups.

4.3.3 FXIII-A levels LVR vs MACE groups.

Comparing the two subgroups LVR+ and MACE+, either as regards the average values of FXIII-A reached in D0 or in D4/D5, no statistically significant differences have been found. Moreover, the average consumption of factor XIII is roughly the same in the two groups. However, the mean values reached on the D0 are on average not-significantly lower in the MACE+ (86.3 ± 24.12) subgroup than in the LVR+ subgroup (91.69 ± 24.37); the same results were achieved by comparing the mean value of FXIII-A reached on D4/D5, where the MACE + subgroup reaches mean values of 76.93 ± 24.29 while the LVR + subgroup reaches mean values of 86.73 ± 25.84 (Table 7R, Figure 5R).

Table 7R. The comparison between the average values of FXIII on days D0 and D4/D5 identified in the LVR + and LVR– subgroups.

	LVR+ (n=22)	**MACE+ (n=36)**	**P Value**
D0 (mean ± SD)	91.69 ± 24.37	86.3 ± 24.12	N.S.
D4/D5 (mean ± SD)	86.73 ± 25.84	76.93 ± 24.29	N.S.
Δmean of consumption (95% C.I.)	9.96 (1.41 to 18.42)	10 (1.92 to 18.09)	---

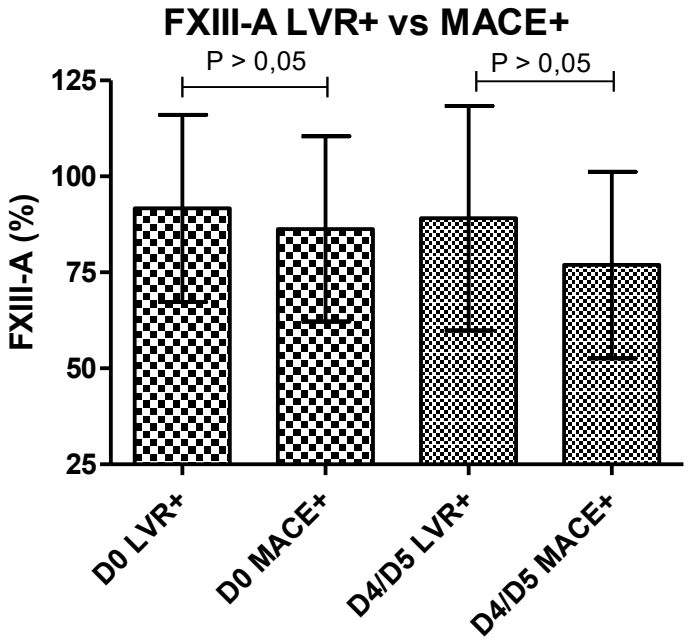

Figure 5R. The figure shows the mean and standard deviation of FXIII–A levels for all patients on the day of STEMI (D0) and the lowest recorded value between the fourth and the fifth day (D4/D5) for the subgroups LVR + and MACE+. The P value is referred to that obtained by t-test for unpaired data.

4.4 Variables with prognostic value for LVR

We evaluated the probability of developing LVR in relation to the classic cardiovascular risk factors and to the values of FXIII-A identified in D0 and in D4/D5. The variables considered were: gender, diabetes, ACE inhibitors/ARBs, Killip class, cTnI and CKMB peaks, and FXIII value in D0 and D4/D5. These parameters considered in the monovariate analysis were found as independent prognostic factors except for FXIII-A values in D0 or in the D4/D5. The prognostic significance of these factors remained significant in the multivariate analysis only as regards gender (P=0.0155) and administration of ACE Inhibitors / ARBs (P=0.041) (Table 8R).

Table 8R. Linear regression analysis for LVR. Only the significant variables were included in the multivariate analysis. * The overall significance level in univariate analysis for Killip class is P=0.0267.

Variable	P-value (univariate)	P-value (multivariate)	Odds ratio	95% CI
Gender (Female)	**0.0086**	**0.0155**	42.804	1.3187 to 13.8935
ACE Inhibitors / ARBs (no administration)	**0.0006**	**0.041**	35.937	1.0534 to 12.2595
Diabetes (yes)	**0.0089**	0.1451	2.508	0.7280 to 8.6404
CK-MB Peak	**0.0006**	0.8131	10.012	0.9914 to 1.0111
Troponin Peak	**0.0007**	0.0677	10.355	0.9975 to 1.0751
KILLIP=2	**0.0144***	0.0503	6.22	0.9974 to 38.7904
KILLIP=4	**0.0958***	0.5127	21.099	0.2256 to 19.7319
FXIII-A D0	0.478	--	--	--
FXIII-A D4/D5	0.597	--	--	--

4.5 Variables with prognostic value for MACE

We also evaluated the probability of developing combined MACE in relation to the classic cardiovascular risk factors and to the values of FXIII-A identified in D0 and in D4/D5. The variables considered were: gender, diabetes, ACE inhibitors/ARBs, cTnI and

CKMB peaks, and FXIII value in D0 and D4/D5. These parameters considered in the monovariate analysis were found as independent prognostic factors except for FXIII-A values in D0 and in D4/D5. The prognostic significance of these factors remained significant in the multivariate analysis only as regards gender (P=0.0025) and administration of ACE Inhibitors / ARBs (P=0.011) (Table 9R).

Table 9R. Linear regression analysis for combined MACE. Only the significant variables were included in the multivariate analysis

Variable	P-value (univariate)	P-value (multivariate)	Odds ratio	95% CI
Gender (Female)	**0.0001**	**0.0025**	83.112	2.1075 to 32.7766
ACE Inhibitors / ARBs (no administration)	**0.0002**	0.1402	27.915	0.7135 to 10.9209
Diabetes (yes)	**0.0001**	**0.011**	61.032	1.5138 to 24.6062
CK-MB Peak	**0.028**	0.7261	0.9981	0.9874 to 1.0089
Troponin Peak	**0.009**	0.0799	10.357	0.9958 to 1.0771
FXIII-A D0	0.1	--	--	--
FXIII-A D4/D5	0.07	--	--	--

4.6 Investigated Polymorphisms

Table 10R shows the seven investigated polymorphisms related to the genes coding for factor XIII (FXIII-A and FXIII-B) and for fibrinogen (FGA and FGB). In table are also shown the minor allele frequency (MAF) values found in the LVR + and LVR– subgroups.

No difference was observed in the prevalence of the analysed polymorphisms between LVR + and LVR– subgroups, with the exception of Val34Leu polymorphism.

In a recessive model, comparing the Leu34-homozygotes with the rest of genotypes combined together, a higher frequency of the Leu34-homozygotes in the LVR+ subgroup have been found compared to LVR– subgroups (13% vs 2%, P=0.037, O.R.=6.32, 95%

C.I: 1.18 to 33.66). By re-analyzing the cohort according to gender, in the same model, we confirmed a higher frequency of the Leu34-homozygotes in the LVR+ subgroup compared to LVR– subgroups (30% vs 2%, P=0.0022, O.R.=21, 95%C.I: 2.99 to 147.10) only in men subgroup but not in that of women. By applying this approach to the other investigated polymorphisms, a statistical significance emerged for His95Arg SNP in a dominant model only within the men subgroup. By comparing the His95-homozyogotes with the rest of genotypes combined together, a higher frequency of the His95-homozyogotes in the LVR– subgroup have been found compared to LVR+ subgroups (89.4 % vs 77.2, P=0.0136, O.R.=6, 95% C.I. 1.44 to 24.91).

Table 10R. Haemostatic gene polymorphisms tested for association with LVR and MACE. MAF: minor allele frequency.

Gene	dbSNP	Functional Variation	MAF LVR+	MAF LVR-
FXIIIA1	Rs5985	Val34Leu	0.27	0.22
	Rs3024477	Tyr204Phe	0.02	0.03
	Rs5982	Pro564Leu	0.18	0.25
	Rs17141831	Intron 13 variant T/C	0.07	0.07
FXIIIB	Rs6003	His95Arg	0.11	0.05
FGA	Rs6050	Thr311Ala	0.2	0.23
FGB	Rs1800790	-455G /A	0.16	0.25

5 DISCUSSIONS and CONCLUSIONS

Nowadays, left ventricular remodelling (LVR) after acute myocardial infarction represents a remarkable clinical event occurring in percentages ranging between 12% and 45%, despite the increasing effectiveness of pharmacological and reperfusion therapies[67]. LVR is the main determinant of survival after STEMI as it predisposes to the development of arrhythmias and heart failure associated with increased mortality and hospitalization [69,70].

In one of our previous studies[50], we ascribed to FXIII-A a prognostic value associated with the onset of Major Adverse Clinical Events (MACE), especially considering death and HF by themselves or combined together. This conclusion derived from the observation and analysis of the dynamics of the residual circulating levels that FXIII-A had in a group of infarcted patients (n=350) in the very early stages after the acute event (D0 and the following 6 days) combining FXIII-A levels with survival at 30 days and after one-year follow-up. Analyzing the whole cohort of patients during the six days of monitoring both as a total group or as subgroups (defined according to the presence/absence of different MACE), these showed particular dynamics in the residual circulating levels of FXIII-A towards reduction of levels, particularly on the fourth and fifth day after infarction (Figure 6) The ROC-analysis returned a threshold level of FXIII-A (74.9%) below which patients showed both in the short (30 days) and in the prolonged (one-year) follow-up a significant decreased survival probability of 1.5 and 2.1 folds respectively ($P = 0.04$ and $P = 0.02$). Furthermore, the sub-analysis performed for the different end-points considered showed an increased probability of developing major adverse events defined as MACEs after the infarction, in particular considering HF and death for those who had FXIII-A below the threshold (<74.9 %) on the fourth/fifth day after infarction[50]. The results obtained by this first tranche of data give us the possibility to patent our methods as a novel prognostic biomarker in acute myocardial infarction (US2016363592 (A1) 2016-12-15).

On the bases of these results, we decided to deepen the knowledge in the role of this multifaceted factor by investigating its contribution in determining heart wall remodeling after infarction considered the worst consequence in AMI survivors.

Out of 145 patients who completed the three-month end-point, 22 have shown LVR. The incidence found in the presents thesis (15.2%) is slightly lower than those stated in other published works ranging from 25 to 45%[67]. In our opinion, and after discussion with Cardiology Unit of the University-Hospital of Ferrara, this could in part due to the very

early period of follow-up (3-months after AMI) we fixed for LVR remodeling evaluation with the aim to check if our approach was able to predict in advance any slight sign of remodeling. Left ventricular remodeling was defined as an increase of ≥ 20% of the tele-diastolic volume (LVEDV) and / or ≥ 15% of the tele-systolic volume (LVESV), in this definition the ejection fraction was not taken into account.

One of the relevant aspects in our population investigated for LVR is the greater incidence in women than in men. Although women are on average slightly older, especially in the remodeled group (66 ± 13.38 years), compared to men (63 ± 8.41 years) (P=N.S.), they are on average over 65 years. It is well known that cardiovascular disease develops 5 to 10 years later in women than in men, thanks to the exposure to endogenous protective estrogens during the fertile period of life, but after 65 years of age CVD becomes the major cause of death in women[71]. This aspect, together with the initial different numerousness among patient recruitment between women (23.8%) and men (76.2%) in our study, in part exacerbates the observed differences affecting in turn also the statistical analysis.

Furthermore, it is interesting to explain and discuss some particular findings and aspects with respect to other risk factors found over-represented in the remodeled group.

As regards the Killip classification is a system used in individuals with a myocardial infarction in order to predict their risk of mortality; individuals with a low Killip class are less likely to die within the first 30 days than individuals with a higher Killip class. Therefore, it is not surprising to note that in the group of remodeled a Killip class >1 is more represented. In addition, the results obtained concerning the administration of ACE inhibitors and ARBs can be interpreted in the same way as those related to the Killip class. That is they had different rates among remodelled and non-remodeled. As a result of myocardial infarction, patients under hypotension or cardiogenic shock (Killip = 4) are contraindicated to receive these drugs. Considering that the LVR + group had a greater incidence with regard to the Killip class> 1 it is comprehensible that these patients, at least initially, are not elective for such drugs, justifying the different incidence found. Likewise, data on diabetes mellitus, which showed a significantly higher prevalence in the LVR+ subgroup compared to the LVR– subgroup, gave us an indication that the LVR+ group is at higher baseline risk. However, diabetes is known to be considered as an independent predictor of LVR after acute myocardial infarction[70,72], for this reason the incidence found in the different subgroups account for our conclusion affecting in part the final result.

As well documented in the literature, the parameters such as CKMB and Troponin I directly reflect the severity of the necrotic damage occurred as a result of the infarct, so it is understandable to think that the extent of damage to the heart muscle is higher in those who have reshaped by explaining the significant differences find between the two groups.
Altogether these single interpretations are in line with the results sprung out from the multivariate analysis. None of these risk factors remained significant in the multivariate analysis, except for the female gender and diabetes, testifying that in our patient cohort they do not constitute independent risk factors. Unfortunately, the FXIII dynamics also do not correlate with LVR establishment in statistical multivariate analysis.
Regarding the dynamics of FXIII levels, in our previous work we observed a progressive reduction in the first four/five days after infarction and then a recovery in the following days, identifying the mean values at 30 days (D30) higher than those found in D0. The interesting difference between D0 and D30 is ascribable to an earlier consumption of FXIII during the first moments of the acute phase of the infarct, before to have a blood sample available to test, and the consumption continues during the following days. The peak of consumption is realized between the fourth and fifth day and then values resume a progressive ascent. For this reason, in the present work we selected and evaluated only the first, fourth and fifth days after AMI and we took into consideration the value at D0 and the lowest value recorded between the fourth and fifth day called D4/D5.
By observing the entire cohort of patients analyzed during the three-month follow-up, LVR and subsequent development of the MACE during the extended follow-up of one year, we confirmed different dynamics among subgroups and the described trend of consumption. Within the subgroup of the LVR, comparing the levels of FXIII in D0 and in D4/D5, significant and interesting differences emerged. The average levels in D0 were significantly lower in the remodeled subgroup (LVR+; FXIII-A 91.96%±24.37) than in the non-remodeled subgroup (LVR–; FXIII-A 96.19%±26.51) while the average levels in D4/D5 overlapped. This observation strongly advices that the first hours during AMI are crucial in determining the fate of the myocardial wall and the future prognosis. The data obtained in the MACE subgroup are really interesting. Not only the dynamics of consumption have been confirmed, but the differences between those who reached at least one endpoints after one year (MACE+) and those who did not (MACE–), are evident on both days considered. In D0, the MACE– subgroup showed an average of FXIII-A of 98.33%±29.58 while in MACE+ it reached 86.3%±24.12. However, we get the most interesting results, in D4/D5 where those in the MACE– subgroup reached an average of FXIII-A of 89.47%±28.76 and those in the MACE+ subgroup reached 76.93%±24.29. It

is worth of note that this value is similar to the cut-off value (FXIII-A in D4 <73.5%) found in our previous work[50], where we demonstrated that below this cut-off patients were more prone to early die o to early develop heart failure. Accordingly, this was the threshold (in day 4) for the FXIII dynamics to acquire an informative prognostic value for the development of selected MACEs (i.e. heart failure or death).

Based on this interpretation, it would seem that the MACE+ subgroup tends toward a more severe prognosis than the equivalent subgroup accounting just LVR+ patients. However, the MACE+ subgroup contains all the LVR+ and takes into consideration other adverse events. Considering this, it is reasonable to think that within the group of those who remodelled we may not have considered at least some subjects who instead entered in the MACE+ subgroup. In other words, the physically detected LVR by the speckle-tracking within the short 3-months follow-up could lost those who remodeled later and become manifest afterwards showing the clinical phenotype of heart failure. Accordingly, for our instrumental definition of remodeling we chose a time window of just 3-months and this determined the exclusion of some borderline values that would be included in the LVR+ category simply by extending the time window considered. In addition our cohort of patients also included previous infarcted subjects, these may already have undergone structural changes as a result of the previous infarct and therefore any subsequent changes in the wall geometry could be lost or hardly appreciable to be defined as “remodeled”.

At this point, two interpretative keys can be proposed. The monitoring of FXIII-A is confirmed as prognostic circulating biomarker for the development of major adverse cardiovascular events after AMI also including ventricular remodeling but not strong enough to predict LVR as unique end-point. Though plausible, we rather think that the low percentage of LVR+ patients, due to a relatively small case-group and a too short time follow-up, co-determined such final result. In confirmation of this, the introducing of additional end-points and a larger follow-up improved the prognostic power of the methodological approach.

In addition, the fact that a consistent rate of patients already had a former infarction strongly affected the detection power of “pure” remodeling.

The completion of the study, by including the analysis of the remaining already enrolled patients but not yet characterized for remodeling, could improve the statistical power of the analyses and make clear some of these doubts.

As far as the genetic analysis is concerned, we state that with the exception of the Val34Leu polymorphism we did not achieve appreciable significance for the whole group

of selected SNPs. In the present work, we found a relation regarding the Val34Leu in a recessive model identifying a higher frequency of the Leu34-homozygotes in the LVR+ subgroup. By re-analyzing the cohort dividing men and women into further subgroups, we confirmed a higher Leu 34-homozygotes frequency in LVR+ only in men subgroup but not in that of women. Applying this approach to the other investigated polymorphisms, significance emerged in a dominant model only for the His95Arg SNP within the men subgroup identifying a higher frequency of the His95-homozygotes in the LVR- subgroup. It is necessary to underline that our cohort of patients consists of STEMI subjects and the great complexity of the events leading to ventricular remodeling may not be sufficiently influenced by some genetic polymorphisms often considered strong genetic factor for the AMI onset, and scarcely investigated in the prognosis after AMI.

Overall, the results related to post-infarction FXIII-A levels are in line with previous works that ascribe to FXIII-A a role in tissue healing and in myocardial tissue regeneration after an acute infarct event [40,42,43,73]. These observations lead us to claim that the FXIII is strongly necessary during cardiac damage at the injury site to perform functions related to the organization of a proper three-dimensional scaffold of fibrin useful for an optimal and early healing of the damaged tissue. When a myocardial infarction occurs, the clinical outcome also depends on the extent of the lesion and the modalities of healing[74]. Accordingly, FXIII-A carries out its action in all those mechanisms like angiogenesis, synthesis of fibrin and collagen, migration and cell proliferation, as well as the modulation/inhibition of MMPS, which are aimed at tissue repair and that are responsible for the formation of a stable and elastic scar[38].

We are therefore of the idea that FXIII-A molecule might help and support endogenous physiological processes allowing the self-healing of a injured tissue during the very early phases of damage and that the complete understanding of the role of this multitasking factor could be also useful for potential therapeutic approaches.

6 BIBLIOGRAPHY

1. Mackay, J., Mensah, G. A., Mendis, S. & Greenlund, K. *The atlas of heart disease and stroke*. *World Health Organization* (2004).

2. Wilkins, E. *et al.* European Cardiovascular Disease Statistics 2017 edition. *Eur. Hear. Network, Brussels* 192 (2017). doi:978-2-9537898-1-2

3. Thygesen, K. *et al.* Third universal definition of myocardial infarction. *Eur. Heart J.* **33,** 2551–2567 (2012).

4. Ross, R. Atherosclerosis--an inflammatory disease. *N. Engl. J. Med.* **340,** 115–26 (1999).

5. Kalogeris, T., Baines, C. P., Krenz, M. & Korthuis, R. J. Cell Biology of Ischemia/Reperfusion Injury. *Int. Rev. Cell Mol. Biol.* **298,** 229–317 (2012).

6. Pasupathy, S., Air, T., Dreyer, R. P., Tavella, R. & Beltrame, J. F. Systematic review of patients presenting with suspected myocardial infarction and nonobstructive coronary arteries. *Circulation* **131,** 861–870 (2015).

7. Ago, L. & Realized, E. Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature. *Circulation* **59,** 607–609 (1979).

8. Thygesen, K. *et al.* Universal definition of myocardial infarction: Kristian Thygesen, Joseph S. Alpert and Harvey D. White on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. *Eur. Heart J.* **28,** 2525–2538 (2007).

9. Goldmann, B. U., Newby, L. K. & Ohman, E. M. Cardiac markers for decision making in acute ischemic syndromes. *ACC Current Journal Review* **9,** 43–45 (2000).

10. Medicine, C. *et al.* Textbook of Cardiovascular Medicine. *Congenital Heart Disease* **288,** 1628 (2002).

11. Apple, F. S. & Collinson, P. O. Analytical characteristics of high-sensitivity cardiac troponin assays. *Clinical Chemistry* **58,** 54–61 (2012).

12. Menon, V. *et al.* Thrombolysis and adjunctive therapy in acute myocardial infarction: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. in *Chest* **126,** (2004).

13. Gore, J. M. *et al.* Stroke after thrombolysis. Mortality and functional outcomes in the GUSTO-I trial. Global Use of Strategies to Open Occluded Coronary Arteries. *Circulation* **92,** 2811–2818 (1995).

14. Weaver, W. D. *et al.* Comparison of primary coronary angioplasty and intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review. *Jama* **278,** 2093–8 (1997).

15. Levine, G. N. *et al.* 2011 ACCF/AHA/SCAI guideline for percutaneous coronary intervention a report of the American College of Cardiology Foundation/American

Heart Association Task Force on Practice Guidelines and the Society for Cardiovascular Angiography and Interventions. *Circulation* **124,** (2011).

16. Banning, A. P. *et al.* Percutaneous coronary intervention in the UK: recommendations for good practice 2015. *Heart* **101,** 1–13 (2015).

17. Boersma, E. Does time matter? A pooled analysis of randomized clinical trials comparing primary percutaneous coronary intervention and in-hospital fibrinolysis in acute myocardial infarction patients. *Eur. Heart J.* **27,** 779–788 (2006).

18. Buch, P. *et al.* Temporal decline in the prognostic impact of a recurrent acute myocardial infarction 1985 to 2002. *Heart* **93,** 210–5 (2007).

19. Musumeci, G., Di Lorenzo, E. & Valgimigli, M. Dual antiplatelet therapy duration: what are the drivers? *Curr. Opin. Cardiol.* **26 Suppl 1,** S4-14 (2011).

20. Frangogiannis, N. G. Targeting the inflammatory response in healing myocardial infarcts. *Curr. Med. Chem.* **13,** 1877–93 (2006).

21. Cleutjens, J. P. M., Kandala, J. C., Guarda, E., Guntaka, R. V. & Weber, K. T. Regulation of collagen degradation in the rat myocardium after infarction. *J. Mol. Cell. Cardiol.* **27,** 1281–1292 (1995).

22. Pfeffer, M. A. & Braunwald, E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* **81,** 1161–1172 (1990).

23. Gilson, W. D. *et al.* Borderzone Contractile Dysfunction Is Transiently Attenuated and Left Ventricular Structural Remodeling Is Markedly Reduced Following Reperfused Myocardial Infarction in Inducible Nitric Oxide Synthase Knockout Mice. *J. Am. Coll. Cardiol.* **50,** 1799–1807 (2007).

24. Akasaka, Y. *et al.* Myocardial apoptosis associated with the expression of proinflammatory cytokines during the course of myocardial infarction. *Mod. Pathol.* **19,** 588–598 (2006).

25. French, B. A. & Kramer, C. M. Mechanisms of postinfarct left ventricular remodeling. *Drug Discovery Today: Disease Mechanisms* **4,** 185–196 (2007).

26. Burchfield, J. S., Xie, M. & Hill, J. A. Pathological ventricular remodeling: Mechanisms: Part 1 of 2. *Circulation* **128,** 388–400 (2013).

27. Dorn, G. W. Novel pharmacotherapies to abrogate postinfarction ventricular remodeling. *Nat. Rev. Cardiol.* **6,** 283–291 (2009).

28. Weber, K. T. Extracellular matrix remodeling in heart failure: A role for de novo angiotensin II generation. *Circulation* **96,** 4065–4082 (1997).

29. Sutton, M. G. S. J. & Sharpe, N. Left Ventricular Remodeling After Myocardial Infarction : Pathophysiology and Therapy. *Circulation* **101,** 2981–2988 (2000).

30. Ariëns, R. A. S., Lai, T. S., Weisel, J. W., Greenberg, C. S. & Grant, P. J. Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. *Blood* **100,** 743–754 (2002).

31. Adány, R. & Bárdos, H. Factor XIII subunit A as an intracellular transglutaminase.

Cell. Mol. Life Sci. **60,** 1049–60 (2003).

32. Board, P. G., Webb, G. C., McKee, J. & Ichinose, A. Localization of the coagulation factor xiii a subunit gene (F13a) to chromosome bands 6p24→p25. *Cytogenet. Genome Res.* **48,** 25–27 (1988).

33. Webb, G. C., Coggan, M., Ichinose, A. & Board, P. G. Localization of the coagulation factor XIII B subunit gene (F13B) to chromosome bands 1q31-32.1 and restriction fragment length polymorphism at the locus. *Hum. Genet.* **81,** 157–160 (1989).

34. Gemmati, D. *et al.* Coagulation factor XIIIA (F13A1): Novel perspectives in treatment and pharmacogenetics. *Curr. Pharm. Des.* **22,** 1449–1459 (2016).

35. Lewis, S. D., Shafer, J. A., Janus, T. J. & Lorand, L. Regulation of Formation of Factor XIIIa by Its Fibrin Substrates. *Biochemistry* **24,** 6772–6777 (1985).

36. E. L. HETHERSHAW, A. L. C. L. C. and H. P. The effect of blood coagulaton factor XIII on fibrin clot structure and fibrinolysis. *J. Thromb. Haemost.* 197–205 (2014). doi:10.1111/jth.12455

37. Ritchie, H., Lawrie, L. C., Crombie, P. W., Mosesson, M. W. & Booth, N. a. Cross-linking of plasminogen activator inhibitor 2 and alpha 2-antiplasmin to fibrin(ogen). *J. Biol. Chem.* **275,** 24915–20 (2000).

38. Inbal, A. & Dardik, R. Role of coagulation factor XIII (FXIII) in angiogenesis and tissue repair. *Pathophysiol. Haemost. Thromb.* **35,** 162–5 (2006).

39. Richardson, V. R., Cordell, P., Standeven, K. F. & Carter, A. M. Substrates of Factor XIII-A: roles in thrombosis and wound healing. *Clin. Sci.* **124,** 123–137 (2013).

40. Zamboni, P. Factor XIII Contrasts the Effects of Metalloproteinases in Human Dermal Fibroblast Cultured Cells. *Vasc. Endovascular Surg.* **38,** 431–438 (2004).

41. Nahrendorf, M. *et al.* Factor XIII deficiency causes cardiac rupture, impairs wound healing, and aggravates cardiac remodeling in mice with myocardial infarction. *Circulation* **113,** 1196–1202 (2006).

42. Nahrendorf, M., Weissleder, R. & Ertl, G. Does FXIII deficiency impair wound healing after myocardial infarction? *PLoS One* **1,** (2006).

43. Vanhoutte, D. & Heymans, S. Factor XIII: The cement of the heart after myocardial infarction? *European Heart Journal* **29,** 427–428 (2008).

44. Mukherjee, R. *et al.* Targeted Myocardial Microinjections of a Biocomposite Material Reduces Infarct Expansion in Pigs. *Ann. Thorac. Surg.* **86,** 1268–1276 (2008).

45. Li, X.-H. *et al.* Effects of intramyocardial injection of platelet-rich plasma on the healing process after myocardial infarction. *Coron. Artery Dis.* **19,** 363–370 (2008).

46. Wehberg, K. E. *et al.* Intramyocardial injection of autologous platelet-rich plasma combined with transmyocardial revascularization. *Cell Transplant.* **18,** 353–359 (2009).

47. Alkjaersig, N., Fletcher, A. P., Lewis, M. & Ittyerah, R. Reduction of coagulation factor XIII concentration in patients with myocardial infarction, cerebral infarction, and other thromboembolic disorders. *Thromb. Haemost.* **38,** 863–873 (1977).

48. Kohler, H. P. *et al.* Factor XIII A-subunit concentration predicts outcome in stroke subjects and vascular outcome in healthy, middle-aged men. *Br. J. Haematol.* **118,** 825–832 (2002).

49. Kucher, N., Schroeder, V. & Kohler, H. P. Role of blood coagulation factor XIII in patients with acute pulmonary embolism. Correlation of factor XIII antigen levels with pulmonary occlusion rate, fibrinogen, D-dimer, and clot firmness. *Thromb. Haemost.* **90,** 434–438 (2003).

50. Gemmati, D. *et al.* Factor XIII-A dynamics in acute myocardial infarction: A novel prognostic biomarker? *Thromb. Haemost.* **114,** 123–132 (2015).

51. Kohler, H. P., Ariens, R. A. S., Whitaker, P. & Grant, P. J. Common coding polymorphism in the FXIII A-subunit gene (FXIIIVAL34LEU) affects cross-linking activity [3]. *Thrombosis and Haemostasis* **80,** 704 (1998).

52. Wartiovaara, U. *et al.* Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. *Atherosclerosis* **142,** 295–300 (1999).

53. Catto, A. J. *et al.* Association of a common polymorphism in the factor XIII gene with venous thrombosis. *Blood* **93,** 906–8 (1999).

54. Guella, I. *et al.* Common variants in the haemostatic gene pathway contribute to risk of early-onset myocardial infarction in the Italian population. *Thromb. Haemost.* **106,** 655–664 (2011).

55. Kohler, H. P., Futers, T. S. & Grant, P. J. Prevalence of three common polymorphisms in the A-subunit gene of factor XIII in patients with coronary artery disease. Association with FXIII activity and antigen levels. *Thromb. Haemost.* **81,** 511–515 (1999).

56. Yee, V. C. *et al.* Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7296–7300 (1994).

57. Gallivan, L., Markham, A. F. & Anwar, R. The Leu564 factor XIIIA variant results in significantly lower plasma factor XIII levels than the Pro564 variant [10]. *Thrombosis and Haemostasis* **82,** 1368–1370 (1999).

58. van Wersch, J. W., Peters, C. & Ubachs, J. M. Coagulation factor XIII in plasma of patients with benign and malignant gynaecological tumours. *Eur J Clin Chem Clin Biochem* **32,** 681–684 (1994).

59. Reiner, a P. *et al.* Polymorphisms of coagulation factor XIII subunit A and risk of nonfatal hemorrhagic stroke in young white women. *Stroke.* **32,** 2580–2586 (2001).

60. Komanasin, N. *et al.* A novel polymorphism in the factor XIII B-subunit (His95Arg): Relationship to subunit dissociation and venous thrombosis. *J. Thromb. Haemost.* **3,** 2487–2496 (2005).

61. Reiner, A. P. *et al.* Genetic variants of coagulation factor XIII, postmenopausal estrogen therapy, and risk of nonfatal myocardial infarction. *Blood* **102,** 25–30 (2003).

62. Gemmati, D. *et al.* Factor XIIIA-V34L and factor XIIIB-H95R gene variants: effects on survival in myocardial infarction patients. *Mol. Med.* **13,** 112–20 (2007).

63. Carter, A. M., Catto, A. J. & Grant, P. J. Association of the alpha-fibrinogen Thr312Ala polymorphism with poststroke mortality in subjects with atrial fibrillation. *Circulation* **99,** 2423–2426 (1999).

64. Tybjærg-Hansen, A. *et al.* A common mutation (G-455→A) in the β-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease: A study of 9,127 individuals based on the Copenhagen City Heart Study. *J. Clin. Invest.* **99,** 3034–3039 (1997).

65. Bi, S., Wang, D., Li, G., Wen, S. & Pan, S. Beta-fibrinogen promoter -455 G/A (HaeIII) polymorphism prediction of plasma fibrinogen but not of ischemic cerebrovascular disease. *Chin Med Sci J* **19,** 1–5 (2004).

66. Kreutz, M., Schock, G., Kaiser, J., Hochstein, N. & Peist, R. PyroMark?? instruments, chemistry, and software for Pyrosequencing?? analysis. *Methods Mol. Biol.* **1315,** 17–27 (2015).

67. Huttin, O. *et al.* Prediction of left ventricular remodeling after a myocardial infarction: Role of myocardial deformation: A systematic review and meta-analysis. *PLoS ONE* **11,** (2016).

68. Hicks, K. A. *et al.* 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials. *Circulation* **132,** 302–361 (2015).

69. Abate, E. *et al.* Value of three-dimensional speckle-tracking longitudinal strain for predicting improvement of left ventricular function after acute myocardial infarction. *Am. J. Cardiol.* **110,** 961–967 (2012).

70. Lacalzada, J. *et al.* Left ventricular global longitudinal systolic strain predicts adverse remodeling and subsequent cardiac events in patients with acute myocardial infarction treated with primary percutaneous coronary intervention. *Int J Cardiovasc Imaging* **31,** 575–584 (2015).

71. Maas, A. H. E. M. & Appelman, Y. E. A. Gender differences in coronary heart disease. *Netherlands Hear. J.* **18,** 598–603 (2010).

72. D'Andrea, A. *et al.* Global longitudinal speckle-tracking strain is predictive of left ventricular remodeling after coronary angioplasty in patients with recent non-st elevation myocardial infarction. *Int. J. Cardiol.* **153,** 185–191 (2011).

73. Nahrendorf, M. *et al.* Transglutaminase activity in acute infarcts predicts healing outcome and left ventricular remodelling: Implications for FXIII therapy and antithrombin use in myocardial infarction. *Eur. Heart J.* **29,** 445–454 (2008).

74. Ertl, G. & Frantz, S. Healing after myocardial infarction. *Cardiovascular Research* **66,** 22–32 (2005).