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Complete List of Authors:	Gemmati, Donato; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Zeri, Giulia; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Orioli, Elisa; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Mari, Rosella; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Moratelli, Stefano; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Vigliano, Marco; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Marchesini, Jlenia; University of Ferrara, Operative Unit of Cardiology, Dpt. Medical Sciences Grossi, Maria; University of Ferrara, Operative Unit of Cardiology, Dpt. Medical Sciences Pecoraro, Alessandro; University of Ferrara, Operative Unit of Cardiology, Dpt. Medical Sciences Cuneo, Antonio; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Ferrari, Roberto; University of Ferrara, Operative Unit of Cardiology, Dpt. Medical Sciences Pinotti, Mirko; University of Ferrara, Dpt. Life Sciences and Biotechnology Serino, Maria; University of Ferrara, Ctr. Haemostasis & Thrombosis, Haematology Section, Dpt. Medical Sciences Ansani, Lucia; University of Ferrara, Operative Unit of Cardiology, Dpt. Medical Sciences
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Extra Table

'What is known on this topic'

FXIII has extraordinary properties in wound healing. Myocardial infarction lesion shares with classical wound several aspects. (Constitutive) low FXIII levels associate with heart rupture and severe heart failure after infarction. Interesting findings have been reported on FXIII utility in the healing of injured heart.

'What this paper adds'

A significant transient FXIII drop in blood was observed in the majority of patients during acute myocardial infarction. Patients who died or experienced post-MI heart failure had higher FXIII-A consumptions in the acute phase. A putative FXIII threshold has been recognized with prognostic value. The risky indiscriminate FXIII treatment in MI suggested by some authors could be avoided by monitoring FXIII levels in the acute phase to recognize those really needing it.

Factor XIII-A dynamics in acute myocardial infarction: a novel prognostic biomarker?

Gemmati Donato[1], Zeri Giulia[1], Orioli Elisa[1], Mari Rosella[1], Moratelli Stefano[1], Vigliano Marco[1], Marchesini Jlenia[2], Grossi Maria Elena[2], Pecoraro Alessandro[2], Antonio Cuneo[1], Ferrari Roberto[2], Pinotti Mirko[3], Serino M. Luisa[1], Ansani Lucia[2].

[1]Centre of Haemostasis & Thrombosis, Haematology Section, Department of Medical Sciences, and [2]Operative Unit of Cardiology, University-Hospital S. Anna, Ferrara, and [3]Department of Life Sciences and Biotechnology; University of Ferrara, Italy.

Corresponding author: Gemmati Donato, Ctr. Hemostasis & Thrombosis, Hematology Section, Dpt. of Medical Sciences, University of Ferrara, Ferrara, Italy;
tel (+39) 0532.237291
fax (+39) 0532.209010
email: d.gemmati@unife.it

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Abstract

After acute myocardial infarction (MI) the damaged heart have to be repaired. Factor XIII (FXIII) is considered a key molecule in favouring heart healing. FXIII deficiency was associated to cardiac rupture and anomalous remodelling in MI. During MI, FXIII contributes firstly to the intracoronary thrombus formation and shortly after to heal the myocardial lesion. To quantify the real contribution of FXIII in this process, and to explore its possible prognostic role, we monitored the FXIII-A subunit levels in 350 acute MI patients during the first six days (d_0-d_5) plus a control at 30-60 days (d_{30}). A one-year follow-up was performed for all the patients. A transient drop in the FXIII-A mean level was noted in the whole cohort of patients (FXIII-A_{d0} 99.48±30.5 vs FXIII-A_{d5} 76.51±27.02; P<0.0001). Interestingly, those who developed post-MI heart failure showed the highest drop (FXIII-A_{d5} 52.1±25.2) and they already presented with low levels at recruitment. Similarly, those who died showed the same FXIII-A dynamic (FXIII-A_{d5} 54.0±22.5). Conversely, patients who remained free of major adverse cardiac events, had lower consuming (FXIII-A_{d0} 103.6±29.1 vs FXIII-A_{d5} 84.4±24.5; P<0.0001). Interestingly, the FXIII-A drop was independent from the amount of injury assessed by TnT and CKMB levels. The survival analysis ascribed an increased probability of early death or heart failure inversely related to FXIII-A quartiles (FXIII-A_{25th}<59.5%; HR=4.25; 2.2-5.1; P<0.0001). Different FXIII-A dynamics and levels could be utilized as early prognostic indicators during acute MI, revealing the individual potential to heal and suggesting tailored treatments to avoid heart failure or its extreme consequence.

Key words: Factor XIII; myocardial infarction; myocardial healing; prognosis; myocardial infarction biomarkers.

Introduction

The presence of effective tools in acute myocardial infarction (MI) care but not of similarly valid options in chronic treatment of MI survivors, has led to a reduced acute mortality, though the long-term mortality rate or hospitalization due to severe heart failure remain high (1-3). After MI, the damaged heart tissue starts a complex series of processes aimed at repairing, and eventually replacing, the lesion by scar tissue (1, 4, 5). Pre-existing extracellular matrix (ECM) proteins are digested by metalloproteinases (MMPs) and new matrix is laid down (1, 6). The first step in the reparative processes implies the formation of a three-dimensional fibrin meshwork aimed at providing a provisional scaffold/platform for endogenous neo-vessels formation, cell recruiting/spreading, and at limiting lesion expansion. The creation of a robust and elastic fibrin meshwork favouring cell proliferation, as well as neo-vessel formation, strongly depends on a circulating enzyme, (coagulation factor XIII; FXIII), contributing to myocardial healing and recovery after injury.

FXIII is a plasma transglutaminase, consisting of two enzymatic A-subunits (FXIII-A) and two non-catalytic B-subunits (FXIII-B) forming the hetero tetrameric structure A2B2 (7). FXIII-A plays a critical role in generating a stable haemostatic plug, in wound healing, in tissue repair, and in angiogenesis in vivo and in vitro (7-12). In addition, FXIII-A is present in platelets, monocytes, and macrophages, all components deeply involved in infarct healing (8, 13-15). Extraordinary direct evidences of the essential role of FXIII-A in acute and chronic infarct scar stability come from an experimental animal model with genetically reduced FXIII-A levels (16). Authors found that 100% of FXIII-A deficient mice died within 5 days after induced MI due to left ventricular rupture, and that intravenous FXIII replacement therapy reversed the adverse outcome though, the cardiac magnetic resonance (MRI) showed worse left ventricular remodelling with low heart performances. Afterwards, other studies supported the extraordinary role of FXIII in the post-MI healing fate (17-19), suggesting also a FXIII supplementary therapy and even intramyocardial injection of FXIII-modifiable biomaterial (20, 21). In addition, by utilizing specific FXIII-substrates, they have been developed promising noninvasive molecular imaging approaches to monitor myocardial healing or thrombus formation/fibrinolysis in vivo (6, 18, 22, 23).

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. Two studies in the late ’70 investigated in detail the changes of FXIII and fibrinogen levels after ischemic events, ascribing this phenomenon mainly to extra- or intra-vascular coagulation reflecting the degree and duration of the

concomitant coagulopathy not excluding the possibility that fibrin deposition in the infarcted area could be a pathophysiological reaction to injury and inflammation and a possibly repair mechanism (24, 25). More recently, it is reawakened the interest towards FXIII fluctuations (or of its derivatives) during the acute venous or arterial accidents by comparing the earliest acute phase with the final re-established steady state or comparing the presence/absence of the ischemic event to make attempts at ascribing to this (epi)-phenomenon diagnostic/prognostic information (26-30). However, no definitive or conclusive results have been so far produced on the extent of FXIII level/consumption as a predictor of the different clinical outcome. On the contrary, the role of FXIII levels and/or of the associated gene variants in the risk of MI or in other atherothrombotic diseases has been definitively investigated (32-34) and interesting results, showing correlations between particular FXIII genotypes and FXIII levels, have been reported in patients undergoing coronary artery disease (35, 36).

In the present paper, we investigated a cohort of 350 consecutive acute MI patients and performed a detailed monitoring (every 24h) of the dynamics of FXIII-A circulating levels during the first six days after MI, followed by a one-year follow-up of patients to ascribe possible prognostic tasks to this interesting phenomenon.

Materials and methods

Patients

From January 2009 to December 2011, we recruited 350 acute MI patients (whole group; mean age 68.2±12.95 years; 72.8% men) admitted to the Coronary Care Unit (CCU) of the University-Hospital of Ferrara. Acute MI was defined according to the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction (37), as a rise and/or fall of cardiac biomarkers (cardiac troponin T -cTnT, CKMB measured by mass assay) with at least one of the followings: 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 the ECG, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, identification of an intracoronary thrombus by angiography. Patients with ST-elevation myocardial infarction (STEMI) received primary percutaneous coronary intervention (PCI) within 90 minutes of hospital admission, in case of symptoms ≤12 hours in duration and in case of symptoms lasting 12 to 24 hours if pain consisted at the time of admission. Patients with non ST-elevation myocardial infarction (NSTEMI) underwent coronary angiography within 2 to 72 hours from hospital admission, according to the ESC recommendations for invasive

evaluation and revascularization of NSTE-ACS. All patients received standard medical therapy according to the ESC guidelines for the treatment of acute MI unless contraindicated, including aspirin, clopidogrel, glycoprotein IIb/IIIa inhibitors (tirofiban or abiciximab), unfractioned or low molecular weight heparin, beta-blockers, statins, renin and/or angiotensin blockers. The baseline demographic, clinical, echocardiographic, and angiographic test results were collected in all patients. The study was approved by the local ethics committee and all patients gave written informed consent to enter the study.

Blood samples

Blood was collected in Trisodium Citrate Coagulation tubes at admission (d_0) and every 24h for the additional five days (d_1-d_5) from the acute confirmed MI event. Control samples were drawn at least after 30-days (d_{30}; range: 30-60 days) to have basal FXIII-A levels far from the acute ischemic event. Additional blood samples (extended time) were not available for the patients under study. To exclude possible further *in vitro* enzyme degradation/activation additional comparative samples were drawn in EDTA plus Aprotinin tubes. Plasma was obtained by blood centrifugation (2500gx10m), and different aliquots were stored at -80°C.

FXIII-A level measurements

FXIII-A antigen levels were assessed by means of a Latex Reagent (HemosIL Factor XIII Antigen) which is a suspension of uniform size polystyrene latex particles coated with rabbit polyclonal antibodies, highly specific for the A-subunit of FXIII according to the manufacturer's instructions (Instrumentation Laboratory, Milan, Italy). FXIII-A was tested by Automated Coagulation Analyzer - Instrumentation Laboratory - ACL Futura Plus at all the recruited time considered.

Follow-up and description of endpoints

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. 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 hemorrhage, and death due to other cardiovascular causes. Cardiovascular origin of death was established clinically or at autopsy. An HF event is defined as hospitalization 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 for Cardiovascular and Stroke End Point Events in Clinical Trials for CDISC (Draft Definitions for CDISC August 20, 2014; http://www.cdisc.org/system/files/all/standard/Draft%20Definitions%20for%20CDISC%20August%2020,%202014.pdf).

Statistics

Continuous data were presented as means ± SD, with the significance of differences judged by *t*-test. Categorical variables were summarized in terms of number and percentages with the significance of differences judged by Chi-Square test. Fisher's exact test (two-tailed) was used as appropriate. FXIII-A levels were adjusted for confounders (sex, age and smoking). Survival curves were constructed by the Kaplan-Meier method and survival among groups was compared using the Log-Rank test. MACE were retrospectively analyzed as single variable or combined by means of logistic regression analyses. Spearman analysis tested correlation coefficients between FXIII-A and cardiac biomarker levels. The recognition of the FXIII-A threshold(s) at any period of time considered (d_0-d_5) was obtained by means of the Receiver Operating Characteristic (ROC) analysis, utilizing the continuous rating scale powered by http://www.rad.jhmi.edu/jeng/javarad/roc/JROCFITi.html and the points for plotting pasted into the Excel program. Probability was considered significant at a level of $P \leq 0.05$. Analysis was performed using IBM SPSS Statistics 21 Developer.

Results

Patient's characteristics

Table 1 shows the main baseline characteristics of the MI patients under study. The NSTEMI group showed a higher percentage of classical cardiovascular risk factors compared with the STEMI group. Overall, 13.1 % of patients had Killip class > 1 at entry (15.1% in STEMI *vs* 8.1% in NSTEMI; $P = 0.10$).

FXIII-A levels in the different patient's subgroups

Whole group

Figure 1 (and supplementary table ST1 for details), shows the mean and SD of FXIII-A

antigen levels assessed at the different times considered: day of recruitment (d_0), the next five days (d_1-d_5), and far from the acute MI event (d_{30}) in the whole group (n=350) of patients considered. A low rate of patient drop-out was recorded being 0% at d_0-d_2 reaching the highest value at d_{30} being anyhow very low (table ST1). Among the whole cohort of MI patients, a remarkable FXIII-A level drop was observed starting from d_1 with the lowest value reached at d_5 (d_0 *vs.* d_5; P<0.0001). It is worth noting the higher value of mean and median at d_{30} compared to d_0 (P=0.02).

MACE+ vs MACE– patients

Among the whole cohort of MI patients, 77 cases experienced at least one of the two major adverse cardiac events considered during the one-year follow-up (i.e. MACE+, n=77, 22.0% and MACE–, n=273, 78.0%). Comparing those patients who experienced MACE during the follow-up with the MACE– patients, the analysis showed that the FXIII-A drop was significantly stronger among the formers (Figure 2). It's noteworthy the observation that MACE+ patients presented significantly lower FXIII-A levels at d_0 and at any considered time compared to the MACE– subgroup (P<0.0001 at any point analyzed).

Heart failure vs death in MACE+ patients

Low FXIII-A levels after MI might cause inefficient myocardial scar formation that can lead to heart failure or even death because of the anomalous post-MI remodelling or heart rupture respectively (16, 19). Guided by the observation of significantly lower levels of FXIII-A found in the MACE+ subgroup, we further investigated the relationship between FXIII-A levels and the occurrence of post-MI heart failure or death (Figure 3). Those who developed HF during the follow-up (n=39; 11.1%) showed a more remarkable reduction of FXIII-A mean levels with the trend characterized by a faster lowering level at day-5. On the other hand they showed appreciable and almost normal recovery at day-30 (Figure 3, left panel). Similarly, those patients who died (n=38; 10.9%) due to severe infarction complications, presented at day-0 with a slightly lower FXIII-A mean value than that observed among HF cases, and reached the day-5 lowest levels slightly faster. Unfortunately, day-30 was not collected because of large part of these patients died before/or around that time or were definitely not available due to concomitant treatments or complications (Figure 3, right panel).

STEMI vs NSTEMI and FXIII-A consumption

When we compared STEMI and NSTEMI patients we observed that the formers were characterized by a less pronounced FXIII-A drop than the others, and the difference was mainly related to mean FXIII-A levels at day-5 (e.g. $STEMI_{d0}$, 99.8±26.6 *vs* $NSTEMI_{d0}$, 97.99±30.4, P=NS; $STEMI_{d5}$, 79.1±24.1 *vs* $NSTEMI_{d5}$, 70.2±28.1, P<0.01). Potentially, a lower FXIII-A consumption observed in a patient could be related to an associated faster reperfusion intervention of the culprit coronary, that could reflect the higher percentage of PCI performed among STEMI compared to NSTEMI (93.6% *vs* 78.8% respectively; P<0.0001). Accordingly, comparing all the PCI patients (n=313) versus the few who did not receive PCI treatment (n=37) the results matched (FXIII-A mean level: PCI_{d0}, 99.76±24.5 *vs* no-PCI_{d0}, 96.49±29.5, P=NS; PCI_{d5}, 76.9±27.2 vs no-PCI_{d5}, 68.9±25.5, P=0.01). Unfortunately, these findings are strongly correlated and mutually influenced since the STEMI group contains the quite totality of PCI (i.e. 93.6%) and the PCI group includes more than 75% of STEMI. Therefore, we could just speculate and hypothesize that prompt PCI could save more FXIII-A molecule.

Ejection fraction (EF) and FXIII-A levels stratified by MACE

We then analysed FXIII-A mean levels at day-5 and EF in patients who experienced the different adverse events and compared them with the MACE- group (Table 2). As expected, the lowest values of EF were observed among the HF and Death groups, and they were characterized by the lowest FXIII-A mean levels. Higher mean FXIII-A values were found among the MACE− subgroup and comparisons of both HF and Death resulted in significant differences.

Clinical outcome

After one-year follow-up, we observed an overall number of 77 (22.0%) adverse events including 39 (50.6%) heart failures and 38 (49.4%) deaths. Among these, 48 events were in the STEMI-group and 29 in the NSTEMI-group (62.3% and 37.6% respectively). In regard to the overall number of adverse events after the 30 days follow-up, we recorded a total of 41 (11.7%) including 11 (26.8%) heart failures and 30 (73.2%) deaths. Among these, 26 events were in the STEMI-group and 15 in the NSTEMI-group (63.4% and 36.6% respectively). Cases who died had older mean age (73.9 ± 10.7 *vs* 65.5 ± 13.7; *P* = <0.0001),

more often had previous MI (44.23% *vs* 20.1%, *P* = <0.0001), displayed higher Killip class at entry (28.8% *vs* 9.1%, P<0.0001), and were often male (73.9% *vs* 63.2%, P=0.061).

Relation between infarct size and FXIII-A consuming

The strong and consolidated correlation demonstrated between infarct size and selected cardiac biomarker parameters (38, 39), prompted us to investigate whether the FXIII-A consuming could be dependent or influenced by the amount of cardiac injury assessed as the troponin T and the CKMB mass levels at 3 days after MI (TnT72h and CKMB72h). These specific time-points were selected among those suggested in literature (38, 39) and resulting appreciable among those available in our analysis. We performed the complete analyses of all the time-points available (t0-t96h) and the selected timings were the more significant before the biomarker level curve reverted. Accordingly, table 3 shows TnT72h and CKMB72h in the STEMI and in the NSTEMI group stratified by FXIII-A_{d5} levels. Summarizing, the significant highest marker mean levels were reserved to the STEMI/MACE+ subgroups, ascribing always higher mean levels to the presence of MACE. Conversely, FXIII-A feels mainly, and at a similar extent, the presence of MACEs regardless which group (STEMI or NSTEMI) they belong to. Anyway, to confirm the possible independent role that FXIII-A could have in predicting poor prognosis (i.e. HF or death) both TnT72h and CKMB72h were included in the general regression model, and this accounting maintained the significant association (see below). Accordingly, the Spearman analyses failed in recognizing correlation between FXIII-A_{d5} and TnT72h or CKMB72h in the whole group of cases (r^2=0.011 and r^2=0.006 respectively) or in the STEMI subgroup (r^2=0.029 and r^2=0.018 respectively).

Predictive role of FXIII-A level

In an explorative analysis, aimed at providing prognostic elements, we investigated whether definite FXIII-A levels could help in predicting a particular clinical endpoint (i.e. HF or death). For this purpose, we analysed by the ROC procedure the FXIII-A levels of the whole cohort of patients considering the reached combined endpoint heart failure or death. The results were appreciable and supplementary table 2 (ST2) shows in detail all the relative findings together with the FXIII-A thresholds at any of the d_0-d_5 time analyzed. It is to note that at any considered time the p-value was significant with appreciable AUC and sensitivity/specificity ratios. As an example, the ROC curve at day-4 (FXIII-A_{d4} cut-off= 73.5%) is provided in supplementary figure 1 (SF1). Interestingly, this FXIII-A cut-off value almost matched with the 50th percentile of the distribution in the whole cohort of patients

(FXIII-A_{50th}: 74.2%), thus prompting us to evaluate a possible FXIII-A dosage-effect on survival by stratifying the analysis on FXIII-A quartiles. The cumulative percentage of death and HF at one-year follow-up was 22.0% (n=77), and it was 29.7% and 14.28% respectively among cases with FXIII-A below and above the median value at day-4 (OR=2.54; CI95%, 1.49-4.32). Accordingly, the probability to die or to experience HF increased as the FXIII-A level considered decreased (Figure 4). Finally, the worst prognosis was reserved to those cases with FXIII-A below the 25th percentile (FXIII-A_{25th}<59.5%) and the associated analysis yielded a risk to experience heart failure or death at one-year as higher as about four folds (HR=3.96; CI95%, 2.14-6.61; P<0.0001). A sub-analysis at day-30, slightly increased the risk (HR=4.25; CI95%, 2.2-5.1; P<0.0001). Finally, in the linear regression model (univariate), male sex, age, diabetes, smoke, previous MI, EF<50%, Killip-class>1, TnT72h, and FXIII-A_{d4}<73.5% were significantly correlated with the combined endpoint (death or HF) at one-year follow-up. By incorporating in the multivariate analysis all the significant variables, age, previous-MI, EF<50%, Killip-class>1, TnT72h, and FXIII-A_{d4}<73.5% were significant putative predictors (Table 3).

Discussion

Several lines of evidence support an active role of FXIII-A in the post-MI healing and in the scar formation processes. FXIII-A contrasts the collagen deficit deposition and the restrained MMPs activity caused by the exaggerated and prolonged inflammation during MI. Both these situations affect in turn scar stability and neutrophil/macrophage recruitment (13-16). Accordingly, FXIII supplementary therapy and even intramyocardial injection of FXIII-modifiable biomaterial have been suggested (16-21). In addition, recent papers dealt with the role that platelet rich plasma has in the healing of MI injury (40-42). Platelets contain FXIII-A and a wide range of growth factors, and after activation they release a huge variety of pro-healing molecules at the injury site. By organizing a robust and elastic tri-dimensional fibrin network and influencing the ECM components, FXIII becomes essential for additional important tasks such as adult staminal cell recruitment, neo-angiogenesis, collagen deposit and in turn myocardial healing, being FXIII a molecule placed at the intersection of several crucial pathways (8, 43). A so organized durable fibrin/ECM network is essential requisite to contrast adverse infarct healing responsible for its extreme consequence the heart rupture or the development of severe HF.

These information prompted us to investigate variations in the circulating FXIII-A levels in patients during acute MI in the attempt to suggest FXIII-A as a novel prognostic

biomarker. The experimental data herein collected showed that an acute (d_0-d_5) and transient fall in FXIII-A levels virtually occurs, albeit to a different extent, in the whole cohort of MI patients. This is compatible with both coronary thrombus formation, in which activated FXIII-A cross-links fibrin, and the subsequent myocardial healing processes and scar formation. Interestingly, we found that patients undergoing excessive FXIII-A consuming at the time of MI were more prone to die or to develop HF. Accordingly, those presenting with constitutive low or borderline FXIII-A level might be even at higher risk.

First of all, the comparison of the dynamic of FXIII-A in the MACE− and in the MACE+ patients strongly points toward this relationship, and this was further reinforced by analyzing separately those who died or those who developed HF. As a matter of fact, among HF patients, the mean FXIII-A levels were significantly lower than those of MACE− group, and completely resembled those of the deceased cases. Huger thrombus formation, wider/deeper myocardial lesion extension, and/or constitutively low circulating FXIII values necessarily influences FXIII-A consuming and possibly prognosis. For these reasons, we investigated if the FXIII-A consuming could be dependent on the amount of injury assessed by CKMB or TnT release. As expected, there was a strong association between biomarker levels and the different kind of MI (i.e. STEMI>NSTEMI) and different prognosis (i.e. MACE+>MACE−), but no significant correlation was observed between biomarker and FXIII-A levels. Nevertheless, CKMB and TnT were included in the regression model to evaluate the independent prognostic role of FXIII-A. The existence of constitutively low FXIII-A levels among the patients under study was indeed suspected by the fact that cases with low FXIII-A levels were also found far from the acute phase (e.g. in HF at d_{30}) when the steady state should be re-established. Similarly, among the FXIII-A_{d0} we observed even lower FXIII-A levels, but this fact could be explained by the concomitant infarct condition. Accordingly, FXIII-A starts to be consumed concomitantly to the heart attack before patients arrive to the UCC. This fact explains in part why the levels of FXIII-A_{d30} resulted often higher than those of FXIII-A_{d0}. Unfortunately, the present hypothesis could not be proven in the deceased cases because of the lack of the day-30sample, nevertheless it is to note the lower FXIII-A mean level both group had at day-0. In a speculative hypothesis, this observation supports the idea that presenting with non-optimal FXIII-A levels during the early phase of acute MI could increase the risk of severe complications mainly involving the integrity of myocardial wall. The fact that these two groups had practically overlapped FXIII-A profile but different severity in prognosis could be in part explained by the fact that the deceased patients had higher additional risk factor at entry. Conversely, those cases with

better prognosis (i.e. MACE-) showed higher FXIII-A mean levels at the entry and they were apparently less affected by the FXIII-A fall. It is so assumable that these patients could not have had evident heart wall damage responsible for severe HF or death during the follow-up. Our data are in part consistent with those obtained by Nahrendorf in a mouse model (16, 18), and further they give additional information on the detailed acute monitoring of FXIII-A. The authors found that 100% of mice with genetically-determined reduced FXIII-A levels died within 5 days after induced MI of the left ventricular rupture, and that the adverse outcome could be reversed by intravenous FXIII supplementary therapy. It is worth noting that not only $FXIII^{-/-}$ homozygous KO mice died (FXIII-A, <5%) but also the $FXIII^{-/+}$ heterozygous mice having appreciable, albeit reduced, FXIII-A levels (i.e. FXIII-A, ~70%). Another extremely important finding was that FXIII supplementary therapy improved the survival to that of the wildtypes but did not prevent the anomalous heart wall remodelling. Summarizing, the totality of FXIII-A deficient mice (homozygotes and heterozygotes) died due to left ventricular rupture and the reconstituted $FXIII^{-/-}$ KO mice showed: ì. enhanced post-MI remodelling at MRI, ìì. significantly thinner scar thickness of the infarct area, and ìiì. increased left ventricular end-diastolic volume. The pivotal role of FXIII-A in driving the clinical outcome was further strengthened by the higher FXIII activity observed in the infarct area of wild-type mice as compared to the remote myocardium, an activity that was not detected in the $FXIII^{-/-}$ mice. In addition, direct evidence reported that FXIII-A levels were diminished in patients with infarct rupture (17-19) and that heparin and FXIII modulate collagen synthesis in opposite way influencing in turn healing and survival (18).

As a rule, residual FXIII-A level also depends on how much and how long was the consumption and the time, and this could be influenced by the persistence of the coronary occlusion. This led us to evaluate the relationship between residual FXIII-A levels in different kinds of MI (i.e. STEMI vs NSTEMI). Then, it was verified the hypothesis that a prompt reperfusion (prerogative of the STEMI) could prevent waste of FXIII-A. Comparison of data in the STEMI with the NSTEMI revealed a slight lower FXIII-A consumption in the formers, similarly to what observed comparing patients who underwent PCI *vs* those who did not. This could suggest that the FXIII-mediated healing phase, and not only the coronary thrombus formation, has a role in the dynamic of FXIII-A.

To provide information on possible associations between FXIII-A and post-MI heart performance, we compared in each specific subgroup the FXIII-A levels and the respective rates of ejection fraction. As expected, patients who died or those who experienced HF showed the lowest EF rate and this was interestingly coupled with the lowest FXIII-A levels.

Finally, we focused on the survival analysis by 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; the lower the FXIII-A level, the higher the risk. Accordingly, the worst prognosis was reserved to those cases with FXIII-A below the 25th percentile with an increased risk of about 4-folds to reach the combined endpoint at both 30-days or one-year follow-up. We would like to point out that we have chosen values at day-4 just as an explorative example and in the attempt to have prognostic information as earlier as possible but at a time-point in which FXIII-A consuming has been reasonably established. It is to note that similar results were obtained at day-5 (data not shown). Although, this FXIII-A value is assumable to be enough to guarantee healing in normal condition, it could not be sufficient to sustain healing during the acute MI when a prolonged and intensive activity is needed to prevent wall damage. These findings are in part consistent with those previous reported in mice (16, 18) or in patients (17-19). Although they are two completely different models or contest, both figures point out the fact that optimal outcome after MI demands optimal FXIII-A levels. Accordingly, low FXIII-A levels might be considered a predictor/marker of the severity of the post-MI outcome. The existence of a prognostic FXIII-A threshold necessarily is going to further encourage investigations aimed at better defining it and at including FXIII-A among the conventional monitoring of acute biomarkers in MI. On the other hand, if further confirmed, these information would boost research in evaluating if FXIII supplementation might contrast severe infarction complications (17-21) only in those patients with "reduced" FXIII-A levels, thus avoiding its indiscriminate use and the potentially risky procoagulant impact.

In conclusion, for the first time we provided evidence that FXIII-A level dynamics in the early phases of MI might help clinicians to predict the infarction evolution, and particularly the post-MI damage responsible for HF or death, thus providing elements for "personalized" therapeutic interventions based on FXIII-A levels. Only one interesting paper recently investigated in detail the dynamics of FXIII activity thought with completely different aims and in congenital heart defects (44). New strategies aimed to furnish a cardiac matrix with superior and modified quality is the goal in myocardial healing and FXIII is a candidate molecule to maintain structural and functional integrity after MI.

Limitations of the Study

The present study reserves some limitations. First of all, the lack of additional FXIII measurements, such as the activity, and the antigen levels of the FXIII-A2B2 complex and of the FXIII-B, as well as the investigation of additional different coagulation factors. In addition, we did not consider the FXIII-A levels during the few days after day-5 which were available in our survey for a very limited number of patients only. These comparisons would have added precious information on the pathomechanism of this phenomenon. Accordingly, our results strongly suggest additional future detailed studies in the field.

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Conflict of interest

None declared.

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Legends to Tables and Figures

Table 1. PCI= percutaneous coronary intervention; STEMI= patients showing ST-segment elevation MI at enrolment; NSTEMI= patients not showing ST-segment elevation MI at enrolment; EF= Ejection Fraction.

Supplementary Table 1. (ST1) Patients (%), indicates the percentage of patients available at any specified day. The associated number of patients is reported in the first row showing the day in which findings are referred to (d_0-d_5 and d_{30}).

Table 2. *P-value shows the comparisons of both HF and Death sub-group with the MACE– group.

Supplementary Table 2. (ST2) AUC, indicates Area Under the Curve. In bold is depicted the day-4, to which the ROC-curve in supplementary figure 1 (SF1) is referred.

Table 3. All the values showed are mean ± SD. * and ** indicates statistically significant differences, P<0.0001 and P<0.01 respectively comparing STEMI vs NSTEMI group and MACE+ vs MACE– subgroup.

Table 4. Linear regression analysis for the combined end-point (dead or HF); in bold are reported the significant p-values. Only the significant variables were included in the multivariate analysis.

Figure 1. Dynamics of FXIII-A (mean and SD) at the scheduled times in the whole cohort of MI patients. P<0.00001 comparing d_0 and d_5 mean levels, and P=0.02 comparing d_0 and d_{30} mean levels.

Figure 2. Dynamics of FXIII-A (mean and SD) at the scheduled times in those cases who during the follow-up experienced any kind of MACE vs those who didn't. Comparing mean levels between MACE+ and MACE–, P<0.0001 at any point analyzed.

Figure 3. Dynamic of FXIII-A (mean and SD) at the scheduled time in those patients who experienced heart failure (left) and in those who deceased during the follow-up (right). In these latter, d_{30} was not reported due to the partial and scanty availability of samples.

Figure 4. Survival analysis (death or HF) at one-year follow-up stratified by FXIII-A_{d4} quartiles; the dashed line indicates the cumulative analyses performed in the whole group.

Supplementary Figure 1. (SF1) ROC curve of FXIII-A values at day 4 in MI patients. The endpoint considered was HF or death.

Table 1. Main baseline characteristics in the whole MI group, in the STEMI and NSTEMI subgroups.

Characteristics	All cases (n=350)	STEMI (n=251)	NSTEMI (n=99)	P
Age (*y, SD, range*)	68.2±12.95 (31-80)	67.1±13.50 (31-80)	71.05±11.33 (38-80)	<0.01
Male (*n, %*)	255 (72.8)	185 (73.7)	70 (70.7)	NS
PCI (*n, %*)	313 (89.42)	235 (93.6)	78 (78.8)	<0.0001
Hyertension (*n, %*)	232 (66.3)	163 (64.9)	69 (69.7)	NS
Dyslipidemia (*n, %*)	125(35.7)	80 (31.8)	45 (45.45)	0.02
Obesity (*n, %*)	40 (10.6)	27 (10.8)	13 (13.2)	NS
Diabetes (*n, %*)	82 (23.42)	47 (18.7)	35 (35.4)	0.002
Smoking (*n, %*)	189 (54.0)	140 (55.8)	49 (49.5)	NS
Familiarity (*n, %*)	115 (32.9)	82 (32.7)	33 (33.4)	NS
Previous MI (*n, %*)	97 (27.7)	58 (23.2)	39 (39.4)	0.01
KIllip class >1 (*n, %*)	46 (13.1)	38 (15.1)	8 (8.1)	NS
EF% ≥ 50% (*n, %*)	142 (40.6)	96 (38.24)	46 (46.55)	NS
EF (*% ± SD*)	44.9±11.27	44.56±10.82	45.6±12.34	NS

Table 2. FXIII-A levels and EF % according to the different MACE.

	HF	Death	MACE−	P-value*
EF, *mean±SD (range)*	38.4±10.4 (20-60)	39.5±14.4 (15-60)	47.5±10.5 (35-72.0)	<0.0001
FXIII-A_{d5}, *mean±SD (range)*	52.1±25.2 (20.0-64.2)	54.0±22.5 (18.1-60.0)	83.5±23.53 (46.0-136.6)	<0.0001

Table 3. Cardiac biomarkers (TnT72h and CKMB72h mass) stratified by FXIII-A_{d5} levels.				
	STEMI (n=251)		**NSTEMI (n=99)**	
TnT72h, *ng/mL*	*3.23±2.1		1.24±0.9	
	MACE+ (n=48)	**MACE- (n=203)**	**MACE+ (n=29)**	**MACE- (n=70)**
	*5.02±3.5	2.81±1.5	*2.55±1.7	0.70±0.7
CKMB72h, *ng/mL*	*64.75±43.9		20.1±16.9	
	MACE+	**MACE-**	**MACE+**	**MACE-**
	**84.61±68.2	60.08±47.3	**32.85±22.44	14.69±12.8
FXIII-A_{d5}, *%±SD*	79.1±24.1		70.2±28.1	
	*54.2±25.7	84.8±24.6	*50.7±30.1	77.86±26.9

Table 4. Linear regression analysis for the combined end-point (dead or HF).		
	P-value (univariate)	**P-value (multivariate)**
Sex (male)	**0.023033**	0.120674
Age	**<0.00001**	**0.000606**
Diabetes	**0.000148**	0.281156
Smoke	**0.005504**	0.944806
previous-MI	**0.003956**	**0.044997**
EF<50%	**0.001166**	**0.016027**
Killip>1	**<0.00001**	**0.000013**
TnT72h	**0.001589**	**0.000733**
CKMB72h	0.08107	--
FXIII-A<73.5%	**0.000003**	**0.000125**

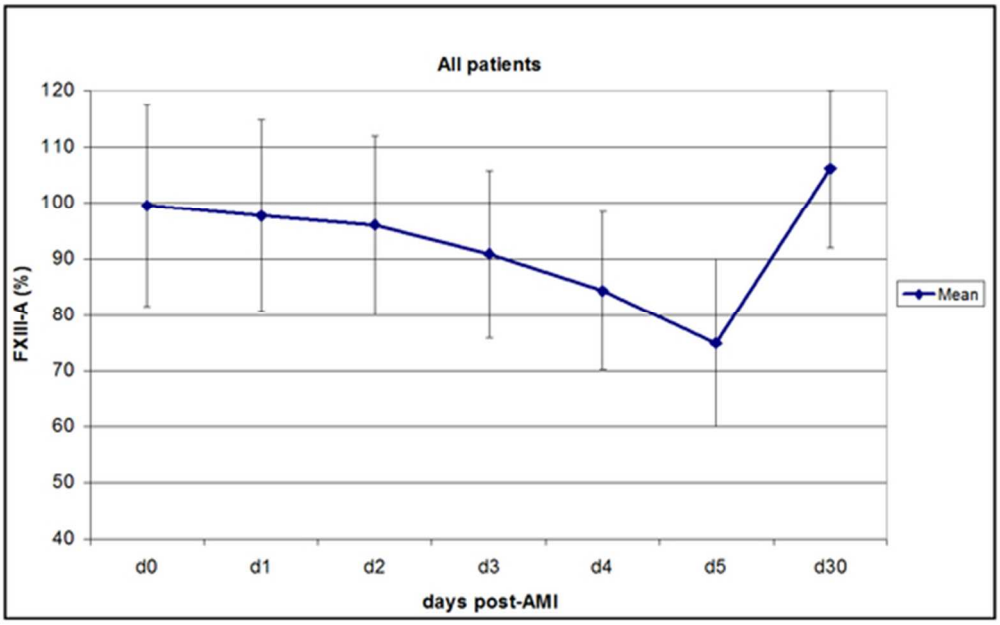

Fig.1
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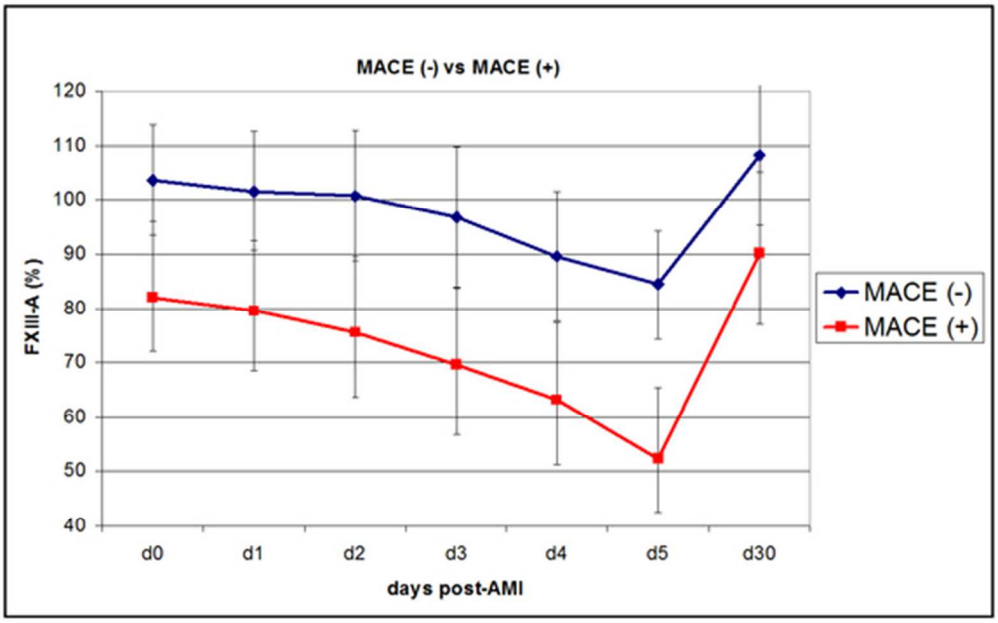

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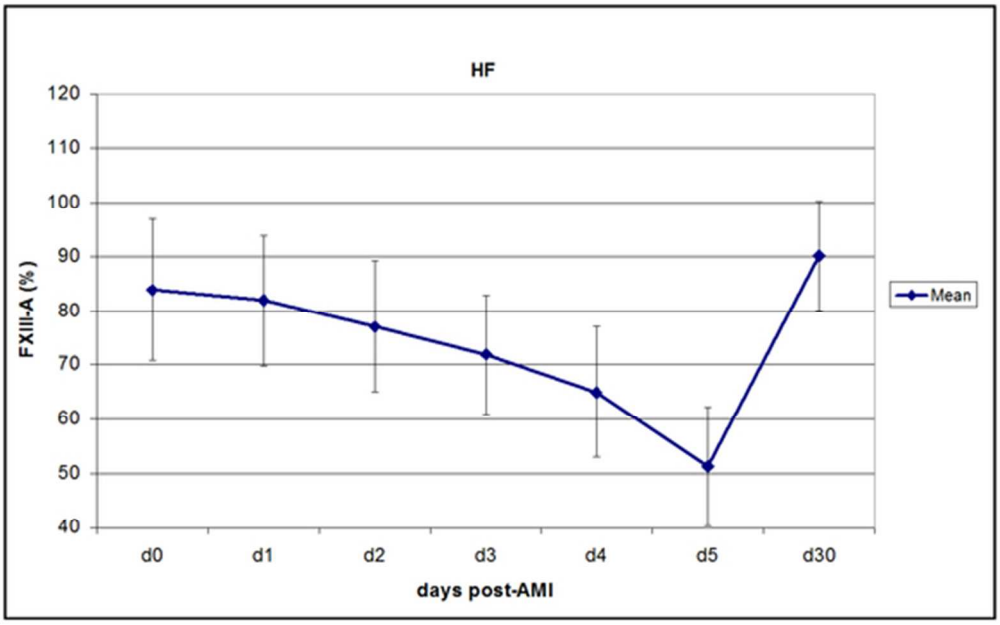

Fig.3 (left)
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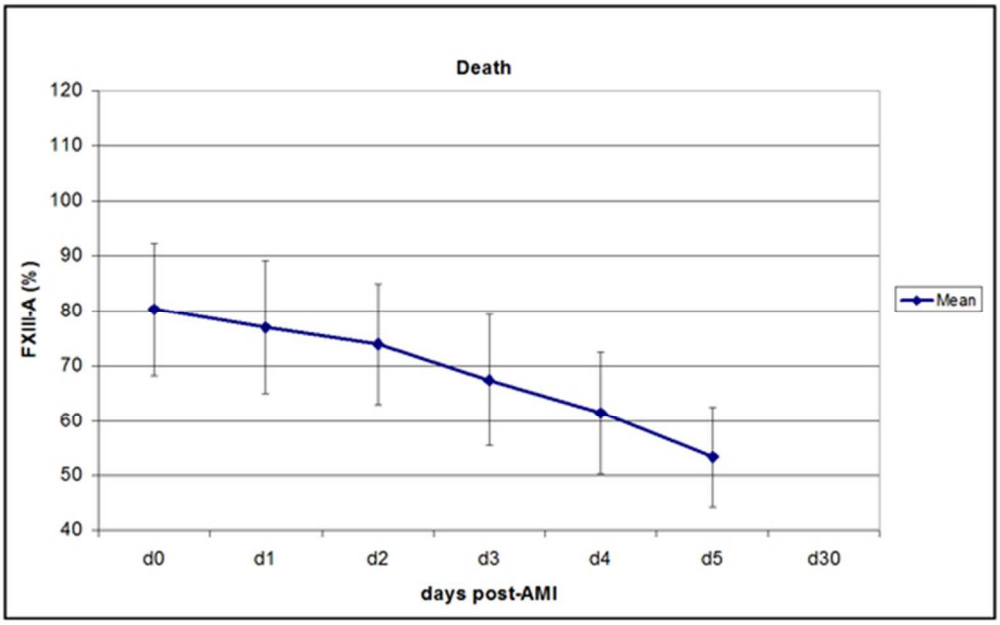

Fig.3 (right)
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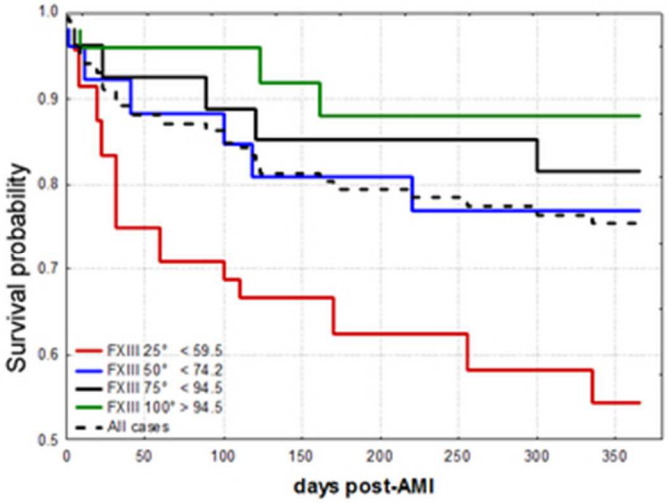

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