Effect of Eplerenone on Extracellular Cardiac Matrix Biomarkers in Patients With acute ST-elevation Myocardial Infarction without Heart Failure: Insights from the Randomized Double-Blind REMINDER Study

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Abstract

Objective: Aldosterone stimulates cardiac collagen synthesis. Circulating biomarkers of collagen turnover provide a useful tool for the assessment of cardiac remodeling in patients with an acute myocardial infarction (MI).

Methods: The REMINDER trial assessed the effect of eplerenone in patients with an acute ST-elevation Myocardial Infarction (STEMI) without known heart failure (HF), when initiated within 24 h of symptom onset. The primary outcome was almost totally (>90%) driven by natriuretic peptide (NP) thresholds after 1-month post-MI (it also included a composite of cardiovascular death or re-hospitalization or new onset HF or sustained ventricular tachycardia or fibrillation or LVEF ≤40% after 1-month post-MI). This secondary analysis aims to assess the extracellular matrix marker (ECMM) levels with regards to: 1) patients` characteristics; 2) determinants; 3) and eplerenone effect.

Results: Serum levels of ECMM were measured in 526 (52%) of the 1012 patients enrolled in the REMINDER trial. Patients with procollagen type III N-terminal propeptide (PIIINP) above the median were older and had worse renal function ($p<0.05$). Worse renal function was associated with increased levels of PIIINP (standardized $\beta\approx0.20$, $p<0.05$). Eplerenone reduced PIIINP when the levels of this biomarker were above the median of 3.9 ng/mL (0.13±1.48 vs. -0.37±1.56 ng/mL, p=0.008). Higher levels of PIIINP were independently associated with higher proportion of NP above the prespecified thresholds (HR=1.95, 95%CI=1.16-3.29, p=0.012).

Conclusions: Eplerenone effectively reduces PIIINP levels when baseline values were above the median. Eplerenone may limit ECMM formation in post-MI without HF.

Key-words: myocardial infarction; extracellular cardiac markers; eplerenone.

Introduction

Acute myocardial infarction (AMI) is a major cause of deleterious cardiac remodeling harboring an increased risk for left ventricular dysfunction and heart failure (HF). The effect of adverse remodeling on cardiac structure and function assessed by routinely available imaging methods (such as cardiac ultrasound) and laboratorial results (such as natriuretic peptides) may detect only late stages of adverse remodeling and cannot assess if the myocardial changes are a consequence of impairment in the extracellular collagen matrix markers (ECMM) or in the cardiac myocytes[1, 2]. In this regard, circulating biomarkers of collagen turnover may provide a useful and validated tool to reliably assess this ECMM turnover, as demonstrated in patients with systolic dysfunction either in chronic heart failure with reduced ejection fraction (HF-REF) or post-MI[1, 3, 4].

The REMINDER (early eplerenone treatment in patients with acute ST-elevation myocardial infarction without heart failure) trial[5] was designed to assess the impact of eplerenone on cardiovascular (CV) outcomes in patients with STEMI without known HF. This setting may allow to assess the impact of the mineralocorticoid receptor antagonist (MRA) eplerenone for limiting "myocardial fibrosis" in patients at risk for HF but without overt HF.

Alterations of the ECMM and cardiac remodelling play a major role in the development and evolution of the cardiac alterations that lead to HF[6]. In this regard, several potential circulating biomarkers (BMs) for assessment of cardiac fibrosis have been proposed[6, 7]. Particularly, the BMs of ECMM formation measured in the REMINDER study were: procollagen type III N-terminal propeptide (PIIINP), collagen type I C-terminal telopeptide (ICTP), and procollagen type I N-terminal propeptide (PINP), and also Galectin-3. Of these, the PIIINP (involved in the collagen biosynthesis) has been the most robustly associated with myocardial fibrosis and validated thoroughly[8-10].

The main aims of this secondary analysis of the REMINDER trial are to assess the ECMM marker levels with regards to: 1) patients` characteristics; 2) factors associated with increased levels; 3) and eplerenone effect.

Methods

Study Design and Patient Population

The design and main results of the REMINDER trial have been previously reported[5] and is registered at ClinicalTrials.gov with the number: NCT01176968.

In short, the REMINDER trial was a randomized, placebo-controlled, double-blind trial, assigning 1012 patients with acute STEMI and without HF history to receive either eplerenone (25–50 mg once daily) or placebo (n =506 in each group) in addition to standard therapy. Eligible subjects were identified for inclusion following emergency room/ambulance evaluation and diagnosis of acute STEMI in the absence of a clinical diagnosis of HF. Randomization had

to take place as early as possible following diagnosis and the first dose of study drug administered within 24 h of the onset of symptoms of acute MI and preferably within 12 h. The primary endpoint was the composite of CV mortality, re-hospitalization, or extended initial hospital stay due to diagnosis of HF, sustained ventricular tachycardia or fibrillation, ejection fraction ≤40% at 1-month or later post-randomization, or elevated BNP/NT-proBNP at 1-month. Natriuretic peptide (NP) elevation was defined as BNP levels ≥200 pg/mL or NT-proBNP values ≥450 pg/mL (in patients aged less than 50 years), ≥900 pg/mL (in patients aged 50 to 75 years), or ≥1800 pg/mL (in patients older than 75 years). The primary endpoint occurred in 92 patients (18.2%) in the eplerenone group and in 149 patients (29.4%) in the placebo group (adjusted hazard ratio, HR [95% confidence interval, CI] =0.58 [0.45–0.76], p =0.0001). The primary endpoint was essentially (91%) driven by the NP thresholds that were higher in the placebo group. The "hard" endpoint of CV mortality or HF hospitalization or extended hospitalization due to HF occurred only in 9 (1.8%) patients in the eplerenone group vs. 13 (2.6%) patients in the placebo group which is largely underpowered to drive any conclusion on major CV events. Adverse event rates were low (<6%) and similar in both groups.

This present substudy was conducted in the 526 patients participating in the REMINDER trial with ECMM measurements. The selection of patients in whom ECMM were measured was performed in a random fashion. In the present subanalysis the NP thresholds represented 92% (n=126) of the 137 recorded events.

Blood samples for all the biomarkers of collagen turnover were analyzed in 1 laboratory. Plasma NPs and other neurohormones were analyzed in different laboratories.

A transthoracic echocardiogram was performed at the 6-month follow-up and at the end of study visit.

Blood Sampling

Blood samples were drawn at baseline (i.e., within the first 24h after STEMI diagnosis) and at 6 months of follow-up. All samples were centrifuged immediately at 3000 rpm for 10 minutes and stored at -80°C until assay analysis. A minimum of 2 samples were available per patient: 1 at baseline and 1 at 6-month follow-up. All samples were transported to the central laboratory and assayed in 1 batch.

Laboratory Analysis of the Biomarkers

The measured "collagen" biomarkers were: PIIINP, ICTP, PINP, and Galectin-3. All assays were performed by technicians blinded to clinical data and subject randomization. Commercial radioimmunoassays (Orion Diagnostica®) were used to measure PIIINP, ICTP, PINP, and Galectin-3). The sensitivity (lowest concentration different from zero) was 0.3 ng/mL for PIIINP, 2.0 ng/mL for PINP, 0.4 µg/L for ICTP, and 3 ng/mL for Galectin-3. Normal serum ranges were provided by the assay manufacturer were 2.3 to 6.3 ng/mL for PIIINP; 19 to 83

ng/mL for PINP in women and 22-87 ng/mL in men; 2.1 to 5.6 ng/mL for ICTP in women and 2.1 to 5.0 ng/mL in men; and <22.1 ng/mL for Galectin-3. Inter-assay variations for PIIINP, PINP, ICTP, and Galectin-3 were <12% and their intra-assay variations were <10%.

Statistical Analysis

Continuous variables were all inspected by histogram visualization and described as mean ± standard deviation if normally distributed or median (percentile $_{25\text{-}75}$) if distribution was skewed. Categorical variables were described as absolute numbers (n.) and proportions (%). The studied biomarkers were divided by their median values in order to be compared by "lower" vs. "higher" values. Normality assumptions were verified by histogram assessment. Missing values proportion was overall low and no multiple imputation was performed.

Linear regression models were performed to assess the relation between the studied biomarkers (as outcome variables) and several explanatory variables selected from demographic (age, gender), clinical (heart rate, systolic blood pressure, body mass index), laboratorial (estimated glomerular filtration rate, hemoglobin, sodium, potassium, urea, albumin, total bilirubin, alanine aminotransferase, aldosterone, cortisol), medical history (diabetes, hypertension, atrial fibrillation, previous myocardial infarction), concomitant treatments (angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, β-blockers, lipid lowering therapies, diuretics), and biomarker (natriuretic peptides, troponins) variables. Linear regression assumptions were met. In particular normality was handled by Log_2 transformation of skewed variables and "normality" re-verification on histogram. Colinearity was assessed by colinearity tables and tolerance verification (diastolic blood pressure and aspartate aminotransferase were excluded from the models due to high correlation [>0.7] with systolic blood pressure and alanine aminotransferase, respectively). Observed versus expected probability P-P padronized residuals regression plot was checked for linearity assessment and plot of padronized residuals versus predicted padronized values was also checked for heteroscedasticity exclusion (all values were within the 3 to -3 range). The first model was entered with 200x bootstrapping, then a backward selection model was processed. The variables retained in the final model with a p-value <0.05 are presented in the tables. Due to a high proportion of missing values (≈70%), natriuretic peptides (median) were ran (as independent variable) in individual models, but they were not significantly associated with any of the outcome variables. Due to imprecision in the associations regarding natriuretic peptides, we present in tables any association with a p-value of less than 0.1.

To determine predictors of having a "fibrosis markers" above the median we developed logistic regression models. These models used clinical and laboratory variables with a p-value <0.2 as entry criteria. The first model was a forward conditional model eliminating progressively the variables with weaker association and retaining in the final model those variables with a p <0.05. The second model used a stepwise backward selection process. Both

models provided similar final results. Logistic regression assumptions were checked and multicollinearity excluded. Linear relationship between continuous independent variables and the logit transformation of the dependent variable was verified by plotting the means vs. the β estimates in quintiles. All presented models met the logistic regression assumptions. The computation of ECMM on their median values was not a prespecified analysis.

The natriuretic peptide threshold outcome was analyzed by Cox proportional hazard regression models. An interaction term between the biomarker of interest and treatment allocation (as "dummy" variable) was tested within the Cox model. In the multivariable models, the covariates for adjustment were chosen from demographic (age and gender), clinical (heart rate, body mass index, diabetes, hypertension, previous myocardial infarction), laboratorial (estimated glomerular filtration rate determined by the CKD-EPI formula[11] and aspartate aminotransferase), and pharmacological (ACEi/ARBs, β-blockers, diuretics, and treatment allocation i.e. eplerenone or placebo) variables. The "rule of thumb" of 1 variable per 10 events was met. Proportional hazard assumption was verified graphically using "log-log" plots. The studied variables had a linear association with the outcome.

We also performed a Principal Components Analysis (PCA) in order to determine which variable(s)/biomarker(s) that explain the largest proportion of the variability in the data. We used the direct oblimin rotation method and fixed the number of retained components to two.

Additional analysis of covariance (one-way ANCOVA) were also performed to assess the treatment effect on PIIINP levels adjusted on age, gender and estimated glomerular filtration rate.

All analysis were performed using R® software (The R Foundation for Statistical Computing).

Results

Baseline Characteristics of the Study Population

The characteristics of the population analyzed in the present study are depicted in **Table 1**, where the characteristics of the patients with levels of PIIINP below vs. above the median are also described. Patients with PIIINP levels above the median were older, had lower eGFR, higher ICTP and PINP levels (all $p < 0.05$). **Table 1**. The comparison of patients according to the median levels of Galectin-3, ICTP, PINP, and Aldosterone are presented in the **Supplemental Material Tables 1 to 4**. The patients` characteristics according to the median PIIINP levels at 6-months are presented in the **Supplemental Table 5**.

The characteristics of the patients with vs. without ECMM markers measurements, as well as the characteristics of the patients randomized to eplerenone or placebo, are well balanced. **Supplemental Table 6** and **Supplemental Table 7**. The correlation between ECMM

markers is generally weak, with higher correlations between PIIINP and ICTP (=0.29). **Supplemental Table 8**.

The baseline levels of the ECMM markers did not differ between eplerenone and placebo groups. **Supplemental Table 9**.

Linear and Logistic regression models

Linear regression models using ECMM marker as outcome variables are presented in **Table 2**. The proportion of the total variability in the outcome variables was little explained by the variables inserted in the models, ranging from 11% (adjusted R^2 =0.11) for PIIINP to 23% for Galectin-3 (adjusted R^2 =0.23). The levels of PIIINP were likely to increase in the context of lower eGFR (β =0.23), higher heart rate (β =0.15), and higher total bilirubin (β =0.11). Galectin-3 levels were likely to increase in the context of lower eGFR (β =0.34), higher cortisol and aldosterone levels (β =0.18 and β =0.13, respectively), and higher ALT (β =0.12). Levels of ICTP possibly also increase in the context of worse renal function, higher BMI, bilirubin, heart rate, and diuretic use. PINP levels were likely to be higher in females, and decrease with ageing, aldosterone, beta-blockers use, hypertension and diabetes history. **Table 2**.

Logistic regression models (using ECMM markers above the median as reference) are presented in the **Supplemental Table 10.** and show similar associations to those observed in the linear regression models.

Eplerenone Effect and Biomarkers

Eplerenone decreased PIIINP levels (from baseline to 6 months) when the baseline PIIINP levels were above the median of 3.9 ng/mL (absolute Δ PIIINP =0.13±1.48 in the placebo group vs. -0.37±1.56 in the eplerenone group; p =0.008). **Figure 1** and **Table 3**. PINP levels were also decreased by eplerenone treatment when PINP baseline values were above the median. **Table 3**. As expected feedback mechanism eplerenone treatment increased aldosterone levels. **Supplemental Table 11**.

An analysis of covariance (one-way ANCOVA) was also performed to assess the eplerenone effect on PIIINP levels according to their baseline "median" value adjusted on age, gender and estimated glomerular filtration rate, providing overlapping results with those presented in univariate analysis. **Supplemental Table 12.**

Natriuretic Peptide Associations

The NP driven primary outcome associations are depicted in the **Table 3**. Linear PIIINP and Galectin-3 were independently associated with the NP thresholds outcome (HR; 95%CI =1.95; 1.16-3.29; p =0.012 and 2.21; 1.49-3.28; p <0.001, respectively), whereas ICTP and PINP were not. **Table 3**. The associations considering the biomarkers median levels and also those of aldosterone are presented in the **Supplemental Table 13**.

Eplerenone effect did not vary by baseline median ECMM levels (i.e. no significant "interactions" were found). **Figure 2**.

Principal Components

The PCA identified PIIINP as explaining the majority of the variance in the data (38.1%), followed by ICTP (25.7%); PIIINP was also the first component to be retained after oblimin rotation (=0.75), followed by ICTP (=0.72).

Discussion

This is the first study describing mid-term kinetics of ECMM collagen biomarkers in patients with acute MI without overt HF. In the present manuscript, we demonstrate that higher levels of ECMM markers are partially dependent on factors such as renal function, heart rate and age. Importantly, eplerenone independently reduced the levels of PIIINP and PINP when these were above the median. These findings suggest that MRAs can limit the "pro-fibrotic" ECMM deposition and may have a role in limiting adverse cardiac remodelling independently of age, gender or renal function.

Tissue repair through the synthesis of new ECMM by fibroblasts may be beneficial, particularly after a MI. However, prolonged activation of this process results in excess scar tissue formation and increased ECMM deposition, leading to an "excessive fibrosis" pattern that may severely compromise the myocardial tissue and impair electrical conduction favoring the advent of arrhythmia, particularly atrial fibrillation[12]. Fibrosis is itself a "dynamic tissue"[13] in which collagen synthesis is an ongoing process involving metabolically active myofibroblasts[12]. Serum PIIINP has been found to be correlated to myocardial collagen type III in HF patients of ischemic etiology and idiopathic dilated cardiomyopathy (DCM)[9, 10]. Collagen scar formation after acute MI causing left ventricular systolic dysfunction could also be quantified by measurements of serum PIIINP concentrations[14]. Moreover, in patients with DCM the reduction of the myocardial collagen achieved by the treatment with the MRA spironolactone was also accompanied by a significant reduction in the serum levels of PIIINP[9]. In HF-REF patients with severe symptoms (findings from the Randomized Aldactone Evaluation Study: RALES), higher baseline PIIINP (levels superior to the median of 3.9 ng/mL) were associated with increased mortality rates (HR; 95%CI =2.36; 1.34-4.18) and PIIINP levels decreased in spironolactone treated patients from baseline to 6 months (whereas remained stable in placebo-treated patients). Of notice, in this subanalysis of the RALES trial the death rate reduction achieved by spironolactone treatment was only observed in patients with PIIINP levels above the median, whereas it was "neutral" in patients with PIIINP levels below the median (HR; 95%CI = 0.44; 0.26–0.75 vs. 1.11; 0.66–1.88). In post-MI patients with HF-REF (findings from the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study: EPHESUS)[3], eplerenone treatment also consistently reduced PIIINP levels. In 134 patients with acute anterior STEMI a strategy of early aldosterone blockade with intravenous potassium canrenoate (the active metabolite of spironolactone) improved LVEF and reduced LV end-diastolic volume in

comparison to controls. In this study, cardiac aldosterone extraction was suppressed and the plasma PIIINP levels were also significantly reduced in the aldosterone antagonist group[15]. All these findings overlap, to a great extent, those reported in the present REMINDER trial subanalysis, in which only patients with PIIINP levels above the median of 3.9 ng/mL had significant reduction of this biomarker.

The median baseline levels of PIIINP in the REMINDER trial patients were similar to those reported in post-MI patients from EPHESUS (≈4.2 ng/mL) and HF patients from RALES (≈3.9 ng/mL), and lower than those reported in a setting of dilated cardiomyopathy patients (≈6.1 ng/mL)[10], suggesting that patients with dilated cardiomyopathy have may have higher collagen turnover and ECMM matrix formation in concordance to the severity of disease presentation and prognosis.

ICTP and Galectin-3 were not reduced by eplerenone treatment. Galectin-3 is a binding protein related to the collagen deposition of fibroblasts that is likely upregulated by the renin-angiotensin-aldosterone system (RAAS)[16, 17], but its direct correlation to the extent of cardiac fibrosis is lacking[18]. In the present study aldosterone and cortisol were slightly associated to Galectin-3 levels, suggesting that the RAAS may have a role for Galectin-3 activation also in the post-MI without HF context. The association of ICTP to histologically proven myocardial fibrosis has also been inconclusive, and this molecule also does not seem to respond to MRA therapy in other contexts[9, 10]. Whether PINP is associated (or not) with myocardial fibrosis is unknown[6, 8], and its associations to morbidity and mortality outcomes are also lacking[1, 3]. In the present analysis PINP levels decrease with ageing, aldosterone, hypertension and diabetes and were also reduced by eplerenone as compared to placebo. These ECMM fibrosis markers are not "heart-specific" and are present in other organs such as the kidney and liver[19], the present analysis also offers insight in this regards, since variables such as eGFR, bilirubin and ALT are often retained in the final linear regression models as associated to the studied ECMM markers. Moreover, in some populations (e.g. hypertrophic cardiopathy) ECMM were not correlated to myocardial collagen deposition[7], hence in the present analysis we cannot conclude that "cardiac fibrosis" was reduced by MRA, we can only state that some markers associated with collagen turnover were reduced by eplerenone treatment.

Importantly, in the REMINDER trial several biomarkers were studied for research purposes. However, in clinical practice this is unlikely to be feasible. The PCA analysis demonstrates that PIIINP is also the biomarker that accounts for the greatest proportion of the variability in the data; therefore, it should be the biomarker to be used in this setting.

In resume, the results of our study with eplerenone are consistent with and extend the results of previous experimental and clinical observations with aldosterone antagonists in HF and after MI, and suggest that the beneficial effect of aldosterone antagonists on ECMM remodeling may contribute to the clinical benefits of this therapy.

Clinical and Research Implications

A strategy of early aldosterone antagonism using potassium canrenoate in the first health-care contact for a MI (even without potassium or creatinine results were made available), followed by oral spironolactone was performed in the ALBATROSS trial (Early Aldosterone Blockade in Acute Myocardial Infarction). This "open-label" trial enrolled 1603 acute MI patients (STEMI and NSTEMI) to MRA or "standard therapy", and did not show significant between-group differences regarding the primary outcome of death, resuscitated cardiac arrest, significant ventricular arrhythmia, indication for implantable defibrillator, or new or worsening HF at 6-month follow-up. Of notice, the death rate (non-prespecified exploratory outcome) was significantly lower in the STEMI subgroup (n =1229; HR; 95%CI =0.20; 0.06-0.70; p for interaction =0.01)[20]. However, it should be highlighted that both REMINDER and ALBATROSS were largely underpowered to detect significant treatment effect differences in morbidity and mortality. In ALBATROSS, the primary outcome occurred in only 95 (11.8%) and 98 (12.2%) patients in the treatment and control groups, respectively, and considering the same events in REMINDER the proportion was even lower (as described in the Methods section). The event rate would have been insufficient to ascertain treatment differences event if these trials had been performed together. The post-MI CV mortality and severe LV systolic dysfunction event rate has been declining steadily due to improvements in reperfusion times and techniques, and concomitant treatments, including devices. However, adverse remodelling still occurs thoroughly, leading to high and apparently unchanged rates of HF[21]. Designing an event-driven trial in the setting of low event-rates is challenging as it requires several thousands of patients, a complex structure and unaffordable costs (especially for investigator-initiative trials). Hence, using clinically meaningful endpoints[22] such as NPs and measures of myocardial remodelling (e.g. LVEF, LV volumes, strain analysis, or magnetic resonance imaging) may shorten the sample size to a few hundreds of patients and allow to determine the treatment effect. From this point of view, STEMI patients without HF with the characteristics of those enrolled in the REMINDER trial benefit from early introduction of eplerenone treatment as it decreases NPs, that are also a sensitive and specific marker of HF. Altogether, these findings suggest that MRAs may be an important therapy for HF prevention in post-MI. To date no data on biomarkers from the ALBATROSS trial have been made available, but do to its pharmacologic characteristics, intravenous potassium canrenoate may be interest in the MI setting due to its rapid onset of action (<30 min) reaching high plasma concentrations, whereas eplerenone may take up to 2 hours and spironolactone more than 24 hours, both reaching much lower plasma concentrations[23, 24], and making these oral drugs less interesting when a rapid aldosterone blockade is required.

The ECMM marker PIIINP has shown reproducible results and may be useful for trials with adaptive design where "responders" to therapy may be better selected along the trial based on the levels of this marker. However, "situation-specific" thresholds should be better determined and these assumptions require prospective validation.

Limitations

Several limitations should be noticed in the present analysis. First, this is a prespecified secondary analysis of the REMINDER trial, however the computation of ECMM on their median values was not prespecified in the protocol and some of the associations reported herein may result from multiple testing chance findings. Moreover, no validation cohort is available at this moment to reproduce these results, but this also strengthens the originality of the present work. Additionally, the characteristics of the patients analyzed herein are similar to those without ECMM markers determination, suggesting that randomization was well-balanced and that these results may be generalizable to the entire population of the trial. To detect a 0.3 ng/mL difference in Log PIINP levels between eplerenone and placebo groups assuming a Log standard deviation of 0.4 ng/mL, a sample size of 76 patients (38 per group) would be required for a 90% power and an alpha of 5%. Hence this study was also adequately powered to detect differences in biomarker levels by treatment allocation. Second, this study does not allow to thrive conclusions on major adverse cardiovascular events, but such a study in this population is also unlikely to be performed due to a low event rate in this population (as explained in the above section). Third, no baseline/randomization transthoracic echocardiogram was performed, hence we cannot provide association between these markers and the baseline echocardiographic variables. Moreover, echocardiographic changes (from baseline to 6 months) cannot be computed in the present dataset either. These parameters could also provide information on cardiac remodelling. Fourth, no cardiac magnetic resonance imaging (MRI) was performed, this exam could provide useful information on myocardial fibrosis and would allow to assess the correlation between myocardial fibrosis and circulating ECMM in this population. In a previous report from 50 patients with hypertrophic cardiopathy (compared to 25 controls) the cardiac contribution to peripheral levels of byproducts of collagen synthesis was insignificant and peripheral levels of these biomarkers did not accurately reflect myocardial collagen content (as evaluated by cardiac MRI) in those patients[7]. Fifth, information regarding vessel involvement and lesions extension is not available in the dataset, and this could also influence the levels of ECMM. Sixth, ECMM were only measured at baseline and 6 months, hence we could not explore the kinetics of these biomarkers in the early post-MI period. Lastly, renal function is associated with the concentrations of ECMM in this context as is likely to be one of the strongest variables driving these markers` concentration in the present context, this reinforces the need for caution in interpreting these results as they may not reflect cardiac collagen

turnover.

Conclusions

This is the first study describing mid-term kinetics of ECMM collagen biomarkers in patients with acute MI without overt HF. Eplerenone independently reduced PIIINP levels at 6 months when the baseline values were above the median of 3.9 ng/mL. These findings suggest that MRAs can limit the "pro-fibrotic" ECMM deposition.

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Table 1. Baseline (randomization) Characteristics of the Study Population with available "fibrosis biomarkers" and comparison with regards to median PIIINP levels (comparison according to the median values of the other biomarkers are presented in the Supplemental Material)

Variables	N	Total	PIIINP ≤3.9 ng/mL	PIIINP >3.9 ng/mL	p-value
Demographics					
Age (years)	526	57.7 ± 10.7	56.7 ± 10.1	58.6 ± 11.2	0.039
Female sex, n. (%)	526	99 (18.8 %)	44 (16.7 %)	55 (21.0 %)	0.22
White race, n. (%)	526	507 (96.4 %)	251 (95.1 %)	256 (97.7 %)	0.13
Clinical					
Heart rate (bpm)	518	72.7 ± 15.8	71.5 ± 15.4	73.9 ± 16.2	0.091
SBP (mmHg)	526	125.5 ± 17.6	125.5 ± 18.0	125.5 ± 17.3	0.99
DBP (mmHg)	526	76.2 ± 11.6	76.2 ± 12.4	76.2 ± 10.7	0.96
BMI (Kg/m^2)	526	27.9 ± 4.1	27.7 ± 4.1	28.0 ± 4.1	0.39
Laboratory					
eGFR ($ml/min/1.73m^2$)	526	87.3 ± 17.5	90.1 ± 16.3	84.5 ± 18.4	<0.001
Urea (mg/dL)	519	15.9 ± 5.2	15.5 ± 4.5	16.3 ± 5.7	0.078
Serum potassium (mmol/L)	526	4.1 ± 0.5	4.0 ± 0.4	4.1 ± 0.5	0.54
Serum sodium (mmol/L)	526	138.6 ± 3.0	138.3 ± 2.9	138.9 ± 3.2	0.040
Hemoglobin (g/dL)	525	14.3 ± 1.4	14.4 ± 1.3	14.2 ± 1.5	0.19
Albumin (g/dL)	414	4.0 ± 0.5	4.1 ± 0.4	4.0 ± 0.6	0.36
AST (IU/L)	481	50.0 (27.0 - 139.2)	54.3 (28.0 - 151.0)	45.0 (25.0 - 132.0)	0.16
ALT (IU/L)	493	32.4 (21.0 - 49.0)	33.0 (23.0 - 48.6)	31.8 (20.0 - 50.0)	0.46
Total bilirubin (mg/dL)	450	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.51
Aldosterone (mmol/L)	525	0.2 (0.1 - 0.3)	0.2 (0.1 - 0.3)	0.2 (0.1 - 0.3)	0.23
Medical History					
Previous MI, n (%)	526	31 (5.9 %)	12 (4.5 %)	19 (7.3 %)	0.20
Hypertension, n (%)	526	244 (46.4 %)	119 (45.1 %)	125 (47.7 %)	0.60
Diabetes, n (%)	526	65 (12.4 %)	30 (11.4 %)	35 (13.4 %)	0.51
Atrial fibrillation, n (%)	526	8 (1.5 %)	2 (0.8 %)	6 (2.3 %)	0.18
Concomitant treatments at randomization					
ACEi/ARBs, n (%)	526	349 (66.3 %)	175 (66.3 %)	174 (66.4 %)	1.00
β-blockers, n (%)	526	373 (70.9 %)	183 (69.3 %)	190 (72.5 %)	0.44
Lipid lowering agents, n (%)	526	516 (98.1 %)	257 (97.3 %)	259 (98.9 %)	0.34
Diuretics, n (%)	526	39 (7.4 %)	16 (6.1 %)	23 (8.8 %)	0.25
PCI or Thrombolysis, n (%)	526	456 (86.7 %)	223 (84.5 %)	233 (88.9 %)	0.001
Randomized to eplerenone, n (%)	526	266 (50.6 %)	136 (51.5 %)	130 (49.6 %)	0.73
Biomarkers					

BNP (ng/dL)	51	103.2 (58.0 - 225.0)	104.6 (59.5 - 214.5	93.6 (43.0 - 303.5)	0.73
NT-pro BNP (ng/dL)	114	262.1 (81.0 - 769.6)	262.1 (76.0 - 759.9	258.0 (138.2 - 988.8)	0.43
NPs above the median, n (%)	165	82 (49.7 %)	47 (50.0 %)	35 (49.3 %)	1.00
Troponin I (ng/dL)	281	6.4 (0.5 - 45.5)	6.8 (0.7 - 50.0)	5.8 (0.3 - 40.5)	0.24
Troponin T (ng/dL)	210	0.5 (0.1 - 2.8)	0.4 (0.1 - 2.1)	0.9 (0.1 - 3.2)	0.063
Troponins above the median, n (%)	491	245 (49.9 %)	119 (48.8 %)	126 (51.0 %)	0.65
PIIINP (ng/mL)	526	3.9 (3.3 - 4.7)	3.3 (2.5 - 3.6)	4.7 (4.3 - 5.3)	<0.001
Galectin-3 (ng/mL)	523	11.9 (9.6 - 15.8)	12.0 (9.5 - 16.0)	11.8 (9.7 - 15.1)	0.80
ICTP (µg/L)	526	3.7 (3.0 - 4.5)	3.3 (2.8 - 3.9)	4.0 (3.4 - 4.9)	<0.001
PINP (ng/mL)	526	31.0 (23.0 - 42.0)	28.5 (21.0 - 39.0)	34.0 (24.0 - 45.0)	<0.001
Study outcome					
Primary composite endpoint*	526	137 (26.0 %)	59 (22.3 %)	78 (29.8 %)	0.059

Legend: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; AST, aspartate amino-transferase; ALT, alanine amino-transferase; ACEi/ARBs, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; PCI, percutaneous coronary intervention; BNP, brain natriuretic peptide; NT-pro BNP, N-terminal pro brain natriuretic peptide; NPs, natriuretic peptides; PIIINP, procollagen type III N-terminal propeptide; ICTP, type I collagen C-terminal telopeptide; PINP, procollagen type I N-terminal propeptide.

*CVM or re-hospitalization or new onset HF or sustained ventricular tachycardia or fibrillation or LVEF ≤40% after 1-month post-MI or BNP above 200 pg/mL or NT-proBNP above 450 pg/mL (in patients aged below 50) or above 900 pg/mL (in patients aged 50 to 75 years), or above 1800 pg/mL (in patients older than 75 years) after 1-month post-MI.

Biomarkers groups are based on the median values (i.e. below vs. above the median).

Table 2. Linear regression models for baseline (randomization) Extra-cellular Matrix Markers as dependent variables

Biomarkers / Final Models	**Adjusted R^2**	**Standardized β**	**Non-standardized coefficient (95% CI)**	**p-value**
		Log **PIIINP**		
Overall model fit	0.11	-	-	<0.001
Constant	-	-	1.26 (0.95 to 1.58)	<0.001
eGFR (per 10 ml/min decrease)	-	0.23	0.05 (0.03 to 0.07)	<0.001
Heart rate (per 10 bpm increase)	-	0.15	0.04 (0.01 to 0.06)	0.003
Total Bilirubin (per 1 mg/dL increase)	-	0.11	0.14 (0.03 to 0.26)	0.018
ACEi/ARBs (yes)	-	-0.09	-0.10 (-0.19 to -0.00)	0.049
		Log **Galectin-3**		
Overall model fit	0.23	-	-	<0.001
Constant	-	-	2.40 (1.83 to 2.97)	<0.001
eGFR (per 10 ml/min decrease)	-	0.34	0.07 (0.05 to 0.09)	<0.001
Log Cortisol (per 1 *Log* unit increase)	-	0.18	0.10 (0.03 to 0.17)	0.004
Log Aldosterone (per 1 *Log* unit increase)	-	0.13	0.08 (0.01 to 0.16)	0.027
Log ALT (per 1 *Log* unit increase)	-	0.12	0.07 (0.01 to 0.13)	0.019
		Log **ICTP**		
Overall model fit	0.14	-	-	<0.001
Constant	-	-	2.11 (1.39 to 2.83)	<0.001
eGFR (per 10 ml/min decrease)	-	0.24	0.05 (0.03 to 0.07)	<0.001
Body Mass Index (per 5 kg/m² increase)	-	0.12	0.01 (0.00 to 0.02)	0.022
Total Bilirubin (per 1 mg/dL increase)	-	0.11	0.13 (0.00 to 0.25)	0.045
Heart rate (per 10 bpm increase)	-	0.10	0.02 (0.00 to 0.05)	0.040
Diuretics (yes)	-	0.10	0.15 (0.00 to 0.29)	0.050
Log ALT (per 1 *Log* unit increase)	-	-0.12	-0.07 (-0.13 to -0.01)	0.026
Log Cortisol (per 1 *Log* unit increase)	-	-0.11	-0.06 (-0.12 to -0.00)	0.036
		Log **PINP**		
Overall model fit	0.12	-	-	<0.001
Constant	-	-	3.68 (3.37 to 4.00)	<0.001
Gender (female)	-	0.16	0.19 (0.07 to 0.31)	0.003
Age (per 10-year increase)	-	-0.16	-0.07 (-0.12 to -0.02)	0.004
Log Aldosterone (per 1 *Log* unit increase)	-	-0.16	-0.12 (-0.20 to -0.05)	0.002

β-blockers (yes)	-	-0.13	-0.18 (-0.32 to -0.04)	0.011
Hypertension history (yes)	-	-0.12	-0.11 (-0,205 to -0,012)	0.027
Diabetes (yes)	-	-0.12	-0.17 (-0.31 to -0.02)	0.026

Legend: eGFR, estimated glomerular filtration rate; ACEi/ARBs, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; ALT, alanine aminotransferase; PIIINP, procollagen type III N-terminal propeptide; ICTP, type I collagen C-terminal telopeptide; PINP, procollagen type I N-terminal propeptide.

Table 3. Treatment effect on Extra-cellular Matrix Markers according to their baseline "median" levels

Biomarker variable	**Placebo**	**Eplerenone**	**p-value**
PIIINP >3.9 ng/mL			
Absolute Δ PIIINP	0.13±1.48	-0.37±1.56	0.008
Relative Δ PIIINP	0.04±0.29	-0.05±0.26	0.011
PIIINP ≤3.9 ng/mL			
Absolute Δ PIIINP	0.84±1.22	0.81±1.12	0.84
Relative Δ PIIINP	0.39±0.80	0.40±0.87	0.89
Galectin-3 >11.9 ng/mL			
Absolute Δ Galectin-3	-5.06±9.07	-4.17±8.29	0.42
Relative Δ Galectin-3	-0.22±0.30	-0.17±0.27	0.19
Galectin-3 ≤11.9 ng/mL			
Absolute Δ Galectin-3	0.32±2.12	0.81±2.60	0.091
Relative Δ Galectin-3	0.05±0.23	0.11±0.30	0.058
ICTP >3.7 µg/L			
Absolute Δ *ICTP*	-0.16±1.71	-0.28±1.74	0.58
Relative Δ *ICTP*	-0.01±0.34	-0.03±0.33	0.76
ICTP ≤3.7 µg/L			
Absolute Δ *ICTP*	0.55±0.88	0.48±1.07	0.54
Relative Δ *ICTP*	0.21±0.34	0.24±0.80	0.75
PINP >31 ng/mL			
Absolute Δ PINP	-4.02±17.66	-8.27±14.99	0.041
Relative Δ PINP	-0.05±0.37	-0.17±0.30	0.008
PINP ≤31 ng/mL			
Absolute Δ PINP	6.83±11.46	5.61±10.22	0.35
Relative Δ PINP	0.37±0.63	0.34±0.75	0.75

Legend: Δ, delta: 1) absolute =6 month value – baseline value, 2) relative =(6 month value – baseline value)/baseline value; PIIINP, procollagen type III N-terminal propeptide; ICTP, type I collagen C-terminal telopeptide; PINP, procollagen type I N-terminal propeptide.

Table 4. Natriuretic peptide thresholds outcome associations for the studied Extra-cellular Matrix Markers

Biomarkers	**Model 1 HR (95%CI)**	**p-value**	**Model 2 HR (95%CI)**	**p-value**	**Model 2 C-index**
"Linear" Models					
Log PIIINP (per 1 Log unit increase)	2.23 (1.34-3.70)	0.002	1.95 (1.16-3.29)	0.012	0.67
Log Galectin-3 (per 1 Log unit increase)	2.79 (2.00-3.88)	<0.001	2.21 (1.49-3.28)	<0.001	0.71
Log ICTP (per 1 Log unit increase)	1.26 (0.77-2.07)	0.35	0.92 (0.57-1.47)	0.71	0.66
Log PINP (per 1 Log unit increase)	0.72 (0.51-1.01)	0.057	0.76 (0.53-1.09)	0.14	0.67

Model 1: adjusted on treatment "random" allocation (i.e. eplerenone or placebo as "dummy" variable). All tested "interactions" between "linear" biomarker*treatment allocation were non-significant ($p > 0.1$).

Model 2: adjusted on age, gender, heart rate, body mass index, estimated glomerular filtration rate, aspartate aminotransferase, diabetes, hypertension, previous myocardial infarction, ACEi/ARBs, β-blockers, diuretics, and treatment allocation.

Legend: PIIINP, procollagen type III N-terminal propeptide; ICTP, type I collagen C-terminal telopeptide; PINP, procollagen type I N-terminal propeptide.