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**Determinants of anti-fibrotic response to mineralocorticoid receptor antagonist therapy: insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) and Early Eplerenone Treatment in Patients with Acute ST-elevation Myocardial Infarction without Heart Failure (REMINDER) trials**

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**Determinants of anti-fibrotic response to mineralocorticoid receptor antagonist therapy: insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) and Early Eplerenone Treatment in Patients with Acute ST-elevation Myocardial Infarction without Heart Failure (REMINDER) trials**

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## **Abstract**

*Introduction:* After myocardial infarction complicated by heart failure, eplerenone (compared to placebo) significantly decreases amino-terminal propeptide of type III procollagen (PIIINP). Determining the subset of patients who are more prone to have a decrease in PIIINP and those who may respond better to the anti-fibrotic effects of mineralocorticoid receptor antagonists (MRA) therapy may be relevant for a personalized treatment approach. The aim of this study was to identify predictors of a PIIINP decrease and assess potential subgroups of "responders" to eplerenone.

*Methods:* Clinical factors and biomarkers were evaluated as predictors of a PIIINP decrease from randomization to month 9 in 323 patients from the biomarker substudy of Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS). Additionally, the association between PIIINP decrease and the composite of cardiovascular (CV) death or CV hospitalization were also explored. External validation was performed in the REMINDER trial.

*Results:* Female sex, eplerenone, reperfusion therapy, potassium < 4 mmol/L, circulating levels of PIIINP  $\geq 3.6$  ng/mL and PINP  $\geq 27$  ng/mL predicted a PIIINP decrease (AUC=0.75). Randomization PIIINP showed a significant interaction with the treatment allocation: patients with PIIINP  $\geq 3.6$  ng/mL had a better response (decrease in PIIINP) to eplerenone (OR for PIIINP  $\geq 3.6 = 2.9$ , 95%CI=1.46-5.89, p=0.003) and OR for PIIINP < 3.6 = 1.09, 95%CI=0.55-2.2, p=0.8; interaction<sub>p</sub>=0.026). These findings were internally robust using another statistical approach (LOESS). External validation showed good discrimination (AUC=0.70). There was a tendency towards a lower rate of CV death/CV hospitalizations in patients with decreased PIIINP (adjusted HR=0.52, 95%CI=0.26-1.02, p=0.058).

*Conclusion:* In patients who had a myocardial infarction, clinical factors used in combination and treatment with eplerenone were associated with a PIIINP decrease. Interestingly, higher randomization PIIINP levels might help in identifying patients more prone to have an "anti-fibrotic response" when treated with MRAs.

*Key-words:* collagen; myocardial infarction; eplerenone.

## **Introduction**

After acute myocardial infarction (MI) complicated by heart failure (HF), eplerenone therapy has been shown to improve morbidity and mortality outcomes<sup>1,2</sup>. These beneficial effects of eplerenone (and other mineralocorticoid receptor antagonists) were independent from their diuretic and potassium-sparing effects<sup>3</sup>. In addition, eplerenone reduces the levels of circulating biomarkers associated with collagen synthesis in patients who had a MI<sup>4-6</sup>; including the blood levels of amino-terminal propeptide of type III procollagen (PIIINP) that have correlated with “in-situ” myocardial fibrosis<sup>7,8</sup>. Nonetheless, the subset of patients that may have a more pronounced “anti-fibrotic” response is yet to be determined. Identifying the patients that may respond better to the “anti-fibrotic” effects of eplerenone may be relevant for a personalized treatment approach, as an early treatment with a mineralocorticoid receptor antagonist (MRA) may limit the excessive collagen deposition in the myocardium and consequently reduce the “adverse remodeling” after a MI. Studying biomarkers correlated with myocardial fibrosis may help in selecting patients more prone to an effective “anti-fibrotic” response.

The aims of this study were to identify predictors of a decrease in PIIINP and assess potential subgroups of “anti-fibrotic responders” to eplerenone in MI patients. A subset of patients in whom circulating biomarkers, including PIIINP, were determined at randomization and during follow-up<sup>4,5</sup>, were used for the present analyses, that incorporated two randomized controlled trials: the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS, NCT01319344)<sup>2</sup>, used for derivation, and the Impact Of Eplerenone On Cardiovascular Outcomes In Patients Post Myocardial Infarction (REMINDER, NCT01176968), used for replication<sup>9</sup>.

## **Methods**

### **Study design and patient population**

The EPHESUS trial included 6642 patients, enrolled 3-14 days after MI who had a left ventricular ejection fraction (LVEF)  $\leq 40\%$ , HF or both<sup>1,2</sup>. In EPHESUS, HF was defined as at least one of the following: pulmonary rales, pulmonary congestion on chest radiography, or a third heart sound. Patients were randomly assigned to eplerenone (up to 50 mg/day) or placebo. Addition of eplerenone to optimal medical therapy was demonstrated to reduce all-cause mortality and the composite endpoint of cardiovascular (CV) death or CV hospitalizations<sup>1,2</sup>. In the biomarker substudy of EPHESUS, circulating plasmatic biomarker determinations were performed in 476 patients at randomization (i.e. 3 to 14 days after MI diagnosis), and months 1, 3, 6 and 9. The selected biomarkers PIIINP, amino-terminal propeptide of type I procollagen (PINP), type I collagen telopeptide (ICTP) and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) were measured. Commercial radioimmunoassays were used to measure PIIINP, PINP, and ICTP (Orion Diagnostica, Espoo, Finland). TIMP-1 was measured with ELISA kits (Amersham

Biosciences, Orsay, France). Further details on blood sampling and biomarker assays have previously been reported<sup>4</sup>. This substudy demonstrated that PIIINP levels significantly dropped in patients treated with eplerenone (compared with placebo)<sup>4</sup>, an effect that remained significant after 9 months of treatment. For the present “derivation” study, aimed to determine which patients in whom a sustained “anti-fibrotic” response could be observed, we included those with available PIIINP measurements both at randomization and month 9 (N=323).

External validation was performed in 526 patients included in the biomarker substudy of the REMINDER trial<sup>9</sup>, that included patients with an acute STEMI in the absence of a clinical diagnosis of HF. In REMINDER patients were randomized to eplerenone (25-50 mg/day) or placebo. The primary outcome was time-to-first of cardiovascular death, re-hospitalization or extended initial hospital stay due to HF or sustained ventricular tachycardia or ventricular fibrillation, as well as LVEF $\leq$ 40% or elevated natriuretic peptides after 1-month. Eplerenone treatment was associated with a lower risk for the primary endpoint compared to placebo, an effect that was driven (>90%) by a drop in natriuretic peptides. In REMINDER, blood samples (plasma) were drawn at baseline (i.e. <24 hours after STEMI diagnosis) and after 6 months.

The study protocols of EPHEBUS and REMINDER were approved by the institutional review board or ethics committee at each site, and all patients provided written informed consent to participate in the respective studies.

### **Definition of PIIINP decrease**

A decrease in PIIINP during follow-up was defined as an absolute PIIINP reduction between randomization and month 9 (adjusting for randomization PIIINP levels).

### **Statistical analyses**

Continuous variables are expressed as mean  $\pm$  SD and categorical variables as frequencies and proportions. For comparison of means and proportions, the Student’s t-test and the chi-square test were used, respectively.

To identify predictors of a decrease in PIIINP, a clinical model was constructed using backward logistic regression, as follows: 1) log-linearity of continuous variables was assessed visually by inspecting the shape of the distribution of the beta-estimates vs. the median by quintiles with regard to the outcome of interest (i.e. PIIINP decrease). If deemed appropriate based on log-linearity, continuous clinical and biomarker variables were dichotomized (except for the LOESS procedure); 2) relevant clinical parameters on demographics, medical history, baseline physical examination, laboratory tests and HF medication were assessed in univariate analyses and those with  $p < 0.1$  were considered for the multivariate model. To

test whether renal function influenced the model, estimated glomerular filtration rate (eGFR) at admission and change in eGFR from randomization to 6 months (eGFR was not available at month 9) were “forced into the model”; 3) a multivariable backward selection procedure was then performed (a forward procedure was also performed providing the same results). On top of this clinical model, biomarkers were added one-by-one to determine which independently predicted a decrease in PIIINP. A total of 17 biomarkers were considered (*Supplemental table 1*). Correlations between the biomarkers (and PIIINP decrease) were also studied. The diagnostic ability of the models was verified using receiving operating characteristics (ROC) curves. Furthermore, a prespecified interaction between randomization PIIINP and eplerenone treatment was assessed based on previous studies<sup>5, 10</sup>.

The internal robustness of the model was performed using a LOESS procedure which is a nonparametric technique that uses local weighted regression to fit a smooth curve through points in a scatter plot. Eplerenone and placebo treated patients were ordered by ascending log randomization PIIINP (x-axis) and plotted against the log change in PIIINP from randomization to month 9 (y-axis). This was followed by application of the Kolmogorov–Smirnov test for non-parametric distributions to detect the randomization PIIINP level where the difference in log PIIINP decrease between eplerenone and placebo became significant (two-tailed test,  $\alpha=0.05$ ). Next, patients in the eplerenone group with randomization PIIINP concentrations equal/above and below this level were defined as responders and non-responders, respectively. Due to a smaller sample size, a forward as opposed to a backward logistic regression procedure was performed to assess whether the predictors of eplerenone response were similar to the predictors for a decrease in PIIINP performed in the primary analysis.

External validation of the prediction model for a decrease in PIIINP was performed in the REMINDER study. The diagnostic ability of the prediction model for a decrease in PIIINP was verified using a ROC curve.

To explore associations between a decrease in PIIINP and the primary endpoints of EPHEBUS (all-cause mortality and CV death/CV hospitalizations) Kaplan–Meier estimates and Cox proportional hazards models were used. In the multivariable model, adjustments were made for the variables that were identified as predictors of a PIIINP decrease during follow-up (eplerenone treatment, reperfusion therapy, gender and randomization potassium levels).

Statistical analyses were performed using SPSS 24 (IBM inc., Armonk, NY) and R (The R Foundation for Statistical Computing, Vienna, Austria). A  $P<0.05$  was considered significant except for the analyses involving interactions ( $P<0.025$ ). No multiple imputation was performed. Given that the present investigation was “hypothesis generating” and not a “discovery” study, we did not correct for potential false discoveries.

## Results

### Baseline characteristics

The characteristics of the patients with a decrease in PIIINP versus those with a stable or increased PIIINP during follow-up is depicted in the *Table 1*. Patients with a decrease in PIIINP were more often female, received eplerenone treatment more frequently, and also had a Q-wave MI and received reperfusion therapy more often. Patients with a decrease in PIIINP had higher circulating levels of randomization PIIINP, PINP, TIMP-1, interleukin-6 (IL-6) and CRP compared to patients with a stable/increased PIIINP (*Supplemental table 1*).

### Predictors of PIIINP decrease

Crude and adjusted odds ratio of the predictors for PIIINP decrease are depicted in the *Table 2*. Eplerenone treatment, reperfusion therapy, randomization potassium < 4 mmol/L, and female sex were independent predictors of PIIINP decrease in a model that included only the clinical variables (AUC=0.65). Estimated glomerular filtration rate was not a significant PIIINP drop predictor, and forcing randomization eGFR into the model yielded similar results to the model without eGFR (data not shown). The clinical model strongly improved after the addition of PIIINP  $\geq 3.6$  ng/mL and PINP  $\geq 27$  ng/mL (AUC=0.75).

Randomization PIIINP showed a significant interaction with the treatment allocation, whereby patients with PIIINP  $\geq 3.6$  ng/mL showed a better response (defined as a PIIINP decrease) to eplerenone (OR for PIIINP  $\geq 3.6$  ng/mL = 2.9, 95% CI 1.46-5.89,  $p=0.003$ ) and OR for PIIINP < 3.6 ng/mL = 1.09, 95% CI 0.55-2.2,  $p=0.81$ ; interaction  $p=0.026$ ). The interplay between randomization PIIINP and eplerenone with regard to a decrease in PIIINP is shown in *Figure 1*. Both randomization PIIINP and a decrease in PIIINP were only weakly correlated ( $r=0.38$ ) and no correlations above 0.5 between the studied biomarkers were observed (*Supplemental figure 1*). The time point of randomization after the index MI was associated with baseline PIIINP levels: patients randomized early after the index MI had lower baseline PIIINP levels compared to patients randomized at a later time point (PIIINP 3.99, 4.27 and 4.51 ng/mL for patients randomized at days 3-7, 8-10 and 10-14 after the index MI ( $p=0.023$ , one-way ANOVA, between groups)).

### Internal and external validation of the prediction model

In the primary analysis, predictors for a decrease in PIIINP were studied in patients with PIIINP measurements both at randomization and month 9, irrespective of treatment allocation. Using a LOESS procedure, patients who experienced a significant decrease in PIIINP with eplerenone (compared to placebo) were identified by plotting the randomization PIIINP levels against the decrease in PIIINP during

follow-up, a significantly greater decrease in PIIINP was observed in eplerenone-treated patients, but only when randomization PIIINP was  $\geq 3.2$  ng/L (*Figure 3*). Eplerenone-treated patients with randomization PIIINP  $\geq 3.2$  ng/mL were therefore further defined as “responders” and those with PIIINP  $< 3.2$  ng/mL as “non-responders”. Baseline characteristics of responders and non-responders are depicted in *Supplemental table 3*. Responders to eplerenone were more often female, received reperfusion therapy, and more often had a randomization PINP  $\geq 27$  ng/mL and a PIIINP  $\geq 3.6$  ng/mL (*i.e.* the same characteristics that predicted a PIIINP decrease in the model including the complete study population; *Table 3*). Again, addition of PIIINP and PINP improved the clinical model (AUC=0.65 for the clinical model to AUC=0.80 for the model incorporating clinical variables and biomarkers).

External validation of the prediction model was performed in the REMINDER dataset with good discriminative ability (AUC=0.70; *Supplemental table 4*).

We used the 9-month measurement in EPHEBUS to be consistent with the ongoing HOMAGE trial (NCT02556450). In REMINDER only 6-month PIIINP was available. However, in EPHEBUS at month 6, eplerenone also reduced the PIIINP levels: ANCOVA regression with change in PIIINP from baseline to month 6 as outcome variable and eplerenone as independent variable (adjusting for the baseline PIIINP levels) gives a  $\beta$  (95%CI) = -0.47 (-0.81 to -0.13),  $p=0.007$ .

### **PIIINP decrease and outcome**

There was a tendency towards a lower rate for the endpoint of CV death/CV hospitalizations in patients with a decrease in PIIINP compared to those with a stable/increase in PIIINP (respectively N=16 (9%) vs. N=21 (15%); adjusted HR =0.52, 95% CI 0.26-1.02,  $p=0.058$ , *Figure 2A*). There was no significant difference for all-cause death (respectively N=2 (1%) vs. N=5 (4%); adjusted HR =0.27, 95% CI 0.05-1.47,  $p=0.13$ , *Figure 2B*).

### **Discussion**

To the best of our knowledge, the present study is the first to identify a subset of patients who had an MI, that may respond better to the “anti-fibrotic” effects of eplerenone. The main findings of this study are: 1) a decrease in PIIINP from randomization to month 9 may be predicted by a model that includes eplerenone treatment, reperfusion therapy, randomization potassium  $< 4$  mmol/L, female sex, randomization PIIINP  $\geq 3.6$  ng/mL and PINP  $\geq 27$  ng/mL, suggesting that a decrease in the collagen content may be predicted using clinical and biomarker variables; 2) a significant interaction was observed between randomization PIIINP levels and treatment allocation, whereby patients with a randomization PIIINP  $\geq 3.6$  ng/mL more often experienced a decrease in PIIINP with eplerenone treatment, suggesting



that a subset of potential “anti-fibrotic responders” to eplerenone may be better identified with the use of randomization PIIINP levels.

Dysregulation of the extracellular cardiac matrix plays an important role in progressive adverse cardiac remodeling with fibrosis<sup>11</sup>. In particular, increased accumulation of collagen in the cardiac interstitium by altered metabolism of collagen fibers types I and III (both involved in collagen synthesis) is the hallmark of myocardial fibrosis<sup>12</sup>. Many circulating biomarkers have been proposed as non-invasive markers of myocardial fibrosis, although only two have been associated with histologically-proven fibrosis: PIIINP which is formed during the conversion of procollagen type III to collagen type III, and C-terminal propeptide of procollagen type I (PICP) which is formed during the conversion of procollagen type I into collagen type I<sup>8</sup>. Both biomarkers may therefore reflect collagen synthesis. However, these biomarkers are not cardiac-specific and may be affected by renal function, bone metabolism, and hepatic function, for example<sup>13</sup>; suggesting that the eventual modification of these biomarkers by the use of certain drugs, such as MRAs, may not reflect the change in the cardiac collagen content but the change in collagen in other organs.

Previous reports showed that MRA treatment was associated with a decrease in collagen biomarkers, PIIINP in particular<sup>4, 10, 14</sup>. Hence, a decrease in PIIINP was used as a surrogate of anti-fibrotic response in the present study. Multivariate analyses identified eplerenone treatment, reperfusion therapy, randomization potassium <4 mmol/L, female sex, and randomization PINP  $\geq 27$  ng/mL and PIIINP  $\geq 3.6$  ng/mL as independent predictors of a decrease in PIIINP.

Iraqi *et al.*, using the EPHEBUS data, previously demonstrated a significant decrease in PIIINP in patients treated with eplerenone<sup>4</sup>. In the REMINDER trial, eplerenone was also associated with a decrease in PIIINP, but only when randomization PIIINP levels were  $>3.9$  ng/mL<sup>5</sup>. In anterior STEMI, intravenous potassium canrenoate (the active metabolite of spironolactone) also decreased circulating PIIINP<sup>15</sup>. Moreover, a decrease in PIIINP was also observed with spironolactone in severe HFrEF<sup>10</sup>. MRA treatment reduced the amount of fibrosis (as assessed by endomyocardial biopsy) and was associated with a decrease in PIIINP in patients with dilated cardiomyopathy<sup>16</sup>.

In the present study, patients who received reperfusion therapy were more likely to experience a decrease in PIIINP. Small observational studies have previously suggested that PIIINP decreases after thrombolysis and successful percutaneous coronary interventions for acute MI<sup>17, 18</sup>. A decrease in PIIINP observed after reperfusion may reflect a lesser degree of adverse remodeling.

Randomization potassium <4 mmol/L was an independent predictor of a decrease in PIIINP. A recent post-hoc analysis of the Eplerenone in Mild Patients Hospitalization and Survival Study in HF (EMPHASIS-HF) demonstrated that a potassium <4 mmol/L amplified the eplerenone treatment effect (compared with placebo)<sup>19</sup>. With an overactivation of the renin angiotensin aldosterone system (associated

or not with an insufficient blockade), one could hypothesize that the beneficial effects of MRA treatment may be more pronounced.

In the present study, female sex predicted a decrease in PIIINP. In a study involving chronic systolic HF, female sex was an independent predictor of beneficial reverse remodeling (defined as  $\geq 15\%$  reduction in LV end-systolic volume index)<sup>20</sup>. A protective effect of estrogens in women and/or a detrimental effect of androgens in men was suggested although sex differences in therapy response and adherence were also likely. Further studies on sex differences in expression of mineralocorticoid system and in MRA treatment response are needed in this setting.

In the present study, PINP  $\geq 27$  ng/mL independently predicted a decrease in PIIINP. PINP is considered to be reflective of type I collagen synthesis and has been shown to decrease after MRA treatment<sup>4, 10</sup>. However, a correlation between plasma PINP and myocardial fibrosis on endomyocardial biopsy was not been observed so far<sup>21</sup>. Hence, given the currently available evidence, PINP lacks validation as a circulating biomarker of myocardial fibrosis<sup>8, 22</sup>.

An important finding was an interaction between randomization PIIINP and treatment allocation where patients with randomization PIIINP  $\geq 3.6$  ng/mL more often had a decrease in PIIINP with eplerenone. A decrease in PIIINP after MRA treatment was also observed in other cohorts (as above discussed).

The use of another statistical approach (LOESS) led to similar identification of anti-fibrotic responders to eplerenone (as defined by a significant decrease in PIIINP in the eplerenone compared to the placebo group). A similar randomization PIIINP cut-off level of  $\geq 3.2$  ng/mL (compared to  $\geq 3.6$  ng/mL in the primary analysis) also identified eplerenone-treated patients with a significant decrease in PIIINP compared to the placebo group. We suggest that these eplerenone-treated patients with higher randomization PIIINP may have more collagen content, and consequently may have more substrate for eplerenone to act on. In the group of eplerenone-treated patients, similar predictors of PIIINP decrease were also identified (similar to that observed in the primary analysis), thus reinforcing the internal robustness of our findings.

Importantly, external validation of the prediction model for a decrease in PIIINP in REMINDER also yielded good calibration of the model, despite the patients enrolled in the REMINDER trial did not present with HF; suggesting that an anti-fibrotic response with eplerenone may be observed even in the absence of HF.

Higher PIIINP levels have been associated with histologically-proven myocardial fibrosis and adverse outcome in acute MI patients<sup>7, 8, 23</sup>. However, no studies to date had examined the prognostic value of PIIINP changes, with this study being the first to demonstrate a putative tendency towards improved outcomes in MI patients with a PIIINP decrease. However, event rates in this substudy were low

precluding any definitive conclusions regarding PIIINP changes and outcome associations. Larger studies need to further assess the prognostic value of changes in PIIINP and outcome in MI patients.

### **Implications for clinicians and future research**

Current ACC/AHA and ESC guidelines recommend MRA treatment (in addition to treatment with an ACE-inhibitor and a beta-blocker) in all symptomatic HFrEF patients and after a MI with an LVEF $\leq$ 40% or diabetes<sup>24-26</sup>. This recommendation is based on trials showing a beneficial effect of MRA on top of standard treatment. However, it is likely that not all patients benefit equally<sup>19, 27</sup>. In a prespecified analysis of the RALES trial, the beneficial treatment effect of spironolactone was confined only to patients with baseline PIIINP levels above the median<sup>10</sup>. In this study, we demonstrate that MI patients with higher randomization PIIINP and who received eplerenone were more likely to show a PIIINP decrease. In the current era of personalized treatment, it would be interesting to prospectively determine whether PIIINP levels could help determine which patients respond better to MRAs. A prospective study with an adaptive design and/or stratification based on randomization PIIINP could be valuable in ascertaining whether randomization PIIINP levels identify “responders” to eplerenone. A cut-off level between 3.2 (as observed with LOESS) and 3.6 ng/mL (as observed in the primary analysis) would appear appropriate when considering a randomization PIIINP cut-off level for such future studies in MI patients. The ongoing HOMAGE trial (NCT02556450) might determine whether the MRA spironolactone may reduce the markers of collagen synthesis and also improve clinical and echocardiographic parameters in patients at risk for developing HF.

### **Limitations**

Several limitations should be acknowledged in the present study. First, the substudy population differed from the main EPHEBUS cohort with respect to baseline characteristics, the timing of treatment initiation and outcomes<sup>2</sup>. Extrapolation of the results of this study to the complete EPHEBUS patient population should therefore be made with caution. Second, the sample size was relatively small and  $\pm$ 30% of patients had missing PIIINP levels at month 9. However, findings were robust in both internal and external validation analyses. Third, the event rates were low, precluding any definitive conclusions regarding PIIINP changes and outcome associations. Larger studies need to assess the prognostic value of PIIINP changes and outcome in MI patients. Fourth, carboxy-terminal propeptide of procollagen type I (PICP) was not measured in EPHEBUS. After a MI, there a significant rise in PICP levels may occur<sup>28</sup>, and further studies could assess the PICP changes in MI patients treated with aldosterone antagonists. For example, a simultaneous decrease in myocardial collagen content and of plasma PICP was observed after spironolactone treatment in dilated cardiomyopathy patients<sup>16</sup>. Fifth, PIIINP was correlated with

interstitial fibrosis in explanted hearts<sup>7</sup>, but further replication (including with myocardial fibrosis determined by MRI) is required. Sixth, a detailed cardiac function characterization (e.g. chamber volumes, diastolic function parameters) was not available in these datasets; in consequence we cannot ascertain whether the PIIINP levels correlate (or not) with the cardiac structure and function. Lastly, the timing of measurement may influence the levels of PIIINP. In EPHEBUS the samples were drawn at the time of randomization (i.e. from 3 to 14 days after the MI), whereas in REMINDER the samples were drawn at baseline (i.e. within the first 24h after the MI). The increase in PIIINP levels in the days after the index MI, as observed in EPHEBUS, suggests a slow release of PIIINP into the circulation. Observing a consistent eplerenone effect in both cohorts suggests that PIIINP may be decreased by this drug regardless of the timing of measurement. For avoiding additional “adverse remodeling” and excessive scar formation after a MI, the sooner eplerenone is initiated the better<sup>29</sup>.

## **Conclusion**

In patients who had a myocardial infarction, eplerenone treatment, reperfusion therapy, potassium <4 mmol/L, female sex, PINP  $\geq 27$  ng/mL and PIIINP  $\geq 3.6$  ng/mL predicted a PIIINP decrease from randomization to 9 months. Eplerenone-treated patients with higher randomization PIIINP might have experienced a more pronounced decrease in PIIINP levels. Determination of randomization PIIINP levels may help in identifying a subset of “anti-fibrotic responders” to eplerenone.

## **Conflicts of interest**

The EPHEBUS and REMINDER trials were sponsored by Pfizer. Dr. Zannad and Dr. Pitt were members of the steering committee for EPHEBUS. Dr. Stienen, Ferreira, Zannad and Rossignol are supported by a public grant overseen by the French National Research Agency (ANR) as part of the second “Investissements d’Avenir” program FIGHT-HF (reference: ANR-15-RHU-0004) and by the French PIA project “Lorraine Université d’Excellence”, reference ANR-15-IDEX-04-LUE, and by the Contrat de plan Etat-lorraine and FEDER lorraine. Dr. Pitt reports is a consultant for Bayer, Astra Zeneca, Sanofi, KBP Biosciences\*, Sarfez\*, Relypsa/ Vifor\*, Tricida\*, Stealth Peptides.\*=stock options. He holds a patent for site-specific delivery of eplerenone to the myocardium (US patent # 9931412). All other co-authors report no conflicts of interest.

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## Figure legends

- Graphical abstract** Predictors of an antifibrotic response after MI complicated by HF.  
Legend: several clinical factors and biomarkers predicted a PIIINP decrease after a MI complicated by HF. There was a significant interaction between baseline PIIINP levels and eplerenone treatment: patients with baseline PIIINP  $\geq 3.6$  mmol/L treated with eplerenone had the best response (PIIINP decrease).
- Figure 1** Interplay between randomization PIIINP and eplerenone relative to the observed decrease in PIIINP  
Legend: PIIINP, amino-terminal propeptide of type III procollagen
- Figure 2** Kaplan Meier curves for cardiovascular (CV) death or CV hospitalizations (A) and all-cause death (B) according to change in PIIINP
- Figure 3** Locally weighted scatterplot smoothing (LOESS) procedure to identify patients with a treatment response to eplerenone  
Legend: This figure depicts randomization log PIIINP levels (x-axis) and log of the change in PIIINP from randomization to month 9 (y-axis). Each dot represents a patient in the eplerenone (red) and placebo (blue) group. Patients treated with eplerenone and placebo were ordered based on their randomization log PIIINP levels. The red (eplerenone) and blue (placebo) lines depict the mean log change in PIIINP during follow-up. A significant difference in mean log change is observed in patients with a randomization log PIIINP level  $\geq 0.505$  which is 3.20 ng/mL.

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Table 1 Baseline characteristics of patients with a decrease versus stable/increase in PIIINP

	Decrease in PIIINP (N=184)	Stable/increase in PIIINP (N=139)	p-value
Female sex, n (%)	58 (32 %)	25 (18 %)	0.006
Eplerenone treatment, n (%)	101 (55 %)	59 (42 %)	0.027
Age, yrs, mean±SD	62±11	60±11	0.21
BMI, kg/m <sup>2</sup> , mean±SD	27.6±4.2	27.7±4.3	0.81
Diabetes mellitus, n (%)	51 (28 %)	39 (28 %)	0.51
History of hypertension, n (%)	109 (59 %)	92 (66 %)	0.20
History of HF, n (%)	13 (7 %)	17 (12 %)	0.11
Previous HF admission, n (%)	3 (2 %)	9 (7 %)	0.023
Previous MI, n (%)	35 (19 %)	35 (25 %)	0.18
Smoking status, n (%)			
Current	62 (34 %)	56 (40 %)	0.43
Never	80 (44 %)	57 (41 %)	
Former	42 (23 %)	26 (19 %)	
Heart rate, bpm, mean±SD	73±9	71±9	0.78
DBP, mmHg, mean±SD	74±10	74±9	0.85
SBP, mmHg, mean±SD	119±16	119±15	0.94
Q-wave MI, n (%)	149 (82 %)	98 (72 %)	0.029
Killip class			
1	20 (11 %)	15 (11 %)	0.026
2	146 (79 %)	95 (68 %)	
3	17 (9 %)	24 (17 %)	
4	1 (1 %)	5 (4 %)	
LVEF, %, median (IQR)	36 (32 - 38)	37 (32 - 39)	0.18
Atrial fibrillation, n (%)	19 (11 %)	10 (7 %)	0.30
Reperfusion therapy, n (%)	89 (48 %)	51 (37 %)	0.036
Baseline laboratory levels, mean±SD			
Potassium, mmol/L	4.3±0.4	4.4±0.4	0.023
Baseline eGFR, mL/min/1.73m <sup>2</sup>	82±29	86±39	0.33
Hemoglobin, g/dl	13.2±1.6	13.5±1.5	0.083
Serum sodium, mmol/L	140±4	140±4	0.79
Baseline medication, n (%)			
ACEi or ARB	153 (83%)	118 (85%)	0.76
BB	142 (77 %)	117 (84%)	0.12
Statins	82 (45 %)	72 (52 %)	0.20
Antiplatelet therapy	42 (23 %)	34 (25 %)	0.73
ASA	162 (88 %)	124 (89 %)	0.74
Loop diuretic	75 (40.8 %)	59 (42.4 %)	0.76
ACEi/ARB, BB, ASA, statin	49 (27 %)	48 (35 %)	0.13

Legend: BMI, body mass index; IQR, interquartile range; HF, heart failure; MI, acute myocardial infarction; DBP, diastolic blood pressure; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate, ACEi, ACE-inhibitor; ARB, angiotensin receptor blocker, ASA, acetylsalicylic acid, BB, beta-blocker,

Table 2 Crude and adjusted odds ratios of predictors for a decrease in PIIINP during follow-up

	UNIVARIATE ANALYSES		MULTIVARIATE ANALYSES			
			Clinical model (AUC 0.65)		Clinical model + biomarkers (AUC 0.75)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
<b>CLINICAL VARIABLES</b>						
Female sex	2.10 (1.24 – 3.62)	0.006	2.19 (1.26 – 3.81)	0.005	1.97 (1.09 - 3.54)	0.024
Eplerenone	1.65 (1.06 - 2.58)	0.027	1.65 (1.04 - 2.63)	0.035	1.73 (1.05 - 2.85)	0.033
Q-wave MI	1.80 (1.06 - 3.06)	0.030				
Reperfusion therapy	1.62 (1.03 - 2.54)	0.036	1.57 (0.98 - 2.51)	0.061	1.33 (0.80 - 2.20)	0.27
Potassium < 4 (mmol/L)	2.37 (1.33 - 4.25)	0.004	2.26 (1.23 - 4.16)	0.008	2.45 (1.28 - 4.69)	0.007
Hemoglobin < 11.9 (g/dl)	2.06 (1.15 - 3.67)	0.015				
<b>BIOMARKERS</b>						
PIIINP $\geq$ 3.6 (ng/mL)	4.79 (2.98 - 7.70)	<0.001			4.17 (2.51 - 6.93)	<0.001
PINP $\geq$ 27 (ng/mL)	2.33 (1.46 - 3.73)	<0.001			1.99 (1.16 - 3.41)	0.012
TIMP-1 $\geq$ 1230 (ng/mL)	1.95 (1.22 - 3.11)	0.005				
CRP $\geq$ 25 (mg/L)	1.64 (0.99 - 2.71)	0.056				

Interaction analyses: PIIINP $\geq$ 3.6 \* Eplerenone = 0.026. Others were non-significant.

Legend: MI, myocardial infarction; PIIINP, amino-terminal propeptide of type III procollagen; PINP, amino-terminal propeptide of type I procollagen; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; CRP, C-reactive protein; CI, confidence interval; AUC, area under the curve.

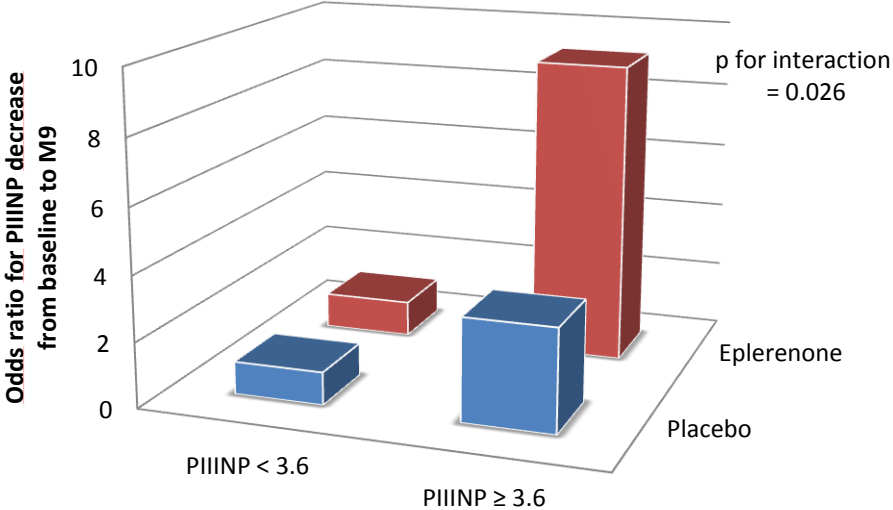
Table 3 Adjusted odds ratios of predictors of a treatment response to eplerenone after application of a locally weighted scatterplot smoothing (LOESS) procedure.

	<b>MULTIVARIATE ANALYSES</b>			
	Clinical model (AUC 0.65)		Clinical model + biomarkers (AUC 0.80)	
	OR (95% CI)	p-value	OR (95% CI)	p-value
<b>CLINICAL VARIABLES</b>				
Female sex	2.05 (1.13 – 3.72)	0.019	1.85 (0.93 – 3.69)	0.081
Reperfusion therapy	2.65 (1.52 – 4.63)	0.001	2.23 (1.17 – 4.23)	0.014
Potassium<4 (mmol/L)				
<b>BIOMARKERS</b>				
PIIINP≥3.6 (ng/mL)			9.93 (4.53 – 21.77)	<0.001
PINP≥27 (ng/mL)			1.97 (1.01 – 3.86)	0.048

A forward multivariate logistic regression analysis yielded similar clinical predictors as those observed in the model for the prediction of a decrease in PIIINP during follow-up (Table 2). Addition of PIIINP and PINP improved the clinical model. Details on the LOESS procedure are described in the legend of Fig. 3.

Legend: PIIINP, amino-terminal propeptide of type III procollagen; PINP, amino-terminal propeptide of type I procollagen

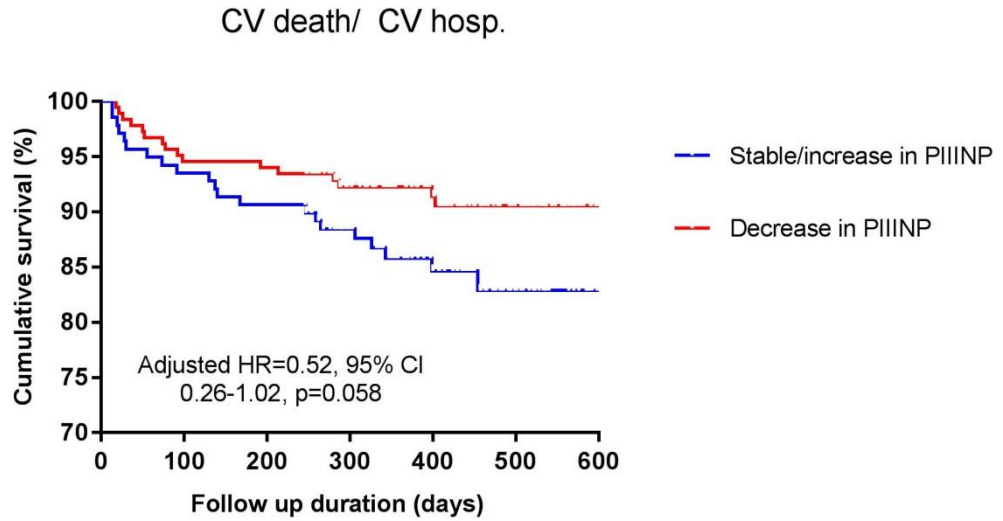
Figure 1 Interplay between baseline PIIINP and eplerenone relative to the observed decrease in PIIINP



Legend: PIIINP, amino-terminal propeptide of type III procollagen.

Figure 2 Kaplan Meier curves for cardiovascular (CV) death or CV hospitalizations (A) and all-cause death (B) according to change in PIIINP

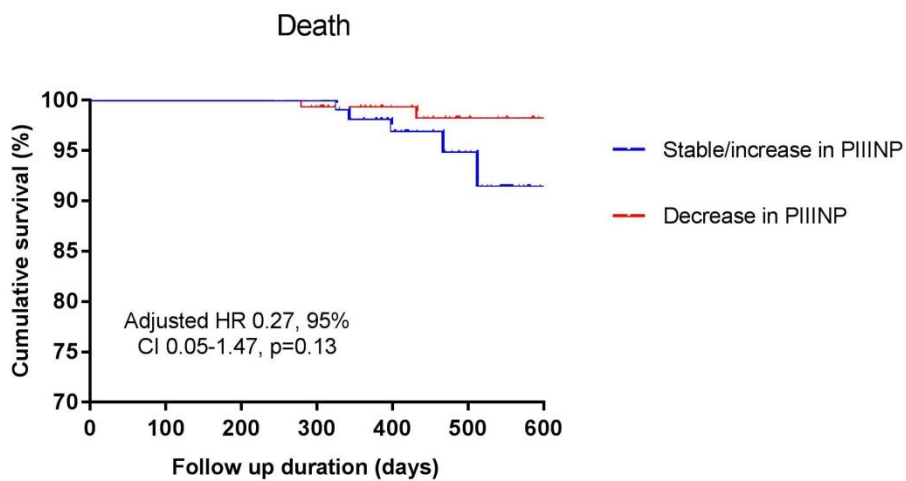
A.



**Nb. of patients at risk**

Stable/increase in PIIINP	139	131	127	108	74	31	1
Decrease in PIIINP	184	175	175	147	109	56	5

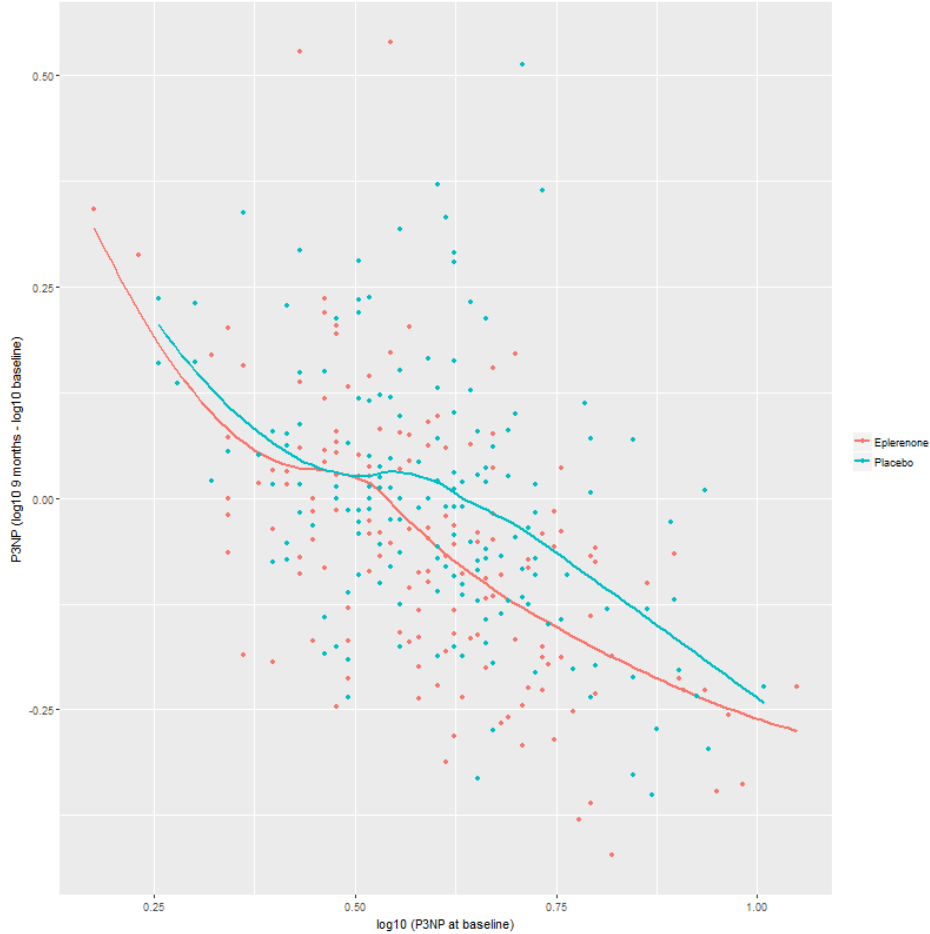
B.



**Nb. of patients at risk**

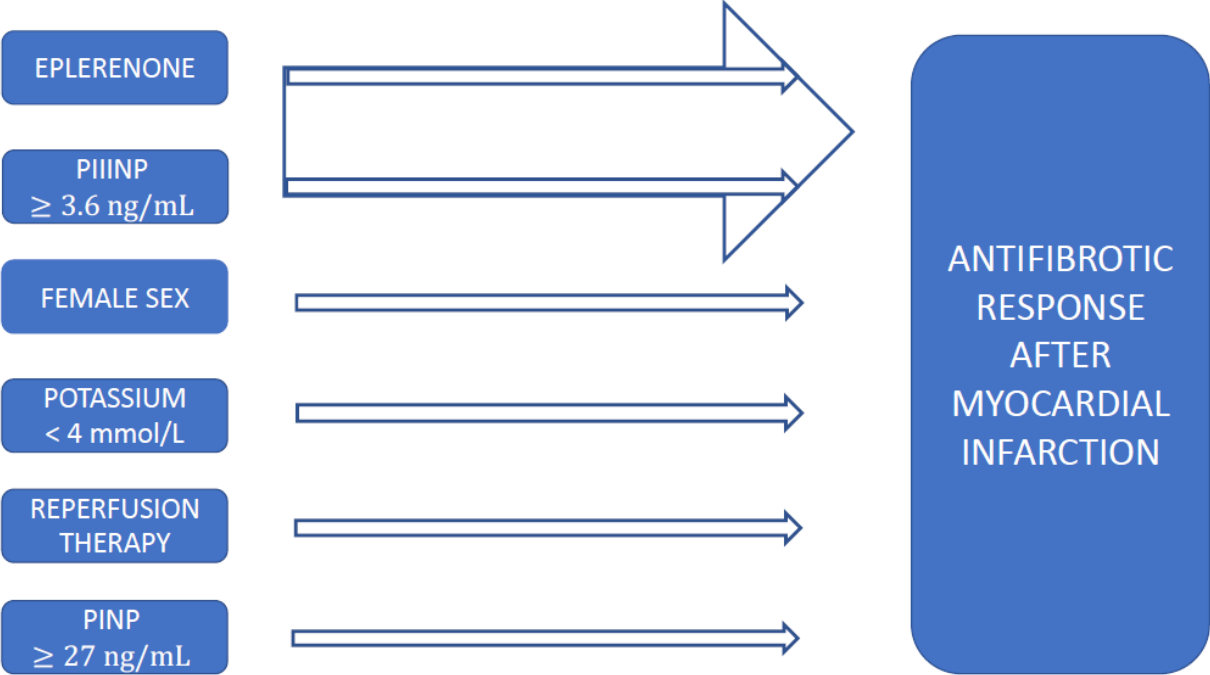
Stable/increase in PIIINP	139	139	139	121	81	36	2
Decrease in PIIINP	184	184	184	158	118	61	5

Figure 3 Locally weighted scatterplot smoothing (LOESS) procedure to identify patients with a treatment response to eplerenone



This figure depicts baseline log PIIIINP levels (x-axis) and log of the change in PIIIINP from baseline to month 9 (y-axis). Each dot represents a patient in the eplerenone (red) and placebo (blue) group. Patients treated with eplerenone and placebo were ordered based on their baseline log PIIIINP levels. The red (eplerenone) and blue (placebo) lines depict the mean log change in PIIIINP during follow-up. A significant difference in mean log change is observed in patients with a baseline log PIIIINP level  $\geq 0.505$  which is 3.20 ng/mL.

Graphical abstract





Supplemental table 1

Biomarkers at baseline in patients with a decrease versus stable/increase in PIIINP during follow-up

	Decrease in PIIINP (N=184)	Stable/increase in PIIINP (N=139)	p-value
PINP, ng/mL, mean±SD	36.7±14.7	32.5±16.6	0.019
PIIINP, ng/mL, mean±SD	4.7±1.6	3.5±1.1	<0.001
ICTP, ng/mL, mean±SD	6.5±3.4	6.1±2.9	0.20
TIMP, ng/mL, mean±SD	1198.9±346.2	1121.2±353.9	0.050
hCRP, mg/L, mean±SD	34.5±48.3	23.7±34.6	0.035
BNP, pg/mL, mean±SD	183±102.8	174.6±106.9	0.47
Troponin T, ng/mL, median (IQR)	0.5 (0.2 - 1.2)	0.9 (0.2 - 1.8)	0.081

Legend: PIIINP, amino-terminal propeptide of type III procollagen; PINP, amino-terminal propeptide of type I procollagen; ICTP, type I collagen telopeptide; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; hCRP; human C-reactive protein; BNP, B-type natriuretic peptide. Parametric distributed data are compared using independent student t test; non-parametric data using Mann-Whitney U test.

Supplemental table 2

## Time point of randomization after index myocardial infarction

	<b>Time point of randomization after index myocardial infarction</b>		
	<i>Days 3-7</i>	<i>Days 8-10</i>	<i>Days 10-14</i>
Baseline PIIINP levels, ng/mL	3.99*	4.27*	4.51*

\* significant difference between groups (p=0.023, one-way ANOVA)

Supplemental table 3

## Responders vs. non-responders to eplerenone treatment

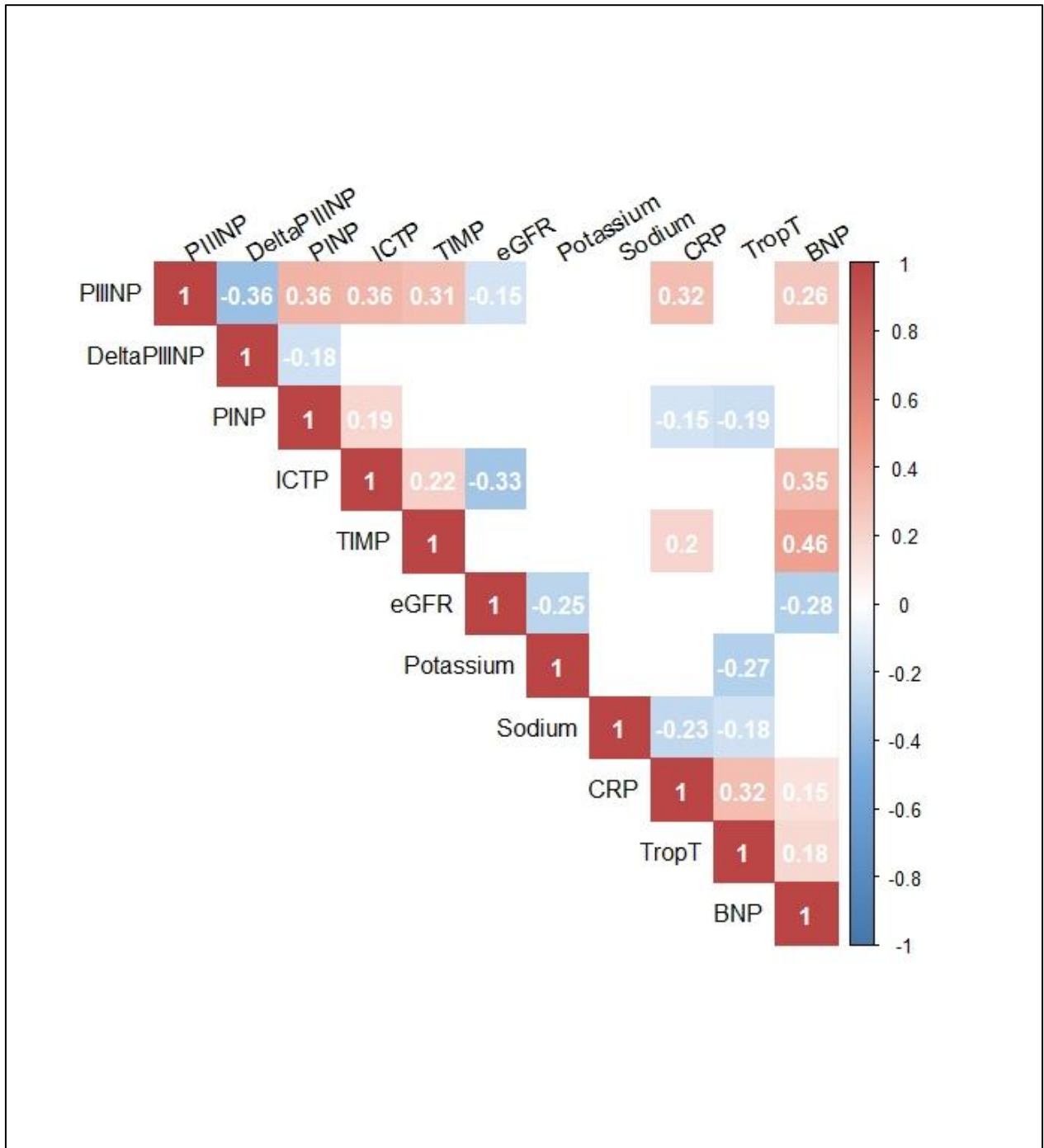
	Responders (N=84)	Non-responders (N=156)	p-value
Female sex, n (%)	31 (36.9 %)	35 (22.4 %)	0.017
Eplerenone treatment, n (%)	84 (100.0 %)	156 (100.0 %)	NA
Age, years, mean±SD	64±10	61±12	0.11
Diabetes mellitus, n (%)	26 (31.0 %)	45 (28.8 %)	0.73
History of hypertension, n (%)	51 (60.7 %)	90 (57.7 %)	0.65
History of HF, n (%)	8 (9.5 %)	13 (8.3 %)	0.76
Previous HF admission, n (%)	3 (3.6 %)	5 (3.2 %)	1
Previous AMI, n (%)	23 (27.4 %)	33 (21.2 %)	0.28
Smoking status, n (%)			
Current	24 (28.6 %)	63 (40.4 %)	0.078
Never	43 (51.2 %)	57 (36.5 %)	
Former	17 (20.2 %)	36 (23.1 %)	
Heart rate, bmp, mean±SD	73±9	73±10	0.88
DBP, mmHg, mean±SD	73±11	74±10	0.52
SBP, mmHg, mean±SD	120±16	118±15	0.44
Q-wave MI, n (%)	67 (79.8 %)	117 (76.0 %)	0.5
Killip class			
1	8 (9.5 %)	15 (9.6 %)	0.17
2	66 (78.6 %)	109 (69.9 %)	
3	10 (11.9 %)	25 (16.0 %)	
4	0 (0.0 %)	7 (4.5 %)	
LVEF, %, mean±SD	34±5	35±5	0.30
Reperfusion therapy, n (%)	44 (52.4 %)	46 (29.5 %)	<0.001
Baseline laboratory levels, mean±SD			
Potassium, mmol/L	4.5±0.4	4.4±0.5	0.090
eGFR (mL/min/1.73m <sup>2</sup> )	79±28	80±30	0.78
Hemoglobin (g/dl)	13.1±1.6	13.4±1.5	0.30
Serum sodium (mmol/L)	140±4	140±4	0.87
PIIINP≥3.6 (ng/mL)	75 (89%)	61 (41%)	<0.001
PINP≥27 (ng/mL)	63 (75%)	75 (51%)	<0.001
Baseline medication, n (%)			
ACEi or ARB	72 (85.7 %)	126 (80.8 %)	0.34
Beta-blocker	22 (26.2 %)	34 (21.8 %)	0.44
Statins	43 (51.2 %)	68 (43.6 %)	0.26
Antiplatelet therapy	21 (25.0 %)	23 (14.7 %)	0.050
ASA	80 (95.2 %)	139 (89.1 %)	0.11
Loop diuretic	35 (41.7 %)	73 (46.8 %)	0.45
ACEi/ARB, BB, ASA, statin	28 (33.3 %)	45 (28.8 %)	0.47

Legend: BMI, body mass index; HF, heart failure; MI, acute myocardial infarction; DBP, diastolic blood pressure; SBP, systolic blood pressure; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate, ACEi, ACE-inhibitor; ARB, angiotensin receptor blocker, ASA, acetylsalicylic acid, BB, beta-blocker, PIIINP, amino-terminal propeptide of type III procollagen; PINP, amino-terminal propeptide of type I procollagen

	<b>Multivariate logistic model (AUC 0.70)</b>	
	OR (95% CI)	p-value
Female sex	0.81 (0.50 – 1.31)	0.39
Eplerenone	1.31 (0.90 – 1.92)	0.16
Reperfusion therapy	0.66 (0.38 – 1.16)	0.15
Potassium<4 (mmol/L)	0.98 (0.67 – 1.44)	0.94
PIIINP $\geq$ 3.6 (ng/mL)	4.67 (3.01 – 7.24)	<0.001
PINP $\geq$ 27 (ng/mL)	1.14 (0.77 – 1.70)	0.52

Legend: PIIINP, amino-terminal propeptide of type III procollagen; PINP, amino-terminal propeptide of type I procollagen.  
OR, odds ratio; CI, confidence interval.

Supplemental figure 1 Correlation heatmap for biomarkers and clinical variables at baseline



The figure shows the Pearson correlation between various fibrosis biomarkers and clinical variables. Only significant correlations with p-value below 0.05 are shown. Blank cells depict non-significant correlations. There was no correlation >0.5 observed between the different variables. On the right-scale the correlation coefficients are depicted, blue for negative correlations and red for positive ones.