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1 **Serum microRNAs and antifibrotic response to eplerenone in acute myocardial**  
2 **infarction complicated by systolic dysfunction**

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25 *Disclosures*

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1 **Abstract**

2 *Background*

3 After myocardial infarction (MI) complicated by heart failure (HF), eplerenone reduced  
4 serum concentrations of amino-terminal propeptide of type III collagen (PIIINP) and carboxy-  
5 terminal propeptide of type I collagen (PICP). Determining a subgroup who are more prone to  
6 decrease their collagen content and to respond better to the antifibrotic effects of  
7 mineralocorticoid receptor antagonists (MRA) may be relevant for a personalized treatment  
8 approach. Whether circulating microRNAs may identify a subgroup that have experienced a  
9 more pronounced antifibrotic effect of eplerenone as measured by a PICP and PIIINP  
10 decrease is unclear.

11 *Methods*

12 A set of circulating microRNAs linked to cardiac fibrosis (mir-1, mir-21, mir-29a, mir-29b,  
13 mir-101, mir-122, mir-133a) were measured at baseline in 198 patients in the biomarker  
14 substudy of Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival  
15 Study (EPHESUS). Associations between baseline microRNA levels and changes in both  
16 PIIINP and PICP from baseline to month 9 were studied using multivariable analysis of  
17 covariance, adjusting for age, sex, history of hypertension and diabetes mellitus, prescription  
18 of ACE-inhibitors or angiotensin receptor blockers, baseline PIIINP or PICP, and eplerenone  
19 treatment. Furthermore, a treatment-by-microRNA interaction was studied.

20 *Results*

21 From the selected microRNAs, only mir-133a was associated with a PICP decrease ( $\beta$ -6.43,  
22 95%CI-12.71 to -0.15,p=0.045). None of the microRNAs was associated with a PIIINP  
23 change. The microRNAs did not predict an effect of eplerenone on PICP and PIIINP changes.

24 *Conclusion*

25 Although serum mir-133a was associated with PICP change, none of the microRNAs  
26 previously linked to cardiac fibrosis predicted an antifibrotic response to eplerenone. Further  
27 study is needed to identify other suitable targets for a personalized treatment approach.

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## 1 **Background**

2 Eplerenone reduced morbidity and mortality in patients with an acute myocardial infarction  
3 (MI), complicated by systolic dysfunction, HF or diabetes mellitus in the Eplerenone Post-  
4 Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS)<sup>1</sup>. In post-  
5 hoc analyses of the trial, eplerenone reduced the serum concentrations of amino-terminal  
6 propeptide of type III collagen (PIIINP) and carboxy-terminal propeptide of type I collagen  
7 (PICP)<sup>2, 3</sup>. These biomarkers have been linked to collagen synthesis and associated with  
8 histopathological myocardial fibrosis<sup>4</sup>. Hence, it has been postulated that the beneficial  
9 treatment effect of eplerenone (and other mineralocorticoid receptor antagonists - MRAs)  
10 could be, at least partially, mediated by its antifibrotic effect. Identifying the subset of patients  
11 who may respond better to the antifibrotic effects of MRA therapy is pertinent for a  
12 personalized treatment approach. We have previously shown in an EPHESUS substudy that  
13 patients with higher baseline PIIINP levels experienced a better treatment response to  
14 eplerenone<sup>5</sup>. Circulating microRNAs (short RNA sequences that regulate gene expression)  
15 have also been linked to collagen turnover<sup>4, 6</sup>. We thus hypothesised that a few selected  
16 microRNAs could identify a subgroup of patients who might have experienced a more  
17 pronounced antifibrotic effect of eplerenone as measured by a PICP and PIIINP decrease.

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## 19 **Methods**

20 In this biomarker substudy of EPHESUS, a set of circulating microRNAs previously linked to  
21 cardiac fibrosis (mir-1, mir-21, mir-29a, mir-29b, mir-101, mir-122, mir-133a (as reviewed by  
22 Thum<sup>6</sup>) were measured at baseline in 198 patients. A detailed account of the methods of this  
23 biomarker substudy has been previously published<sup>2</sup>. The number of patients included in this  
24 specific substudy was lower than the original biomarker substudy (n=476) since not all  
25 patients had enough sample volume left for the microRNA measurements.

1 Total RNA was isolated from 150 µl plasma samples using miRNeasy Serum/Plasma  
2 Advanced Kit (Qiagen, Hilden, Germany). Reverse transcription reactions were performed  
3 using the Reverse Transcription TaqMan MicroRNA Reverse Transcription Kit (Applied  
4 Biosystems®, Darmstadt, Germany). For each reaction, 2.5 µl of RNA was used. All  
5 procedures were performed according to the manufacturers' instructions. After qPCR, starting  
6 quantities (N0) of each microRNA were normalized to N0 to the geometric mean of cel-miR-  
7 39-3p and miR- 486- 5p ( $\text{Relative quantity} = \frac{N0_{\text{miR}}}{\sqrt{(N0_{\text{cel-miR-39-3p}} * N0_{\text{miR- 486- 5p}})}}$ ).

8 Associations between baseline microRNA levels and changes in both PIIINP and  
9 PICP from baseline to month 9 were studied using multivariable analysis of covariance,  
10 adjusting for age, sex, baseline PIIINP or PICP, history of hypertension or diabetes mellitus,  
11 prescription of ACE inhibitors or angiotensin receptor blockers and eplerenone treatment.  
12 Furthermore, a treatment-by-microRNA interaction was studied.

13

## 14 **Results and discussion**

15 A total of 96 patients (49%) were randomized to eplerenone treatment. Baseline levels of  
16 microRNAs did not differ between patients treated with eplerenone versus placebo (all p-  
17 values >0.1). MicroRNAs were not significantly correlated with baseline PICP and PIIINP  
18 (including when the correlations were assessed in the eplerenone and placebo groups,  
19 separately), although some microRNAs showed a moderate correlation between them (data  
20 not shown). Only mir-133a was associated with a change in PICP ( $\beta$ -6.43, 95% CI-12.71 to -  
21 0.15,  $p=0.045$ , *Table*).

22 None of the microRNAs measured at baseline was associated with a change in PIIINP. The  
23 microRNAs did not predict an effect of eplerenone on PICP and PIIINP changes (*Table*).

24 There were no significant associations between the microRNAs and clinical outcomes (all-  
25 cause mortality and cardiovascular (CV) death / CV readmissions) (data not shown).

1           Mir-133a, one of the most abundant miRNAs in the heart, plays a role in the early  
2 phase after MI and subsequent cardiac remodeling and is increased in MI patients<sup>7</sup>. In this  
3 study, higher circulating mir-133a levels were independently associated with a decrease in  
4 PICP, which may suggest an antifibrotic response. In line with our findings, increased  
5 baseline mir-133a levels were associated with reverse cardiac remodeling in dilated  
6 cardiomyopathy<sup>8</sup>. Interestingly, mir-133a was also previously found to be a direct target of  
7 the collagen 1 (Col1A1) gene where its downregulation was associated with Col1A1  
8 overexpression and hence myocardial fibrosis<sup>9</sup>. Despite not being able to identify a subset of  
9 patients more likely to respond to eplerenone (probably due to the small sample size and lack  
10 of power to study interactions), further research is needed to study the utility of mir-133a as a  
11 fibrosis marker.

12           Some additional limitations should be acknowledged. First, the substudy population  
13 differed from the main EPHESUS cohort with respect to baseline characteristics, the timing of  
14 treatment initiation, and outcomes<sup>2</sup>. Extrapolation of the results of this study to the whole  
15 EPHESUS population should, therefore, be made with caution. Second, a detailed  
16 characterization of cardiac function and fibrosis (e.g., with cardiac MRI) was not available in  
17 our data; in consequence, cardiac fibrosis was indirectly assessed by the evaluation of  
18 collagen synthesis biomarkers, that have been shown to correlate directly with cardiac fibrosis  
19 in previous research<sup>4</sup>. Third, the microRNAs selected for this study had been previously  
20 associated with cardiac fibrosis; in consequence, we cannot exclude that other unmeasured  
21 microRNAs could predict an antifibrotic response. Lastly, event rates were very low in this  
22 present study, precluding any conclusions regarding the associations between microRNAs,  
23 eplerenone treatment and clinical outcomes such as hospitalizations or death.

24           In conclusion, although serum mir-133a was independently associated with PICP  
25 change, none of the circulating microRNAs previously linked to cardiac fibrosis predicted an

1 antifibrotic response to eplerenone treatment in patients after an acute MI complicated by left  
2 ventricular dysfunction and/or heart failure. Further study is needed to identify other suitable  
3 targets for a personalized treatment approach.

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**Table 1. Multivariable linear regression analyses on associations between microRNAs and PICP and PIIINP changes with a treatment-by-miR interaction**

	$\Delta$ PICP (M0-M9)				$\Delta$ PIIINP (M0-M9)			
	$\beta$	95% CI	p-value	Interaction <sup>p</sup> for treatment*miR	$\beta$	95% CI	p-value	Interaction <sup>p</sup> for treatment*miR
<b>Model</b>								
Age	-0.29	-0.82 to 0.24	0.28		0.01	-0.01 to 0.02	0.53	
Male sex	13.94	0.47 to 27.41	0.04		-0.44	-0.78 to -0.10	0.01	
Hypertension	13.67	1.68 to 25.67	0.03		0.48	0.18 to 0.78	0.002	
Diabetes mellitus	-17.89	-30.38 to -5.40	0.005		-0.13	-0.46 to 0.20	0.44	
ACEi or ARB use	-1.40	-17.55 to 14.75	0.86		0.05	-0.34 to 0.44	0.78	
Eplerenone treatment	-13.43	-24.53 to -2.33	0.02		-0.49	-0.78 to -0.20	0.001	
Baseline PIIINP					-0.59	-0.69 to -0.49	<0.001	
Baseline PICP	-0.77	-0.88 to -0.65	<0.001					
<b>Model + individual MiRNAs</b>								
MiR-1	0.03	-4.70 to 4.75	0.99	0.97	-0.01	-0.15 to 0.15	0.98	0.31
MiR-21	0.70	-8.77 to 10.16	0.89	0.78	-0.20	-0.48 to 0.08	0.17	0.98
MiR-29a	-2.15	-12.58 to 8.29	0.69	0.17	-0.04	-0.36 to 0.28	0.82	0.73
MiR-29b	-2.50	-10.85 to 5.84	0.55	0.59	0.03	-0.25 to 0.31	0.83	0.89
MiR-101	-4.72	-18.17 to 8.74	0.49	0.26	-0.19	-0.59 to 0.22	0.36	0.12
MiR-122	-0.31	-4.75 to 4.13	0.89	0.24	-0.01	-0.13 to 0.14	0.89	0.45
MiR-133a	-6.43	-12.71 to -0.15	0.045	0.08	-0.10	-0.30 to 0.10	0.33	0.54

Legend: PICP, carboxy-terminal propeptide of procollagen type I; PIIINP, amino-terminal propeptide of type III procollagen;  $\Delta$ PIIINP (M0-M9), absolute change in PIIINP from baseline to month 9;  $\Delta$ PIIINP (M0-M9), absolute change in PIIINP from baseline to month 1; MiR, microRNA ; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker

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