

Effect of Galectin 3 on Aldosterone-Associated Risk of Cardiovascular Mortality in Patients Undergoing Coronary Angiography



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Recent experimental studies have suggested that galectin-3 has an interaction with aldosterone, and modifies its adverse effects. We therefore aimed to elucidate whether the relationship between plasma aldosterone concentrations (PACs) and long-term fatal cardiovascular (CV) events would depend on plasma galectin-3 levels. A total of 2,457 patients (median age: 63.5 [interquartile range (IQR) = 56.3 to 70.6] years, 30.1% women) from the Ludwigshafen Risk and Cardiovascular Health study, with a median follow-up of 9.9 (IQR = 8.5 to 10.7) years, were included. We tested the interaction between aldosterone and galectin-3 for CV-mortality using a multivariate Cox proportional hazard model, reporting hazard ratios (HRs) with 95% confidence intervals (95% CIs). Adjustments for multiple CV risk factors as well as medication use were included. Mean PAC was 79.0 (IQR = 48.0 to 124.0) pg/ml and there were 558 (16.8%) CV deaths. There was a significant interaction between PAC and galectin-3 ($p = 0.021$). When stratifying patients by the median galectin-3, there was a significant association between aldosterone and CV-mortality for those above (HR per 1 standard deviation = 1.14; 95% CI [1.01 to 1.30], $p = 0.023$), but not below the cut-off value (HR per 1 standard deviation = 1.00; 95% CI [0.87 to 1.15], $p = 0.185$). In conclusion, the current study demonstrates for the first time a modifying effect of galectin-3 on the association between aldosterone and CV-mortality risk in humans. These findings indicate that galectin-3 is an intermediate between aldosterone and adverse outcomes. © 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license. (<http://creativecommons.org/licenses/by/4.0/>) (Am J Cardiol 2020;127:9–15)

Aldosterone is a steroid hormone secreted by the supra-renal glands and has classically been known to regulate salt and fluid homeostasis. After its first description by Simpson in 1953,¹ it took nearly four decades until its direct cardiovascular (CV) effects were recognized.² Since then a broad basis of evidence suggests that high aldosterone is a major factor for CV disease. Part of what may be caused by the strong association with arterial hypertension, heart failure and vascular health.^{3–6} This seems to be due to a maladaptation of the renin angiotensin aldosterone system.^{7,8}

Similar to aldosterone, galectin-3 (Gal-3), a marker of fibrosis and inflammatory activity, has been associated with an increased mortality.^{9,10} Recently, Calvier et al describe that Gal-3 was required for the inflammatory and fibrotic reaction in response to aldosterone.^{11,12} This finding suggest an interaction between Gal-3 and aldosterone.¹³ From a clinical point of view this observation is important as Gal-3 together with aldosterone might be used to identify high-risk patients and might help to guide future interventional studies. However, whether this interaction between

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aldosterone and Gal-3 exists in a human population remains to be shown. Therefore, we set out to examine if the association of aldosterone with CV mortality would depend on plasma Gal-3 levels.

Methods

Study population and design

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study is a prospective cohort study of consecutive patients referred to invasive coronary angiography. It was designed to investigate genetic and environmental risk factors of coronary artery disease (CAD) and CV disease. A detailed design of the LURIC study has been described previously.¹³ The baseline examination was performed between July 1997 and January 2000 at a tertiary care center in Germany (Herz-Zentrum Ludwigshafen). Inclusion criteria were the availability of a coronary angiogram, clinical stability and Caucasian origin. Informed written consent was obtained from all study participants and study approval was obtained from the appropriate ethics committee at the "Landesärztekammer Rheinland-Pfalz" (Mainz, Germany). The LURIC study complies with the Declaration of Helsinki.

Baseline examination

Angiographic CAD was diagnosed in patients with $\geq 50\%$ stenosis of at least 1 of 20 possible coronary segments using the maximal luminal narrowing.¹⁴ Diabetes mellitus was diagnosed if HbA1c was above 6.4%, or fasting glucose was ≥ 7.0 mmol/L, if glucose exceeded 11.1 mmol/L, or if patients received antidiabetic medication. Arterial hypertension was defined as present if the patient was on antihypertensive medication, had a median systolic and/or diastolic blood pressure ≥ 140 and/or ≥ 90 mm Hg out of 5 readings at rest during the hospitalization or if a significant history of hypertension was documented.

Sampling of biochemical parameters

Blood sampling was performed with patients resting in the supine position between 06:10 and 10:30h (mean sampling time, 07:22h), before coronary angiography and routine laboratory parameters were obtained. Remaining blood samples were snap-frozen for further determinations and stored at -80°C until analysis. Plasma aldosterone concentration (PAC) was determined using a radioimmunoassay (Active aldosterone; Diagnostic Systems Laboratories, Beckman Coulter Inc., Fullerton, CA). Intra- and interassay coefficients of variation were 3.6% to 8.3% and 7.3% to 10.4%, respectively. The reference interval is stated as 30 to 160 pg/ml. Gal-3 concentration was measured in plasma samples taken at baseline with the ARCHITECT galectin-3 assay on an ARCHITECT analyzer (Abbott Diagnostics, Abbott Park, IL), as recently described.¹⁵ The lower limit of detection was 1.01 ng/ml with an intra-assay and interassay variability of 3.2% and 0.8%, respectively.¹⁶

Endpoint ascertainment

Information regarding mortality was obtained from local registries. Two experienced clinicians classified the cause of

death by independently examining death certificates and medical records. Relevant information was retrieved for all of the included patients. Only in 20 patients the cause of death remained unclear and was accounted as all-cause mortality.

Statistical methods

We tested for a normal distribution by using visual inspection of the histograms, Q-Q plots and the skewness and kurtosis (-1 to +1). Variables displaying a non-normal (skewed-) distribution were log₁₀-transformed as indicated herein with the prefix "log," when appropriate. Patients with missing covariates were excluded from the present analysis. We grouped the study participants into tertiles according to their PACs levels for the purpose of studying baseline characteristics. Group comparisons were performed by Kruskal-Wallis nonparametric analysis of variance, Chi-square tests or analysis of variance (ANOVA), where appropriate. The baseline correlation between PAC and Gal-3 was tested by Spearman's correlation and a multivariate linear regression analysis. Subsequently, we performed a Cox proportional hazard regression analysis investigating the association of Gal-3 and PAC with CV mortality with adjustments for established risk factors. After detecting the first order interaction between PAC and Gal-3 we additionally included an interaction term in the Cox regression models. For all analysis, we prespecified 3 models containing each time increasing numbers of covariates. A basic model included age and gender as covariates. Model 1 additionally included body mass index, diabetes mellitus, low-density-lipoprotein cholesterol, high-density-lipoprotein cholesterol, mean systolic office blood pressure, cystatin C, smoking status, and N-terminal pro-brain natriuretic peptide. In model 2, we further added the covariates medication (angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor blockers, oral anticoagulation, respiratory medication [theophyllin/bronchodilator use], -blockers, calcium channel blockers, digitalis, aspirin, insulin-therapy and/or statins). All models include the main effects (PAC and Gal-3) as well as the interaction term (aldosterone*Gal-3). All hazard ratios refer to standardized variables (z-scores) and thus represent increased risk per change in 1 standard deviation. Assumptions for Cox proportional hazard analysis were assessed by correlation coefficients (co-linearity), Schoenfeld and Martingale residuals. The respective Kaplan-Meier estimates for PAC tertiles are reported with a log-rank test.

Sensitivity analysis

To minimize the risk of residual confounding we repeated the final model (prespecified as Model 2) and added adjustments for renin, parathyroid hormone levels, and left-ventricular ejection fraction. A sensitivity analysis was performed by using a Cox regression analysis with a competing risk model, proposed by Fine and Gray¹⁷ enabling us to take into account competing risk events (e.g., non-CV related cause of death).

Subgroup analysis

Finally, to better explore the potential modifying effect of Gal-3 on the relationship between PAC and risk of CV

mortality, we repeated the analyses by stratifying the cohort according to the median value of Gal-3. Statistical analysis was performed with SPSS (version 20, SPSS, Inc, Chicago, IL), R (version 3.1.1, The R Foundation) and STATA 12 (Stata Corp, College Station, TX). A two-sided p value of less than 0.05 was considered to indicate statistical significance.

Results

Participants and measurements

A total of 2,457 patients who underwent coronary angiography had PAC and Gal-3 measurements available. From the original cohort 696 had to be excluded due to missing values. Single vessel disease was detected in 19% of the patients, whereas multivessel disease was diagnosed in 49% of the studied population (Table 1). No correlation between PAC and Gal-3 was observed in the spearman correlation ($r = -0.009$; $p = 0.664$), and in the linear regression analysis (beta coefficient = -0.02 ; $p = 0.353$).

Table 1
Baseline characteristics according to tertiles of plasma aldosterone concentration

Variable	Aldosterone tertiles				p value
	Whole cohort n = 2,457	T1 n = 846 (34%)	T2 n = 823 (33%)	T3 n = 788 (32%)	
Aldosterone (ng/L)	79.0 (48.0-123.8)	38.6±11.5	79.9±14.0	152.0 (124.0-199.0)	NA
Galectin-3 (ng/ml)	14.5 (11.4-18.4)	14.0 (11.1-17.9)	14.2 (11.4-18.3)	14.8 (11.7-18.9)	0.566
10 year CV mortality	16.9%	15.2%	17.3%	17.9%	0.550
Age (years)	62.7±10.6	64±11	63±10	61±11	<0.001
Women	30.3%	30.1%	28.9%	31.4%	0.442
Body mass index (kg/m ²)	27.5±4.1	27.3±4.0	27.6±4.1	27.7±4.1	0.073
Waist Hip Ratio (WHR)	0.96±0.08	0.97 (0.92-1.01)	0.97 (0.91-1.02)	0.96 (0.91-1.00)	0.007
Systolic blood pressure (mm Hg)	141±24	139±23	142±24	142±25	0.042
Diabetes mellitus	39.9%	40.4%	40.1%	39.3%	0.872
Coronary artery disease	75.6%	72.2%	65.6%	65.4%	0.001
Previous myocardial infarction	39.5%	44.7%	40.1%	37.5%	0.003
LVEF (%)	58.7±17.4	61 (46-72)	66 (48-74)	64 (45-75)	0.083
Heart rate (bpm)	68.8±12.3	68±12	68±12	70±13	0.001
Daily activity 1-11 scale	5.9±1.4	5.9±1.8	5.9±1.8	5.8±1.7	0.558
Total cholesterol (mg/dl)	192±39	185±37	194±39	200±39	0.001
LDL cholesterol (mg/dl)	116±34	114±33	118±43	120±35	<0.001
HDL cholesterol (mg/dl)	38±10	38±11	39±11	40±11	<0.001
Triglycerides (mg/dl)	144 (107 – 199)	132 (101 – 185)	142 (107 – 189)	157 (113 – 219)	<0.001
Cystatin C (mg/L)	0.98±0.8	0.97±0.3	0.98±0.3	1.04±0.5	0.010
NTproBNP (ng/ml)	293 (105-868)	325 (123-966)	272 (99-832)	244 (8-726)	<0.001
Renin (pg/ml)	19 (10-41)	15 (8-31)	18 (10-37)	28 (13-64)	<0.001
Gamma GT (U/L)	17 (10-29)	16 (10-30)	17 (11-27)	17 (11-30)	0.179
Glucocorticoid	2.4%	0.02%	1.9%	1.9%	0.986
Insulin treatment	5.0%	5.2%	6.2%	3.7%	0.025
Statins	40.8%	53.1%	45.2%	40.8%	0.001
ACE-inhibitor	49.9%	57.3%	51.0%	49.5%	0.001
Angiotensin receptor blocker	5.1%	4.4%	4.0%	5.2%	0.378
Aspirin/other antiplatelet	68.1%	75.7%	70.0%	67.7%	<0.001
Warfarin/VKA	7.3%	6.2%	6.1%	7.6%	0.308
Theophyllin/bronchodilator	7.1%	6.1%	6.8%	6.3%	0.819
Beta blocker	58.8%	68.1%	61.5%	58.7%	<0.001
Calcium antagonist	18.0%	13.2%	16.9%	17.5%	0.013
Digitalis	17.3%	14.0%	14.8%	16.7%	0.206

Reporting mean ± SD, median (IQR) or %. ACE indicates angiotensin-converting enzyme; HDL indicates high-density lipoprotein; LVEF indicates left-ventricular ejection fraction; LDL indicates low-density lipoprotein; NTproBNP indicates pro brain natriuretic peptide. VKA, vitamin K antagonists. Data are shown as %, mean (SD), or median (IQR) as appropriate. p values refer to tests across the tertiles.

Mortality rates and Cox proportional hazard analysis

After a median follow-up of 9.9 (IQR 8.5–10.7) years, a total of 558 patients had died due to fatal CV events. Multivariate Cox proportional hazard analysis revealed a 1.3 times increased risk for CV mortality per increment of 1 standard deviation of the log-aldosterone*Gal-3 interaction term (Model 2: hazard ratio = 1.30; 95% confidence interval [1.04 to 1.62], Figure 3S). Similar results were observed when using the competing risk model from Fine and Gray²⁸ instead of the standard Cox regression (Supplement 1). Stability of the results was seen throughout the different models (Table 2).

Analysis of aldosterone in galectin-3 subgroups

We examined the relationship between PAC and CV mortality in each of the Gal-3 subgroups (i.e., above vs below the median cut-off value of 14.5 ng/ml). Kaplan-Meier cumulative survival estimates were 10.7, 10.7, and 10.6 years ($p = 0.742$) for the aldosterone tertiles (T1, T2,

Table 2
Cox proportional hazard for log aldosterone*galectin-3 interaction term

Model	Proportional hazards for log aldosterone*galectin-3 interaction term and CV mortality		
	Entire cohort (n = 2,457)		
	Hazard ratio (HR)	95% Confidence interval	p value
Basic	1.33	0.73 – 2.43	0.358
1	2.16	1.13 – 4.11	0.019*
2	2.15	1.13 – 4.12	0.021*
2 + PTHi	1.97	1.03 – 3.77	0.040*
2 + Renin	2.35	1.21 – 4.56	0.012*
2 + LVEF	4.12	1.57 – 10.80	0.004*

Basic Model includes age, sex, aldosterone, galectin-3, and aldosterone*galectin-3 interaction term. Model 1 additionally includes BMI, diabetes, LDL, HDL, systolic BP, cystatin C, smoking status at baseline and NTproBNP. Model 2 further includes treatment with: cortisone, insulin, statins, ACEi, ARBs, aspirin, oral anticoagulation, inhaled bronchodilators or theophylline, -blockers, calcium channel blockers and digitalis (*= p <0.05).

and T3 respectively) in patients with low Gal-3 and 10.8, 10.2, and 10.3 years (p = 0.019) in patients with high Gal-3 (Figures 1S and 2S). There was a significant association between PAC and CV mortality in patients above but none among those below the median level of Gal-3 (Table 3). Similar results were observed when using aldosterone tertiles for the Cox regression analysis in patients with high (p for group difference = 0.001) and low Gal-3 (p = 0.567), (Figures 1 and 2). Further adjustment for additional CV risk factors (parathyroid hormone levels, renin, and left-ventricular ejection fraction) did not materially affect the results.

Discussion

We demonstrate a significant interaction of Gal-3 on aldosterone-associated risk for fatal CV events. Gal-3 seems to modify the risk for CV mortality conferred by elevated plasma aldosterone. Accordingly, the association between PAC and CV mortality was observed only in patients with elevated Gal-3 concentrations. This indicates that the prognostic impact of PAC on CV risk appears to be modified by higher Gal-3. Prior investigations described remodeling of the vasculature and the myocardium, but also increased CV mortality risk associated with elevated PAC.^{3,8} Similar to aldosterone, Gal-3 has been described as a biomarker of increased CV risk, it has been suggested as a

surrogate for myocardial and vascular fibrosis and progression of CV disease.^{9,10} To date, the exact mechanisms as to how both molecules might impact on the CV risk remains to be elucidated.¹⁸ However, considering previous literature, it has been hypothesized that aldosterone and Gal-3 mutually depend on each other.¹⁹ Calvier et al recently demonstrated that Gal-3 was required for the inflammatory and fibrotic reaction in response to aldosterone administration.^{11,12} Their findings addressed this question by demonstrating that Gal-3 knock-out mice experienced no negative effects from aldosterone injections.¹¹ This was confirmed by Liao et al that described a reduction in Gal-3 in hypertensive patients after surgical removal of their aldosterone producing adenomas.²⁰ Lin et al also described that aldosterone induced the secretion of Gal-3 in macrophages and fibroblasts.^{21,22} This might be related to aldosterone induced changes of the pH (increased renal acid excretion),²³ as the acid-base balance influences Gal-3 ligand binding.²⁴ Another potential pathway might be related to galectin-3 interactions with tissue calcification.²⁵ Following this hypothesis, elevated concentrations of Gal-3 could be considered necessary for invoking the adverse effects of aldosterone. This would be in line with our results, but such a mechanism cannot be proven or refuted with our results as used an observational study design. The main difference in these previous study to our current investigation is that they used animal models and addressed Gal-3 tissue expression, which not necessarily can be compared with Gal-3 plasma levels.²⁶ Our study sheds further light on this field, as it is the first clinical study to describe a modifying effect of Gal-3 on the relationship between aldosterone and CV mortality. The findings of the present study have several potential clinical implications. First, it improves our understanding of the interplay between pro-fibrotic signaling and the renin-angiotensin-aldosterone system. Second, it might improve risk stratification in patients referred to coronary angiography, especially those with PAC measurements already available (e.g., as a screening for primary aldosteronism).²² Furthermore, in the near future we might hopefully be able to identify Gal-3 or another marker that helps us trigger an earlier or later initiation of MRA or aldosterone synthase inhibitor treatment, given their risk/benefit profile.²⁷ It should be noted that already some analysis of patients taking MRAs investigated the interaction with Gal-3, but results were inconclusive so far.^{26,28,29}

Table 3
Cox proportional hazard for log aldosterone, stratified according to the median of galectin-3 levels

Model	Proportional hazards for log aldosterone and CV mortality	
	Galectin-3 (hazard ratio (HR) 95% confidence interval p value)	
	High (>14.5 ng/ml) n = 1,308	Low (<14.5 ng/ml) n = 1,271
Basic	1.99 (1.17-3.37), P=0.011*	1.49 (0.89-2.50), P=0.133
1	1.86 (1.10-3.14), P=0.022*	1.60 (0.95-2.69) P=0.079
2	1.90 (1.09-3.30), P=0.023*	1.45 (0.84-2.53) P=0.185

Basic model includes age, sex, aldosterone, galectin-3, and aldosterone*galectin-3 interaction term. Model 1 additionally includes BMI, diabetes, LDL, HDL, systolic BP, cystatin C, smoking status at baseline, and NTproBNP. Model 2 further includes treatment with: cortisone, insulin, statins, ACEi, ARBs, aspirin, oral anticoagulation, inhaled bronchodilators or theophylline, -blockers, calcium channel blockers, and digitalis. The cohort was stratified according to the median of galectin-3 (14.5 ng/ml; *= p <0.05).

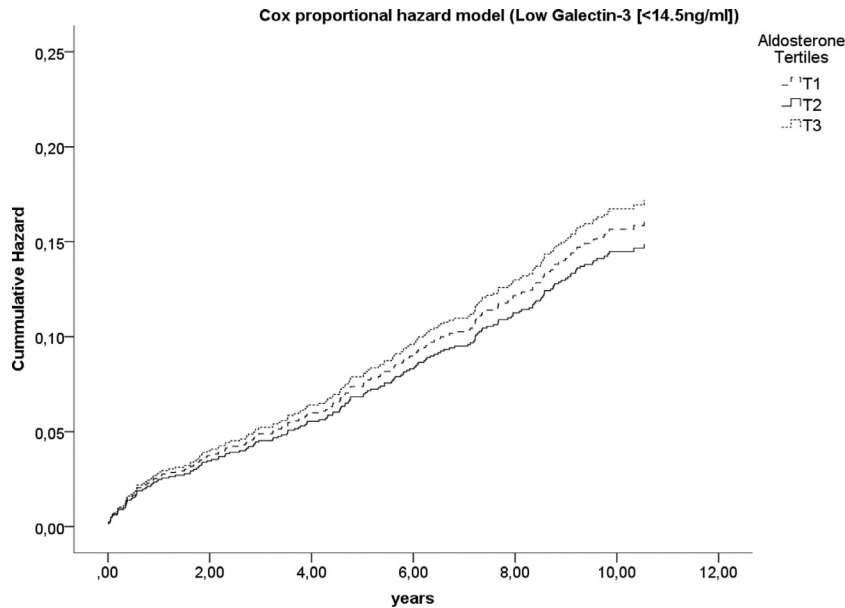


Figure 1. Cox proportional hazard analysis of aldosterone tertiles and cardiovascular (CV) mortality adjusted for various established confounders (adjusted for age, sex, BMI, diabetes, LDL, HDL, systolic BP, cystatin C, smoking status at baseline, NTproBNP, treatment with: cortisone, insulin, statins, ACEi, ARBs, aspirin, oral anticoagulation, inhaled bronchodilators or theophylline, -blockers, calcium channel blockers, and digitalis) in patients with low galectin-3 (below the median <14.5 ng/ml). There was no statistically significant difference between the tertiles ($p = 0.567$).

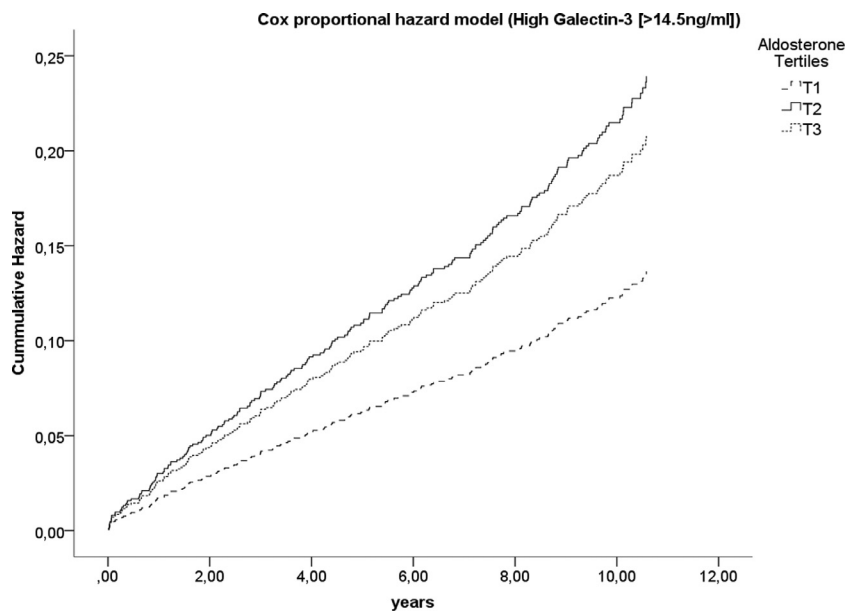


Figure 2. Cox proportional hazard analysis of aldosterone tertiles and CV mortality adjusted for various established confounders (adjusted for age, sex, BMI, diabetes, LDL, HDL, systolic BP, cystatin C, smoking status at baseline, NTproBNP, treatment with: cortisone, insulin, statins, ACEi, ARBs, aspirin, oral anticoagulation, inhaled bronchodilators or theophylline, -blockers, calcium channel blockers, and digitalis) in patients with high galectin-3 (above the median >14.5 ng/ml). There was a statistically significant difference between the tertiles ($p = 0.001$). There was a significantly decreased risk of hazard ratio 0.67, 95%CI (0.41 to 0.84; $p = 0.004$) for the first tertile compared with the second. There was no statistically significant difference between the third and the second tertile (HR 1.07, 95%CI [0.77 to 1.49]; $p = 0.673$).

Limitations

The present study recruited almost exclusively Caucasian patients in Germany, which limits the generalizability of the current study findings. Second, we lack data on 24-hour urinary sodium to estimate salt intake. Although a salt-rich diet is associated with adverse outcomes it suppresses aldosterone remarkably.³⁰ In an effort to account

for the distinct circadian pattern of aldosterone secretion, blood sampling was scheduled during morning hours. Another drawback is that even though we adjusted for medication intake, it is well known that especially angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blocker, but also other antihypertensive medication influence PAC. Therefore this medication should ideally be

stopped before blood sampling. Considering the presence of coronary artery diseases, arterial hypertension and stroke in our cohort this was not feasible from a clinical and ethical point of view. Finally, Gal-3 is increased in different states of organ disease, which cannot be discounted, even after adjusting for a broad panel of possible confounders.²⁹

Conclusion

The present analysis highlights the potential interaction of aldosterone with Gal-3. Foremost, myocardial fibrosis and remodeling amplified by the presence of high Gal-3 concentrations could be a potential mechanism that explains part of the deleterious actions of aldosterone. If confirmed in additional studies, this interaction could prove useful in aiding risk stratification and help identifying new treatment options.

Disclosures

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Supplementary materials

Supplementary material associated with this article can be found in the online version at <https://doi.org/10.1016/j.amjcard.2020.04.017>.

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