ORIGINAL ARTICLE

Galectin-3 and aldosterone as potential tandem biomarkers in pulmonary arterial hypertension

Laurent Calvier,[1] Ekaterina Legchenko,[1] Lena Grimm,[1] Hannes Sallmon,[2] Adam Hatch,[3] Brian D Plouffe,[3] Christoph Schroeder,[4] Johann Bauersachs,[5] Shashi K Murthy,[3,6] Georg Hansmann[1]

For numbered affiliations see end of article.

Correspondence to
Professor Dr Georg Hansmann, Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Carl-Neuberg-Str. 1, Hannover 30625, Germany; hansmann.georg@mh-hannover.de, georg.hansmann@gmail.com

LC, EL and LG contributed equally.

Received 5 July 2015
Revised 31 October 2015
Accepted 6 November 2015

ABSTRACT

Background Several studies have identified circulating biomarkers to be associated with the presence and severity of pulmonary arterial hypertension (PAH). Recent evidence supports a role for galectin-3 (Gal-3) and the mineralcorticoid aldosterone in left ventricular failure. However, studies on aldosterone together with Gal-3 in PAH are lacking.

Objective We investigated a novel Aldosterone-galectin-3 (Gal-3) tandem and several other potential PAH biomarkers and their association with the disease severity.

Methods A total of 57 patients, 41 with idiopathic PAH. (IPAH) and 16 with PAH associated with connective tissue disease (CTD), and 8 age-matched, non-relative controls were studied. Gal-3, aldosterone and other potential protein plasma concentrations were measured by single ELISA and multi-array MSD (Meso Scale Discovery) technology.

Results Gal-3 values were increased in both patients with IPAH (12.2±0.6 ng/mL; p<0.05) and with PAH-CTD (14.1 ±1.6 ng/mL; p<0.05) versus control (8.5±0.9 ng/mL), while aldosterone was significantly elevated in IPAH only (248.5 ±38.8 pg/mL vs control 71.9±18.2 pg/mL; p<0.05). In addition, aldosterone, Gal-3, and N-terminal pro-brain natriuretic peptide (NT-proBNP) values were all higher in patients in WHO functional class II–III versus PAH functional class I or controls. The vascular injury marker intercellular adhesion molecule 1 (ICAM-1) was increased in IPAH and PAH-CTD versus controls (559.5±18.2 pg/mL and 734.1 ±59.4 pg/mL vs controls 394.8±39.3 pg/mL, p<0.05, p<0.0001, respectively), whereas vascular cell adhesion molecule 1 (VCAM-1) and proinflammatory, anti-angiogenic interleukin-12 (IL-12) were elevated in PAH-CTD only (879.5±110.0 pg/mL and 391.2±70.3 pg/mL vs controls 489.8±44.6 pg/mL, p<0.01, and 102.1±15.2 pg/mL, p<0.01, respectively).

Conclusions Heightened Gal-3 and aldosterone plasma concentrations in PAH patients indicate a role for Gal-3 signalling in the pathobiology of IPAH and PAH-CTD, and may serve as biomarkers for functional status and progression of disease.

INTRODUCTION

Pulmonary arterial hypertension (PAH) and associated heart failure is a complex, multifactorial disease with a poor prognosis.[1–3] None of the current therapies has been shown to be universally effective, nor have these therapies been able to reverse advanced pulmonary vascular disease or to prevent right heart failure in advanced PAH.[2 3] The progressive nature and heterogeneous aetiology of PAH underlines the critical need for both early diagnosis and accurate stratification to allow tailored, efficient therapies.[4]

Several studies have identified circulating biomarkers associated with PAH, including markers of endothelial dysfunction, vascular injury and remodelling, myocardial damage, inflammation, and oxidative stress. N-terminal pro-brain natriuretic peptide (NT-proBNP), endothelin-1, interleukin-6, interferon-α, C reactive protein (CRP), serotonin, as well as endothelial progenitor cells, and recently identified pulmonary hypertension (PH)-relevant microRNAs, belong to the most validated markers.[5 6]

A role for aldosterone in pulmonary vascular remodelling and fibrosis has been suggested by increased plasma and tissue concentrations of aldosterone in experimental models, and also in patients with PAH.[7–10] Aldosterone contributes to the activation of pathways that decrease bioavailable nitric oxide levels, induce inflammation, and promote cell proliferation, migration, extracellular matrix remodelling, and fibrosis. Moreover, clinical mineralocorticoid receptor (MR) blockade in addition to endothelin-1 receptor inhibition has been associated with a better prognosis in clinical PAH.[11]

The molecular mechanisms by which aldosterone exerts its cardiovascular effects are complex.[12] Recent evidence indicates a critical role for galectin-3 (Gal-3), a β-galactoside-binding lectin, in inflammation, fibrosis, and heart failure, downstream of aldosterone.[13 14] Accordingly, Gal-3 is already established as a biomarker for cardiac fibrosis, and chronic left ventricular heart failure.[15 16] Consequently, Gal-3 has been approved by the US Food and Drug Administration as a new biomarker for additive risk stratification in heart failure, and received a class IIb recommendation in the most recent American Heart Association/American College of Cardiology (AHA/ACC) guidelines.[17]

However, to date, combined clinical studies on the role of both Gal-3 and aldosterone in PAH are lacking. Here, we investigate several biomarkers in PAH associated with vascular injury and myocardial fibrosis, and identify Gal-3 and aldosterone as potential tandem biomarkers in patients with idiopathic PAH (IPAH) or PAH associated with connective tissue disease (PAH-CTD).

METHODS

Study population

During the research conferences of the Pulmonary Hypertension Association in California (2010) and

To cite: Calvier L, Legchenko E, Grimm L, *et al*. *Heart* 2016;**102**: 390–396.

Florida (2012), 57 patients (IPAH, n=41; PAH-CTD, n=16) and eight healthy, age- and gender-matched, non-relative controls were recruited. Exclusion criteria were male sex, sleep apnoea, liver disease, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis. All participants completed a questionnaire regarding their demographic data (tables 1 and 2) and medication (table 2). Written informed consent was obtained from each subject. The study has been approved by the internal review board of Northeastern University (IRB # 11-07-19). Detailed characteristics of each individual PAH patient can be found in table 2.

Biomarker assays

EDTA whole blood samples were collected through peripheral venipuncture in non-fasting subjects, processed to plasma (ie, immediate centrifugation for 10 min at 1300 *g*), and stored at −80°C. Gal-3 and aldosterone concentrations were measured in one thaw step by BGM Gal-3 ELISA (BG Medicine, Waltham, Massachusetts, USA) and by aldosterone ELISA (RE52301, IBL International, Hamburg, Germany), respectively, according to the manufacturers' instructions. For assessment of NT-proBNP, the singleplex sandwich electrochemiluminescent immunoassay by Meso Scale Discovery was used, according to the manufacturer's protocol. All other molecules were measured using Meso Scale Discovery's Multi-Array technology (MSD, Angiogenesis Panel 1 Human, Cytokine Panel 1 Human, Proinflammatory Panel 1 Human, Vascular Injury Panel 2 Human). Signal intensities were measured with the MESO QuickPlex SQ 120 instrument (Meso Scale Discovery, Rockville, Maryland, USA) and analysis was performed using the Discovery Workbench software V.4.0 (Meso Scale Discovery, Rockville, Maryland, USA), according to the manufacturer's instructions. All protein markers of which more than 50% of the measurements fell into the detection range of the standard curves were considered valid.

Statistical analysis

Statistical analyses were performed with GraphPad Prism V.6 software. Data are expressed as mean±SEM or median values with IQR as appropriate. Data were tested for normal distribution with D'Agostino-Pearson omnibus, Shapiro-Wilk, and Kolmogorov-Smirnov tests. For comparison of data, one-way analysis of variance (ANOVA) or Kruskal-Wallis tests were used, corrected for multiple comparisons by Dunn's test, as appropriate. Values of $p<0.05$ were considered significant.

Table 1 Characteristics of control subjects and PAH patients studied

	Control	PAH (total)	IPAH	CTD
N	8	57	41	16
Age (years)	49.3 (33–67)	47.2 (19–77)	44.6 (19–74)	53.8 (28–77)
Male sex (n)	0	0	0	0
Height (m)	1.69	1.63	1.63	1.62
Weight (kg)	73.7	70.3	69.0	73.5
BMI (kg/m^2)	25.8	26.5	25.9	27.0
Race/ethnicity				
White	7	32	23	9
Black	0	2	0	2
Asian	1	1	0	1
Hispanic	0	5	5	0
Other	0	17	13	4

Values represent the number of subjects or mean values, as appropriate. Control subjects were age and gender matched to study subjects.
BMI, body mass index; CTD, pulmonary arterial hypertension associated with connective tissue disease; IPAH, idiopathic pulmonary arterial hypertension; PAH, pulmonary arterial hypertension.

RESULTS

Demographic characteristics

Demographic characteristics of the study subjects by groups are listed in table 1. Detailed information on each individual PAH patient including age, weight, body mass index, PAH diagnosis (IPAH, PAH-CTD), WHO functional class (FC) and medication is provided in table 2.

Plasma concentrations of Gal-3, aldosterone and NT-proBNP in PAH patients

In order to test whether Gal-3, aldosterone and NT-proBNP plasma concentrations were increased in patients with PAH versus controls, ELISAs were performed in control (n=8), IPAH (n=41), and CTD (n=16) plasma. Aldosterone plasma concentrations were elevated only in IPAH (248.5±38.8 pg/mL vs control 71.9±18.2 pg/mL; $p<0.05$), while the difference between controls and PAH-CTD patients (147.8±28.7 pg/mL vs control 71.9±18.2 pg/mL) did not reach statistical significance (figure 1A, B). Gal-3 concentrations were significantly increased in patients with IPAH (12.2±0.6 ng/mL) and PAH-CTD (14.1±1.6 ng/mL) versus controls (8.5±0.9 ng/mL; $p<0.05$; figure 1C, D). In addition, NT-proBNP values were increased in both IPAH and PAH-CTD patients as compared to controls (1575±291 ng/mL; $p<0.05$; and 1544±380 ng/mL; $p<0.05$, respectively; figure 1E, F).

Subsequently, we evaluated whether plasma concentrations of aldosterone, Gal-3, and NT-proBNP differed between PAH patients, grouped by WHO FC (FC I, n=10; FC II–III, n=53). Only one patient fulfilled the criteria for WHO FC IV and was, therefore, excluded from the analysis. Aldosterone, Gal-3, and NT-proBNP concentrations were all significantly increased in FC II-III PAH versus controls. A significant difference between FC II-III and FC I PAH was found only for Gal-3 and NT-proBNP but not for aldosterone plasma concentrations (figure 2A-B, C-D, E-F, respectively).

Other markers of vascular injury and inflammation

For our analysis we used several panels of the Meso Scale Discovery's Multi-Array assays in control (n=5), IPAH (n=41), and CTD (n=16) plasma. Quantitative measurements were considered reliable when more than 50% of the measurements fell within the linear range of the respective standard curves. Such markers included intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), serum amyloid A (SAA), CRP, interleukin-12 (IL-12), fibroblast growth factor 2 (FGF-2) (figure 3), as well as interleukin-16 (IL-16), vascular endothelial growth factor (VEGF), VEGF-C, and VEGF-D (figure 4). Plasma ICAM-1 concentrations were increased in patients with IPAH and PAH-CTD versus controls (559.5±18.2 pg/mL and 734.1±59.4 pg/mL vs controls 394.8±39.3 pg/mL, $p<0.05$, $p<0.0001$, respectively). Of note, the average plasma ICAM-1 concentration was significantly higher in PAH-CTD than in IPAH patients ($p<0.01$; figure 3A). In addition, VCAM-1 and IL-12 values were elevated in PAH-CTD patients versus controls (879.5±110.0 pg/mL and 391.2±70.3 pg/mL vs controls 489.8±44.6 pg/mL, $p<0.01$, and 102.1±15.2 pg/mL, $p<0.01$, respectively; figure 3B, E), but not in IPAH patients. The vascular injury/inflammatory markers SAA and CRP plasma concentrations tended to be higher in IPAH and PAH-CTD versus controls (ns; figure 3C, D). FGF-2, that has

Table 2 Individual PAH patient characteristics, including medication.

Age (yrs)	Weight (kg)	BMI (kg/m^2)	WHO Class	Medication*
Diagnosis†: IPAH				
63	59	26.2	1	SIL, **TREPIN**, VAL, O2
31	70	28.4	1	AMB, SIL, **TREPIN**, DIG, O2
33	64	22.7	1	NIF, WAR
33	57	20	1	SIL, WAR, PPI
33	91	28,1	1	THY, PRE, **TREPIV**, O2, intravenous immunoglobuline, IRO, WAR, VEN, AMI, SPI, LIS, TOR, BOS, PPI, TRAM, PRC
49	59	21,7	1	BOS, FUR, NIF, WAR, SAL, SER
40	94	34,5	1	AMB, AML, FUR, TAD
26	58	19,4	1	AML, FUR, SIL, WAR
41	59	20.9	2	BOS, SIL, NIF, WAR
50	49	19.9	2	BOS, TAD, ASA, SIM, SER
47	70	28.4	2	BOS, **TREPIV**, HCT, WAR, ESC, GAB
19	54	21.9	2	SIL, **TREPIV**, FUR, ALD, DIG, WAR, IRO, PPI
74	84	29.8	2	SIL, THY, WAR, SD, CEL
50	102	36.1	2	BOS, SIL, FUR, ALD, WAR, FLU, FEX, PPI
60	75	31.2	2	AMB, TRI, ALD, NAP
44	61	23.8	2	**EPIV**, ALD, FLU, BUP, GAB
38	78	26.1	2	BOS, TAD, **TREPIV**, FUR
49	86	30.5	2	AMB, AML, **TREPIN**, TOR, HCT, PRA, PPI, O2
31	68	22.2	2	**PGI**, WAR, ESC, GAB, PPI
34	51	22.7	2	BOS, SIL, **TREPSC**, FUR, DIG, IRO, HYD, PRE
48	68	25	2	AMB, TAD, **TREPPO**, CAV, WAR, BUM, RIS, PPI, TRAM, HYD, Calcium, CET, TEM, TRA, ALP
43	88	35	2	BOS, SIL, SPI, FUR, ATO, WAR, ILO
68	53	19,5	2	NIF, WAR, SIM, FLU, PPI, ASA, ALP, IRO, PRC, COD, RAN
52	71	27,4	2	BOS, TAD, SPI, FUR, MEF, SIT, LIS, DIG, CLG, DUL, LOV, THY, KCL, FAM
37	109	33,6	2	TAD, AMB, WAR, CET, RAN, SER, GUA, FLT
48	66	24,8	2	**TREPIN**, BOS, DIG, TAD, DIL
21	61	23	2	SIL, AMB, WAR, DIG, PPI, FUR, SPI, Depo Provera
31	56	21,9	2	SIL, AMB, **TREPPO**, PPI, TOP, SOL, DIG
65	51	24,6	2	NIF, THY, SIL, VYT
60	66	24,8	2	**TREP**, AMB, TAD, GAB, DIG, SPI, SER
39	64	27,7	2	**TREP**, BOS, FUR, PPI, SPI
29	56	19,4	2	AMB, TAD, TOP, DAB, FUR, IRO
44	38	14	2	FSA, AMT, AZE, WAR, DIG, FUR, HCT, PPI, MTZ, CIC, O2, PRM, **TREPIV**, SIL, SPI, TRAM, TRC, LEV
69	75	28,2	2	AMB, DIL, CLO, LIS, MEF, PIO, FUR, RAN, ROS, SIT, AMT, REP, DAB, MET
45	52	20,3	2	**TREPIN**, BOS, FUR, WAR
32	88	34,4	2	PRC, BOS, Calcium, IMB, Immodium, LOR, O2, RAN, THY, **TREPIV**
65	84	27,4	2	DIL, RAL, RAN, FLT, BOS, SPI, Finacea, ASA, CLB, CIT, BME, **TREPIN**
44	54	19,8	3	SIL, AMB, IMB, **EPIV**, Calcium, THY, TOR, PPI, DIL, MON, CET, SPI
38	70	28,4	3	BOS, WAR, NOR, ARG, AML, HCT, SD, MOM
48	86	37,2	3	**EPIV**, BOS, TAD, FUR, PPI, IRO, FA
59	86	31.6	3	AMB, SIL, **TREPIN**, FUR, ALD, WAR, SIM, IRO, OM3, PPI
Diagnosis: CTD*				
50	107	37.9	2	BOS, SIL, DIL, FUR, WAR, O2
62	64	26.3	2	SIL, MTX, PPI
77	72	27.1	2	BOS, NIF, HCT, THY, PRED, MTX, OM3
58	70	27.3	2	TAD, HCT, WAR, IRO
50	60	26.0	2	AMB, AML, FUR, ALD, DIG, ALL, COL, URS, O2
51	54	23.4	2	BOS, SIL, DIL, THY, PRED
52	104	38.2	2	BOS, SIL, **TREPIN**, FUR, BUM, THY, INS, DUL, PRE
57	84	30.9	2	NIF, **TREPIV**, MTX, PRED, FA, PPI
46	91	36,9	2	SIL, QUE, WAR, **TREPIN**, PPI, HXC, LOZ
60	68	22,7	2	**TREPIN**, AMB, SIL, FUR
57	52	20,3	2	AMB, FUR, BUP, CIT, PPI, PEN, ZOL, FEX
57	64	26,0	2	BOS, TAD, FUR, SPI, O2
40	82	26.8	3	BOS, SIL, AML, **TREPIV**, FUR, ALD, PRED, LOS, CLO, ATE
66	83	31.2	3	BOS, SIL, FUR, ALD, THY, LIS, PRED, AZA, PPI, PAR, O2
28	58	21,8	2	**TREPIV**, SIL, WAR, HXC, FUR, PRC/OXC, MMF, FLU, THY, PRED, FA, PPI, RAN, ZOL
50	63	25,2	4	PPI, DOM, SIL, AMB, FUR, AML, FA, PRE, LOR

*Medications (prostanoids are in bold): ALD=aldactone PO; ALL=allopurinol PO; AMB=ambrisentan PO; AML=amlodipine PO; ALP=alprazolam PO; AMI=amiloride PO; AMT=amitryptilin PO; ARG=l-arginine PO; ASA=acetylsalicylic acid; ATE=atenolol PO; ATO=atorvastatine PO; AZA=azathioprine PO; AZE=azelastine PO; BME=betamethasone PO; BOS=bosentan PO; BUM=bumetanide PO; BUP=bupropion PO; CAV=carvedilol PO; CET=cetirizine PO; CIC=ciclenosid PO; CIT=citalopram PO; CLB=clobetasol topical; CLG=clopidogrel PO; CLO=clonidine PO; COD=codein PO; COL=colchicin PO; DAB=dabigatranexilat PO; DIG=digoxin PO; DIL=diltiazem; DIP=dipyridamole; DOM=domperidone PO; DUL=duloxetine PO; EPIV=epoprostenol IV; ESC=escitalopram PO; FA=folic acid PO; FAM=famotidin PO; FEX=fexofenadine PO; FLT=fluticasone INH; FLU=fluoxetin PO; FSA=fluticasone/salmeterol INH; FUR=furosemide PO; GAB=gabapentin PO; GUA=guifenesin PO; HCT= hydrochlorothiazide PO; HXC=hydroxychloroquine PO; HYD=hydrocodone; ILO=iloprost INH; IMB=imatinib PO; INS=insulin; IRO=iron PO; LEV=levabuterol INH; LIS=lisinopril PO; LOR=loratadin PO; LOS=losartan PO; LOV=lovastatin PO; MED=mededrine; MEF=metformin PO; MET=metoprolol PO; MMF=mycophenolat- mofetil PO; MOM=mometasone PO; MON=montelukast inhal.; MTX=methotrexate PO; MTZ=metronidazole PO; NIF=nifedipine; NOR=norethindrone PO; O2=oxygen by nasal canula; OM3=omega-3-acid ethyl esters PO; OXC=ocycodon PO; PAR=paroxetine PO; PGI=prostanoid; PPI=proton pump inhibitor PO; PRA=pravastatin PO; PRC=paracetamolo PO; PRE=pregabalin; PRED=prednisone PO; PRM=promethazin PO; QUE= quetiapin PO; RAL=raloxifen PO; RAN=ranitidine PO; RIS=risedronate PO; SAL=salmeterol INH; SD=PH study drug; SER=sertraline PO; SIL=sildenafil PO; SIM=simvastatine PO; SIT=sitagliptin PO; SOL=solifenacinsuccinat PO; SPI=spironolactone PO; TAD=tadalafil PO; TEM=temazepam PO; THY=levothyroxine PO; TOP=topiramat PO; TOR=torsemide PO; TRA=trazodone; TRC=triamcinolon PO; TRAM=tramadole; TREP=trepostinil; TREPIV=trepostinil IV; TREPIN=trepostinil inhal.; TREPPO=trepostinil PO ; TREPSC=trepostinil subcutaneously; TRI=triamterene PO; URS=ursodiol PO; VAL=valsartan PO; VEN=venlaxafine PO; VYT=ezetimibe/simvastatin PO; WAR=warfarin PO; ZOL=zolpidem PO

†Clinical Classification of Pulmonary Hypertension (World Symposium on PH; Nice 2013) as described in Simonneau G, J Am Coll Cardiol 2013. Parenteral prostacyclin administration (intravenous, inhaled, subcutaneous) in the far right column is indicated in bold letters. The definition of WHO functional class of PAH can be found in Rubin LJ. Diagnosis and management of pulmonary arterial hypertension: ACCP Evidence-Based Clinical Practice Guidelines. Introduction. Chest 2004;126:7S–10S.

BMI, body mass index; CTD, PAH associated with connective tissue disease (WSPH Nice PH category 1.4.1); DTI, PAH drug and toxin induced (Nice PH category 1.3); HPAH, heritable PAH (Nice PH category 1.2.); IPAH, idiopathic PAH (Nice PH category 1.1); IV, intravenous; PAH, pulmonary arterial hypertension; PO, oral; SC, subcutaneous; SSRI, selective serotonin reuptake inhibitor.

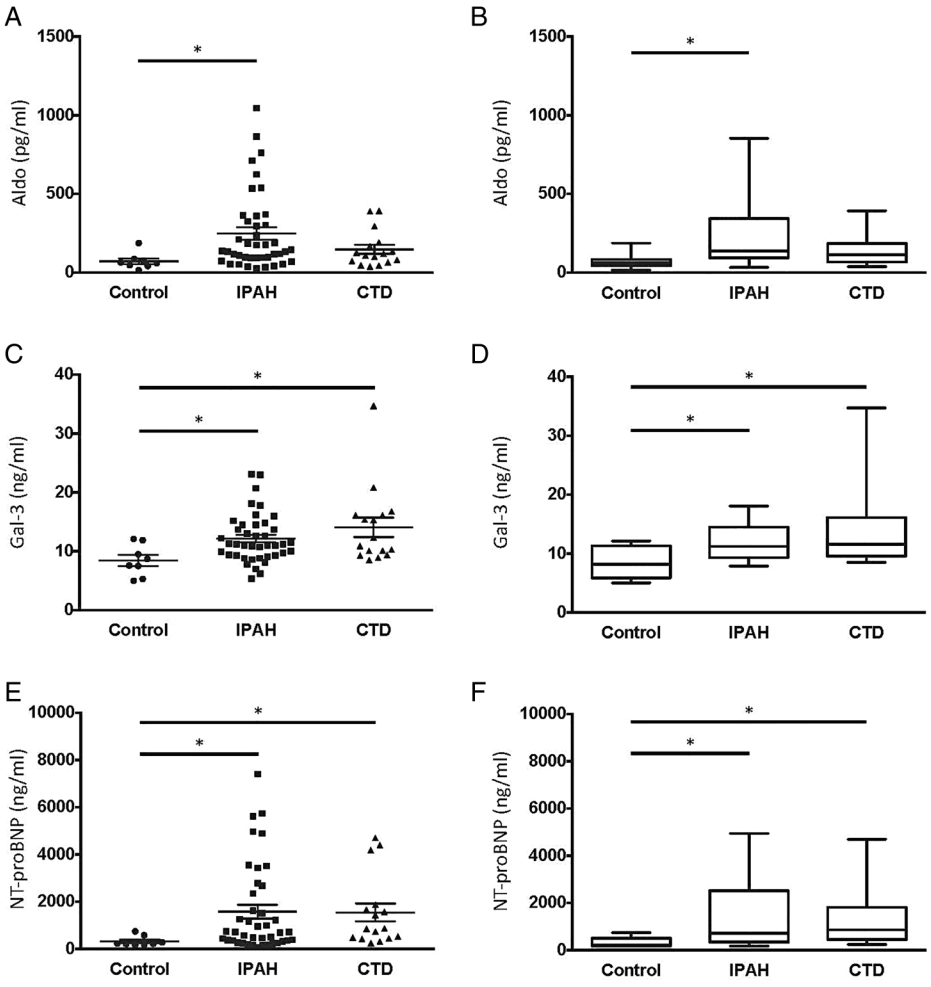

Figure 1 Galectin-3 (Gal-3), aldosterone, and N-terminal pro-brain natriuretic peptide (NT-proBNP) plasma concentrations are elevated in pulmonary arterial hypertension (PAH) patients versus controls. The scatter plots on the left and the box and whiskers plots on the right provide data for aldosterone (A and B), galectin-3 (Gal-3) (C and D), and NT-proBNP (E and F) plasma concentrations in control subjects (n=8), patients with idiopathic pulmonary hypertension (IPAH, n=41), and PAH associated with connective tissue disease (CTD, n=16). The scatter plots show the mean±SEM; the box and whiskers plots show the median with IQR±10–90th centile. *p<0.05, **p<0.01.

previously been shown to be involved in the pathobiology of PAH,[18] also showed a trend toward increased plasma concentrations in IPAH patients versus controls (ns; figure 3F). For the other protein biomarkers measured in PAH and control plasma (IL-16, VEGF, VEGF-C, VEGF-D), no statistically significant differences or clear trends were found (figure 4).

DISCUSSION

Biomarkers hold the potential to provide valuable insights into the pathobiology and disease progression of PAH—a heterogeneous, fatal disease leading to pressure overload and ultimately failure of the right ventricle. Prior biomarker studies identified several plasma proteins being altered in PAH.[19] However, none of these molecules exhibited all characteristics of an ideal biomarker (eg, high specificity and sensitivity, correlation with disease progression and therapy, etc). Thus, a multiparameter approach has been proposed to adequately reflect the complex and diverse pathogenetic mechanisms of clinical PAH.[6]

This is the first report to show that both plasma aldosterone and Gal-3 are increased in PAH, associated with WHO functional class, thus pointing to the potential of aldosterone/Gal-3 to indicate disease severity. Gal-3 is involved in several pathological and physiological processes—for example, the regulation of organ development, immune reactions, tumorigenesis, tumour growth, and metastasis.[20] In the heart, Gal-3 augments fibrosis and modulates immune responses, which confers to maladaptive cardiac remodelling.[21] Extracellular matrix remodelling is a key component of fibrosis, and increased Gal-3, which is released by macrophages, is likely to reflect profibrotic activity.[21] Since advanced pulmonary hypertensive vascular disease is characterised by the development of vascular and cardiac fibrosis, increased Gal-3 concentrations in PAH may reflect vascular and right ventricular fibrosis.[22]

Gal-3 is activated by aldosterone in vascular smooth muscle cells of the systemic circulation.[13] Here, we demonstrate that aldosterone is significantly increased in IPAH, but not in PAH-CTD patients, indicating the involvement of aldosterone-independent pathways in CTD, for example, inflammation-mediated induction of Gal-3, possibly mediated by VCAM-1 and IL-12 (figure 2).[20] Independently of the aldosterone pathway, Gal-3 is expressed by activated macrophages and endothelial cells, attracting inflammatory cells and inducing fibrosis.[20] This aldosterone-independent Gal-3 secretion might be particularly active in PAH-CTD patients. Interestingly, we did not find a direct correlation between plasma Gal-3 and aldosterone in IPAH patients. Therefore, one might speculate that aldosterone-independent pathways are also relevant in IPAH-associated Gal-3 upregulation. However, we provide data from a single time point, so that further studies addressing the temporal courses of aldosterone and Gal-3 values, and their possible correlation, are needed. Further limitations of this study include uncontrolled dietary and volume status at the time of plasma acquisition.

Both aldosterone and Gal-3 correlated with advanced disease states (as indicated by WHO-FC). It is therefore likely that longitudinal assessments of aldosterone and Gal-3 may help identify patients with more advanced PAH. Aldosterone can be

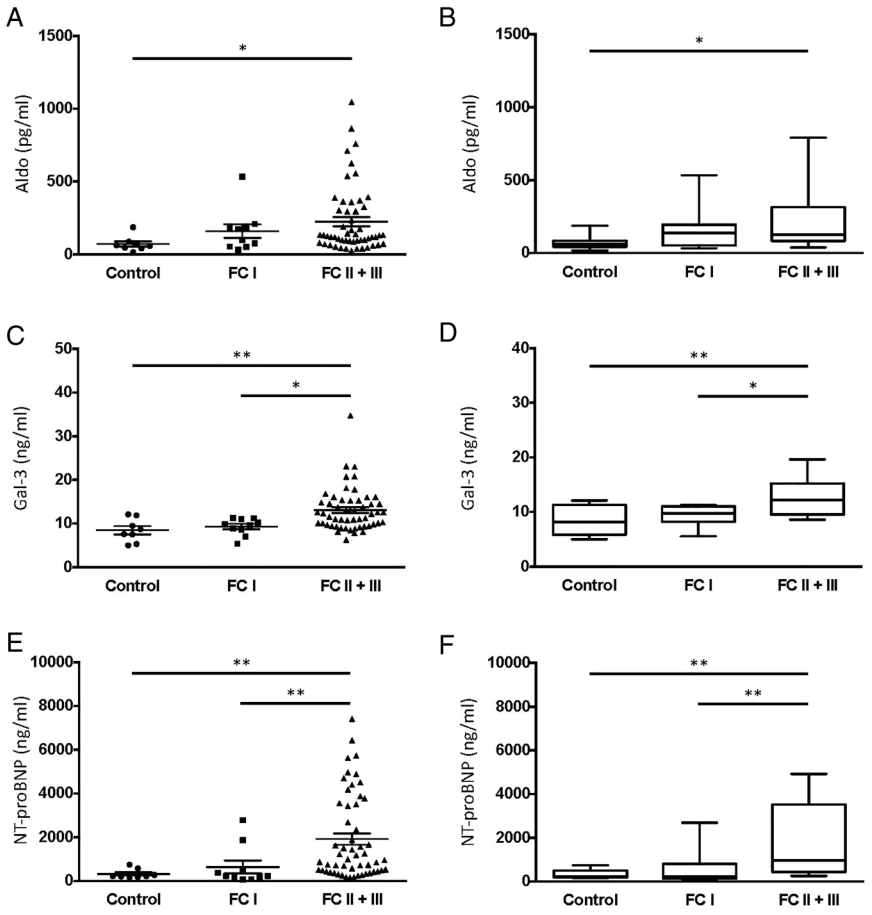

Figure 2 Galectin-3 (Gal-3), aldosterone, and N-terminal pro-brain natriuretic peptide (NT-proBNP) plasma concentrations increase with pulmonary arterial hypertension (PAH) severity. The scatter plots on the left and the box and whiskers plots on the right provide data for aldosterone (A and B), galectin-3 (Gal-3) (C and D), and NT-proBNP (E and F) plasma concentrations according to WHO functional classes (FC). The scatter plots show the mean±SEM; the box and whiskers plots show the median with IQR±10–90th centile. Control, n=8; FC I, n=10; FC II+III, n=53. *p<0.05, **p<0.01.

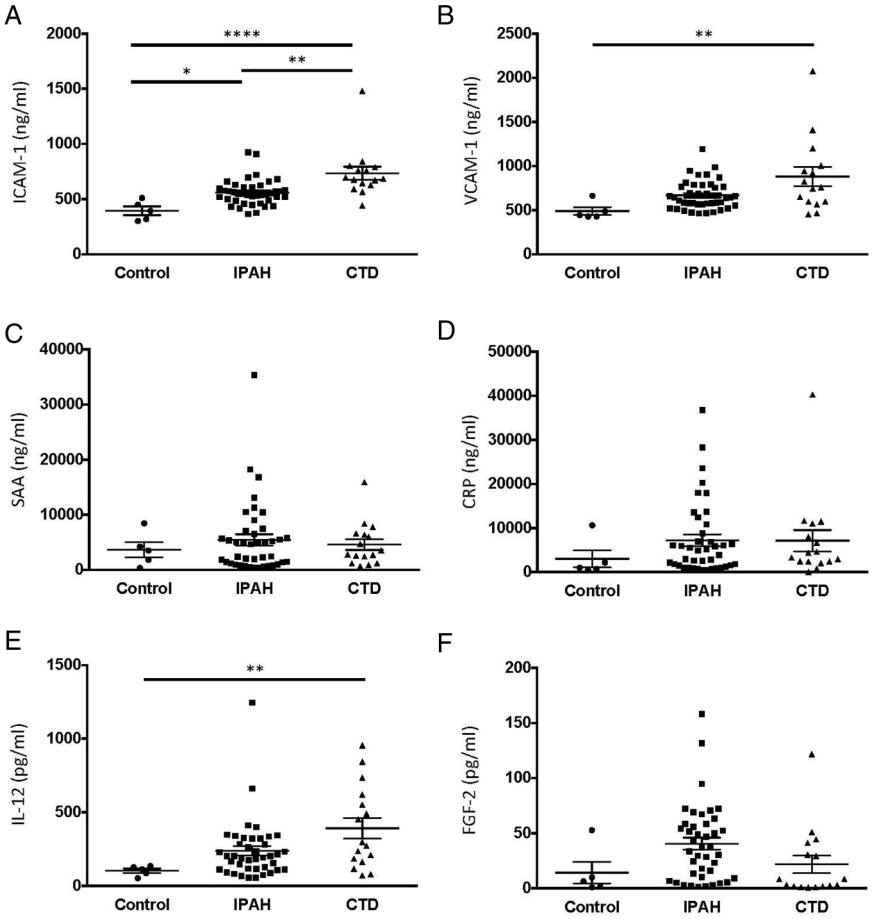

Figure 3 Selected potential biomarker plasma concentrations in pulmonary arterial hypertension (PAH) patients. The scatter plots provide data for intercellular adhesion molecule 1 (ICAM-1) (A), vascular cell adhesion molecule 1 (VCAM-1) (B), serum amyloid A (SAA) (C), C reactive protein (CRP) (D), interleukin-12 (IL-12) (E), and fibroblast growth factor 2 (FGF-2) (E) plasma concentrations in control subjects (n=5), patients with idiopathic pulmonary hypertension (IPAH, n=41), and connective tissue disease-associated pulmonary hypertension (CTD, n=16). Mean±SEM; *p<0.05, **p<0.01, ****p<0.0001.

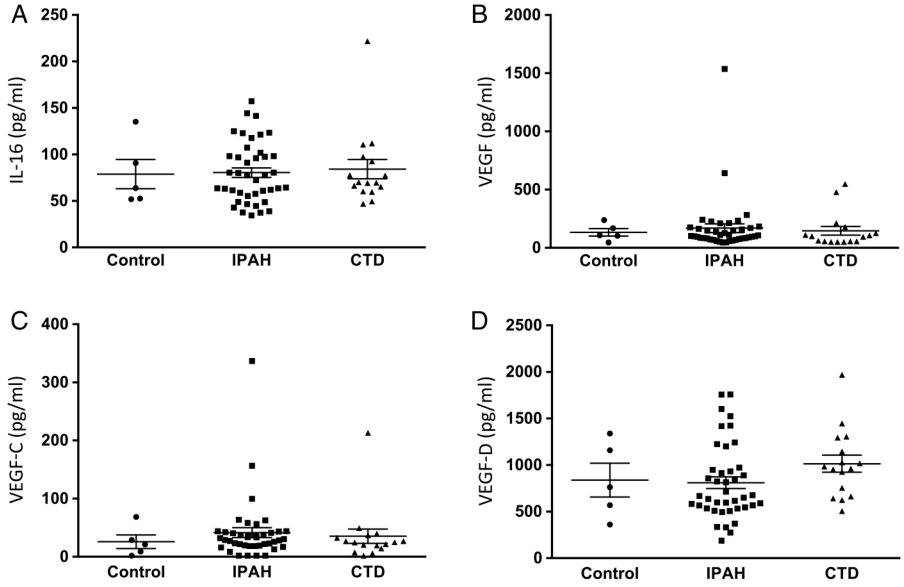

Figure 4 Selected potential biomarker concentrations in pulmonary arterial hypertension (PAH) patients. The scatter plots provide expression level data for interleukin-16 (IL-6) (A), vascular endothelial growth factor (VEGF) (B), VEGF-C (C), and VEGF-D (D) in control subjects (n=5), patients with idiopathic pulmonary hypertension (IPAH, n=41), and connective tissue disease-associated pulmonary hypertension (CTD, n=17).

targeted by well-established MR antagonists such as spironolactone, which raises the possibility of treating selected patients with aldosterone antagonists.[10 23] In fact, cardiac remodelling and dysfunction appears to be responsive to MR blockade (spironolactone) treatment in clinical studies, such as the ARIES and TOPCAT trials.[11 24]

Because plasma aldosterone is not increased in PAH-CTD, while Gal-3 upregulation is observed in both IPAH and PAH-CTD, PAH patients might benefit from direct Gal-3 blockade. These issues therefore need to be addressed in future studies.

Beyond the aldosterone/Gal-3 tandem, we found that ICAM-1 was elevated both in IPAH and PAH-CTD versus controls. Interestingly, in our study, increased IL-12 and VCAM-1 concentrations were only found in PAH-CTD but not in IPAH patients, which indicates PAH-subtype specific roles for these molecules, probably related to the degree of inflammation, vascular injury and anti-angiogenesis. In serum from PAH patients, IL-12 expression values were found to be moderately elevated in IPAH and strongly elevated in PAH-CTD.[25] In contrast, patients with chronic thromboembolic pulmonary hypertension, who do not show perivascular infiltration of T and B lymphocytes in the lungs, had no significant changes in the circulating concentrations of IL-12, indicating that IL-12 elevation is not a direct response to elevated pulmonary arterial pressure.[25] The association of circulating VCAM-1 and ICAM-1 with PH was also reported in patients with sickle cell disease.[26] Knockdown of BMPR-II increased ICAM-1 and VCAM-1 expression in HUVECs (human umbilical vein endothelial cells).[27] These data suggest that VCAM-1 and ICAM-1 induction reflects endothelial activation and dysfunction occurring in PAH.

Taken together, we provide evidence for an involvement of Gal-3 and aldosterone in the pathobiology of human PAH and right ventricular dysfunction, and suggest their potential application as a new tandem in clinical PAH. This study provides the rationale for further clinical and experimental studies investigating the aldosterone/Gal-3 axis in the pathogenesis of PAH. Larger prospective studies including hemodynamics are needed to assess the suitability of aldosterone/Gal-3 as tandem biomarkers for disease progression and treatment response in different PAH aetiologies.

Key messages

What is already known on this subject?
The mineralocorticoid receptor agonist aldosterone is involved in pulmonary hypertensive vascular disease and cardiovascular fibrosis. We have previously demonstrated in animal models and in vitro that galectin-3 (Gal-3) is a key mediator of cardiac and renal fibrosis induced by aldosterone.

What might this study add?
This study demonstrates for the first time that the axis aldosterone/galectin-3 (Gal-3) is relevant in pulmonary arterial hypertension (PAH), by showing that plasma levels of both are associated with PAH severity.

How might this impact on clinical practice?
Galectin-3 (Gal-3) is already established as a biomarker for cardiac fibrosis, and chronic left heart failure. This work suggest potential applications for aldosterone/galectin-3 (Gal-3) as a new tandem biomarker for clinical PAH.

Author affiliations
[1]Department of Pediatric Cardiology and Critical Care, Hannover Medical School, Hannover, Germany
[2]Department of Neonatology, Charité University Medical Center, Berlin, Germany
[3]Department of Chemical Engineering, Northeastern University, Boston, Massachusetts, USA
[4]Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany
[5]Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany
[6]Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, Massachusetts, USA

Acknowledgements We thank Dr Paolo Galuppo for his advice on the aldosterone ELISA.

Contributors GH conceived the study, and obtained IRB approval, samples and written consent. AH, BDP, HS and SKM participated in the study design, IRB approval and sample collection. LC, EL, and LG performed the biochemical assays and statistical analysis. LG, HS, and GH structured and analysed the clinical data sets. GH and HS wrote the paper. HS, LC, EL, JB and GH edited the manuscript.

Funding GH currently receives grant support from the German Research Foundation (DFG; HA 4348/2-1), Kinderherzen e.V (W-H-001-2014), and Stiftung KinderHerz

(2511-6-13). LC is the recipient of a postdoctoral research stipend from the European Section of the Aldosterone Council (ESAC). SKM was supported by grant R01 EB009327 from the US National Institutes of Health. The contribution of the COST Action ADMIRE BM1301 is acknowledged (JB).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Northeastern University, Boston (IRB # 11-07-19).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Tuder RM, Archer SL, Dorfmüller P, *et al*. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol* 2013;62:D4–12.
2 Galiè N, Humbert M, Vachiery JL, *et al*. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J* 2015 Aug 29. pii: ehv317.
3 Abman SH, Hansmann G, Archer S, *et al*. Pediatric pulmonary hypertension: guidelines from the American Heart Association and American Thoracic Society. *Circulation* 2015;132:2037–99.
4 Sutendra G, Michelakis ED. Pulmonary arterial hypertension: challenges in translational research and a vision for change. *Sci Transl Med* 2013;5:208sr5.
5 Hansmann G, Plouffe BD, Hatch A, *et al*. Design and validation of an endothelial progenitor cell capture chip and its application in patients with pulmonary arterial hypertension. *J Mol Med* 2011;89:971–83.
6 Pezzuto B, Badagliacca R, Poscia R, *et al*. Circulating biomarkers in pulmonary arterial hypertension: update and future direction. *J Heart Lung Transplant* 2015;34:282–305.
7 Maron BA, Leopold JA. The role of the renin-angiotensin-aldosterone system in the pathobiology of pulmonary arterial hypertension (2013 Grover Conference series). *Pulm Circ* 2014;4:200–10.
8 Maron BA, Opotowsky AR, Landzberg MJ, *et al*. Plasma aldosterone levels are elevated in patients with pulmonary arterial hypertension in the absence of left ventricular heart failure: a pilot study. *Eur J Heart Fail* 2013;15:277–83.
9 Maron BA, Zhang Y-Y, White K, *et al*. Aldosterone inactivates the endothelin-B receptor via a cysteinyl thiol redox switch to decrease pulmonary endothelial nitric oxide levels and modulate pulmonary arterial hypertension. *Circulation* 2012;126:963–74.
10 Maron BA, Leopold JA. Emerging concepts in the molecular basis of pulmonary arterial hypertension: part II: neurohormonal signaling contributes to the pulmonary vascular and right ventricular pathophenotype of pulmonary arterial hypertension. *Circulation* 2015;131:2079–91.
11 Maron BA, Waxman AB, Opotowsky AR, *et al*. Effectiveness of spironolactone plus ambrisentan for treatment of pulmonary arterial hypertension (from the [ARIES] study 1 and 2 trials). *Am J Cardiol* 2013;112:720–5.
12 Bauersachs J, Jaisser F, Toto R. Mineralocorticoid receptor activation and mineralocorticoid receptor antagonist treatment in cardiac and renal diseases. *Hypertension* 2015;65:257–63.
13 Calvier L, Miana M, Reboul P, *et al*. Galectin-3 mediates aldosterone-induced vascular fibrosis. *Arterioscler Thromb Vasc Biol* 2013;33:67–75.
14 Calvier L, Martinez-Martinez E, Miana M, *et al*. The impact of galectin-3 inhibition on aldosterone-induced cardiac and renal injuries. *JACC Heart Fail* 2015;3:59–67.
15 Edelmann F, Wachter R, Schmidt AG, *et al*. Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction: the Aldo-DHF randomized controlled trial. *JAMA* 2013;309:781–91.
16 Edelmann F, Holzendorf V, Wachter R, *et al*. Galectin-3 in patients with heart failure with preserved ejection fraction: results from the Aldo-DHF trial. *Eur J Heart Fail* 2015;17:214–23.
17 Yancy CW, Jessup M, Bozkurt B, *et al*. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013;62:e147–239.
18 Izikki M, Guignabert C, Fadel E, *et al*. Endothelial-derived FGF2 contributes to the progression of pulmonary hypertension in humans and rodents. *J Clin Invest* 2009;119:512–23.
19 Cracowski J-L, Leuchte HH. The potential of biomarkers in pulmonary arterial hypertension. *Am J Cardiol* 2012;110:32S–8S.
20 Gruson D, Ko G. Galectins testing: new promises for the diagnosis and risk stratification of chronic diseases? *Clin Biochem* 2012;45:719–26.
21 Sharma UC, Pokharel S, van Brakel TJ, *et al*. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 2004;110:3121–8.
22 Nishi Y, Sano H, Kawashima T, *et al*. Role of galectin-3 in human pulmonary fibrosis. *Allergol Int* 2007;56:57–65.
23 Azibani F, Fazal L, Chatziantoniou C, *et al*. Aldosterone mediates cardiac fibrosis in the setting of hypertension. *Curr Hypertens Rep* 2013;15:395–400.
24 Shah AM, Shah SJ, Anand IS, *et al*. Cardiac structure and function in heart failure with preserved ejection fraction: baseline findings from the echocardiographic study of the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist trial. *Circ Heart Fail* 2014;7:104–15.
25 Larsen KO, Yndestad A, Sjaastad I, *et al*. Lack of CCR7 induces pulmonary hypertension involving perivascular leukocyte infiltration and inflammation. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L50–9.
26 Kato GJ, Martyr S, Blackwelder WC, *et al*. Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. *Br J Haematol* 2005;130:943–53.
27 Kim CW, Song H, Kumar S, *et al*. Anti-inflammatory and antiatherogenic role of BMP receptor II in endothelial cells. *Arterioscler Thromb Vasc Biol* 2013;33:1350–9.