

Anti- β 1-Adrenoreceptor auto-Antibodies in elderly heart failure patients

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1. ABSTRACT

An autoimmune reaction directed against the cardiac β 1-adrenergic receptor (β 1-ADR) leading to the generation of autoantibodies (AA) against this G-coupled receptor has been described in patients with heart failure (HF). Agonist-like β 1-ADR-AA are associated with morbidity in HF patients and even predict mortality. Standardised and valid diagnostic tools to detect β 1-ADR-AA in clinical routine are

lacking. We used a novel ELISA approach to investigate β 1-ADR-AA in a cohort of 574 HF patients of the CIBIS-ELD trial with follow up. The CIBIS-ELD trial compared the titration of bisoprolol and carvedilol to recommended target doses in regard to BB tolerability in patients aged 65 years and older. Patient with left ventricular (LV) ejection fraction (EF) < 50% or LV diameter end diastolic (DED) >55 cm showed sig-

nificantly higher levels of β 1-ADR-AA. Although not yet fully validated, this ELISA allowed for a negative correlation of β 1-ADR-AA with the EF at baseline and at the follow up, β 1-ADR-AA further correlated positively with basal heart rate at follow up 12 weeks later. β 1-ADR-AA levels thus determined significantly increased under titration with beta-blockers ($p < 0.0.1$). Changes in β 1-ADR-AA between F-Up and baseline were significantly higher in patients who used beta blockers ($p = 0.0.16$) before study inclusion. The type of beta-blocker (BB) titrated in this study did not affect log β 1-ADR-AA levels at baseline ($p = 0.1.32$), follow-up ($p = 0.0.58$), nor the change ($p = 0.4.26$). β 1-ADR-AA levels were estimated using a novel, commercially available ELISA. Although not yet fully validated, this ELISA allowed for pathophysiological insights: β 1-ADR-AA levels thus determined significantly increased under titration with beta-blockers ($p < 0.0.1$), irrespective of type of BB. Higher levels of β 1-ADR-AA at baseline are associated with higher heart rates, lower ejection fraction and enlarged left ventricles. The relevance of the β 1-ADR-AA biomarker should be further evaluated.

2. INTRODUCTION

β 1-Adrenoreceptors (β 1-ADR) mediate the positive inotropic, chronotropic, bathmotropic, dromotropic, and lusitropic effects of endogenous neuro-hormonal stress on cardiac tissues (1), (2). Beta Blockers (BB) shield the heart from such catecholaminergic stress – i.e. by blockade of the cardiac β 1-ADR - and have emerged as a cornerstone of optimum adjusted medical therapy of Heart Failure (HF) (3) and remain of paramount importance in the routine clinical treatment of patients suffering from this debilitating syndrome.

Recent advances in the understanding of HF pathophysiology suggest a contributing role of autoimmune mechanisms in the development and progression of HF (4). Autoimmune reactions directed against the cardiac β 1-ADR may lead to the generation of autoantibodies (β 1-ADR-AA) against this G-coupled receptor (5). Molecular mimicry i.e. leads to β 1-ADR-AA in patients with Chagas cardiomyopathy (6), (7). β 1-ADR-AA may interfere with ligand binding and receptor conformation, i.e. increasing cAMP stimulation (8) or amplifying the effect of catecholamines (9).

β 1-ADR-AA have previously been associated with cardiac dilatation as well as pump failure (10) in dilatative (DCM) (10), (11), (8) and ischemic cardiomyopathy (ICM) (10) but not necessarily in HF of other aetiologies (11). Aside from association, agonistic-like β 1-ADR-AA were found to predict mortality in idiopathic cardiomyopathy (12). Passive transfer of agonist-like β 1-ADR-AA induced heart failure in rodents (13). These findings indicate the

specific role of such AA in the pathophysiology and development of HF and have furthermore opened a window of opportunity of therapeutic approaches in our patients. Removing the AA from the bloodstream by means of immunoadsorption - as a non-pharmacologic concept - has already shown to significantly improve cardiac performance and clinical status in patients (14), (15). Pharmacologic concepts such as aiming at the partial abrogation of β 1-ADR-AA by use of cardio selective BB (16), and binding and neutralizing of β 1-ADR-AA by aptamers (17), or the cyclic peptide COR-1 (18) have been reported.

Furthermore, diagnostic and therapeutic implications have recently received growing attention in the HF community (4). Standardised and valid assessments of β 1-ADR-AA may prove pivotal for an understanding of pathophysiology, diagnosis, and risk-stratification in HF patients. In a situation of interference of β 1-ADR-AA with established, life-saving BB therapies, an option to monitor therapy response may be required. Autoimmune diseases such as grave's or myasthenia gravis have taught us that the development of suitable assays is especially complex in the setting of autoimmunity (19). β 1-ADR-AA were measured by intracellular cAMP by fluorescence resonance energy transfer using a highly sensitive cAMP sensor, a very sensitive method which is not suitable in clinical routine (20). Recently a cell-based competition ELISA has been introduced (21). Hence, to prospectively address cardiac autoimmunity new diagnostic studies (i.e. ET-ICS (22)) are currently recruiting.

In the present analysis, we used a solid-phase ELISA for the detection of antibodies (IgG) targeted against the g-protein-coupled β 1-adrenoreceptor from baseline and follow-up blood samples collected within the CIBIS-ELD (23) trial on selective vs. non-selective beta-blocker titration in HF patients. The ELISA used within this analysis is (i) not a peptide-ELISA, (ii) it is not yet fully validated regarding sensitivity and specificity compared to functional bioassays, and (iii) should therefore be considered a putative surrogate marker for β 1ARAAB. In this secondary post-hoc analysis we investigated the association between β 1-ADR-AA - as the novel Biomarker – and HF characteristics, severity and possible implications of beta-blocker therapy. The present study serves to provide clinical correlation for this marker.

3. METHODS

3.1. Subjects and intervention

The CIBIS-ELD trial design (24) and its primary results (23) have been reported previously. In principle, the trial compared the titration of bisoprolol and carvedilol to recommended target doses in regard to BB tolerability. Patients aged 65 years and older with

clinical, chronic stable heart failure with either reduced or preserved ejection fraction were randomized to either BB. At baseline all patients were BB naïve or receiving 25% or less of the target dose. BB dose was titrated according to a standardized protocol every two weeks over a 12-week period, starting with 1.2.5 mg bisoprolol daily or 3.1.25 mg carvedilol twice daily. Target doses were 10 mg bisoprolol daily or 25 mg carvedilol twice daily. Adverse events were recorded at every visit; treatment-related events were defined according to a blinded committee.

3.2. Data collection and definitions

Data on demographics, medical history, electrocardiogram, echocardiography, New York Heart Association (NYHA) functional class, and laboratory parameters were collected at baseline and after up-titration at 12 weeks. Glomerular filtration rate (GFR) was calculated using the MDRD formula. Serum β 1-ADR-AA were measured with a commercially available sandwich ELISA (CellTrend GmbH Luckenwalde, Germany) according to the instruction manual. The microtiter 96-well polystyrene plates were coated with extracts from transfected Chinese Hamster Ovary (CHO) cells overexpressing the human β 1-ADR. To conserve conformational epitopes of the receptor as much as possible, a concentration of 1 mM calcium chloride was maintained during all steps of the assay. Duplicate samples of a 1:100 serum dilution were incubated at 48°C for 2 hours. After washing steps, plates were incubated for 60 minutes with a 1:20 000 dilution of horseradishperoxidase-labeled goat anti-human IgG (Jackson, Bar Harbor, ME, USA) used for detection. In order to obtain a standard curve, plates were incubated with test sera from an anti- β 1-ADR antibody positive index patient. All tests were done in duplicate. The detection threshold of β 1-ADR-AA was set at 2.5. U. All sera were coded and assessed for β 1-ADR-AA by individuals who had no information regarding the patients' characteristics.

3.2. Data analysis

This is secondary a post-hoc analysis. Baseline variables are presented as frequencies and percentages for binary variables, or means and standard error (SE), i.e. in combination with a median-split by log-transformed β 1-ADR-AA. Data are presented overall and separately in the respective groups. Comparisons of all groups were performed by Fisher's exact test or Wilcoxon test, respectively. For beta blockers, heart rate, ECG, systolic and diastolic blood pressure, 6 min walk test, NYHA class and echocardiographic parameters, the relationships between pre- and post-titration measurement were examined. Quantitative measures were analyzed by Kendall's rank correlation. R (R Core Team (2015). R:

A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.) was used as statistical software.

3.3.Ethics

The CIBIS-ELD trial (ISRCTN 34827306 at controlled-trials.com) was approved by the institutional ethical review boards of all participating centres. The study conforms with the principles outlined in the Declaration of Helsinki. All patients provided written informed consent prior to enrolment.

4. RESULTS

CIBIS-ELD included 876 patients in the primary analysis (431 bisoprolol, 445 carvedilol) and 782 in the follow-up analysis (386/396) (23). We report here on a sample of 574 patients (286 bisoprolol, 288 carvedilol) of the CIBIS-ELD trial population with available serum analysis. Baseline characteristics of the population according to a median split of log β 1-ADR-AA levels are displayed in Table 1.

At baseline, the proportion of women in the group with lower β 1-ADR-AA values was significantly higher than in the setting of higher β 1-ADR-AA, while age distribution was found to be similar in both groups. Regarding the clinically deemed aetiology of HF, NYHA functional class, 6-minutes-walk-test distance, NT-pro-BNP levels and history of HF hospitalization in the past 12 months, we report a similar clinical profile in the respective groups with lower or higher β 1-ADR-AA levels.

Patients with higher levels of baseline log β 1-ADR-AA had a significantly lower left ventricular (LV) ejection fraction (EF). When LVEF was classified categorically according to reduced vs. normal (LVEF \leq / $>$ 55, $p=0.0.035$) and severely impaired vs. preserved (LVEF \leq 35/ \geq 50%, $p=0.0.05$) significant differences in log β 1-ADR-AA came to display (Figure 1). Similar results were observed in regard to LV end diastolic (DED) >55 cm ($p=0.0.012$) (Figure 2), and systolic (SED) diameter, where dilatation was seen in the group with higher levels of β 1-ADR-AA. Comparable results were observed for end-diastolic intra-ventricular septum thickness (IVSED) < 11 mm ($p=0.0.113$) and end-diastolic posterior wall thickness < 11 mm ($p=0.0.140$) at baseline, follow-up and change. Thinner left ventricular walls were described when higher levels of log β 1-ADR-AA were present.

Heart rate was lower in patients with lower β 1-ADR-AA levels. We report a significant association between baseline β 1-ADR-AA and resting heart rate (from ECG) at baseline (Table 2), at follow up 12 weeks later ($r=0.1.41$, $p= 0.0.04$) as well as in regard

Table 1. Baseline characteristics; all patients and median split [$< 50\% >$] by log β1-AAK levels

Variable	VarN° of patients	all patients descriptive Value	N° of patients low β1-AAK	low β1-AAK descriptive Value	N° of patients high β1-AAK	high β1-AAK descriptive Value	high vs. low p=
	(n=)	(mean±sd; percent)	(n=)	(mean±sd; percent)	(n=)	(mean±sd; percent)	
Demographics & Heart Failure							
Age [years]	574	72±5.4	287	73±5.5	287	72±5.4	0.083
Sex [female]	574	198 (34%)	287	111 (39%)	287	87 (30%)	* 0.043
Systolic blood pressure [mmHg]	574	138±21.3	287	140±21.3	287	136±21.1	* 0.037
Diastolic blood pressure [mmHg]	574	81±11.7	287	81±11.6	287	81±11.7	0.671
LVEF < 50 %	569	379 (67%)	286	175 (61%)	283	204 (72%)	** 0.006
NYHA Class	574		287		287		0.607
I		21 (4%)		11 (4%)		10 (3%)	
II		392 (68%)		201 (70%)		191 (67%)	
III		161 (28%)		75 (26%)		86 (30%)	
NT-pro-BNP quantile	545		270		275		0.060
1 st		137 (25%)		81 (30%)		56 (20%)	
2 nd		136 (25%)		59 (22%)		77 (28%)	
3 rd		136 (25%)		65 (24%)		71 (26%)	
4 th		136 (25%)		65 (24%)		71 (26%)	
Heart Failure Etiology	563		282		281		0.333
Hypertension		194 (34%)		106 (38%)		88 (31%)	
Coronary Artery Disease		261 (46%)		126 (45%)		135 (48%)	
Cardiomyopathy		44 (8%)		18 (6%)		26 (9%)	
Other		64 (11%)		32 (11%)		32 (11%)	
Peripheral Edema	573	107 (19%)	287	66 (23%)	286	41 (14%)	* 0.014
6 Minute Walk Test walk distance [m]	538	332±106	266	332±103	272	331±109	0.913
Hospitalization for heart failure during the past 12 months	572	202 (35%)	285	90 (32%)	287	112 (39%)	0.067
Quality of life	494		246		248		
SF-36 physical score		39±9.7		39±9.8		38±9.5	0.628
SF-36 psychosocial score		46±11.9		46±11.7		46±12.2	0.918
Medical History							
Atrial Fibrillation	563	261 (46%)	282	126 (45%)	281	135 (48%)	0.333
Arterial Hypertension	574	481 (84%)	287	253 (88%)	287	228 (79%)	** 0.006
Hyperlipoproteinemia	573	370 (65%)	287	196 (68%)	286	174 (61%)	0.067
Diabetes mellitus	574	153 (27%)	287	84 (29%)	287	69 (24%)	0.186
Renal dysfunction [GFR < 60ml]	553	211 (38%)	278	105 (38%)	275	106 (39%)	0.861
Hyperuricemia	574	51 (9%)	287	27 (9%)	287	24 (8%)	0.770
COPD	574	45 (8%)	287	26 (9%)	287	19 (7%)	0.352
pAVD	573	37 (6%)	286	24 (8%)	287	13 (5%)	0.064
Smoking	574		287		287		0.215
Non-smoker		326 (57%)		163 (57%)		163 (57%)	
Ex-smoker		199 (35%)		105 (37%)		94 (33%)	
Smoker		49 (9%)		19 (7%)		30 (10%)	
Anthropometrics							
Weight [kg]	574	78±14	287	78±13.4	287	79±14.6	0.414
Waist [cm]	552	100±13.2	269	100±12.6	283	100±13.8	0.808
Hip [cm]	552	106±10.9	269	106±10.9	283	106±10.9	0.974
BMI [kg/m ²]	574	27.8±4.6	287	28±4.6	287	27.7±4.6	0.430
Transthoracic Echocardiography							
Left ventricular ejection fraction [%]	574	42±13.7	287	43±13.9	287	40±13.3	* 0.010
LVDED [mm]	569	56±10.2	285	55±9.9	284	57±10.5	** 0.009
LVDES [mm]	556	43±11.6	276	41±11.1	280	44±11.8	** 0.001

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IVSED [mm]	569	11.3±2.61	285	11.5±2.79	284	11±2.39	* 0.017
PWED [mm]	568	10.6±2.29	285	10.8±2.42	283	10.3±2.13	* 0.013
E-Wave [cm/s]	535	77±45	272	77±48	263	77±43	0.917
e'-Wave [cm/s]	504	7.8±4	259	7.7±4.2	245	8±3.9	0.357
E/e'	502	11.9±8.7	259	11.9±8.2	243	11.9±9.2	0.977
LAVI [ml/m ²]	528	34±15.4	267	34±15.6	261	33±15.3	0.561
Deceleration Time [ms]	529	220±86	269	219±86	260	221±85	0.830
Fractional shortening [%]	556	25.6±10.6	276	26.7±10.7	280	24.4±10.3	* 0.010
Elektrocardiogram							
Heart Rate [bpm]	574	73±14.6	144	71±14.2	144	74±15	* 0.047
Sinusrhythm	563	194 (34%)	282	106 (38%)	281	88 (31%)	0.333
PQ interval [ms]	449	171±33	233	172±33	216	169±33	0.265
QRS interval [ms]	572	104±26.9	286	104±26.2	286	104±27.7	0.977
QT interval [ms]	570	393±53	285	397±47	285	389±58	+ 0.050
Left Bundle Branch Block	563	74 (13%)	281	37 (13%)	282	37 (13%)	1.000
Laboratory Markers							
Serum sodium [mmol/l]	539	141±3.6	272	141±3.7	267	141±3.6	0.633
Serum potassium [mmol/l]	541	4.4±0.51	274	4.4±0.49	267	4.4±0.53	0.870
Creatinine [mg/dL]	553	101±34	278	99±29.2	275	103±38	0.162
HbA1c [%]	286	6.5±1.15	146	6.5±1.05	140	6.4±1.24	0.517
N-terminal Propeptide BNP [pg/mL]	545	1404±2543	270	1301±2131	275	1506±2892	0.348
hs-CRP [mg/dL]	428	0.52±0.79	207	0.42±0.58	221	0.61±0.93	* 0.017
log β1-ADR-AA [U/mL]	574	0.99±0.283	287	0.77±0.142	287	1.21±0.208	** 0.001
Beta blocker therapy							
Pre-treated with beta blocker [yes]	574	348 (61%)	287	170 (59%)	287	178 (62%)	0.550

* P= 0.05, † P<0.05, ** P<0.01

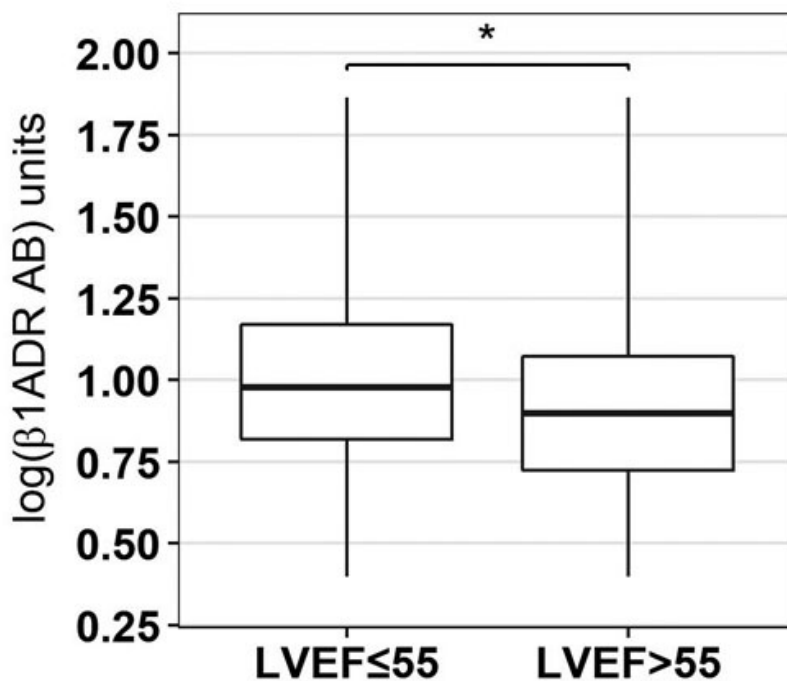


Figure 1. log β1-ADR-AA classified according to LVEF ≤/ > 55% (p=0.0035)

Table 2. Kendall's rank correlation coefficient [τ] and statistical significance [p] between log- β 1-ADR-AK and clinical characteristics.

Variable	Baseline [τ]	Baseline [p]	Change [τ]	Change [p]
Weight [kg]	0.015	0.589	-0.017	0.538
Systolic blood pressure [mmHg]	- 0.090	** 0.003	0.047	0.336
Diastolic blood pressure [mmHg]	- 0.037	0.208	0.019	0.541
6 Minute Walk Test walk distance [m]	- 0.006	0.823	0.044	0.381
Transthoracic Echocardiography				
Left ventricular ejection fraction [%]	- 0.116	** 0.001	0.034	0.526
LVDED [mm]	0.072	* 0.011	-0.086	0.107
LVDES [mm]	0.088	** 0.001	-0.093	0.093
IVSED [mm]	- 0.044	0.135	0.031	0.598
PWED [mm]	- 0.057	0.054	-0.05	0.369
LAES [mm]	- 0.050	0.082	0.112	* 0.036
Tissue Doppler E-Wave	0.047	0.119	-0.123	* 0.022
Tissue Doppler A-Wave	- 0.087	** 0.007	-0.14	* 0.015
Deceleration Time [ms]	0.011	0.695	-0.005	0.921
Electrocardiogram				
ECG Heart Rate [bpm]	0.062	* 0.028	-0.011	0.818
PQ interval [ms]	- 0.100	0.757	-0.013	0.835
QRS interval [ms]	0.011	0.696	-0.119	* 0.024
QT interval [ms]	- 0.049	0.082	-0.05	0.339
Laboratory Markers				
HbA1c [%]	- 0.011	0.788	-0.029	
N-terminal Propeptide BNP [pg/mL]	0.052	0.072	-0.098	0.097
hs-CRP [mg/dL]	0.060	0.065	0.033	0.697

P<0.05, ** P<0.01

to change in HR during the study period ($r=0.0.99$, $p=0.0.45$). Change in β 1-ADR-AA did not correlate with change in HR. Systolic blood pressure tended to be higher in a situation of lower β 1-ADR-AA levels at baseline.

In a clinical models of heart failure with reduced (HF_rEF, LVEF < 50%) and preserved ejection fraction (HF_pEF, LVEF >50% and NTproBNP >125 (pg/mL)), we observed higher β 1-ADR-AA levels in HF_rEF ($1.0.5\pm 0.2.77$ vs. $0.9.5\pm 0.3.3$, $p=0.0.13$).

β 1-ADR-AA levels partially differed between different etiologies of heart failure. Hypertension and coronary artery disease ($0.9.6\pm 0.2.8$ and $1.0.2\pm 0.2.86$, $p=0.0.285$) showed significantly different β 1-ADR-AA levels. Hypertension and cardiomyopathy ($0.9.6\pm 0.2.8$ and $1.0.2\pm 0.2.53$, $p=0.0.979$) or coronary artery disease and cardiomyopathy ($1.0.2\pm 0.2.86$ and $1.0.2\pm 0.2.53$, $p=0.6.789$) did not show statistically significant differences.

We observed a significant increase of β 1-ADR-AA levels under titration with beta-blockers ($p<0.0.1$) between baseline and after 12 weeks of follow-up (Figure 3). Levels of β 1-ADR-AA were independent of pre-use of beta blockers at the time of inclusion into the study. Changes in β 1-ADR-AA between follow-up and baseline were significantly higher in patients who used beta blockers ($p=0.0.16$) before study inclusion. The type of beta-blocker titrated in this study did not affect log β 1-ADR-AA levels at baseline ($p=0.1.32$), follow-up ($p=0.0.58$), nor the change ($p=0.4.26$). Further, in repeated measurement ANOVA, the type of beta-blocker titrated did not affect log β 1-ADR-AA levels ($p=0.0.81$), nor their change ($p=0.4.09$), while sex influenced values ($p=0.0.05$), but not change ($p=0.6.28$). Beta-blocker dosage did not affect β 1-ADR-AA levels.

Graphical display of correlation between log β 1ADR AB and HR, LVEF, LVDED and systolic blood pressure are provided in Figure 4-7.

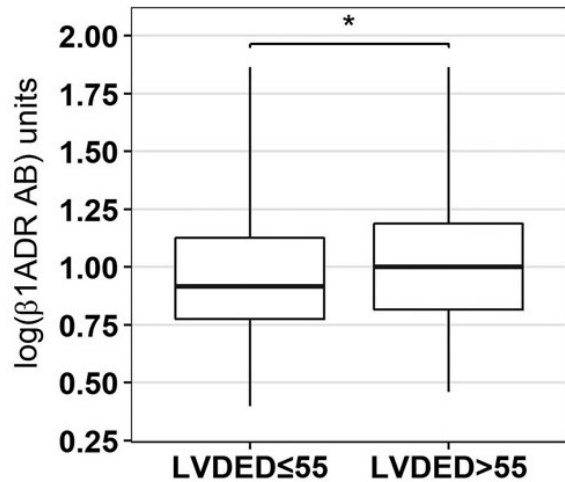


Figure 2. log β1-ADR-AA classified according to LVDED) ≤/ > 55 mm (p=0.0012)

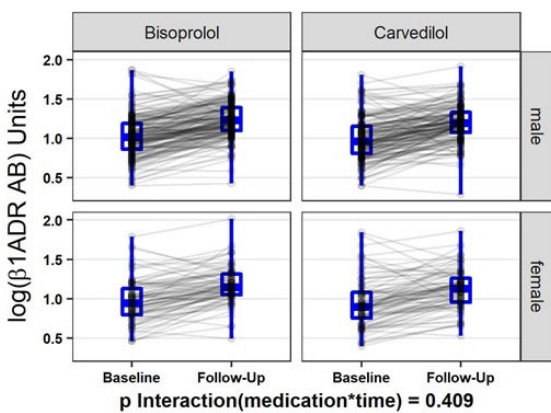


Figure 3. Repeated measurement ANOVA for change in log-β1-ADR-AA by sex and Medication

5. DISCUSSION

In this paper we assess levels of β1-ADR-AAs under titration of a cardio-selective vs. non-selective beta-blocker using a novel commercially available ELISA. To date, this is the largest assessment of β1-ADR-AA in clinical heart failure patients. It should be noted that sensitivity and specificity of the assay employed has not yet been completely established. Nevertheless, this assay exhibits a clear association between β1-ADR-AAs and cardiac dilatation as well as pump failure, which confirms to previous results obtained by others with functional assays currently considered a gold standard (20, 21, 22). We propose therefore that it can be considered as a valid surrogate marker for β1-ADR-AA. β1-ADR-AA were found to be higher in a setting of reduced LVEF, and higher in

the setting of dilated ventricles. β1-ADR-AA correlated positively with heart rate, but were not modified by the usage or dosage of beta-blockers.

5.1. Study population

Patients included in this analysis represent a typical heart failure population suffering from clinical HF irrespective of LVEF and of different aetiologies. In the CIBIS-ELD main trial, overall tolerability of titrating bisoprolol vs. carvedilol to target dose in elderly patients with heart failure was comparable. The pattern of intolerance, however, was different: bradycardia occurred more often in the bisoprolol group, whereas pulmonary adverse events occurred more often in the carvedilol group (23). The currently analysed study population represents a 65% share of the CIBIS-ELD trial patients. Patient distribution in the current sample was comparable to the main trial in regard key parameters, e.g. sex, age, NYHA class, BMI, blood pressure, heart rate, LVEF, and comorbidities (23).

5.2. B1-adrenergic autoantibodies in heart failure

Antibodies specific for the β1-ADR are found in patients with HF of various aetiologies (25). The mechanisms causing the autoimmune response in predisposed individuals are complex and not fully understood (26). Although the role of certain AA remains unclear, these antibodies actually contribute to the pathogenesis of HF (4). The epitopes most frequently targeted by the group of β1-ADR-AA are located in the first and second extracellular loops of the β1-ADR (27), (28). In regard to therapeutic options, immuno-adsorption (14), (15) and cardio-selective beta-blockade (16) have been investigated in this setting for more than a decade. Novel approaches, such as aptameres (5) and cyclic peptides such as the COR-1 peptide (18) and β1EC2-CP (29) are currently being debated.

5.3. Aetiology of heart failure

Interestingly, the previously described association between β1-ADR-AA and cardiac dilatation as well as pump failure was confirmed in the present heart failure population. In addition to previous work (10), (11), where altered levels of β1-ADR-AA were restricted to DCM/ICM, but also found in a broader population of HF with different etiologies. Holthoff *et al* have described β1-ADR-AA in 60% of patients with dilatative cardiomyopathy, 8% of healthy volunteers and 17% of patients with hypertensive heart disease (21). Our findings may indicate a role of auto-immune response that is not restricted to DCM alone, hence in line previous works on Chagas' cardiomyopathy (6) or ischemic heart disease (30), but not with others (11),

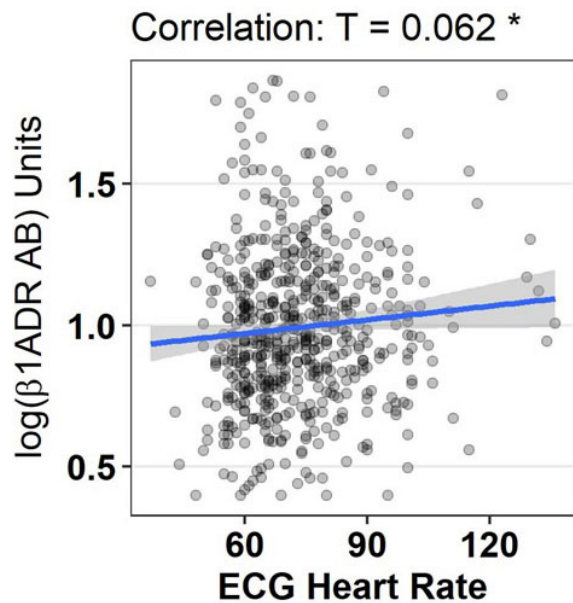


Figure 4. Graphical display of correlation between log β 1ADR AB and heart rate obtained by electrocardiogram

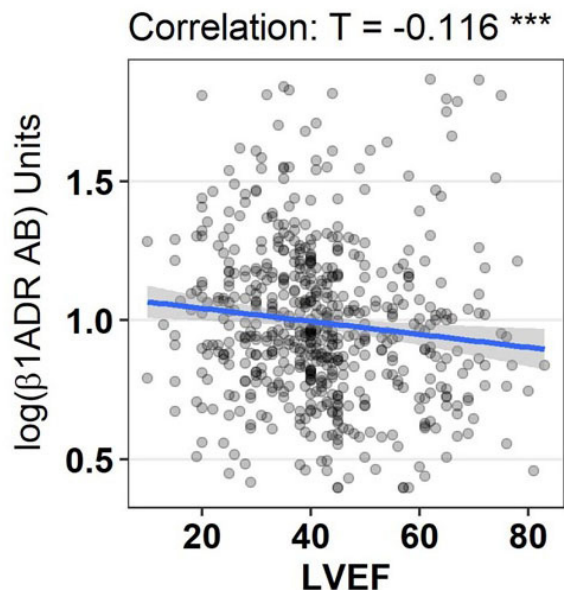


Figure 5. Graphical display of correlation between log β 1ADR AB and left ventricular ejection fraction

5.4. Heart Rate

Resting heart rate has been identified a relevant risk factor and promising treatment target in patients suffering from heart failure (31), (32). Hence, the correlation observed between baseline β 1-ADR-AA and resting heart rate at baseline, at follow up 12 weeks later as well as in regard to change in HR during the study period ($r=0.099$, $p=0.045$) may bear significant clinical consequences. Interestingly,

when looking at the change in HR and a change in β 1-ADR-AA between baseline and follow-up, this correlation was lost. This may partially be explained by the presence of a cocktail of β 1-ADR-AA with different functional (agonist vs. antagonist) entities involved in our observation, of which at least part of the detected AA are agonistic in function. Similar cocktails have recently described by Stavrakis *et al* (JACCS 54, 1309, 2009) using functional assays.

5.5. Beta blocker therapy

While we did observe an increase in β 1-ADR-AA levels under titration over time, we did not observe a relevant effect of the respective study medication titration on β 1-ADR-AA levels. Although treatment in this study differed in regard to a β 1-cardioselective beta-blockade with bisoprolol vs. a non-selective blockade with carvedilol, we assume that both compounds addressed the β 1-ADR similarly. This is in line with the main findings of the CIBIS-ELD trial (23). Interestingly, *receptor-distal* mechanisms have been discussed to enhance myocardial performance despite ongoing β 1-ADR antagonist occupancy (33). Bisoprolol (34) and carvedilol (35) have been used in animals to reduce G protein-coupled receptor kinase-2 expression. Jahns *et al* have used bisoprolol to interfere with the adenylyl cyclase response to β 1-ADR-AA (13). In fact, beta-blocker therapy may not be sufficient to ameliorate the β 1-ADR-AA induced end-organ damage. Novel double-acting therapeutic strategies that scavenges harmful anti- β 1EC2-antibodies and also selectively deplete memory B-cells involved in the production of such antibodies by immuno-modulating cyclo-peptides alone or as an add-on to β 1-blockade may represent a promising new therapeutic option in such immune-mediated heart failure (29).

5.6. ELISA

The presently used and commercially available ELISA used to detect AA targeted against the cardiac β 1-ADR allowed for routine quantification of β 1-ADR-AA levels. Nikolaev *et al* (20) have previously developed a rapid method for the detection of activating autoantibodies directed against the β 1-ADR in HF. Their approach by also measuring β 1-ADR-mediated increase in intracellular cAMP by fluorescence resonance energy transfer (FRET) using a highly sensitive cAMP sensor prove to be fast and highly sensitive (20). In the presently used ELISA, it remains unclear if a conformational change in the g-protein-coupled receptor also results in an increased production of cAMP. Hence, some claim studies on g-protein-coupled receptor AA in cardiovascular disease inconclusive and misleading unless corroborated by measurements of IgG interactions with the respective native membrane-bound receptor(s) and/or by an assessment of their impact on receptor

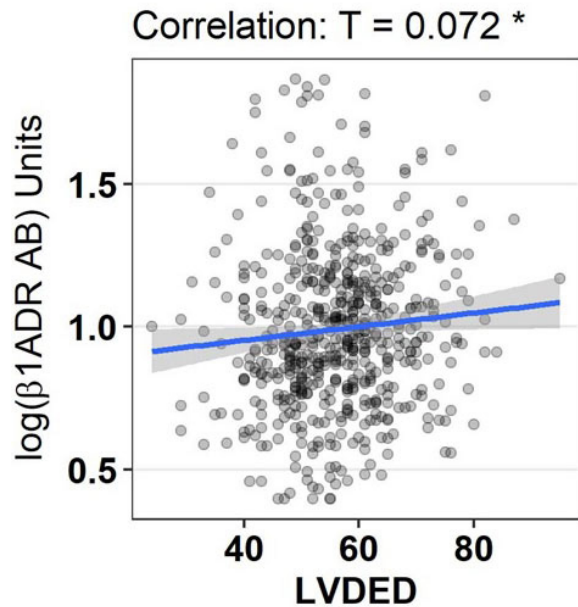


Figure 6. Graphical display of correlation between log β 1ADR AB left ventricular end diastolic diameter

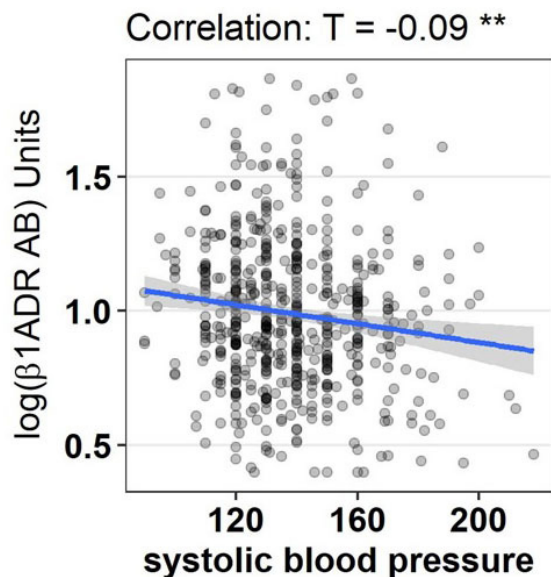


Figure 7. Graphical display of correlation between log β 1ADR AB and systolic blood pressure

function, which includes their effects on receptor-mediated signalling (36, 37). Refining this approach, data from native cell-based competition ELISA (21) is suggestive of an AA agonist-like modulation of the β 1-ADR conformation (38). Aptamers (5) and cyclic peptides such as the COR-1 peptide (18) have been discussed (4) to optimize the solid phase of ELISA such as the ELISA routinely used. Interestingly, Holthoff's novel whole-cell-based competition ELISA yielded a markedly lower rate of false-positives compared with peptide-based assays (21).

The currently used ELISA does not address whether the detected AA, act in agonist-like, neutral or antagonist-like functions. Possibly, the presently used ELISA quantifies a *cocktail* of β 1-ADR-AA with different functional (agonist vs. antagonist) or non-functional entities. It should be noted that sensitivity and specificity of the assay employed has not yet been completely established. Nevertheless, the values for β 1-ADR-AAs measured with this assay exhibit a clear-cut association with cardiac dilatation as well as pump failure, which conforms to previous results obtained by others with functional assays currently considered a gold standard. We propose therefore that these results can be considered as a clinically valid surrogate marker for β 1-ADR-AA. Our data show clinical effects which may partially be explained by β 1-ADR-AA of which at least part of are agonistic in function. Future studies will have to clarify this important question. To further prospectively address cardiac autoimmunity and the specific cardio-noxious role β 1-ADR-AA bear as disease-modifier or disease-triggers, Jahns *et al* currently conduct a trial on aetiology, titre-course, and survival (ETICS) (22) in HF patients.

6. LIMITATIONS

Although data and blood samples of the CIBIS-ELD trial were prospectively collected, the character of the present secondary post-hoc analysis is a hypothesis generating description and warrants further clarification. While the used ELISA can quantify the β 1-ADR-AA serum levels, the quality of the AA; i.e. agonist-like or antagonist-like functions, remains unclear.

7. CONCLUSION

This novel ELISA, utilizing β 1-ADR in its physiological condition, offers the possibility to measure β 1-ADR-AA in clinical routine. The ELISA allowed for pathophysiological insights: β 1-ADR-AA levels significantly increased under titration with beta-blockers ($p < 0.0.1$), irrespective of type of BB. Higher levels of β 1-ADR-AA at baseline are associated with higher heart rates, lower ejection fraction and enlarged left ventricles, indicating a role of these antibodies in the heart failure.

The specificity of the new ELISA has to be further investigated to elucidate the quality of detected antibodies in respect to their agonist vs. antagonist like functions. Studies assessing Etiology, Titre-Course, and effect on Survival (ETICS) of beta1-adrenoceptor antibodies are needed to further elucidate the described phenomenon.

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