

## Effects of ivabradine therapy on heart failure biomarkers

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### Abstract

**Background:** Heart rate (HR) reduction is associated with improved outcomes in patients with heart failure (HF) and biomarkers can be a valuable diagnostic tool in HF management. The primary aim of our study was to evaluate the short-term (6 months) effect of ivabradine on N-terminal pro B-type natriuretic peptide (NT-proBNP), CA-125, and cystatin-C values in systolic HF outpatients, and secondary aim was to determine the relationship between baseline HR and the NT-proBNP, CA-125, cystatin-C, and clinical status variation with ivabradine therapy.

**Methods:** Ninety-eight patients (mean age:  $65.81 \pm 10.20$  years; 33 men), left ventricular ejection fraction  $< 35\%$  with Simpson method, New York Heart Association (NYHA) class II–III, sinus rhythm and resting HR  $> 70$ /min, optimally treated before the study were included. Among them, two matched groups were formed: the ivabradine group and the control group. Patients received ivabradine with an average (range of 10–15) mg/day during 6 months of follow-up. Blood samples for NT-proBNP, CA-125, and cystatin-C were taken at baseline and at the end of a 6-month follow-up in both groups.

**Results:** There was a significant decrease in NYHA class in the ivabradine group ( $2.67 \pm 0.47$  vs.  $1.85 \pm 0.61$ ,  $p < 0.001$ ). When ivabradine and control groups were compared, a significant difference was also found in NYHA class 6 months later ( $p = 0.013$ ). A significant decrease was found in HR in the ivabradine and control groups ( $84.10 \pm 8.76$  vs.  $68.36 \pm 8.32$  bpm,  $p = 0.001$ ;  $84.51 \pm 10$  vs.  $80.40 \pm 8.3$  bpm,  $p = 0.001$ ). When both groups were compared, a significant difference was also found in HR after 6 months ( $p = 0.001$ ). A significant decrease was found in cystatin-C ( $2.10 \pm 0.73$  vs.  $1.50 \pm 0.44$  mg/L,  $p < 0.001$ ), CA-125 ( $30.09 \pm 21.08$  vs.  $13.22 \pm 8.51$  U/mL,  $p < 0.001$ ), and NT-proBNP ( $1,353.02 \pm 1,453.77$  vs.  $717.81 \pm 834.76$  pg/mL,  $p < 0.001$ ) in the ivabradine group. When ivabradine and control groups were compared after 6 months, a significant decrease was found in all HF parameters (respectively; cystatin-C:  $p = 0.001$ , CA-125:  $p = 0.001$ , NT-proBNP:  $p = 0.001$ ). Creatinine level was significantly decreased and glomerular filtration rate (GFR) was significantly increased in the ivabradine group ( $1.02 \pm 0.26$  vs.  $0.86 \pm 0.17$ , creatinine:  $p = 0.001$ ;  $79.26 \pm$

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$\pm 18.58$  vs.  $92.48 \pm 19.88$ ,  $GFR: p = 0.001$ ). There was no significant correlation between NYHA classes (before and after ivabradine therapy) and biochemical markers, or HR.

**Conclusions:** In the outpatients with systolic HF, persistent resting HF  $> 70/\text{min}$  with optimal medical therapy, the NT-proBNP, CA-125, and cystatin-C reductions were obtained with ivabradine treatment. Measurement of NT-proBNP, CA-125, and cystatin-C may prove to be useful in biomarker panels evaluating ivabradine therapy response in HF patients. (Cardiol J 2015; 22, 5: 501–509)

**Key words:** systolic heart failure, ivabradine, NT-proBNP, CA-125, cystatin-C

## Introduction

Heart failure (HF) is a clinical syndrome characterized by the inability of the heart to pump enough blood to supply the body's demands [1]. Enhanced heart rate (HR), which is generally observed in patients with congestive HF, attenuates the decrease in cardiac output or preserves cardiac output at the cost of impaired left ventricular (LV) filling, increased myocardial  $O_2$  consumption, and reduced coronary perfusion time. The developments of LV dysfunction and/or congestive HF were observed after persistent tachycardia [2–5].

Epidemiological and clinical studies indicate that a higher resting HR in sinus rhythm is associated with increased morbidity and mortality in the general population and in patients with cardiovascular disease. HR reduction is associated with improved outcomes in patients with HF, because some of the beneficial effects of  $\beta$ -blockade may be attributed to HR reduction and the effects of  $\beta$ -blockers on cardiac function and survival are correlated with the magnitude of the HR reduction [6–8]. However, some patients cannot tolerate target dosages of  $\beta$ -blockers and, when resting HRs remain elevated despite efforts to optimize  $\beta$ -blocker dose, there is potential benefit from further HR reduction. Ivabradine is a selective inhibitor of the cardiac pacemaker *If* current, which modulates pacemaker activity in the sino-atrial node, providing pure HR reduction without modifying atrioventricular or intraventricular conduction or contractility and has no effect on blood pressure. HR reduction with ivabradine improves LV filling by prolonging diastolic time and increases stroke volume [9–11]. It improves event-free survival, exercise capacity, and quality of life in patients with systolic HF and resting HR  $> 70/\text{min}$  [12, 13]. However, it remains unclear what the effect of ivabradine is on HF markers. Recently, new biomarkers might have an additional

contribution to improving the prognostic assessment in patients with HF. The objective of our study was, accordingly, to evaluate in stable outpatients with systolic HF the short-term (6-month) effect of ivabradine on HF markers.

## Methods

### Patients

Ninety-eight ambulatory, clinically stable symptomatic outpatients with systolic chronic HF ( $\geq 4$  weeks), who had been hospitalized for worsening HF in the 12 months before inclusion, on optimized standard medical therapy were consecutively included and randomly allocated to the ivabradine group and non-ivabradine group between October 2013 and August 2014.

### Inclusion criteria

Patients with chronic HF, on optimized medical therapy according to European Society of Cardiology guidelines, with LV ejection fraction (LVEF)  $< 35\%$  according to the Simpson method, New York Heart Association (NYHA) class II–III, and sinus rhythm and resting HR  $> 70/\text{min}$  were eligible for inclusion in the study.

### Exclusion criteria

Patients who presented with the following, were excluded from the study: acute decompensation (acute coronary syndromes and acute HF); hemodynamically significant valve disease; cerebrovascular events during the previous 6 months; dysfunctional prosthetic heart valve; obstructive or non-obstructive cardiomyopathy; uncorrected congenital heart disease; active myocarditis; a history of resuscitation from sudden death; an absence of stable sinus rhythm, severe arrhythmias; HR  $< 60$  bpm; sick sinus syndrome; second-degree and third-degree atrioventricular block; severe obesity (body mass index  $> 36$  kg/m<sup>2</sup>); established or suspected pulmonary diseases (vital capacity  $< 80\%$  or

forced expiratory volume in 1 s < 80% of age specific and sex-specific reference values); hemoglobin  $\leq$  11 g/dL; treatment with non-dihydropyridine calcium-channel blockers, class I antiarrhythmic agents, strong inhibitors of cytochrome P450-3A4, or QT interval-prolonging medications; acute and chronic kidney failure; pregnancy; hypo- and hyperthyroidism or acute infections.

### Study protocol

This was a 6-month, open-label, blinded, parallel-group, interventional, prospective-cohort study. The baseline evaluation comprised physical examination; NYHA class; 12-lead electrocardiography (ECG); blood sampling for laboratory measurements, including cystatin-C, CA-125, and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Echocardiographic imaging was performed using Vivid 7 equipment (GE Vingmed Ultrasound AS, Horten, Norway) with phased-array 2.5-MHz multi-frequency transducers. Accordingly, all patients underwent screening echocardiography to determine their suitability for the trial by the evaluation of strict criteria for EF. Images were saved in digital format and stored on a secure server for offline analysis. LVEF was determined using a modified Simpson biplane method. All patients were receiving standard HF therapy for their comorbidities (diuretics, angiotensin-converting enzyme inhibitors (ACEI), angiotensin II antagonists, aldosterone receptor blockers, and  $\beta$ -adrenoreceptor blockers), at their maximum doses tolerated in both groups. Investigators maintained participants as closely as possible to target dosages, notably with  $\beta$ -blockade. Patients in the ivabradine group were allocated to ivabradine at a starting dose of 5 mg twice daily; doses were adjusted upward or downward (2.5, 5, or 7.5 mg twice daily) at every visit according to HR at rest and tolerability. To check for the presence of bradycardia, patients were seen for the first time on the 7th day after the initiation of treatment. In case of a resting HR < 50 bpm or the occurrence of signs or symptoms related to bradycardia, the dose of ivabradine was to be reduced to 2.5 mg twice daily, or if these persisted after dose reduction, the study medication was to be withdrawn. At least monthly visits to the clinic were scheduled after the first visit on the 7th day after the initiation of treatment. At 6 months, a full clinical evaluation, including NYHA class assessment and laboratory workup including NT-proBNP, cystatin-C and CA-125 determination, was performed in both groups. The variation in relation to the baseline value (for

the HR) was determined as absolute (initial final) and relative (percent) variation. The study adhered to the Declaration of Helsinki and was approved by the institutional Ethics Committee. Informed consent was obtained from all subjects before involvement in the study.

### NT-proBNP measurement

Peripheral venous blood samples were drawn between 8 and 9 am after a 30-min rest in the supine position. Plasma samples were frozen at  $-70^{\circ}\text{C}$  until assay. NT-proBNP was determined with an Elecsys 20.10 bench-top analyzer (Roche Diagnostics, Meylan, France) with proBNP reagent pack (Roche Diagnostics). BNP was measured with BNP Triage reagent pack (Biosite Inc. San Diego, CA, USA).

### Cystatin-C measurement

Cystatin-C serum levels were measured using a sandwich enzyme-linked immunoassay kit (Biovendor Research and Diagnostic Products) from peripheral venous blood samples.

### CA-125 measurement

Serum levels of CA-125 were determined with use of the commercially available Tumor Markers CA 125 AxSYM<sup>®</sup> System (Abbott Laboratories; Abbott Park i2000, Ill) from peripheral venous blood samples like BNP and cystatin-C.

### Statistical analysis

Continuous variables were expressed as mean  $\pm$  standard deviation (SD) and categorical variables as percentages. To compare the changes from baseline to 6 months, a paired Student's *t*-test was used, and the  $\chi^2$  test was used in the comparison of percentage data. Independent samples T-test was used to compare data between two different groups. The relationship between parameters was determined with the Pearson correlation coefficient. A *p* value of < 0.05 was considered statistically significant. All tests were 2-tailed and performed using a commercially available package (SPSS for Windows, version 17.0, SPSS Inc).

## Results

### Clinical characteristics

Ninety-eight patients (65 female, 33 male) were studied. The patients in ivabradine group had a mean age of  $65.24 \pm 8.70$  years, and were overweight. At baseline, there were no differences in LVEF, NHYA class, hypertension, diabetes mel-

**Table 1.** Clinical and demographic characteristics of the study population.

	Ivabradine group	Non-ivabradine group	P
<b>Demographics</b>			
Number of patients	49	49	> 0.05
Age [years]	65.24 ± 8.70	66.38 ± 11.57	0.58
Male/female	16/33 (33/67%)	17/32 (35/65%)	0.83
Weight [kg]	78.59 ± 8.67	75.34 ± 10.16	0.09
NYHA class II/III	16/33 (33/67%)	21/28 (43/57%)	0.29
LVEF [%]	26.42 ± 5.28	27.75 ± 5.24	0.21
Heart rate	84.10 ± 8.76	84.51 ± 10	0.79
<b>Concomitant diseases</b>			
Hypertension	30 (61%)	33 (67%)	0.52
Diabetes mellitus	17 (34%)	11 (22%)	0.18
Dyslipidemia	15 (31%)	13 (27%)	0.65
Ischemic heart disease	47 (96%)	43 (88%)	0.14
<b>Treatment at inclusion</b>			
Furosemide	47 (96%)	46 (94%)	0.64
Spironolactone	35(71%)	32 (65%)	0.51
Beta-blocker	44 (90%)	46 (94%)	0.46
ACEI/ARB	45 (92%)	42 (86%)	0.33
Statin	44 (90%)	40 (82%)	0.24
CRT-D	7 (14%)	9 (18%)	0.58

ACEI — angiotensin-converting enzyme inhibitor; ARB — angiotensin receptor blocker; CRT-D — cardiac resynchronization therapy-ICD (implantable cardioverter defibrillator); LVEF — left ventricular ejection fraction; NYHA — New York Heart Association

litus, ischemic heart disease history, between the ivabradine and non-ivabradine groups. Concomitant medication was as follows: diuretic (96–94%) and adrenergic  $\beta$ -blocker (90–94%) agents were the most commonly employed in both groups; 68% also received mineralocorticoid receptor antagonists (MRA medications) and 88% ACEIs/angiotensin receptors blockers in the ivabradine group. In total, 16 patients had cardiac pacemakers (CRT-D) (Table 1).

It is important to note that 14 patients developed congestive HF and 3 patients developed shortly paroxysmal atrial fibrillation once under optimal medical treatment in the non-ivabradine group during 6 months of follow-up. Sinus rhythm was achieved by intravenous metoprolol treatment. Nine patients had remaining bundle branch block on ECG in the non-ivabradine group. In the ivabradine group, 9 patients developed congestive HF and 7 of 9 had cardiac pacemaker (CRT-D). During the 6-month follow-up there were no deaths. No patients were dropped out of the study in both groups. Eight patients had the furosemide dosage increased and 3 patients had furosemide dosage decreased in the non-ivabradine group. Also,

5 patients stopped spironolactone due to potassium increase. In the ivabradine group, 4 patients had the furosemide dosage increased and 2 patients had furosemide dosage decreased. Of the patients on  $\beta$ -blockers (87%), 26 achieved the target dose in the ivabradine group and 29 achieved it in the non-ivabradine group.

Patients received ivabradine with an average  $10.30 \pm 3.29$  mg/day during the 6 months of follow-up. There was a significant decrease in NYHA class between the groups at baseline (before ivabradine) and at 6 months later (after ivabradine) ( $2.67 \pm 0.47$  vs.  $1.85 \pm 0.61$ ,  $p < 0.001$ ). When ivabradine and non-ivabradine groups were compared, a significant difference was also found in NYHA class after 6 months ( $p = 0.013$ ). A significant decrease was found in HR in the ivabradine and non-ivabradine groups ( $84.10 \pm 8.76$  vs.  $68.36 \pm 8.32$  bpm,  $p = 0.001$ ;  $84.51 \pm 10$  vs.  $80.40 \pm 8.3$  bpm,  $p = 0.001$ ). When both groups were compared, a significant difference was also found in HR after 6 months ( $p = 0.001$ ) (Table 2). A significant decrease was found in cystatin-C ( $2.10 \pm 0.73$  vs.  $1.50 \pm 0.44$ ,  $p < 0.001$ ), CA-125 ( $30.09 \pm 21.08$  U/mL vs.  $13.22 \pm 8.51$  U/mL,

**Table 2.** New York Heart Association (NYHA) class, cardiac and biochemical parameters of the study population.

	Ivabradine treatment			Non-ivabradine group			P — between groups	
	Baseline (before ivabradine)	6 months (after ivabradine)	P	Baseline	6 months	P	Baseline	6 months
NYHA class	2.67 ± 0.47	1.85 ± 0.61	<b>0.001*</b>	2.57 ± 0.50	2.26 ± 0.70	0.060	0.08	<b>0.013*</b>
Heart rate [bpm]	84.10 ± 8.76	68.36 ± 8.32	<b>0.001*</b>	84.51 ± 10	80.40 ± 8.3	<b>0.001*</b>	0.83	<b>0.001*</b>
Cystatin-C [mg/L]	2.10 ± 0.73	1.50 ± 0.44	<b>0.001*</b>	2.28 ± 0.69	2.25 ± 0.49	0.62	0.21	<b>0.001*</b>
CA-125 [U/mL]	30.09 ± 21.08	13.22 ± 8.51	<b>0.001*</b>	36.69 ± 23.89	33.98 ± 19.94	0.058	0.15	<b>0.001*</b>
NT-proBNP [pg/mL]	1,353.02 ± 1,453.77	717.81 ± 834.76	<b>0.001*</b>	1,383.42 ± 1,064.62	1,323.26 ± 979.26	0.12	0.90	<b>0.001*</b>
WBC [k/uL]	9.09 ± 2.07	8.96 ± 2.30	0.627	7.87 ± 3.11	7.29 ± 1.95	0.19	<b>0.025*</b>	<b>0.001*</b>
Platelet [k/uL]	266.02 ± 67.81	248.00 ± 69.01	0.004	223.61 ± 78.72	215.36 ± 66.47	<b>0.007*</b>	<b>0.005*</b>	<b>0.019*</b>
Hg [g/dL]	13.31 ± 1.48	13.34 ± 1.42	0.837	12.99 ± 1.68	13.43 ± 1.11	<b>0.018*</b>	0.32	0.71
Htc [%]	39.90 ± 4.08	40.69 ± 3.62	<b>0.017*</b>	40.74 ± 4.47	40.01 ± 3.28	0.1	0.33	0.33
MPV [fL]	8.07 ± 1.13	7.97 ± 1.34	0.427	10.73 ± 1.12	9.81 ± 1.62	<b>0.001*</b>	<b>0.001*</b>	<b>0.001*</b>
PDW [%]	17.74 ± 1.00	17.79 ± 1.35	0.825	17.53 ± 1.33	17.22 ± 1.45	0.056	0.38	0.047
RDW [%]	15.96 ± 1.50	16.05 ± 2.37	0.729	15.86 ± 1.31	15.70 ± 1.29	0.36	0.73	0.36
Glucose [mg/dL]	150.77 ± 61.37	141.79 ± 58.51	0.205	145.46 ± 86.62	136.51 ± 52.42	0.13	0.72	0.33
Creatinine [mg/dL]	1.02 ± 0.26	0.86 ± 0.17	<b>0.001*</b>	0.94 ± 0.25	1.00 ± 0.20	0.069	0.18	<b>0.001*</b>
GFR	79.26 ± 18.58	92.48 ± 19.88	<b>0.001*</b>	78.65 ± 20.45	71.12 ± 16.54	<b>0.001*</b>	0.87	<b>0.001*</b>
LDL-C [mg/dL]	107.10 ± 30.85	109.59 ± 31.38	0.614	79.91 ± 25.77	96.12 ± 25.76	0.001	<b>0.001*</b>	0.022
TG [mg/dL]	161.18 ± 74.44	158.51 ± 61.26	0.721	150.75 ± 38.08	164.14 ± 43.43	<b>0.015*</b>	0.40	0.60

\*Statistically significant; NT-proBNP — N-terminal pro B-type natriuretic peptide; Hg — hemoglobin; Htc — hematocrit; MPV — mean platelet volume; PDW — platelet distribution width; RDW — red cell distribution width; GFR — glomerular filtration rate; LDL-C — low density lipoprotein cholesterol; TG — triglyceride

$p < 0.001$ ), and NT-proBNP ( $1,353.02 \pm 1,453.77$  vs.  $717.81 \pm 834.76$  pg/mL,  $p < 0.001$ ) in the ivabradine group. But there was no significant decrease in the non-ivabradine group in terms of cystatin-C ( $2.28 \pm 0.69$  vs.  $2.25 \pm 0.49$  mg/L,  $p = 0.62$ ), CA-125 ( $36.69 \pm 23.89$  vs.  $33.98 \pm 19.94$  U/mL,  $p = 0.058$ ) and NT-proBNP ( $1,383.42 \pm 1,064.62$  vs.  $1,323.26 \pm 979.26$  pg/mL,  $p = 0.12$ ). When ivabradine and non-ivabradine group were compared after 6 months, a significant decrease was found in all HF parameters (respectively; cystatin-C:  $p = 0.001$ , CA-125:  $p = 0.001$ , NT-proBNP:  $p = 0.001$ ) (Table 2).

White blood cell count was significantly lower in the control group at baseline and after 6 months compared to the ivabradine group (baseline:  $p = 0.025$ , 6 months:  $p = 0.001$ ). Platelet count was significantly lower in the ivabradine than in the non-ivabradine group (ivabradine:  $p = 0.004$ , control:  $p = 0.007$ ). Also, platelet count was significantly lower at baseline and at 6 months when ivabradine and non-ivabradine groups were compared (baseline:  $p = 0.005$ , 6 months:  $p = 0.019$ ). There was no statistical difference in hemoglobin level, glucose level, platelet distribution width and red cell distribution width value between the groups at 6-month follow-up. But a significant increase was found in hematocrit level after the ivabradine therapy ( $p = 0.017$ ). Mean platelet volume value was significantly lower in the non-ivabradine group ( $p = 0.001$ ), but mean platelet volume value was significantly higher at baseline ( $p = 0.001$ ) and at 6 months ( $p = 0.001$ ) in the non-ivabradine group compared to the ivabradine group. In addition, there was no significant change in fasting glucose level. Creatinine level was significantly decreased and glomerular filtration rate (GFR) was significantly increased in the ivabradine group ( $1.02 \pm 0.26$  vs.  $0.86 \pm 0.17$ , creatinine:  $p = 0.001$ ;  $79.26 \pm 18.58$  vs.  $92.48 \pm 19.88$ , GFR:  $p = 0.001$ ). However, GFR was significantly decreased and no significant decrease was found in terms of creatinine in the non-ivabradine group ( $78.65 \pm 20.45$  vs.  $71.12 \pm 16.54$ , GFR:  $p = 0.001$ ;  $0.94 \pm 0.25$  vs.  $1.00 \pm 0.20$ , creatinine:  $p = 0.069$ ) (Table 2).

There was no significant correlation between NYHA classes (before and after ivabradine therapy) or between biochemical markers, or HR (NYHA class before and after, cystatin-C before and after; before:  $p = 0.73$ , after:  $p = 0.98$ ; NYHA class before and after, CA-125 before and after; before:  $p = 0.06$ , after:  $p = 0.76$ ; NYHA class before and after, NT-proBNP before and after; before:  $p = 0.16$ , after:  $p = 0.73$ ; NYHA class before and after, HR before and after; before:  $p = 0.35$ , after:  $p = 0.07$ ).

HR variation was 3.00 vs. 35.00 bpm. There was no significant correlation between HR variation and NT-proBNP, cystatin-C in the ivabradine group ( $p = 0.92$ ,  $p = 0.56$ , respectively), yet a significant correlation between HR variation and CA-125 was found in the ivabradine group ( $p = 0.02$ ). Also, a significant correlation was found between HR variation and NT-proBNP in the non-ivabradine group ( $p = 0.003$ ) (Table 3).

## Discussion

This study demonstrates that in outpatients with systolic HF on optimized medical therapy and resting HR  $> 70$ /min, the expected HR reduction with ivabradine addition significantly decreases NT-proBNP, cystatin-C and CA-125 after 6 months.

HR reduction with  $\beta$ -blockers or with ivabradine improves LV performance and has a positive effect on LV remodeling, reducing the risk of hospitalization and improving survival [10, 14, 15]. Busseuil et al. [16] demonstrated that selective HR reduction with ivabradine prevented the detrimental effects of hypercholesterolemia on LV myocardial performance. Ivabradine attenuated LV diastolic dysfunction, reduced left atrial and LV structural remodeling (interstitial fibrosis), and reduced LV collagen type I in hypercholesterolemic rabbits. Ivabradine also improved left atrium fractional shortening. Circulating angiotensin II levels were significantly lowered by ivabradine and correlated with HR. Aldosterone levels also correlated with HR during treatment and were lower with ivabradine [16]. Results in experimental studies are consistent with the effects of ivabradine on cardiac remodeling [11]. In a rat model of chronic mild HF, ivabradine preserved cardiac output and improved LV function and geometry [17]. These changes were linked to modifications in the extracellular matrix and in cardiac myocyte function. Ivabradine also had beneficial effects on the global cardiac remodeling process involved in HF, including optimization of energy consumption, reverse electrophysiological and structural cardiac remodeling [18]. Similar effects with ivabradine have been found by other workers in a rat model of chronic severe HF, including reductions in fibrosis, local renin-angiotensin-aldosterone system stimulation, and sympathetic drive, as well as improvement in endothelial function [19, 20].

The results of the SHIFT trial showed that treatment with ivabradine added to conventional therapy for HF was associated with an 18% reduction in the relative risk for the primary composite

**Table 3.** Relationship between heart rate and biochemical markers.

	Ivabradine treatment						Non-ivabradine group					
	Heart rate (before ivabradine)		Heart rate (after ivabradine)		Heart rate variation		Heart rate — baseline		Heart rate — 6 months later		Heart rate variation	
	r	p	r	p	r	p	r	p	r	p	r	p
NT-proBNP*	0.02	0.89	0.06	0.66	0.01	0.92	-0.22	0.11	0.08	0.58	<b>0.42</b>	<b>0.003<sup>#</sup></b>
Cystatin-C**	0.10	0.49	<b>0.36</b>	<b>0.01<sup>#</sup></b>	0.08	0.56	-0.08	0.56	0.04	0.76	-0.6	0.68
CA-125***	0.20	0.16	0.04	0.75	<b>0.32</b>	<b>0.02<sup>#</sup></b>	-0.08	0.54	0.07	0.60	0.13	0.35

\*Statistically significant; \*Comparison of heart rate (before and after ivabradine) and NT-proBNP (before and after ivabradine); \*\*Comparison of heart rate (before and after ivabradine) and cystatin-C (before and after ivabradine); \*\*\*Comparison of heart rate (before and after ivabradine) and CA-125 (before and after ivabradine); NT-proBNP — N-terminal pro B-type natriuretic peptide

endpoint of cardiovascular death or hospitalization for worsening HF [10]. It also had a positive effect on LV remodeling in the echocardiographic sub-study of the BEAUTIFUL (morBidity-mortality EvAIUa-Tion of the *If* inhibitor ivabradine in patients with coronary disease and left ventricular dysfunction) study [21].

NT-proBNP is a marker for the presence of LV systolic dysfunction and a prognostic marker for morbidity and mortality in HF. It is the inactive split product of the BNP [22]. NT-proBNP levels often decline after initiation and up-titration of HF therapy such as vasodilators and aldosterone blockers [23–25]. Persistently elevated (or rising) levels of NT-proBNP are predictive of poor outcome [26]. Several factors may influence the effect of drugs on the NT-proBNP values: the baseline value, severity of HF, and age [23]. Sargento et al. [27] found that the NT-proBNP reduction obtained by short-term ivabradine treatment correlates closely with the degree of HR reduction in systolic HF patients.

Cystatin-C, a cysteine protease inhibitor produced at a constant rate by nearly all human cells, is freely filtered by the renal glomerulus and excreted into the bloodstream. This protein has a low molecular weight and does not form a complex with other secreted proteins in the blood [28]. Cystatin-C independently predicts adverse events in chronic HF and is also an independent risk factor in the prognosis of patients with HF [29]. Shlipak et al. [30] showed the prognostic value of cystatin-C in stable HF patients who had lower EF < 35%. Dupont et al. [31] found that cystatin-C was a strong predictor of adverse events in stable chronic HF, independent of traditional risk factors and BNP. Beside this, we found a significant increase in GFR and a significant decrease in creatinine level in the ivabradine group. This finding indicates that

ivabradine treatment may improve renal perfusion in systolic HF in addition to cystatin-C reduction.

The CA-125 antigen as an ovarian cancer marker exhibits low specificity. Its elevated concentrations occur in different types of cancer and in non-gynecological diseases [32]. Elevated concentrations of this antigen also occur in HF and are connected with the patients' poor prognosis [33]. Fluid overload and the inflammatory process are interactional and they mutually magnify their activities and secretion during the exacerbation of chronic HF. It has become increasingly clear that mechanical stress and inflammatory stimuli together initiate CA-125 synthesis [34]. In the course of HF, CA-125 is positively correlated with the NYHA functional class, the presence of pleural effusion, and pulmonary capillary wedge pressure [35, 36]. Nunez et al. [37] found significant differences in CA-125 levels in patients with and without HF; patients with acute HF exhibited a 7-fold increase in mean CA-125 serum levels in comparison with control subjects. Also, Folga et al. [38] found that elevated values of NT-proBNP and CA-125 were independent death risk factors in advanced HF patients.

NT-proBNP, CA-125 and cystatin-C values were found to be higher in HF patients in different studies [27, 31, 35]. In this study, we have tried to assess the utility of old and new HF biomarkers with ivabradine therapy in HF patients. HF biomarkers were decreased after ivabradine therapy in 6-month follow-ups. In addition, NYHA class and HR were decreased after ivabradine therapy in Sargento et al. [27] study at 3-month follow-up. However, we did not find a significant relationship between NYHA class decrease and biomarker (NT-proBNP, CA-125 and cystatin-C) decreases after ivabradine therapy. Also, we found no correlation

between HR variation and NT-proBNP, cystatin-C. Conversely, a significant correlation between HR variation and CA-125 was found.

To the best of our knowledge, the present study is the first to disclose ivabradine effect on HF markers including NT-proBNP, CA-125, and cystatin-C. We found that the levels of all of these HF markers were decreased after ivabradine therapy at 6-month follow-up. This indicates that we may evaluate ivabradine therapy response in HF patients by following changes in blood levels of HF markers.

### Limitations of the study

This was a single-center trial, which limits generalization but allows stronger trial control and adherence to study protocol. The intervention period was short. It is probable that a longer follow-up would show more changes in variables such as NT-proBNP, CA-125, and cystatin-C and that a correlation would be found between HR variation and biomarkers. Therefore, more studies are required to evaluate the effects observed in a larger number of patients for a longer period.

### Conclusions

According to our study, in outpatients with systolic HF, persistent resting HF > 70/min with optimal medical therapy, NT-proBNP, CA-125, and cystatin-C reduction was obtained with ivabradine treatment. Only CA-125 correlated with HR variation. Measurement of NT-proBNP, CA-125, and cystatin-C may prove to be useful in biomarker panels evaluating ivabradine therapy response in HF patients.

**Conflict of interest:** None declared

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