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Clinical value of detecting autoantibodies against β_1-, β_2-, and α_1-adrenergic receptors in carvedilol treatment of patients with heart failure

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Abstract

Objective To determine the possible association of anti-β_1-adrenergic receptors (anti-β_1-AR), anti-β_2-AR and anti-α_1-AR with carvedilol treatment in patients with heart failure (HF). **Methods** A total of 267 HF patients were prospectively enrolled. Blood samples were measured by an enzyme-linked immunosorbent assay. All of the patients received carvedilol for their HF. Each patient was followed up for six months and their cardiac function was measured. **Results** The final analysis encompassed 137 patients comprising 65 patients with three autoantibodies (positive group) and 72 patients without all three autoantibodies but with one or two autoantibodies (negative group). The frequency and geometric mean titer of anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR were significantly lower in the group without all three autoantibodies after six months of carvedilol treatment (all $P < 0.01$; from 100% to 57%, 50%, and 49%, respectively; and from 1: 118, 1: 138, and 1: 130 to 1: 72, 1: 61, and 1: 67, respectively). Furthermore, 28 patients in the positive group demonstrated complete ablation of autoantibodies. In addition, left ventricular remodelling and function was significantly improved by the use of carvedilol combined with the standard treatment regime for six months in the positive group ($P < 0.01$) when compared to the negative group ($P < 0.05$). **Conclusions** Carvedilol treatment significantly decreases frequency and geometric mean titer in patients with all three autoantibodies, even up to complete ablation, and significantly improved cardiac function and remodelling. The effect of carvedilol is probably correlated to the presence of all three autoantibodies.

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Keywords: Adrenergic receptors; Autoantibodies; Cardiac function; Carvedilol; Heart failure

1 Introduction

The development of various cardiovascular diseases may lead to the same ultimate sequence as heart failure (HF). Over the past ten years, the morbidity and mortality of cardiovascular disease has decreased significantly, but the incidence and mortality of HF has increased.[1,2] In recent years, the treatment of HF has been constantly updated, for instance the 2013 American College of Cardiology Foundation/American Heart Association (ACC/AHA) guidelines have been updated, and β-blockers are now recommended for all present HFs unless contraindicated in order to reduce morbidity and mortality.[3]

Carvedilol is the β-blocker most often used for the management of patients with HF.[4,5] The Carvedilol Or Metoprolol European Trial (COMET) revealed that carvedilol improves cardiac performance to a greater extent than metoprolol when administered to patients with HF.[6] Furthermore, the multi-parametric comparison of CARvedilol *vs.* NEbivolol *vs.* BIsoprolol in moderate heart failure (CARNEBI) trial suggests that there are no significant differences in clinical evaluation [New York Heart Association (NYHA) classification, Minnesota questionnaire], laboratory founding [including kidney function and B-type natriuretic peptide (BNP)], echocardiography, and lung mechanics.[7] The Multicenter Automatic Defibrillator Implantation Trial-Cardiac Resynchronization Therapy (MADIT-CRT) suggests that novel beneficial and synergistic effects of carvedilol are observed in patients with cardiac resynchronisation therapy with defibrillator (CRT-D) and left bundle branch block (LBBB).[8] Furthermore, numerous studies have shown that HF patients have difficulties in reaching the optimal β-blocker doses. Hence, it is important to identify factors that can impact the effects of carvedilol.

HF is the main pathogenesis of ventricular remodelling and the excessive activation of neuroendocrine hormones. A

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large number of mechanical, molecular, immunological, and ischemic mechanisms are involved in this process. Among these, autoantibodies against β_1, β_2, and α_1 adrenergic receptors (ARs) (anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR) play an important role in the pathology of HF.[9–11] β_1-AR autoantibodies could stabilise the active conformation of the β_1-AR-molecule, which is associated with the activation state of the receptor, and β_1-AR autoantibodies inhibit the binding of radio ligands to the receptor. In addition to activating the receptor, anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR also interfere with receptor cycling and desensitisation to cause either the blunting or sensitisation of the receptor for endogenous catecholamines.[12] Such strong binding and the subsequent stabilisation of AR by their autoantibodies leads to the enhancement of the effects of the AR in HF and thus promotes the deterioration of HF. The antagonists of α_1-AR can potentially treat HF, which indicates that α_1-AR plays a negative role in HF. Thus, its autoantibody may be important for the diagnosis and treatment of HF.[11]

Cardiac function (contractility) is tightly controlled by the activity of β-AR, which is located in the membranes of cardiac myocytes. It is well-established that β-AR belongs to the family of G-protein coupled receptors and the three subtypes, β_1, β_2, and β_3-ARs have been found to induce various changes in HF owing to diverse HF etiology. The cardiac β_2 receptor is presently known to signal and function in a substantially different manner than the β_1 receptor. For example, the β_1 receptor appears to be pro-apoptotic while the β_2 receptor appears to be anti-apoptotic in the heart. The role of α_1-AR in cardiac contractility remains a matter of debate, which is in contrast to their fully documented role in cardiac hypertrophy/remodelling. Studies have revealed an α_1-AR-induced rise in intracellular (Ca^{2+}) activated hypertrophy-promoting gene expression programs in the cardiac myocyte rather than contraction.[13–16]

The present study explored the possible association of anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR with left ventricular (LV) function in HF patients treated with carvedilol and the clinical value of autoantibodies against β_1-, β_2-, and α_1-ARs for the treatment of patients with HF.

2 Methods

2.1 Subjects

From March 2016 to December 2017, a total of 267 HF patients underwent a physical examination, chest radiography, a cardiogram, and echocardiography. All patients performed two six-minute walk tests and the second scores of the pre- and post-treatment walk tests were used as the baseline values. Almost half of the patients did not meet the inclusion/exclusion criteria. Furthermore, four subjects only agreed to provide the autoantibody detection result. Finally, a total of 137 HF patients were included in the present study. These patients were divided into two groups: those with all three autoantibodies (positive group, $n = 65$) and those with only one or two autoantibodies (negative group, $n = 72$).

The inclusion criteria for HF patients were as follows: (1) patients with stable NYHA class II-III heart function after treatment with ACEI, diuretics, β-receptor blockers, and/or digoxin; (2) patients who had chronic cardiac insufficiency, which was defined as a left ventricular ejection fraction (LVEF) of < 45%, and (3) patients with the ability to complete the study visits. The exclusion criteria were as follows: (1) patients who could not use an ACEI; (2) patients with a heart rate of < 55 beats/min under a clearheaded and quiescent condition (kept awake and quiet); (3) patients with an atrioventricular block of > 1°, sick sinus syndrome, and β-receptor blocker hypersensitivity disease; (4) patients with hepatic and renal dysfunction; (5) patients with haemoglobin, creatinine, glutamic pyruvic transaminase, and potassium levels above the normal limit and a creatinine clearance of ≤ 30 mL/min (calculated using the Cockcroft-Gault formula); (6) patients who had a stroke within the past three months; and (7) patients with a systolic blood pressure of > 160 mmHg or a diastolic blood pressure of > 95 mmHg despite antihypertensive therapy.

2.2 Materials

Three peptides that corresponded to the amino acid sequence of the second extracellular loop of human β_1-, β_2-, and α_1-AR were synthesised by Genomed (Genomed Synthesis, Inc., CA, USA). The sequences are presented in Table 1.[17–19] The peptide was judged to be pure on the basis of a HPLC analysis on a Vydac C-18 column and via an amino acid analysis on an automated amino acid analyser (Beckman Instruments, Inc., Palo Alto, CA).[20]

2.3 The enzyme-linked immunosorbent assay (ELISA) protocol

All experiments provided a double hole. An ELISA

Table 1. Amino acid sequences of human β_1, β_2, and α_1 adrenoreceptors.

Adrenoreceptor	Position	Sequence
β_1	197-222	H-W-W-R-A-E-S-D-E-A-R-R-C-Y-N-D-P-K-C-C-D-F-V-T-N-R
β_2	172-197	H-W-Y-R-A-T-H-Q-E-A-I-N-C-Y-A-N-E-T-C-C-D-F-F-T-N-Q
α_1	192-218	G-W-K-E-P-V-P-P-D-E-R-F-C-G-I-T-E-E-A-G-Y-A-V-F-S-S-V

protocol previously described by Fu, *et al*.[21] was used to screen for the presence of autoantibodies. Briefly, peptides in a Na_2CO_3 solution (PH = 11.0) were coated on microtiter plates and saturated overnight with phosphate-buffered saline supplemented with foetal bovine serum. Then, different dilutions of serum samples (1: 20–1: 160) were added to the microtiter plates overnight at 4°C. The microtiter plates were washed three times with PBS and a peroxidase substrate ammonium salt was added to the wells and reacted for 30 min. Then, the photometric qualities were evaluated at 405 nm.

2.4 Administration of carvedilol

All patients received the standard regimen for HF: ACEI (perindopriltert-butylamine tablets: 2 mg q.d.), diuretics (spironolactone tablets: 20 mg q.d.) and digoxin (digoxin: 0.125 mg q.d.). In addition, all the patients received an initial daily dose of 3.125 mg carvedilol (b.i.d.). This was subsequently uptitrated over a two- to four-week period by doubling the amount to 25 mg (b.i.d.). The target heart rate and blood pressure were 60 beats/min and 90/60 mmHg, which are the acceptable heart rate and blood pressure of HF patients undergoing long-term treatment. The 25 mg twice daily target dose was maintained until the end of the study.

2.5 Follow-up

Each patient was designated to one specific investigator from whom that patient received follow-up examinations at least once a month for six months. Patients were encouraged to schedule *ad-hoc* appointments if needed. The data that was collected included the following: heart rate, blood pressure, weight, pulmonary rales, cardiac function, peripheral oedema, and current dose of the treatment regimen. Adverse drug reactions were also recorded.

2.6 Data analysis

The data was checked for normality using the Kolmogorov-Smirnov test. The quantitative data was expressed as mean ± SD and analysed using *t*-test. Positive was defined as: (sample A – blank A)/(negative control A – blank A) ≥ 2.1 and was analysed using a chi-square test. The antibody titer was reported as a geometric mean. $P < 0.05$ was considered statistically significant (two-tailed). The analysis was performed using the SPSS 18.0 software package. The sample size was calculated as approximately 55 subjects for each group (based on LVEDD, LVESD, and LVEF, $\alpha = 0.05$, $1-\beta = 0.8$).

3 Results

3.1 Clinical characteristics

The basic characteristics of the patients in the positive group are shown in Table 2. The cardiac functions of all patients in the two groups are presented in Table 3. The clinical characteristics of these HF patients were as follows: (1) age: 53 ± 14 *vs.* 54 ± 18 years; (2) echocardiography parameters: left ventricular end-diastolic diameter (LVEDD): 65.6 ± 5.7 *vs.* 66.4 ± 8.4 mm; left ventricular end-systolic diameter (LVESD): 53.9 ± 6.8 *vs.* 56.4 ± 9.3 mm; LVEF: 33.9% ± 7.03% *vs.* 32.0% ± 8.24%, and (3) NYHA: 2.9 ± 0.5 *vs.* 3.0 ± 0.7. There were no differences between these two groups.

Table 2. The basic characteristics of the patients in the positive and negative groups.

Type	Positive group (Negative group)			Age, yrs	Weight, Hg
	Men	Women	Total		
HHD	11 (12)	9 (10)	20 (22)	55 ± 16 (54 ± 15)	61.2 ± 4.8 (60.8 ± 5.2)
ICD	10 (9)	9 (12)	19 (21)	52 ± 18 (53 ± 16)	62.7 ± 6.8 (61.5 ± 4.5)
DCM	7 (8)	8 (9)	15 (17)	56 ± 12 (57 ± 14)	67.9 ± 10.6 (66.9 ± 7.6)
RHD	6 (6)	5 (6)	11 (12)	55 ± 13 (52 ± 13)	54.8 ± 6.8 (57.6 ± 5.2)

Data are presented as mean ± SD unless other indicated. DCM: dilated heart disease; HHD: hypertensive heart disease; ICD: ischemic heart disease; RHD: rheumatic heart disease.

Table 3. Cardiac functions of all the patients in the positive and negative groups.

Groups	*N* (Female/Male)	Age, yrs	LVEDD, mm	LVESD, mm	LVEF, %	Cardiac function (NYHA)
Positive group	65 (29/36)	53 ± 14	65.6 ± 5.7	53.9 ± 6.8	33.9 ± 7.0	2.9 ± 0.5
Negative group	72 (33/39)	54 ± 18	66.4 ± 8.4	56.4 ± 9.3	32.0 ± 8.2	3.0 ± 0.7

Data are presented as mean ± SD unless other indicated. LVEDD: left ventricular end-diastolic dimension; LVESD: left ventricular end-systolic dimension; LVEF: left ventricular ejection fraction; NYHA: New York Heart Academy.

3.2 Carvedilol tolerance, titration time, and the final carvedilol dosages, heart rate (HR) and blood pressure (BP) of the two groups

(1) Carvedilol tolerance: 30.54 ± 11.45 *vs.* 30.24 ± 10.89 mg/day, (2) titration time: 41 ± 3 *vs.* 42 ± 2 days, (3) carvedilol dosages: 30.54 ± 11.45 *vs.* 30.24 ± 10.89 mg/day, (4) HR: 58 ± 4 *vs.* 61 ± 3 beats/min, and (5) BP: 92 ± 6/61 ± 4 *vs.* 93 ± 4/60 ± 5 mmHg. There were no differences between these two groups.

3.3 ELISA results

The positive rate of anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR in all of the patients were 41.5%, 37.2%, and 41.8%, respectively. The titers of anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR in all of the patients were 1: 95, 1: 112, and 1: 86, respectively. In addition, the correlation between these three autoantibodies was also analysed. Of all the patients, 34.0% (48/137) of the patients had two autoantibodies, while 46.1% (65/137) had three autoantibodies (Figure 1).

3.4 Left ventricular remodelling and function changes after carvedilol treatment

After six months of carvedilol treatment in combination with the standard treatment for HF (ACEI, diuretics, and digoxin), the LVEDD and LVESD values decreased from 65.6 ± 5.7 to 57.5 ± 7.3 mm ($P < 0.01$) and 53.9 ± 6.8 to 43.1 ± 8.2 mm ($P < 0.01$), respectively, in the positive group; while they decreased from 66.4 ± 8.4 to 64.0 ± 7.4 mm ($P < 0.05$) and 56.4 ± 9.3 to 52.9 ± 8.3 mm ($P < 0.05$), respectively, in the negative group. Similarly, LVEF markedly increased from 33.9% ± 7.0% to 50.4% ± 10.9% ($P < 0.01$) in the positive group and increased from 32.0% ± 8.2% to 41.7% ± 7.4% in the negative group ($P < 0.05$, Figure 2). The trends concerning the LVEDD, LVESD, and LVEF of the two groups were shown in Figure 3.

The results of the study participants' six-minute walk before and after treatment were illustrated and show that carvedilol combined with the standard treatment for HF is associated with a significant increase in the distance covered during these timed six-minute walks. For the positive group,

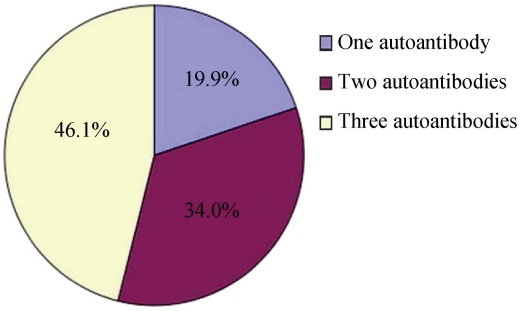

Figure 1. The distribution of the three autoantibodies in patients with heart failure.

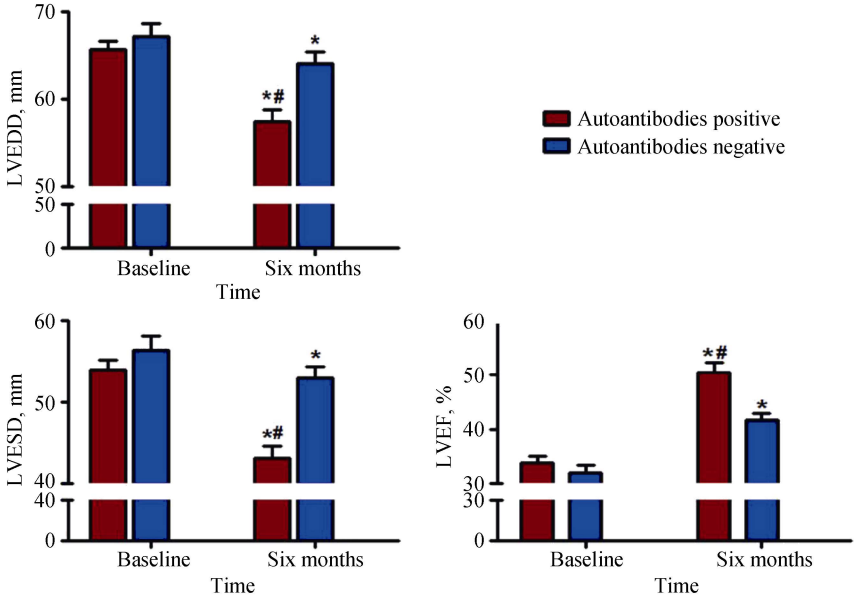

Figure 2. The comparison of echocardiographic data after carvedilol treatment. In patients with heart failure, after six months of treatment with carvedilol, the LVEDD and LVESD decreased more compared to those in the group of patients without all three autoantibodies. In addition, the LVEF changed more in the group with all three autoantibodies. The group of patients with all three autoantibodies exhibited a significant improvement in response to the carvedilol therapy. $^{\#}P < 0.01$ *vs.* baseline, $^{*}P < 0.05$ *vs.* Control. LVEDD: left ventricular end-diastolic dimension; LVESD: left ventricular end-systolic dimension; LVEF: left ventricular ejection fraction.

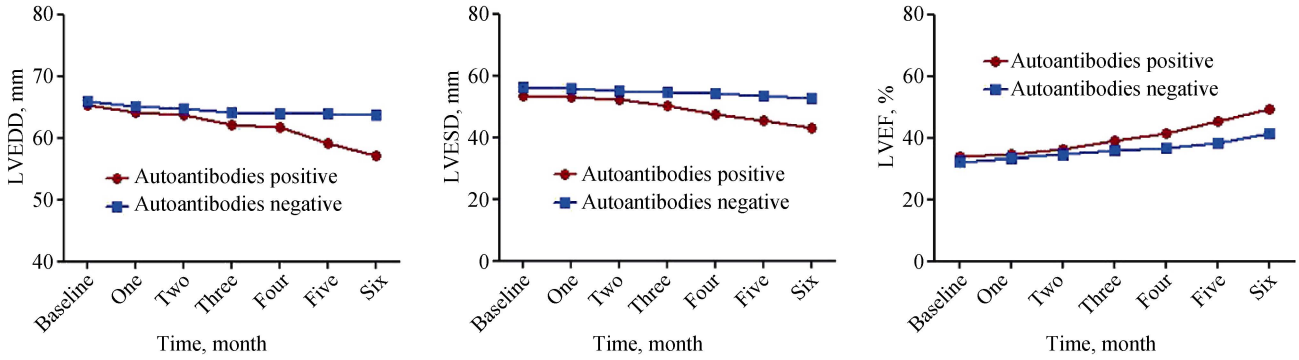

Figure 3. The trends of the LVEDD, LVESD, and LVEF for the two groups after one to six months post-baseline. LVEDD: left ventricular end-diastolic dimension; LVESD: left ventricular end-systolic dimension; LVEF: left ventricular ejection fraction.

the distance walked increased from 296.18 ± 90.23 to 419.26 ± 71.98 m ($P < 0.01$). For negative group, the distance walked increased from 301.50 ± 53.04 to 382.50 ± 53.40 m ($P < 0.01$). However, the positive group exhibited a more pronounced improvement ($P < 0.05$, 419.26 ± 71.98 *vs.* 382.50 ± 53.40 m). In addition, the clinical lab data, including haemoglobin, creatinine, glutamic pyruvic transaminase, and potassium levels, remained stable throughout the first year.

3.5 Changes in autoantibodies' positive rate and titers after carvedilol treatment

The frequency of anti-β_1-AR decreased from 100% to 57% in the positive group, $P < 0$ .01 and from 100% to 76% in the negative group after carvedilol treatment and the geometric mean titers changed from 1: 118 to 1: 72 in the positive group (Table 4). The frequency of anti-β_2-AR decreased from 100% to 50% in the positive group, $P < 0.01$ and from 100% to 69% in the negative group and the geometric mean titers changed from 1: 138 to 1: 61 in the positive group (Table 4). The frequency of anti-α_1-AR decreased from 100% to 49% in the positive group ($P < 0.01$) and from 100% to 72% in the negative group. The geometric mean titers became significantly lower, changing from 1: 130 to 1: 67 in the positive group (Table 4). This result indicates that β_2-AR and α_1-AR changed more after carvedilol treatment and thus would be more effective targets of carvedilol.

It is noteworthy that 28 patients demonstrated that complete ablation of autoantibodies six months after the initiation of carvedilol treatment in the positive group. In addition, the geometric mean titer of the remaining patients in the positive group significantly decreased compared to the baseline value.

4 Discussion

The present study is to find that the proportion of patients in the positive group is significantly higher than the patients in the negative group. Meanwhile, it was found that after six months of carvedilol treatment, patients in the positive group exhibited greater improved changes in LV remodelling and heart function compared to those in the negative group.

4.1 Autoantibody against adrenergic receptors and cardiac function

The activation of the sympathetic nervous system (SNS) and the inhibition of the parasympathetic system have long been recognised as manifestations of the clinical syndrome of HF. AR mediates the central and peripheral actions of the primary sympathetic neurotransmitter, norepinephrine and the primary adrenal medullary hormone, epinephrine. In the heart, the two main ARs are the β-ARs and α_1-ARs, which comprise about 90% and 10% of the total cardiac ARs, respectively.

Table 4. The changes of the positive rate and titers of the autoantibodies in the follow-up.

	Positive group		Negative group	
	Before % (titers)	After % (titers)	Before % (titers)	After % (titers)
β_1	100 (1 : 118)	57 (1 : 72)*	100 (1 : 132)	76 (1 : 113)†
β_2	100 (1 : 138)	50 (1 : 61)*	100 (1 : 132)	69 (1 : 110)†
α_1	100 (1 : 130)	49 (1 : 67)*	100 (1 : 127)	72 (1 : 113)†

The positive rate of anti-β_1-AR, anti-β_2-AR and anti-α_1-AR is changed more in the patients of autoantibodies positive after six months of carvedilol treatment; the titers of the three autoantibodies decreased more than the negative group. *$P < 0.01$ *vs.* baseline; $^{\dagger}P < 0.05$ *vs.* control group.

In HF patients, it was found that there are more patients in the positive group than patients in the negative group. These observations strongly suggest that interactions between adrenergic receptors are needed to improve the clinical utility of β-AR and α-AR antagonists in HF. Hence, it is critical to understand the consequences of either the activation or inhibition of AR in the hypertrophic growth of the heart.[23,24] Previous studies have shown that autoantibodies against α-AR display a selective $\alpha_{1D/1A}$ antagonistic activity on the corresponding receptor and influence cardiac function.[25,26] Another study has revealed that during the development of heart failure, the densities of pulmonary β1-AR and β2-AR decrease while the levels of anti-β_1-AR anti-β_2-AR autoantibody increase.[27] With this understanding, more efficient pharmacologic agents can be developed to prevent the progression of this major health problem.

4.2 The relationship between carvedilol treatment and autoantibodies

The present study makes two novel observations. Firstly, LV remodelling and heart function are directly associated with the different response to carvedilol in combination with digoxin, diuretic, or ACEI treatment for six months between the two group. This reveals that patients in the positive group exhibit greater improved LV remodelling and cardiac function compared to patients in the negative group. Secondly, it is demonstrated that the mean titer values are significantly reduced or in some cases, completely disappear in approximately 50% of patients in the positive group. These results suggest that carvedilol can selectively block the over activation of β_1-, β_2-, and α_1-adrenergic receptors. Therefore, it can be suggested that for those with all three antibodies, carvedilol can be effective because it can target all three antibodies. In addition, patients without all three autoantibodies require a longer titration time compared to patients with the expression of all three autoantibodies, which also proves that carvedilol is more effective in patients with all three antibodies due to its mechanisms. It was considered that the down-regulation and desensitisation of autoantibodies by exposing receptors specifically to autoantibodies might have induced the different titration rates.[11,28] For this reason, the investigators propose that autoantibody reactivity can be a useful clinical marker in determining the optimal dosage of a selective receptor blocker. The COMET study showed that most patients with NYHA class II–III and an LVEF of < 35% reach the target dose of carvedilol.[29] However, in the present study, although most patients in the positive group reached the target dose, only a few of the patients in the negative group reached the target dose. Furthermore, it was found that patients without all three autoantibodies are less tolerant to carvedilol.

4.3 Predictive value of autoantibodies against β_1-, β_2-, and α_1-AR

The changed transduction and down-regulation of receptors is one of the causes of molecular defect in progressive HF.[30] The cardiac receptor is the most important receptor that regulates heart function. Its binding with the receptor promotes cyclicadenosine monomphsopate production, resulting in increased cardiac contractility and cardiac output.[31] Anti-β_1-AR not only bound to their receptors in the myocardium but can induce receptor-mediated responses.[32] Carvedilol, uniquely among β-blockers, has a strong central sympatholytic effect,[33] which, by reducing neuronal norepinephrine release, can significantly affect cardiac β receptor activity and, in turn, affect the levels of the autoantibodies raised against these receptors. However, this does not affect the already reduced β_1-AR levels in failing human hearts. That is, it neither upregulates these nor reduces these further.[34–36] Therefore, carvedilol in combination with digoxin, diuretics, or ACEI can inhibit the overstimulation of receptors and reduce the progression of cardiac damage.

Based on this, the observed reductions in the level of autoantibodies as a result of carvedilol in the present study might be carvedilol-specific and these autoantibodies in HF patients may serve as biomarkers of carvedilol's therapeutic efficacy. In addition, autoantibody reactivity is a potential clinical marker in determining the optimal dosage of a selective receptor blocker. Therapies for HF and the activation of the immune system may improve as drug standardisation becomes more sophisticated and the autoantibody titer should decrease.

The present study has some limitations. A patient without any autoantibodies was not among the patients studied, so the effect of each single autoantibody needs to be verified in future studies. In addition, there was no control group of subjects taking a placebo, which should also be included in future studies. In addition, as the follow-ups only lasted for six months, data on readmission for HF was not collected.

4.4 Conclusions

Carvedilol, as a nonselective β-receptor blocker and a selective α_1-receptor blocking agent, significantly decreases the frequency and geometric mean titer in patients with anti-β_1-AR, anti-β_2-AR, and anti-α_1-AR, even to complete ablation. Patients who were positive for these three autoantibodies experienced more pronounced improvements in cardiac function and remodelling after six months of treatment with carvedilol. The effects of carvedilol are probably correlated to the existence of all three autoantibodies.

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