The effect of autoantibody against M2-muscarinic acetylcholine receptor in peripartum cardiomyopathy patients on digoxin additional to standard treatment

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Abstract

Background: To evaluate the effects of autoantibodies against the M2-muscarinic receptor (anti-M2-R) on digoxin additional to standard treatment in peripartum cardiomyopathy (PPCM) patients.

Methods: 86 PPCM patients were separated into anti-M2-R negative or positive group according to the anti-M2-R reactivity. All the patients received digoxin additional to standard treatment regimen. Echocardiography was performed at baseline and after 5 years treatment. Serum digoxin concentration (SDC) were performed every 3 to 6 months. All-cause mortality, cardiovascular mortality and re-hospitalization for heart failure were compared after 5 years of follow-up.

Results: There were 82 patients completed the final data analysis, including 38 in the anti-M2-R (+) group and 44 in the anti-M2-R (-) group. The heart rate of the positive group was higher than that of the negative group at baseline (102.3 ± 6.3 vs. 95.9 ± 6.8 , $p < 0.001$). The initial SDC of patients in the positive group was higher than that of patients in the negative group with the same dose of digoxin (1.21 ± 0.41 vs. 0.73 ± 0.16 ng/mL, $p < 0.001$). Patients in the anti-M2-R (-) group had better tolerance to metoprolol and digoxin ($p < 0.05$). All the PPCM patients showed prominent improvement in cardiac function, especially in the anti-M2-R (-) group. Re-hospitalization for heart failure was decreased in the negative group, but not of all-cause or cardiovascular mortality.

Conclusions: Patients negative for anti-M2-R showed better tolerance to metoprolol and digoxin. Anti-M2-R maybe a predictor for vagus nerve overactivation and is associated with poor response to digoxin treatment in PPCM patients.

Background

Peripartum cardiomyopathy (PPCM) is a rare idiopathic dilated cardiomyopathy defined by the signs and symptoms of heart failure (HF) in the last month of pregnancy through the fifth month postpartum [1]. The definition of PPCM states that there must be no previously known structural heart disease, and echocardiographic parameters must achieve one of the following: left ventricular ejection fraction (LVEF) $< 45\%$, fractional shortening $< 30\%$, or both, with a possible additive left ventricular end diastolic dimension > 2.7 cm/m² body surface area [2]. This disease is associated with a high morbidity and mortality but its aetiology remains unknown [1].

The main pathogenesis of HF are ventricular remodeling and the imbalance of endogenous neuroendocrine hormone systems. The excessive activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system has been recognized by most doctors. Angiotensin-converting enzyme inhibitors (ACEIs), β receptor blockers, and mineralocorticoid receptor antagonists have become the golden triangle for HF. However, the importance of the vagus nervous system is relatively unfamiliar. The M2-muscarinic acetylcholine receptor is the most important receptor for the vagus nervous system to act on the heart. Autoantibodies against the M2-muscarinic receptor (anti-M2-R), have been found in HF patients of various etiologies including PPCM [3-5], which can interfere with

radioligand binding on the target receptor, display agonist-like activities on the M2 receptors, and hence modulate cardiac function [6].

Digitalis is a positive inotropic agent, also has the function of vagus nervous system stimulation. Digoxin is the most commonly used oral digitalis in HF patients. So it could neutralize the over activation of the sympathetic system and RAAS, which is similar to the effect of anti-M2-R. Therefore, what is the clinical sense of anti-M2-R? Are there any different responses to digoxin between patients negative and positive for anti-M2-R? If so, do patients negative for anti-M2-R have better improvement in cardiac function? In this study, we investigate the presence of anti-M2-R and cardiac function in response to digoxin additional to standard treatment regimen for HF (ACEI, β receptor blocker, furosemide and spironolactone) in PPCM patients.

Materials And Methods

Study population

This was an prospective observational study, which began in January 1998 and ended in December 2019. A total of 86 consecutive newly diagnosed PPCM patients were enrolled at the HF clinic of Beijing Chaoyang Hospital. We obtained the demographic data and related information by in-person interview using the structured questionnaire. The inclusion criteria were as follows: (1) age, between 18 and 40 years old, (2) cardiac function, New York Heart Association functional classes (NYHA) II-IV, (3) symptoms of HF, happened in the last month of pregnancy or during the first 5 months postpartum, (4) no other identifiable causes for HF, and (5) LVEF < 45% by transthoracic echocardiography. Exclusion criteria were as follows: (1) clinical conditions with increased levels of autoantibody, such as rheumatoid arthritis, HIV, and evidence for sepsis, (2) moderate-severe anemia, (3) metabolic disorders such as thyroid disease, or (4) moderate-severe hepatic or renal dysfunction. The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Chaoyang Hospital, Beijing, China. All the PPCM patients provided written informed consent before study entry.

Serum anti-M2-R detection

About 2 mL of blood was withdrawn from the antecubital vein of each patient when enrolled into this study and after five years treatment. Serum samples were separated by centrifugation at 3,000 rpm for 10 min and stored at -20°C . Peptide corresponding to the sequence of the second extracellular loop of human M2-muscarinic receptor (amino acid sequence number 169-193: V-R-T-V-E-D-G-E-C-Y-I-Q-F-F-S-N-A-A-V-T-F-G-T-A-I), was synthesized by Genomed (Genomed Synthesis, Inc., San Francisco, CA, USA) with the solid-phase method of Merrifield. The purity of the peptide was 98%, on the basis of HPLC analysis on a Vydac C-18 column. Levels of serum anti-M2-R was measured with SA-ELISA and positive was defined as a ratio of (sample A - blank A)/(negative control A - blank A) ≥ 2.1 [7]. Titers of autoantibodies was the highest when this ratio ≥ 2.1 with serum diluted from 1:20 to 1:160. The coefficient of variation of intra-assay and inter-assay were less than 5%.

Beginning of the standard pharmacological regimen

All the patients received digoxin additional to standard therapy regimens (perindopril or losartan, metoprolol, furosemide, and spironolactone). Perindopril was taken at an initial dose of 2 mg/day, and then uptitrated according to the blood pressure. If perindopril wasn't tolerated, losartan was used instead. Metoprolol was taken at an initial dose of 12.5 mg/day that was up-titrated over a 2–4-week period by doubling the twice-daily amount to the maximum tolerated dose or a target of 100 mg/day [2]. The maximum tolerated heart rate and blood pressure were 60–75 bpm and 120/65 ± 10/5 mmHg, respectively. The initial dosage of furosemide was 10-20 mg/day, which allowed to increase if a patient displayed signs or symptoms of HF progression. The dosage of spironolactone was 10-20 mg/day. Digoxin was taken at an initial dose of 0.125 mg/day and then adjusted according to the serum digoxin concentration (SDC). The target SDC was 0.5-0.9 ng/mL as suggested by the ACCF/AHA guideline for the management of HF [8]. If the SDC was 0.9-1.5 ng/mL, the dosage of digoxin was reduced to 0.0625mg/day. The dosage of digoxin was reduced to 0.0625mg every other day when the SDC was higher than 1.6 ng/mL. In addition, patients were advised to control their salt intake and body weight.

Follow-up examination

All patients were assigned to a fixed investigator for up to 5 years of follow-up after they were included in the study. The primary endpoint events were all-cause mortality, cardiovascular mortality, and re-hospitalization for HF. Patients received follow-up once a month for the first year and every 3–6 months for up to 5 years or until the primary endpoint. Echocardiography, 6-minute walk tests, and clinical laboratory tests including SDC were performed regularly. We collected heart rate, blood pressure, body weight, cardiac function, the presence of peripheral edema, drug dosages during the examinations. Subjects were also questioned and examined for the presence of any adverse drug reaction.

Statistical methods

Quantitative data are presented as mean ± SD, and categorical data are presented as percentage. Titers of serum anti-M2-R was reported as the geometric mean. For two groups comparison, we used Student's *t* test or Mann-Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. Chi-square statistics and log-rank test were used for all-cause mortality, cardiovascular mortality, and hospitalization for HF. Data on the titration of metoprolol were fit to a variable slope sigmoidal equation ($Y = \text{Initial Dose} + (\text{Maximum Dose} - \text{Initial Dose}) / (1 + 10(\text{LogEC}_{50} - X) * \text{Slope}))$), in which the independent variable (*X*) is the log of the time of the dosage value (*Y*). The LogEC₅₀ denotes the time that corresponds to halfway between the minimum and maximum dosages. All the tests were 2-tailed. *P* < 0.05 was considered to be statistically significant. Data were analyzed using SPSS 20.0 (SPSS, Chicago, Illinois, USA).

Results

Study characteristics

All the 86 patients were diagnosed as PPCM for the first time. 30 patients were primiparous, and 25 patients had multiple gestation. There were 30 patients who had pregnancy-induced hypertension, and 18 patients had gestational diabetes mellitus. There were 44 patients with symptoms in the postpartum period. At baseline, 33 patients were in NYHA functional class II, 38 patients were in class III, and 15 patients were in class IV.

According to the anti-M2-R reactivity, 46 patients were assigned to the negative group and the other 40 patients were assigned to the positive group. The baseline characteristics of the two groups were shown in Table 1. Four patients lost to follow-up during the first year (2 patients in the negative group and 2 patients in the positive group), and the other 82 patients completed the final data analysis, including 38 patients (38/40, 95.0%) in the positive group and 44 patients (44/46, 95.7%) in the negative group. Of all the parameters, only the mean resting heart rate of the negative group was higher than that of the positive group. We posit that anti-M2-R maybe influence heart rate via the activation of the vagus nervous system.

Table 1 Clinical characteristics of PPCM patients

	Anti-M2-R (+) group n = 40	Anti-M2-R (-) group n = 46	P value
Age (years)	29.1 ± 4.7	29.3 ± 4.1	0.835
Multiple gestation	12	13	0.859
Multiparity	26	30	0.983
Pregnancy complication			
Pregnancy induced hypertension	16	14	0.353
Preeclampsia	9	7	0.387
Gestational diabetes mellitus	9	9	0.739
Postpartum diagnosed PPCM	21	23	0.817
Blood pressure (mmHg)			
Systolic	136.7 ± 15.1	136.4 ± 15.2	0.927
Diastolic	88.5 ± 10.8	86.7 ± 10.5	0.437
Heart rate (bpm)	95.9 ± 6.8	102.3 ± 6.3	< 0.001
NYHA functional class	2.75 ± 0.74	2.83 ± 0.71	0.630
Echocardiographic data			
LVEDD (mm)	58.9 ± 7.0	58.7 ± 7.8	0.900
LVESD (mm)	46.9 ± 6.9	47.2 ± 7.3	0.845
LVEF (%)	38.9 ± 6.1	39.4 ± 4.4	0.668
6-min walk distance (m)	190.0 ± 82.3	195.2 ± 75.8	0.763

Abbreviations: PPCM, peripartum cardiomyopathy; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction. Unpaired Student's t-test were made between the two groups.

Drug dosage

All the patients received digoxin and standard pharmacological regimen for HF, which includes perindopril, metoprolol, spironolactone, and furosemide. There were no differences between patients with anti-M2-R (-) and those with anti-M2-R (+) regarding the dosage of perindopril, spironolactone and furosemide. The dosages of metoprolol and digoxin in the negative group was higher than that in the positive group, shown in Table 2.

Table 2. Dosages of drugs for PPCM patients

	Anti-M2-R (+) group	Anti-M2-R (-) group	P-value
Perindopril, mg	3.3 ± 1.0	3.2 ± 1.0	0.879
Metoprolol, mg	26.6 ± 4.2	38.3 ± 4.9	< 0.001
Spironolactone, mg	18.8 ± 3.3	18.9 ± 3.1	0.886
Furosemide, mg	19.3 ± 2.7	18.7 ± 3.4	0.365
Digoxin, mg	0.08 ± 0.04	0.12 ± 0.02	< 0.001

There were no differences between patients with anti-M2-R (-) and patients with anti-M2-R (+) regarding the dosages of perindopril, spironolactone and furosemide (all $p > 0.05$). The mean dose of metoprolol and digoxin for anti-M2-R (-) patients was higher than that for anti-M2-R (+) patients ($p < 0.001$).

Titration of metoprolol

Patients in the anti-M2-R (-) group demonstrated a better tolerance and a more rapid rate of up-titration of metoprolol than those in the anti-M2-R (+) group. During the 5 years treatment, the maximum tolerated dose of metoprolol for the anti-M2-R (-) group was 38.3 ± 4.9 mg b.i.d., which was higher than 26.6 ± 4.2 mg b.i.d. for the anti-M2-R (+) group ($p < 0.001$), as shown in Fig.1. The mean time to maximum tolerated dose of metoprolol was 67.1 ± 10.8 days in the anti-M2-R (-) group, which was shorter than 82.8 ± 11.7 days in the anti-M2-R (+) group ($p < 0.001$).

Serum digoxin concentration and the dose of digoxin

SDC was tested every three to six months. The target SDC was 0.5-0.9 ng/mL. We prescribed digoxin at an initial dose of 0.125 mg daily. The mean SDC was significantly higher in patients with anti-M2-R (+) than those negative for anti-M2-R for the first time detection (1.21 ± 0.41 vs. 0.73 ± 0.16 ng/mL, $p < 0.001$). The mean maintenance dose of digoxin was 0.12 ± 0.02 mg/day in the anti-M2-R (-) group, significantly higher than 0.08 ± 0.04 mg/day of the anti-M2-R (+) group ($p < 0.001$).

Dynamic variation of the serum anti-M2-R

Sera positive for anti-M2-R was found in 46.5% (40/86) of the PPCM patients at enrollment. In positive cases, the mean titers of anti-M2-R was 1:121. With five years of treatment, the positive rate of serum

anti-M2-R was 9.0% (7/78) and the mean titers of anti-M2-R was 1:59, which were significantly decreased compared to baseline (all $p < 0.001$) (Fig.2).

Adverse events

There were no obvious effects on blood glucose, serum lipid, or hepatic and renal function during the 5-year treatment and follow-up. Only one patient in the positive group showed symptom of digoxin intoxication such as somewhat weakness and nausea. The SDC of this patient was 2.5 ng/mL, and the dosage of digoxin was reduced to 0.0625 mg every other day, and the above symptoms disappeared subsequently.

Cardiac function and 6-minute walk test

Clinical data, NYHA functional class, echocardiographic results, and 6-minute walk distance at baseline and 5-year were determined, shown in Table 3. With long-term low dose of digoxin additional to standard pharmacological treatment for HF, the left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) decreased from 58.7 ± 7.8 to 47.5 ± 4.5 mm and 47.2 ± 7.3 to 33.1 ± 5.5 mm in the anti-M2-R (-) group, respectively, and from 58.9 ± 7.0 to 49.9 ± 4.7 mm and 46.9 ± 6.9 to 36.2 ± 6.5 mm in the anti-M2-R (+) group, respectively. Meanwhile, the LVEF increased from 39.4 ± 4.4 to $62.7 \pm 6.8\%$ in the anti-M2-R (-) group and from 38.9 ± 6.1 to $57.7 \pm 7.4\%$ in the anti-M2-R (+) group. It is worth noting that the improvement of the structural and functional of left ventricular including 6-min walk distance in anti-M2-R (-) patients was better than that in anti-M2-R (+) patients. The LVEF returned to normal in 91% (71/78) of patients with five years of treatment. Laboratory data, including levels of hemoglobin, creatinine, glutamic pyruvic transaminase, and potassium were stable throughout the follow-up.

Table 3. Summary of the effect of treatment on cardiac function

	Anti-M2-R (+) group		Anti-M2-R (-) group	
	Baseline n = 40	5-Year n = 35	Baseline n = 46	5-Year n = 43
Blood pressure (mmHg)				
Systolic	136.7 ± 15.1	$117.0 \pm 7.4^*$	136.4 ± 15.2	$116.0 \pm 7.2^*$
Diastolic	88.5 ± 10.8	$66.2 \pm 6.2^*$	86.7 ± 10.5	$65.7 \pm 5.1^*$
Heart rate (bpm)	95.9 ± 6.8	$68.9 \pm 2.1^*$	102.3 ± 6.3	$68.2 \pm 2.5^*$
NYHA functional class	2.75 ± 0.74	$1.29 \pm 0.46^*$	2.83 ± 0.71	$1.14 \pm 0.35^*$
Echocardiographic data				
LVEDD (mm)	58.9 ± 7.0	$49.9 \pm 4.7^*$	58.7 ± 7.8	$47.5 \pm 4.5^*\$$
LVESD (mm)	46.9 ± 6.9	$36.2 \pm 6.5^*$	47.2 ± 7.3	$33.1 \pm 5.5^*\$$
LVEF (%)	38.9 ± 6.1	$57.7 \pm 7.4^*$	39.4 ± 4.4	$62.7 \pm 6.8^*\#$
6-min walk distance (m)	190.0 ± 82.3	$461.7 \pm 76.3^*$	195.2 ± 75.8	$498.9 \pm 61.6^*\$$

*: $p < 0.01$ between baseline and 5-year of each group; #: $p < 0.01$ between the anti-M2-R (-) group and the anti-M2-R (+) group at 5-year; §: $p < 0.05$ between the anti-M2-R (-) group and the anti-M2-R (+) group at 5-year. Abbreviations: LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction.

Primary endpoint events

During the 5-year follow-up, four patients died during hospitalization and the deaths were due to the progression of HF. One patient was in the anti-M2-R (-) group and the other three deaths were in the anti-M2-R (+) group. There were sixteen patients re-hospitalized for acute exacerbation of HF, including 4 patients in the anti-M2-R (-) group and 12 patients in the anti-M2-R (+) group ($p = 0.01$). Although it is not advised, three patients in the anti-M2-R (-) group with normalized LVEF had subsequent pregnancies safely without PPCM recurrence. There were no differences in all-cause mortality or cardiovascular mortality between the two groups ($p > 0.05$), as shown in Fig.3.

Discussion

Major Findings

In this study, we observed 82 PPCM patients with long-term low dose of digoxin additional to standard therapy for HF. We observed some characteristics. (I) We found the heart rate of anti-M2-R (+) patients was lower than that in anti-M2-R (-) patients. (II) Patients in the anti-M2-R (-) group had better tolerance to metoprolol and digoxin compared to patients positive for anti-M2-R. (III) With the same dosage of digoxin, the mean SDC was higher in anti-M2-R (+) patients than that in the anti-M2-R (-) patients. (IV) LVEF $\geq 50\%$ was observed in 91.0% of the patients at 5 years. Anti-M2-R (-) patients showed greater reduction of left ventricular diameter and improvement in cardiac function compared to positive patients. (V) Four patients died due to the progression of HF. Risk of hospitalization for HF was decreased in the negative group. However, there were no differences in all-cause mortality or cardiovascular mortality between the two groups.

Digoxin and HF

Digoxin is a traditional drug for HF treatment for more than 200 years. It enhances LVEF by inhibiting the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity on the cardiomyocytes and enhancing myocardial contractility. The effects of digoxin on multiple neurohormones imply that long-term treatment with digoxin can provide benefits to HF patients through inhibiting the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the afferent fibers of the vagus nerve, thus increasing the activity of vagus nervous system and inhibiting the sympathetic activity [9]. Digoxin can also increase the sensitivity of the carotid sinus baroreceptor to further reduce the activity of the sympathetic and RAAS [10].

It is demonstrated that digoxin was able to improve maximum exercise endurance of patients with mild-moderate stable HF and reduce acute exacerbation of HF whether the basic treatment used diuretics alone or diuretics in combination with ACEIs [11-13]. Subsequent studies on digoxin demonstrated that additional use of 0.25 mg/day digoxin on the basis of diuretics and ACEIs could reduce the all-cause and HF exacerbation-induced hospitalization, although it could not reduce the overall mortality of HF patients [14]. A meta-analysis reported that digoxin could reduce the hospitalization rate and relieve clinical symptoms, and it is believed that HF patients could still benefit from the use of digoxin [15].

It is important to note that the SDC was closely correlated with the prognosis of HF. Ahmed et al [16] reported that 0.5-0.9 ng/mL of SDC could reduce the mortality, all-cause hospitalization and HF-associated hospitalization, while $\text{SDC} \geq 1.0$ ng/mL could only reduce the HF-associated hospitalization without impact on the mortality. Similarly, Rathore et al [17] also demonstrated that 0.5-0.8 ng/mL of SDC could reduce the HF-associated mortality. Low-dose of digoxin (≤ 0.125 mg/day) was the strongest predictive factor of the low SDC [16]. Digoxin should be added to maintain the SDC of 0.5-0.9 ng/mL in the early stage of treatment in patients with severe HF, or those whose symptoms are still present after ACEIs and β receptor blockers have begun working [8].

Xu et al [18] have recruited a total of 756 chronic HF patients with reduced LVEF. All the patients received digoxin additional to standard treatment regimen. The SDC was maintained at 0.5–0.9 ng/mL. A clinical follow-up for up to 15 years was performed and it demonstrated well safety and efficacy to long-term low dose of digoxin. Hou et al [19] found the SDC was higher in the anti-M2-R (+) group compared to anti-M2-R (-) group with similar dose of digoxin in chronic HF patients, similar to this study. In this study, all the PPCM patients reacted well to long-term low dose of digoxin treatment. Symptoms of digoxin intoxication occurred when the SDC was significantly elevated, almost about 2.5 ng/mL. Regularly testing of the SDC was essential when using digoxin.

Anti-M2-R and PPCM

The M2 receptor is the main muscarinic acetylcholine receptor expression on cardiomyocytes [20,21]. Anti-M2-R was detected in the idiopathic dilated cardiomyopathy (IDCM) patients firstly [7]. It could induce ventricular enlargement and thinning of the walls, the typical changes of IDCM and PPCM in humans, by monthly immunization peptides in accordance to the sequence of the second extracellular loop of the M2 receptor in rabbits [22,23]. Gimenez et al [24] have immunized mouse by using plasmid DNA encoding entire M2 receptor proteins, which leads to cardiac remodeling and contractile dysfunction. Ribeiro et al [25] found immunization with plasmids encoding M2 receptor epitopes impairs cardiac function in mice and induces autophagy in the myocardium, indicating novel roles for the anti-M2-R. Our previous study suggested anti-M2-R not only existed in IDCM patients, but also in HF caused by different causes, including PPCM. We posit that serum anti-M2-R may be related to cardiac structural and functional changes, which need further studies to clarify [3].

Regulation of the autonomic system has an important influence on the progression of PPCM. Elevated activities of sympathetic system is associated with an adverse prognosis. Activation of the vagus

nervous system seems to be a double-edged sword. With the extensive use of ACEIs and β receptor blockers, activities of sympathetic system was attenuated mainly, but the effects was poorly to the standard pharmacological regimen in a number of patients. For these patients, the tension of vagus nervous system may be activated pathologically. In anti-M2-R (+) patients, the chronic interaction between anti-M2-R and the M2 receptor causes a pathological activation of cardiac vagus nervous system. Therefore, these patients showed a slower heart rate and lower maximum tolerated dose of metoprolol and digoxin compared to anti-M2-R (-) patients. In the anti-M2-R (-) group, the enlarged left ventricular chamber could return to normal more rapidly, which maybe partially referred to different dosages of metoprolol and digoxin between the two groups.

The prognosis of PPCM is better than that of other cardiomyopathies with reduced LVEF. We found the LVEF of 71 patients (91.0%) return to normal at 5 years, similar to previous studies [26,27]. The prognosis of PPCM is different from other cardiomyopathies, it maybe linked to the removal of hormonal toxins by the delivery of placenta and the termination of lactation. The all-cause and cardiovascular mortality of the anti-M2-R (-) group was lower than those in the positive group, but not statistically, which maybe attributed to the small sample size. Hospitalization for HF was decreased in the anti-M2-R (-) group. Therefore, we speculated that anti-M2-R may take part in the pathological process of PPCM and may be an index for prognosis. Early screening of serum anti-M2-R may have predictive value for the improvement of cardiac function with long-term low dose of digoxin additional to standard treatment in PPCM patients.

Conclusions

PPCM patients, with long-term low dose of digoxin additional to standard treatment regimen, have a better prognosis, especially in the anti-M2-R (-) patients. Patients negative for anti-M2-R showed greater tolerance to metoprolol and digoxin. The improvement in cardiac function may partially be related to the regulation of autonomic nervous system.

Abbreviations

ACEI: Angiotensin-converting enzyme inhibitors; Anti-M2-R: Autoantibodies against the M2-muscarinic receptor; HF: Heart failure; IDCM: Idiopathic dilated cardiomyopathy; LVEDD: Left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; LVESD: Left ventricular end-systolic diameter; NYHA: New York Heart Association; PPCM: Peripartum cardiomyopathy; RAAS: Renin-angiotensin-aldosterone system; SDC: Serum digoxin concentration.

Declarations

Ethics approval and consent to participate: The present study was performed in compliance with the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee and the

Prescription and Therapeutic Committee of Beijing Chaoyang Hospital, Capital Medical University (Beijing, China). All the patients provided written informed consent prior to enrolment.

Consent for publication: The authors confirm that written consent from each patient has been obtained to publish the manuscript.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: LZ and XYL conceived and designed the study and were responsible for performing data collection, analysis and interpretation and giving final approval of the version to be published. GLM was responsible for drafting the manuscript and revising it critically for important intellectual content, and made substantial contributions to follow-up of the patients and analysis of data. YY and YDW performed the echocardiographic examination and were responsible for follow-up of the patients. WS and YSL were responsible for collection of patient and laboratory data, data interpretation and revision of the manuscript regarding content. LC and TYL were responsible for performing the study and the data analysis. FS and CS performed the ELISA for anti-M2-R. All authors read and approved the final version of the manuscript for publication.

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Figures

Figure 1

Non-linear fit of metoprolol titration data. With up-titration of metoprolol, the mean dosage of metoprolol in the anti-M2-R (-) group was 38.3 ± 4.9 mg b.i.d., compared with 26.6 ± 4.2 mg b.i.d. in the anti-M2-R (+) group ($p < 0.001$). The mean time to maximum titration in the anti-M2-R (-) group was 67.1 ± 10.8 days, compared with 82.8 ± 11.7 days in the anti-M2-R (+) group ($p < 0.001$).

Figure 2

Frequencies and titers of serum anti-M2-R. The frequencies and geometric mean titers of serum anti-M2-R were obviously decreased with treatment of the PPCM patients. a: Frequency. b, Geometric titer. *: $p < 0.001$ for comparison between baseline and 5-year of frequencies and geometric mean titers of anti-M2-R. Abbreviations: PPCM, peripartum cardiomyopathy

Figure 3

Endpoint events in both groups over 5 years. There were no differences in all-cause mortality or cardiovascular mortality between the positive group and the negative group during the 5 years of follow-up. Hospitalization for heart failure was decreased in the negative group. a: all-cause mortality. b, Cardiovascular mortality. c, Hospitalization for heart failure.