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Original article

Association of autoantibodies against the M2-muscarinic receptor with long-term outcomes in peripartum cardiomyopathy patients: A 5-year prospective study

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ABSTRACT

Background: Peripartum cardiomyopathy (PPCM) is characterized by heart failure. Our previous study found that autoantibodies against the M2-muscarinic receptor (anti-M2-R) are increased in PPCM patients. We aimed to evaluate the association of anti-M2-R on prognosis of PPCM patients with standard treatment.

Methods: Synthetic peptides corresponding to the M2 receptor served as the target antigens in an enzyme-linked immunosorbent assay experiment. They were used to screen the sera of 80 PPCM patients, who were separated into anti-M2-R-negative and positive groups according to their anti-M2-R reactivity. Clinical assessment and echocardiography examination were performed at baseline and after 5 years with a standard treatment regimen. The endpoint events were compared after 5 years of follow-up.

Results: There were 76 PPCM patients who completed the final data analysis, including 36 in the anti-M2-R (+) group and 40 in the anti-M2-R (−) group. Both groups showed improvement in the left ventricular end-diastolic and end-systolic dimensions and the ejection fraction with standard treatment regimens for 5 years (all $p < 0.001$). Patients in the anti-M2-R (−) group had greater tolerance and were more rapidly titrated to metoprolol, and they had better improvement in cardiac function than patients in the anti-M2-R (+) group ($p < 0.05$). Patients in the anti-M2-R (−) group had a marked decrease in re-hospitalization ($p < 0.05$), but not in all-cause mortality or cardiovascular mortality. Being positive for anti-M2-R increased the risk of PPCM (OR = 4.7, 95% CI 1.8–12.2, $p = 0.002$).

Conclusions: PPCM patients, especially anti-M2-R (−) patients, have a relatively better prognosis than other patients. We posit that the presence of anti-M2-R may be involved in the pathogenesis of PPCM.

Introduction

Peripartum cardiomyopathy (PPCM) is a relatively rare and progressive cardiac disease that occurs with idiopathic dilated cardiomyopathy. PPCM is defined by clinical manifestations of signs and symptoms of heart failure 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/m^2 body surface area [2]. PPCM can occur all over the world, and the most notable risk factors for PPCM are maternal age, race, multiple gestation, and co-occurrence of preeclampsia [3]. Although the mechanism of PPCM is still unclear, increasing evidence indicates that autoimmunity may play an important role in the development of PPCM [4,5].

Autoimmune disorders have been shown to play a critical role in the pathogenesis of heart failure. Autoimmune responses and autoreactive autoantibodies, such as autoantibodies against β1, β2, α1-adrenergic receptors, autoantibodies against angiotensin II

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type 1 receptor (anti-AT1-R), and autoantibodies against M2-muscarinic receptor (anti-M2-R), have been found in heart failure patients of various etiologies [6]. Further studies have shown that these autoantibodies can interfere with radioligand binding on the target receptor, display various agonist-like activities on the corresponding cardiac receptors, and hence modulate cardiac function [7]. We found that heart failure patients with different reactivities to autoantibodies against β1-adrenergic receptor (anti-β1-R) and anti-AT1-R had different responses to β-blockers and angiotensin-converting enzyme inhibitor (ACEI), respectively [8,9].

We found the positive rate was 59.5% for anti-β1-R and 45.9% for anti-M2-R in PPCM patients [10]. The excessive activation of the sympathetic nervous system in heart failure patients has been accepted by most doctors. β-blockers play a fundamental role in the treatment of heart failure. However, the importance of the parasympathetic nervous system is still unclear, and the relationship between anti-M2-R and the treatment and prognosis of PPCM is largely unknown. So, what is the clinical value of anti-M2-R? Are there any different responses to β-blockers between anti-M2-R positive and negative patients? If so, do patients negative for anti-M2-R have greater improvement in heart function than patients who are positive for anti-M2-R?

In this study, we evaluated the benefit of long-term standard treatment regimens for heart failure (ACEI, β-blocker, furosemide, spironolactone, and/or digoxin) on PPCM patients. We also researched the presence of anti-M2-R and heart function improvement in response to β-blocker in PPCM patients.

Materials and methods

Study population

This was a single center, prospective, observational study, which began in January 1998 and ended in December 2017. A total of 80 consecutive patients with newly diagnosed PPCM were enrolled at the heart failure clinic of Beijing Chao-Yang Hospital. During the same period, we selected 80 normal pregnant women from the obstetric ward of our hospital and 80 non-pregnant age-matched women from the physical examination center as control groups. The demographic data and information of related risk factors were obtained by in-person interview using a structured questionnaire, including socioeconomic status (good/poor). Good socioeconomic status refers to patients who came from a city and who had a university degree. All of the other patients were defined as poor socioeconomic status. The inclusion criteria of the PPCM group were as follows: (1) age between 18 and 40 years old, (2) New York Heart Association functional classes (NYHA) II-IV, (3) symptoms of congestive heart failure developed in the last month of pregnancy or during the first 5 months postpartum, (4) no other identifiable causes for heart failure, and (5) LVEF $<$45% by transthoracic echocardiography. Exclusion criteria were as follows: (1) clinical conditions other than cardiomyopathy with increased autoantibody levels observed by screening the serum for rheumatoid arthritis, human immunodeficiency virus, and evidence for sepsis, (2) moderate-severe anemia (hemoglobin concentration $<$9 g/dL), (3) metabolic disorders affecting lipoprotein metabolism such as thyroid disease, or (4) moderate-to-severe hepatic or renal dysfunction.

Serum anti-M2-R detection

Blood (2 mL) was withdrawn from the antecubital vein of each subject before the initiation of therapy and separated by centrifugation at 3,000 rpm for 10 min. Serum samples were stored at -20 °C until needed for assays. The serum anti-M2-R was measured with an enzyme-linked immunosorbent assay (ELISA) using a synthetic peptide corresponding to the sequence of the second extracellular loop of the human M2 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). The peptide was synthesized by Genomed (Genomed Synthesis, Inc., San Francisco, CA, USA). The purity of the peptide, determined by high performance liquid chromatography using a Vydac C-18 column, was 98%. An ELISA protocol, previously described by Fu et al., was used for screening. The intra-assay and inter-assay coefficients of variation were less than 5%. The detection range of absorbance (A) was up to 2.5. Further dilution was done when the absorbance was over the upper limit. Positive was defined as a ratio of (sample A – blank A)/(negative control A – blank A) $\geq$2.1. The autoantibody titer was highest when this ratio $\geq$2.1, which occurred when the antibody was diluted from 1:20 to 1:160 [11].

Beginning of the standard pharmacological regimen

All of the PPCM patients received standard therapy regimens (furosemide 10–20 mg/day, perindopril 2–4 mg/day or losartan 25–50 mg/day, spironolactone 10–20 mg/day, and metoprolol at an initial daily dose of 12.5 mg/day that was then up-titrated over a 2–4-week period by doubling the twice-daily amount to the maximum tolerated dose) [1]. The maximum tolerated heart rate and blood pressure were 60–75 bpm and 120/65 $\pm$ 10/5 mmHg, respectively. The dose of furosemide was permitted to be increased if a patient displayed signs or symptoms of heart failure progression. In addition, patients were strongly advised to reduce their salt intake to approximately 5 g of sodium chloride daily, to restrict their intake of food, and to control their body weight. The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Beijing, China. All of the participants provided written informed consent prior to admission into the study.

Follow-up examination

All of the patients were assigned to one of the designated study investigators and received follow-up examinations for 5 years after the initiation of the study. The primary endpoint events were all-cause mortality, cardiovascular mortality, and re-hospitalization for heart failure. Patients were encouraged to schedule interim appointments and follow up at least once per month for the first 12 months and then every 3–6 months for up to 5 years or until the primary endpoint. Concurrently, echocardiography, 6-minute walk tests, and clinical laboratory tests were also performed. Data collected during the examinations included heart rate, blood pressure, body weight, the presence of rales during the pulmonary exam, cardiac murmur, the presence of peripheral edema, drug doses, and the presence of any adverse drug reactions, based on the physician record system.

Statistical methods

Quantitative data are presented as the mean $\pm$ SD, and categorical data are presented as percentage. Antibody titer was reported as the geometric mean. For two-group comparison, we used the 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 heart failure. Univariate and multivariate analyses were performed to evaluate the association of anti-M2-R and onset of PPCM by the stepwise logistic regression model, in which age, socioeconomic status, parity, multiple gestation, and pregnancy-

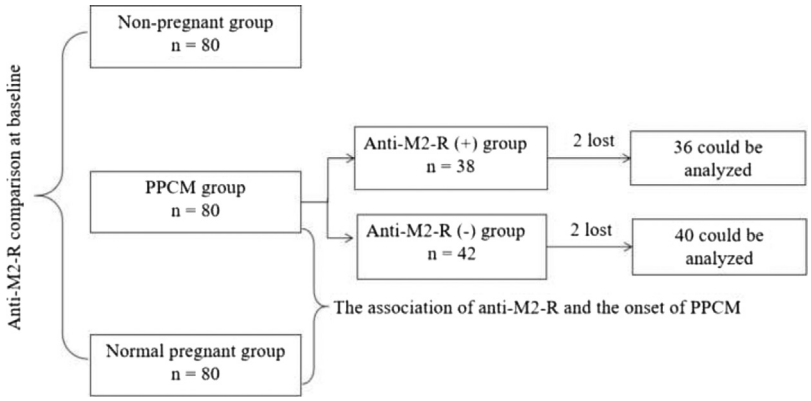

Fig. 1. Enrollment and outcomes. This study was divided into three sections of observational studies. In the first case-control study, serum levels of anti-M2-muscarinic receptor (anti-M2-R) were detected in 240 subjects including 80 PPCM patients, 80 normal pregnant women, and 80 non-pregnant controls. In the second case-control study, the association of anti-M2-R and the onset of PPCM was assessed between the 80 PPCM patients and 80 normal pregnant women. In the third section, a prospective study was performed to examine the possible correlation between serum anti-M2-R and clinical prognosis in the 80 PPCM patients with standard treatment. PPCM, peripartum cardiomyopathy.

induced hypertension were adjusted. Cut-offs for entry and departure for the logistic regression model were 0.05 and 0.10. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the association of anti-M2-R and the risk of PPCM. Metoprolol titration data were fit to a variable slope sigmoidal equation [Y = initial dose + (maximum dose − initial dose)/(1 + 10 (LogEC50 − X) × 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 of the tests were 2-tailed. Data were analyzed using SPSS 20.0 (SPSS, Chicago, IL, USA). A value of $p < 0.05$ was considered to be statistically significant.

Results

Study characteristics

This study was divided into three sections of observational studies (Fig. 1). The characteristics of the study population, including patients with PPCM, the normal pregnant group, and the non-pregnant group, are summarized in Table 1. Compared with the non-pregnant group and the normal pregnant group, PPCM patients had a higher heart rate and blood pressure ($p < 0.001$). Left ventricular dimension was significantly enlarged, and LVEF was obviously decreased ($p < 0.001$). In the PPCM group, 80 patients were diagnosed as having PPCM for the first time. In this group, 28 patients were primiparous, and 24 patients had multiple gestation. There were 29 patients who had pregnancy-induced hypertension, and 17 patients had gestational diabetes mellitus. There were 42 patients with symptoms in the postpartum period, all within three months after delivery. At baseline, 31 patients were in NYHA functional class II, 34 patients were in class III, and 15 patients were in class IV.

Based on anti-M2-R reactivity, 38 patients were assigned to the anti-M2-R (+) group, and 42 patients were assigned to the anti-M2-R (−) group. The baseline characteristics of the two groups are shown in Table 2. Four patients were lost to follow-up (2 patients in the positive group and 2 patients in the negative group). There were 76 patients who completed the data analysis in December 2017, including 36 patients (36/38, 94.7%) in the positive group and 40 patients (40/42, 95.2%) in the negative group.

Cardiac function and 6-min walk test

In the PPCM group, clinical data, NYHA functional class, echocardiographic results, and 6-min walk distance at baseline and five years were determined, as shown in Table 3. Upon standard pharmacological treatment for heart failure, the left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) significantly decreased from 59.0 ± 7.9 to 47.7 ± 4.7 mm and 46.6 ± 8.4 to 33.1 ± 5.6 mm in the anti-M2-R (−) group, respectively, and from 58.8 ± 7.1 to 50.2 ± 4.7 mm and 46.7 ± 6.9 to 36.4 ± 6.5 mm in the anti-M2-R (+) group, respectively. Consistently, the LVEF markedly increased from 39.3 ± 4.5% to 62.7 ± 6.2% in the anti-M2-R (−) group and from 39.4 ± 5.9% to 57.3 ± 7.4% in the anti-M2-R (+) group. Notably,

Table 1
Clinical characteristics of study population.

	Non-pregnant group $n = 80$	Normal pregnant group $n = 80$	PPCM group $n = 80$
Age (years)	28.3 ± 4.5	28.5 ± 4.3	29.2 ± 4.3
Blood pressure (mmHg)			
Systolic	112.6 ± 7.5	111.2 ± 7.0	135.7 ± 14.9*
Diastolic	70.4 ± 5.6	71.6 ± 5.8	87.7 ± 10.7*
Heart rate (bpm)	76.4 ± 8.2	88.2 ± 10.1#	99.2 ± 7.4*
Echocardiographic data			
LVEDD (mm)	44.6 ± 4.7	45.0 ± 5.0	58.9 ± 7.5*
LVESD (mm)	27.9 ± 4.0	28.2 ± 3.6	47.1 ± 7.2*
LVEF (%)	70.2 ± 4.9	69.4 ± 5.8	39.3 ± 5.1*

PPCM, peripartum cardiomyopathy; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction. One-way ANOVA and unpaired Student's t-test were made among the three groups or between two groups.
* $p < 0.001$ among the three groups.
# $p < 0.001$ between the normal pregnant group and the non-pregnant group.

Table 2
Clinical characteristics of PPCM patients.

	Anti-M2-R (+) group $n=38$	Anti-M2-R (−) group $n=42$	p-Value
Age (years)	29.3 ± 4.7	29.0 ± 3.9	0.738
Multiple gestation	11	13	0.845
Multiparity	24	28	0.742
Pregnancy complication			
Pregnancy-induced hypertension	15	14	0.568
Pre-eclampsia	9	7	0.433
Gestational diabetes mellitus	9	8	0.613
Postpartum diagnosed PPCM	20	22	0.982
NYHA functional class	2.8 ± 0.8	2.8 ± 0.7	0.808
Blood pressure (mmHg)			
Systolic	136.2 ± 14.9	135.2 ± 15.0	0.766
Diastolic	88.3 ± 10.9	87.2 ± 10.7	0.651
Heart rate (bpm)	95.8 ± 6.9	102.2 ± 6.6	<0.001
Echocardiographic data			
LVEDD (mm)	58.8 ± 7.1	59.0 ± 7.9	0.905
LVESD (mm)	46.7 ± 6.9	46.6 ± 8.4	0.954
LVEF (%)	39.4 ± 5.9	39.3 ± 4.5	0.933
6-min walk test (m)	190.3 ± 84.4	196.9 ± 78.0	0.720

Anti-M2-R, anti-M2-muscarinic receptor; PPCM, peripartum cardiomyopathy; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.
Unpaired Student's t-test was made between the two groups.

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

	Anti-M2-R (+) group		Anti-M2-R (−) group	
	Baseline $n=38$	5 years $n=33$	Baseline $n=42$	5 years $n=39$
Blood pressure (mmHg)				
Systolic	136.2 ± 14.9	115.0 ± 9.3*	135.2 ± 15.0	115.4 ± 8.4*
Diastolic	88.3 ± 10.9	66.3 ± 6.1*	87.2 ± 10.7	65.4 ± 5.4*
Heart rate (bpm)	95.8 ± 6.9	68.9 ± 2.0*	102.2 ± 6.6	68.3 ± 2.1*
NYHA functional class	2.8 ± 0.8	1.3 ± 0.5*	2.8 ± 0.7	1.2 ± 0.4*
Echocardiographic data				
LVEDD (mm)	58.8 ± 7.1	50.2 ± 4.7*	59.0 ± 7.9	47.7 ± 4.7*,§
LVESD (mm)	46.7 ± 6.9	36.4 ± 6.5*	46.6 ± 8.4	33.1 ± 5.6*,§
LVEF (%)	39.4 ± 5.9	57.3 ± 7.4*	39.3 ± 4.5	62.7 ± 6.2*,#
6-min walk test (m)	190.3 ± 84.4	460.6 ± 77.9*	196.9 ± 78.9	497.7 ± 65.0*,§

Anti-M2-R, anti-M2-muscarinic receptor; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.
* $p<0.01$ between baseline and 5 years of each group.
# $p<0.01$ between the anti-M2-R (−) group and the anti-M2-R (+) group at 5 years.
§ $p<0.05$ between the anti-M2-R (−) group and the anti-M2-R (+) group at 5 years.

anti-M2-R (−) patients had greater improvements in left ventricular remodeling and heart function than anti-M2-R (+) patients after treatment. Normalization of LVEF (≥50%) was observed in 90.3% of patients (65/72) at five years. The distance covered in the 6-min walk test was more increased in the anti-M2-R (−) group compared to the anti-M2-R (+) group after five years of treatment. Clinical laboratory data, including hemoglobin, creatinine, glutamic pyruvic transaminase, and potassium levels remained stable throughout the follow-up.

Drug dosage

All of the PPCM patients received standard pharmacological regimen, which includes perindopril, metoprolol, spironolactone, and diuretics (3.2 ± 1.0 mg of perindopril, 32.5 ± 7.0 mg b.i.d. of metoprolol, 18.2 ± 3.9 mg of spironolactone, and 18.8 ± 3.3 mg of furosemide). There were no differences between the anti-M2-R (−) group and the anti-M2-R (+) group on the dosage of perindopril, spironolactone, or furosemide. The maximum tolerated dose of metoprolol in the anti-M2-R (−) group was higher than that in the anti-M2-R (+) group (38.1 ± 4.7 mg b.i.d. vs. 26.6 ± 4.3 mg b.i.d., $p<0.001$).

Titration of metoprolol

During the first year of treatment, the maximum tolerated dose of metoprolol for the anti-M2-R (−) group was 38.1 ± 4.7 mg b.i.d., which was higher than 26.6 ± 4.3 mg b.i.d. for the anti-M2-R (+) group ($p<0.001$), as shown in Fig. 2. The mean time to maximum tolerated dose of metoprolol in the anti-M2-R (−) group was 67.2 ± 10.1 days, which was shorter than the 83.1 ± 11.8 days required in the anti-M2-R (+) group ($p<0.001$), indicating that anti-M2-R (−) patients have greater tolerance and a more rapid rate of up-titration of metoprolol.

Dynamic variation of anti-M2-R

Sera positive for anti-M2-R was found in 47.5% (38/80) of the PPCM group, 10.0% (8/80) of the normal pregnant group ($p<0.001$ vs. the PPCM group), and 8.8% (7/80) of the non-

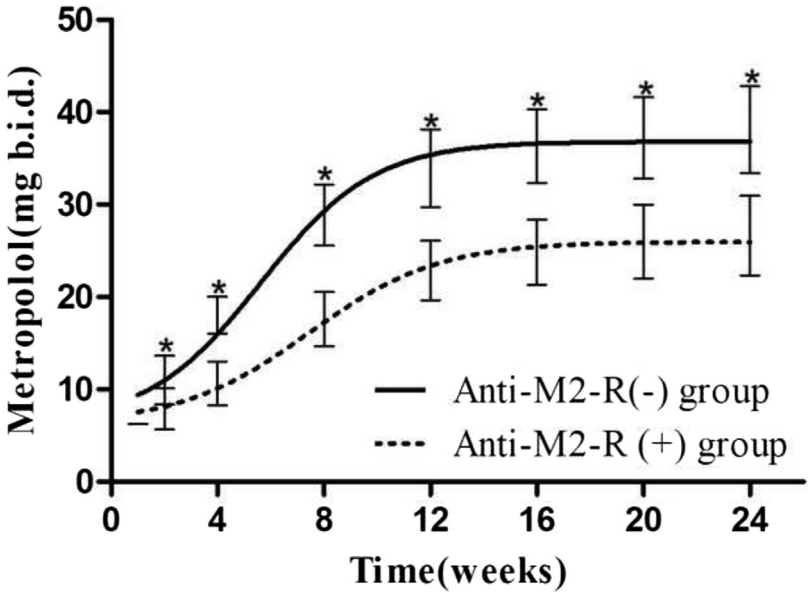

Fig. 2. Non-linear fit of metoprolol titration data. With up-titration of metoprolol, the mean metoprolol dose in the anti-M2-muscarinic receptor (anti-M2-R) (−) group was 38.1 ± 4.7 mg b.i.d., compared with 26.6 ± 4.3 mg b.i.d. in the anti-M2-R (+) group ($p < 0.001$). The mean time to maximum titration in the anti-M2-R (−) group was 67.2 ± 10.1 days, compared with 83.1 ± 11.8 days in the anti-M2-R (+) group ($p < 0.001$).

pregnant group ($p < 0.001$ vs. the PPCM group) at baseline. There was no difference in positive rate between the normal pregnant group and the non-pregnant group. In positive cases, the mean titers of anti-M2-R in the PPCM group was 1:121, whereas the titers were 1:47 ($p < 0.001$ vs. the PPCM group) in the normal pregnant group and 1:36 in the non-pregnant group ($p < 0.001$ vs. the PPCM group). After five years of treatment, the positive rate of anti-M2-R was 9.7% (7/72), and the geometric titer of anti-M2-R was 1:66, which was significantly decreased compared to baseline (all $p < 0.001$) (Fig. 3).

Primary endpoint events

During the five years of follow-up, four patients died during hospitalization and the deaths were due to the progression of heart failure. One patient was in the anti-M2-R (−) group and the other three deaths were in the anti-M2-R (+) group with high titers of autoantibodies. There were 15 patients who were re-hospitalized for exacerbation of heart failure, of which 4 patients were in the anti-M2-R (−) group and 11 patients were in the anti-M2-R (+) group ($p = 0.02$). Although it is not advised, two 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 and cardiovascular mortality between the two groups ($p > 0.05$), as shown in Fig. 4.

The association of anti-M2-R and the onset of PPCM

To identify the association of anti-M2-R and the onset of PPCM, we compared the positive rates of serum anti-M2-R between 80 PPCM cases and 80 normal pregnant controls. A multivariate analysis was done to evaluate the effect of the following variables on the onset of PPCM: anti-M2-R (positive vs. negative), advanced maternal age (<35 years vs. ≥35 years), multiple gestation (yes vs. no), pregnancy-induced hypertension (yes vs. no), and poor socioeconomic status (yes vs. no). Among these, anti-M2-R (OR = 4.7, 95% CI, 1.8–12.2, $p = 0.002$) was the one independent predictor for the onset risk of PPCM. This result is shown in Table 4.

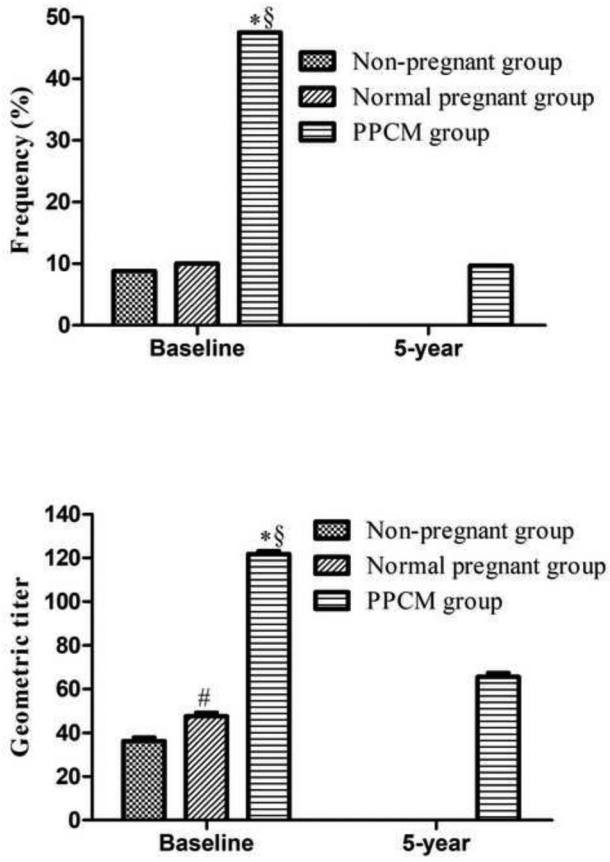

Fig. 3. Frequencies and titers of anti-M2-muscarinic receptor (anti-M2-R). Frequencies and geometric mean titers of anti-M2-R were significantly higher in the PPCM group than in the normal pregnant group and the non-pregnant group. In the PPCM group, the frequency and geometric mean titer of anti-M2-R were obviously decreased with treatment. * $p < 0.001$ for the PPCM group compared to the normal pregnant group and non-pregnant group at baseline; # $p < 0.001$ for the normal pregnant group compared to the non-pregnant group at baseline; § $p < 0.001$ compared between baseline and 5-year in the PPCM group. PPCM, peripartum cardiomyopathy.

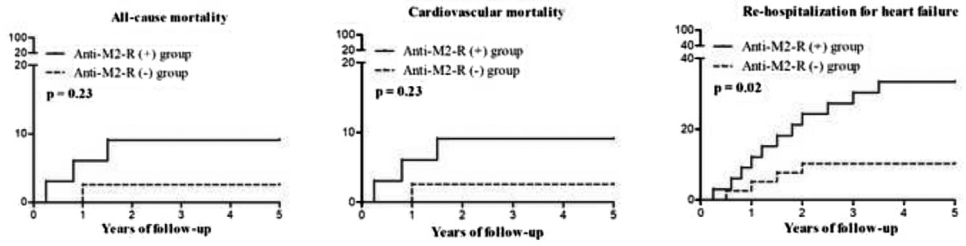

Fig. 4. Endpoint events in both groups over 5 years. There were no differences in all-cause mortality or cardiovascular mortality between the positive group and the negative group during the 5 years of follow-up. Hospitalization for heart failure was decreased in the negative group. Anti-M2-R, anti-M2-muscarinic receptor.

Table 4
Univariate and multivariate analysis for the onset of peripartum cardiomyopathy.

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
Advanced maternal age	2.8	1.1–7.2	0.031	1.1	0.2–5.1	0.903
Multiple gestations	3.9	1.6–9.2	0.002	1.7	0.2–11.3	0.595
Multiparity	1.2	0.6–2.2	0.623			
Poor socioeconomic status	3.4	1.5–7.4	0.002	1.3	0.2–6.9	0.755
Positive for anti-M2-R	8.1	3.5–19.1	<0.001	4.7	1.8–12.2	0.002

Anti-M2-R, anti-M2-muscarinic receptor; OR, odds ratio; CI, confidence interval.
p-Value from multivariable logistic regression analysis adjusted for advanced maternal age, multiple gestations, poor socioeconomic status, and pregnancy-induced hypertension.

Discussion

Major findings

Over the past 20 years, we observed 76 PPCM patients with standard therapy for heart failure. There are some novel findings. (I) Four of the 76 patients died due to progression of heart failure. Cardiac function of the other 72 patients improved with 5 years of follow-up. LVEF ≥50% was observed in 90.3% of the patients at 5 years. (II) We demonstrated that the frequency and titer of serum anti-M2-R in PPCM patients were higher than in a normal pregnant group and a non-pregnant group. (III) Anti-M2-R (−) patients showed rapid up-titration and higher maximum tolerated dose of metoprolol than did patients positive for the autoantibodies. Anti-M2-R (−) patients showed greater reduction of left ventricular diameter and improvement in cardiac function compared to positive patients. (IV) The frequency and titer of serum anti-M2-R in the PPCM group decreased remarkably with treatment. (V) Hospitalization for heart failure was decreased in the negative group. However, there were no differences in all-cause mortality or cardiovascular mortality between the two groups.

Anti-M2-R and heart failure

The M2 receptor, which belongs to the family of the G-protein-coupled receptors, is the main muscarinic acetylcholine receptor present on myocardial tissue [12,13]. Anti-M2-R were first detected in idiopathic dilated cardiomyopathy (IDCM) [11]. Monthly immunization of rabbits using synthetic peptides corresponding to the sequence of the second extracellular loop of the M2 receptor could induce cardiac morphological changes including enlarged ventricles and thinner walls, the typical changes of IDCM and PPCM in humans [14,15]. Gimenez et al. [16] have attempted to conduct mouse immunization by using plasmid DNA encoding entire M2 receptor proteins, which can lead to contractile dysfunction and cardiac remodeling. Further study revealed some ultrastructural alterations suggesting autophagy and mitophagy in mice immunized with a plasmid encoding an M2 receptor epitope, suggesting novel roles for the anti-M2-R [17]. Our previous study showed that anti-M2-R not only existed in IDCM patients, but also in heart failure caused by different cardiac diseases, such as ischemic cardiomyopathy, hypertensive heart disease, and rheumatic valvular heart disease. Moreover, the serum levels of anti-M2-R have no significant differences among different etiologies. We posit that serum anti-M2-R may be related to cardiac structural and functional changes, which need further studies to clarify [6].

During the five years follow-up period, we observed the interesting phenomenon that serum anti-M2-R gradually decreased or disappeared with standard treatment regimen. In our previous studies, we found similar phenomenon that serum anti-β1-R and anti-AT1-R decreased or disappeared with standard treatment, which may be related to the use of β-blocker and ACEIs and the improvement in heart function [8,9]. However, the specific mechanism is still unclear.

Autoimmune mechanisms in PPCM

Multiple etiologies have been identified as potentially leading to PPCM. Abnormal prolactin metabolism caused by oxidative stress-related improper cleaving of the hormone into an active, antiangiogenic form disturbs cardiomyocyte angiogenesis, leading to heart failure [18]. Fms-like tyrosine kinase-1 (Flt1) is a membrane-bound decoy receptor for vascular endothelial growth factors (VEGFs). In the mouse PPCM models, neutralization of VEGF by sFlt1 was sufficient to induce cardiac failure. Several dysregulated immune responses have been associated with the development of PPCM. Fetal cells gain access to the maternal circulation, but maternal immunity usually destroys them. These fetal chimera may escape the weakened maternal immunity and nestle in the maternal myocardium. After delivery, the foreign pathogens will be attacked by normal maternal immunity [19]. High titers of autoantibodies against selected cardiac tissue proteins have been found in the majority of women with PPCM [4]. It has been reported that the role of humoral immunity and class G and all subclass immunoglobulins against cardiac myosin heavy chain were raised in PPCM [20]. Our previous study first detected circulating anti-M2-R in PPCM patients, while there were many issues that still need to be clarified [10].

Autonomic nerve regulation in PPCM

Autonomic regulation has an important influence on the progression of PPCM. Although elevated sympathetic activity is associated with an adverse prognosis, a high level of parasympathetic activation confers cardioprotection by several potential mechanisms. Parasympathetic activation seems to be a double-edged sword. Parasympathetic stimulation is reduced after depolarizations during adrenergic activation, potentially attenuating β-adrenergic-mediated increases in myocyte contractility, and depresses cardiac function [21,22]. With the broad use of ACEIs and β-blockers, sympathetic tone was attenuated, but some patients still reacted poorly to the standard pharmacological regimen. For these patients, the parasympathetic tone may be activated pathologically. In the anti-M2-R (+) patients, the chronic interaction between anti-M2-R and M2 receptor cause a pathological activation of the cardiac parasympathetic system. As a consequence, these patients have a slower heart rate and lower maximum tolerated dose of metoprolol compared to anti-M2-R (−) patients. During the 5 years of follow-up, the heart function and enlarged left ventricular chamber may return to normal levels more rapidly in anti-M2-R (−) patients. As to the different dosages of metoprolol in the two groups, the possibility that the improvement in cardiac function depended on the different dosages of metoprolol cannot be excluded.

Anti-M2-R and the prognosis of PPCM

The prognosis of PPCM is generally better than that of other cardiomyopathies of reduced systolic function. In this study, normalization of LVEF was observed in 65 patients (90.3%) at 5 years, similar to previous studies [23,24]. The improvement of PPCM is different from other cardiomyopathies, as it may be related to the removal of hormonal toxins with the delivery of the placenta and the termination of lactation. 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. This seems to improve more in the anti-M2-R (−) group after five years of treatment. Therefore, we hypothesized that anti-M2-R may participate in the pathological changes of PPCM and may be a quantifiable index for prognosis. In this study, we further investigated the association between anti-M2-R and the onset of PPCM. Early detection of serum anti-M2-R may have predictive value for cardiac function improvement with long-term standard treatment in PCCM patients.

Conclusions

Anti-M2-R was prevalent in a cohort of PPCM patients. PPCM patients, with standard treatment regimens, have a relatively better prognosis. Patients in the anti-M2-R (−) group showed greater tolerance to metoprolol and improvement in cardiac function, which may be related to regulation of the autonomic nervous system. We propose that anti-M2-R may be involved in the pathogenesis of PPCM. Further studies are needed to dissect the underlying mechanisms for this observation.

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Conflict of interest

There is no conflict of interest.

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