samples were assayed by immunoblotting. For depletion of CD protein, LV supernatant was incubated with a CD neutralizing antibody (Nr. 06-467, Upstate) and subsequently protein A-agarose (Roche) to sediment and remove CD from supernatant.

Real-Time PCR and Immunoblot Analyses

Real-time measurements of PCR amplification were performed by using the Stratagene MX4000 multiplex QPCR System using the SYBR green dye method (Brilliant SYBR Green Mastermix-Kit, Stratagene) as described (Klein et al., 2005). Primer sequences are listed in the Supplemental Experimental Procedures. Protein expression levels were determined by western blotting according to standard procedures; antibodies are listed in the Supplemental Experimental Procedures.

Statistical Analyses

Data are presented as mean ± SD. Differences between groups were analyzed by Mann-Whitney test, log-rank test, Student's t test, or ANOVA followed by Bonferroni as appropriate. A two-tailed p value of <0.05 was considered to indicate statistical significance.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, Supplemental References, ten figures, and ten tables and can be found with this article online at http://www.cell.com/cgi/content/full/128/3/589/DC1/.

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Evaluation of Bromocriptine in the Treatment of Acute Severe Peripartum Cardiomyopathy

A Proof-of-Concept Pilot Study

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Background—Peripartum cardiomyopathy (PPCM) is a potentially life-threatening heart disease that occurs in previously healthy women. We identified prolactin, mainly its 16-kDa angiostatic and proapoptotic form, as a key factor in PPCM pathophysiology. Previous reports suggest that bromocriptine may have beneficial effects in women with acute onset of PPCM.

Methods and Results—A prospective, single-center, randomized, open-label, proof-of-concept pilot study of women with newly diagnosed PPCM receiving standard care (PPCM-Std; n=10) versus standard care plus bromocriptine for 8 weeks (PPCM-Br, n=10) was conducted. Because mothers receiving bromocriptine could not breast-feed, the 6-month outcome of their children (n=21) was studied as a secondary end point. Blinded clinical, hemodynamic, and echocardiographic assessments were performed at baseline and 6 months after diagnosis. Cardiac magnetic resonance imaging was performed 4 to 6 weeks after diagnosis in PPCM-Br patients. There were no significant differences in baseline characteristics, including serum 16-kDa prolactin levels and cathepsin D activity, between the 2 study groups. PPCM-Br patients displayed greater recovery of left ventricular ejection fraction (27% to 58%; $P=0.012$) compared with PPCM-Std patients (27% to 36%) at 6 months. One patient in the PPCM-Br group died compared with 4 patients in the PPCM-Std group. Significantly fewer PPCM-Br patients (n=1, 10%) experienced the composite end point of poor outcome defined as death, New York Heart Association functional class III/IV, or left ventricular ejection fraction <35% at 6 months compared with the PPCM-Std patients (n=8, 80%; $P=0.006$). Cardiac magnetic resonance imaging revealed no intracavitary thrombi. Infants of mothers in both groups showed normal growth and survival.

Conclusions—In this trial, the addition of bromocriptine to standard heart failure therapy appeared to improve left ventricular ejection fraction and a composite clinical outcome in women with acute severe PPCM, although the number of patients studied was small and the results cannot be considered definitive. Larger-scale multicenter and blinded studies are in progress to test this strategy more robustly. (***Circulation.*** **2010;121:1465-1473.)**

Key Words: cardiomyopathy ■ heart failure ■ hormones ■ parturition ■ pregnancy

Peripartum cardiomyopathy (PPCM) is characterized by new onset of heart failure between 1 month before and 5 months after delivery in previously healthy women.[1] The clinical presentation and management of PPCM and its outcome have been reviewed recently.[1,2] Only 23% to 54% of patients show recovery of cardiac function within 6 months.[3–6] Investigation of a large cohort of PPCM patients demonstrated that this condition is associated with a proinflammatory response, as evidenced by elevated plasma levels of tumor necrosis factor-α, Fas-Apo-1, interleukin-6, and C-reactive protein (CRP).[5,7,8]

Editorial see p 1463
Clinical Perspective on p 1473

We recently reported that enhanced oxidative stress in a mouse model for PPCM (mice with a cardiac-specific deletion for signal transducer and activator of transcription-3) triggers the activation of cathepsin D, a ubiquitous lysosomal

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This study is a proof-of-concept study and was initiated before the new Declaration of Helsinki 2008 was published. Therefore, it has not been registered as a clinical trial on a publicly accessible Web site.

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Table 1. Baseline Characteristics, Treatment, and 6-Month Results for 20 PPCM Patients

Patient	Group	Age, y	Parity, n	Symptom Onset Postpartum, d	Carvedilol Dose, mg BID	Enalapril Dose, mg/d	Furosemide Dose, mg/d	Aldactone Dose, mg/d
1	PPCM-Std	23	2	25	6.25	10	80	25
4	PPCM-Std	21	2	18	12.5	10	80	25
5	PPCM-Std	22	1	20	6.25	5	80	25
9	PPCM-Std	46	3	21	12.5	10	120	50
10	PPCM-Std	24	2	26	25	10	80	25
12	PPCM-Std	21	1	26	6.25	5	80	0
13	PPCM-Std	24	1	22	25	10	80	25
16	PPCM-Std	44	6	28	12.5	5	80	0
17	PPCM-Std	18	1	12	6.25	5	80	0
20	PPCM-Std	38	3	7	12.5	10	80	25
2	PPCM-Br	22	2	8	6.25	5	80	25
3	PPCM-Br	38	3	14	6.25	5	80	12.5
6	PPCM-Br	24	1	26	12.5	5	80	25
7	PPCM-Br	22	2	7	6.25	5	80	25
8	PPCM-Br	18	2	24	6.25	5	80	25
11	PPCM-Br	24	2	7	6.25	10	120	25
14	PPCM-Br	23	1	4	25	5	80	50
15	PPCM-Br	28	1	30	25	5	80	25
18	PPCM-Br	22	1	2	6.25	5	80	25
19	PPCM-Br	18	1	3	12.5	5	120	0

LVEDD indicates LV end-diastolic diameter; CHF, congestive heart failure; and NR, not reported.

enzyme that subsequently cleaves serum prolactin into its antiangiogenic and proapoptotic 16-kDa form.[9] This is associated with endothelial inflammation, impaired cardiomyocyte metabolism, and reduced myocardial contraction, suggesting that oxidative stress, inflammation, and prolactin may be interconnected and responsible for initiating PPCM.

Similarly, we found evidence for increased oxidative stress, enhanced cathepsin D activity, and increased prolactin cleavage in patients with acute PPCM.[9] More recently, we documented a close correlation between N-terminal brain natriuretic peptide (NT-proBNP; a marker of ventricular wall stress and heart failure), prolactin, and markers of oxidative stress (oxidized low-density lipoprotein) and inflammation (interferon-γ), further supporting the detrimental role of the oxidative stress–prolactin axis.[10]

Importantly, blockade of prolactin with the dopamine-2D agonist bromocriptine prevented the onset of PPCM in mice and in 6 women at high risk of this condition as a result of documented PPCM in a previous pregnancy.[9] Several case reports have also described seemingly beneficial effects from the addition of bromocriptine to standard heart failure therapy in patients with acute PPCM.[9,11,12] Although these preliminary results suggesting beneficial effects of bromocriptine treatment in patients with acute PPCM appear promising, concerns have been raised about the risk of thrombotic complications, including cerebral vascular incident and myocardial infarction, related to bromocriptine therapy[13–16] and the consequences for the children of these patients because the mothers are unable to breast-feed.[17]

The present work summarizes data from the first randomized study to assess the efficacy of bromocriptine on recovery of left ventricular (LV) function, symptom status, and other clinical measures in patients presenting within the first month postpartum with new-onset symptomatic PPCM and an LV ejection fraction (LVEF) <35%. The progress of the newborn children over the 6-month follow-up period was also studied. All open-label efficacy assessments were made by independent blinded investigators.

Methods

Study Design and Patient Recruitment

This study was approved by the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa, and complies with the Declaration of Helsinki. All patients and control subjects gave written informed consent before study entry. Twenty consenting consecutive patients diagnosed with PPCM and fulfilling the inclusion criteria were enrolled in the study. All patients were included and randomized with a computer-generated randomization list within 24 hours of diagnosis.

The study was conducted at the Chris Hani Baragwanath Hospital. Patients were referred from local clinics, secondary hospitals, and the Department of Obstetrics at the Chris Hani Baragwanath Hospital. History of preexisting cardiac symptoms and signs, occurrence of preeclampsia, and mode of delivery were obtained from the patient and confirmed by examination of the obstetric card carried by each patient. Symptoms and signs were recorded during first presentation at the cardiac unit at the Chris Hani Baragwanath Hospital (baseline) and after a follow-up period of 6 months. Clinical assessment, echocardiography, and blood analysis were performed at baseline and at 6 months. Cardiac magnetic resonance imaging (MRI) was obtained 4 to 6 weeks after diagnosis in patients receiving bromocriptine.

Inclusion criteria were symptoms of congestive heart failure that developed in the last month of pregnancy or during the first month postpartum, no other identifiable cause for heart failure, and LVEF

Table 1. Continued

Prolactin at Baseline, μg/L	Prolactin at 6 mo, μg/L	NYHA Class at Baseline	NYHA Class at 6 mo	LVEDD at Baseline, mm	LVEDD at 6 mo, mm	LVEF at Baseline, %	LVEF at 6 mo, %	Prespecified End Point of Poor Outcome
54	60	III	III	46	43	33	40	Yes
11	NR	II	NR	61	NR	28	NR	Yes (died 1 mo after baseline of sudden death)
9	NR	IV	NR	65	NR	18	NR	Yes (died 1 mo after baseline of CHF)
16	16	IV	III	62	60	24	22	Yes
50	48	II	II	60	62	19	24	Yes
50	9	II	I	59	52	34	50	No
5	NR	II	NR	62	NR	34	NR	Yes (died 3 mo after baseline of CHF)
233	7	III	III	57	43	32	44	Yes
52	NR	IV	NR	59	NR	14	NR	Yes (died on index admission)
30	8	II	II	60	74	32	37	No
135	8	IV	I	33	44	34	58	No
122	6	II	I	65	59	29	37	No
22	7	II	I	68	65	30	62	No
56	7	II	I	54	51	27	72	No
4	6	II	I	56	48	30	56	No
91	25	III	I	63	51	30	58	No
55	8	IV	I	55	47	33	60	No
18	13	II	I	49	34	32	75	No
NR	NR	III	NR	55	NR	18	NR	Yes (died on index admission)
5	12	III	I	54	56	8	48	No

<35% by transthoracic echocardiography. Exclusion criteria were systolic blood pressure >160 or <95 mm Hg or diastolic >105 mm Hg; clinical conditions other than cardiomyopathy that could increase plasma levels of inflammatory markers such as sepsis, autoimmune disease, or HIV positivity; significant liver disease (defined as liver transaminase levels >2 times the upper limit of normal); history of peptic ulcer disease; history of psychiatric disorders; impaired renal function (defined as urea and/or creatinine >1.5 times the upper limit of normal); and any clinical condition that, according to the investigators, precluded inclusion in the study such as ischemic heart disease or malignancy.

All patients received treatment with the diuretic furosemide and the angiotensin-converting enzyme (ACE) inhibitor enalapril. Patients with an LVEF <25% or LV thrombus received anticoagulation therapy with warfarin for 6 months. Carvedilol was added after resolution of overt heart failure. Enalapril and carvedilol doses were titrated upward as tolerated during the first 4 weeks after diagnosis and then remained unchanged throughout the remainder of the 6-month study period. Furosemide dose was decreased as indicated according to clinical assessment during the 6-month study period. The 10 patients randomized to standard therapy (PPCM-Std group) were treated as outlined above. The 10 patients randomized to standard therapy plus bromocriptine (PPCM-Br) received bromocriptine 2.5 twice daily for 2 weeks followed by 2.5 mg daily for 6 weeks in addition to standard heart failure therapy. After the initial screening and baseline visits, monthly outpatient visits were scheduled for clinical assessment and evaluation of medication compliance.

Echocardiography, Cardiac MRI, Assessment of New York Heart Association Functional Class, and Noninvasive Blood Pressure Measurements

Patients were diagnosed by specialist physicians and cardiologists working at the Chris Hani Baragwanath Hospital. Patients were included in this trial within 24 hours after diagnosis once the diagnosis was confirmed by a cardiologist (K.S.), who repeated the echocardiography. Two-dimensional and targeted M-mode echocardiography with Doppler color-flow mapping was performed with either a Hewlett Packard Sonos 5500 (Royal Philips Electronics, Amsterdam, the Netherlands) or a VIVID i (General Electric Company, Fairfield, Conn) echocardiography machine. Systolic and diastolic LV dimensions were measured according to the American Society of Echocardiography guidelines.[18] Measurements of LV dimensions and function were determined by use of the average of ≥3 cycles. Mitral effective regurgitant orifice area and Doppler parameters of diastolic function were measured according to American Society of Echocardiography guidelines.[19,20] Echocardiography was recorded on video or a compact disk and stored within the Soweto Cardiovascular Research Unit Division for further reference, audit purposes, and repeat blinded analysis by a single operator.

Cardiac MRI was performed 4 to 6 weeks after diagnosis in patients receiving bromocriptine to detect possible mural thrombi. Studies were performed with a 1.5-T MRI scanner (General Electric, Milwaukee, Wis) with a cardiac-dedicated phased-array coil. The cardiac MRI studies were ECG triggered by standard software. Studies consisted of steady-state free precession and spin echo. Short-axis, transverse, and coronal views were obtained. Steady-state free-precession sequences were performed to assess regional wall motion abnormalities and LVEF. Slice thickness was 8 mm with no gap, 256×256 matrix, 400-mm field of view, and 1.6×1.6×8-mm voxel size. The total time required for the investigation was 30 to 45 minutes. Gadolinium enhancement was not studied. Ventricular parameters were assessed in a standard manner by 1 observer using commercially available software (CAAS MRV, Pie Medial Imaging, Maastricht, the Netherlands). The cardiac MRI studies were assessed by 2 independent experienced observers who determined the presence or absence of intracavitary thrombi.

New York Heart Association (NYHA) functional class of each patient at baseline and follow-up visits was evaluated by a physician who was provided clinical data but was blinded to treatment allocation and was unaware of the results of the laboratory tests. Heart rate and systolic and diastolic blood pressures were measured noninvasively with a Critikon Dinamap Vital Signs Monitor 1846

and calculated as mean values from 5 readings. Measurements were made after a 30-minute resting period in patients in the sitting position with 2-minute intervals between successive measurements.

Research-Specific Blood Tests

Blood (8 mL) was withdrawn from an antecubital vein, collected in prechilled tubes containing EDTA acid or clot activator, and mixed rapidly. Plasma or serum was separated by centrifugation at 2500 rpm for 7 minutes within 10 minutes of collection. Aliquots were stored at −80°C for possible future analysis. High-sensitivity CRP (hsCRP) was measured as described previously.[5,7,8] In addition, prolactin, NT-proBNP, full blood count, liver function, and creatinine were measured. Serum levels of 16-kDa prolactin were measured by immunoprecipitation followed by Western blotting. Cathepsin D activity was assayed with the Sensolyte 520 Cathepsin D Assay Kit (MoBiTec) as previously described.[9]

Analysis of Outcome

The prespecified combined end point of poor outcome was defined as death, NYHA functional class III/IV, or LVEF <35% at 6 months as previously described.[8]

Assessment of Children

Standard growth monitoring charts issued by the South African Department of Health and maintained by primary physicians were obtained for the newborn children of mothers included in this study. These charts listed the weight of each child at birth and at regular intervals to 6 months and beyond. Weights were plotted on World Health Organization weight-for-age Child Growth Standard charts for girls and boys.[21,22]

Statistical Analysis

Data were analyzed with the SAS version 9.1 statistical program (SAS Institute Inc, Cary, NC). Results are expressed as mean±SD or median (range). Comparison between groups at baseline and within groups (baseline to 6 months) of class variables was analyzed by χ^2 test or the Fisher exact test when adequate. NT-proBNP data were log transformed. To assess differences between the 2 treatment groups, we analyzed mean changes (baseline to 6 months) in all continuous variables with a t test or an exact Wilcoxon 2-sample test when distribution was not normal. For within-group comparisons, a paired t test or a sign test when distribution was not normal was performed. Significance was assumed at a 2-sided value of $P<0.05$.

Results

Baseline Characteristics and Treatment

Ninety-three patients with suspected PPCM were screened to recruit 20 consecutive patients with confirmed PPCM who were HIV negative and presented within 1 month postpartum. As depicted in Tables 1 and 2, the baseline characteristics of patients in the PPCM-Br and PPCM-Std groups were similar in terms of age, parity, NYHA functional class, systolic and diastolic blood pressures, heart rate, LV end-diastolic and end-systolic dimensions, and LVEF. Median prolactin and median NT-proBNP levels were comparable, whereas serum levels of 16-kDa prolactin and cathepsin D activity were elevated to a similar degree in all patients (Figure 1).

Treatment with standard heart failure medications was similar between the PPCM-Br and PPCM-Std groups (Table 1). Median dose of enalapril in the PPCM-Br group was 5 mg/d (range, 5 to 10 mg/d) and in the PPCM-Std group was 10 mg/d (range, 5 to 10 mg/d). Median dose of carvedilol in the PPCM-Br group was 6.25 mg twice daily (range, 6.25 to 25 mg) and in the PPCM-Std group was 12.5 mg twice daily (range, 6.25 to 25 mg). Median dose of furosemide at 6 months was 80 mg/d (range, 80 to 120 mg). All patients, including those with normalized LV systolic function, continued on medical therapy with ACE inhibitor and carvedilol during the 6-month study period. Cardiac transplantation or implantation of a LV assist device is not performed in state hospital patients in the Gauteng province of South Africa.

Table 2. Baseline Characteristics of PPCM-Br Versus PPCM-Std Patients

	PPCM-Br (n=10)*	PPCM-Std (n=10)*	P
Clinical parameters			
Age, y	24±6	28±10	0.60
Parity, n (range)	1.5 (1–3)	2 (1–6)	0.52
Systolic blood pressure, mm Hg	116±23	110±19	0.50
Diastolic blood pressure, mm Hg	70±16	76±18	0.45
Heart rate, bpm	102±13	108±15	0.34
NYHA functional class, n (%)			1.00
II	5 (50)	5 (50)	
III/IV	5 (50)	5 (50)	
Echocardiographic parameters			
LVEDD, mm	55±10	59±5	0.25
LVESD, mm	46±9	52±6	0.16
LVEF, %	27.2±8.1	26.9±7.6	0.87
Mitral regurgitation (grade)	2.1±0.6	1.9±0.6	0.70
Mitral ERO, cm^2	0.45±0.13	0.44±0.18	0.90
Laboratory parameters			
Hemoglobin, g/dL	13.0±2.2	11.8±1.9	0.22
Creatinine, μmol/L†	71 (6–109)	66 (5–96)	0.43
hsCRP, mg/L†	7.8 (1.1–58.0)	6.0 (4.0–115.3)	0.86
Prolactin, μg/L†	49.9 (3.8–135.0)	30.0 (5.1–233.0)	0.87
Log NT-proBNP	8.54±1.14	8.45±1.24	0.88

LVEDD indicates LV end-diastolic diameter; LVESD, LV end-systolic diameter; and ERO, effective regurgitant orifice.

*Values are mean±SD unless otherwise specified.

†Values are median (range).

Hemodynamic and Echocardiographic Parameters

Changes in systolic and diastolic blood pressures and heart rate from baseline to 6 months were not significantly different between the 2 treatment groups. In contrast, recovery of LVEF between baseline and 6 months was greater in the PPCM-Br group (31%) than in the PPCM-Std group (9%; $P=0.012$; Table 3 and Figure 2). Furthermore, the degree of mitral regurgitation significantly improved in the PPCM-Br group compared with the PPCM-Std group ($P=0.013$), as did several parameters of diastolic function (Table 3). No significant differences were observed in LV end-diastolic and end-systolic dimension change from baseline to 6 months between the 2 groups (Table 3).

NYHA Functional Class

All 9 surviving patients in the PPCM-Br group recovered to NYHA functional class I at 6 months. In contrast, all patients

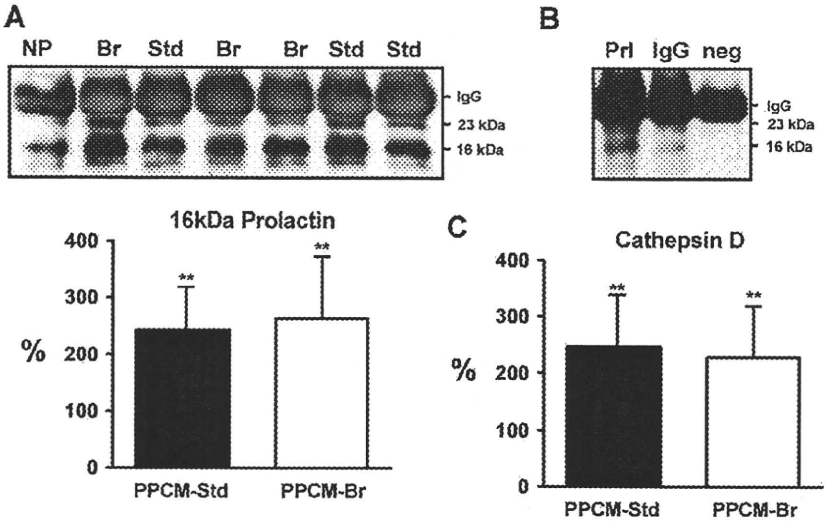

Figure 1. Analysis of prolactin subforms and cathepsin D activity in baseline serum probes from PPCM patients. A, Representative Western blot showing 16-kDa prolactin immunoprecipitated from serum probes of PPCM patients and from serum of a nulliparous (NP) control. Bar graph depicts 16-kDa prolactin serum levels in PPCM-Std (Std; n=7) and PPCM-Br (Br; n=8) vs the mean value of NP (n=3), which was set at 100% (**P<0.01 vs NP). B, The specificity of the immunoprecipitation (IP) was confirmed with anti-prolactin antibodies (Prl), nonspecific immunoglobulin G (IgG), and no antibody (neg) in a PPCM serum probe, followed by detection of 16-kDa prolactin by Western blot. C, Bar graph depicting cathepsin D activity in serum probes from PPCM patients (PPCM-Std, n=8; PPCM-Br, n=9) at baseline and in NP (n=7). Mean value of NP was set at 100% (**P<0.01 vs NP).

from the PPCM-Std group who survived 6 months were in NYHA functional class II (3 patients) or III (3 patients) (Tables 1 and 4).

Survival

The single patient who died in the PPCM-Br group presented in severe heart failure and survived only 7 days. All 9 remaining patients in the PPCM-Br group survived 6 months. Four patients in the PPCM-Std group died during the 6-month follow-up period: 1 died of heart failure during the index admission, 2 died of heart failure 4 to 12 weeks after diagnosis, and 1 experienced sudden cardiac death 1 month after baseline assessment.

Laboratory Parameters

There was a difference in change of log NT-proBNP levels from baseline to 6 months of borderline statistical significance in the PPCM-Br patients compared with the PPCM-Std patients (P=0.05), whereas the reductions in prolactin and hsCRP levels at 6 months were similar between the 2 groups (Table 5).

Table 3. Comparison of Hemodynamic and Echocardiographic Parameters in PPCM-Br and PPCM-Std Patients at Baseline and 6 Months

	PPCM-Br Baseline (n=10)*	PPCM-Br 6 Months (n=9)*	PPCM-Std Baseline (n=10)*	PPCM-Std 6 Months (n=6)*	P†
Clinical parameters					
Systolic blood pressure, mm Hg	116±23	118±13	110±19	115±9	0.78
Diastolic blood pressure, mm Hg	70±16	74±9	76±18	73±6	0.77
Heart rate, bpm	102±13	64±7	108±15	79±15	0.22
Echocardiographic parameters					
LVEDD, mm	55±10	51±9	59±5	56±12	0.50
LVESD, mm	46±9	34±10	52±6	45±11	0.18
LVEF, %	27±8	58±11	27±8	36±11	0.0007
Mitral regurgitation (grade)	2.1±0.6	0.22±0.44	1.9±0.6	1.5±1.0	0.0042
Mitral ERO, cm^2	0.45±0.13	0.11±0.03	0.44±0.18	0.34±0.18	0.02
Left atrial diameter, cm	3.54±0.25	3.36±0.53	3.83±0.62	3.93±0.83	0.25
Mitral E velocity, cm/s	86±19	66±24	89±23	85±24	0.53
Mitral A velocity, cm/s	32±7	48±19	33±6	45±12	0.80
Mitral E velocity/A velocity ratio	2.82±0.76	1.63±1.13	2.73±0.68	1.94±0.67	0.82
Deceleration time, ms	118±26	197±59	136±30	168±36	0.08
Mitral medial annular (E′) TDI velocity, cm/s	7.0±1.3	12.4±2.4	6.5±1.1	7.3±2.5	0.014
E/E′ (medial annular velocity)	12.5±3.0	5.4±2.5	14.0±4.6	12.4±4.6	0.08
Mitral lateral annular (E′) TDI velocity, cm/s	7.2±1.1	12.4±2.5	6.6±0.97	7.3±2.5	0.007
E/E′ (lateral annular velocity)	12.0±2.0	5.4±2.5	13.8±4.2	12.1±3.9	0.051

Abbreviations as in Table 2, plus TDI indicates tissue Doppler imaging.
*Values are mean±SD.
†Comparing the change from baseline to 6 months in PPCM-Br and PPCM-Std patients.

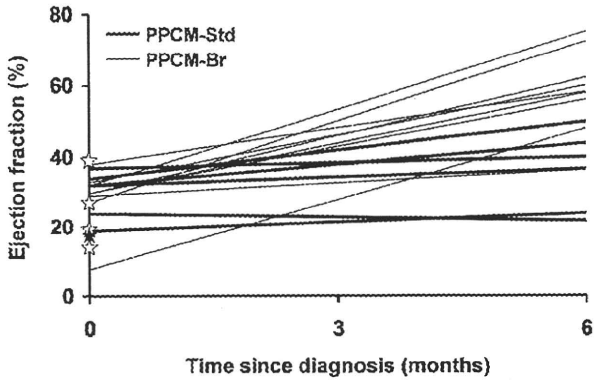

Figure 2. Change in LVEF from baseline to 6 months among survivors. Stars represent baseline LVEF for patients who died during the study period.

Combined Measure of Poor Outcome

The combined measure of poor outcome that included LVEF <35% (surviving PPCM-Br, 0 of 9 [0%] versus surviving PPCM-Std, 2 of 6 [33%]), NYHA functional class III/IV at 6 months (surviving PPCM-Br, 0 of 0 [0%] versus surviving PPCM-Std, 3 of 6 [50%]), or death within 6 months (PPCM-Br, 1 of 10 [10%] versus PPCM-Std, 4 of 10 [40%]) revealed that the PPCM-Br patients had better outcome than the PPCM-Std patients (P=0.006; Figure 3).

Thrombi and Thromboembolism

No adverse effects, including thromboembolism, were reported in either group. Cardiac MRI was performed at 4 to 6 weeks after diagnosis in 8 of the 10 patients in the PPCM-Br group to assess for thrombus formation. MRI results were not available for 1 patient who died before becoming stable enough for the MRI, and the images acquired for a second patient were not of sufficient quality for reliable assessment. None of the remaining patients had intracavitary thrombi (Figure 4).

Infant Growth Curves and Survival

All 21 children of the PPCM-Br and PPCM-Std patients showed normal growth curves when plotted on the World Health Organization standard weight-for-age growth charts (Figure 5A and B). Although the survival of all 21 children through the 6-month follow-up period was verified, weight-for-age data at 6 months were available for only 13 children.

Table 4. Comparison of NYHA Functional Class in PPCM-Br and PPCM-Std Patients at Baseline and 6 Months

	PPCM-Br at Baseline (n=10), n (%)	PPCM-Br at 6 mo (n=9), n (%)	PPCM-Std at Baseline (n=10), n (%)	PPCM-Std at 6 mo (n=6), n (%)	P*
NYHA functional class					0.008
I	0	9 (100)	0	0	
II	5 (50)		5 (50)	3 (50)	
III/IV	5 (50)		5 (50)	3 (50)	

*Comparing the change from baseline to 6 months in PPCM-Br and PPCM-Std patients.

The mothers of 5 children died during the course of the study and family members could not provide the children's growth charts, and the growth charts of the 3 other children with missing data were incomplete because of challenges in the delivery of quality care in the primary healthcare system in South Africa. However, all children had weight data up to the age of 3 months, and there were no significant differences in growth curves between the children of the PPCM-Br patients and those of the PPCM-Std patients.

Discussion

This prospective, single-center, randomized, open-label pilot study with blinded efficacy assessments showed that the addition of bromocriptine to standard heart failure therapy in women with PPCM appeared to result in significantly greater improvements in NYHA functional class, LV systolic and diastolic function, and degree of functional mitral regurgitation than seen with standard therapy alone. Bromocriptine seemed to be well tolerated, and no thrombotic complications were observed. Moreover, although bromocriptine stopped lactation and breast-feeding in the PPCM patients, the growth and survival of those infants were normal. However, our study was very small, and these findings are in no way definitive. On the other hand, these findings are encouraging and suggest that a larger study should be considered.

This proof-of-concept pilot study was performed in a group of homogeneous patients in terms of ethnic background, age, time point of diagnosis, and baseline characteristics. Unfortunately, blinding of the study was not possible because the PPCM-Std group continued to nurse their infants while the PPCM-Br group could not breast-feed because of bromocriptine-induced cessation of lactation. However, investigators were blinded for data analysis. We believe that the homogeneous patient cohort, well-balanced baseline characteristics, and blinded assessment of outcomes to some extent compensate for the small size of our study and its open-label design.

The design of the present study was chosen on the basis of our hypothesis that a cleaved form of the hormone prolactin initiates and drives PPCM and that early pharmacological blockade of prolactin with bromocriptine may improve the condition of patients with acute onset of PPCM before irreversible damage caused by cell death, fibrosis, and remodeling. Increased serum levels of 16-kDa prolactin and augmented cathepsin D activity at baseline in PPCM patients included in the present study support this hypothesis. The rationale for the dose and length of bromocriptine therapy was based on previous observations in animal models and a previous pilot study,[11] as well as several case reports in patients with PPCM.[12,23,24] We believe that some of the apparently beneficial effects of bromocriptine result from eliminating the detrimental 16-kDa prolactin form, the harmful effects of which on the heart and the vasculature have been described experimentally.[11,24] In addition, both forms of prolactin promote inflammation,[24] a reaction that seems to be associated with PPCM in this African cohort, because most patients displayed increased serum levels of the inflammatory marker hsCRP.[5]

Table 5. Comparison of Laboratory Parameters in PPCM-Br and PPCM-Std Patients at Baseline and 6 Months

	PPCM-Br at Baseline (n=10)	PPCM-Br at 6 mo (n=9)	PPCM-Std at Baseline (n=10)	PPCM-Std at 6 mo (n=6)	P†
Hemoglobin, g/dL†	13.0±2.2	12.7±1.5	11.8±1.9	13.0±1.4	0.58
Creatinine, μmol/L‡	71 (6–109)	78 (52–113)	66 (5–96)	62 (41–73)	0.86
hsCRP, mg/L‡	7.8 (1.1–58.0)	4.7 (1.0–10)	6.0 (4.0–115.3)	1.8 (1.1–15.1)	0.18
Prolactin, μg/L‡	49.9 (3.8–135.0)	8.0 (5.9–25.0)	30.0 (5.1–233.0)	12.5 (7.4–60.0)	0.72
Log NT-proBNP†	8.54±1.14	5.62±0.80	8.45±1.24	6.64±0.60	0.056

*Comparing the change from baseline to 6 months in PPCM-Br and PPCM-Std patients.
†Values are mean±1SD.
‡Values are median (range).

Apart from its prolactin blocking role, bromocriptine may exert additional "off-target effects" in PPCM patients. For example, effects of bromocriptine on hemodynamic parameters in patients with heart failure were described 30 years ago[25] before treatment with ACE inhibitors and β-blockers was routine. Positive effects of bromocriptine on blood pressure, vascular resistance, and plasma norepinephrine levels have been described.[25] Moreover, bromocriptine has been shown to increase stroke volume index and to decrease LV filling pressure.[25,26] Whether these potential beneficial effects of bromocriptine on hemodynamic parameters play a role in contemporary patients with heart failure who are treated with ACE inhibitors and β-blockers remains to be elucidated.

Bromocriptine may also affect metabolic parameters. We observed that PPCM patients display increased oxidized low-density lipoprotein serum levels compared with healthy postpartum women,[9] suggesting impaired antioxidative defense mechanisms and potential metabolic perturbations. In turn, Wexler and McMurtry [27] reported that, experimentally, bromocriptine treatment reduced triglyceride, free fatty acid, total cholesterol, and glucose levels in isoproterenol-induced heart failure. Whether such parameters play a role in the pathophysiology of PPCM is currently under investigation in experimental models.

In addition, bromocriptine has been shown to inhibit oxidative stress–induced cell death in neuronal cells by dopamine D2 receptor–dependent transactivation of c-Src/endothelial growth factor receptor and downstream PI3K-Akt signaling, which results in upregulation of antiapoptotic Bcl-2.[28] Preliminary data show that bromocriptine treatment increases Akt activation and upregulates Bcl-2 expression in the heart of postpartum mice (D.H.-K., unpublished data, 2010), suggesting that bromocriptine may indeed have direct cardioprotective effects. Taken together, these data show that off-target effects of bromocriptine on metabolism, oxidative stress, and cytoprotection may act in concert with its prolactin-lowering capacity and may help to explain the positive effects of prolonged treatment with bromocriptine beyond an effective prolactin blockade.

We found that the overall mortality rate in the PPCM-Std group was high. Other studies have demonstrated a lower PPCM mortality rate (averaging ≈15%), including our own series of 100 patients[1,5,8] and the prospective long-term study by Fett et al.[4] One explanation for the differences in mortality rate between the present study and our other series of 100 patients might be the inclusion criteria. In the present study, patients were enrolled very early (within 24 hours after diagnosis). This timely enrollment was not possible for the previous cohort of 100 patients. As a consequence, some patients in that study died between diagnosis and enrollment. In addition, our previous study included patients diagnosed

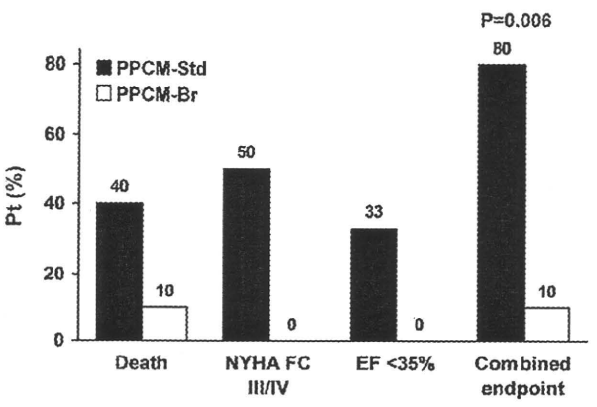

Figure 3. Comparison of 6-month prespecified poor outcome, including death, NYHA functional class (FC) III/IV, and LVEF <35% among survivors, and the combined end point including all 3 of these end points for PPCM-Br vs PPCM-Std patients (Pt).

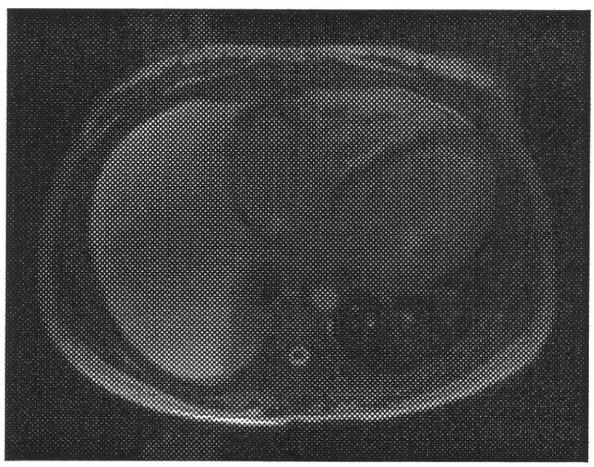

Figure 4. Cardiac MRI (transverse view, steady-state free-precession sequence) in a young African woman 2 months after delivery demonstrates marked dilation of both ventricles and the right atrium. LVEF is 8%.

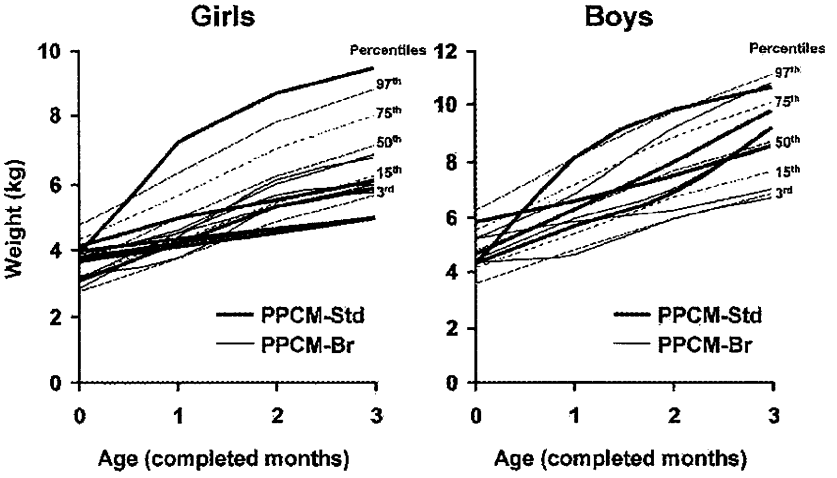

Figure 5. Growth and survival of children of PPCM study mothers from birth to 3 months plotted on World Health Organization growth charts.

between 4 weeks and 5 months postpartum. The development of symptoms >4 weeks postpartum may be a manifestation of milder forms of this disease.

In this study, the cause of death in the PPCM-Std group was either heart failure or sudden cardiac death, with all deaths occurring within 3 months of randomization. In contrast, the 1 patient who died in the PPCM-Br group was admitted with severe heart failure and died 7 days after diagnosis while still in the intensive care unit.

The safety of bromocriptine treatment during pregnancy has already been assessed by a survey of 1400 pregnant women who took bromocriptine primarily in the first few weeks of pregnancy and found no increased rates of abortion or congenital malformations.[29] In the postpartum phase, bromocriptine has been used worldwide since 1980 to suppress lactation. However, concerns have been raised about a potential risk for cerebral and cardiovascular complications, as emphasized in some case reports describing stroke,[13] seizure,[15] coronary artery thrombosis,[15] and coronary artery vasospasm.[14] Although these data were observational, bromocriptine was withdrawn from the market in the United States in 1994 for use as an agent to block lactation.

It is known that the postpartum period is associated with an increased risk of thrombosis and myocardial infarction, probably because of changes in coagulation that may have evolved as a protection from bleeding caused by miscarriage and childbirth.[30] We observed no adverse effects in any of the 9 surviving patients in the PPCM-Br group. However, the number of patients studied was small, and because of poor cardiac function, all patients in the present study received subcutaneous low-molecular-weight heparin during their index admission. Therefore, although the data suggesting that bromocriptine has a prothrombotic effect are not robust, we cannot rule out such an effect.

There has been some concern that PPCM patients in developing countries treated with bromocriptine will no longer be able to breast-feed, which may increase the risk for malnutrition and infection in their infants.[17] The survival rate of infants of the PPCM-Br patients was not affected, and no serious illnesses were reported, although the number of children we studied was very small. Normal weight gain from birth to 3 months was observed in all infants and continued to be normal during the 6-month follow-up period in those for whom data were available. Although this was a small study with only short-term follow-up, our results suggest no disadvantage to the infant of a PPCM patient who could not breast-feed because of bromocriptine treatment. However, we are aware that larger studies in Soweto and other developing areas in the world are needed to support this statement.

Conclusions

In this trial, the addition of bromocriptine to standard heart failure therapy appeared to improve LVEF and a composite clinical outcome in women, although the number of patients studied was small and the results cannot be considered definitive. Larger-scale multicenter and blinded studies are in progress to test this strategy more robustly.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Peripartum cardiomyopathy (PPCM) is a potentially life-threatening heart disease that occurs in previously healthy women. We identified prolactin, mainly its 16-kDa angiostatic and proapoptotic form, as a key factor in PPCM pathophysiology. Blockade of prolactin with the dopamine-2D agonist bromocriptine had previously been shown to prevent the onset of PPCM in mice and in women at high risk of this condition because of documented PPCM in a previous pregnancy. We recruited 20 women with onset of severe acute PPCM during the first month postpartum within 24 hours of diagnosis and randomized them into 2 groups: standard care (PPCM-Std; n=10) or standard care plus bromocriptine for 8 weeks (PPCM-Br, n=10). PPCM-Br patients displayed greater recovery of left ventricular ejection fraction compared with PPCM-Std patients at 6 months. Four PPCM-Std patients died; only 1 PPCM-Br patient did not survive. Significantly fewer PPCM-Br patients met the composite end point of poor outcome defined as death, New York Heart Association functional class III/IV, or left ventricular ejection fraction <35% at 6 months. Because the PPCM-Br mothers could not breast-feed, the outcome of their children was assessed. Infants of mothers in both groups showed normal growth and survival at 6 months. Our findings suggest that the addition of bromocriptine to standard heart failure therapy appears to improve left ventricular ejection fraction, functional class, and survival in women with severe acute PPCM with no obvious detriment to their children.

2010年10月25日（月） 日経新聞 朝刊掲載

妊婦らの心筋症 患者追跡調査へ

循環器病研究センター

国立循環器病研究センターは妊娠中や出産後半年の間に女性に心不全が起こる「周産期心筋症」の患者の調査に乗り出す。発症原因を探り、簡易検査法や診断基準作成などを目指す。

同センターの倫理委員会で研究計画が承認された。周産期心筋症の発症には免疫やホルモン、胎児に血液を送るための心臓の負担増などが関係するとみられる。計画では2年間で全国の患者50人を登録して患者の状態などを追跡。血液や組織を採取して病気の危険因子を調べる。一般の心不全で診断に使わる血液中の特定物質の量が、周産期心筋症の診断も有効かなどを明らかにする。

Ⅱ.分担研究年度終了報告書

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

周産期心筋症全国後方視的症例調査のサブ解析と疾患概念普及のための広報活動

分担研究者　池田　智明　国立循環器病研究センター　周産期・婦人科部

研究要旨　未曾有の少子高齢化が進行するわが国にとって、安心安全な妊娠出産産褥を実現する医療は極めて重要である。周産期医療では、救急体制の不備に加え、難治性周産期疾患も依然として母子の生命予後を脅かしている。とりわけ、周産期心筋症（産褥心筋症）については、その疾患概念すら周知されておらず、かつ妊産褥婦の病気であることから、心不全発症時の初診医が、産科医や一般内科医などの普段心不全診療に不慣れな医師が担当することが圧倒的に多い。その結果適切な診断治療に恵まれないために、重症化する症例も存在する。この現状を踏まえ、産科医が周産期心筋症患者を早期発見できるような指標がないか、全国調査結果のサブ解析を行った。また、疾患概念の普及を目指し、ホームページの作成や各種学会等での発表・講演を行った。

A. 研究目的

周産期心筋症は、母児の命にかかわる重要な疾患であるが、わが国においては、その疾患概念すら周知されていないのが現状である。わが国初の全国調査結果より、60%以上の患者において、心不全発症時の初診医が、普段心不全診療に不慣れな産科医であった。これは、患者が妊産褥婦であるという周産期心筋症の特異性を反映している。また、患者の 40%が妊娠高血圧症候群、各 15%が双胎妊娠または切迫早産を合併しており、約 70%の患者が合併症妊娠として、産科医が注意して診療を行う患者であることも判明した。

そこで、初診医となりやすい産科医が、周産期心筋症患者を早期に診断し、治療へと結びつけるために、どのような因子に注目すればよいかを検討した。

また、昨年に引き続き、疾患概念の普及活動に務めた。

B. 研究方法

早期診断を行えるよう、危険因子、特に妊娠関連高血圧症候群についてのサブ解析と、診断するための簡易検査値について、正常値と異常値を区別するために、正常妊婦での値を計測し、患者群と比較解析した。

周産期心筋症の従来から知られている知見とともに、わが国初の後方視的全国調査からの知見も含め、一般に閲覧しやすい形式：ホームページ形式にて、情報公開した。

また、周産期心筋症モデルマウスとして、GCA-KO マウスについての基礎実験を行った。

（倫理面への配慮）

ホームページ作成に当たっては、全国調査結果についても個人が特定されることがないよう、報告内容に配慮した。

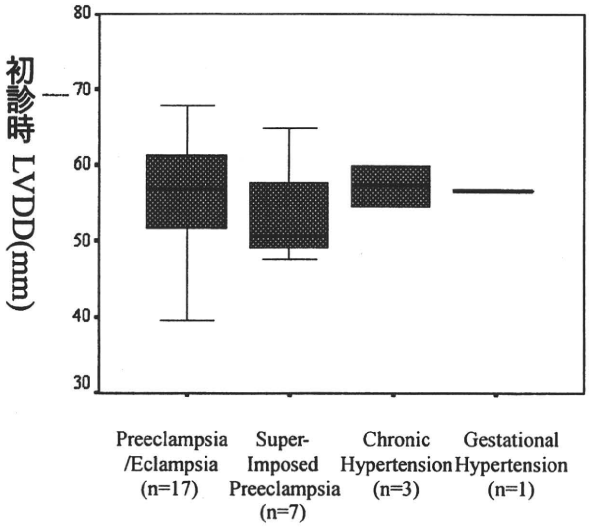
また、平成 22 年 10 月から開始した前方視的症例調査においては、厚生労働省・文部科学省の「疫学研究に関する倫理指針」に従って作成し、事前に本研究の主旨を、アンケート調査施設に充分に説明したうえで実施している。また、得られたいかなる個人情報についても秘密が厳守されることを保証し、統計結果を公開する際には、アンケート質問事項のうち、個人が特定されない項目を集計・解析したもののみ、発表する。

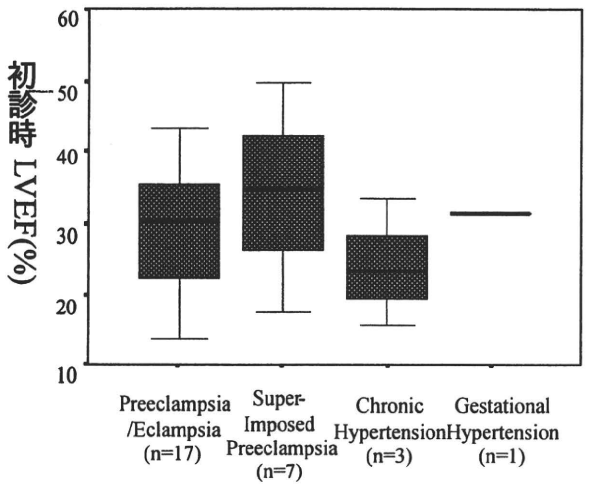
C. 研究結果と考察

・妊娠高血圧症候群のサブ解析

どのような妊娠高血圧症候群がリスクを持つのか、また、予後に影響を及ぼすのかを検討した。

(1)高血圧の種類と初診時心機能の関係





妊娠高血圧症候群の種類と初診時心機能：左室拡張末期径（LVDd）、左室駆出率（LVEF）に相関はなかった。

(2)高血圧や腎障害の重症度と初診時心機能の関係

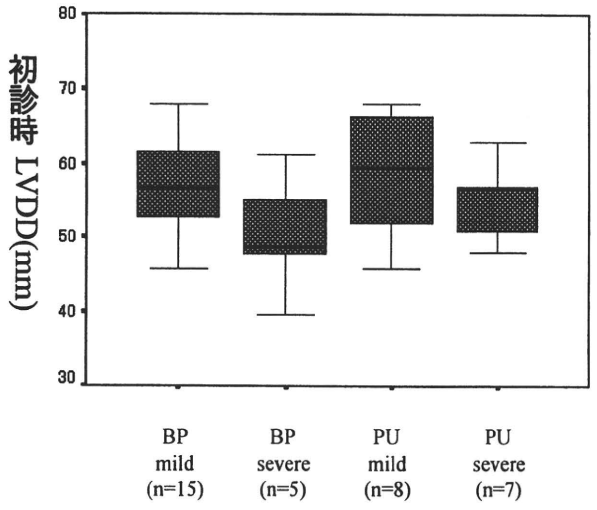
重症度は以下のように定義した。

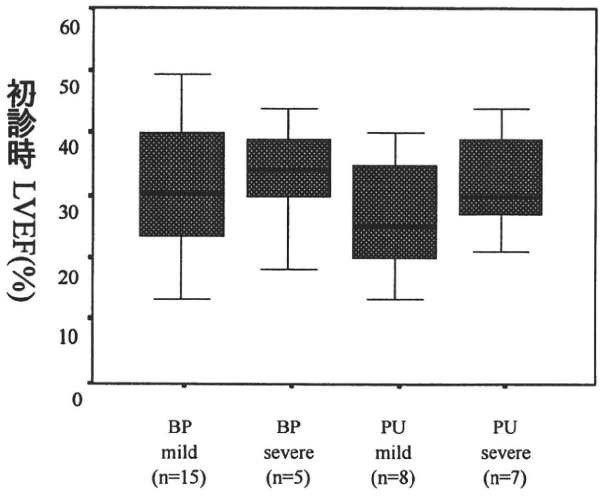
【血圧】**軽症**:収縮期血圧 140mmHg 以上 160mmHg 未満、拡張期血圧 90mmHg 以上 110mmHg 未満

重症：収縮期血圧 160mmHg 以上、拡張期血圧 110mmHg 以上

【腎障害（蛋白尿）】**軽症**：24 時間尿による蛋白尿が 300mg/日以上 2g/日未満

重症：24 時間尿による蛋白尿が 2g/日以上

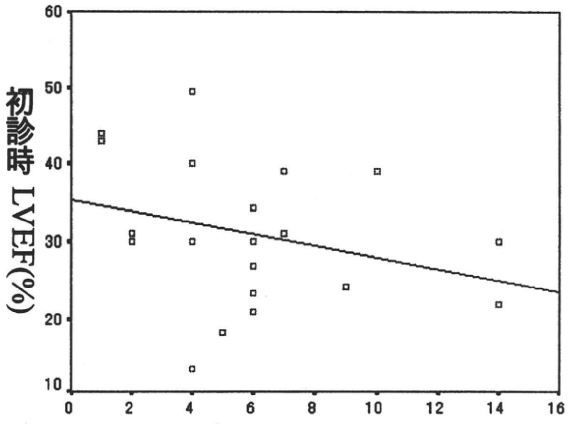




妊娠高血圧症候群の血圧・蛋白尿の重症度と初診時心機能に相関はなかった。

（1）（2）の結果から、妊娠高血圧症候群の種類や重症度と、心機能の重症度とは関連せず、妊娠高血圧症候群が重症化して心機能にも変化を及ぼすのではなく、共通因子をきっかけに、全身内皮機能障害が惹起されれば妊娠高血圧症候群を、心機能障害が惹起されれば周産期心筋症を発症している可能性が考えられた。

(3)妊娠高血圧症候群の診断から心不全診断に至るまでの期間と初診時心機能の関係



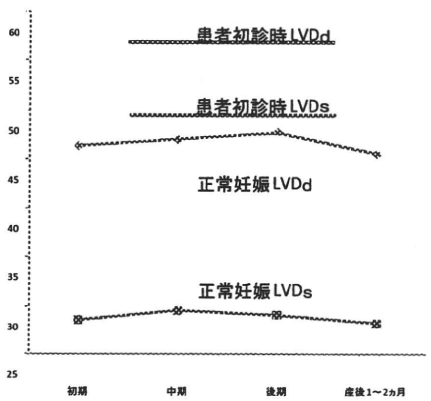
Time from onset of hypertensive disorders complicating pregnancy to diagnosis of PPCM (weeks)

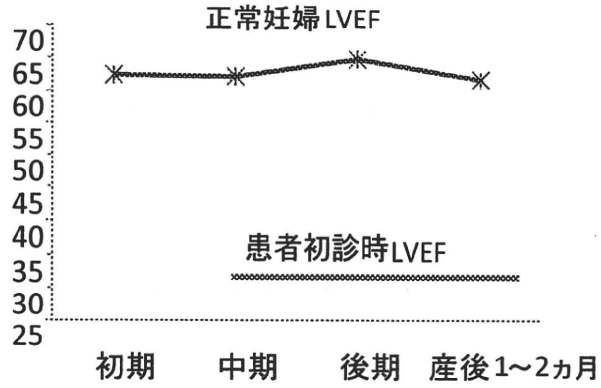
妊娠高血圧症候群の診断から、心不全診断までの期間が長いほど、初診時 LVEF が低下している傾向があった。これは、高血圧発症時から自他覚症状に注意し、早期発見すれば、予後改善につながる可能性が大いに示唆された。

これらの結果は、第２報として、全国調査に協力いただいた施設へ送り、前向き症例登録にご協力いただく先生や希望者にも配布を行っている(添付資料 1)。

・各種検査値の正常妊婦と患者群との比較
正常妊婦においても、軽度の心拡大や BNP 上昇を認める。そこで、心筋症診断においての正常値・異常値を決定するため、正常妊婦での検査値を計測し、検討を開始した。

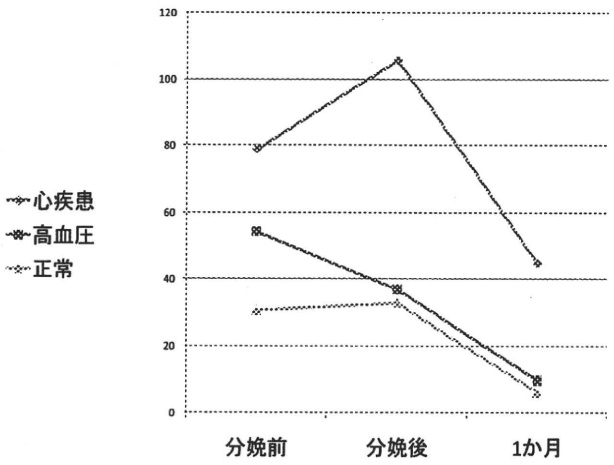
（１） 心エコー上の心機能指標
日本人女性における正常値を特定するために、正常妊婦における心エコー臨床研究を実施中である。下記にこれまでの結果と、周産期心筋症患者における検査値を表示する。





正常妊娠においても心臓は拡大するが、収縮力が妊娠中に低下することはない。拡張・収縮末期径の正常２SD の範囲を特定するため、今後検体数を増やす予定である。

（２） BNP 値



妊娠高血圧症候群を合併すると、正常妊婦に比し、分娩前は血清 BNP 値が増加することが判明した。
しかし、平均で 50pg/ml 未満であり、周産期心筋症患者の平均 1258pg/ml とは大きくかけ離れていることが分かった。しかし、これについても、正常、高血圧合併での２SD 範囲を特定し、周産期心筋症の早期診断に活用できるよう今後検体数を増やす予定である。

・ホームページ・パンフレット作成
一般向けと医療従事者向けホームページを作成した（添付資料2、3)。ホームページ閲覧により、診療内容の問い合わせや、患者・家族からの質問が寄せられるといった反響を得た。新たな患者・家族に対して、また医師以外の医療従

事者向けに周産期心筋症についてのパンフレットを作成した。新規患者発生時にすぐ患者説明に利用できるよう、調査ホームページからダウンロード可能である。

・国際コンソーシアムへの参加

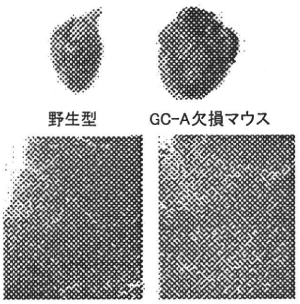
ヨーロッパ心臓病学会周産期心筋症調査会議に事務局として出席し、国際協力体制を構築した。

・学会広報活動

日本心臓病学会などで講演し、疾患概念普及に努めた(添付資料4)。

・周産期心筋症モデルマウスによる基礎実験

国立循環器病研究センター研究所では、ANP(心房性利尿ペプチド)・BNP(脳性利尿ペプチド)の受容体であるGuanylyl cyclase (GC)-A受容体を欠損した遺伝子改変マウスが、高血圧モデルマウスとして研究されている。本研究グループは、この遺伝子改変マウスの雌が、妊娠授乳に伴って血圧上昇と心拡大・心肥大を示し、高血圧合併周産期心筋症モデルとなりえることを見出した(下図)。



GC-A遺伝子多型((CT)n=6 polymorphism)は日本人における高血圧発症因子として報告されており、その保因頻度も一般人口において100-200人に1人と高い。そこで、この基礎実験結果をもとに、平成23年度には、周産期心筋症患者において、家族性拡張型心筋症の主な原因遺伝子とGC-A遺伝子などの解析を行い、当該疾患における遺伝因子解明を目指す。本研究の成果は、疾患特異的な診断検査の開発や、ハイリスク群の早期診断・予防、さらには、病態の解明と新たな治療法開発の基盤になると、期待される。

D. 結論

周産期心筋症にとって、疾患概念の周知はきわめて重要である。そこで、広報活動を積極的に行い、また、心不全専門医でなくとも早期診断できる体制作りを図った。

病因・病態究明の為に、モデル動物による基礎実験を行い、新たな知見を得た。今後、遺伝子検索や新たな治療法へと発展させる予定である。

E. 健康危険情報

該当なし。

F. 研究発表

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3. 神谷千津子、池田智明. 成人先天性心疾患と妊娠・出産管理. 心エコー. 11(8): 818-24. 2010

2. 学会発表

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2. 池田智明「わが国の妊産婦死亡の原因と評価」ＳＳニューイヤーセミナー　1.31/’10　東京

3. 池田智明「妊産婦死亡の原因究明と予防策」大阪産婦人科医会平成21年度第2回研修会 2.20-21/’10　大阪

4. 池田智明　「わが国の妊産婦死亡の原因究明と予防策」平成22年度日本産婦人科学会秋田地方部会　4.29/’10　秋田

5. 神谷千津子、桂木真司、根木玲子、山中薫、佐々木禎仁、上田恵子、池田智明「妊娠高血圧症候群を合併した周産期心筋症患者の予後の解析」第31回日本妊娠高血圧学会 10.15-16/'10 東京

6. 池田智明「妊産婦死亡について―日産婦学会と厚労省の立場から―」平成22年度日産婦学会富山地方部会 11.4/'10 富山

7. 池田智明「妊産婦死亡の原因分析・評価について」第19回（平成22年度）全国支部医療安全担当者連絡会 11.23/'10 東京

G. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし。

2. 実用新案登録

該当なし。

3. その他

研究協力者：

国立循環器病研究センター

生化学部 徳留 健

再生医療部 大谷 健太郎

妊娠関連の心筋症(周産期心筋症 産褥心筋症)の発症に関する全国多施設共同研究結果報告(第二報)

厚生労働科学研究(子供家庭総合研究事業)
「乳幼児死亡と妊産婦死亡の分析と提言に関する研究」
厚生労働科学研究(難治性疾患克服事業)
「周産期心筋症の全国調査に関する研究」

国立循環器病研究センター　周産期・婦人科　池田　智明
心臓血管内科　北風　政史

【はじめに】

妊娠関連の心筋症（周産期心筋症、産褥性心筋症）は、わが国における妊産婦死亡の非常に重要な原因の一つです。重症例では、母体死亡や左心補助装置(LVAS)を装着しての心臓移植待機となったり、数年の経過で心不全に陥ったりする難治性の疾患であります。しかしながら、これまでわが国における実態は明らかではありませんでした。

周産期心筋症全国調査事務局では、厚生労働科学研究の一環として、平成21年に後ろ向き全国アンケート調査を実施しました。ご協力いただいた先生方には、心より御礼申し上げます。結果、わが国における発症率が約2万分娩に1例であること、慢性高血圧症や妊娠高血圧症候群の合併・多胎妊娠・子宮収縮抑制剤の使用などが危険因子であること、全体の1割弱が死亡もしくは心移植待機となること、などが判明し、ご登録いただいた施設の先生方、関係の先生方に結果報告第一報として、昨年送付させていただきました。

(右記ホームページにも掲載しております。http://www.ncvc.go.jp/cvdinfo/pro/peripartum_cardiomyopathy.html)

今回は、最大危険因子である高血圧症について検討したサブ解析結果を第二報として送付させていただきます。

米国においては、発症数が年々増加していることが報告されており、わが国におきましても、今後の増加が見込まれます。また、異型プロラクチン病因説が新たに提唱され、抗プロラクチン療法の試みが始まっております。そこで、調査事務局では、疾患概念の周知促進とともに、わが国における危険因子と予後の更なる検討や病因研究、抗プロラクチン療法の効果検討を課題に、前向き症例登録研究を開始いたしました。

本疾患の病態に迫るとともに、疾患概念の周知と医療連携システムの改善を図り、患者予後の改善へと結び付けられるよう、調査事務局としては継続して努力をしていく所存でおります。

是非とも本研究に引き続きご協力くださいますよう、お願い申し上げます。

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厚生労働科学研究（子ども家庭総合研究事業）
「乳幼児死亡と妊産婦死亡の分析と提言に関する研究」
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