Relaxin-2 and Soluble Flt1 Levels in Peripartum Cardiomyopathy

Results of the Multicenter IPAC Study

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ABSTRACT

OBJECTIVES This study explored the association of vascular hormones with myocardial recovery and clinical outcomes in peripartum cardiomyopathy (PPCM).

BACKGROUND PPCM is an uncommon disorder with unknown etiology. Angiogenic imbalance may contribute to its pathophysiology.

METHODS In 98 women with newly diagnosed PPCM enrolled in the Investigation in Pregnancy Associated Cardiomyopathy study, serum was obtained at baseline for analysis of relaxin-2, prolactin, soluble fms-like tyrosine kinase 1 (sFlt1), and vascular endothelial growth factor (VEGF). Left ventricular ejection fraction (LVEF) was assessed by echocardiography at baseline and 2, 6, and 12 months.

RESULTS Mean age was 30 ± 6 years, with a baseline of LVEF 0.35 ± 0.09. Relaxin-2, prolactin, and sFlt1 were elevated in women presenting early post-partum, but decreased rapidly and were correlated inversely with time from delivery to presentation. In tertile analysis, higher relaxin-2 was associated with smaller left ventricular systolic diameter (p = 0.006) and higher LVEF at 2 months (p = 0.01). This was particularly evident in women presenting soon after delivery (p = 0.02). No relationship was evident for myocardial recovery and prolactin, sFlt1 or VEGF levels. sFlt1 levels were higher in women with higher New York Heart Association functional class (p = 0.01) and adverse clinical events (p = 0.004).

CONCLUSIONS In women with newly diagnosed PPCM, higher relaxin-2 levels soon after delivery were associated with myocardial recovery at 2 months. In contrast, higher sFlt1 levels correlated with more severe symptoms and major adverse clinical events. Vascular mediators may contribute to the development of PPCM and influence subsequent myocardial recovery. (Investigation in Pregnancy Associate Cardiomyopathy [IPAC]; NCT01085955) (J Am Coll Cardiol HF 2016;4:380–8) © 2016 by the American College of Cardiology Foundation.
Peripartum cardiomyopathy (PPCM) is characterized by the development of heart failure late in pregnancy or in the months after delivery (1). Incidence varies geographically and in the United States is estimated at 1 in 1,000 to 4,000 live births (2–6). Presentation, clinical course, and outcomes are heterogeneous and can be associated with significant morbidity and mortality (1). In a recent prospective, multi-center study of 100 women with PPCM (IPAC [Investigations in Pregnancy Associated Cardiomyopathy]), 13% of patients suffered major events or had persistent severe cardiomyopathy (7). Factors associated with poorer outcomes include left ventricular (LV) dilation, severely depressed LV systolic function, black race, later presentation, and greater body mass index (7–10).

The etiology of PPCM is unknown. Suggested causes include genetic predisposition, hormonal abnormalities, abnormal immune response, and inflammation (11,12). Recently, it has been proposed that biological mediators with hemodynamic or vascular effects that are produced in the pregnant or peripartum state may play a role in the pathogenesis of PPCM (13–15). One of these factors is relaxin-2, a hormone produced in the corpus luteum of the ovary and in the heart (16,17). Relaxin-2 increases during pregnancy with hemodynamic and vasoactive effects, including increased cardiac output, plasma volume, heart rate, and renal blood flow and lower vascular resistance (18). In addition, relaxin-2 has anti-inflammatory, angiogenic, and anti-fibrotic properties (16). Both the vasodilatory and angiogenic effects of relaxin-2 are mediated in part by vascular endothelial growth factor (VEGF) (19,20), which may exert a protective effect in heart failure of multiple etiologies (21). This understanding has led to investigation of relaxin as a therapeutic target in heart failure (22) and raises the possibility of a role in treatment of PPCM.

Another biological factor proposed to contribute to PPCM is prolactin, a pituitary hormone that is secreted in the peripartum period and stimulates lactation (23). Under conditions of oxidative stress, prolactin is cleaved proteolytically to a 16-kDa fragment that has deleterious effects on endothelial cells and promotes inflammation and fibrosis. Higher levels of this angiostatic fragment, and the resulting angiogenic imbalance in the myocardium, have been hypothesized to play a role in the pathophysiology of PPCM (24). Another anti-angiogenic factor released from the placenta and endothelial cells in mid to late pregnancy is soluble fms-like tyrosine kinase 1 (sFlt1 or VEGF receptor 1). sFlt1 peaks at delivery and inhibits the activity of VEGF, leading to endothelial dysfunction and further angiogenic imbalance (14,25). Increased levels of sFlt1 may play an important role in the development of PPCM in some women (14,15), and recent data suggest that therapy targeting these pathways can rescue mouse models of PPCM (14,26). We report the analysis of relaxin-2, prolactin, sFlt1, and VEGF levels and their relationship to recovery of LV function and clinical outcomes in the IPAC cohort.

**METHODS**

**COHORT.** As previously reported, 100 women with newly diagnosed PPCM were enrolled at 30 centers (Online Appendix) between December 1, 2009 and September 30, 2012. All women were at least 18 years of age and had no previous history of cardiovascular disease, an estimated clinical LV ejection fraction (LVEF) of ≤0.45, and an evaluation consistent with nonischemic cardiomyopathy. Women with significant valvular disease, coronary disease, bacterial septicemia, ongoing drug or alcohol abuse, history of chemotherapy or chest radiation within 5 years, or a history of a previous cardiomyopathy were excluded.

**PROTOCOL.** The study protocol was approved by the institutional review boards at all participating centers, and informed consent was obtained from all subjects. At the time of enrollment, demographic information, including self-designated race, previous clinical evaluation, and current medical therapy, was recorded. Women were followed until 1 year postpartum. All hospitalizations and major cardiac events including death, cardiac transplantation, or implantation of a LV assist device (LVAD) were recorded.

**LV FUNCTION.** All subjects had an echocardiogram to assess LVEF at the time of enrollment and at 6 and 12 months post-partum. In addition, women enrolled early (within 6 weeks post-partum, n = 66) had a repeat assessment of LV function at 2 months. LV volumes and LVEF were assessed in a core laboratory (University of Pittsburgh) using biplane Simpson’s rule with manual tracing of digital images. LV end-diastolic diameter and LV systolic diameter were assessed in the parasternal long axis view. A subset of studies (22 of 310, 7%) were not available for assessment by the core laboratory due to format, and the LVEF calculated locally was used.

**ABBREVIATIONS AND ACRONYMS**

- BP = blood pressure
- GLM = general linear model
- LV = left ventricular
- LVAD = left ventricular assist device
- LVEF = left ventricular ejection fraction
- NYHA = New York Heart Association
- PPCM = peripartum cardiomyopathy
- sFlt1 = soluble fms-like tyrosine kinase 1
- VEGF = vascular endothelial growth factor

**SEE PAGE 389**
BIOMARKER ASSAYS. Serum was collected on 98 of 100 subjects at the time of entry, shipped overnight at room temperature to the core laboratory (University of Pittsburgh), and stored at −80 °C until the time of analysis. The following enzyme-linked immunoassays were obtained from R&D Systems (Minneapolis, Minnesota): Human Prolactin (Cat. No. DPRLO0) was run with 50 μl of undiluted sample per well in duplicate; Human Relaxin (Cat. No. DRL200) was run with 50 μl of undiluted sample per well in duplicate; and Human sFlt1 (Cat. No. DVR100B) was run with 100 μl of a 1:2 dilution per well in duplicate. Prolactin, relaxin, and sFlt1 enzyme-linked immunoassays were read at 450 nm on a Packard SpectraCount instrument, ALPCO (Salem, New Hampshire). VEGF was run on a MSD (Meso Scale Diagnostics, Rockville, Maryland). Human cytokine panel 1 was run on a multiplex plate (Cat. No. K15050D) with 50 μl of a 1:2 diluted sample per well in duplicate, read on an MSD QuickPlex SQ120 instrument and analyzed using the MSD software.

STATISTICAL ANALYSIS. The association of biomarker level with days post-partum was analyzed by Spearman correlation for non-normally distributed variables. To further evaluate the decrease in biomarker levels with increasing days post-partum, subjects were divided into quartiles based on days post-partum to enrollment and mean biomarker levels in quartiles compared by a general linear model (GLM) analysis of variance. For examination of demographics, medical therapy and clinical characteristics by time post-partum (quartiles), GLM and Fisher exact tests were used to compare continuous and categorical variables between groups, respectively.

BIOMARKER TERTILE ANALYSIS AND CHANGE IN LVEF OVER TIME. Because data were collected from 30 centers, we examined the effect of the 30 centers on LVEF at 2 months using variance components analysis and observed that center of enrollment had minimal effect on LVEF at 2 months (Var [center] = <1%; estimated using restricted maximum likelihood estimation and analysis of variance methods). Analysis subsequently ignored the effect of center of enrollment on this outcome. Similar analyses for all outcomes showed minimal effect of center; therefore, site of enrollment was excluded from further modelling considerations. To analyze the association of biomarkers on the improvement of LVEF over time, the mean LVEF at 4 time points (early, and at 2, 6, and 12 months) was compared for the entire cohort (n = 98) by tertiles of biomarker level, comparing the mean LVEF at each time point by GLM for subjects with high, intermediate, and low biomarker levels. In addition, given that for relaxin-2, prolactin, and sFlt1, the highest biomarker levels were primarily noted early, the tertile analysis was repeated for each biomarker just in subjects presenting early, that is, the first quartile (days 0 to 11 post-partum).

NEW YORK HEART ASSOCIATION FUNCTIONAL CLASS AND EVENT-FREE SURVIVAL. For comparison of New York Heart Association (NYHA) functional class, mean biomarker levels for subjects in each class were compared by GLM. For outcomes, an event was defined as death, cardiac transplantation, or LVAD implantation. Event-free survival was compared by tertile of biomarker level using the Kaplan-Meier log-rank method.

RESULTS

TIME POST-PARTUM. Subjects were enrolled from 0 to 95 days post-partum. The demographics of the IPAC cohort and levels of relaxin-2, prolactin, sFlt1, and VEGF are listed in Table 1 by quartiles of days post-partum to presentation (0 to 11, 12 to 24, 25 to 51, and 52 to 95 days). Subjects presenting earlier post-partum were less likely to be black and had a significantly higher NYHA functional class. Relaxin-2, prolactin, and sFlt1 were correlated significantly and inversely with the days post-partum to presentation; VEGF was not (Spearman correlations for relaxin-2, r = -0.42, p = 0.00001; prolactin, r = -0.51, p < 0.00001; sFlt1, r = -0.39, p = 0.0002; VEGF, r = -0.07, p = 0.48). The comparison of biomarker levels by quartiles of days post-partum to entry demonstrated significantly higher levels of relaxin-2 and sFlt1 in subjects presenting in the first quartile (0 to 11 days). Relaxin-2 and sFlt1 levels declined rapidly in the later quartiles to levels similar to those reported in non-post-partum subjects. Similar findings were evident for prolactin while no such relationship was evident for VEGF (Figure 1).

MYOCARDIAL RECOVERY AND REMODELING. For the entire cohort (n = 98), GLM analysis by tertiles of relaxin-2 level (lowest, <2.5; intermediate, 2.5 to 6.4; highest, >6.4 pg/ml) demonstrated that higher relaxin-2 levels were associated with a higher LVEF at 2 months (p = 0.01) (Figure 2A, Table 2). Analysis of heart rate and blood pressure (BP) by relaxin-2 tertiles revealed no differences either at entry (mean heart rate in low, intermediate, and high relaxin tertiles: 83 ± 17, 87 ± 12, 87 ± 19, p = 0.38; systolic BP: 110 ± 15,
111 ± 18, 114 ± 18, p = 0.35; diastolic BP: 67 ± 11, 71 ± 14, 73 ± 13, p = 0.10) or at 2 months (mean heart rate in low, intermediate, and high relaxin tertiles: 78 ± 17, 78 ± 11, 71 ± 12, p = 0.07; systolic BP: 107 ± 16, 107 ± 14, 106 ± 13, p = 0.88; diastolic BP: 68 ± 9, 67 ± 12, 65 ± 10, p = 0.22).

No differences were apparent by relaxin tertiles in medical therapy. At entry, 81% of subjects were on an angiotensin-converting enzyme inhibitor. Of the patients studied, 61% were on lisinopril with no differences in mean dose by relaxin tertile (lisinopril daily dose at entry by low, intermediate, high relaxin tertile: 12 ± 10, 10 ± 11, 11 ± 15 mg, p = 0.79). At 2 months, 86% were on an angiotensin-converting enzyme inhibitor, including 64% on lisinopril. There remained no differences in dose by relaxin tertile (low, intermediate, high relaxin tertiles: 14 ± 10, 11 ± 11, 12 ± 16 mg, p = 0.66). At entry, 88% were on a beta-blocker. 68% were on carvedilol with no significant differences in dose by relaxin tertile (carvedilol daily dose at entry by low, intermediate, high relaxin tertiles: 13 ± 6, 16 ± 12, 18 ± 16 mg, p = 0.17). At 2 months, 94% were on a beta-blocker, including 71% on carvedilol. There were no significant differences in dose at 2 months by relaxin tertile (low, intermediate, and high relaxin tertiles: 17 ± 12, 18 ± 11, 25 ± 19 mg, p = 0.09).

Given that relaxin-2 levels were predominantly increased in subjects presenting in the first quartile (0 to 11 days, n = 25), we repeated the previous analysis specifically in this subset presenting early post-partum. GLM analysis comparing 2 month LVEF by tertiles of relaxin-2 levels (lowest, intermediate, highest, >22 pg/ml) demonstrated that the association between relaxin-2 and the 2-month LVEF was more pronounced and remained significant (p = 0.02) (Figure 2B).

In the entire cohort, higher relaxin-2 levels were also associated with smaller LV systolic diameter (p = 0.006) at presentation with a trend toward lower LV end-diastolic diameter (p = 0.06); however, this was not evident in the subset presenting early (LV systolic diameter [p = 0.30] and LV end-diastolic diameter [p = 0.74]). Comparison of the mean LVEF over time by tertiles of prolactin, sFlt1, and VEGF levels did not demonstrate any relationship in either the overall IPAC cohort or in the quartile of subjects presenting early.

**SYMPTOMS AND EVENT-FREE SURVIVAL.** At the time of enrollment, sFlt1 levels were significantly higher in women with more severe heart failure based on NYHA functional class (p = 0.01) (Figure 3). Six subjects either died or required LVAD
support during the first year post-partum, and mean sFlt1 levels were higher in these subjects (592 ± 595 vs. 223 ± 393 pg/ml, p = 0.03). Event-free survival was significantly poorer for subjects in the highest sFlt1 tertile (p = 0.004) (Figure 4). In contrast, relaxin-2, prolactin, and VEGF were not associated with NYHA functional class or event-free survival.

**DISCUSSION**

Recent laboratory data suggest that vascular biomarkers may play a role in the development and progression of PPCM. In a well-defined cohort of women with newly diagnosed PPCM, we found that higher relaxin-2 levels were associated with more rapid myocardial recovery as evidenced by significantly higher LVEF at 2 months post-partum. Higher sFlt1 levels were associated with more severe functional limitation and major adverse clinical events. This is the first report of an association of relaxin and sFlt1 with outcomes in PPCM.

The presence of higher levels of relaxin-2 in patients with less LV remodeling and greater likelihood of recovery at 2 months suggests that it may play a cardioprotective role in PPCM. Relaxin is a naturally occurring human peptide that exerts systemic vasodilatory effects through stimulation of endothelin B receptors on vascular endothelial cells. Additional beneficial effects include sodium
and water excretion, reduced inflammation, and cardioprotection. Given the known physiologic activities of relaxin, possible mechanisms of benefit in PPCM include lower vascular resistance, decreased inflammation, increased angiogenesis, and decreased fibrosis. These findings raise the possibility of using recombinant human relaxin-2 (serelaxin) as a therapeutic agent to facilitate recovery in PPCM. Serelaxin has been shown to improve dyspnea in patients with acute heart failure (22) and is currently being tested in a phase III clinical study. Our data are observational in nature, and it is possible that relaxin-2 is a marker rather than a mediator of LV recovery. In this scenario, higher levels of relaxin-2 may indicate a good prognosis in women with PPCM, whereas lower levels may direct closer follow-up.

Higher levels of sFlt1 in patients with more advanced heart failure symptoms and higher likelihood of progression to death or LVAD support the proposed role of sFlt1 in the pathogenesis of PPCM as well as the potential for therapy directed at this pathway. sFlt1 is an antiangiogenic factor released from the placenta during the peripartum phase. Translational work from Patten et al. (14) has shown that sFlt1 and other VEGF inhibitors combine to create an anti-angiogenic environment that impairs both systolic and diastolic function.
These data also help to explain why pre-eclampsia and multiple gestations have been observed to be risk factors for the development of PPCM (27,28). The source of sFlt1 in PPCM subjects presenting late is not known, but placental remnants and other circulating cells have been postulated (29,30). Our findings suggest a potential role for sFlt1 in risk stratification for patients presenting with PPCM. The number of events in our study was small, however, and larger cohorts of PPCM will be needed to define the independent prognostic power of sFlt1 as well as the time-dependent nature of this circulating factor.

The findings of both relaxin-2 and sFlt1 lend credence to the concept of PPCM as a disorder, at least in part, of impaired angiogenesis. The discordance of demonstrated impact of relaxin-2 and sFlt1 on LV remodeling and recovery and clinical heart failure events is interesting. Given that the vasodilatory and proangiogenic effects of relaxin-2 are mediated in part by VEGF and that sFlt1 inhibits VEGF activity, this points to a common pathway of imbalance in angiogenesis driving PPCM and the associated clinical outcomes. The balance of these cardioprotective and pathologic mediators may be crucial in determining occurrence of PPCM and clinical outcomes for these patients. Further study may aid in developing a prognostic framework using these markers.

In this cohort, we observed no relationship between prolactin and LV recovery or adverse clinical events. Although therapeutic intervention with bromocriptine, an inhibitor of prolactin release, has been reported to improve clinical outcomes for PPCM in a small pilot investigation (31), a large non-randomized German registry of 115 women with PPCM (in which 67% were treated with bromocriptine) reported no differences in the percentage of women with full recovery based on bromocriptine therapy (32). A randomized trial currently underway should help determine the role of bromocriptine therapy (33). Although as expected prolactin levels tended to be higher in the 15% of this cohort who were breast feeding at the time of enrollment, no difference in LVEF was observed during follow-up (7). The 16-kDa prolactin cleavage product was not measured in the current study, and we cannot draw conclusions about its potential role in either the pathogenesis of PPCM or subsequent myocardial recovery.

**STUDY LIMITATIONS.** There was significant variability in the time to presentation within the IPAC cohort, which may confound the results based on referral bias. Analysis of subjects enrolled in the first quartile based on days post-partum was chosen as a way to address this issue. In addition, within the cohort, there were patients that had significant outlying values for sFlt1 and VEGF, which could cloud detectable associations with clinical outcomes.

**CONCLUSIONS**

In the IPAC cohort, higher relaxin-2 levels, particularly when obtained early post-partum, were associated with early myocardial recovery and a higher LVEF at 2 months. In contrast, increased sFlt1 levels were associated with higher NYHA functional class and a greater risk of major adverse events. These findings support the concept that PPCM is, at least in part, a disorder of vascular homeostasis and suggest that these biomarkers may not only assist in the determination of prognosis in PPCM, but also serve as targets for future therapy.

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COMPETENCY IN MEDICAL KNOWLEDGE: PPCM is a cardiomyopathy with unknown etiology and diverse clinical outcomes. Recently, vascular biomarkers have been proposed to play a role in the development and progression of PPCM. In this cohort, elevated relaxin-2 was associated with early myocardial recovery and elevated sFlt1 with adverse clinical outcomes. These findings support the idea that vascular homeostasis may play a role in PPCM. These mediators may provide prognostic information and future targets for therapy in PPCM.

PERSPECTIVES

TRANSLATIONAL OUTLOOK: Support for a vascular component to the pathophysiology of PPCM highlights the need for further study of vascular biomarkers in this disorder. Whether these agents can serve as prognostic indicators in patients with PPCM needs to be determined. They may also serve as potential therapeutic targets in PPCM.

REFERENCES


**KEY WORDS** cardiomyopathy, heart failure, hormones, pregnancy and post-partum

**APPENDIX** For a list of the members of the IPAC Investigators and their institutions, please see the online version of this article.