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Heart Failure

Imbalanced Angiogenesis in Peripartum Cardiomyopathy
— Diagnostic Value of Placenta Growth Factor —

Alexandre Mebazaa, MD, PhD; Marie-France Seronde, MD, PhD; Etienne Gayat, MD, PhD; Kemi Tibazarwa, MD, PhD; Dilly O.C. Anumba, MD; Najla Akrout, MD; Malha Sadoune; Jamela Sarb, MD; Mattia Arrigo, MD; Justina Motiejunaite, MD; Said Laribi, MD, PhD; Matthieu Legrand, MD, PhD; Lydia Deschamps, MD; Loubina Fazal, PhD; Lila Bouadma, MD, PhD; Corinne Collet, PhD; Philippe Manivet, PhD; Alain Cohen Solal, MD, PhD; Jean-Marie Launay, MD, PhD; Jane-Lise Samuel, MD, PhD; Karen Sliwa, MD, PhD

Background: Concentrations of the anti-angiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) are altered in peripartum cardiomyopathy (PPCM). In this study we investigated changes in the angiogenesis balance in PPCM.

Methods and Results: Plasma concentrations of sFlt-1 and the pro-angiogenic placenta growth factor (PlGF) were determined in patients with PPCM during the post-partum phase (n=83), in healthy women at delivery (n=30), and in patients with acute heart failure (AHF; n=65). Women with cardiac failure prepartum or associated with any form of hypertension, including pre-eclampsia, were excluded. Compared with non-pregnant women, in women with AHF and PPCM, median PlGF concentrations were greater (19 [IQR 16–22] and 98 [IQR 78–126] ng/mL, respectively; P<0.001) and the sFlt-1/PlGF ratio was lower (9.8 [6.6–11.3] and 1.2 [0.9–2.8], respectively; P<0.001). The sFlt-1/PlGF ratio was lower in PPCM than in normal deliveries (1.2 [0.9–2.8] vs. 94.8 [68.8–194.1], respectively; P<0.0001). The area under the curve for PlGF (cut-off value: 50ng/mL) and/or the sFlt-1/PlGF ratio (cut-off value: 4) to distinguish PPCM from either normal delivery or AHF was >0.94. Median plasma concentrations of the anti-angiogenic factor relaxin-2 were lower in PPCM and AHF (0.3 [IQR 0.3–1.7] and 0.3 [IQR 0.3–1] ng/mL, respectively) compared with normal deliveries (1,807 [IQR 1,101–4,050] ng/mL; P<0.001).

Conclusions: Plasma of PPCM patients shows imbalanced angiogenesis. High PlGF and/or low sFlt-1/PlGF may be used to diagnose PPCM.

Key Words: Angiogenesis; Peripartum cardiomyopathy (PPCM); Placental growth factor; Relaxin-2

The incidence and prevalence of pregnancy-related heart disease is increasing worldwide.[1] One of the conditions that is increasingly being recognized as an important contributor to early (<2 days postpartum) and late (up to 1 year postpartum) maternal death is peripartum cardiomyopathy (PPCM).[2] PPCM most commonly presents with acute heart failure in the weeks following pregnancy, leading to urgent hospitalization.

Editorial p????

Despite improvements in diagnosis and management, PPCM continues to have significant morbidity and mortality.[3,4] For reasons unknown, the time of onset of symptoms of PPCM varies according to region and ethnic group, with Black Africans presenting almost exclusively postpar-

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UMR-S 942 INSERM, Lariboisière Hospital, Paris (A.M., M.-F.S., E.G., N.A., M.S., M.A., J.M., S.L., M.L., L.F., P.M., A.C.S., J.-M.L., J.-L.S.); Paris Diderot University, Sorbonne Paris Cité, Paris (A.M., E.G., M.S., M.L., L.F., A.C.S., J.-L.S.); Department of Anaesthesiology and Critical Care (A.M., E.G., N.A., M.A., J.M., M.L., J.-L.S.), Department of Emergency Medicine (S.L.), Biochemistry Department (C.C., P.M., J.-M.L.), Department of Cardiology (A.C.S.), Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, Paris; Department of Cardiology EA3920, University Hospital Jean Minjoz, Besancon (M.-F.S.), France; Hatter Institute for Cardiovascular Research in Africa and MRC Inter-Cape Heart Unit, Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town (K.T., K.S.); Soweto Cardiovascular Research Unit, University of the Witwatersrand, Johannesburg (K.T., K.S.), South Africa; Gynecology, Obstetric, Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield Medical School, Sheffield (D.O.C.A., J.S.), UK; Department of Pathology (L.D.), Department of Medical ICU (L.B.), Bichat Hospital, AP-HP, Paris; and Paris Descartes University, Paris (J.-M.L.), France
The last two authors contributed equally as senior authors (J.-L.S., K.S.).
Mailing address: Karen Sliwa, MD, PhD, Hatter Institute for Cardiovascular Research in Africa, Faculty of Health Sciences, University of Cape Town, Private Bag X3, Observatory, 7935, South Africa. E-mail: Karen.Sliwa-Hahnle@uct.ac.za

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Table 1. Clinical Characteristics of PPCM Patients, Controls, and Parturients

	Healthy non-pregnant (n=29)	Pregnant (n=10)	At delivery (n=30)	PPCM patients (n=83)	AHF non-pregnant subjects (n=65)
Age (years)	28 (24–31)	28 (25–38)	33 (30–37)	28 (24–34)	54 (48–61)
LVEF (%)	60 (56–66)	–	–	30 (22–35)	30 (20–40)
Parity	3 (2–3)	–	–	2 (1–3)	–
SBP (mmHg)	120 (114–125)	–	–	111 (107–122)	128 (105–158)
DBP (mmHg)	70 (70–78)	–	–	70 (69–79)	77 (69–95)
Creatinine (μmol/L)	–	–	–	78 (70–94)	105 (77–131)

Data are presented as the median (interquartile range). All subjects in all groups except the acute heart failure (AHF) group were women. In the AHF group, 45% of subjects were female. Additional information on AHF non-pregnant subjects is provided in Table S1. DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; PPCM, postpartum cardiomyopathy; SBP, systolic blood pressure.

tum.[5] In addition, the incidence, contributing comorbidities (e.g., hypertension and pre-eclampsia), and outcome seem to be variable, depending on the geographical region, ethnic background, and the inclusion criteria of the given study.[5,6] It was recently suggested that PPCM may be associated with increased placental production of soluble fms-like tyrosine kinase-1 (sFlt-1), as in pre-eclampsia, for which it has long been thought to be associated.[7] The anti-angiogenic factor sFlt-1 reduces free circulating concentrations of the angiogenic factors placenta growth factor (PlGF) and vascular endothelial growth factor (VEGF) and blunts the beneficial effects of these angiogenic factors on the maternal endothelium.[8] Plasma concentrations of pro-angiogenic factors (PlGF or VEGF) in PPCM were not measured in previous studies, which would have enabled assessment of the sFlt-1/PlGF balance, nor did previous studies measure plasma relaxin-2, another anti-angiogenic marker.[7] Furthermore, expression levels of angiogenic factors in myocardial tissue in PPCM has also not been described.

We hypothesized that the angiogenesis balance may be altered in PPCM during the postpartum phase. Thus, the aim of the present study was to assess whether plasma concentrations of pro- and anti-angiogenic factors were dysregulated in patients presenting with PPCM compared with control groups and, if so, whether this could facilitate early diagnosis.

Methods

Plasma samples in the postpartum phase were collected prospectively from PPCM patients (n=83) seen at the Chris Hani Baragwananth Hospital, Soweto, the Groote Schuur Hospital, Cape Town (South Africa), or referred to the Assistance Publique-Hôpitaux de Paris, France. Inclusion criteria were as described previously,[9] namely: (1) age ≥16 and ≤40 years; (2) symptoms of congestive heart failure that developed in the last months of pregnancy or during the first 5 months postpartum; (3) no other identifiable cause for heart failure; (4) left ventricle ejection fraction ≤45% by transthoracic echocardiography; and (5) sinus rhythm. Exclusion criteria were: (1) significant organic valvular heart disease; (2) systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg; (3) clinical conditions other than cardiomyopathy that could increase plasma concentrations of inflammatory markers; and (4) severe anemia (hemoglobin <9 g/dL). No patients with prepartum PPCM were included in the present study because in South Africa PPCM presenting prepartum is very rare; in addition, patients with postpartum cardiac failure associated with any form of hypertension, including pre-eclampsia, were excluded because we have shown previously that they have different characteristics.[6] Clinical assessment, echocardiography, and blood analysis for PPCM patients were performed at the time of admission and hospitalization. Plasma samples were also collected from healthy non-pregnant women (n=29), healthy pregnant women (n=10; 20–36 weeks gestation), and healthy women after delivery (within 24 h of delivery; n=30). Plasma samples from relatively young patients with acute heart failure (AHF; n=65; almost half of whom were women) from the "Biomarcoeurs" cohort[10] were collected at admission. None of the patients was using heparin when blood samples were taken.

The primary objective of the present study was to focus on women after delivery and to assess whether plasma concentrations of factors contributing to the angiogenic balance, especially the sFlt-1/PlGF ratio, can distinguish between women with PPCM and those with a normal delivery, as well as between women with PPCM and those patients with other, non-pregnancy-related, causes of AHF.

The present study was approved by the relevant human research ethics committees of the participating institutes and complies with the Declaration of Helsinki. All study participants, including healthy women, provided written informed consent before entering into the study. The present study is a sub-study of the cohort registered with ClinicalTrials.gov (ID: NCT01374880).

Biomarker Testing

During initial patient examination, and within 4 h of the unscheduled admission for acute dyspnea, blood samples were collected in plastic tubes containing EDTA, and aliquots of EDTA-plasma samples were stored in a standardized manner at −80°C until further analysis. Prior to analysis, samples were centrifuged through Nanosep microconcentrator filters (Pall Filtron, Northborough, MA, USA) to remove proteins. Plasma concentrations of the different plasma biomarkers were quantified using commercially available kits. N-Terminal pro B-type natriuretic peptide (NT-proBNP) and sFlt-1 were quantified on a Roche Cobas E601 analyzer (Roche, Meylan, France), whereas copeptin and mid-regional pro-adrenomedullin (MR-proADM)[9] were quantified on a Kryptor compact analyzer (Thermo-Fisher Scientific, Pittsburgh, PA, USA). An ELISA was used to determine plasma concentrations of soluble ST2 (sST2), as described previously[4] (Presage

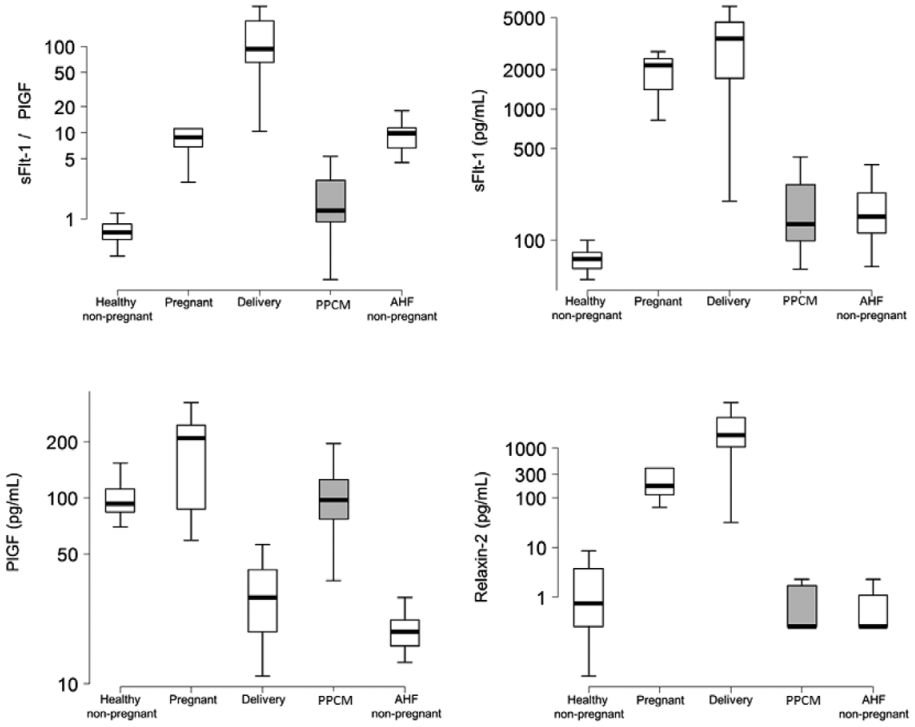

Figure 1. Comparison of plasma soluble fms-like tyrosine kinase-1 (sFlt-1), placenta growth factor (PlGF), the sFlt-1/PlGF ratio, and relaxin-2 in healthy and diseased peripartum women, as well as in acute heart failure (AHF) patients. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range. PPCM, postpartum cardiomyopathy.

Table 2. Plasma Concentrations of Various Angiogenic and Cardiovascular Biomarkers in the Different Groups

	Healthy non-pregnant (n=29)	Pregnant (n=10)	At delivery (n=30)	PPCM (n=83)	AHF non-pregnant (n=65)
NT-proBNP (pg/mL)	34 (20–49)	–	55 (19–178)	4,323 (1,361–7,379)*	3,156 (706–6,175)
VEGF (pg/mL)	92 (84–109)	207 (82–345)	91 (76–142)	269 (123–404)*	221 (143–267)
sFlt1/VEGF ratio	0.8 (0.6–0.9)	9 (4.4–12.1)	33.4 (19.1–47.1)	0.6 (0.3–1.3)*	1 (0.7–1.2)†
sST2 (ng/mL)	25.1 (18.5–29.5)	–	131.1 (96.2–208.6)	38.4 (21.9–72.4)*	82.2 (55–127.2)†
MR-proADM (nmol/L)	0.4 (0.4–0.6)	–	0.2 (0.1–0.4)	0.7 (0.4–1.1)*	1.1 (0.8–1.7)†
Copeptin (pmol/L)	10 (6.4–14.6)	–	12.6 (4.8–28.2)	15.6 (8.5–23.8)	16.2 (10.1–34.1)

Data are presented as the median (interquartile range). All subjects in all groups except the acute heart failure (AHF) group were women. In the AHF group, 45% of subjects were female. *P<0.05 compared with delivery (Mann-Whitney test); †P<0.05 compared with the postpartum cardiomyopathy (PPCM) group (Mann-Whitney test). Of note, there were no associations observed between pro- and anti-angiogenic factors and either left ventricular ejection fraction or N-terminal pro B-type natriuretic peptide (NT-proBNP) in PPCM patients (for placenta growth factor [PlGF], ρ=–0.08 [–0.32, 0.16] and –0.16 [–0.42, 0.13], respectively; for soluble fms-like tyrosine kinase-1 (sFlt-1), ρ=0.08 [–0.16, 0.32] and –0.02 [–0.30, 0.27], respectively; and for relaxin-2, ρ=0.19 [–0.11, 0.47] and 0.23 [–0.08, 0.51], respectively). MR-proADM, mid-regional pro-adrenomedullin; sST2, soluble ST2; VEGF, vascular endothelial growth factor.

ST2 Assay Kit; Critical Diagnostics, San Diego, CA, USA), VEGF, PlGF, and relaxin-2 (R&D Systems, Minneapolis, MN, USA). Details of the ELISA quantification of plasma markers used in the present study are described in **Table S1**. Blinded measurements were performed in 2 separate laboratories (U-942 INSERM [Paris, France] and the Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield Medical School [Sheffield, UK]). Results showed a high level of agreement (R^2=0.78; 95% confidence interval [CI] 0.67–0.86; **Figure S1**).

Analyses of Myocardial Biopsies

Analyses were performed on myocardial biopsies taken at the time of heart transplantation from 6 donor hearts not suitable for transplantation (controls), 13 explanted hearts from patients transplanted for end-stage heart failure with idiopathic dilated cardiomyopathies (DCM; n=11), DCM lasting more than 24 months before explantation, and from PPCM patients (n=2; 17 days and 20 months after onset of the disease). Clinical data have been described previously.[11] Quantative polymerase chain reaction (qPCR) was used to measure the mRNA expression of BNP, PlGF,

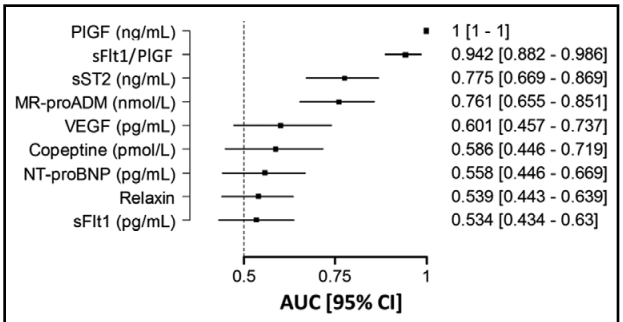

Figure 2. Results of receiver operating characteristic analyses for the diagnosis of PPCM among women in the peripartum phase. AUC, area under the curve; CI, confidence interval; MR-proADM, mid-regional pro-adrenomedullin; NT-proBNP, N-terminal pro B-type natriuretic peptide; sST2, soluble ST2; VEGF, vascular endothelial growth factor. Other abbreviations as in Figure 1.

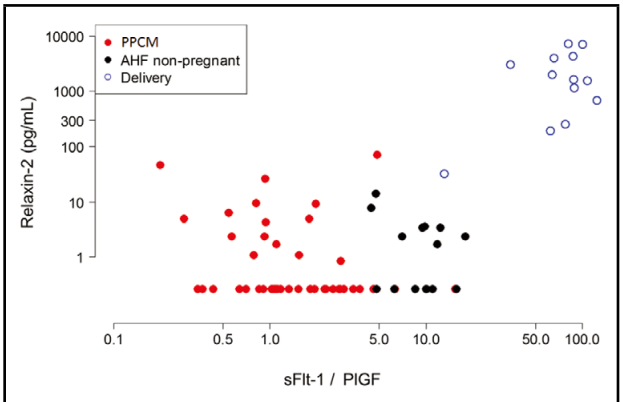

Figure 3. Diagnostic properties of plasma relaxin-2 and the soluble sFlt-1/PlGF ratio in PPCM. Combining these 2 biomarkers for the diagnosis of PPCM in peripartum women had a specificity of 0.95, a sensitivity of 0.74, a positive predictive value of 0.92 and a negative predictive value of 0.82. Abbreviations as in Figure 1.

VEGF, relaxin, Flk1, Flt1 and sFlt1. Protein expression of Flk1, Flt1, VEGFa, PlGF and relaxin-2 was evaluated using immunoblotting. Tissue localization of the proteins was determined by immunofluorescence. For further details see **Supplementary Methods**.

Statistical Analysis

Results are expressed as median values with the interquartile range (IQR) or as counts and percentages. Comparisons of biomarkers among predefined groups of patients in the postpartum phase or not (see above for details) were made using Kruskal-Wallis or Mann-Whitney tests. The discriminative ability of each biomarker was evaluated by the c-statistic, identical to the area under the receiver operating characteristics (ROC) curve. Two outcomes were considered: (1) PPCM vs. delivery; and (2) PPCM vs. AHF non-pregnant. Statistical analyses were performed using R statistical software (http://www.r-project.org/). Two-sided $P<0.05$ was considered significant.

Results

The clinical characteristics of the different study groups, namely healthy non-pregnant women, healthy peripartum women (i.e., antepartum [median 29 weeks gestation] and at delivery [<24 h]), women with PPCM (median admission time 4.0 [IQR 3.4–4.6] weeks after delivery; all breastfeeding), and subjects with AHF, are given in **Table 1** and **Table S2**.

Plasma Concentrations of Angiogenic Factors During Pregnancy and at Delivery

Plasma concentrations of the pro-angiogenic (PlGF, VEGF) and anti-angiogenic biomarkers (sFlt-1 and relaxin-2), as well as the sFlt-1/PlGF ratio, were all higher in pregnant women than in healthy non-pregnant women (**Figure 1**; **Table 2**). At delivery, PlGF and VEGF concentrations decreased, whereas sFlt-1 and relaxin-2 concentrations remained high and the sFlt-1/PlGF ratio was increased compared with values during pregnancy (**Figure 1**; **Table 2**).

Plasma Concentrations of Angiogenic and Cardiovascular Factors in Women With PPCM

Compared with non-pregnant AHF subjects, women with PPCM had similar high levels of NT-proBNP (**Table 2**), but different pro-angiogenic and anti-angiogenic profiles. Indeed, compared with AHF subjects, women with PPCM had much higher PlGF concentrations (median [IQR] 19 (16–22) and 98 (78–126) ng/mL, respectively; $P<0.001$), similar levels of VEGF and sFlt-1, and a lower sFlt-1/PlGF ratio (9.8 [6.6–11.3] and 1.2 [0.9–2.8], respectively; $P<0.001$; **Figure 1**; **Table 2**). Subjects in the AHF and PPCM groups had very low plasma concentrations of relaxin-2 (**Figure 1**), but concentrations of sST2 and MR-proADM were lower in the PPCM than AHF group (38.4 [21.9–72.4] vs. 82.2 [55–127.2], respectively, for sST2 [$P<0.0001$]; 0.7 [0.4–1.1] vs. 1.1 [0.8–1.7], respectively, for MR-proADM [$P=0.00012$]). Of note, circulating concentrations of the biomarkers evaluated herein did not differ according to age or sex in the AHF non-pregnant group (**Table S3**). No association was observed between pro- and anti-angiogenic factors and either left ventricular ejection fraction (LVEF) or NT-proBNP in PPCM patients.

Compared with women at delivery, women with PPCM had higher PlGF and VEGF concentrations (median [IQR] 97.5 [77.5–125.5] vs. 29 [19.2–40.8] ng/mL for PlGF [$P<0.0001$]; 268.5 [123–403.8] vs. 91 [6–141.5]) ng/mL for VEGF [$P<0.001$]) and lower sFlt-1 concentrations (132.2 [99–265] vs. 3454.5 [1,716–4,566] pg/mL; $P<0.0001$), leading to a lower sFlt-1/PlGF ratio in the PPCM group (1.2 [0.9–2.8] vs. 95 [69–194]; $P<0.0001$). Relaxin-2 concentrations were lower in the PPCM group (0.3 [0.3–4.3] ng/mL) than at normal delivery (1,807 [1,101–4,050] ng/mL; $P<0.001$; **Figure 1**), as were sST2 concentrations (**Table 2**).

Diagnostic Properties of Biomarkers Studied in PPCM vs. Non-Pregnant AHF

As indicated in **Figures 2**,**3** and **Table S4**, sFlt-1/PlGF and PlGF have striking diagnostic value in distinguishing PPCM from healthy women with a normal delivery or non-pregnancy-related AHF. ROC analyses comparing PPCM with non-pregnant AHF showed that the sFlt-1/PlGF ratio can discriminate PPCM from AHF (threshold at a cut-off value of 4). Similarly, PlGF discriminated between PPCM and AHF (threshold at a cut-off value of 32 ng/mL). The specificity and sensitivity of the sFlt-1/PlGF

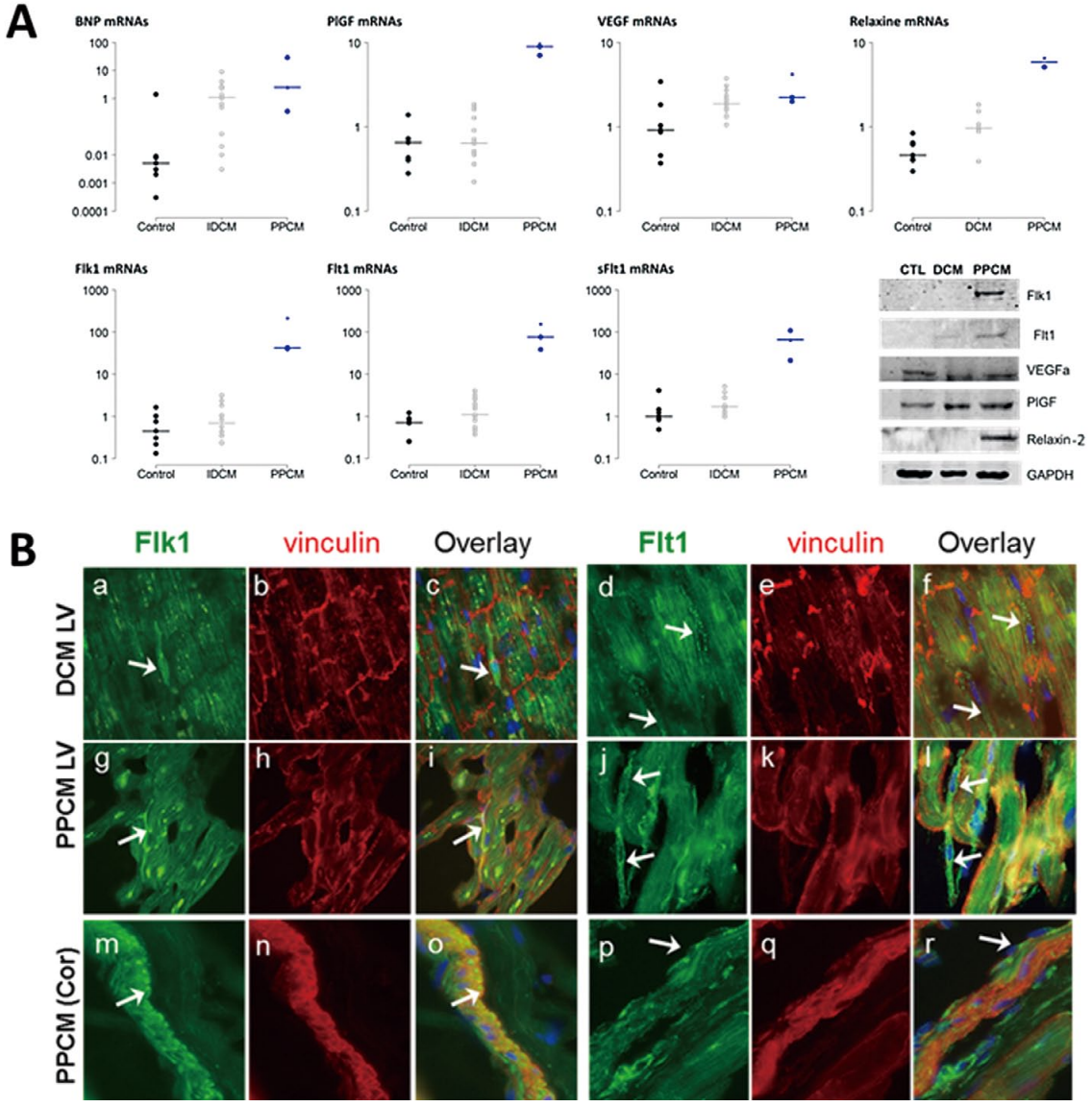

Figure 4. Expression and distribution of angiogenic and cardiovascular markers in cardiac tissues. (**A**) Relative mRNA expression of different markers in the control (CTL), idiopathic dilated cardiomyopathy (DCM) and PPCM groups, as determined using quantitative polymerase chain reaction. All data are for left ventricle (LV) samples, except for the asterisks in the PPCM group, which are from the right ventricle (RV). BNP, B-type natriuretic peptide; Flk1, fetal liver kinase 1; Flt1, fms-like tyrosine kinase-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Other abbreviations as in Figures 1,2. A representative immunoblot for Flk-1, Flt-1, VEGF, PlGF, and relaxin-2 expression in LV samples clearly shows strong expression of all markers in PPCM tissue in contrast with tissues from the other groups. (**B**) Double-immunolabeling of the LV myocardium and coronary artery (Cor) in samples from the DCM and PPCM groups. Arrows indicate strong positive labeling of cells for Flt-1 or Flk-1. Note the strong Flt-1 labeling in endothelial cells of PPCM tissue.

ratio in diagnosing PPCM was 1.0 and 0.87–1.0, respectively (**Table S4**). As seen in **Figure 2**, sST2 and MR-proADM also exhibited good diagnostic value in diagnosis PPCM, although their sensitivity and specificity were much lower than those of PlGF or the sFlt-1/PlGF ratio. **Figure 3** further shows that, when combined, sFlt-1/PlGF and relaxin-2 levels may define 3 clusters of patients: PPCM, AHF and delivery.

Myocardial biopsies from 2 explanted PPCM subjects were compared with myocardial biopsies of explanted subjects with DCM or control hearts. In biopsies from PPCM subjects, increased mRNA and protein expression was found for PlGF, sFlt-1, and relaxin-2 in myocardium and sFlt-1 in the endothelium (**Figure 4** and **Table S5**).

Discussion

Impaired Angiogenesis in the Plasma of PPCM Patients

The present study revealed an angiogenic imbalance in favor of angiogenic factors in PPCM patients. Indeed,

PlGF was markedly higher and the sFlt-1/PlGF ratio markedly lower in PPCM than healthy women at the time of delivery. Furthermore, plasma sFlt-1 was low in the present PPCM cohort compared with women at delivery, and this is in line with plasma Flt-1 concentrations recently measured in peripartum cardiomyopathy patients.[7] sFlt-1 is known to antagonize interactions of PlGF and VEGF with their endothelial receptors by binding to them. Accordingly, low plasma concentrations of sFlt-1 measured in the present study could explain the high plasma concentrations of the angiogenic factors PlGF and VEGF in PPCM patients. A low sFlt-1/PlGF ratio in PPCM patients was unlikely to be related to the AHF episodes because non-pregnant AHF subjects had a very high sFlt-1/PlGF ratio, almost 10-fold higher than that in PPCM patients.

An angiogenic imbalance in PPCM has been previously observed in experimental studies: Patten et al[7] demonstrated that mice that lack cardiac peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α), a powerful regulator of angiogenesis, develop profound PPCM. Importantly, pro-angiogenic therapy with recombinant VEGF allowed for amelioration of PPCM. In contrast, Seno et al[12] reported that an anti-PlGF neutralizing antibody prevented pressure overload-induced cardiac dysfunction in an sFlt-1-knockout mouse model. These experimental studies indicate that therapies targeting angiogenic imbalance in PPCM may be successful. This requires further exploration.

Relaxin-2 is a naturally occurring peptide initially identified as a reproductive hormone and a key player in maternal hemodynamic and renal adjustments that accommodate pregnancy.[13] Intravenous serelaxin (a relaxin-2 analog) has recently been shown to improve clinical symptoms and organ function when given at the time of hospital admission to patients with AHF.[14] In the present study, plasma concentrations of relaxin-2 (measured in 2 independent laboratories) were very low in patients admitted with AHF, regardless of whether they were non-pregnant AHF or PPCM patients. This contrasts with findings of a previous study, which reported increased plasma relaxin-2 concentrations in stable chronic heart failure, especially in patients with New York Heart Association Class IV heart failure.[15] Although relaxin-2 has been shown to stimulate VEGF expression,[13] mechanisms regulating relaxin-2 expression (except placental overexpression during pregnancy) and release in the plasma are unknown. Interestingly, plasma concentrations of relaxin-2 have been shown to be very high during normal human pregnancy (ranging between 500 and 1,500 pg/mL), with peak values in the first trimester that decline in the second and third trimesters.[16] This observation is consistent with the markedly increased production of relaxin from gestational tissue. Outside of pregnancy, as demonstrated in the present study, plasma relaxin concentrations decline sharply and are primarily derived from non-gestational tissue, such as the myocardium.

Myocardial biopsies of PPCM patients performed in the present study show a global increase in the mRNA and protein expression of all pro- and anti-angiogenic factors measured in PPCM compared with control and DCM patients. However, pronounced differences in sFlt-1 and relaxin-2 (*RLN2*) mRNA expression in cardiac tissues of PPCM and of AHF patients contrasts with the lack of difference in circulating sFlt-1 and relaxin-2 concentrations between these 2 groups. This may suggest that the contribution of cardiac sFlt-1 or relaxin-2 to plasma levels is minor. In contrast, myocardial expression and circulating concentrations of PlGF were consistently increased in PPCM compared with AHF and control subjects, suggesting that, in PPCM, the heart may be an important source of circulating PlGF. This needs to be confirmed in further studies.

Little information is available on the myocardium in human PPCM. Previous histological studies demonstrated structural remodeling of myocardial capillaries in PPCM patients suggestive of endothelial damage and disturbed microcirculation.[17] The findings of the present study further confirm the importance of angiogenic imbalance in PPCM.

In summary, the present study showed that PPCM was associated with altered angiogenesis in both the plasma and hearts of PPCM patients.

Clinical Relevance

There is an increased awareness of PPCM promoted by a dedicated working group at the Heart Failure Association of the European Society of Cardiology (www.escardio.org) and the International Registry on Peripartum Cardiomyopathy, which is part of the EURObservational Research Programme, with more than 450 PPCM patients from 40 countries recruited thus far (http://www.eorp.org).[18] In addition, the number of original and review publications on PPCM on PubMed has increased substantially over the past 20 years. However, PPCM remains a diagnosis of exclusion: all patients should undergo investigations to identify any alternative etiology for heart failure. In this context, there is a need for diagnostic biomarkers. The present study showed that PPCM patients presenting post partum, the most frequent form of PPCM, have a unique biomarker feature: PlGF and/or the sFlt-1/PlGF ratio, readily available biomarkers in obstetric hospitals, may, in the days or weeks following delivery, discriminate PPCM from other causes of acute dyspnea, whether they are related to acute decompensated heart failure or not. Other cardiovascular biomarkers, namely copeptin, MR-proADM, and sST2, performed less well in the diagnosis of PPCM.

Study Limitations

In the present study, PPCM patients were included from a limited number of countries and future studies should have a wider geographic representation. Expanding the study to a larger population of different ethnic backgrounds, including patients presenting prepartum and with contributing comorbidities, such as pre-eclampsia, is now achievable in the context of the EURObservational Research Programme. In countries with a high rate of pregnant women with a history of heart disease, acute dyspnea during the peripartum phase may be related to decompensation of cardiac disease or to PPCM. It would have been of interest to assess angiogenesis in women after delivery who had been admitted for acute dyspnea not related to PPCM; however, such patients and such presentation are rare and so difficult to include in a study. It has to be noted that that the patients with PPCM in the present study were diagnosed during the postpartum and not the antepartum period. In order to determine whether the biomarkers identified as possible diagnostic tools for PPCM in the present study are more widely applicable to the general population, future

studies should include all patients admitted for acute dyspnea during the peripartum phase and assess plasma PlGF and/or the sFlt-1/PlGF ratio in these patients. The number of myocardial biopsies for PPCM in the present study was extremely low. However, for many biomarkers tested, mRNA and protein expression differed considerably in PPCM compared with DCM and controls subjects. Despite this, larger studies are needed in the future to confirm the findings presented herein.

One important limitation of the present study is related to the highly specific physiological changes that take place during the peripartum phase, which involves upregulation and fast clearance of pregnancy-related hormones, growth factors, and cytokines. In particular, concentrations of PlGF, sFlt-1 and relaxin-2 are markedly elevated towards the end of pregnancy and in the first days after delivery, but return to normal thereafter.[19] In the present study, we did not obtain plasma samples from "normal" pregnant women 4 weeks after delivery. Therefore, the differences observed in the aforementioned serum markers may be due, at least in part, to differences in the timing of blood sampling between the immediate postpartum controls and the PPCM patients, in which sampling was performed at the time of diagnosis. Therefore, the generally lower relaxin-2 concentrations in the serum of PPCM patients could also be explained by the later timing of blood sampling in relation to delivery and may simply reflect normal clearance of the hormone postpartum. However, persistence of high PlGF concentrations and very low values for the sFlt-1/PlGF ratio in PPCM patients is unusual in the postpartum phase and may be markers of PPCM. In order to better assess the postpartum time course of these biomarkers, future studies should compare women in the days, weeks, and months following delivery and, ideally, include breastfeeding and non-breastfeeding women. The effects of lactation on physiological regression of postpartum left ventricular hypertrophy,[20] the role of prolactin products,[21] and reverse remodeling under healthy conditions and in diseased hearts deserve further research, because these factors may have implications for a number of cardiovascular diseases in women who are pregnant.

Another important limitation of the present study is the lack of echocardiographic characteristics other than LVEF. It would be interesting to describe echocardiographic profiles of cardiac dysfunction in PPCM patients and to investigate correlations with pro- and anti-angiogenic factors.

Conclusions

The present study showed impaired angiogenesis in the plasma and myocardial tissues of PPCM patients. Compared with other biomarkers that have been shown to be altered in PPCM,[22] such as microRNAs,[23] both PlGF and/or the sFlt-1/PlGF ratio are readily available biomarkers in obstetrics that can be used to ascertain a diagnosis of PPCM in acute dyspneic patients. The results of the present study highlight the close interaction between the cardiovascular system and the placenta, providing further evidence for the already described cardioplacental syndrome.[24]

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Conflict of Interest

A.M. reports being on advisory boards for Bayer, Cardiorentis, and The Medicines Company and receiving lecture fees from Alere, Edwards, Orion, Novartis, Vifor, and Thermo-Fisher. A.C.S. has been a consultant for Novartis. A patent application is pending on biomarkers in cardiovascular disease during the peripartum period. The patent belongs to the Assistance Publique-Hôpitaux de Paris (France), to which A.M. and J.-M.L. belong, and the University of Cape Town (South Africa), to which K.S. belongs. The 3 inventors (J.-M.L., K.S., A.M.) may benefit from the patent application. The remaining authors have no conflicts of interest to declare.

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Supplementary Files

Supplementary File 1

Supplementary Methods

Figure S1. Comparison of relaxin-2 measures between INSERM U942 Laboratory (Paris, France) and the Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield Medical School (Sheffield, UK).

Table S1. Characteristic of the ELISA quantification used throughout the study

Table S2. Additional characteristics of non-pregnant AHF patients (n=65)

Table S3. Comparisons of biomarkers among non-pregnant AHF patients according to sex and age

Table S4. Specificity and sensitivity of biological markers to discriminate PPCM from non-pregnant AHF subjects

Table S5. Clinical characteristics of the 2 PPCM patients who underwent myocardial biopsies

Please find supplementary file(s);
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